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19 baseflow S isotope data revealed significant bedrock S in the hydrologic export at Bisley (with a
20 distinctive $\delta^{34}\text{S}$ values of $1.6\pm 0.7\text{‰}$), but not at Icacos. Pore water data supported the co-
21 occurrence of at least three major biological S-fractionating processes in these soils: plant
22 uptake, oxidative degradation of organic S and bacterial sulfate reduction. The rates and relative
23 importance of these processes varied in time and space. Vegetation litter was 3 to 5‰ depleted in
24 ^{34}S compared to the average pore water, providing evidence for fractionation during uptake and
25 assimilation. Out of all abiotic soil forming factors, climate, especially the high rainfall, was the
26 main driver of S biogeochemistry in the LEF by dictating the types and rates of processes.
27 Topography appeared to impact S cycling by influencing redox conditions: C, N and S content
28 decrease downslope at all sites, and the Bisley lower slope showed strongest evidence of
29 bacterial sulfate reduction. Parent material type did not impact the soil S cycle significantly. To
30 compare the fate of S and N in the soil, we used an advection model to describe the isotopic
31 fractionation of total S and N associated with downward movement of organic matter in both
32 dissolved and solid fractions. This model worked well for N, but the assumption of a constant
33 fractionation factor α with depth failed to describe S transformations. This result revealed a
34 fundamental difference between N and S cycling in these soils, indicating an apparent greater
35 sensitivity of S isotopes to fluctuating redox conditions.

36

37

38 **Abbreviations**

39

40 COS: carbonyl sulfide

41 CZO: Critical Zone Observatory

42 DMS: dimethyl sulfide

43 DOS: dissolved organic sulfur

44 LEF: Luquillo Experimental Forest

45 LTER: Long-Term Ecological Research

46 MAP: mean annual precipitation

47 masl: meters above sea level

48 MAT: mean annual temperature

49 MSA: methanesulfonic acid

50 NAD83: North American Datum of 1983

51 NRCS: National Resources Conservation Service

52 nss: non-seasalt

53 SOS: soil organic sulfur

54 USDA: United States Department of Agriculture

55 USGS: United States Geologic Survey

56

57 1. INTRODUCTION

58 The terrestrial biogeochemical cycle of an element is driven by inputs, outputs, and
59 transformations within the plant/soil system (e.g. Vitousek and Stanford, 1986). Sulfur (S), like
60 nitrogen (N), has a complex biogeochemical cycle for two main reasons. First, S exists in a wide
61 range of valence states (from -2 to +6), and can participate in intricate biochemical reactions,
62 some of which are challenging to elucidate (Norman et al., 2002; Brunner and Bernasconi, 2005;
63 Bradley et al., 2011; Sim et al., 2011). Second, S is an essential nutrient, but soils can suffer both
64 from S deficiency (Acquaye and Beringer, 1989; Tabatabai, 1984), and from S excess (via
65 pollution, or naturally sulfide-rich bedrocks) (Likens et al., 2002). Unlike N however, for which
66 both the natural and the human-impacted cycles have been well studied, S has been studied
67 mostly in industrialized regions subject to high deposition rates (e.g. Novak et al., 2001; Likens
68 et al., 2002; Marty et al., 2011), and we still lack a basic understanding of the natural controls on
69 the S cycle in less perturbed environments. Based on N studies (e.g. Austin and Vitousek, 1998;
70 Amundson et al., 2003), it is likely that the soil formation factors identified by Jenny (1941)
71 (climate, organisms, topography, parent material and time) play a role in the soil S cycle,
72 however no previous research has addressed these controls systematically.

73 A potential impact of topography was observed in Costa Rica, where Bern and Townsend (2008)
74 found that hillslope soils had higher $\delta^{34}\text{S}$ values compared to alluvial soils. Climate should have
75 a first order impact on redox-sensitive elements, as mean annual precipitation (MAP) impacts
76 elemental input and loss rates, and the dominant biological processes in the soil, while mean
77 annual temperature (MAT) impacts the rates of biotic processes such as bacterial sulfate
78 reduction (Bruchert et al., 2001; Canfield et al., 2006; Turchyn et al., 2010). Previous research
79 found that wet tropical forest soils may emit hydrogen sulfide due to dissimilatory sulfate

80 reduction (Delmas and Servant, 1983; Delmas et al., 1978; Newman et al., 1991), however this
81 was shown not to be an important process in Costa Rica, where most of the S research on pristine
82 wet tropical forest soils to date has focused (Bern et al., 2007; Bern and Townsend, 2008).
83 Hillslope soils in Costa Rica depend exclusively on atmospheric S inputs, show minimal
84 variation in concentration and isotope values with depth, and an overall slight enrichment in ^{34}S
85 compared to the precipitation. This isotopic enrichment may be due to emissions of highly
86 depleted biogenic S gases by vegetation, or due to oxidative degradation of organic S (Bern et
87 al., 2007; Bern and Townsend, 2008). Fractionation during plant uptake is negligible (Bern and
88 Townsend, 2008). The largest S isotope fractionations observed in nature occur during microbial
89 dissimilatory sulfate reduction, which can deplete the sulfide products by up to 70‰ (Brunner
90 and Bernasconi, 2005). Despite their generally small magnitude (Table A1), other biological
91 fractionations sensitive to climate are also significant because they all operate in the same
92 direction: microbes and plants preferentially utilize the lighter isotope during metabolism,
93 therefore $\delta^{34}\text{S}$ values tend to decrease in products compared to the substrates (see Table A2 for a
94 compilation of input $\delta^{34}\text{S}$ values). An overview of the soil S cycle, based on current knowledge,
95 is illustrated in Fig. A1.

96 Here we use stable isotopes to investigate the geochemical cycling of S in the wet montane forest
97 ecosystem of the Luquillo Experimental Forest (LEF) in Puerto Rico, and compare it to the
98 better-known N cycle. At the LEF, previous studies have focused on rates of chemical
99 weathering and solute loss (e.g. White et al., 1998; Murphy et al., 1998; Schulz et al., 1999; Buss
100 et al., 2008), and on the sources and cycling of nutrients such as N and P (e.g. Silver et al., 1994;
101 Pett-Ridge et al., 2009; Buss et al., 2010). Sulfate and/or total S concentrations have been
102 measured in precipitation (McDowell et al., 1990; Asbury et al., 1994; White et al., 1998;

103 Heartsill-Scalley et al., 2007), soil and saprolite (Cox et al., 2002; White et al., 1998; Stanko-
104 Golden and Fitzgerald, 1991), and major streams (White et al., 1998; McDowell and Asbury,
105 1994; Bhatt and McDowell, 2007). Here we integrate and expand on these previous studies and
106 present novel stable S and N isotope data for the coupled precipitation-soil-vegetation system, to
107 address two yet unanswered questions: (1) how do soil forming factors, particularly bedrock and
108 topography, impact S biogeochemistry in a tropical environment? and (2) are the N and S
109 biogeochemical cycles coupled, or at least similar, in tropical soils? These questions have
110 importance for understanding spatial patterns of nutrient dynamics and feedbacks, and for setting
111 a baseline for studying the response of tropical soils to climate or land use change.

112

113 **2. SITE DESCRIPTION**

114 The Luquillo Experimental Forest (LEF) (18°18'N, 65°50'W, referenced to the NAD83 datum)
115 is a Long-Term Ecological Research (LTER) and Critical Zone Observatory (CZO) site, with a
116 long history of biogeochemical research (e.g. Scatena, 1989; McDowell et al., 1990; Silver et al.,
117 1994; White et al., 1998). MAP in this warm and humid tropical forest increases with elevation,
118 ranging from about 2500 mm to over 5000 mm at the highest elevation of 1074 m (Scatena,
119 1989; McDowell and Asbury, 1994). Rainfall is significant year round, with January through
120 April being the driest period (Heartsill-Scalley et al., 2007). Precipitation occurs mostly as
121 frequent, short, high intensity events (White et al., 1998; Scatena, 1989; Buss et al., 2010).
122 Convective boundary layer storms with strong orographic effects are most common (White et al.,
123 1998), but northeasterly trade winds, winter cold fronts, tropical storms, depressions and
124 hurricanes also affect the region (Heartsill-Scalley et al., 2007). MAT is 22-23°C at both sites
125 (White et al., 1998; Murphy and Stallard, 2012).

126 Our study focused on two LEF watersheds instrumented by the USGS (White et al., 1998; Buss
127 et al., 2011; Buss and White, 2012): Icacos (mature Colorado forest on quartz diorite) and Bisley
128 (mature Tabonuco forest on volcanoclastics) (Fig. 1). The Icacos site is located on the shoulder of
129 the Guaba ridge in the catchment of the Guaba stream, a tributary of Rio Icacos (see White et al.,
130 1998, for a detailed description of the site). The site is above the average cloud condensation
131 level at 600 m (Cox et al., 2002). Icacos soils are a somewhat poorly drained mix of Ultisols
132 (Boccheciamp, 1977) and Inceptisols (Huffaker, 2002; Soil Survey Staff, Web Soil Survey). The
133 profile we investigated was a Plinthic Haplohumult, closely matching the Los Guineos series
134 (very fine, kaolinitic, isothermic Humic Haplodox). The Bisley site is located within the Rio
135 Mameyes drainage system (Scatena, 1989), at an elevation below the average cloud condensation
136 level. Soils are well drained on convex slopes, and somewhat poorly drained on concave slopes
137 (Murphy et al., 2012). The soils are mapped as clay-rich Ultisols (Scatena, 1989; Johnston, 1992;
138 Cox, et al, 2002; Soil Survey Staff, Web Soil Survey). The profile we investigated was a Typic
139 Haplohumult, corresponding to the Humatus series.

140 Each site has soil water and gas samplers augered in a transect from a hillslope nose downslope
141 to the adjacent stream channel – a toposequence. In 1992, at Icacos, White et al. (1998) installed
142 ceramic cup suction lysimeters at three topographic locations on the Guaba Ridge: local ridgetop,
143 steep hillslope (~50% grade, downhill from the ridgetop) and ridge shoulder (moderate slope,
144 ~25% grade, uphill from the local ridgetop) – sites LG-1, LG-2 and LG-3, respectively. At
145 Bisley, Buss et al. (2011) developed a similar installation on a ridgetop, upper slope and lower
146 slope (riparian) (sites B1S1, B1S2 and B1S4 respectively). The approximate coordinates for the
147 Icacos and Bisley lysimeter fields (referenced to the NAD83) are N18°16.9', W65°47.4' and
148 N18°18.9', W65°44.7' respectively. Additionally, ground water wells were installed at both sites

149 (coordinates: N18°16.94', W56°47.34' at Icacos, N18°18.93', W65°44.75' at Bisley, WGS84
150 datum; Buss and White, 2012). The two sites have similar S deposition chemistry.

151

152 **3. METHODS**

153 **3.1 Sample collection**

154 Field investigations followed standard field methods (Schoeneberger et al., 2002). At the highest
155 topographic position, trenches were excavated to 2 m and sampled by horizon. At all hillslope
156 positions, two 1.5 m-deep hand-augered cores and fresh leaf litter from the forest floor were
157 collected. During the initial field campaign in June 2010, all operational lysimeters were
158 sampled. Subsequently, four lysimeters in the upper 2 m of the soil at each subsite were sampled
159 monthly from February 2011 until February 2012, whenever water was present. The aim was to
160 collect water at 15, 60, 150 and 180 cm; however, if functional lysimeters were unavailable at
161 those depths at a site, we used the closest functional ones instead (Table A3).

162 Precipitation (openfall) was sampled monthly from June 2010 to March 2012, and then again in
163 June 2012, at a station 2 km east of the Icacos site on Pico del Este (elevation 1051 m, MAP
164 4436 mm averaged over the period 1970-1994; Garcia-Martino et al. 1996). The collectors were
165 emptied weekly, thus each sample is the average deposition for the preceding week. This method
166 of collecting openfall reflects rainfall plus the coarse particulate fraction that settles out via
167 gravity (McDowell et al., 1990), but not the more abundant fine particulate and gaseous fraction
168 scavenged by the forest canopy.

169 Baseflow stream water was collected from the Guaba and Bisley streams in February, March and
170 June 2012, and a groundwater sample was collected in August 2012 at a depth of ~6.1 m from a

171 well at the Icacos site. It was not possible to retrieve enough water for isotope analyses from a
172 well at Bisley (9.6 m deep).

173

174 **3.2 Sample processing and analysis**

175 Soils were stored at room temperature in sealed bags. For total soil S isotope and chemical
176 analysis, splits of the samples were dried at 60°C overnight, sieved to <2 mm, and ground with a
177 mortar and pestle. Plant-available sulfate was extracted from splits of unprocessed samples by
178 shaking for a minimum of 4 hours in a 1:7 soil to deionized water mixture, centrifuging for 30
179 minutes at 3000 rpm, and then filtering the supernatant to 0.7 µm glass microfiber filters. Litter
180 samples were kept frozen then freeze-dried and pulverized in a ball mill.

181 Rain and pore water samples were shipped frozen from Puerto Rico to Berkeley, then filtered in
182 the lab to 0.7 µm within a few days of sampling, and stored refrigerated until further use. Only
183 water samples with a volume greater than 500 ml were processed for isotope measurements to
184 ensure sufficient quantities of sulfate. For sulfate isotope analysis, filtered water samples were
185 heated in a warm water bath, and a 1M BaCl₂ solution was added in excess (in a quantity equal to
186 approximately 10% of the sample volume). After 24 hours, the samples were acidified with a
187 few drops of 1N HCl to dissolve carbonates, then filtered again on a 1.6 µm filter to collect the
188 BaSO₄ precipitate. Because the samples were low in S, it was impossible to remove the BaSO₄
189 precipitate, therefore the entire surface of the filter was scraped off for combustion in the
190 elemental analyzer/mass spectrometer. The S content of blank filter samples was below the
191 detection limit.

192 The anion content of the soil extracts and water samples was determined on a Dionex ICS-1500
193 ion chromatograph with an IonPac AS9-HC 4 mm column, a 9 mM sodium bicarbonate eluent at
194 a flow rate of 1.0 mL/min and an international seven anion standard from Dionex. The analytical
195 precision of the instrument, in the range of values measured, was +/-10%.

196 The C and N contents (% dry weight) and stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of soil and litter
197 samples were determined via elemental analyzer/continuous flow isotope ratio mass
198 spectrometry using a CHNOS Elemental Analyzer (Vario ISOTOPE Cube, Elementar, Hanau,
199 Germany) coupled with an IsoPrime100 IRMS (Isoprime, Cheadle, UK) at the Center for Stable
200 Isotope Biogeochemistry, University of California, Berkeley. The reference material NIST SMR
201 1547 (peach leaves) was used as a calibration standard. Long-term external precision for C and N
202 isotope analyses was 0.10‰ and 0.15‰, respectively.

203 The total S concentration and $\delta^{34}\text{S}$ values of soils and litter, and the $\delta^{34}\text{S}$ value of sulfate in water
204 samples, were determined using the SO_2 EA-combustion-IRMS method on a GV Isoprime
205 isotope ratio mass spectrometer coupled with an Eurovector Elemental Analyzer (model
206 EuroEA3028-HT) at the Laboratory for Environmental and Sedimentary Isotope Geochemistry
207 (LESIG), University of California at Berkeley. Briefly, a small amount of powdered sample
208 containing a minimum of 2 μg S mixed with V_2O_5 catalyst was thermochemically decomposed
209 with copper wires at 1020°C, and the isotopic composition of the resulting SO_2 gas was
210 measured. Water vapor was removed with a $\text{Mg}(\text{ClO}_4)_2$ trap and CO_2 was eluted out using a
211 dilutor. Several replicates of the international standard NBS127 and two lab standards (both pure
212 BaSO_4) were run with each batch of samples. The long-term analytical precision of this method
213 is better than 0.2‰.

214 Due to the lack of certified soil and organic material standards for S isotope analyses, we
215 selected several different material types and cross-validated the results from the LESIG lab
216 against measurements from other labs. The results, displayed in Table A4, show that the
217 averages obtained by this lab agree well with those from other labs, and with good precision.
218

219 **3.3 Models and calculations**

220 The isotope composition is reported using standard δ notation:

$$221 \quad \delta(\text{‰}) = \left(\frac{R_{\text{Sample}}}{R_{\text{Standard}}} - 1 \right) \cdot 1000, \quad (1)$$

222 where R is the ratio of the rare to the common isotope. For $\delta^{34}\text{S}$, R is the ratio of ^{34}S to ^{32}S and
223 the standard is Canyon Diablo Troilite (CDT). Isotope fractionation can be described by the
224 fractionation factor α :

$$225 \quad \alpha = \frac{R_{\text{Product}}}{R_{\text{Substrate}}}, \quad (2)$$

226 the enrichment factor ε :

$$227 \quad \varepsilon = (\alpha - 1) \cdot 1000 \quad (2)$$

228 and the Δ value:

$$229 \quad \Delta = \delta_{\text{Product}} - \delta_{\text{Substrate}} \cong 1000 \cdot \ln \frac{R_{\text{Product}}}{R_{\text{Substrate}}}. \quad (4)$$

230 Following other studies (e.g. Brenner, 1999; Baisden et al., 2002; Sanderman and Amundson,
231 2008), we examined the content and isotopic composition of organic matter with depth,
232 assuming that its transport occurs through largely advective processes (i.e. vertical transport of a

233 solute or particulate matter in the liquid phase), and that during transport, decomposition creates
 234 aqueous or gaseous products that leave the system. At steady state, in soils where aboveground
 235 inputs far exceed belowground inputs, the advection/decomposition equations for the abundant
 236 and rare isotopes are (after Brenner, 1999):

$$237 \quad \frac{\delta[^{32}\text{S}]}{\delta t} = 0 = v \frac{\delta[^{32}\text{S}]}{\delta z} - k^{32}\text{S} \quad (5)$$

$$238 \quad \frac{\delta[^{34}\text{S}]}{\delta t} = 0 = v \frac{\delta[^{34}\text{S}]}{\delta z} - k\alpha^{34}\text{S} \quad (6)$$

239 where v is the advection coefficient (in cm/yr), k is the first order organic matter decay constant
 240 (in yr^{-1}) and α is the fractionation factor. In terms of the heavy to light isotope ratio R , the
 241 general solution becomes:

$$242 \quad \frac{R(z)}{R(0)} = e^{-\frac{kz}{v}(\alpha-1)} \quad (7)$$

243 where $R(z)$ and $R(0)$ are the $^{34}\text{S}/^{32}\text{S}$ ratios of the soil at depth z and of the inputs respectively.
 244 Denoting the fraction of total S remaining at depth z compared to the surface inputs as f :

$$245 \quad \frac{S(z)}{S(0)} = e^{-\frac{kz}{v}} = f \quad (8)$$

246 we can rewrite Eqn. 7 as:

$$247 \quad \frac{R(z)}{R(0)} = f^{(\alpha-1)} \quad (9)$$

248 In δ notation, Eqn. 9 is equivalent to:

$$249 \quad \delta(z) = \varepsilon \cdot \ln f + \delta(0) \quad (10)$$

250 Furthermore, at fractionation factors α close to unity, Eqn. 9 converts to (Ewing et al., 2008;
251 Amundson et al., 2012):

$$252 \quad \delta(z) = (\delta_i(z) + 1000) \cdot f(z)^{(\alpha-1)} - 1000 \quad (11)$$

253 To model this for N and S, we assigned the parameters as follows: $\delta(z=0)$ equals the $\delta^{15}\text{N}$ or $\delta^{34}\text{S}$
254 value of the A horizon; for any subsequent horizons, $\delta_i(z) = \delta(z-I)$; and $f(z)$ equals the ratio of
255 total soil N or S at depth z to the total soil N or S content of the uppermost horizon. We
256 calculated the α values that best fit the data.

257 We calculated any S contributed to pore water by rock weathering as the amount measured in
258 pore water minus the amount contributed by rainfall corrected for evapotranspiration (after
259 White et al., 2009):

$$260 \quad S_{weathering} = S_{pore\ water} - S_{rainfall} \left[\frac{Cl_{pore\ water}}{Cl_{rainfall}} \right] \quad (12)$$

261 We calculated the sulfate flux Q through the regolith (soil+saprolite) with the equation:

$$262 \quad Q = q_h \Delta c \quad (13)$$

263 where q_h is the vertical infiltration rate or field flux density (product of the hydraulic
264 conductivity and the hydraulic gradient), and $\Delta c = c_f - c_i$ is the change in concentration
265 between two depths (White et al., 1998). White et al. (1998) reported a vertical infiltration rate of
266 1 m/yr at Icacos. Here we used the hydraulic field flux density values from Buss et al. (2011),
267 which are corrected for evapotranspiration: 1.28 m/yr at Icacos and 1.62 m/yr at Bisley. Sulfate
268 fluxes were calculated to and from a given depth interval by multiplying q_h times the difference
269 in the average sulfate concentration between the lysimeter sampled at that depth (c_f) and the one

270 directly above it (c_i). For the topmost lysimeters, the starting concentration c_i is the sulfate
271 concentration in precipitation. The isotopic enrichment associated with additions or losses in
272 pore water was expressed as the difference between the $\delta^{34}\text{S}$ value of the two lysimeter samples
273 (i.e. the Δ value, Eqn. 4)

274

275 **4. RESULTS**

276 **4.1 Field observations**

277 Both excavated soil profiles, located at the highest topographic location of each transect, had
278 high clay contents, reddening and loss of rock structure (Table 1). The granitic soil at Icacos had
279 about 5% less clay than the volcanic-derived soil at Bisley. In addition, the clay content of the
280 Icacos soil sharply declined below 111 cm, whereas it remained high to the depth of excavation
281 (158 cm) at Bisley. In the upper 16-17 cm, both soils were rich in humus and highly mixed by
282 earthworms. Below 17 cm, significantly darker patches or zones in the soil profile suggest that
283 humus had also accumulated in some subsurface horizons. Below the well-mixed biotic horizons,
284 clay content increased and the soil color indicated gleying, suggesting at least periodic reducing
285 conditions. The Icacos soil had a prominent zone of plinthite (red areas enriched in Fe (III) oxide
286 adjacent to gray, Fe (III)-depleted zones, formed in response to fluctuating water tables and
287 redox conditions) above a 10 cm-thick layer displaying Mn oxide stains. In general, both soils
288 showed evidence of reducing conditions due to periodic saturation, and adequate C for microbial
289 metabolism and oxygen consumption.

290

291 **4.2 Vegetation litter and soil chemistry**

292 C, N and S contents and stable isotope values vary widely among vegetation types (Table 2).
293 Palo Colorado (*Cyrilla racemiflora*, the dominant tree at Icacos) leaves have a C:N ratio twice
294 that of Tabonuco (*Dacryodes excelsa*, the dominant tree at Bisley) leaves (54:1 versus 27:1),
295 higher C:S (308:1 versus 253:1), but lower N:S (6:1 versus 9:1) (Table 2). The difference is even
296 more pronounced in the O horizon (C:N ratio of 78:1 at Icacos versus 29:1 at Bisley, C:S ratio
297 522:1 versus 317:1, and N:S ratio 7:1 versus 11:1). As expected, mineral soil C and N content
298 declines exponentially with depth at all sites. C and N content also decreases downslope, with
299 highest values on the ridges (surface values of $3.99 \pm 0.20\%$ C and $0.22 \pm 0.01\%$ N at Icacos, and
300 $4.31 \pm 0.22\%$ C and $0.35 \pm 0.02\%$ N at Bisley). Total soil S and water extractable sulfate (Fig. 2)
301 concentrations are highest in the surface horizons. Sulfate declines exponentially with depth, to
302 <1 mg S/kg. In contrast, total S – largely in organic forms – decreases irregularly with soil depth,
303 and has subsurface accumulations that correspond to the visual humus increases in some clay-
304 rich horizons. The mineral soil has a lower S content than the O horizon. Total soil S content (in
305 mg/kg) is highest on ridgetops in the top 10 cm, but decreases downslope when averaged over
306 the upper meter. The highest S concentrations occur in the Bisley ridgetop soil (625 ± 27 mg S/kg
307 in surface samples and 346 ± 7 at the bottom of the profile), and the lowest in the Bisley lower
308 slope soil (189 ± 16 mg/kg at the surface, 12 ± 3 mg/kg at the bottom). C:S ratios decrease nearly
309 exponentially with depth, from 130:1 to 3:1 at Icacos, and from 81:1 to 4:1 at Bisley (Fig. 2).
310 The decrease is less pronounced in the Bisley lower slope site. Similarly, N:S ratios generally
311 decrease with depth from 7.2:1 to 0.1:1 at Icacos, and from 9.2:1 to 0.5:1 at Bisley. N:S ratios are
312 highest throughout at the Bisley lower slope site, and remain relatively constant with depth.

313 The $\delta^{13}\text{C}$ values of Palo Colorado leaves are slightly higher than those of Tabonuco (-33.2 versus
314 -36.0‰), but the O horizons both have similar $\delta^{13}\text{C}$ values (-30‰). Despite higher $\delta^{15}\text{N}$ values in

315 plant litter at Icacos (0.97 compared to -0.51‰), $\delta^{15}\text{N}$ values of the O horizon are more negative
316 at Icacos than at Bisley (-1.4 compared to -0.04‰). As expected, soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values
317 exceed those in plant litter and the O horizon, and generally increase with depth. Surface (top 10
318 cm) $\delta^{13}\text{C}$ values are independent of topographic position. Surface soil $\delta^{15}\text{N}$ values are highest in
319 the Bisley soils, and increase downslope at Bisley ($4.56 \pm 0.5\text{‰}$ on the ridgetop, $6.45 \pm 0.6\text{‰}$ on
320 the upper slope and $6.8 \pm 0.8\text{‰}$ on the lower slope), but are unaffected by topographic location at
321 Icacos. The two sites have similar litter (15.4 and 14.9‰) and O horizon (12.9 and 13.3‰) $\delta^{34}\text{S}$
322 values. The mineral soil is enriched in ^{34}S compared to the O horizon (depth-weighted average
323 soil $\delta^{34}\text{S}$ values between 13.6 and 18.9‰, depending on the site) (Fig. 2). The difference
324 between the $\delta^{34}\text{S}$ values of litter and the top 10 cm of the soil ranges from -2.1 to 1.4‰.

325

326 **4.3 Water chemistry**

327 The precipitation chemistry data integrate wet and coarse-sized dry inputs to the ecosystem.
328 Total precipitation chemistry varied widely, with sulfate concentrations ranging from 4.6 to 38.8
329 μM , and $\delta^{34}\text{S}$ values from 10.7 to 20.5‰ (Table 3). Precipitation sulfate $\delta^{34}\text{S}$ values are
330 uncorrelated with time of the year or sulfate concentration. The volume-weighted average
331 precipitation $\delta^{34}\text{S}$ value is $16.1 \pm 2.8\text{‰}$.

332 The sea salt sulfate was estimated according to:

$$333 \textit{seasalt SO}_4 = 0.052 \cdot Cl \quad (14)$$

334 where 0.052 is the sulfate to chloride ratio in seasalt (Keene et al., 1986). Calculations suggest
335 that sea salt contributes 37% of the precipitation sulfate, with a range from 12 to 74%.

336 Conversely, over a quarter of the precipitation sulfate (26-88%) is of non-seasalt origin (Table 3)

337 – assuming that S is as effectively transported as Cl. Our estimates however refer only to the
338 coarse non-seasalt aerosol fraction, not to the fine aerosol fraction scavenged by the canopy.

339 The Guaba stream sulfate concentration ($14.6 \pm 0.5 \mu\text{M}$) resembles that of the volume-weighted
340 precipitation average. The sulfate concentration of the Bisley stream ($39.4 \pm 0.3 \mu\text{M}$) is two and a
341 half times greater than that of the Guaba, and just slightly larger than the precipitation maximum.
342 Guaba $\delta^{34}\text{S}$ values ($19.5 \pm 1.6\text{‰}$) overlap with the precipitation average, and resemble the values
343 of the groundwater (Table 4). On the other hand, the Bisley stream $\delta^{34}\text{S}$ ($1.6 \pm 0.7\text{‰}$) is much
344 closer to the $\delta^{34}\text{S}$ of volcanic or basalt S (see Table A2).

345 Pore water sulfate concentrations and isotope ratios (Fig. 4) fluctuate with depth and season,
346 though less so at Bisley than at Icacos. Averaged Icacos pore water sulfate concentrations
347 resemble those in groundwater, the Guaba stream, and volume-weighted precipitation averages,
348 except for higher values near the soil surface. The Bisley pore waters also resemble the
349 precipitation average, except for higher sulfate concentrations at 183 cm in the saprolite. All
350 Bisley pore water samples contain significantly less sulfate than the Bisley stream. Average pore
351 water sulfate $\delta^{34}\text{S}$ values vary little with depth. The $\delta^{34}\text{S}$ values of the Icacos samples exceed the
352 volume-weighted precipitation average, but not the groundwater and baseflow values. The Bisley
353 pore water samples are significantly enriched in the heavy isotope compared to the volume-
354 weighted precipitation average and the Bisley stream. We found no contribution of bedrock S to
355 soils. Relating the pore water S (Fig. 4) and precipitation S and Cl data (Table 3) with Eq. 12
356 indicates negative weathering S sources (e.g. net loss). Only the saprolite at Bisley (right below
357 the soil zone) revealed between 9 to 44% rock-derived S contributions.

358

359 5. DISCUSSION

360 5.1 The tropical S cycle

361 Previous work has determined that both sites are likely near quasi-steady state with respect to the
362 biogeochemical cycling of C, N, and S (e.g. Chestnut et al., 1999; Stallard, 2012b). C cycling
363 rate constants have not been determined, but based on MAT, extrapolating from other studies, an
364 organic matter turnover time of ~10 years for the most rapidly cycling pools is likely
365 (Sanderman et al., 2003). As a starting assumption, the S cycle in Puerto Rico is assumed to
366 resemble the one illustrated in Fig. A1. At steady state, the balance between the inputs and losses
367 will determine the $\delta^{34}\text{S}$ value of soil S.

368

369 5.1.1 S sources to the LEF soils

370 Previous stream chemistry research (McDowell and Asbury, 1994; Stallard, 2012a) has shown
371 that atmospheric sources provide the bulk of S in this rainforest soil. Our results, based on pore
372 water chemistry, are consistent with this interpretation. Multiplying the MAP times the average
373 sulfate content of rain water (Table 3) suggests an atmospherically-derived S flux of 2.2 g
374 S/(m²yr) at Icacos, and 1.8 g S/(m²yr) at Bisley, consistent with the results of McDowell and
375 Asbury (1994) based on stream composition.

376 At the LEF, atmospherically-derived S has five main potential sources: seasalt, marine non-
377 seasalt sulfate, volcanic ash input, Saharan dust, and anthropogenic emissions. Out of the 16
378 precipitation samples that were large enough for S isotope measurements, the non-seasalt sulfate
379 $\delta^{34}\text{S}$ values ranged between 6.3 and 11.6‰ for 6 samples (Table 3), which is less than the typical

380 range of 12.5 to 18.7‰ for sulfate produced by DMS oxidation (Calhoun et al., 1991). Three
381 other samples had a total sulfate $\delta^{34}\text{S}$ value less than the average of precipitation over the
382 Atlantic Ocean (13.3‰; Chukrov et al., 1980). The fact that seasalt and DMS fail to fully
383 account for the total S indicates significant non-marine sources at certain times of the year.

384 Volcanic sources of sulfate (average $\delta^{34}\text{S}$ of 5‰) are unlikely given that the closest active
385 volcano is Soufrière Hills on the Island of Montserrat (16°45'N, 62°12'W), about 500 km to the
386 southeast, and no significant volcanic activity was reported over the study period. Saharan
387 mineral dust is known to deliver nutrients such as K, Mg (McDowell et al., 1990), Ca (Heartsill-
388 Scalley, et al, 2007) and P (Pett-Ridge, 2009) to the LEF soils. While Saharan dust reaching
389 Puerto Rico does contain measurable sulfate (Reid et al., 2003; Stallard, 2012a), its quantitative
390 importance is unclear. Heartsill-Scalley et al. (2007) found insignificant differences between
391 sulfate fluxes during Saharan dust times (April-September) and the rest of the year at Bisley,
392 concluding that dust contributes minimal sulfate. Here we observed a slight increase in the
393 sulfate concentrations (Table 3) during April-September (volume-weighted average of 16.6 vs.
394 14.7 μM respectively), and $\delta^{34}\text{S}$ values were slightly lower during April-September than the rest
395 of the year (volume-weighted averages of 15.6 and 17.1‰ respectively). The $\delta^{34}\text{S}$ value of
396 sulfate from Saharan marine evaporite deposits (17-19‰) is indistinguishable from that of the
397 marine non-seasalt input (Brandmeier et al., 2011). However, Saharan dust collected over the
398 North Atlantic Ocean has lower $\delta^{34}\text{S}$ values that range between 11 and 13‰ (Gravenhorst,
399 1978). To explain this, it has been suggested that the depleted S in Saharan dust is probably not
400 of Saharan soil origin, but rather anthropogenic SO_2 oxidized on the dust (Savoie et al., 1989;
401 Harris et al., 2012), although Saharan soil $\delta^{34}\text{S}$ values have not been measured. It is thus possible

402 that at least some of the precipitation samples reflect inputs of long-range sources of
403 anthropogenic S.

404 The low $\delta^{34}\text{S}$ values of samples collected during winter months (January and March) may also
405 reflect anthropogenic inputs. Anthropogenic S (and N) can reach the LEF from North America
406 via Northern cold fronts (McDowell et al., 1990), which deliver 5% of the rainfall at LEF (Scholl
407 et al., 2009). Northern Hemisphere contaminant deposition peaks in January, April and May,
408 when the cold fronts are strongest (Stallard, 2012a).

409 Flux chamber measurements found that soils at LEF take up carbonyl sulfide (COS) from the
410 atmosphere (Whelan and Rhew, 2012). Although not directly measured, COS $\delta^{34}\text{S}$ value has
411 been estimated to be 11‰ (Newman et al., 1991); however, this COS flux is three orders of
412 magnitude smaller than the sulfate input, and therefore unlikely to significantly affect the
413 isotopic composition of soil S.

414

415 *5.1.2 S transformations during transport through soil*

416 Rain infiltration rates in the surface soils are greater at Icacos (2-9 cm/min) than at Bisley (0.07-
417 1.5 cm/min), and exceed typical rainfall intensity (0.013 cm/min) at both sites (McDowell et al.,
418 1992). This suggests that, despite the high MAP, soil surfaces rarely become waterlogged. Once
419 in the soil, the rainwater travels downwards mostly via macropores (White et al., 1998), and
420 follows preferred drainage patterns downslope. At Icacos, subsurface water flow is generally
421 deep, below the rooting zone (McDowell et al., 1992), typically along fractures at the saprolite-
422 bedrock interface (White et al., 1998; Kurtz et al., 2011). On the other hand, soil water at Bisley
423 flows within the rooting zone (McDowell et al., 1992). As a result, McDowell et al. (1992)

424 proposed that oxidative processes (degradation of S and nitrification) are segregated in space
425 from reductive processes (plant uptake of S and denitrification) at Icacos, but can coexist at
426 Bisley due to highly variable redox conditions over only fractions of a cm.

427 The depth trends in solid phase S (Figs. 2 and 3) indicate that microbial decomposition may be
428 releasing sulfate depleted in ^{34}S compared to the soil S. For example, in temperate regions the
429 soil solution has lower sulfate $\delta^{34}\text{S}$ values than the precipitation (Fuller et al., 1986; Novak et al.,
430 1995; Zhang et al., 1998; Alewell and Gehre, 1999; Alewell et al., 1999). However, we found
431 that during most months, sulfate was enriched by up to 5.6‰ in ^{34}S relative to the solids (Fig. 3).
432 This appears to be due to dissimilatory sulfate reduction in the aqueous phase. Plant uptake also
433 increases pore water $\delta^{34}\text{S}$ values, however it is unlikely to affect the deeper samples due to the
434 rooting patterns in these soils. Other mechanisms such as the adsorption/desorption of sulfate
435 (Table A1), produce small isotopic fractionation, and are likely not important here.

436 Our mass balance calculations show that all soils lose pore water sulfate near the surface
437 (between 1.3 and 1.7 g S/(m²yr)), due to uptake by vegetation, and possibly due to sulfate
438 reduction (thin dashed line in Fig. 5). This sulfate loss is associated with an enrichment in the
439 heavier isotope in pore water by 1.1 to 3.2‰, which is consistent with biological sulfate uptake.
440 The zone of apparent uptake is only 15 cm thick in the lower slope (riparian) soil at Bisley, but is
441 approximately 60 cm thick for all other soils. Below this zone, small losses or gains of sulfate
442 occur with nearly no isotopic fractionation, consistent with oxidative degradation. Our data
443 suggest that the oxidative degradation of organic S, resulting in an influx of sulfate to the pore
444 water with slightly lower $\delta^{34}\text{S}$ values, might occur all the way down to the saprolite. The soil-
445 saprolite boundary was identified in the field, based on textural and color changes. For most of
446 these soils, there appears to be a net source of sulfate to pore water in the saprolite region, which

447 could be due to dissolution of sulfide minerals from embedded bedrock corestones. Further work
448 is needed to confirm this. The net result is that as water travels through the soil, it loses sulfate
449 and becomes enriched in the heavy isotopes of S (Fig. 5). The net amount of sulfate lost over the
450 entire soil depth (i.e. losses minus additions occurring above the soil-saprolite boundary) is
451 similar for all soils on quartz diorite and for the volcanoclastic ridgetop soil (between 1.4 and 1.7
452 $\text{gS}/(\text{m}^2\text{yr})$). The slope sites on the volcanoclastic parent material lose significantly less sulfate
453 ($0.8\text{-}0.9 \text{ gS}/(\text{m}^2\text{yr})$). The net enrichment in heavier S isotopes is lower on steeper slope sites,
454 potentially as a result of decreased plant uptake due to less dense vegetation on the steeper
455 slopes.

456

457 *5.1.3 Hydrologic S export*

458 Stream sulfate concentrations at baseflow (Table 4) reflect export from soil. McDowell and
459 Asbury (1994) found that N and S concentrations vary minimally with discharge, therefore our
460 baseflow measurements are likely representative of S loss during an entire hydrological year. To
461 estimate the net export of sulfate from the watershed, we multiplied our measured average
462 stream sulfate concentration by the average runoff estimates for the Guaba (3630 mm/yr) and
463 Bisley (2007.5 mm/yr) streams from Stallard and Murphy (2012) and Schellekens et al. (2004)
464 respectively. Our values, $1.7\pm 0.1 \text{ g S}/(\text{m}^2\text{yr})$ at Icacos, and $2.5\pm 0.2 \text{ g S}/(\text{m}^2\text{yr})$ at Bisley (Fig. 5),
465 mirror the $2.4 \text{ g}/(\text{m}^2\text{yr})$ hydrologic export estimated by McDowell and Asbury (1994).

466 At Icacos, stream S export is $0.5 \text{ g}/(\text{m}^2\text{yr})$ less than the inputs, which means that S accumulates
467 in soil organic matter and/or was released in gaseous form or via sediment losses (Fig. 5). In
468 contrast, the stream S export at Bisley exceeds atmospheric inputs by $0.7 \text{ g}/(\text{m}^2\text{yr})$, possibly

469 indicating bedrock sources. Indeed, pyrite and other S-minerals, likely of hydrothermal origin,
470 are present throughout drilled bedrock cores (to 37 m deep) from the Bisley and, to a much lesser
471 extent, in the Icacos watersheds (Buss and White, 2012; Buss et al., 2013). Although this
472 difference between S inputs and exports may be due to the fact that our input values are based on
473 measurements at a higher elevation rather than directly at the sites, the results are consistent with
474 other studies. Stream S export in the Mameyes stream, into which the Bisley stream drains, also
475 exceeds atmospheric inputs (Stallard and Murphy, 2012); rock S may thus be an important
476 component to the hydrologic export at Bisley. At Bisley, N outputs also exceed inputs,
477 suggesting unaccounted-for inputs or a slow depletion of soil organic N (Chestnut et al., 1999).

478

479 **5.2 Controls on the tropical soil S cycle**

480 Our study design allowed us to investigate how four of the soil forming factors (Jenny, 1941)
481 affect the soil $\delta^{34}\text{S}$ values. The impact of time could not be assessed in this study, as both sites
482 are of approximately the same age (soil residence time equals the ratio of soil thickness to
483 denudation rates; Brown et al., 1995).

484 *5.2.1 Climate*

485 The high MAP is a defining characteristic in this ecosystem, affecting the types and rates of
486 processes. Bern et al. (2007) and Bern and Townsend (2008) found that, in similar ecosystems in
487 Costa Rica and Hawaii, dissimilatory sulfate reduction is not a major process despite the high
488 MAP. In the LEF, S isotopes suggest that sulfate reduction occurs, but is spatially and temporally
489 variable.

490 Given the higher elevation at the Icacos site, rainfall and dust deposition patterns are somewhat
491 different than at Bisley (e.g. Scholl et al., 2009), resulting in a higher sulfate input at Icacos.

492 Icacos receives 700 mm more rainfall per year on average, and derives ~5% of its total
493 precipitation input from cloud water (Pett-Ridge et al., 2009). As a result, we observed higher
494 extractable sulfate concentrations at the soil surface, and greater variability in Cl and sulfate
495 concentrations in pore water. Because Cl and sulfate originate from atmospheric deposition, the
496 differences in their concentrations between the two sites reflect differences in rainfall, dust
497 deposition and/or evapotranspiration rates. The slight differences in MAP also elicit differences
498 in vegetation, which impacts soil C, N and S.

499

500 *5.2.2 Organisms*

501 Although we could not detect a difference between the two sites despite the different vegetation
502 cover types, our isotope data show evidence for extensive biological cycling, including both
503 organic S oxidative degradation and dissimilatory sulfate reduction. The co-occurrence of these
504 two processes in LEF soils is supported by similar results for N (Pett-Ridge et al., 2006) and by
505 other studies showing the co-occurrence of saturated and unsaturated processes due to spatial
506 segregation in these soils (McSwiney et al., 2001).

507 Compared to the soil, vegetation litter is approximately 1‰ depleted in ^{34}S (Fig. 7). Differences
508 between soil and vegetation $\delta^{34}\text{S}$ were also observed in Costa Rica (Bern et al., 2007), and have
509 been reported for some temperate sites as well. Such a small difference between the $\delta^{34}\text{S}$ of total
510 S in soils and vegetation litter might be regarded as evidence for little to no isotopic fractionation
511 during plant uptake and assimilation. However, since plants take up S mostly as sulfate, litter
512 $\delta^{34}\text{S}$ must be compared with rain and pore water $\delta^{34}\text{S}$ to evaluate this issue. Our data show that
513 the fresh litter is 3 to 5‰ depleted in ^{34}S compared to the average pore water (Fig. 3). This
514 suggests that plants discriminate against the heavier isotope in the process of sulfate uptake and

515 assimilation. A distinctive, albeit relatively small, fractionation during S assimilation is common
516 also for other life forms. For instance, S metabolism studies with bacteria, algae and yeast (Table
517 A1) found that assimilated S can be up to 2.8‰ depleted compared to its source (Kaplan and
518 Rittenberg, 1964). Another potential source of fractionation occurred during S retranslocation
519 and the beginning of decomposition of the fresh leaves. Plants also fractionate S isotopes via H₂S
520 emissions when stressed by high S inputs, (Trust and Fry, 1992), but given the low S loading at
521 our sites, we suspect plant H₂S emissions by plants are insignificant.

522

523 *5.2.3 Topography*

524 Our sites were chosen to capture the topographic variation in weathering and pore waters from
525 ridge to valley. Topography is known to impact N cycling in different climates (e.g. Robertson et
526 al., 1988; Raghubanshi, 1992; Roy and Singh, 1994; Chestnut et al., 1999). In the LEF, previous
527 studies have shown that changing redox conditions with topographic location affect N₂O
528 production (McSwiney et al., 2001). We found that the C, N and S content (mg/kg soil) of the
529 mineral soil in the upper meter decrease downslope at all sites, while soil surface $\delta^{15}\text{N}$ values
530 decrease downslope at the Bisley site only. The lower slope site at Bisley has the lowest C, N
531 and S content, and the highest $\delta^{15}\text{N}$ and lowest $\delta^{34}\text{S}$ values, indicating that reduction processes
532 are important. Soils at this site are wetter and more gray in color than those at the other sites,
533 where $\delta^{34}\text{S}$ values seem unaffected by topography.

534

535 *5.2.4 Lithology*

536 Soil and pore water $\delta^{34}\text{S}$ values (e.g. Fig. 3) are closer to input chemistry (Table 2) than to
537 typical values for reduced S minerals in rocks (Table A2), reflecting little to no contribution of
538 bedrock S to soils. Compared to the volcanoclastic bedrock, the quartz diorite contains few S
539 minerals and only in a few discrete zones (e.g., Buss et al., 2008; 2013; Buss and White, 2012),
540 but we found no significant difference in $\delta^{34}\text{S}$ values in the deep soils at the two sites that could
541 indicate a lithologic impact. In contrast, once the water leaves the weathered soil and enters
542 saprolite, lithologic inputs are significant at Bisley (below 180 cm) and in the stream at baseflow,
543 given the very low $\delta^{34}\text{S}$ value ($1.6\pm 0.7\text{‰}$). It then appears that bedrock S is lost to the
544 groundwater before the saprolite converts to soil. Indeed, the 37+ m of saprolite in Bisley
545 contains abundant, highly fractured bedrock corestones containing sulfide minerals (Buss et al.,
546 2013). In contrast, the Icacos baseflow $\delta^{34}\text{S}$ value ($19.5\pm 1.6\text{‰}$) is similar to that of atmospheric
547 inputs, signifying minimal lithologic input of S.

548

549 **5.3 Comparison of the S and N cycles in tropical soils**

550 To understand the degree of coupling or similarity between the S and N cycles, we studied the
551 isotope effects during transport through soil of the dissolved and solid organic matter. Advection
552 is likely the main transport process here, because inputs occur mainly at or near the surface: roots
553 and earthworms diffusively mix the upper 16-17 cm (Table 1), and plant litter is added to the O
554 horizons. An advection model supports that a linear relationship should exist between soil δ
555 values and $\ln f$ (Eqn. 10). We found a linear relationship between $\delta^{15}\text{N}$ and $\ln(N\text{‰})$, but not
556 between $\delta^{34}\text{S}$ and $\ln(S\text{‰})$ (Fig. 6). There are therefore some key differences between N and S
557 biogeochemistry in these soils. The advection model assumes constant v , k and α with depth

558 (Baisden et al., 2003), so one or more of these parameters must vary for S. Since v and k are
559 parameters that should be similar for N and S, the S isotope fractionation factor α is most likely
560 the reason for the contrast between N and S.

561 We calculated the fractionation factors for the soils examined through trenching. For N, the
562 depth-insensitive fractionation factors (Eqn. 11) are all smaller than 1, showing enrichment in the
563 heavier isotope due to oxidative degradation of the organic matter (in the topmost (A) horizon)
564 as it is moved downward in the soil profile. For S, since α changes with depth, we computed the
565 fractionation factor α between soil layers by applying Eqn. 11 to each layer using the layer above
566 as the S input. The results show large variations in α with depth (Fig. A2 in the Appendix), with
567 both: (1) α values less than 1, indicative of oxidative degradation, or successive cycles of
568 oxidative degradation and assimilation, and (2) α values greater than 1, indicative of
569 dissimilatory sulfate reduction, which leads to depleted reduced S compounds that are removed,
570 thus enriching the soil in ^{34}S .

571 Thus, these results suggest a decoupling of, or differences in, the soil N and S cycles, particularly
572 in terms of redox reactions. Denitrification should be thermodynamically favored over
573 dissimilatory sulfate reduction, since it has a higher Gibbs free energy yield (Zehnder and
574 Stumm, 1988). Thus, the greater apparent redox sensitivity of S compared to N is unexpected.
575 The most likely explanation is that the isotope fractionation effects associated with S reduction
576 are enhanced compared to those of denitrification due to the greater relative biological demand
577 for N than for S. S is in greater biological excess than N in these soils, as evidenced by the
578 generally low N:S ratios (Fig. 4). If N becomes limiting, the observable fractionation will be
579 greatly reduced due to near complete consumption of the nitrate by any biological fractionating
580 process, unless reduced N trace gasses leave the system. Because S is likely in low biological

581 demand, it is less likely to be completely consumed by any process, and therefore the observed
582 isotope effects will be greater. Furthermore, sulfate adsorbs on iron and aluminum oxides and,
583 instead of being lost in gaseous form, reduced S species are generally reoxidized and/or
584 assimilated into organic matter (Alewell and Novak, 2001), which can subsequently re-
585 experience oxidative degradation and reduction. In contrast, nitrate is poorly absorbed in soils,
586 and reduced N species easily leave the soil in gaseous forms. In general, it appears that S
587 isotopes reflect an integrative view of multiple cycles of reduction and oxidation processes.

588

589 **6. CONCLUSIONS AND OUTLOOK**

590 This study of S biogeochemistry in two Puerto Rican watersheds (Icacos and Bisley) combined a
591 comparative analysis of stable N and S isotope measurements in the soil with S isotope
592 measurements in atmospheric inputs, pore water, stream water at baseflow and groundwater. In
593 most ecosystems, the LEF included, S cycling depends entirely on the steady supply of sulfate
594 from a variety of atmospheric sources. This Puerto Rican rainforest currently receives a portion
595 of its S from anthropogenic sources in North America and the eastern side of the Atlantic. We
596 found that stream S export is 0.5 g/(m²yr) less than the inputs at Icacos, but 0.7 g/(m²yr) more at
597 Bisley. This suggests that there may be yet-unaccounted loss pathways at Icacos (e.g. gaseous
598 emissions or sediment transport), and input pathways at Bisley (e.g. lithogenic). The
599 volcaniclastic rock likely delivers S to the stream, but not to the soil, and therefore the soil-plant
600 system depends on atmospheric inputs. We found no indication that parent material impacts S
601 biogeochemistry within the soils. Topography, on the other hand, affects the S cycle through
602 redox conditions. Climate is the main abiotic factor driving S cycling in the LEF, especially the
603 high MAP, which determines the types and rates of processes. Biological cycling is extensive,

604 and our data support the co-occurrence of three major S-fractionating processes in these soils:
605 plant uptake, oxidative degradation of organic S and dissimilatory bacterial sulfate reduction.
606 The rate and importance of these processes vary in time and space, and their co-occurrence
607 dampens their individual signals. An advection/decomposition model agreed well with the N
608 data, however the assumption of a constant fractionation factor α with depth failed for S. This is
609 a fundamental difference between N and S cycling in these soils, likely because S is in less
610 biological demand and more likely recycled than lost in gaseous forms compared to N.

611 The ecosystem's dependence on atmospheric S inputs implies that any changes in the amount of
612 rain or the amount of sulfate in rain will drive the system out of its present steady state. In the
613 LEF, climate change and deforestation may decrease orographic rains, which are responsible for
614 29-35% of the precipitation in this rainforest (Scholl et al., 2009). Such decreases in MAP could
615 begin to deplete the soil organic S (and N) pool. In addition to changing rain patterns,
616 deforestation also has a more direct effect on S cycling since vegetation assimilates and retains
617 atmospheric sulfate in the soil. Disturbances in vegetation cover may thus accelerate S losses and
618 decrease the soil organic S pool.

619

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1

TABLES

2 Table 1: Field data for the Icacos and Bisley soils from pits dug at the highest topographic location (ridge
3 shoulder and ridgetop respectively). Nomenclature according to NRCS guidelines (Schoeneberger et al.,
4 2002).

Horizon	Top [cm]	Bottom [cm]	Color	Texture	Clay %	Structure	Roots	Features
<i>Icacos</i>								
O	3	0						
A1	0	7.5	10YR 4/6	cl	34	3 f,c sbk	2vf, 2f, 1m, 1c	
A2	7.5	16	10YR 5/6	cosc	37	2 f,c sbk	1vf, 1f, 1c	
Btg1	16	30	10YR 7/2, 7/6; 7.5YR 6/8	cosc	40	3 c,vc sbk	1vf	
Btg2	30	45	10YR 7/2, 7/6; 7.5YR 5/8; 5YR 5/8	sc	>40	2 m,c sbk	1vf	
Btv	45	75	5YR 5/8; 10YR 6/8; 10YR 4/4	c (or vfsc)	>40	2 c,vc sbk	1vf	plinthite; dark spots with humus and Mn
Bt1	75	85	10YR 2/1; 7.5YR 6/4; 5YR 4/4; 2.5YR 4/6	cosc	35	2 m,c sbk	0	Mn-rich zone
Bt2	85	102	2.5YR 4/6	cl	38	2 m,c sbk	0	white quartz and mica-rich; smooth
Btg3	102	111	5YR 8/1; 5YR 6/6	c	>40	2 m sbk	0	
Crt	111	127	2.5YR 3/4, 5YR 5/8, 7.5YR 7/5	grls	3	rock texture	0	oxidized saprolite with black Mn oxides
<i>Bisley</i>								
O	2	0	10YR 5/6				mat below litter	highly mixed
A1	0	10	10YR 5/6	cl	38- 40	3 f,m sbk	3vf, 3f, 2m, 2c	highly mixed
A2	10	17	10YR 6/8, 5/6	c	45	3 f sbk	2vf, 2f, 1m, 1c	
Btg1	17	40	10YR 7/8; 7.5YR 6/8	c	>45	2 c abk	1vf, 1f, 1c	
Bt1	40	66	5YR 6/8; 10YR 5/6	c	>45	2 c abk → 2 f abk	1vf, 1f	
Bt2	66	95	2.5YR 6/8, 6/6; 10YR 7/8	c	>45	2 c sbk → 2 m sbk	1vf, 1f	white saprolite flakes mixed in; some reduced spots
Bt3	95	102	10R 5/6; 7.5YR 5/6	c	>45	2 m,c sbk	1vf	flakes of saprolite
Btg2	102	142	5YR 5/6; 10R 5/6; 7.5YR 6/8	c	40- 45	2 m,c sbk	1vf	
Crt	142	158	10R 5/8	cl	36	2 c sbk	1vf	many white flakes of kaolinized grus

5 Table 2: The C, N and S composition of vegetation and O horizon (litter layer) at the Icacos and Bisley
6 sites. Analytical error is 0.1‰ for C and N isotopes and 5% of the value for C and N concentration;
7 analytical error for S it is 0.6‰ for isotopes and 350 mg/kg for concentration.

Sample type *	C %	$\delta^{13}\text{C}$ [‰]	N %	$\delta^{15}\text{N}$ [‰]	S [mg/kg]	$\delta^{34}\text{S}$ [‰]	C:N	C:S	N:S
<i>Icacos:</i>									
ground ferns	39.40	-32.9	1.37	-0.41	2266	13.9	29	174	6:1
Bromeliads (family Bromeliaceae)	45.63	-29.9	0.62	-2.5	1076	13.8	74	424	6:1
Heliconia (family Heliconiaceae)	48.01	-28.5	1.08	1.0	1018	13.2	45	471	11:1
ferns	44.90	-32.3	1.51	1.8	2078	13.8	30	216	7:1
Sierra Palm (<i>Prestoea Montana</i>)	45.18	-30.2	1.49	-2.9	7150	15.5	30	63	2:1
Colorado (<i>Cyrilla racemiflora</i>)	51.51	-33.2	0.95	0.97	1671	15.4	54	308	6:1
O horizon (3-0 cm)	50.41	-29.7	0.64	-0.63	965	12.9	78	522	7:1
<i>Bisley:</i>									
Tabonuco (<i>Dacryodes excelsa</i>)	45.38	-36.0	1.67	-0.51	1791	14.9	27	253	9:1
Sierra Palm (<i>Prestoea Montana</i>)	43.44	-31.7	1.41	-1.7	4354	16.0	31	100	3:1
O horizon (2-0 cm)	32.97	-30.0	1.13	-0.04	1040	13.3	29	317	11:1

8 * All samples are whole leaves or leaf fragments collected from the forest floor in May 2010. Samples were run at
9 least in duplicates, with very good precision and quality control.

10

11 Table 3: Anion chemistry of the East Peak precipitation samples. Missing isotope values indicate samples
12 that had insufficient S due to low sulfate concentration and/or low sample volume.

Sampling date	Cl [μM]	SO ₄ [μM]	SO ₄ $\delta^{34}\text{S}$ [‰]	% nss-SO ₄ **	Calculated nss-SO ₄ $\delta^{34}\text{S}$ [‰] ***
6/1/10	18	7.3		87	
7/6/10	46	11.1	12.6	78	10.3
8/3/10	164	19.4	18.3	56	16.1
9/7/10*	76	10.9	17.9	64	16.1
10/5/10*	35	12.4	15.8	85	14.9
11/2/10	110	13.6	16.6	58	13.5
12/14/10*	37	9.5		80	
1/25/11	118	26.2		77	
2/8/11	158	12.8		36	
3/8/11	148	10.4	18.5	26	11.1
4/5/11	226	38.8	10.7	70	6.3
5/17/11	18	5.4		83	
6/7/11	19	8.2	12.3	88	11.0
7/19/11	196	23.1	17.6	56	14.9
8/9/11	56	10.8	17.2	73	15.7
9/6/11	27	4.6	18.0	69	16.7
10/11/11	61	13.4		76	
11/8/11*	38	13.2	20.5	85	20.5

1/3/12	113	13.9	14.7	58	10.1
1/31/12	164	12.7	21.4	33	22.3
3/13/12*	125	14.4	15.8	55	11.6
6/26/12	166	24.5	16.0	65	13.2
average (volume-weighted)	116 ± 65	15.9 ± 7.9	16.1 ± 2.8	63 ± 18	13.4 ± 4.0

- 13 * These samples were not included in the volume-weighted averages due to missing volume information.
 14 ** The non-seasalt (nss) fraction calculations assume all Cl is of marine origin and use the SO₄/Cl in seasalt ratio
 15 from Keene et al. (1986).
 16 *** Calculations assume linear mixing of seasalt and non-seasalt sulfate
 17

18 Table 4: Anion chemistry of the Guaba (tributary of Rio Icacos) and Bisley streams compared to
 19 groundwater sampled at the Icacos site. The streams were sampled at or near baseflow.

Sampling date	Cl [μ M]	SO ₄ [μ M]	SO ₄ δ^{34} S [‰]
<u>Guaba stream:</u>			
2/2/12	175	13.9	20.2
3/8/12	206	14.9	17.7
6/20/12	165	14.8	20.6
average	182 ± 21	14.6 ± 0.5	19.5 ± 1.6
<u>Bisley stream:</u>			
2/2/12	221	39.1	1.9
3/8/12	226	39.5	0.9
6/26/12	214	39.7	2.0
average	220 ± 6	39.4 ± 0.3	1.6 ± 0.7
<u>Icacos groundwater:</u> *			
8/18/12	160	14.6	20.2

20 * LGW1, ~6 m deep

21

FIGURES CAPTIONS

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Figure 1: Map of the study area, showing location of the lysimeter fields. Courtesy of Miguel Leon through the Luquillo Critical Zone Observatory (EAR-0722476).

Figure 2 a-h: Depth trends in total S, C:S, N:S and sulfate S concentration for the Icacos (a-d) and Bisley (e-h) soils. Except for the pit soils, which were sampled according to horizon designation, all other data are averages of two soil cores; error bars represent the range observed among the different core samples.

Figure 3 a-h: Soil S isotopes compared with vegetation litter and pore water averaged over the entire study period at Icacos (a-d) and Bisley (e-h). The sample at 0 cm is the O horizon. Except for the pit soils, which were sampled according to horizon designation, all other data are averages of two soil cores. Error bars for soil, pore water and vegetation litter values represent the range observed among the different soil core samples, different months, and different litter types respectively.

Figure 4 a-d: Depth trends in pore water sulfate concentration and $\delta^{34}\text{S}$ values averaged over the entire sample period at the Icacos (a-b) and Bisley (c-d) sites. The error bars are standard deviations. Vertical lines represent the sulfate concentration or $\delta^{34}\text{S}$ values of volume-weighted average precipitation (AP), groundwater (GW) at the Icacos site, Guaba baseflow (GB) and Bisley baseflow (BB).

23

24 Figure 5 a-f: The fate of atmospheric S in the soils at Icacos (a-c) and Bisley (d-f). Arrows
25 shown represent fluxes in and out of the pore water (not the bulk soil). Solid arrows represent
26 fluxes of sulfate, dashed arrows represent fluxes of other S compounds (e.g. organic S from
27 litterfall and reduced S gases). S in litterfall was calculated using mean litterfall values from
28 Weaver and Murphy (1990) and Zou et al. (1995). S in vegetation uses aboveground biomass
29 estimates from Gould et al. (2006). The hydrologic export calculations use average runoff
30 estimates for the Guaba and Bisley streams from Stallard and Murphy (2012) and Schellekens et
31 al. (2004) respectively. Hydraulic field flux densities for calculating downward S fluxes in pore
32 water are from Buss et al. (2011).

33

34 Figure 6 a-d: δ values versus the natural logarithm of concentration for N and S at Icacos (a-b)
35 and Bisley (c-d). Only significant linear regressions are shown.

36

Figure 1

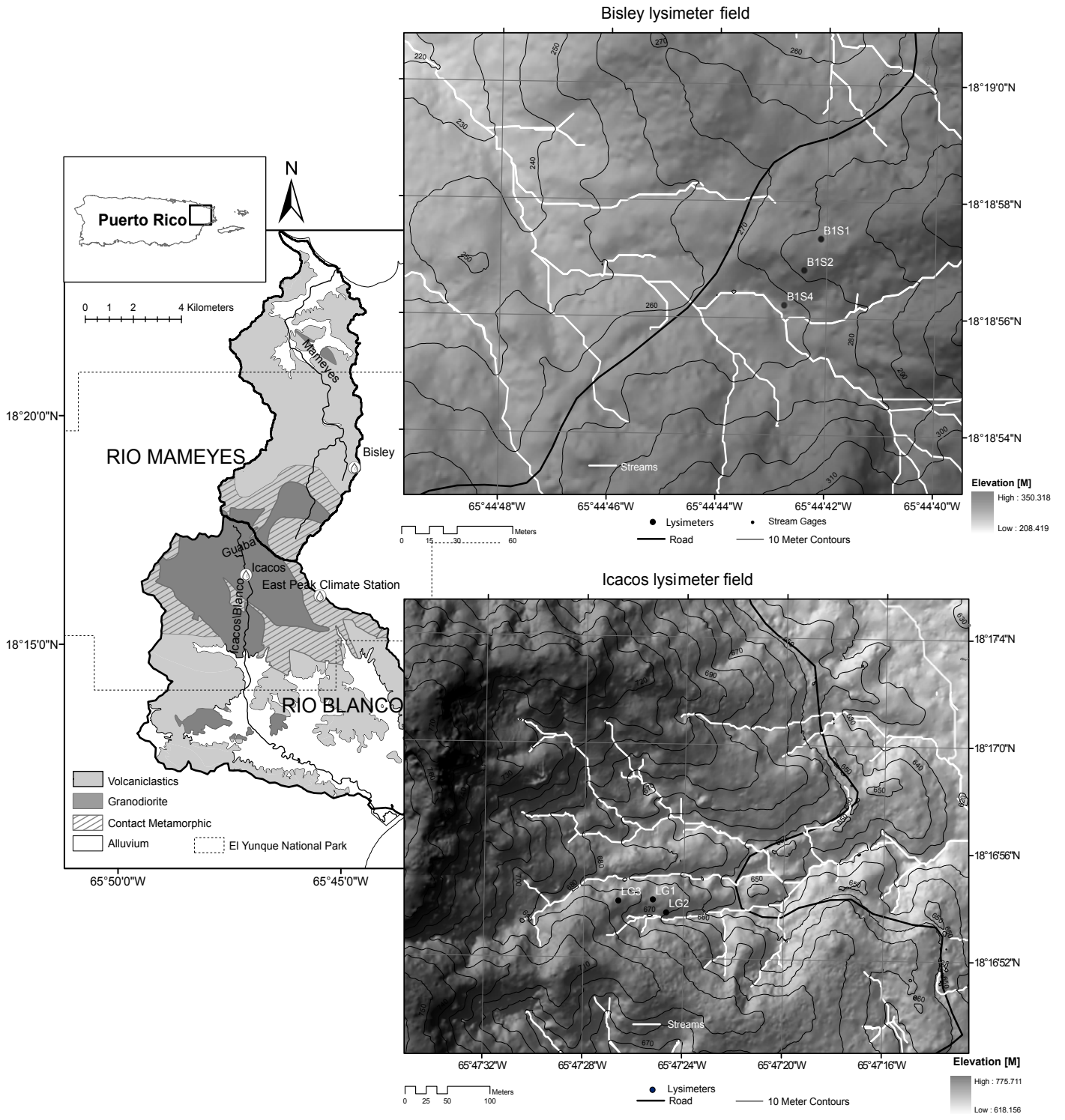


Figure 2

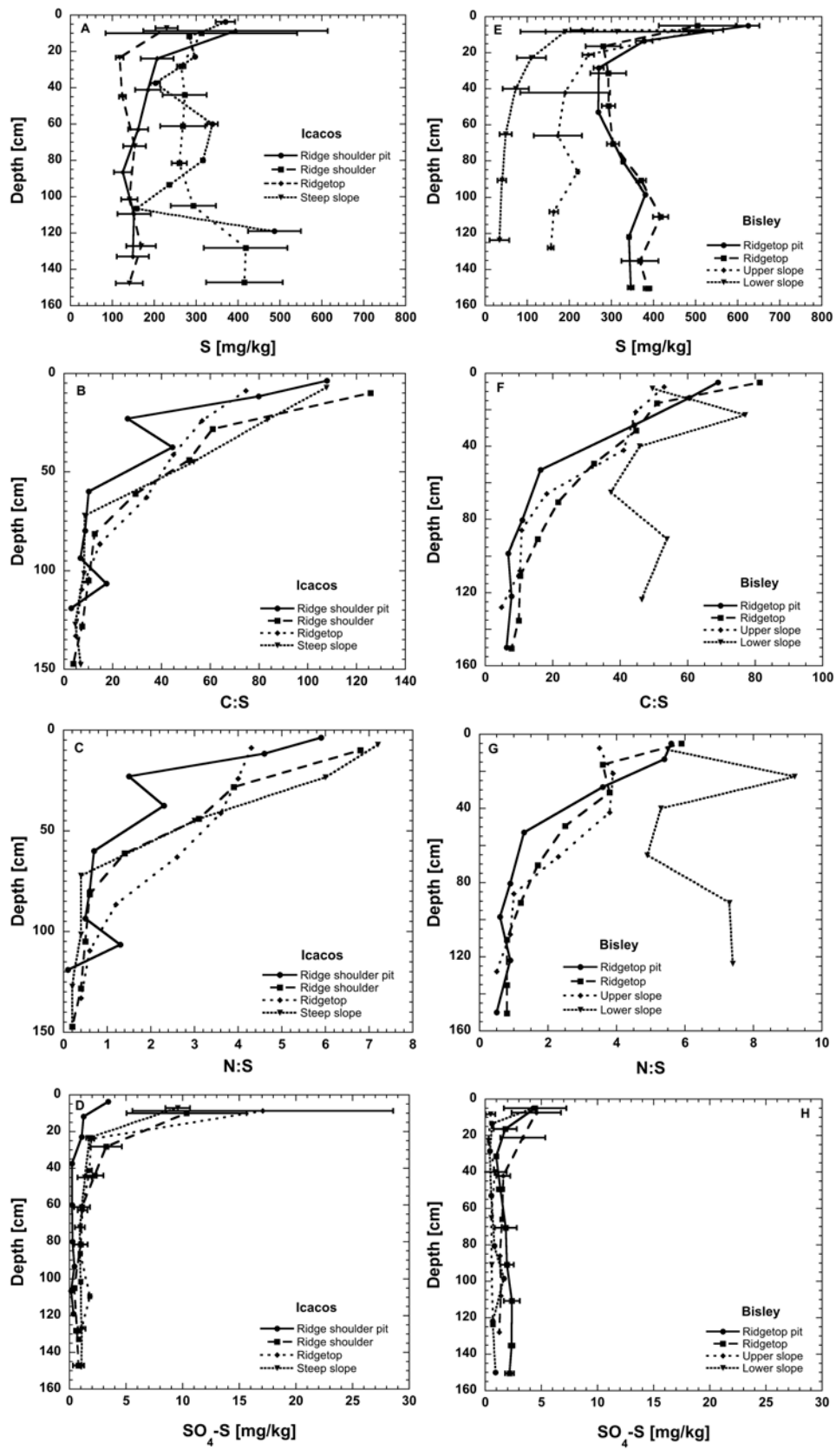


Figure 3

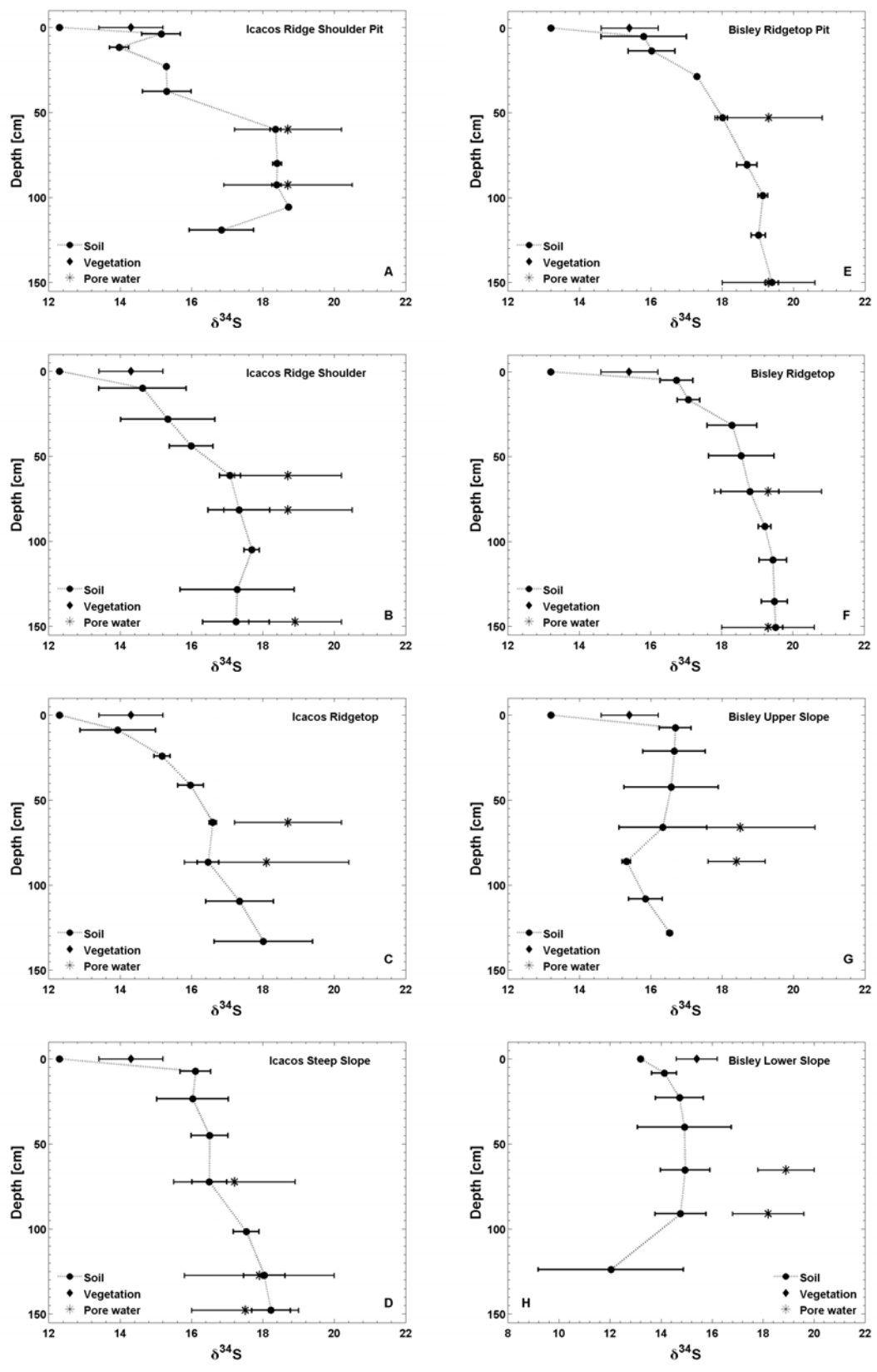


Figure 4

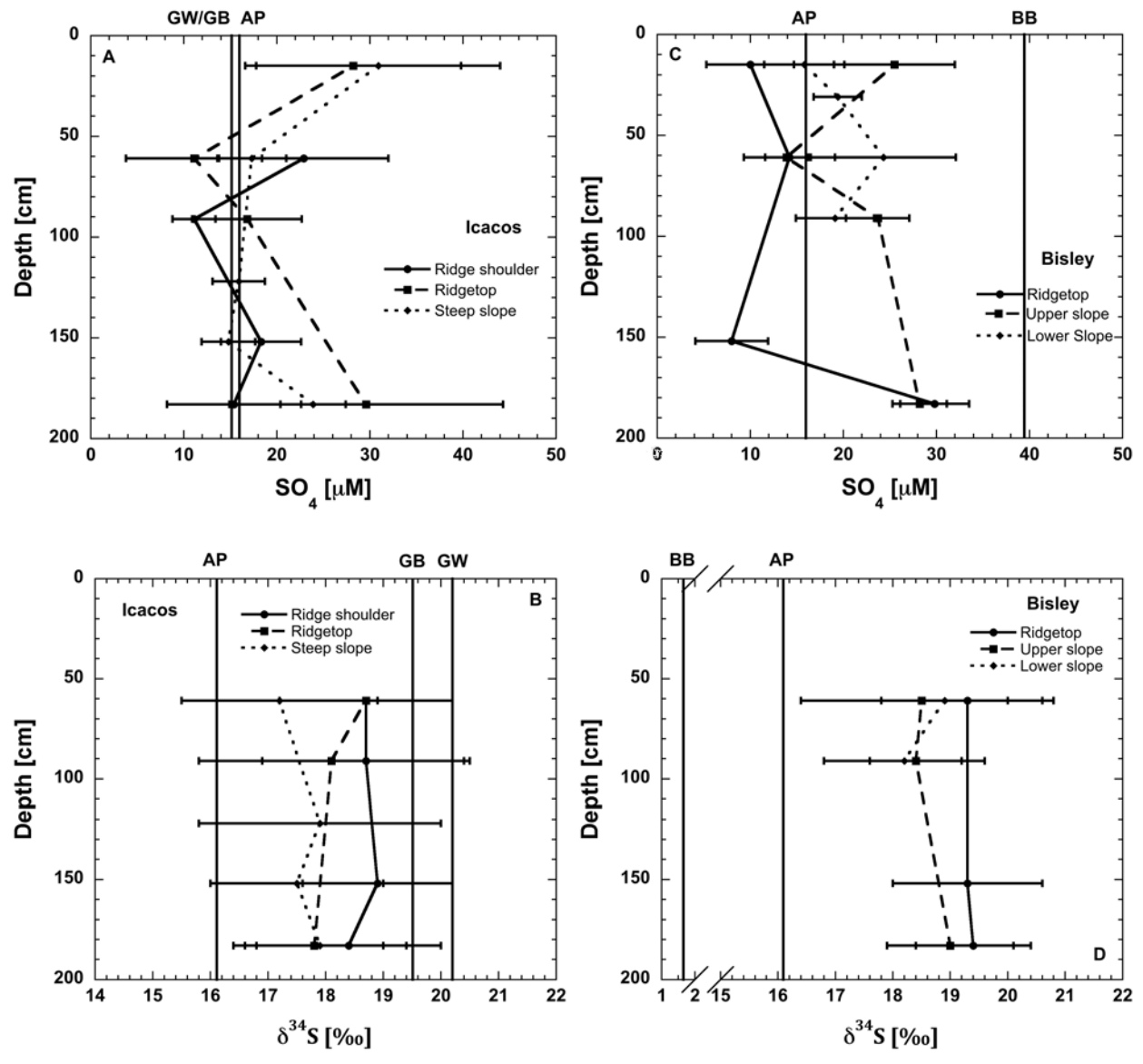


Figure 5

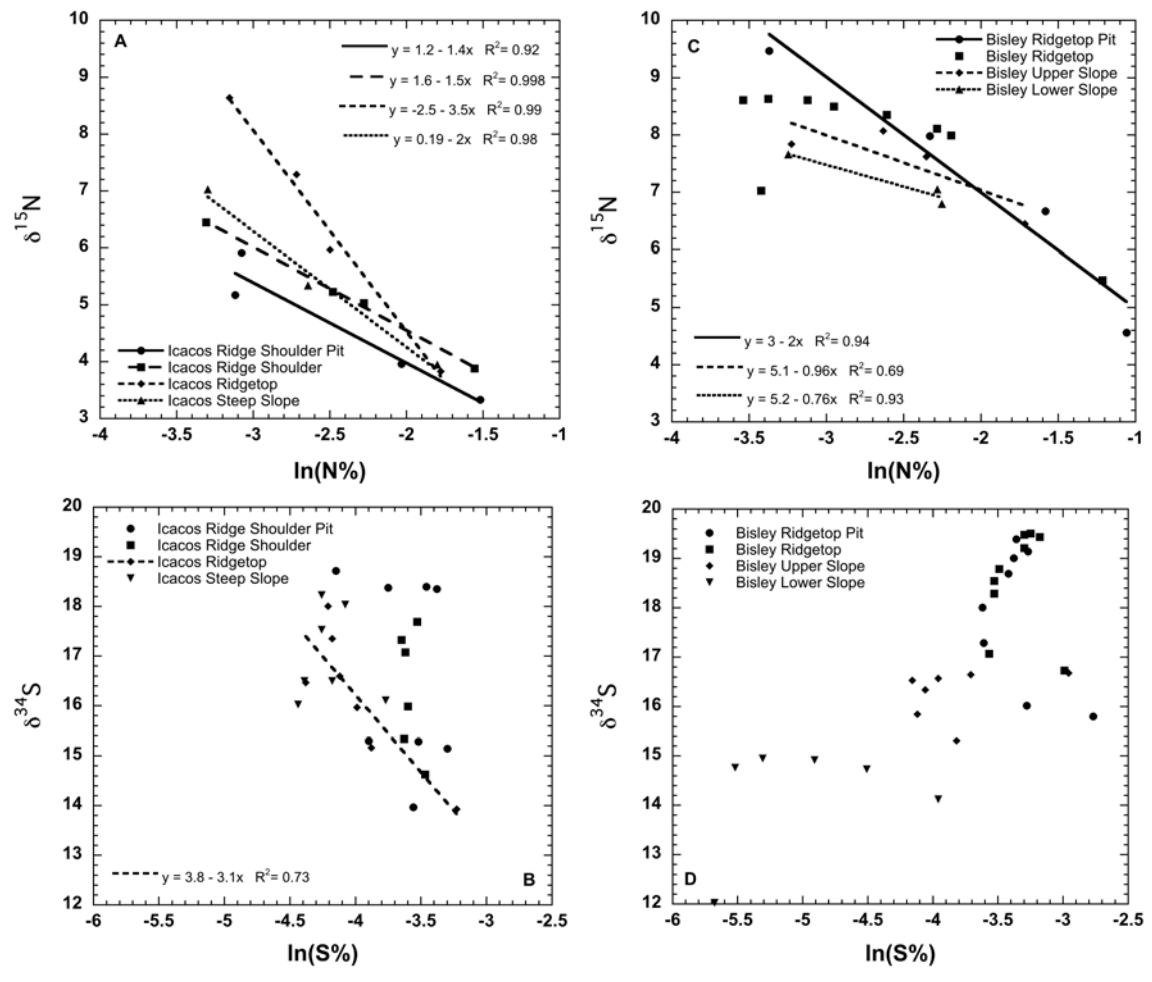
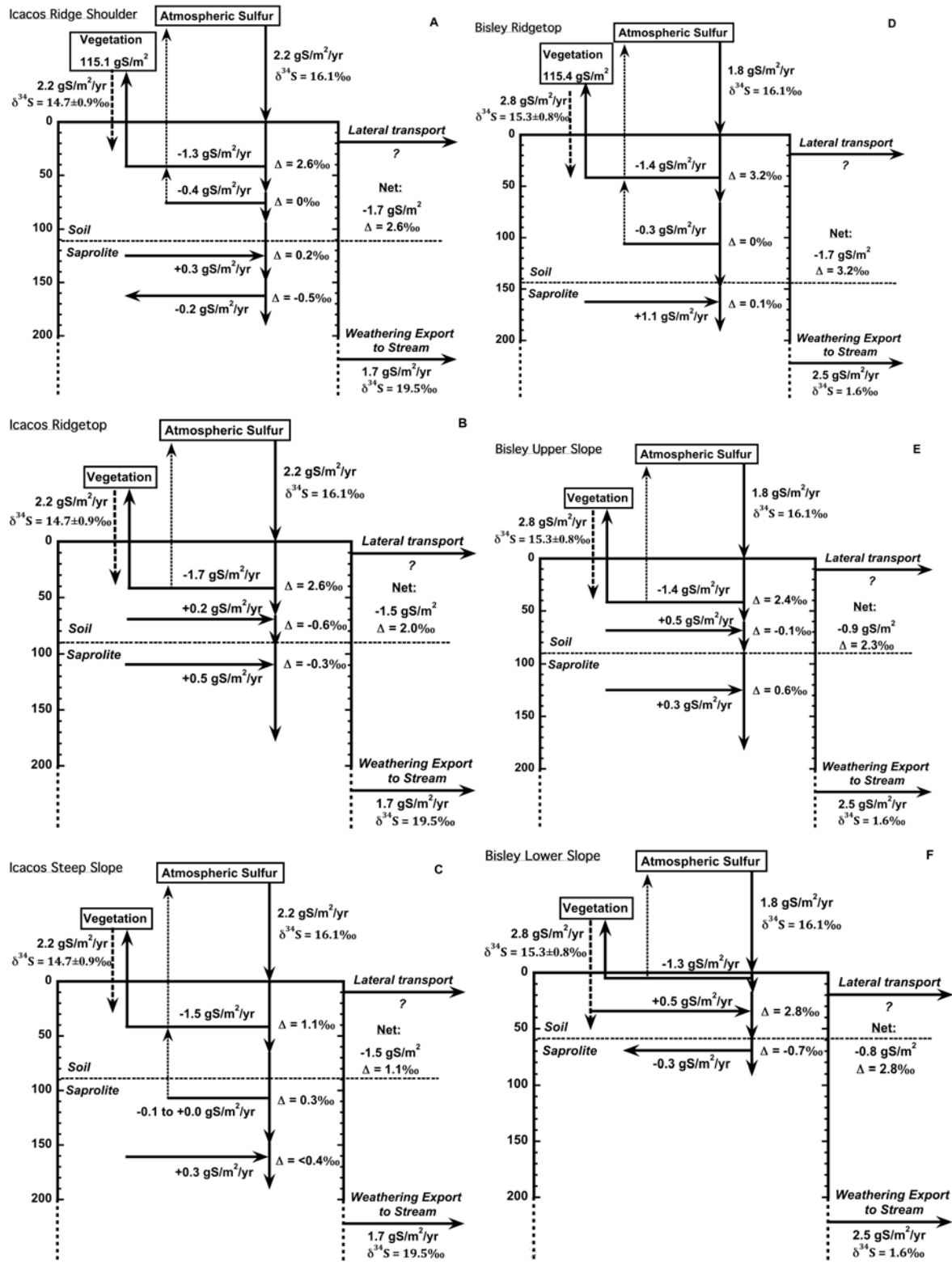


Figure 6



Appendix

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