Disproportionate Contribution of Riparian Inputs to Organic Carbon Pools in Freshwater Systems

Trent R. Marwick,1* Alberto Vieira Borges,2 Kristof Van Acker,1 François Darchambeau,2 and Steven Bouillon1

1Department of Earth and Environmental Sciences, Katholieke Universiteit Leuven (KU Leuven), Celestijnenlaan 200E, 3001 Leuven, Belgium; 2Chemical Oceanography Unit, University of Liège (ULg), Institut de Physique (B5), 4000 Liège, Belgium

ABSTRACT

A lack of appropriate proxies has traditionally hampered our ability to distinguish riverine organic carbon (OC) sources at the landscape scale. However, the dissection of C4 grasslands by C3-enriched riparian vegetation, and the distinct carbon stable isotope signature (δ13C) of these two photosynthetic pathways, provides a unique setting to assess the relative contribution of riparian and more distant sources to riverine C pools. Here, we compared δ13C signatures of bulk sub-basin vegetation (δ13CVEG) with those of riverine OC pools for a wide range of sites within two contrasting river basins in Madagascar. Although C3-derived carbon dominated in the eastern Rianala catchment, consistent with the dominant vegetation, we found that in the C4-dominated Betsiboka basin, riverine OC is disproportionately sourced from the C3-enriched riparian fringe, irrespective of climatic season, even though δ13CVEG estimates suggest as much as 96% of vegetation cover in some Betsiboka sub-basins may be accounted for by C4 biomass. For example, δ13C values for river bed OC were on average 6.9 ± 2.7‰ depleted in 13C compared to paired estimates of δ13CVEG. The disconnection of the wider C4-dominated basin is considered the primary driver of the under-representation of C4-derived C within riverine OC pools in the Betsiboka basin, although combustion of grassland biomass by fire is likely a subsidiary constraint on the quantity of terrestrial organic matter available for export to these streams and rivers. Our findings carry implications for the use of sedimentary δ13C signatures as proxies for past forest-grassland distribution and climate, as the C4 component may be considerably underestimated due to its disconnection from riverine OC pools.

Key words: Riverine organic carbon; Carbon stable isotopes (δ13C); Tropical rivers; C4; C3; Riparian vegetation; Madagascar.

INTRODUCTION

The riparian zone plays a key role in regulating the delivery of solutes to the adjoining inland surface waters (Remington and others 2007; Ranalli and Macalady 2010; Bouwman and others 2013). However, determining the origin of riverine carbon (C) at the landscape scale is often hampered by a lack of appropriate proxies distinguishing various landscape units and their constituent organic carbon
(OC) pools. Riverine OC pools constitute an amalgam of allochthonous (for example, vegetation and soils) and autochthonous (for example, phytoplankton, benthic algae, aquatic macrophytes) C sources, with the relative contributions of each to riverine OC pools increasingly disentangled through multi-proxy approaches, such as the use of C stable isotopes (δ13C) in combination with elemental (for example, C:N) and particulate OC (POC):Chlorophyll a (Chla) ratios (Hamilton and Lewis 1992; Kendall and others 2001; Bernardes and others 2004; Finlay and Kendall 2007; Tamooh and others 2012), and more recently the incorporation of GIS-based toolsets (Ballester and others 2013).

In freshwaters with negligible algal production, the predominant driver of riverine OC δ13C signatures is the proportion of organic matter (OM) derived from terrestrial vegetation following the C3 photosynthetic pathway (woody plants and trees, temperate grasses; δ13C ~ −27‰) compared to the less fractionating C4 photosynthetic pathway (largely tropical and subtropical grasses; δ13C ~ −13‰) (Hedges and others 1986; Bird and others 1994).

Following from these observations, it has been suggested that marine sedimentary sequences adjacent to major river mouths in the tropics may store extensive records of basin vegetation evolution in the form of terrestrial bulk OC δ13C signatures (Mariotti and others 1991). Indeed, others have employed stable isotope techniques to reconstruct paleo-vegetation distribution and paleo-climate regime from OM buried within various environments, including lacustrine and fluvial deposits (Cerling and others 1988; Mora and others 2002) as well as nearshore (Santschi and others 2007) and offshore (dos Santos and others 2013) marine deposits. Yet, such studies often assume no inherent differences in sediment entrainment or transport between C3- and C4-derived carbon from source to sink (Wynn and Bird 2007). In fact, notwithstanding the considerable contribution of C4 biomass to sub-Saharan vegetation cover (~31%; Still and Powell 2010), few studies have identified substantial contribution from C4-derived C in riverine POC or dissolved OC (DOC) pools of grasslands or savannah grasslands (Mariotti and others 1991; Bird and others 1992; Bird and Pousai 1997; Wynn and Bird 2007; Tamooh and others 2012). The predominately perennial connection of the C3-enriched riparian zone to active (surface and ground-) water channels, relative to the seasonally parched and disconnected C4-dominated grasslands, has been identified as a key driver of riverine organic C pools within subtropical and tropical savannah/grassland ecosystems globally (for example, Congo [Mariotti and others 1991]; Amazon [Bird and others 1992]; Australian rivers [Bird and Pousai 1997]; Cameroonian rivers [Bird and others 1994, 1998]).

Despite these findings, little systematic analysis of the proportional contribution of these landscape units to riverine organic C pools has been conducted (for example, Ballester and others 2013). Biomes with distinct spatial segregation of the C3 and C4 photosynthetic pathways should provide a unique scenario with which to assess the proportional contribution of different landscape units to riverine C pools with the use of C stable isotopes. Such settings, for example, include tropical and subtropical C4-dominant grasslands (grasslands as defined by Torello-Raventos and others 2013) partially disconnected from adjacent streams and rivers by a comparatively C3-enriched riparian fringe (for example, see Mariotti and others 1991; Bird and others 1994).

A region where the spatial partitioning of C3 and C4 biomass is astoundingly apparent is within the Hauts-Plateaux grasslands and savannahs of central Madagascar, with estimates that greater than 75% of the island’s surface area is covered by these vegetation biomes (Bond and others 2008). Previous research (Ralison and others 2008) conducted in the Betsiboka basin, which drains much of the Hauts-Plateaux region, reports that C of C4 origin accounts for 30% of the riverine DOC and POC pools in the upper part of the estuary, which was considerably lower than expected given estimates that C4 vegetation comprised more than 80% of basin vegetation land cover. Here, we build on their observations by exploring δ13C of riverine POC (δ13CPOC) and DOC (δ13CDCDOC) as a function of bulk sub-basin vegetation δ13C (δ13CVEG) and soil δ13C signatures in the Betsiboka basin. For comparison, we also report results for an eastern Malagasy drainage basin, the Rianila, characterised by C3 rainforest.

**MATERIALS AND METHODS**

**Basin Characteristics**

**The Betsiboka Basin**

The Betsiboka basin drains between 49,000 to 63,000 km2 of central and western Madagascar (Figure 1A), forming north of the Malagasy capital, Antananarivo, at the confluence of the Jabo and Amparihibe rivers (at 938 metres above sea level [m.a.s.l.]) in the Hauts-Plateaux. This region is often cited as one of the most erosive landscapes identified globally, with annual yields of 20,000 to
40,000 t km\(^{-2}\) (World Bank 1998; USAID 1998), although Cox and others (2009) suggest more modest yields of 32 t km\(^{-2}\) y\(^{-1}\) estimated from cosmogenic \(^{10}\)Be data of river sediments. Lavakas (meaning ‘holes’ in Malagasy) are mass failure features common to the Hauts-Plateaux which lead to considerable loss of arable land, elevated suspended and river bed sediment loads (Cox and others 2003, 2004, 2009), and subsequent alluviation of streams, rivers and estuaries (Zavada and others 2009). Relative to their limited spatial extent, lavakas contribute up to 84% of terrestrial sediment input to Malagasy rivers (Cox and others 2009). At 180 km downstream of the confluence with the Ikopa River, the principle tributary of the Betsiboka basin, the Betsiboka River flows into the Bombetoka estuary. Average discharge for the Betsiboka and Ikopa rivers, upstream of their confluence, is about 301 and about 77 m\(^3\) s\(^{-1}\), respectively (Figure 2A).

Following the recent classification system proposed by Torello-Raventos and others (2013), the terms grasslands and savannah grasslands, with dominance of C\(_4\) grass species, would be appropriate vegetation classifiers for the Hauts-Plateaux region of the Betsiboka basin. Charcoal in regional stratigraphic records suggests natural development and maintenance of the Malagasy grasslands by fire (Burney 1987a, b, c), though the present fire regime is heavily influenced by anthropogenic practices such as pasture management for cattle grazing, with estimates of a quarter to a half of Malagasy grasslands (by land area) are burned annually (Kull 2012). Primary C\(_3\) vegetation is sparse throughout (approximately 4% of basin area; see Du Puy and Moat 1996, 1999), with C\(_3\)-enriched riparian fringes scattered throughout the basin. During the mid-phase of lavaka evolution (Wells and others 1991; Wells and Andriamihaja 1993, 1997) a C\(_3\)-enriched forest complex may establish within the lavaka in response to the localised micro-climate (Figure 3).

The Rianila Basin

The Rianila basin drains approximately 7,800 km\(^2\) and is an archetypal basin of the eastern slopes of
Madagascar (Figure 1B), rising in the Betsimisaraka Heights at 1510 m.a.s.l. Du Puy and Moat (1996, 1999) approximate 61% of the Rianila basin is vegetated by low- or mid-latitude humid evergreen primary forest. Due to the north–south orientated ridge segregating eastern from central and western Madagascar, a rain-shadow effect results in considerably elevated annual rainfall in eastern regions relative to the rest of the island (Figure 1C).

August to October tend to be the driest months, yet trade-winds from the southeast can provide consistent rainfall throughout these months. Annual precipitation ranges between 2,500 and 3,000 mm, resulting in an average discharge of 408 m$^3$ s$^{-1}$ at Brickaville (nt. excludes downstream contribution from the Iaroka basin) (Aldegheri 1972) (Figure 2B).

**Sampling and Analytical Techniques**

Sampling was conducted in the Betsiboka and Rianila basins between July and September 2010 (dry season) and January to February 2012 (wet season). Within the Betsiboka, 43 sites were sampled during the dry season with repeated samples at 33 sites in the wet season, whereas 16 sites were sampled in the Rianila basin during both seasons. Riverine physico-chemical parameters (pH, dissolved oxygen, conductivity) were measured using a combination of a YSI Professional Plus (Pro Plus, Quattro cable bulkhead) and YSI Professional Optical Dissolved Oxygen (Pro ODO) instruments. Calibrations for pH were performed daily using National Bureau of Standards (NBS) buffers of pH 4 and 7.

A Niskin bottle was used to gather riverine solute samples 0.5 m below the surface, or using a rope and bucket when sampling from bridges. For bulk POC concentrations and C stable isotope composition ($\delta^{13}$C$_{POC}$), a known volume of surface water was filtered on 25 mm GF/F filters (pre-combusted overnight at 450°C; nominal porosity = 0.7 μm) and subsequently air dried. Later, filters were exposed to HCl fumes for at least 4 h to remove inorganic C (IC), subsequently re-dried and packed in Ag cups. Soil and sediment samples were only gathered during the dry season. These samples were stored immediately in liquid N$_2$ until return to the laboratory, where they were stored at −20°C. Samples were later dried and homogenised using a mortar and pestle. A weighed subsample was transferred into a Ag cup to which a 10% HCl solution was added to remove carbonates. Samples were then dried at 60°C for 24 h and, if necessary, the HCl procedure was repeated. Leaf litter samples were collected from select sites in each basin below vegetation visually representative of the area. At some sites in the Betsiboka, separate samples were collected from the riparian fringe and the surrounding C$_4$ grasslands. Litter was air dried in the field and stored in air-tight zip-lock bags, and dried...
at 50°C for 24 h in the laboratory oven. Subsamples were ground and homogenised with a portion transferred to Ag cups ready for analysis. Bulk POC and δ¹³C_POC, δ¹³C of soil (δ¹³C_SOIL) and river bed sediments (δ¹³C_SED), as well as leaf litter δ¹³C (δ¹³C_LITTER) were determined on a Thermo elemental analyzer–isotope ratio mass spectrometer (EA-IRMS) system (FlashHT with DeltaV Advantage), and by monitoring the 44, 45 and 46 m/z signal on the IRMS. Quantification and calibration of δ¹³C data were performed with IAEA-C6 and acetanilide which are internally calibrated against international standards. Reproducibility of δ¹³CPOC measurements was typically better than ±0.2‰ whereas relative standard deviations for calibration standards for POC measurements are typically less than 2% and always less than 5%.

DOC samples were obtained by pre-filtering surface water through pre-combusted GF/F filters (0.7 μm), with further filtration through 0.2-μm syringe filters, and preserved with H₃PO₄ in glass vials with Teflon-coated screw caps. Bulk DOC and δ¹³C (δ¹³C_DOC) were measured with either a customised Thermo HiperTOC coupled to a Delta + XL IRMS (Bouillon and others 2006), or by manual injection in a Thermo IsoLink HPLC-IRMS (similar to the method described in Albéric 2011).

Chl a was collected in the field following the procedure of POC above, and used as an index of phytoplankton biomass (Reynolds 2006). Total Chl a measurement was achieved by high performance liquid chromatography (HPLC) for dry season samples. The reproducibility of Chl a by HPLC has been demonstrated to be within 10% (Claustre and others 2004). Samples for HPLC analysis were obtained from filtration of 200–1,000 ml on Whatman GF/F filters, of 0.7 μm nominal pore size. Filters were immediately stored in liquid N₂, and upon return to the lab were kept frozen (at −25°C) until further processing. Pigment extraction was carried out in 3 ml of 90% HPLC grade acetone. After two 15-min sonications separated by an overnight period at 4°C in the dark, pigment extracts were processed through a Waters HPLC system comprising a Waters Nova-Pak C18 column and the ternary gradient of Wright (1991). Calibration was achieved using four-point calibration curves established with a Chl a standard purchased from DHI, Denmark. Wet season Chl a filters, from the Betriboka basin only, were extracted with 1 ml of 90% acetone buffered with saturated MgCO₃, after which they were incubated overnight at 4°C. Extracts were centrifuged at 10,000 rpm for 10 min, followed by measurement on a Perkin Elmer Spectrophotometer Lambda20 at 664 and 750 nm pre- and post-acidi-fication with 750 μl of 0.1 M HCl. Wet season Chl a concentrations were calculated according to Lorenzen (1967). The majority of sites, however, were below the quantification limit of our methodology, hence we used the quantification limit to provide minimum estimates for POC:Chl a ratios (both expressed in mg C) for the wet season dataset. Chl a samples were not collected in the Rianila basin during the wet season due to the unavailability of liquid N₂ for sample preservation.

In-situ pelagic primary production (PPP) was determined by filling two 500-ml polycarbonate bottles with surface water and adding 500 μl of a ¹³C-enriched bicarbonate solution (>99.8% ¹³C NaH¹³CO₃, ~40 mg dissolved in 12 ml of surface
water pre-filtered at 0.2 μm). Bottles were incubated in situ under ambient light and temperature conditions for approximately 2 h, after which a subsample from each bottle was filtered as for POC analysis. The PPP rates were calculated using PPP filters followed the procedures outlined above. Wilcoxon signed rank tests (hereafter SRT) were not normally distributed, Pt–T were replaced by chemical parameters. Where the seasonal observations were not normally distributed, Pt–T were replaced by chemical parameters. Where the seasonal observations were not normally distributed, Pt–T were replaced by chemical parameters. Where the seasonal observations were not normally distributed, Pt–T were replaced by chemical parameters. Where the seasonal observations were not normally distributed, Pt–T were replaced by chemical parameters.

For the analysis of δ13C DIC–PPP, a 2 ml helium (He) headspace was created, and H3PO4 was added to convert all DIC species to CO2. After overnight equilibration, part of the headspace was injected in the stream of an elemental analyser–isotope ratio mass spectrometer (EA–IRMS; ThermoFinnigan FlashHT and ThermoFinnigan DeltaV Advantage). The output δ13C was corrected for isotopic equilibration between gaseous and dissolved CO2 as described in Gillikin and Bouillon (2007). Analysis of PPP filters followed the procedures outlined above for POC analysis. The PPP rates were calculated based on Hama and others (1983):

$$\text{PPP} = \frac{\text{POC}_t (%^{13}\text{POC}_t - %^{13}\text{POC}_i)}{t (%^{13}\text{DIC} - %^{13}\text{POC}_i)},$$

where POCt is the particulate OC after incubation, %13POCt and %13POCi the initial and final (that is, after incubation) percentage 13C of the POC, t is the incubation time and %13DIC the percentage 13C of the DIC after the bottles had been spiked. It must be stressed that our PPP data are not depth integrated and are only valid for the upper surface waters, and should be considered to represent the maximum volumetric rates. They are mainly used here, in conjunction with Chla measurements, to constrain an upper limit of autochthonous contribution to riverine OC pools.

All statistical analyses were performed in SigmaPlot v12. Where sites were measured in both dry and wet seasons, paired t tests (hereafter Pt–T) were used to assess significant seasonal changes in the concentrations and δ13C of the OC pools, as well as other biogeochemical parameters. Where the seasonal observations were not normally distributed, Pt–T were replaced by Wilcoxon signed rank tests (hereafter SRT).

**RESULTS**

**Carbon Stable Isotope Composition of Leaf Litter and Soils**

The average δ13C of leaf litter OC (δ13CLITTER) was $-30.2 \pm 0.7\%_{oo}$ ($n = 4$) and $-29.5 \pm 1.1\%_{oo}$ ($n = 6$) in the Rianila basin and the riparian zone of the Betsiboka basin, respectively, agreeing with modeled δ13C of C3 vegetation for these regions based on Kohn (2010; $-30.3 \pm 0.3$ and $-29.3 \pm 0.0\%_{oo}$, respectively). Likewise, our data are comparable with those of Crowley and others (2011), who report an average C3 δ13CLITTER of $-31.6 \pm 1.1\%_{oo}$ in the eastern Malagasy rainforests and $-28.2 \pm 1.3\%_{oo}$ for C3 vegetation in the lower Betsiboka basin. The grasslands of the Betsiboka basin were clearly dominated by C4 species (average δ13CLITTER $-12.2 \pm 0.7\%_{oo}$; $n = 9$).

These C3 and C4 end-members encapsulate the gradient observed in the δ13C of the soil OC (δ13C SOC) pool in the two basins. In the Betsiboka basin, the δ13C SOC ranged between $-25.8\%_{oo}$ (reflecting a predominantly C3 origin) and $-12.0\%_{oo}$ (pure C4 origin), whereas SOC ranged from a pure C3 ($-28.2\%_{oo}$) to mixed C3:C4 ($-16.9\%_{oo}$) origin in the Rianila basin.

**Estimation of Bulk Sub-basin Vegetation δ13C (δ13CVEG) Signatures**

Still and Powell (2010) provide a modelled C4 vegetation distribution map for the African continent and the island of Madagascar based on climate datasets, the MODIS Vegetation Continuous Fields (VCF) product (estimate of annual percent cover of herbaceous, tree and bare soil), and the Global Land Cover Map 2000, and converted this to an isoscape using vegetation δ13C end-members of $-27\%_{oo}$ and $-12\%$ for pure C3 coverage and pure C4 vegetation, respectively.

With the use of the hydrology tool set of ArcMap 10 and SRTM Void Filled digital elevation data (3 arc-seconds; Earth Explorer, [http://earthexplorer.usgs.gov/](http://earthexplorer.usgs.gov/)), we extracted the upstream contributing sub-basin at each sampling location from the Still and Powell (2010) crop-corrected output. Resultant bulk vegetation δ13C (δ13CSVP) values were calculated as the average pixel value within the extracted sub-basin (see Supplementary Material). To permit comparison between the δ13CSVP estimates and riverine δ13C OC pools, the δ13C SP values were normalised to our δ13CLITTER end-members (that is, Rianila: 100% C3 = $-30.2\%_{oo}$, 100% C4 = $-12.2\%_{oo}$ [C4 grasses from Betsiboka]; Betsiboka: 100% C3 = $-29.5\%_{oo}$, 100% C4 = $-12.2\%_{oo}$).

The δ13CSVP values were calculated from 28 sub-basins, with basin area ranging from 17 to 54,019 km² and covering a δ13C spectrum of $-30.0$ to $-17.6\%_{oo}$. As expected, the Rianila sub-basins were dominated by C3 vegetation, with an average bulk δ13CSVP value of $-27.6 \pm 1.7\%_{oo}$ (range $-22.5$ to $-30.3\%_{oo}$).
to $-30.0^{\circ}$ or 57–99\% C$_3$ cover, $n = 16$). The Betsiboka sub-basin average bulk $\delta^{13}C_{SP}$ value ($-18.7 \pm 0.6^{\circ}$, range $-17.6$ to $-19.6^{\circ}$, or 57 and 69\% C$_4$ vegetation cover, $n = 12$) was more mixed than those of the Rianila, though with less C$_4$ influence than was expected from onsite observation.

As originally stipulated by Still and Powell (2010), their outputs were intended for continental analysis of C$_4$ vegetation distribution. Our attempt here to apply them on a regional to local scale highlights one issue with the climatic constraint used to identify areas of potential C$_4$ land cover. Model output is limited in the upper- to mid-basin of the Betsiboka, where a broad region identified in the field, and confirmed through satellite imagery, to be dominated by C$_4$ grasslands has been classified as pure C$_3$ by Still and Powell (2010). We believe this to be a factor of the perennially cool climate of the central highlands not meeting the C$_4$ bioclimatic conditions constraining the model output. Differences in C$_3$ and C$_4$ quantum yield, the ratio of moles of CO$_2$ assimilated relative to moles of photosynthetic active radiation absorbed by a leaf when photosynthesis is limited by low light conditions (Ehleringer and Björkman 1977; Collatz and others 1998), is a key control on the distribution and ecological sorting of the two photosynthetic pathways (Ehleringer 1978).

Measured temperatures where the quantum yield of C$_3$ and C$_4$ photosynthesis are equivalent (that is, the crossover temperature), vary from of 16 to 24°C (Ehleringer and others 1997), with Still and Powell (2010) using a crossover temperature of 21°C. Yet Antananarivo, situated in the upper Betsiboka-Ikopa basin, experiences average annual monthly temperatures of no higher than 21°C, placing this region outside of the climatic constraints identifying areas of potential C$_4$ land cover. We suggest that a lower crossover temperature may be required to adequately model regional C$_4$ distribution in the Malagasy Hauts-Plateaux, where average monthly temperatures remain below 21°C year-round yet C$_4$ grasslands spatially comprise a significant portion of basin vegetation cover.

For these reasons, we developed our own satellite image-based estimate of C$_3$ and C$_4$ distribution

Figure 4. Illustration of the approach used to calculate $\delta^{13}C_{SBE}$ values for select sub-basins of the Betsiboka basin. A Overview of the sub-basin of site B38 (36 km$^2$), showing the sub-basin area (green polygon). B Site B38 sub-basin extracted and C converted to greyscale. C$_3$ vegetation ($\delta^{13}C_{LITTER} = -29.5^{\circ}$) is classified by black pixels, with all other pixels classified as C$_4$ vegetation ($\delta^{13}C_{LITTER} = -12.2^{\circ}$). D C$_4$ grasses typically dominate the undulating valleys of the mid-basin Betsiboka, with C$_3$ vegetation confined to the perennially moist soils of the riparian zone and old lavaka depressions (field-of-view is indicated by the red arrows in A). See Supplementary Materials for detailed techniques and similar examples for other sub-basins (Data sourced from Google Earth with data provided by DigitalGlobe).
($\delta^{13}C_{\text{SBE}}$) for a selection of the Betsiboka sub-basins. Satellite imagery of 21 sub-basins was extracted from Google Earth Pro (see Supplementary Material). Full sub-basin area was extracted for eight sub-basins (for example, Figure 4), whereas variation in image quality and decreased image resolution at larger scales necessitated the need to extract singular or multiple quadrants representative of total vegetation for the remaining sub-basins (see Supplementary Material). Satellite imagery was subsequently converted to greyscale, followed by increases in gamma correction and contrast, with resultant black pixels classified as C$_3$ cover ($\delta^{13}C_{\text{LITTER}} = -29.5\%$) and all remaining pixels classified as C$_4$ cover ($\delta^{13}C_{\text{LITTER}} = -12.2\%$), with the sum average pixel value used to estimate the sub-basin $\delta^{13}C_{\text{SBE}}$ signature.

Bulk $\delta^{13}C_{\text{SBE}}$ estimates ranged between $-22.8$ and $-12.9\%$ (average $\delta^{13}C_{\text{SBE}}$ 14.7 ± 2.2$\%$, n = 21). Where paired $\delta^{13}C_{\text{SP}}$ and $\delta^{13}C_{\text{SBE}}$ values were estimated, $\delta^{13}C_{\text{SBE}}$ values were significantly (Pt–T: P < 0.001) enriched in $^{13}C$ (+4.8 ± 0.9$\%$, n = 4). This leads us to suspect that the $\delta^{13}C_{\text{SP}}$ values in the Betsiboka underestimate C$_4$ vegetation cover, likely due to differences in pixel resolution between the Still and Powell (2010) output and our $\delta^{13}C_{\text{SBE}}$ technique. Based on our $\delta^{13}C_{\text{LITTER}}$ end-members in the Betsiboka basin, $\delta^{13}C_{\text{SBE}}$ values estimate C$_4$ plants may account for up to 96% of land cover within some sub-basins. Consideration of implicit errors of this method is briefly discussed in the Supplementary Materials.

**Bulk Riverine Carbon Concentrations and Carbon Stable Isotope Compositions**

As could be expected, given the swath of grasslands covered by *lavakas* and the resultant sediment entrainment from these erosional features, streams and rivers in the Betsiboka basin were seasonally more sediment-laden than streams and rivers within the more heavily forested Rianila basin. Bulk TSM concentrations in the Betsiboka were significantly higher (SRT: $P < 0.001$, n = 28) during the wet season (range 2.1–3,589.5 mg l$^{-1}$, average 464.0 ± 769.3 mg l$^{-1}$, n = 29) than during the dry season (range 0.8–128.1 mg l$^{-1}$, average 36.8 ± 40.3 mg l$^{-1}$, n = 38), and whilst less variability occurred between seasons in the Rianila basin than the Betsiboka, bulk TSM concentration was also significantly elevated (SRT: $P = 0.011$, n = 16) during the wet season (range 3.6–103.2 mg l$^{-1}$, average 32.4 ± 32.9 mg l$^{-1}$, n = 16) than during the dry season (range 2.0–31.8 mg l$^{-1}$, average 14.5 ± 9.0 mg l$^{-1}$, n = 16).

Bulk riverine POC in the Betsiboka was significantly higher (SRT: $P < 0.001$, n = 28) during the wet season (range 0.4–27.4 mg C l$^{-1}$, average 5.5 ± 6.0 mg C l$^{-1}$, n = 29) than the dry season (range 0.2–2.2 mg l$^{-1}$, average 0.8 ± 0.5 mg l$^{-1}$, n = 38), and was represented by $^{13}C$ ranges of −26.3 to −17.7 and −26.6 to −16.2$\%$, respectively (Figure 5). The slight, yet significant ($P = 0.001$, n = 28), difference in $\delta^{13}C_{\text{POC}}$ between dry season

![Figure 5](image.png)

**Figure 5.** Carbon stable isotope signatures of riverine POC ($\delta^{13}C_{\text{POC}}$) as a function of POC concentrations in the Betsiboka and the Rianila basins. Grey shading represent bounds for 90% C$_3$ ($\delta^{13}C_{\text{LITTER}} = -28.4\%$) vegetation in the Rianila basin and 90% C$_4$ ($\delta^{13}C_{\text{LITTER}} = -13.9\%$) vegetation for both the Rianila and Betsiboka basins, whereas the horizontal dashed line represents 90% C$_3$ ($\delta^{13}C_{\text{LITTER}} = -27.8\%$) vegetation in the Betsiboka basin.

![Figure 6](image.png)

**Figure 6.** Carbon stable isotope signatures of riverine DOC ($\delta^{13}C_{\text{DOC}}$) as a function of DOC concentrations in the Betsiboka and the Rianila basins. Grey shading represent bounds for 90% C$_3$ ($\delta^{13}C_{\text{LITTER}} = -28.4\%$) vegetation in the Rianila basin and 90% C$_4$ ($\delta^{13}C_{\text{LITTER}} = -13.9\%$) vegetation for both the Rianila and Betsiboka basins, whereas the horizontal dashed line represents 90% C$_3$ ($\delta^{13}C_{\text{LITTER}} = -27.8\%$) vegetation in the Betsiboka basin.
(−22.7 ± 2.0%oo, n = 38) and wet season (−21.4 ± 2.3%oo, n = 29) suggests that an increasing quantity of riverine OC is sourced from C4 grasslands during the wet season. Likewise, increased contribution from these landscape units would account for the marginally, yet again significantly, elevated DOC concentrations (Pt–T: P = 0.002, n = 28) and δ13C signatures (SRT: P < 0.001, n = 28) observed during the wet season (bulk DOC range 0.4–2.9 mg C l−1, average 1.3 ± 0.6 mg C l−1; δ13CDOC range −26.2 to −15.4%oo, average δ13CDOC −22.3 ± 1.9%oo; n = 29) relative to dry season (bulk DOC range 0.4–2.6 mg C l−1, average 1.1 ± 0.6 mg C l−1; δ13CDOC range −29.5 to −21.8%oo, average δ13CDOC −24.8 ± 1.8%oo, n = 38) (Figure 6).

Significantly elevated concentrations of POC (Pt–T: P = 0.014, n = 16) and DOC (Pt–T: P = 0.018, n = 16) were also observed in the streams and rivers of the Rianila basin during the wet season (POC: range 0.4–5.1 mg C l−1, average 2.1 ± 1.6 mg C l−1, n = 16; DOC: range 0.3–5.3 mg C l−1, average 2.6 ± 1.4 mg C l−1, n = 16) compared to the dry season (POC: range 0.4–1.8 mg C l−1, average 0.9 ± 0.5 mg C l−1, n = 16; DOC: range 0.7–2.9 mg C l−1, average 1.6 ± 0.7 mg C l−1, n = 16) (Figures 5, 6). Contrary to the enrichment observed in the Betsiboka basin, and evident from the dominance of C3 vegetation within the Rianila basin, riverine POC was significantly 13C-depleted (Pt–T: P = 0.002, n = 16) during the wet season (range −28.0 to −25.8%oo, average −27.3 ± 0.6%oo, n = 16) relative to dry season POC (range −27.6 to −24.6%oo, average −26.5 ± 0.9%oo, n = 16) in the Rianila basin. We speculate this may be attributed to an increased contribution of POC sourced from the wider Rianila basin (that is, less disturbed C3 forest), where there is less influence of human settlement (for example, logging of forest, agriculture, roads) on basin vegetation distribution.

No significant seasonal shift in riverine DOC origin was found in the Rianila basin (wet season δ13CDOC range −28.8 to −23.4%oo, average −27.6 ± 1.2%oo, n = 16; dry season δ13CDOC range −28.8 to −26.4%oo, average −27.8 ± 0.6%oo, n = 16).

**Indicators of In-Situ Aquatic Production**

Pelagic primary production in the Betsiboka basin during the dry season was generally negligible, ranging between 0.01 and 9.42 μmol C l−1 h−1 (average = 0.52 ± 1.54 μmol C l−1 h−1, n = 38), with all but three sites recording PPP rates below 1.0 μmol C l−1 h−1. Wet season PPP rates were not significantly different, ranging between 0.01 and 1.45 μmol C l−1 h−1 (average = 0.35 ± 0.35 μmol C l−1 h−1, n = 29). Despite low PPP rates in the Rianila across both seasons, PPP increased significantly (Pt–T: P ≤ 0.001, n = 14) during the wet season, with a range between 0.01 and 0.22 C l−1 h−1 (average = 0.07 ± 0.06 μmol C l−1 h−1, n = 15) during the dry season and 0.07 to 0.45 C l−1 h−1 (average = 0.20 ± 0.12 μmol C l−1 h−1, n = 15) in the wet season.

Dry season mg POC: mg Chl a ranged from 173 to 6,843 (average = 2,019 ± 1,241) and 1,280 to 4,468 (average = 2,768 ± 963) in the Betsiboka and Rianila basins, respectively. All wet season Chl a measurements from the Betsiboka basin were below the limit of quantification, which resulted in minimum values for mg POC: mg Chl a consistently above 1,000, except at B38 where the mg POC: mg Chl a was 487.

**Discussion**

**Dominance of Terrestrial Organic Carbon**

The major terrestrial OC sources considered here are C3 and C4 vegetation, though it is necessary to account for or dismiss the contribution from autotrophic sources, including phytoplankton, benthic algae and aquatic macrophytes, which in certain conditions can contribute substantially to riverine OC pools in tropical systems (Lewis and others 2001; Davies and others 2008). Several lines of evidence indicate that terrestrial carbon sources dominate in the basins studied here, and that any contributions from aquatic producers are minimal and would not bias our interpretations. First, the PPP rates were extremely low and showed little seasonal variation. Only downstream from the junction of the Ikopa with the Betsiboka, where channel slope rapidly decreases and the channel broadens, does PPP marginally increase (2.1–9.4 μmol C l−1 h−1). Second, low POC:Chl a ratios (∼40; see Abril and others 2002, and references therein) are indicative of large phytoplankton contributions to the POC pool, with increasingly larger ratios where terrestrial-derived POC becomes more dominant (Finlay and Kendall 2007; Findlay 2010). Our data suggest negligible contribution of Chl a to riverine POC, with mg POC:mg Chl a consistently above 1,000 in both basins, and only once was the mg POC:mg Chl a less than 200 (in the main channel of the lower Betsiboka during dry season). Third, the Malagasy streams and rivers here were generally marked by low concentrations of ammonium (NH4 +), nitrate (NO3 −) and phosphate (PO4 3−) across the two campaigns (Rianila median values: NH4 + = 6.3 μmol l−1, NO3 − = 3.0 μmol l−1,
PO$_4^{3-}$ = 0.6 µmol l$^{-1}$; Betsiboka median values: NH$_4^+$ = 4.1 µmol l$^{-1}$, NO$_3^-$ = 2.7 µmol l$^{-1}$, PO$_4^{3-}$ = 0.8 µmol l$^{-1}$; data not presented in detail; see also Figure 9 of Marwick and others 2014).

The absence of substantial aquatic primary production can likely be ascribed to low nutrient and light conditions, through shading, for example, in the forested headwaters of the Rianila, or due to the high turbidity in the Betsiboka basin. Shading by riparian fringe vegetation has long been identified as an over-riding control on instream primary productivity (Feminella and others 1989; Boston and Hill 1991), and more recently in freshwaters of the tropics specifically (Davies and others 2008), whereas increased turbidity and light attenuation within the water column of larger rivers can also limit instream primary production (Vannote and others 1980). We speculate the latter may also be applicable to the grassland headwaters of the Betsiboka basin, which receive significant suspended sediment inputs from lavakas. Additionally, it has also been shown that, even where riparian shading effects are negligible, nutrients may impart substantial influence on instream primary productivity (Peterson and others 1983; Pringle and others 1986; Winterbourn 1990; Hill and others 1995; Borchardt 1996; Mosisch and others 1999), with evidence for nitrogen being the primary limiting nutrient for algal growth previously found in subtropical (Mosisch and others 2001) and tropical freshwaters (Downing and others 1999; Flecker and others 2002; Udy and Dennison 2005). No aquatic macrophytes were observed during the study, an observation consistent with Elouard (2001) who reports few macrophytes for Malagasy freshwaters as a whole. From the above reasoning, we speculate there is negligible contribution of autochthonous C to riverine OC pools of these Malagasy basins, and hypothesise that $\delta^{13}$C signatures of riverine particulate and DOC will be largely representative of the relative proportion of C originating from the two terrestrial end-members, that is, C$_3$ and C$_4$ vegetation.

Disproportionate Contribution of Riparian OM to Riverine OC Pools

A clear pattern emerges when considering the combined data from both basins, with riverine DOC, POC and sediment OC all consistently more $^{13}$C-depleted than expected across the C$_3$–C$_4$ gradient. These relationships are evident irrespective of whether riverine OC fractions are explored as a function of estimated bulk vegetation $\delta^{13}$C (Figure 7A–C) or soil $\delta^{13}$C (Figure 8A–C).

Excluding lesser autochthonous contributions, riverine DOC is sourced from the leaching of plant litter and the degradation of soil OM, whereas the POC fraction, on the other hand, is largely sourced from fragmented plant matter and direct inputs of leaf litter, OM mobilised from surrounding soil profiles, as well as autotrophic production within the freshwaters (Meybeck 1993; Ludwig and others 1996; Finlay and Kendall 2007). Having already

Figure 7. $\delta^{13}$C data on major riverine C pools (A DOC; B POC; C river sediment) along the gradient of sub-basin bulk vegetation $\delta^{13}$C ($\delta^{13}$C$_{SP}$ or $\delta^{13}$C$_{SBE}$). Sediment samples were only gathered during the dry season campaign. The dotted line represents a 1:1 relationship. Vertical dashed lines represent 100% C$_3$ vegetation in the Rianila ($\delta^{13}$C$_{LITTER}$ = $-30.2^{\circ}_{o}$) and Betsiboka ($\delta^{13}$C$_{LITTER}$ = $-29.5^{\circ}_{o}$) and pure C$_4$ ($\delta^{13}$C$_{LITTER}$ = $-12.2^{\circ}_{o}$) vegetation in both basins.
discounted significant contribution of algal C sources to riverine OC pools in the Rianila basin, it is unsurprising that riverine DOC and POC δ¹³C values reflect the dominance of C₃ vegetation in this system (Figure 7A, B). Additionally, recent radiocarbon measurements of DOC in the Rianila basin during the wet season, including two headwater and one main channel samples, were all ¹⁴C-enriched (Δ¹⁴C range +81 to +136‰; own unpublished data) and indicate a modern terrestrial C₃-enriched DOC source for Rianila streams and rivers.

The relationship between soils and riverine OC in the Rianila basin (Figure 8A, B) is weaker than that discussed above for basin vegetation, though can be expected given that riverine δ¹³C values and δ¹³CVEG estimates are integrated compositions of δ¹³C from broad areas, whereas δ¹³CSOIL values are determined from spatially isolated samples. Although δ¹³CSOIL values do highlight the, at times significant (up to −16.9‰), presence of C₄ biomass within the vicinity of Rianila streams and rivers. We speculate that the presence of this C₄ biomass within the riparian fringe and surrounding floodplain, likely associated with the tendency of human settlement and disturbance of the natural vegetation within these areas (including the introduction of C₄ crops and invasive species), results in the slight ¹³C enrichment observed in the riverine POC pool during the dry season. Others have shown that the riparian fringe disproportionately contributes to dry season riverine POC loads in the tropics (Mariotti and others 1991; Bird and others 1992). In the case of the Rianila, elevated rainfall would lead to increased quantities of OM mobilised from the C₃-enriched surrounding forests, subsequently lowering the relatively ¹³C-enriched dry season POC signal.

Considering δ¹³CVEG estimates suggest that C₄ biomass accounts for between 39 and 96% of vegetation cover in the Betsiboka sub-basins, there is no such prominence of C₄ derived C in riverine OC pools (Figure 7A, B). A similar observation has been made for other subtropical and tropical savannah/grassland ecosystems (Mariotti and others 1991; Bird and others 1992, 1998; Bird and Pousai 1997). For example, Mariotti and others (1991) report an average riverine δ¹³CPOC value of −27.5‰ for three savannah dominated tributaries of the Congo River, whereas Bird and others (1992) provide values ranging from −29.9 to −27.0‰ for the cerrado-dominated Araguaia/Tocantins sub-basin of the Amazon network. A seasonal fluctuation in riverine δ¹³CPOC of 3.3‰ was also identified in the Sanaga River basin by Bird and others (1998) and closely linked to runoff intensity, with the lower δ¹³C values present during the wet season, which is comparable to the average deviation of 1.1‰ in streams and rivers of the Betsiboka where we have both dry and wet season δ¹³CPOC data (n = 28). A common feature to each of these basins for which δ¹³CPOC values are listed above, including the Betsiboka basin here, is the C₃-enriched nature of the riparian fringes. It is generally considered, and re-iterated here through our observations in
the Betsiboka basin, that the C4-enriched riparian fringe, being in close proximity to the riverbanks, provides disproportionately (relative to land cover) to dry season riverine OC pools, whereas with the advent of the wet season, the increased mobilisation of OC from the C4-enriched areas farther from the streams and rivers leads to relatively 13C-enriched riverine POC pools (Mariotti and others 1991; Bird and others 1992, 1998; Bird and Pousai 1997).

Although we consider the disconnection of the wider C4-dominated basin from the streams and rivers as the primary driver of the under-representation of C4-derived C within riverine OC pools in the Betsiboka, given the history (Burney 1987a, b, c) and prevalence (Kull 2004, 2012) of fire within the grasslands of Madagascar, the effect of terrestrial biomass loss through combustion by fire needs some consideration. Kull (2004) reports as much as one quarter to one half (of land area) of the Malagasy grasslands may be burned annually. Lehsten and others (2009) estimate African wildfires consume approximately 10% of savannah NPP annually, approximating 0.52–0.59 t C ha−1 y−1 from Lloyd et al. (2008) estimates of African grassland NPP (see discussion below). Clearly, the preferential loss of C4 derived C to the atmosphere as CO2 or fine particulate matter by combustion diminishes the quantity available for export to streams and rivers (Menaut and others 1990) and thereby should be considered a subsidiary control of riverine OC δ13C composition.

Where only a single sampling of riverine OC is logistically possible in tropical basins containing significant C4 biomass, due to the large annual range of riverine δ13CPOC values, the δ13C values of river bed sediments offer a more reliable indication of the average riverine δ13CPOC transported in these systems (Bird and Pousai 1997). Although here we replicate riverine OC measurements in the dry and wet season, it is reasonable to assume our data do not capture the total annual variability of riverine POC and, as such, our river bed sediment δ13C values may be a more reliable indicator of average riverine δ13CPOC.

The limited range of δ13CSED values relative to δ13CSOIL values (Figure 8C) in both the Rianila (average δ13CSED −25.1 ± 2.3‰, n = 16) and Betsiboka (average δ13CSED −23.0 ± 2.1‰, n = 37) is normal given that river bed sediments assimilate δ13C compositions from a considerably broader area relative to the soil measurements (Bird and Pousai 1997). Whereas δ13CSED values in the Rianila basin were largely reflective of our δ13CVEG estimates (Figure 7C), the significant depletion of 13C in river bed sediments of the Betsiboka basin compared to our δ13CVEG estimates (average δ13CSED−δ13CVEG 6.9 ± 2.7‰, n = 32) confirms the suggestions above that there exists a strong disconnection between the terrestrial C4 and riverine OC pools. Such a disconnection may lead to gross over-estimation of basin-scale C3 vegetation cover when extrapolated from δ13C signatures stored in sedimentary deposits of fluvial origin.

Two regional environmental conditions may complicate our interpretations outlined above. First, if net primary productivity (NPP) in C3-enriched riparian fringes is significantly greater relative to NPP of the surrounding grasslands, it may not be surprising to find an under-representation of C4 sourced C (relative to land cover) in riverine OC pools. We do not believe the riparian fringe vegetation in the Betsiboka basin constitutes tropical forests, an African biome for which no published field studies estimating NPP exist (Valentini and others 2014), but rather more closely resemble scrub and woodland savannah as defined by Torrello-Raventos and others (2013). Employing the NPP estimates provided by Lloyd and others (2008), which cover the transition from grassland to woodland savannah in the tropics, grassland NPP may be between 5.2 and 5.9 t C ha−1 y−1, which is comparable with the median estimate of 6.2 ± 1.8 t C ha−1 y−1 reported by Ciais and others (2011). For open savannah woodland in eastern and southern Africa, Lloyd and others (2008) estimate NPP of 7.8 and 5.1 t C ha−1 y−1, respectively, and values of 8.8 and 7.1 t C ha−1 y−1 for eastern and southern savannah woodlands, respectively. It should also be noted that the riparian fringes of the Betsiboka basin are not spatially expansive, and where present often do not extend more than a few tens of metres from the river (see Figure 3: Supplementary Materials). As such, we suspect NPP in the grasslands and riparian fringe of the Betsiboka are within a comparable range, and any differences will have a minor impact on the quantity of terrestrial C available for export to riverine C pools from the respective landscape units. Second, C4 agricultural crops, such as sugar cane, may contribute significantly to riverine OC pools (Bunn and others 1997). The area of sugar cane harvest in both the Betsiboka and Rianila basins equates to less than 0.2% of total basin area (HarvestChoice 2011), and as no major sugar cane agriculture was observed within the vicinity of any of the field sites, we expect it to be a negligible source of C4 carbon to the riverine pools reported here. Whereas, in Malagasy basins paddy rice agriculture, a C3 crop, is often confined to the riparian fringe (Aldegheri
and could potentially skew riverine OC $\delta^{13}C$ values towards the C$_3$ end-member.

**Conclusions**

With their strong partitioning of C$_3$ and C$_4$ vegetation at the landscape scale, subtropical and tropical C$_4$-rich grasslands provide the optimum environment to further explore the relative delivery of terrestrial C from different landscape units to river networks, as well as the investigation of trophic linkages between aquatic consumers and riparian carbon sources (for example, Abrantes and others 2013). Our data on the $\delta^{13}C$ signatures of various riverine OC pools from subtropical basins demonstrate that the C$_3$-enriched riparian vegetation contributes disproportionately to riverine OC pools compared to the spatially dominant, yet seasonally disconnected, C$_4$-dominant grasslands. The prevalence of fire within grassland ecosystems, with consideration they may combust 10% of annual NPP within this biome type, suggests the prevailing fire regime will also exert significant control over the quantity of grassland OM available for export to streams and rivers and, ergo, riverine OC $\delta^{13}C$ values. These observations clearly highlight that the $\delta^{13}C$ of riverine organic C pools may not be an accurate proxy for extrapolating C$_3$ and C$_4$ vegetation cover of a drainage basin, but appear to be strongly biased towards the dominant photosynthetic pathway of riparian fringe vegetation. Our $\delta^{13}C$ values of riverine sediments in a basin dominated by tropical grassland indicate that upon deposition these sediments are not representative of the overall vegetation distribution in the contributing drainage basin, and that the analysis of sedimentary $\delta^{13}C$ signatures for the purpose of paleo-reconstruction of vegetation distribution (Cerling and others 1988; Mora and others 2002; Santschi and others 2007; dos Santos and others 2013) may lead to significant over-estimation of the areal C$_3$ cover within drainage basins characterised by considerable expanses of C$_4$ grassland.

**Acknowledgments**

Funding for this work was provided by the European Research Council (ERC-StG 240002, AFRIVALAL, http://ees.kuleuven.be/project/af rival/). We kindly thank Rabeharisoa Lilia (LRI ESSA, Madagascar) for logistical support in the field, as well as Rabemanantsoa François, Rabemanantsoa Miharisoa and Mahatsindry Randzato Solo for considerable assistance gathering field samples. We also thank Zita Kelemen (KU Leuven), Peter Salaets (KU Leuven), Benjamin Horemans (KU Leuven), Michael Korntheuer (VUB) and Peter van Breughel (NIOZ) for technical and laboratory assistance. Particular thanks to Christopher Still and Rebecca Powell for providing the GIS data layers of their isoscape models. A.V.B. is a senior research associate at the FRS-FNRS (Belgium). Two anonymous reviewers and the handling editor, S. Bunn, provided insightful and constructive comments on an earlier version of this manuscript.

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