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## Variability in soil and foliar stable carbon and nitrogen isotope compositions in the winter rainfall biomes of South Africa

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2                    isotope compositions in the winter rainfall biomes of South  
3                    Africa

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17

## Abstract

18 We present the stable carbon and nitrogen isotope compositions of 204 plants and 99 soils from South  
19 Africa's Fynbos and Succulent Karoo Biomes, from which we assess variability in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  within and  
20 between the biomes. Higher foliar  $\delta^{13}\text{C}$  in the Succulent Karoo ( $-22.4\pm 4.7\text{‰}$ ) primarily reflects a greater  
21 abundance of CAM photosynthesis relative to Fynbos ( $-25.7\pm 3.5\text{‰}$ ). For  $\text{C}_3$  plants within the Fynbos  
22 Biome, there is no statistically significant relationship between  $\delta^{13}\text{C}$  and climate, suggesting that within  
23 the present sampling framework, local-scale variability masks expected climatic controls on  $\text{C}_3$  plant  $\delta^{13}\text{C}$ .  
24 Soil and foliar  $\delta^{15}\text{N}$  are highly variable, but foliar  $\delta^{15}\text{N}$  is significantly higher in the Succulent Karoo ( $7.7\pm 3.9$   
25  $\text{‰}$ ) compared to the Fynbos ( $4.4\pm 3.3 \text{‰}$ ). Soil and foliar  $\delta^{15}\text{N}$  correlate, with soils  $3\pm 1\text{‰}$  more enriched  
26 in  $^{15}\text{N}$ . Both soil and foliar  $\delta^{15}\text{N}$  show stronger correlations with climate than  $\delta^{13}\text{C}$ , and they accord with  
27 the previously observed tendency for higher  $\delta^{15}\text{N}$  with greater aridity. The relationship between  $\delta^{15}\text{N}$  and  
28 mean annual rainfall is, however, relatively steep ( $2.4\pm 0.6\text{‰}$  per 100 mm rainfall), potentially implying  
29 additional controls on  $\delta^{15}\text{N}$  variability. These data provide a benchmarking of contemporary isotopic  
30 variability, anticipated to support future archaeological, palaeoenvironmental and C and N cycling  
31 research.

32 Keywords: CAM plant; climate; photosynthesis; organic matter; Fynbos; Succulent Karoo

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35 Highlights:

- 36 • Foliar and soil  $\delta^{13}\text{C}$  distinct between biomes
- 37 • Foliar  $\delta^{13}\text{C}$  for  $\text{C}_3$  plants in the Fynbos biome is un-correlated with climate
- 38 • Foliar  $\delta^{15}\text{N}$  is highly variable at the plot scale
- 39 • Plot average soil and foliar  $\delta^{15}\text{N}$  negatively correlated with rainfall
- 40 • Foliar isotopic signatures are mirrored in associated soils

## 41 1. Introduction

42 Stable carbon and nitrogen isotope analyses provide key insights into the nature and functioning of  
43 terrestrial ecosystems (Dawson et al. 2002; Craine et al., 2009). Across southern Africa, several studies  
44 have examined variability in foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Vogel and Seely, 1977; Heaton, 1987; Schulz et al. 1996;  
45 Swap et al., 2004; Aranibar et al., 2004; Wang et al. 2010; 2013; Codron et al. 2013; Herrmann et al. 2016;  
46 Carr et al., 2016). These studies show the varying sensitivities of isotopic values to climatic conditions and  
47 biome type, and present opportunities for further investigations into the isotopic and geochemical  
48 responses of plants and soils across this region. The increasingly routine use of stable isotope data in  
49 palaeoenvironmental and archaeological research in this region also provides an important impetus to  
50 benchmark contemporary soil and plant isotopic compositions across the region's biomes (Chase et al.  
51 2012; Sealy et al., 2016),

52 Foliar  $\delta^{13}\text{C}$  varies with plant photosynthetic pathway, *viz*  $\text{C}_3$  (generally trees, shrubs and cool-  
53 season grasses),  $\text{C}_4$  (generally grasses adapted to warm/dry conditions) and Crassulacean Acid Metabolism  
54 (CAM) plants (O'Leary, 1988). The distribution of  $\text{C}_3$  and  $\text{C}_4$  plants in southern Africa reflects rainfall  
55 seasonality and growing season temperature, with a greater abundance of  $\text{C}_4$  grasses in the summer  
56 rainfall (Nama Karoo, Savanna and Grassland) biomes (Vogel and Seely, 1987; Vogel, 1978). Variations in  
57 soil organic matter  $\delta^{13}\text{C}$ , are also known to respond to the abundance of  $\text{C}_4$  grasses (Bond et al., 1994;  
58 Herrmann et al. 2016).

59 In the winter rainfall zone of the western and southwest margins of southern Africa, particularly  
60 in the Fynbos Biome (**Figure S1**), where the vegetation is dominated by  $\text{C}_3$  plants, intrinsic water use  
61 efficiency ( $\text{WUE}_i$ ) is anticipated to generate variability in carbon isotope discrimination ( $\Delta$ ) and foliar  $\delta^{13}\text{C}$   
62 (Cernusak et al. 2013; Farquhar et al., 1989). Globally, one third to one-half of global-scale variation in  $\text{C}_3$   
63  $\Delta$  is explained by mean annual precipitation (Diefendorf et al. 2010; Kohn, 2010). At the regional scale  
64 similar trends are observed along environmental gradients, (e.g. Stewart et al. 1995; Van der Water et al.,  
65 2002; Peri et al. 2012). At the local scale, foliar  $\delta^{13}\text{C}$  variability can be high (e.g. Aranibar et al., 2004; Araya  
66 et al., 2010 Codron et al., 2013), with other biotic and abiotic factors influencing  $\Delta$  (e.g. leaf nutrient  
67 content, taxonomic effects, altitude, irradiance, air temperature, atmospheric  $\text{CO}_2$  content (Cernusak et  
68 al., 2013)). Local environmental factors, and variability associated with plant morphologies, functional  
69 types or life histories can lead to up to 6‰ differences in  $\Delta$  between biomes (Ehleringer and Cooper, 1988;  
70 Hartman and Danin, 2010; Mosher et al., 2020). In this respect, the degree of variability in foliar  $\delta^{13}\text{C}$  and,

71 therefore, carbon isotope discrimination in C<sub>3</sub> plants across the botanically diverse and topographic  
72 complex Fynbos biome remains poorly defined and beginning to address this knowledge gap is a goal of  
73 this paper. Such information will also be of wider utility in the interpretation of faunal and isotopic data  
74 from archaeological sites across this biome, where past environmental changes, potentially involving  
75 shifts in C<sub>3</sub>/C<sub>4</sub> vegetation abundances, and/or more subtle changes in aridity/micro-habitat and C<sub>3</sub> carbon  
76 isotope discrimination, might be inferred (e.g. Sealy et al., 2016; Roberts et al. 2016)

77 Located north of the Fynbos biome, the Succulent Karoo Biome (**Figure S1**) – the world’s most  
78 species rich semi-desert region (Rutherford et al. 2006) – is renowned for an abundance of CAM plants.  
79 CAM photosynthesis, found in ~6% of all vascular plants, is a mechanism for water conservation, whereby  
80 night-time CO<sub>2</sub> fixation and storage is followed by daytime decarboxylation and CO<sub>2</sub> reduction (Osmond,  
81 1978; Winter et al. 2015). In South Africa, CAM plants are especially prominent in the Succulent Karoo  
82 (von Willert et al. 1985; Veste et al. 2001; Veste and Theide, 2004). Isotopically, δ<sup>13</sup>C in CAM plant tissue  
83 varies between species and individuals, depending on the degree of CAM use (Winter, 2019). In the  
84 Succulent Karoo, considerable variability in CAM foliar δ<sup>13</sup>C has been documented (Rundel et al, 1999),  
85 and it is anticipated that the abundance of CAM species in this biome will render it isotopically distinct  
86 from the Fynbos Biome.

87 Stable nitrogen isotope (δ<sup>15</sup>N) data provide insights into the functioning of the N cycle (Robinson,  
88 2001; Pardo and Nadelhoffer, 2010), but variability in soil and foliar δ<sup>15</sup>N can be challenging to interpret  
89 given the number of the fractionating transformations within the N cycle (Craine et al., 2009; 2015a). As  
90 such, the roles of specific environmental variables in controlling foliar / soil δ<sup>15</sup>N are not always clear (Peri  
91 et al. 2012; Pardo and Nadelhoffer, 2010; Díaz et al. 2016). At the regional to global scale, foliar δ<sup>15</sup>N  
92 correlates with aridity (Austin and Salla, 1999; Handley et al. 1999; Amundson et al., 2003; Aranibar et al.  
93 2004; Murphy and Bowman, 2006; Craine et al. 2009; cf. Nel et al. 2018). This is thought to reflect changes  
94 in the δ<sup>15</sup>N of associated soils, and the increased significance of N losses (relative to the size of the soil N  
95 pool) in arid environments (Austin and Vitousek, 1998; Amundson et al., 2003). Foliar δ<sup>15</sup>N can also vary  
96 with the presence and type of symbiotic mycorrhizae, and due to human impacts (Craine et al. 2009; Pardo  
97 and Nadelhoffer, 2010).

98 In southern Africa, foliar δ<sup>15</sup>N has been considered in the context of animal diet reconstructions  
99 (Sealy et al. 1987; Codron et al., 2009), analyses of nitrogen fixation (e.g. Muofhe and Dakora, 1999; Morris  
100 et al. 2020) and in palaeoclimatic reconstructions from rock hyrax middens (Chase et al., 2012). Studies

101 have shown that, in relation to climate, foliar  $\delta^{15}\text{N}$  in southern Africa broadly corresponds with global scale  
102 trends. For example, Heaton (1987) showed a weak negative correlation between foliar  $\delta^{15}\text{N}$  and mean  
103 annual precipitation (MAP) for 33 sites across central and eastern South Africa and western Namibia. Swap  
104 et al. (2004) measured foliar  $\delta^{15}\text{N}$  for  $\text{C}_3$  vegetation from 21 sites across central and eastern South Africa,  
105 Botswana, northwest Namibia and western Zambia, with more than half of the  $\delta^{15}\text{N}$  variation ( $R^2=0.54$ ) in  
106  $\text{C}_3$  plants ( $n=143$ ) explained by MAP, despite a range of substrates and land uses (see also Wang et al.,  
107 2010). Across the uniform substrate of the Kalahari sands, Aranibar et al. (2004) demonstrated a negative  
108 correlation between foliar  $\delta^{15}\text{N}$  (non- $\text{N}_2$  fixing  $\text{C}_3$  trees, shrubs and forbs) and MAP (range of 978-230 mm  
109 per annum), and a negative correlation between non- $\text{N}_2$  fixing foliar  $\delta^{15}\text{N}$  and foliar C/N ratio.  
110 Furthermore, the relationship between foliar  $\delta^{15}\text{N}$  and rainfall was steeper for plants sampled during the  
111 wet season, highlighting the importance of spatial/temporal variability in water availability for soil and  
112 plant  $\delta^{15}\text{N}$  compositions (Wang et al. 2013). Carr et al. (2016) presented foliar  $\delta^{15}\text{N}$  data from seven sites  
113 across a variety of biomes in South Africa and Namibia. Again, a negative correlation between MAP and  
114 foliar  $\delta^{15}\text{N}$  was reported, although the samples were from a limited number of widely spaced sites.

115 In this paper, we seek to build a more systematic understanding of foliar and soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$   
116 variability by employing a paired soil and plant sampling strategy across two South African biomes, *viz.*  
117 the semi-arid to arid winter rainfall Fynbos and Succulent Karoo Biomes. Particularly in the case of  $\delta^{15}\text{N}$ ,  
118 isotopic variabilities in these biomes have not been explored. We seek to build on existing works to assess  
119 foliar  $\delta^{13}\text{C}$  variability in  $\text{C}_3$  and CAM plants within and between these biomes, and to consider patterns in  
120  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in different plant growth forms. The specific objectives are to:

- 121 1) Quantify variability in foliar  $\delta^{13}\text{C}$  /  $\delta^{15}\text{N}$  within and between the Fynbos and Succulent Karoo;
- 122 2) Assess the contribution of CAM photosynthesis to foliar  $\delta^{13}\text{C}$  variability in the Succulent Karoo  
123 Biome;
- 124 3) Assess the degree to which variability in foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is captured in associated soils;
- 125 4) Assess the relationships between climatic parameters and soil/foliar  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

## 126 2. Study region and sample sites

127 Across both biomes, geological substrate, topography, rainfall amount and rainfall seasonality exert  
128 strong controls on vegetation. The Fynbos Biome is a Mediterranean-type ecosystem characterised by a  
129 high diversity of ericaceous, small leaved shrubs, sclerophyllous proteiod shrubs, Cape Reeds  
130 (*Restionaceae*) and geophytes (Cowling et al., 1997). This biome is characterised by woody shrubs ( $\text{C}_3$ ) and

131 the associated climate is predominantly winter rainfall (> 66% of mean annual rainfall in May-September;  
132 Chase and Meadows, 2007), which determines any grasses that are present are C<sub>3</sub> varieties (Vogel, 1978).  
133 Within the Fynbos Biome, the Montane Fynbos eco-region is associated with the uplands (~200-1600 m  
134 above sea level (asl)) of the Cape Fold Belt Mountains and occurs on shallow, low-nutrient sandy soils  
135 derived from the quartzitic Table Mountain Group sandstones (Campbell, 1983).

136 The Lowland Fynbos eco-region (WWF 2020) is located on the coastal plain of the South African  
137 west coast, at altitudes below 200 m asl (**Figure S1; Table 1**). Substrates here comprise less acidic (than  
138 Montane Fynbos) free draining sandy soils (Arenosols) (Carr et al, 2013). The Succulent Karoo Biome is  
139 associated with a wider range of substrates (Milton et al., 1997) and, in contrast to the Fynbos Biome,  
140 soils are generally finer grained, higher in nutrient content (Cowling et al., 1997) and more varied in terms  
141 of texture and clay mineralogy (e.g. Carr et al. 2013). The vegetation is drought-adapted, with trees and  
142 large shrubs rare. Leaf succulents and dwarf shrubs from the Aizoaceae, Crassulaceae and Euphorbiaceae  
143 families are especially abundant (Cowling and Hilton-Taylor, 1999; Milton et al., 1997). The Succulent  
144 Karoo Biome is a winter rainfall desert (in contrast to subtropical, summer rainfall deserts), experiencing  
145 higher summer temperatures and more elevated moisture stress than the Fynbos Biome. This winter  
146 rainfall, although low, contributes to a relatively reliable rainfall regime (Cowling et al., 1999b).

## 147 2.1 Sample sites

148 All plant and soil samples were obtained during a campaign of systematic sampling in April-May 2010 (the  
149 end of the summer dry-season). Mean annual rainfall across the sampling region ranges between  
150 approximately 480 mm a<sup>-1</sup> and 160 mm a<sup>-1</sup>. Rainfall seasonality varies from ~50% during the austral winter  
151 in the northern reaches of the region (SK7) to ~90% winter rain at SV1 in the south; **Figures S1 and S2;**  
152 **Table 1**). Mean annual temperatures range between 14 and 19 °C; maximum monthly temperatures lie  
153 between 27 and 34 °C (Hijmans et al., 2005) depending on elevation and latitude. Aridity index values,  
154 which are lowest in the Succulent Karoo, are between 0.1 and 0.4 and are generally lower in the Lowland  
155 Fynbos than in the Montane Fynbos.

156 Ten plots were surveyed and sampled in the Fynbos Biome (including the Lowland and Montane  
157 eco-regions) and 15 plots in the Succulent Karoo Biome. These sites were previously described with an  
158 emphasis on their soil organic matter compositions in Carr et al. (2013). At each site, a 10 x 10 m  
159 vegetation survey plot was laid out and a small sample of photosynthetic material from each plant was  
160 sampled (usually a leaf, but occasionally a small piece of stem material in the case of stem succulents, or

161 a clearly identifiable and recent piece of leaf litter). Plants were identified with reference to the University  
 162 of Cape Town herbarium collection, although this was not always possible, particularly in cases (e.g.  
 163 Aizoaceae) where flowers were absent at the time of sampling (see **Table S1**). In most cases, we sampled  
 164 one specimen of each identified plant type at each site. Soil samples were obtained from the same plant  
 165 survey plots at four fixed locations (plot corners). Following removal of surficial leaf litter, each 200-400 g  
 166 sample was obtained from the upper 10-15 cm of the soil (where possible, as some soils were rather thin).  
 167 This broadly corresponded with the soil A Horizon, but many soils were lithosols, lacking well-developed  
 168 soil profiles, particularly in the Succulent Karoo Biome (Carr et al., 2013).

169 **Table 1:** Climatic envelopes (means and standard deviations) for the sampling sites within the considered  
 170 biomes/ecoregions (from WorldClim 1.4; Hijmans et al. 2005)

Biome	Number of sites	Altitude range (m)	Mean annual Precipitation (mm)	Mean annual temperature (°C)	Aridity Index	Precip. WetQ (mm)	Temperature of the driest month (°C)
Lowland Fynbos	5	7-119	297 ± 105	17.3 ± 0.6	0.21 ± 0.11	142 ± 44	21.1 ± 0.8
Montane Fynbos	5	227-947	305 ± 45	17.2 ± 1.6	0.20 ± 0.04	150 ± 20	22.4 ± 1.5
Succulent Karoo Biome	15	93-1072	201 ± 51	17.5 ± 1.7	0.13 ± 0.03	85 ± 22	21.4 ± 3.6

171

### 172 3. Methods

173 Freeze-dried plant material was homogenised and encapsulated in tin cups, with each leaf mixture  
 174 measured in triplicate. Soil samples were sieved at 2 mm, sub-sampled using a riffle box and homogenised  
 175 in a ball mill. For total organic carbon (TOC) and  $\delta^{13}\text{C}_{\text{TOC}}$  determination, soils were treated with dilute  
 176 hydrochloric acid, rinsed in deionised water and freeze-dried. TN and soil  $\delta^{15}\text{N}$  measurements were made  
 177 on (freeze dried) untreated soil samples. Plot average TOC and TN values for soils are reported in Carr et  
 178 al. (2013). Here we present results for the individual soil samples and additionally report the  $\delta^{13}\text{C}_{\text{TOC}}$  and  
 179  $\delta^{15}\text{N}$  obtained in the same analyses. Total carbon (TC), Total nitrogen (TN) and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were  
 180 determined using a SerCon ANCA GSL elemental analyser interfaced to a SerCon Hydra 20-20 continuous-



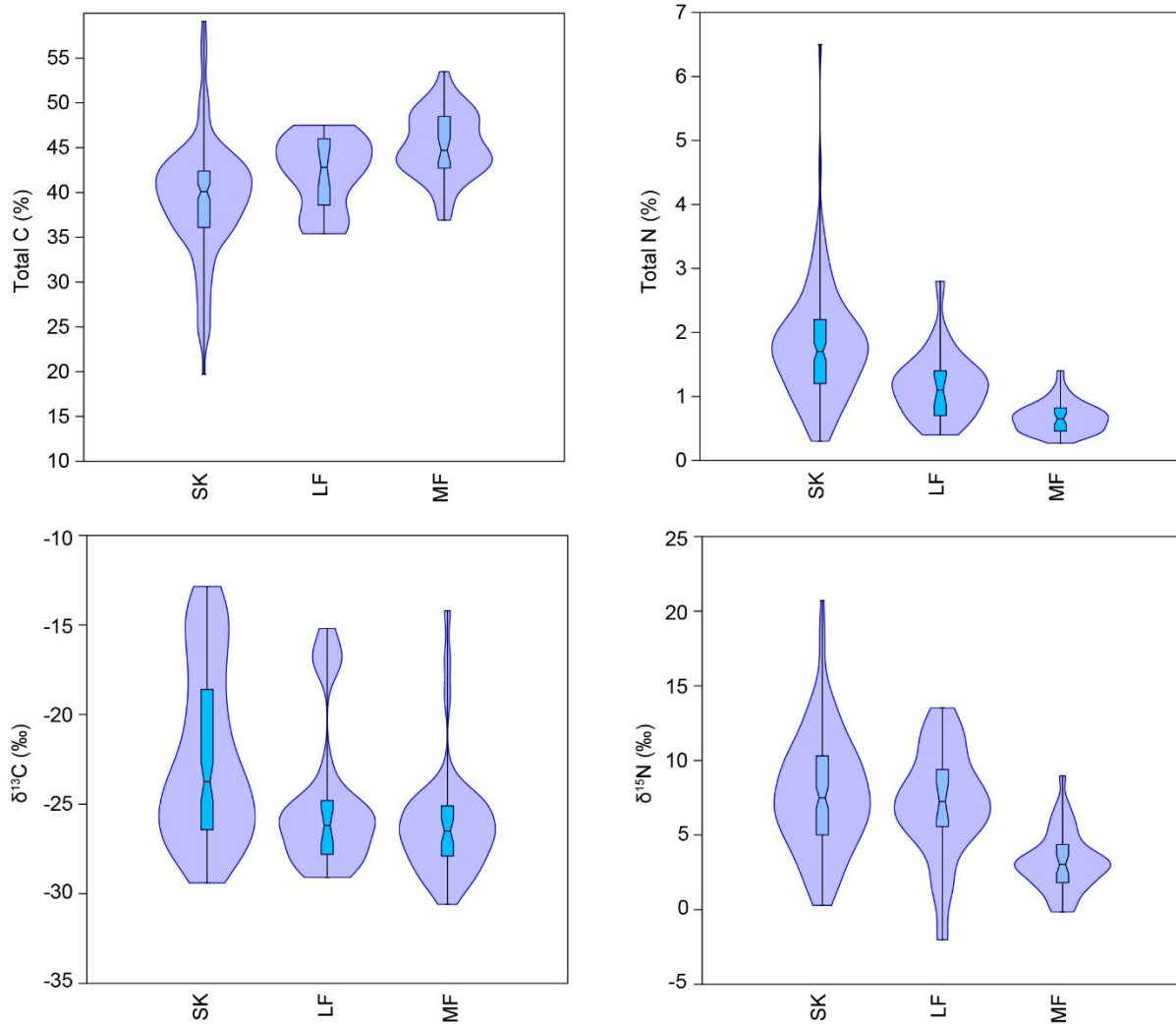
181 flow isotope ratio mass spectrometer. Measurement precision was better than  $\pm 0.1\%$  for  $\delta^{13}\text{C}$  and  $\pm 0.2\%$   
182 for  $\delta^{15}\text{N}$ .

183 Climatic data, interpolated surfaces based on climate data from individual weather stations, were  
184 obtained from WorldClim 1.4 (Hijmans et al., 2005), with site aridity indices from Trabucco and Zomer  
185 (2009). Due to the low density of climate observation stations and high topographic heterogeneity in the  
186 study region, only generalised definitions of climate gradients are feasible. Statistical analyses were  
187 carried out using PAST (Hammer et al., 2001). To explore our data we used non-parametric tests of  
188 difference (Mann-Whitney and Kruskal-Wallis) to assess differences in isotopic values between biomes  
189 or between plant functional types. To consider isotopic-climatic relationships we used site average foliar  
190 and soil data (as will be shown later, inter-sample (plot scale) variability was relatively high) and we report  
191 the Pearson correlation coefficients between isotopic values and climatic parameters from the WorldClim  
192 data. We also inspected the results of Spearman's Rank Order correlations in these instances, although  
193 similar conclusions were drawn from such an analysis. The results of ordinary least squares regression  
194 analysis are reported for some analysis (e.g. plot-wise soil vs. foliar isotopic comparisons).

## 195 4. Results

### 196 4.1 Foliar C and N content

197 Foliar C content varies from 20 % to 59 %, with means and standard deviations of  $45 \pm 4$  % (n=46) for the  
198 Montane Fynbos,  $42 \pm 4$  % (n=24) for the Lowland Fynbos and  $39 \pm 6$  % (n=135) for the Succulent Karoo  
199 Biome (**Figure 1**). The difference between the Succulent Karoo (n=135) and Fynbos Biomes (all Fynbos  
200 samples, n= 70) is statistically significant (Mann Whitney U = 2136, p= < 0.001). Foliar N content from the  
201 Succulent Karoo Biome (mean  $1.8 \pm 0.9\%$ , n=135) is higher (and more varied) than in the Fynbos Biome  
202 (**Figure 1**). This difference is statistically significant whether the Fynbos Biome as-a-whole is compared to  
203 the Succulent Karoo (Mann Whitney U =1233, p = <0.001), or if it is treated as separate eco-regions. Within  
204 the Fynbos Biome, Montane Fynbos Foliar N (mean  $0.7 \pm 0.2$  %, n=41) is significantly lower (Mann Whitney  
205 U= 163, p=<0.0001) than the Lowland Fynbos Foliar N (mean  $1.2 \pm 0.5$  %, n=23). Foliar C/N is highly  
206 variable, but is highest in the Montane Fynbos (average  $76 \pm 27$ , n=41) and lowest in the Succulent Karoo  
207 Biome ( $29 \pm 23$ , n=135). The difference in foliar C/N between the Succulent Karoo Biome (n=135) and the  
208 Fynbos Biome (all samples, n = 64) is significant (Mann Whitney U = 1004, p= < 0.001). The difference  
209 between the Lowland ( $42 \pm 19$ , n= 23) and Montane Fynbos (average  $76 \pm 27$ , n=41) is also significant  
210 (Mann Whitney U = 131, p = <0.001).



211  
 212 **Figure 1:** Violin plots showing variability in foliar total carbon (top left) and total nitrogen (top right), and variability  
 213 in foliar δ<sup>13</sup>C (bottom left) and δ<sup>15</sup>N (bottom right) for the sampled biomes/eco-regions. SK = Succulent Karoo, LF =  
 214 Lowland Fynbos and MF = Montane Fynbos.

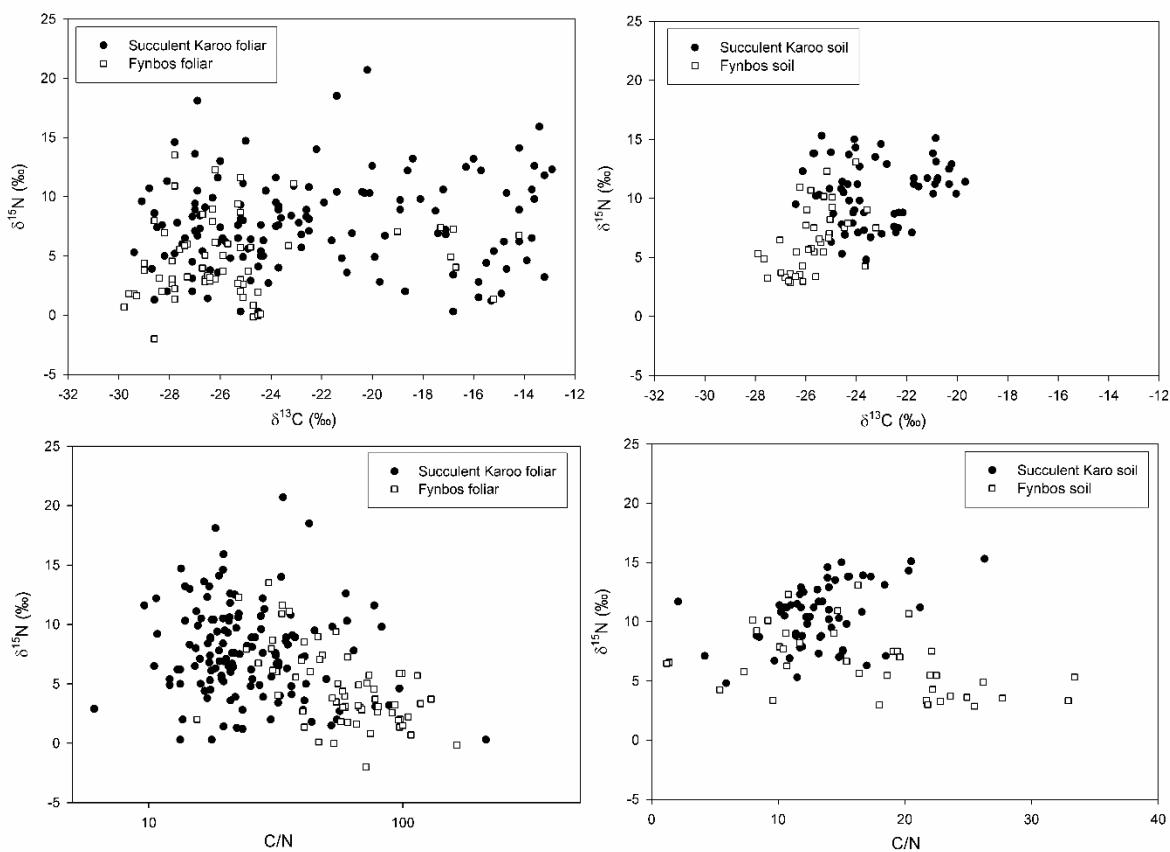
## 215 4.2 Foliar δ<sup>13</sup>C and δ<sup>15</sup>N

216 Foliar δ<sup>13</sup>C across the total dataset ranges between -13.2‰ and -30.6‰. Considered by biome/ecoregion  
 217 the mean values (and standard deviations) are -26.1 ± 3.1‰ (n=45) and -24.9 ± 4.1‰ (n=24) for the  
 218 Montane and Lowland Fynbos respectively, and -22.4 ± 4.7‰ (n=135) for the Succulent Karoo Biome  
 219 (**Figure 1**). Most Lowland and Montane Fynbos foliar δ<sup>13</sup>C is within the anticipated range of C<sub>3</sub> plants, with  
 220 a minority of specimens (7 of 69 specimens) producing values higher than -20‰ (**Figure 1**). Succulent  
 221 Karoo foliar δ<sup>13</sup>C spans a range from -29.4 to -13.2‰. The difference between the Fynbos and Succulent  
 222 Karoo Biomes is statistically significant (i.e. Fynbos vs. Succulent Karoo Biome Mann-Whitney U = 2561, p

223 = < 0.001), while there is no significant difference between the Montane and Lowland Fynbos foliar  $\delta^{13}\text{C}$   
224 (Mann Whitney U = 454, p=0.3). The highest  $\delta^{13}\text{C}$  values (> -14 ‰) in the Succulent Karoo are associated  
225 with both  $\text{C}_4$  photosynthesis (a single specimen of a  $\text{C}_4$  grass and several specimens of *Salsola tuberculata*  
226 (Chenopodiaceae)), and CAM photosynthesis (*Aloë* sp. (Asphodelaceae) and Crassulaceae).

227 Foliar  $\delta^{15}\text{N}$  spans a total range of 22.7‰ (-2.0 to 20.7‰). It is highest and most varied in the  
228 Succulent Karoo Biome (mean  $7.7 \pm 3.9\%$  n = 135) and lowest in the Montane Fynbos eco-region (mean  
229  $3.2 \pm 2.0\%$  n =41). At the plot scale, variation in foliar  $\delta^{15}\text{N}$  is substantial, with a maximum (10x10 m)  
230 sample plot range of 15.4‰ (SK7; n=11). The difference between Succulent Karoo and Fynbos Biomes  
231 (Fynbos sample mean =  $4.6 \pm 3.3\%$  n=64) is significant (Mann Whitney U = 2286, p = < 0.001), as is the  
232 difference between the Montane and Lowland ( $7.1 \pm 3.6\%$  n =23) Fynbos eco-regions (Mann Whitney U  
233 = 152, p< 0.0001). There is no significant difference between foliar  $\delta^{15}\text{N}$  in the Lowland Fynbos and  
234 Succulent Karoo (Mann Whitney U = 1470, p=0.69). The highest measured value (20.7‰ from a woody  
235 succulent of the Aizoaceae family) approaches the highest foliar  $\delta^{15}\text{N}$  values reported globally (21.4‰;  
236 Craine et al., 2015a).

237 Considering the foliar stable isotope dataset in total, there is weak but significant positive  
238 correlation between foliar  $\delta^{13}\text{C}$  and foliar  $\delta^{15}\text{N}$  (Pearson's r = 0.21, p = 0.003), but no such correlations  
239 when the biomes are considered separately (r = 0.10, p = 0.25 for the Succulent Karoo Biome, and r = 0.13,  
240 p=0.30 for the Fynbos) (**Figure 2**). There is no correlation between foliar  $\delta^{15}\text{N}$  and C/N ratio in the  
241 Succulent Karoo Biome. The two variables are correlated in the Fynbos Biome, for both soils and plants  
242 (**Figure 2**).



243

244 **Figure 2:** Scatter plots of foliar (upper left) and soil (upper right)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  split by biome. The  
 245 correlation between soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is statistically significant for the Fynbos Biome (Pearson's  $r = 0.51$ ,  
 246  $p < 0.0001$ ). Bottom: Scatter plots of foliar (left – note log scale x axis) and soil (right) C/N and  $\delta^{15}\text{N}$  split  
 247 by biome. The correlation between foliar C/N and  $\delta^{15}\text{N}$  for the Fynbos Biome is statistically significant  
 248 (Pearson's  $r = -0.52$ ,  $p < 0.0001$ ), as is the correlation for soil C/N and  $\delta^{15}\text{N}$  for the Fynbos Biome (Pearson's  
 249  $r = 0.44$ ,  $p < 0.001$ ). The variables are uncorrelated in the Succulent Karoo Biome.

250

### 251 4.3 Variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ with plant functional type

252 Plants were divided into functional classes following Carr et al (2014) and references therein. The main  
 253 sampled classes are Woody Shrubs ( $n=90$ ), Woody Succulents ( $n = 71$ ), Succulents ( $n=26$ ), and Herbs ( $n=$   
 254  $17$ )) (**Table S1**). For the purposes of this analysis, no differentiation is made between the Lowland and  
 255 Montane Fynbos eco-regions. Woody Succulents ( $n=62$ ) were the dominant functional type sampled in  
 256 the Succulent Karoo, while Woody Shrubs ( $n=52$ ) dominated in the Fynbos Biome. In the Fynbos Biome  
 257 the foliar  $\delta^{13}\text{C}$  values of Woody Shrubs and Herbs are indistinguishable ( $-26.9 \pm 1.6\text{‰}$  ( $n=51$ ) and  $-26.5 \pm$   
 258  $1.4\text{‰}$  ( $n=7$ ) respectively; **Figure S3**). Plants in the Succulent (mean =  $-18.4 \pm 5\text{‰}$  ( $n=5$ )) and Woody

259 Succulent (mean =  $-21.2 \pm 3.8\%$  (n=5)) classes account for the seven high  $\delta^{13}\text{C}$  values previously noted.  
260 These are all likely to be CAM plants (three are identified as *Euphorbia mauritanica* or *Tylecodon*  
261 *paniculata*). In the Succulent Karoo, Woody Succulents and Succulents, exhibit a very wide range of  $\delta^{13}\text{C}$   
262 (**Figure S3**). The mean foliar  $\delta^{13}\text{C}$  values of Succulents ( $-19.4 \pm 4.3\%$  (n=21)) and Woody Succulents ( $-21.6$   
263  $\pm 4.5\%$  (n=62)) are higher than those of the Herbs ( $-25.7 \pm 4.5\%$  (n=9)) and Woody Shrubs ( $-25.1 \pm 3.0\%$   
264 (n=34)). The mean foliar  $\delta^{13}\text{C}$  of Herbs in the Succulent Karoo is indistinguishable from that of Herbs in the  
265 Fynbos Biome. Grasses, which were only sampled in the Succulent Karoo, include both  $\text{C}_3$  and  $\text{C}_4$  varieties  
266 ( $-14.7 \pm 1.4\%$  (n=3) and  $-27.2 \pm 0.7\%$  (n=4)).

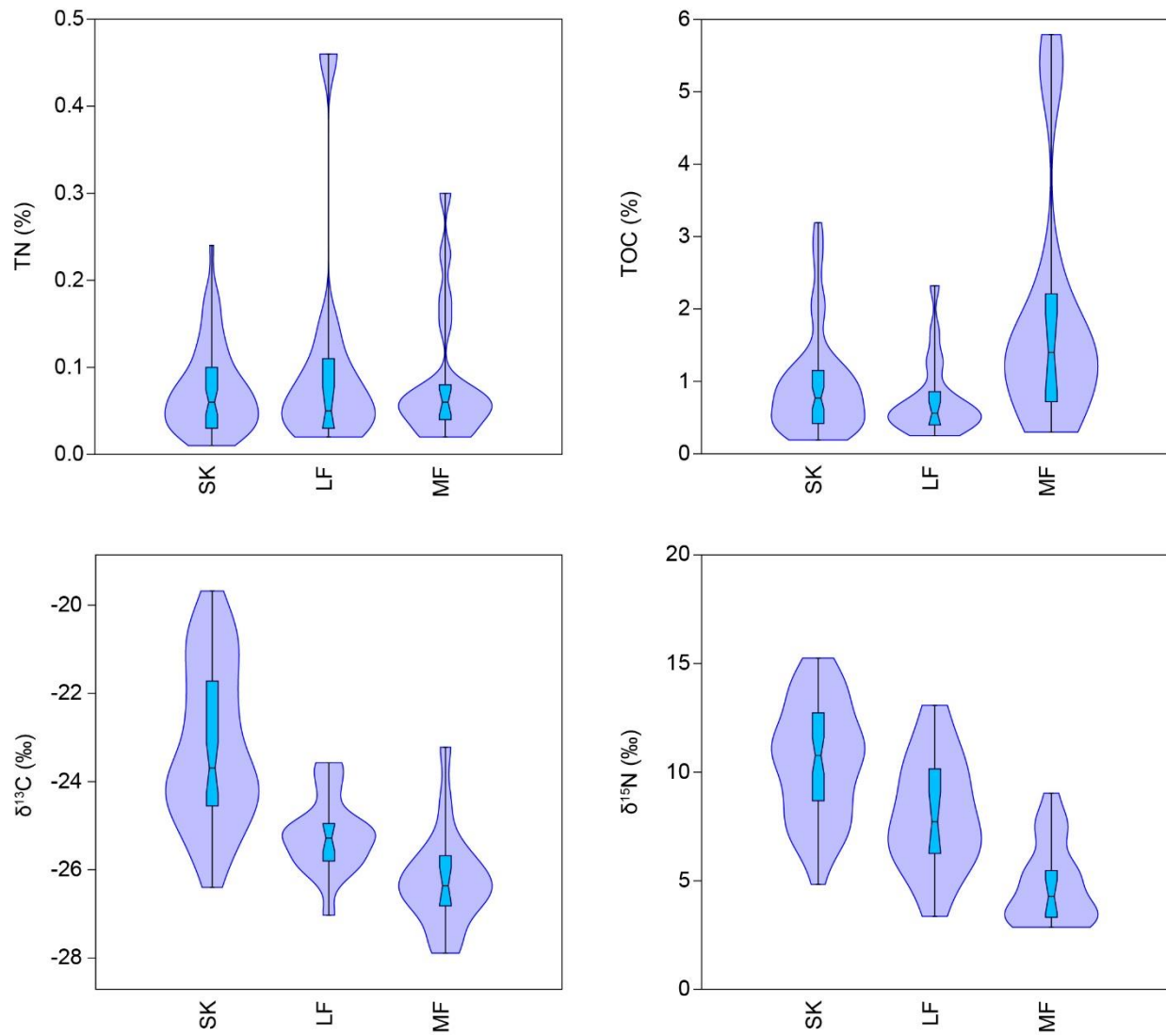
267 There is considerable variability in foliar  $\delta^{15}\text{N}$  within each Fynbos Biome plant functional type  
268 (**Figure S3**), but there are no significant differences in foliar  $\delta^{15}\text{N}$  between groups (Kruskal-Wallis,  $p =$   
269  $0.051$ ).  $\delta^{15}\text{N}$  is also highly variable in the Succulent Karoo Biome and, as in the Fynbos Biome, there are no  
270 significant differences (Kruskal-Wallis,  $p = 0.053$ ) between plant functional type (**Figure S3**).

#### 271 4.4 Soils

272 Summarised (plot average) soil TOC and TN contents were presented previously (Carr et al. 2013) and  
273 these data are here reported on a per sample basis in **Figure 3**. Soil TOC varies between 0.2 and 5.8 %. At  
274 the biome scale, average values are  $0.94 \pm 0.70\%$  (n=60) and  $1.37 \pm 1.32\%$  (n=39) for the Succulent Karoo  
275 Biome and Fynbos Biomes respectively. Lowland Fynbos soil TOC ( $1.89 \pm 1.68\%$ , n=19) is higher than  
276 Montane Fynbos ( $0.88 \pm 0.55\%$ , n=20). The average TN ranges from 0.01% to 0.30%. Overall mean values  
277 are  $0.07 \pm 0.05\%$  (n=60) and  $0.09 \pm 0.11\%$  (n=39) for the Succulent Karoo and Fynbos Biomes respectively.  
278 These indistinguishable soil N values contrast with the higher foliar N contents in Succulent Karoo plants  
279 compared to the Fynbos.

280 Soil  $\delta^{13}\text{C}_{\text{TOC}}$  tracks the foliar data in that, on average, it is highest and most varied in the Succulent  
281 Karoo Biome (mean  $-23.2 \pm 1.8\%$ , n=60) and lowest in the Montane Fynbos (mean  $-26.2 \pm 1.1\%$ , n=19,  
282 compared to  $-25.2 \pm 0.9\%$ , n=20 for the Lowland Fynbos). The isotopic variability within each biome/eco-  
283 region is markedly reduced in comparison the foliar data (**Figures 1 and 3**). Soil  $\delta^{15}\text{N}$  is highest the  
284 Succulent Karoo Biome (mean  $10.5 \pm 2.7\%$ , n=60). As observed for the foliar data, Lowland Fynbos soil  
285  $\delta^{15}\text{N}$  is intermediate ( $8.1 \pm 2.6\%$ , n=20) between the Succulent Karoo and Montane Fynbos ( $4.8 \pm 1.9\%$ ,  
286 n=19). Succulent Karoo soil  $\delta^{15}\text{N}$  is significantly higher than Montane Fynbos (Mann-Whitney  $U = 61$ ,  $p =$   
287  $< 0.001$ ). The difference between Montane and Lowland Fynbos is also significant (Mann-Whitney  $U = 55$ ,  
288  $p = < 0.001$ ), as is the difference between the Succulent Karoo and Lowland Fynbos (Mann-Whitney  $U =$

289 302,  $p < 0.001$ ). As observed in the foliar data, for all samples, across both biomes,  $\delta^{13}\text{C}_{\text{TOC}}$  and  $\delta^{15}\text{N}$  are  
290 weakly, but significantly correlated (Pearson's  $r = 0.51$ ,  $p < 0.001$ ,  $n = 98$ ) (**Figure 2**). When biomes or  
291 ecoregions are considered individually there is a correlation for the Fynbos Biome (Pearson's  $r = 0.51$ ,  
292  $p < 0.001$ ,  $n = 39$ ; **Figure 2**), but no correlation within the Succulent Karoo Biome (Pearson's  $r = 0.11$ ,  $p = 0.4$ ,  
293  $n = 60$ ).



294

295 **Figure 3:** Violin plots showing variation in soil total nitrogen (TN; top left), soil total organic carbon (TOC;  
296 top right) contents, soil  $\delta^{13}\text{C}_{\text{TOC}}$  (bottom left) and soil  $\delta^{15}\text{N}$  (total N; bottom right). SK = Succulent Karoo, LF  
297 = Lowland Fynbos and MF = Montane Fynbos.

## 298 4.5 Soil – plant comparisons at the plot scale

299 Comparing plot average soil  $\delta^{13}\text{C}_{\text{TOC}}$  and foliar  $\delta^{13}\text{C}$  across the total data set, we observe a significant  
300 correlation ( $r^2 = 0.67$ ,  $p = < 0.001$ ) between soil and foliar  $\delta^{13}\text{C}$  (**Figure S4**). The slope of this relationship is  
301 less than 1 ( $0.58 \pm 0.08$ ), and this remains the case when the Fynbos and Succulent Karoo Biomes are  
302 analysed separately ( $0.51 \pm 0.10$  for Succulent Karoo Biome, and  $0.30 \pm 0.15$  for Fynbos (**Figure S4**)).  
303 Turning to nitrogen, plot average soil  $\delta^{15}\text{N}$  and foliar  $\delta^{15}\text{N}$  are positively correlated (**Figure S4**;  $r^2=0.59$ ,  
304  $p<0.0001$ ) with a slope closer to 1 ( $0.84 \pm 0.15$ ) and regression intercept of  $3 \pm 1\%$ . Considered as  
305 individual biomes (**Figure S4**), the correlation is markedly higher in the Fynbos Biome ( $r^2 = 0.80$ , slope  $0.70$   
306  $\pm 0.12$  and intercept  $2.8 \pm 0.7\%$ ) compared to the Succulent Karoo ( $r^2 = 0.34$ , and slope =  $0.60 \pm 0.24$  and  
307 intercept  $5.8 \pm 2.0\%$ ).

308

## 309 5. Discussion

310 These data provide an opportunity to explore variability in, and controls upon, foliar and soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$   
311 across these semi-arid to arid winter rainfall biomes. The discussion focuses on variability at the plot and  
312 biome scales, the relationship to climatic conditions, and the relationships between the isotopic  
313 signatures of plants and associated soils.

### 314 5.1 Controls on foliar $\delta^{13}\text{C}$

315 The highest foliar  $\delta^{13}\text{C}$  values are associated primarily with Succulent and Woody Succulent growth forms  
316 (**Figure S3**). High  $\delta^{13}\text{C}$  values are particularly associated with the Crassulaceae, Aizoaceae and  
317 Asphodelaceae families. Some sampled species (e.g. *Tylocodon paniculatus*, *Euphorbia mauritanica*, *Aloë*  
318 *dichotoma*, *Monolaria moniliformis*) are CAM plants (Mooney et al. 1977; Veste et al, 2001), while other  
319 specimens are from genera known to contain CAM species (Mooney et al. 1977; Rundel et al. 1999).

320 In the Succulent Karoo, the occurrence of CAM plants results in a characteristically broad foliar  
321  $\delta^{13}\text{C}$  distribution, reflecting a mix of  $\text{C}_3$  and obligate/facultative CAM plants (Rundel et al. 1999; Winter, et  
322 al. 2015; **Figure 4**). Isotopic discrimination for Succulent Karoo Biome CAM plants has previously been  
323 considered (Rundel et al. 1999), with specimens largely drawn from the Richtersveld to the north of our  
324 study region. These results are combined with the data from this study (**Figure 4**) after converting to  
325 isotopic discrimination ( $\Delta$ ) as follows:

326

$$\Delta = \frac{\delta^{13}C_{atm} - \delta^{13}C_{leaf}}{1 + \delta^{13}C_{leaf}/1000}$$

327

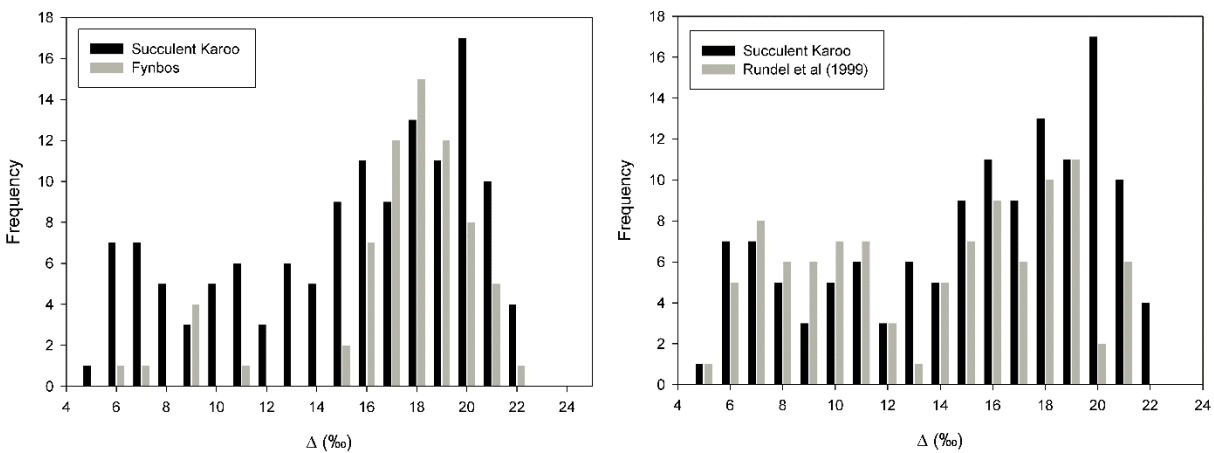
Where  $\delta^{13}C_{atm}$  at the time of sampling (2010) was estimated as -8.2‰ (Keeling et al.

328

<http://cdiac.ess-dive.lbl.gov/trends/co2/iso-sio/iso-sio.html>.

329

The range in  $\Delta$  across the Succulent Karoo Biome, spanning the Richtersveld in the north to the Fynbos Biome in the south, is ~19‰ (**Figure 4**). Compared to Rundel et al. (1999), we observed a larger proportion of specimens with higher discrimination, probably reflecting reduced CAM use under less arid conditions (mean annual precipitation in the Rundel et al. (1999) study sites was ~40-100 mm). The distributions are otherwise quite similar.



334

**Figure 4:** Histograms of isotopic discrimination for this study (Fynbos and Succulent Karoo Biome), and those of Rundel et al. (1999) from the Richtersveld region of the Succulent Karoo Biome – in each case  $C_4$  grasses have not been plotted

338

In terms of taxonomy, the lowest measured  $\Delta$  is observed in the Chenopodiaceae (including *Salsola tuberculata*), Aizoaceae (*Ruschia* sp.), Crassulaceae (e.g. *Tylocodon* sp.), Euphorbiaceae (e.g. *Euphorbia mauritanica*) and Asphodelaceae (*Aloë* sp.) families (**Table S1**). While *Salsola* likely utilises  $C_4$  photosynthesis (Pyankov et al., 2001), the lowest values for the other plants ( $\Delta$  of ~5-7‰ = foliar  $\delta^{13}C$  of ~12-15‰) imply near 100% dark fixation or “strong CAM” (Winter and Holtum, 2002; Winter et al. 2019). However, as a whole, the Succulent Karoo Biome is characterised by a bimodal  $\Delta$  distribution (**Figure 4**) comparable to previous CAM plant  $\Delta$  compilations (Cernusak et al. 2013; Winter and Holtum, 2002; Winter et al. 2015). The distinct reduction in  $\Delta$  at 12-14‰ ( $\delta^{13}C$  values -20 to -19‰), implying <> 40%



346 dark fixation (Winter and Holtum, 2002; Cernusak et al. 2013), is apparent in the Succulent Karoo Biome.  
347 Variability in  $\Delta$  across Succulent and Woody Succulent specimens is also clear (see also **Figure S3**), with  
348 the range of values for a single well-sampled family in the Succulent Karoo (Aizoaceae, **Figure S5**)  
349 demonstrating significant variability in CAM use. Climate is seemingly one factor generating this  
350 variability; the correlation between  $\Delta$  and aridity for this plant family is significant (Aizoaceae  $\Delta$  vs Aridity  
351 index; Pearson's  $r = 0.36$ ,  $p = 0.01$ ,  $n = 48$ ).

352 For Lowland and Montane Fynbos, the  $\delta^{13}\text{C}$  data indicate the expected dominance of  $\text{C}_3$  plants,  
353 although CAM species are present.  $\delta^{13}\text{C}$  from  $\text{C}_3$  plants within arid regions can span a range of  $-20$  to  $-31\text{‰}$   
354 (e.g. Ehleringer, 1989 Ehleringer et al. 1998). Considering only the most likely  $\text{C}_3$  plants (i.e. the Fynbos  
355 Biome samples with the seven aforementioned outliers removed), the  $\delta^{13}\text{C}$  range is approximately  $-23$  to  
356  $-31\text{‰}$ . (**Figure 4**). The highest foliar  $\delta^{13}\text{C}$  measured for an individual (assumed) non-CAM plant (*Montinia*  
357 *caryophyllacea*) is  $-20.3\text{‰}$ . Thus, distinguishing CAM from  $\text{C}_3$  on the basis of foliar isotope data alone is  
358 difficult (Winter and Holtum, 2002). This applies particularly to “weak CAM” (Winter et al. 2019) and is  
359 exemplified here by a specimen of *Monolaria moniliformis* (a “CAM cyler” or Weak CAM; Veste et al.  
360 2001; Winter 2019, respectively) whose foliar  $\delta^{13}\text{C}$  ( $-22.5\text{‰}$ ) is identical to that of some  $\text{C}_3$  shrubs (e.g. a  
361 specimen of *Galenia africana*; SK13 plant 2).

362 Considering plant functional type, while on average isotopic discrimination is consistently lowest  
363 in the Succulent PFT, low discrimination/high  $\delta^{13}\text{C}$  values are observed in all functional types except herbs.  
364 In the Succulent Karoo Biome,  $\Delta$  never exceeds  $21\text{‰}$ , regardless of PFT. Rundel et al. (1999) reported a  $\Delta$   
365 mode of  $17\text{‰}$  for  $\text{C}_3$  plants in their study region, with a range of  $8\text{‰}$  and no correlation with phenology  
366 or morphology. This is comparable to our findings (e.g.  $\text{C}_3$  plant mean of  $\Delta 19 \pm 2\text{‰}$  for the Fynbos Biome  
367 (excluding CAM)). For a single species, Rundel et al. (1999) reported up to  $4\text{‰}$  variability ( $\Delta$  of  $14$ - $18\text{‰}$ ).  
368 Here we observe up to  $6\text{‰}$  variation for the cosmopolitan species *Galenia africana* (Kraalbos), which was  
369 sampled across six sites (Lowland Fynbos and Succulent Karoo Biome) spanning mean annual rainfall range  
370 of  $158 \text{ mm a}^{-1}$ .

## 371 5.2 Relationship to environmental variables – $\delta^{13}\text{C}$

372 **Table 2:** Pearson's correlation coefficients between site-averaged soil and foliar  $\delta^{13}\text{C}$  and climatic data  
373 extracted from WorldClim (Hijmans et al., 2005). MAP = Mean annual precipitation MAT = Mean annual  
374 temperature. Aridity index taken from Trabucco and Zomer (2009). Values in **bold** are significant at  $p$

375 <0.05. The stronger correlations with climatic parameters for plot average soil  $\delta^{13}\text{C}$  most likely reflect  
 376 averaging of individual plant responses and time averaging (see also Carr et al., 2016).

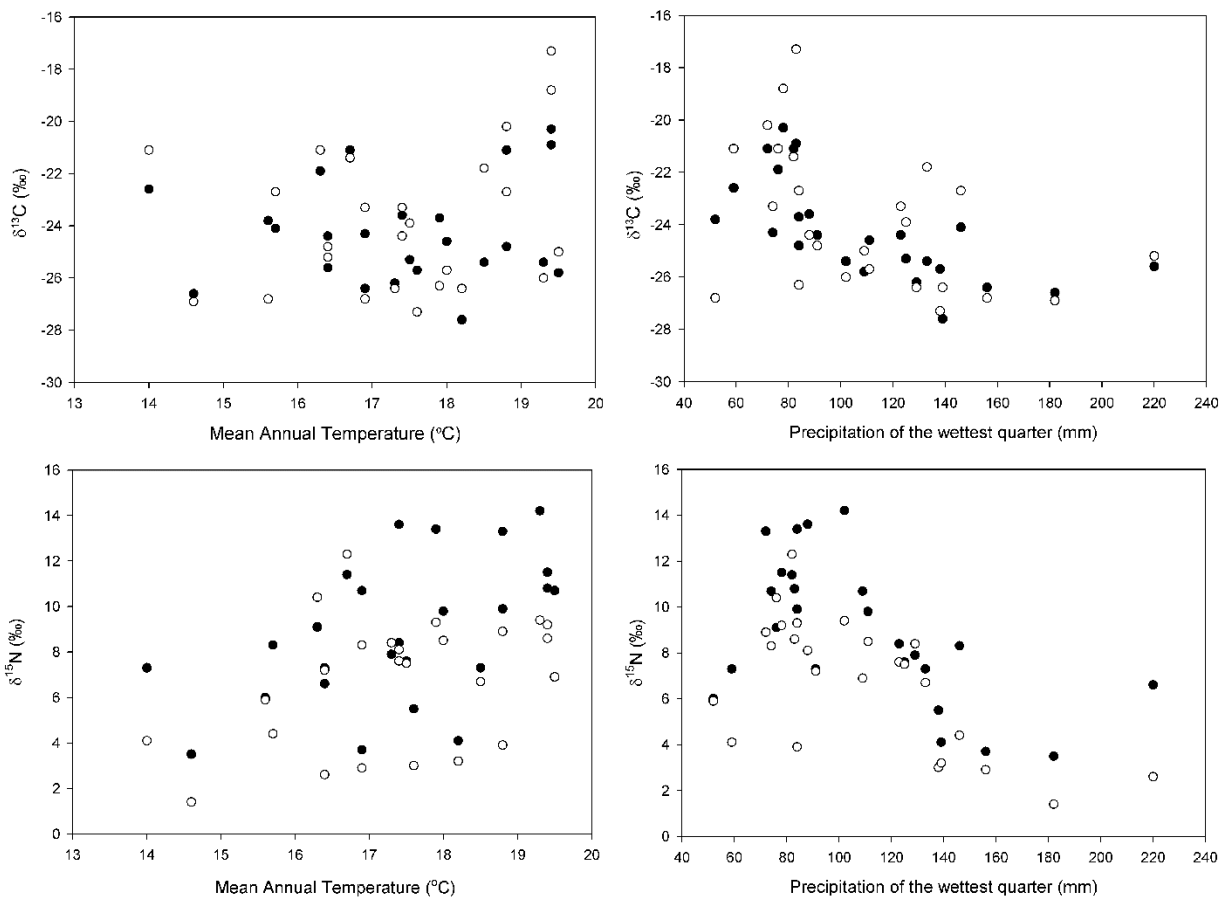
	MAP	MAT	Aridity Index	Temp of the wettest Q	Temp of the driest Q	Precipitation of the Wettest Q	Precipitation of the Driest Q
All plant $\delta^{13}\text{C}$	-0.43	0.23	-0.41	0.11	0.01	-0.45	-0.17
All soil $\delta^{13}\text{C}$	-0.58	0.09	-0.52	0.11	0.00	-0.66	-0.13
SK plant $\delta^{13}\text{C}$	-0.23	0.16	-0.27	-0.12	0.01	-0.13	-0.21
SK soil $\delta^{13}\text{C}$	-0.39	0.01	-0.37	-0.23	0.08	-0.40	-0.21
FB plant $\delta^{13}\text{C}$	-0.23	0.42	-0.16	0.44	0.24	-0.24	-0.20
FB plant $\delta^{13}\text{C}$ (putative CAM removed)	-0.50	0.35	-0.49	0.19	0.40	-0.49	-0.48
FB soil $\delta^{13}\text{C}$	-0.26	0.23	-0.11	0.56	-0.21	-0.32	-0.23

377

378 Overall, plot average soil and foliar  $\delta^{13}\text{C}$  are uncorrelated or weakly correlated with climatic  
 379 parameters (**Table 2**). There are some limitations in this analysis of climatic relationships, notably the  
 380 relatively limited climate space (particularly when biomes are analysed separately) and the constraints of  
 381 the interpolated WorldClim database (Hijmans et al., 2005) (e.g. in the topographically complex Cape Fold  
 382 Belt Mountains)

383 Considering all sites,  $\delta^{13}\text{C}$  from soils and plants is most strongly (negatively) correlated with  
 384 precipitation of the wettest month (**Table 2**). Given the range of foliar  $\delta^{13}\text{C}$  measured, the primary cause  
 385 of this correlation between  $\delta^{13}\text{C}$  and rainfall is most likely photosynthetic pathway, indicating the greater  
 386 occurrence of CAM plants, and occasional  $\text{C}_4$  grasses (e.g. SK2, SK9) in the more arid Succulent Karoo.  
 387 There are no significant correlations when only samples from the Succulent Karoo Biome are considered.  
 388 This most likely reflects the substantial individual plant / soil  $\delta^{13}\text{C}$  variability, driven by the relative  
 389 abundance of CAM and  $\text{C}_3$  specimens within the sample plots, and variability in the degree of night-time

390 CAM fixation within individual specimens (at and prior to sampling; Cernusak et al., 2013). Similarly, for  
 391 the Fynbos Biome alone there are no significant correlations with climate. Excluding the seven outlier  
 392 specimens (almost certainly CAM – see above) (**Table 2**) the strongest (negative) correlation is between  
 393 foliar  $\delta^{13}\text{C}$  and precipitation of the wettest quarter (PWetQ;  $r = -0.49$ ,  $p = 0.15$ ). This is not statistically  
 394 significant and the absence of correlations between foliar  $\delta^{13}\text{C}$  and climate parameters in this biome likely  
 395 reflects a combination of sample numbers, plot scale variability, topographic variability and the relatively  
 396 narrow climatic envelope (**Figure 5**; see also Codron et al. 2013). These likely mask the expected WUE  
 397 signal anticipated in this  $\text{C}_3$  plant dominated biome.

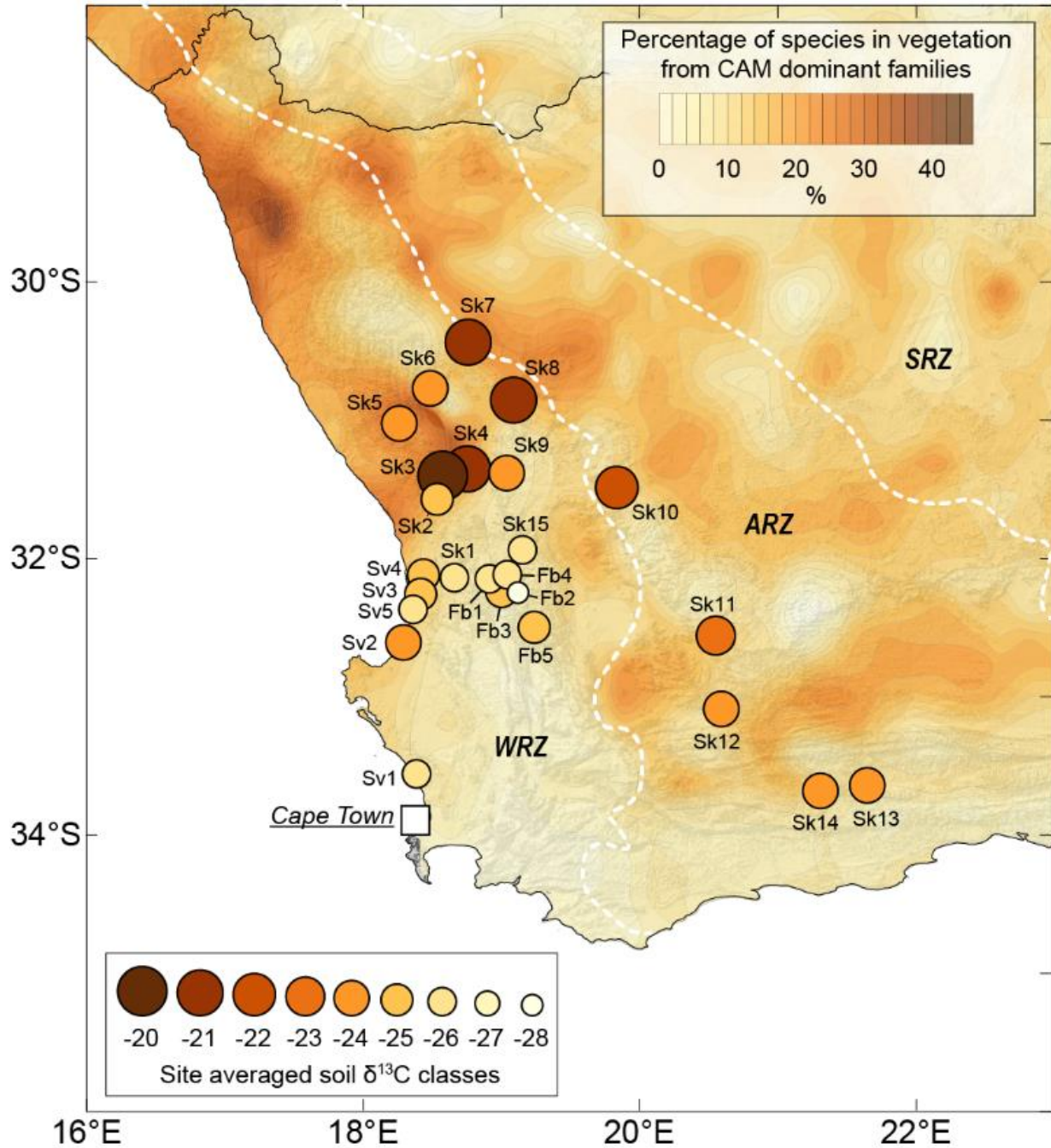


398  
 399 **Figure 5:** Scatter plots considering  $\delta^{13}\text{C}$  in relation to climatic parameters extracted from the WorldClim data. The  
 400 upper two plots show soil (solid fill) and foliar (open symbol)  $\delta^{13}\text{C}$  in relation to mean annual temperature and  
 401 precipitation of the wettest quarter (see **table 2**). The lower two plots show soil (solid fill) and foliar (open symbol)  
 402  $\delta^{15}\text{N}$  in relation to mean annual temperature and precipitation of the wettest quarter (see **table 2**).

### 403 5.3 Soil-plant $\delta^{13}\text{C}$ relationship

404 The correlation between soil and foliar  $\delta^{13}\text{C}$  indicates that variability in foliar  $\delta^{13}\text{C}$  is captured within soil  
405 organic matter (SOM) (**Figures S4**). This is not unexpected, as plant-derived organic matter will be the  
406 main C input to soils (Ehleringer et al. 2000; Wang et al. 2010; Nel et al., 2018). Indeed, in the Succulent  
407 Karoo Biome, plot average soil  $\delta^{13}\text{C}_{\text{TOC}}$  correlates strongly ( $r = 0.88$   $p = < 0.001$ ) with the proportion of  
408 sampled plants with foliar  $\delta^{13}\text{C} \geq 20$  (excluding  $\text{C}_4$  grass samples). While only a crude estimate of the likely  
409 contribution of CAM plant-derived OM to the soils (i.e. it does not take into account biomass or cover  
410 differences), this is markedly stronger than the correlations observed between soil  $\delta^{13}\text{C}_{\text{TOC}}$  and climatic  
411 parameters.

412 Comparing these results more explicitly with vegetation in the study region, **Figure 6** compares  
413 the plot average soil  $\delta^{13}\text{C}_{\text{TOC}}$  values with the percentage of plant species in CAM-rich families (Aizoaceae-  
414 Mesembryanthemaceae, Crassulaceae, Euphorbiaceae), as obtained from the PRECIS database (SANBI,  
415 2003). The highest soil  $\delta^{13}\text{C}$  values broadly accord with this independent measure of CAM plant  
416 abundance, implying contributions to the wider soil and sedimentary organic matter pool in this region  
417 cannot be assumed to be negligible. Comparison of **figures 1 and 3** suggests that time-space averaging in  
418 soils reduces plant-scale isotopic variability and that sedimentary or soil  $\delta^{13}\text{C}_{\text{TOC}}$  values  $> -23$  ‰ are  
419 potentially indicative of a CAM presence (**Figure 3**). The importance of considering CAM as well as  $\text{C}_3:\text{C}_4$   
420 plant abundance within any interpretative (palaeo) framework is clearly implied. The increased presence  
421 of CAM plants in the sampled (winter rainfall) environments is largely driven by aridity (**Table 2**). For a  
422 palaeoenvironmental record, applying such an interpretation would assume inputs from  $\text{C}_4$  grasses could  
423 be excluded (perhaps requiring other lines of evidence). This would be especially important at the margins  
424 of the study region in the winter and year-round rainfall zone transition, where  $\text{C}_4$  grasses are  
425 demonstrably present.



426

427 **Figure 6:** Map showing regional-scale variation percentage of plant species in the vegetation from CAM-dominant  
 428 families (Aizoaceae-Mesembryanthemaceae, Crassulaceae, Euphorbiaceae), as obtained from the PRECIS database  
 429 (SANBI, 2003), along with the mean soil  $\delta^{13}\text{C}_{\text{TOC}}$  from the study sites (as circles). To allow comparison between these  
 430 two datasets their colour ramps are matched. The size of the circles has been varied to allow easier differentiation  
 431 of soil  $\delta^{13}\text{C}_{\text{TOC}}$  values between sites, but does not convey additional information

432 On average soil  $\delta^{13}\text{C}_{\text{TOC}}$  is lower than foliar  $\delta^{13}\text{C}$  and this offset increases at higher foliar  $\delta^{13}\text{C}$  values  
433 (**Figure S6**). In this instance, the variable offset may reflect a single sampling season – the end of the dry  
434 season – meaning that the  $\delta^{13}\text{C}$  of sampled plant tissue, which may integrate the most recent conditions,  
435 became increasingly higher than the more time-averaged soil organic matter, particularly in the most arid  
436 (more CAM, higher foliar  $\delta^{13}\text{C}$ ) Succulent Karoo plots. However,  $^{13}\text{C}$  depleted SOM relative to foliar OM  
437 has been associated with selective preservation of  $^{13}\text{C}$ -depleted fractions of relatively recalcitrant plant  
438 organic matter, such as lignin and lipids (though see Nel et al., 2018). In this instance it is possible to  
439 compare our results with SOM compositions, obtained via pyrolysis-GC/MS analyses of the same soil  
440 samples (Carr et al., 2013). Here we observe no significant correlations between the soil-foliar  $\delta^{13}\text{C}$  offset  
441 and summary variables for this SOM composition analysis. More specifically axes 1 and 2 from a Principal  
442 Component Analysis of the pyrolysis-GC/MS-derived SOM compositions (Carr et al., 2013) were associated  
443 with relative abundances of lignin and leaf cuticle-derived organic matter (Axis 1) or a high (low)  
444 abundances of short to mid-chain length aliphatics (Axis 2). Both however are uncorrelated with the soil-  
445 foliar  $\delta^{13}\text{C}$  offset measured here.

446

#### 447 5.4 $\delta^{15}\text{N}$ variability and controls

448 There is considerable variation in soil and foliar  $\delta^{15}\text{N}$  across the study region. Site averages for soil and  
449 foliar  $\delta^{15}\text{N}$  decrease as Succulent Karoo Biome > Lowland Fynbos > Montane Fynbos (**Figures 1 and 3**).  
450 Although there is significant inter-specimen variation in foliar  $\delta^{15}\text{N}$ , even within the same plot, there are  
451 no significant differences in foliar  $\delta^{15}\text{N}$  between PFTs (**Figure S3**). Furthermore, there is no evidence for  
452 any differences in foliar  $\delta^{15}\text{N}$  with photosynthetic pathway. (Succulent Karoo Biome foliar  $\delta^{13}\text{C}$  < > -20‰  
453 produces identical mean foliar  $\delta^{15}\text{N}$  values ( $\delta^{15}\text{N} = 7.7\text{‰}$ ; < -20 mean vs. > -20‰  $\delta^{15}\text{N} = 7.7\text{‰}$ ; Mann  
454 Whitney U = 1895,  $p = 0.982$ ; **Figure S7**)).

455 An additional source of foliar  $\delta^{15}\text{N}$  variability is the presence of N-fixing plants and plants with  
456 mycorrhizal associations (Craine et al., 2009). The latter are associated with several plant families in the  
457 Fynbos Biome, but less so with succulent-rich families like Aizoaceae (Spriggs et al., 2003). Mycorrhizal  
458 associations are associated with foliar  $\delta^{15}\text{N}$  values up to 6‰ lower than non-mycorrhizal plants (Craine et  
459 al. 2009; 2015a), while N-fixers typically generate foliar  $\delta^{15}\text{N}$  values close to zero (Craine et al. 2015a and  
460 references therein). Although the presence of the former has not been assessed here, our foliar  $\delta^{15}\text{N}$   
461 values are mostly higher than those reported for Fynbos plants with known mycorrhizal associations

462 (Spriggs et al., 2003), with only four  $\delta^{15}\text{N}$  measurements in the present study at or below 0‰. Of these,  
 463 two are from the Fabaceae family (FB5-P4 *Aspalanthus* sp. and FB5-P19 *Rafnia* sp.) and indeed their foliar  
 464  $\delta^{15}\text{N}$  values are commensurately lower (measured  $\delta^{15}\text{N}$  of 0 and 0.1‰) than their (FB5) plot-mean foliar  
 465  $\delta^{15}\text{N}$  (1.4‰). However, one of the lowest measured  $\delta^{15}\text{N}$  values was from a Restionaceae specimen, a  
 466 family not thought to be mycorrhizal (Allsopp and Stock, 1993). Conversely, the  $\delta^{15}\text{N}$  of a sample of *Erica*  
 467 sp. (the Ericaceae family is associated with ericoid mycorrhiza, with reported  $\delta^{15}\text{N}$  values < 0‰; Allsopp  
 468 and Stock, 1993; Spriggs et al. 2003) had a foliar  $\delta^{15}\text{N}$  close to the plot average (2.6‰ compared to the  
 469 FB1 plot mean of 3.0‰). The impact of these effects is therefore difficult to evaluate with the present  
 470 dataset.

471 Considering  $\delta^{15}\text{N}$  in terms of climate (e.g. Handley et al. 1999; Admunson et al. 2003), noting the  
 472 previous caveats regarding gridded climate data,  $\delta^{15}\text{N}$  is most strongly correlated with precipitation of the  
 473 wettest quarter and mean annual precipitation (**Table 3; Figure 5**). Correlations with temperature-related  
 474 parameters are largely non-significant, except for the positive correlation between mean annual  
 475 temperature and soil  $\delta^{15}\text{N}$ , and the negative correlation between aridity and soil  $\delta^{15}\text{N}$ . Correlations are  
 476 weaker when the Succulent Karoo Biome is considered alone, but the narrower climatic envelope and  
 477 reduced sample numbers likely account for this.

478 **Table 3:** Pearson’s correlations between site-averaged soil and foliar  $\delta^{15}\text{N}$  and climatic data extracted from  
 479 WorldClim (Hijmans et al., 2005). Values in bold are significant at  $p < 0.05$ . MAP = Mean annual  
 480 precipitation MAT = Mean annual temperature. Aridity index taken from Trabucco and Zomer (2009).

	MAP	MAT	Aridity Index	Temp of the wettest Quarter	Temp of the driest Quarter	Precipitation of the Wettest Quarter	Precipitation of the Driest Quarter
All sites foliar $\delta^{15}\text{N}$	<b>-0.57</b>	0.38	<b>-0.52</b>	0.18	0.20	<b>-0.62</b>	-0.19
All sites soil $\delta^{15}\text{N}$	<b>-0.60</b>	<b>0.52</b>	<b>-0.55</b>	0.15	0.23	<b>-0.56</b>	-0.35
SK foliar $\delta^{15}\text{N}$	-0.11	0.32	-0.04	-0.19	0.31	-0.15	-0.07
SK soil	-0.28	<b>0.68</b>	-0.33	-0.29	0.46	0.15	<b>-0.54</b>

$\delta^{15}\text{N}$							
<b>FB Foliar</b> $\delta^{15}\text{N}$	<b>-0.70</b>	0.57	-0.56	<b>0.74</b>	0.17	<b>-0.75</b>	<b>-0.65</b>
<b>FB soil</b> $\delta^{15}\text{N}$	-0.43	0.48	-0.26	<b>0.80</b>	-0.06	-0.50	-0.38

481

482

483 It is notable, given the range of potentially fractionating processes in the nitrogen cycle, that  $\delta^{15}\text{N}$  shows  
484 stronger correlations with climate (precipitation, temperature and aridity; **Table 4**) than  $\delta^{13}\text{C}$ . Additionally,  
485 in contrast to  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  shows also shows statistically significant correlations with temperature-related  
486 parameters. Multiple regression (**Table 4**) with temperature and precipitation of the wettest quarter  
487 (these variables are uncorrelated) produces highest combined  $R^2$  (0.48 and  $p < 0.001$ ) for total dataset.  
488 This level of explanation is comparable to several studies (e.g. Cheng et al. 2009; Craine et al., 2009; Perri  
489 et al., 2012; Díaz et al. 2016; Mosher et al. 2020), although other regional-scale studies have noted both  
490 an absence of a relationship between climate and  $\delta^{15}\text{N}$  (e.g. Zhao et al. 2020) or a non-linear relationship  
491 (Wang et al., 2014).

492

493 **Table 5:** Results of the multiple regression analyses for  $\delta^{15}\text{N}$  of all soils and plants with MAP and MAT

	<b>Intercept</b>	<b>MAP slope</b>	<b>MAT slope</b>	<b>R<sup>2</sup></b>	<b>N</b>	<b>p</b>
<b>Soil</b> $\delta^{15}\text{N}$	1.06 ± 6.77	-0.02 ± 0.01	0.72 ± 0.35	0.48	25	0.0007
<b>Foliar</b> $\delta^{15}\text{N}$	4.82 ± 6.75	-0.02 ± 0.01	0.37 ± 0.35	0.38	25	0.0052

494

495 Considering just the relationship between  $\delta^{15}\text{N}$  and precipitation (using MAP for comparison with  
496 published works; soil  $n = 25$ ,  $r^2 = 0.38$   $p = 0.001$ ) the linear regression slope indicates a  $2.4 \pm 0.6\%$  change  
497 in  $\delta^{15}\text{N}$  per 100 mm change in MAP and a  $0.72 \pm 0.35\%$  per °C change in mean annual temperature. The



498 slope for the foliar data is comparable ( $2.1 \pm 0.6\text{‰}$  per 100 mm). For MAP these slope values are higher  
499 than several previous studies (e.g. Perri et al., 2012; Swap et al., 2004), substantially so when compared  
500 to global scale analyses of  $\delta^{15}\text{N}$  vs. MAP, which incorporate wider ranges of MAP (e.g. Hadley et al. 1999;  
501 Craine et al. 2009). For relatively arid environments, Cheng et al. (2009) sampled a range in MAP  
502 comparable to the present study and reported a slope range of 1.1-2.3‰ per 100 mm decrease in MAP,  
503 while in even more arid conditions, over an MAP range of 10-160 mm a<sup>-1</sup>, Díaz et al. (2016) observed soil  
504  $\delta^{15}\text{N}$  to increase by ~5 ‰ (foliar  $\delta^{15}\text{N}$  showing a different relationship). Considering other controls, a  
505 greater abundance of plants using mycorrhizal associations within the otherwise higher rainfall, lower  
506 temperature locales of the Fynbos Biome (particularly the low soil nutrient Montane Fynbos of the  
507 Cederberg Mountains; Campbell, 1983; Muofhe and Dakora, 1999) may account for the relatively steep  
508 MAP- $\delta^{15}\text{N}$  gradient observed across the whole dataset. In this respect it is noteworthy that foliar N and  
509  $\delta^{15}\text{N}$  are significantly correlated within the Fynbos Biome ( $r = 0.43$ ,  $p < 0.0001$ ), but are uncorrelated in  
510 the Succulent Karoo Biome ( $r = -0.01$ ,  $p = 0.9$ ), which would be predicted for a more N-limited Fynbos  
511 biome. A fuller assessment of this requires further study.

512 Non-climatic factors may also be associated with soil and foliar  $\delta^{15}\text{N}$  variability (Craine et al. 2009;  
513 Craine et al. 2015b Nel et al. 2018). A correlation matrix for soil and foliar  $\delta^{15}\text{N}$  with associated soil  
514 properties is shown in **Table 4**. There are substantial differences in soil texture, pH, TOC and TN between  
515 the Fynbos and the Succulent Karoo Biomes (**Figure 3**; **Figure S2**). Apparent correlations between  $\delta^{15}\text{N}$   
516 and TN or TOC may conflate climatic effects (i.e. these are also correlated with climate, via biomass; **Table**  
517 **4**; **Figure S8**). Considering this and accounting for the TOC-MAP (particularly) and TN-MAP relationships  
518 across all sites, we note the overall MAP- $\delta^{15}\text{N}$  relationship remains significant (**Figure S8**). Considering the  
519 biomes separately, while the Fynbos shows no correlation with non-climatic factors, the Succulent Karoo  
520 data imply that soil  $\delta^{15}\text{N}$  (but not foliar  $\delta^{15}\text{N}$ ) is negatively correlated with TN and TOC (soil) and % fines  
521 (**Figure S8**), but uncorrelated with rainfall-related factors (**Table 2**). The latter observations are however  
522 difficult to assess further given the limited climate space in the Succulent Karoo.

523

524

525

526

527 **Table 4:** Correlation coefficients for all site soil and foliar  $\delta^{15}\text{N}$ . % fines and soil pH data taken from Carr et  
 528 al., (2013). Values in bold are significant at  $p < 0.05$ .

	All site soil $\delta^{15}\text{N}$	All site foliar $\delta^{15}\text{N}$	All site $\Delta^{15}\text{N}$	Fynbos site average soil $\delta^{15}\text{N}$	Fynbos Site average foliar $\delta^{15}\text{N}$	FB $\Delta^{15}\text{N}$	Succulent Karoo Biome site average soil $\delta^{15}\text{N}$	Succulent Karoo Biome Site average foliar $\delta^{15}\text{N}$	SK $\Delta^{15}\text{N}$
Soil total N (%)	-0.35	-0.20	0.26	-0.10	-0.07	0.02	<b>-0.70</b>	-0.34	0.41
Soil TOC (%)	<b>-0.49</b>	<b>-0.43</b>	0.15	-0.29	-0.26	-0.06	<b>-0.66</b>	<b>-0.53</b>	0.17
Soil pH	<b>0.37</b>	<b>0.42</b>	0.03	0.28	0.01	-0.42	0.05	0.44	0.41
fines (% < 63 $\mu\text{m}$ )	0.19	0.34	0.19	-0.29	-0.11	0.26	<b>-0.55</b>	0.08	<b>0.70</b>

529

530 Plot average foliar  $\delta^{15}\text{N}$  and soil  $\delta^{15}\text{N}$  are correlated across all sites, with soil  $3 \pm 1$  ‰ more enriched  
 531 in  $^{15}\text{N}$  than the plants (**Figure S4**), consistent with observations elsewhere (Craine et al. 2009; Pardo and  
 532 Nadelhoffer, 2010). As the slope of the relationship between the soil and foliar  $\delta^{15}\text{N}$  is close to 1 ( $0.84 \pm$   
 533  $0.15$ ), these data also imply that the “enrichment factor” (the difference between foliar and soil  $\delta^{15}\text{N}$  i.e.  
 534  $\Delta^{15}\text{N}$ ) is no greater at higher foliar  $\delta^{15}\text{N}$  sites (cf. Craine et al. 2009). This is of potential relevance as  $\Delta^{15}\text{N}$   
 535 can provide insights into N cycling and plant-available N by removing the effects of variations in the  
 536 baseline (soil) signature (Craine et al. 2015a). For the total dataset here, there is no correlation between  
 537  $\Delta^{15}\text{N}$  and any climatic factors (cf. Cheng et al. 2009; Mosher et al., 2020), but interestingly it is correlated  
 538 with % fines for the Succulent Karoo, an observation worth further investigation, particularly given current  
 539 uncertainties surrounding the drivers of the enrichment factor (Craine et al. 2015a).

## 6. Conclusions

Soil and foliar  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  data collected from across the Fynbos and Succulent Karoo Biomes facilitated consideration of a range of environmental variables impacting plant and soil isotopic compositions across these semi-arid to arid winter rainfall biomes. Soil and foliar  $\delta^{13}\text{C}$  for the Fynbos are consistent with a dominantly  $\text{C}_3$  biome, but these new data provide a more robust indication of the degree of variability (the Fynbos  $\delta^{13}\text{C}$  standard deviation, excluding CAM plants, is 1.6‰) within this biome. Additionally, CAM plants with elevated  $\delta^{13}\text{C}$ , while rare, are present in the Fynbos Biome and contribute to foliar isotopic variability (Fynbos foliar  $\delta^{13}\text{C}$  standard deviation including CAM plants is 3.6‰). Indeed, across the total dataset and the Succulent Karoo in particular, CAM is associated with the greatest carbon isotope variability ( $\delta^{13}\text{C}$  range be more than 10‰).  $\delta^{13}\text{C}$  is negatively correlated with mean annual or wet season rainfall, a relationship driven largely by the turnover of vegetation from Fynbos (more humid,  $\text{C}_3$ , rare CAM) to Succulent Karoo (more arid, common CAM). The presence of CAM is apparent in soil  $\delta^{13}\text{C}_{\text{TOC}}$ , and the data available at present suggest soil  $\delta^{13}\text{C}_{\text{TOC}}$  values  $> -23$  ‰ are potentially indicative of CAM presence in the landscape. There are no statistically significant correlations between foliar  $\delta^{13}\text{C}$  ( $\Delta$ ) and climate within the primarily  $\text{C}_3$  Fynbos sites, despite expectations that  $\text{WUE}_i$  would generate such a relationship. This most likely reflects: 1) a limited range of  $\text{WUE}_i$ -induced variability relative to non- $\text{WUE}_i$  factors, 2) the narrow climatic envelope of the study area, and 3) the limited number of samples.

We observe substantial  $\delta^{15}\text{N}$  variability at the plot scale, but overall, soil and foliar  $\delta^{15}\text{N}$  values conform to the hot/dry (higher  $\delta^{15}\text{N}$ ) versus cool/wet (lower  $\delta^{15}\text{N}$ ) paradigm outlined in previous works (e.g. Craine et al. 2009). Both soil and foliar  $\delta^{15}\text{N}$  are correlated with climate (rainfall and temperature). The relationship with climate, particularly with MAP is, however, relatively steep compared to previous compilations. This is not a result of systematic differences in foliar  $\delta^{15}\text{N}$  for succulent growth forms or CAM plants, but mycorrhizal associations, and contrasting soil /nutrient properties between the Fynbos and Succulent Karoo are potential contributors to within and between biome variability. Soil and foliar  $\delta^{15}\text{N}$  are correlated (more strongly than the equivalent analysis of  $\delta^{13}\text{C}$ ) and replicate the  $\sim 3$ ‰ offset between soil and foliar  $\delta^{15}\text{N}$  reported across a range of ecosystems.

Overall, these data provide a starting point for future studies of N and C cycling in these biomes, as well as important baseline isotope ranges for palaeoecological, palaeoclimatic and archaeological research. An understanding of spatial variability in isotopic compositions, and particularly variability within any biome or climatic zone, is clearly necessary for robust interpretations of changing (palaeo)

570 climates, animal diets and human diets (Sealy et al., 1987; Szpak et al. 2013). A challenge in this region,  
571 particularly near to the winter rainfall-year round rainfall transition zone, will be to disentangle the roles  
572 of CAM (aridity) and C<sub>4</sub> (rainfall seasonality) plants within stable isotopic records.

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## 774 Figure Captions:

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776 **Figure 1:** Violin plots showing variability in foliar total carbon (top left) and total nitrogen (top right), and  
777 variability in foliar  $\delta^{13}\text{C}$  (bottom left) and  $\delta^{15}\text{N}$  (bottom right) for the sampled biomes/eco-regions. SK =  
778 Succulent Karoo, LF = Lowland Fynbos and MF = Montane Fynbos.

779 **Figure 2:** Scatter plots of foliar (upper left) and soil (upper right)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  split by biome. The  
780 correlation between soil  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is statistically significant for the Fynbos Biome (Pearson's  $r = 0.51$ ,  
781  $p < 0.0001$ ). Bottom: Scatter plots of foliar (left – note log scale x axis) and soil (right) C/N and  $\delta^{15}\text{N}$  split  
782 by biome. The correlation between foliar C/N and  $\delta^{15}\text{N}$  for the Fynbos Biome is statistically significant  
783 (Pearson's  $r = -0.52$ ,  $p < 0.0001$ ), as is the correlation for soil C/N and  $\delta^{15}\text{N}$  for the Fynbos Biome (Pearson's  
784  $r = 0.44$ ,  $p < 0.001$ ). The variables are uncorrelated in the Succulent Karoo Biome.

785 **Figure 3:** Violin plots showing variation in soil total nitrogen (TN; top left), soil total organic carbon (TOC;  
786 top right) contents, soil  $\delta^{13}\text{C}_{\text{TOC}}$  (bottom left) and soil  $\delta^{15}\text{N}$  (total N; bottom right). SK = Succulent Karoo, LF  
787 = Lowland Fynbos and MF = Montane Fynbos.

788 **Figure 4:** Histograms of isotopic discrimination for this study (Fynbos and Succulent Karoo Biome), and  
789 those of Rundel et al. (1999) from the Richtersveld region of the Succulent Karoo Biome – in each case  $\text{C}_4$   
790 grasses have not been plotted

791 **Figure 5:** Scatter plots considering  $\delta^{13}\text{C}$  in relation to climatic parameters extracted from the WorldClim  
792 data. The upper two plots show soil (solid fill) and foliar (open symbol)  $\delta^{13}\text{C}$  in relation to mean annual  
793 temperature and precipitation of the wettest quarter (see table 2). The lower two plots show soil (solid  
794 fill) and foliar (open symbol)  $\delta^{15}\text{N}$  in relation to mean annual temperature and precipitation of the wettest  
795 quarter (see table 2).

796 **Figure 6:** Map showing regional-scale variation percentage of plant species in the vegetation from CAM-  
797 dominant families (Aizoaceae-Mesembryanthemaceae, Crassulaceae, Euphorbiaceae), as obtained from  
798 the PRECIS database (SANBI, 2003), along with the mean soil  $\delta^{13}\text{C}_{\text{TOC}}$  from the study sites (as circles). To  
799 allow comparison between these two datasets their colour ramps are matched. The size of the circles has  
800 been varied to allow easier differentiation of soil  $\delta^{13}\text{C}_{\text{TOC}}$  values between sites, but does not convey  
801 additional information

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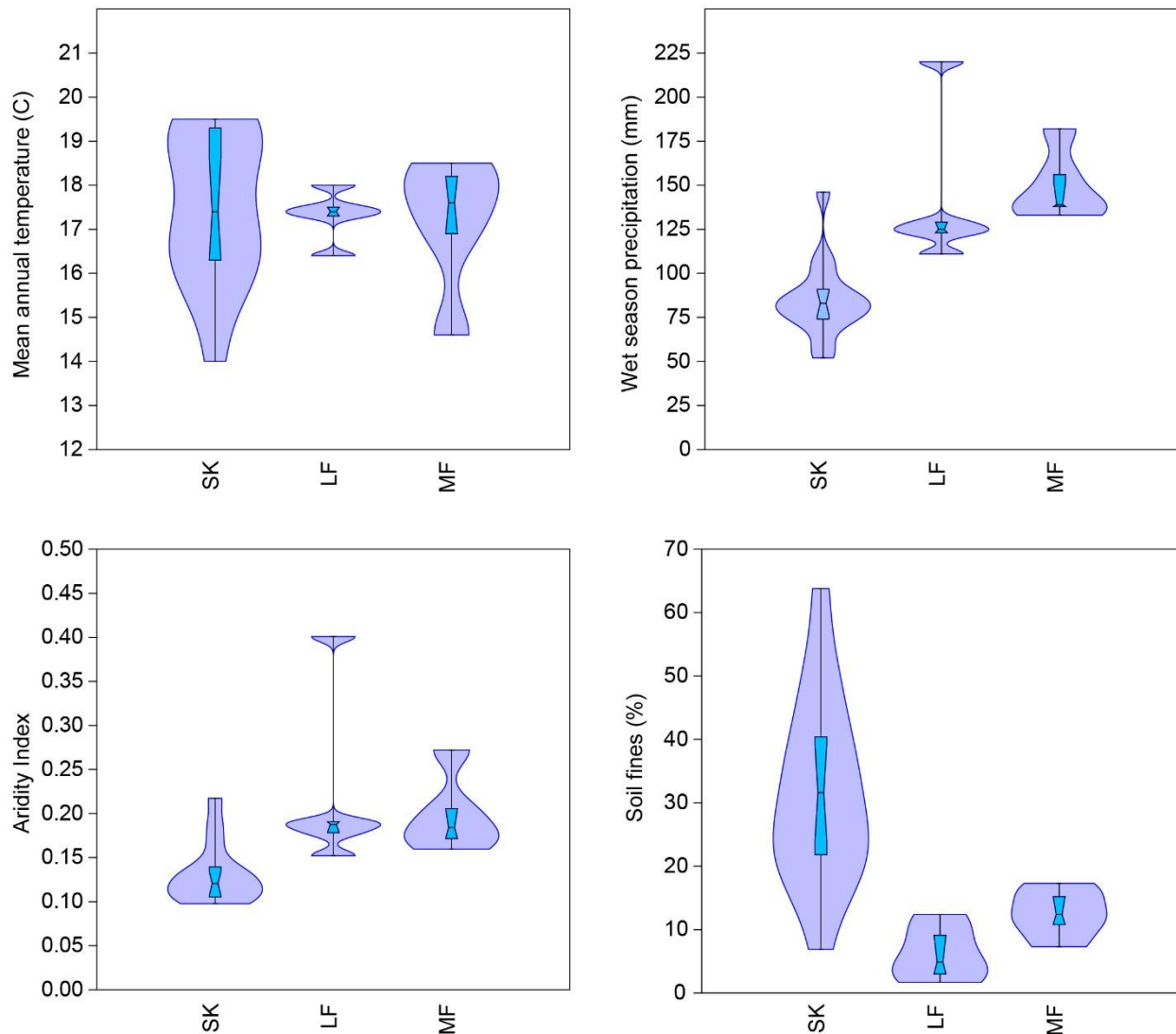
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## Supplementary figures for Carr et al - Variability in soil and foliar stable carbon and nitrogen isotope compositions in the winter rainfall biomes of South Africa

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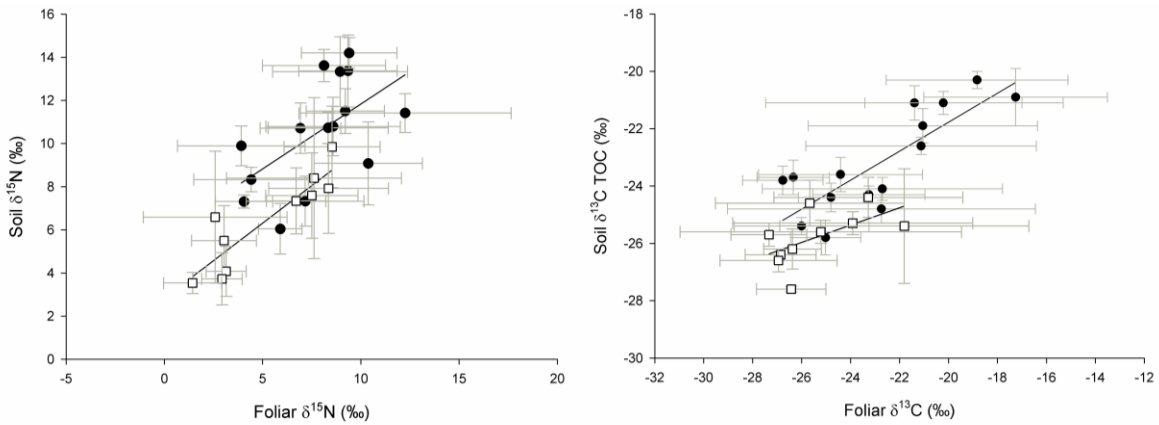


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814 **Figure S1:** Violin plots showing variability in climatic conditions (from Hijmans et al., 2005) and soil texture  
815 variability (% < 63  $\mu\text{m}$  – see Carr et al., 2013) for the sampled biomes/eco-regions. SK = Succulent Karoo,  
816 LF = Lowland Fynbos and MF = Montane Fynbos. The Lowland Fynbos outlier for aridity and temperature  
817 is the near coastal site SV1 (see also **Figure 1**).

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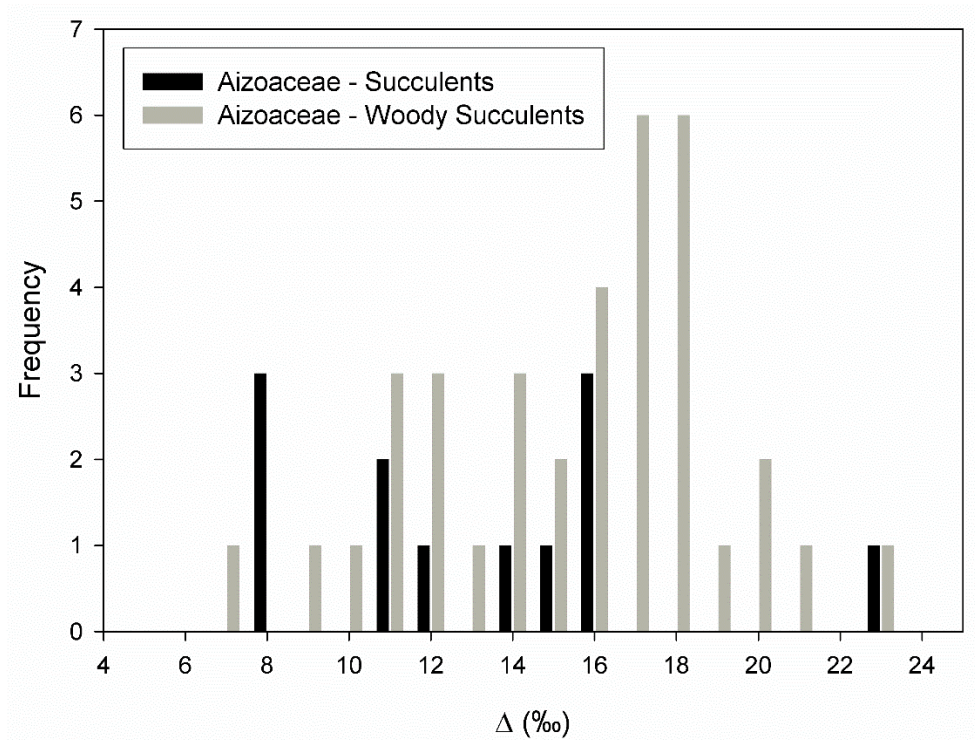
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821 **Figure S2:** Soil vs plant  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  split by biome (Succulent Karoo in closed circles and Fynbos in open  
822 squares). Linear regression results for the individual biomes are as follows:  $\delta^{15}\text{N}$  Fynbos  $y = 0.70x + 2.8$   
823  $r^2=0.80$ ;  $\delta^{15}\text{N}$  Succulent Karoo:  $y = 0.60x + 5.8$   $r^2=0.33$ ;  $\delta^{13}\text{C}$  Fynbos  $y = 0.30x - 18.1$   $r^2=0.32$ ;  $\delta^{13}\text{C}$  Succulent  
824 Karoo  $y = 0.51x - 11.7$   $r^2=0.66$

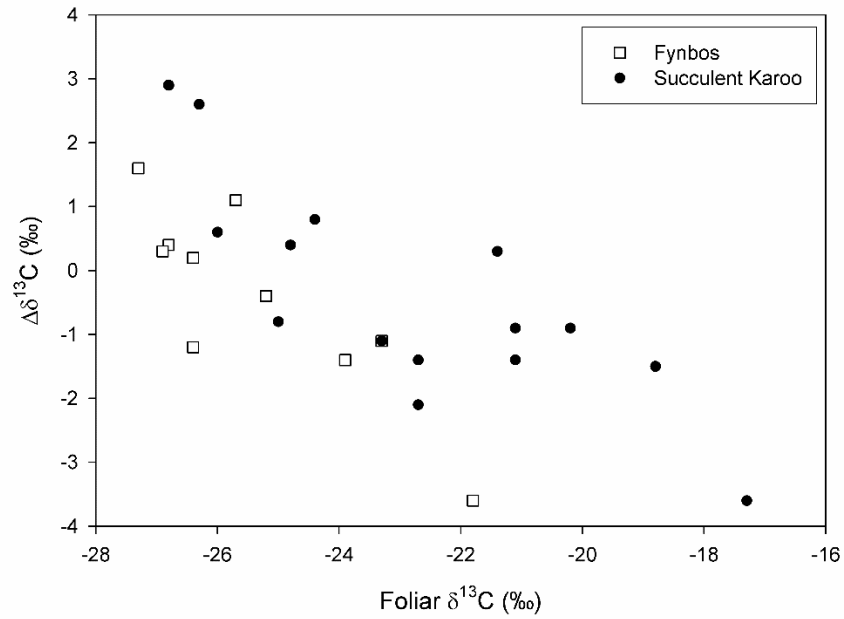
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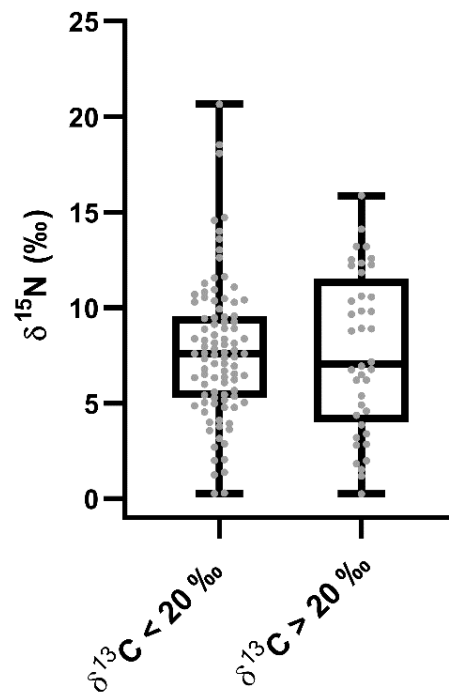
827 **Figure S3:** The distribution of  $^{13}\text{C}$  ( $\Delta$ ) discrimination for Succulents and Woody Succulents of the  
828 Aizoaceae family, sampled across the succulent Karoo biome (n=48).

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831 **Figure S4:** The magnitude of soil-foliar  $\delta^{13}\text{C}$  offset ( $\Delta\delta^{13}\text{C}$ ) (obtained from plot average data) is negatively  
 832 correlated with foliar  $\delta^{13}\text{C}$ .

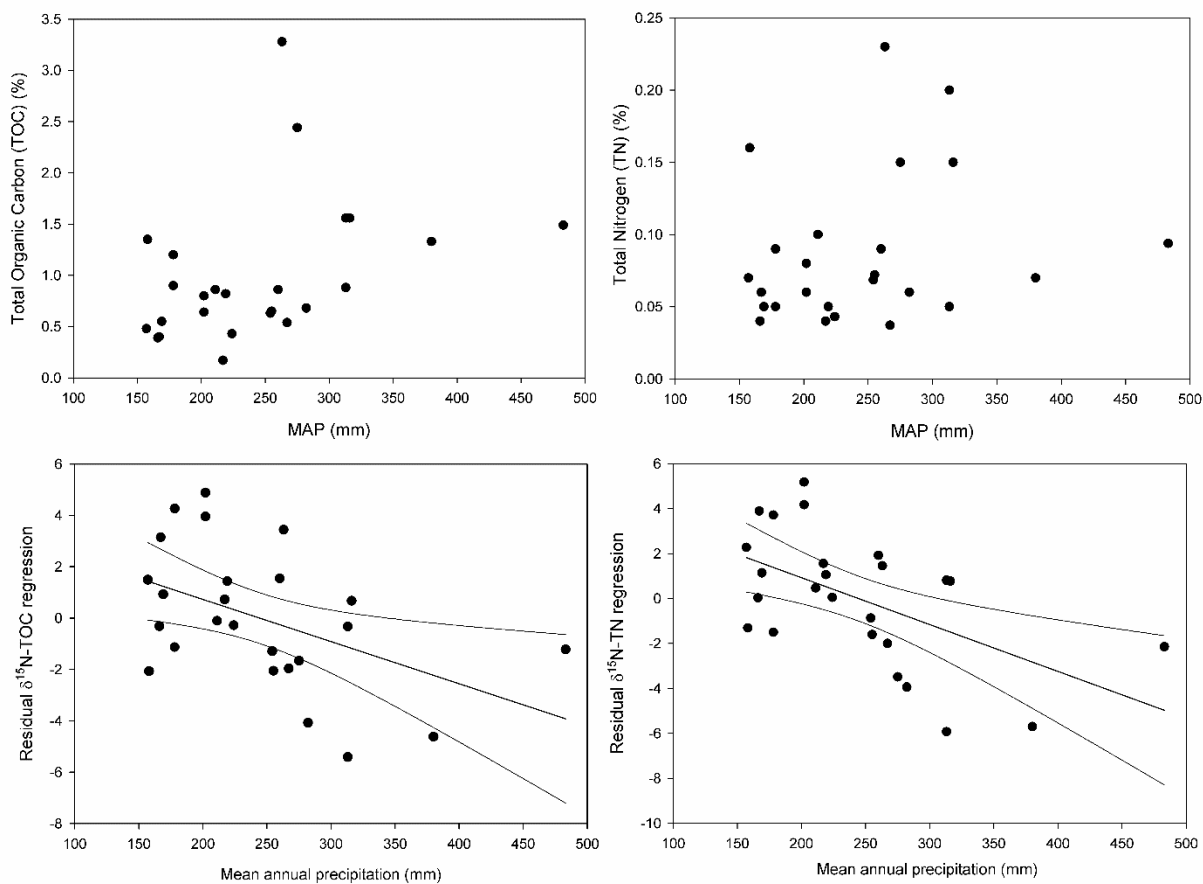


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834 **Figure S5:** Box and whisker plot of foliar  $\delta^{15}\text{N}$  in the Succulent Karoo Biome, split for plants  $\delta^{13}\text{C} < > -20$   
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839 **Figure S6:** Top row: Scatter plots of plot average soil TOC and TN and mean annual precipitation (MAP)

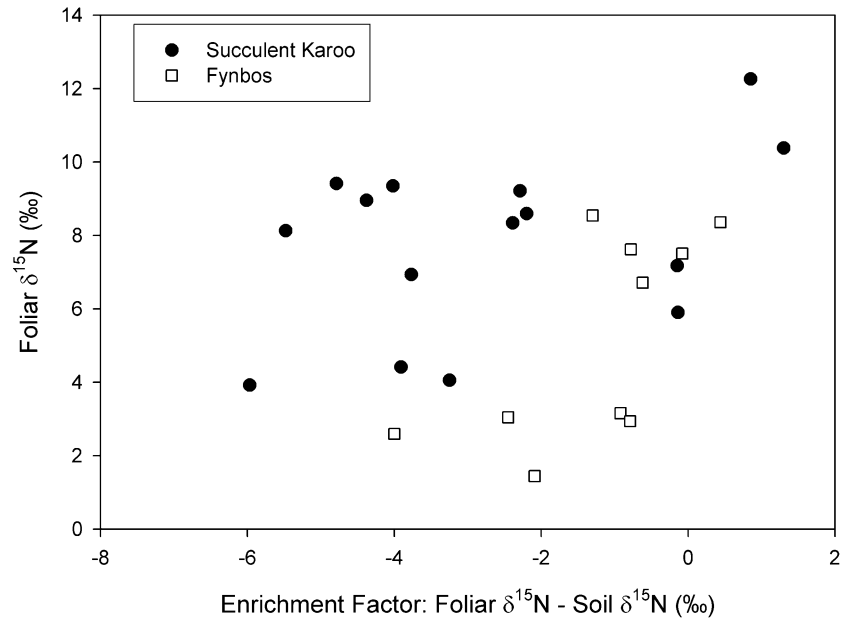
840 Bottom row: regression analysis the residuals from the  $\delta^{15}\text{N}$  vs TOC and  $\delta^{15}\text{N}$  vs TN analyses with mean

841 annual precipitation. The regression outputs for TOC and TN are  $y = -0.02x + 4.0$  ( $r^2 = 0.22$ ) and  $y = -$

842  $0.02x + 5.1$  ( $r^2 = 0.31$ ) respectively.

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845 **Figure S7:** Leaf minus soil δ<sup>15</sup>N (Δδ<sup>15</sup>N or “enrichment factor”), as derived from site average values,  
 846 plotted by biome relative to site average foliar δ<sup>15</sup>N.

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