IX Field School on Soil Zoology and Ecology
Karaganda 2025

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Stable isotope tracing and mapping of invertebrates

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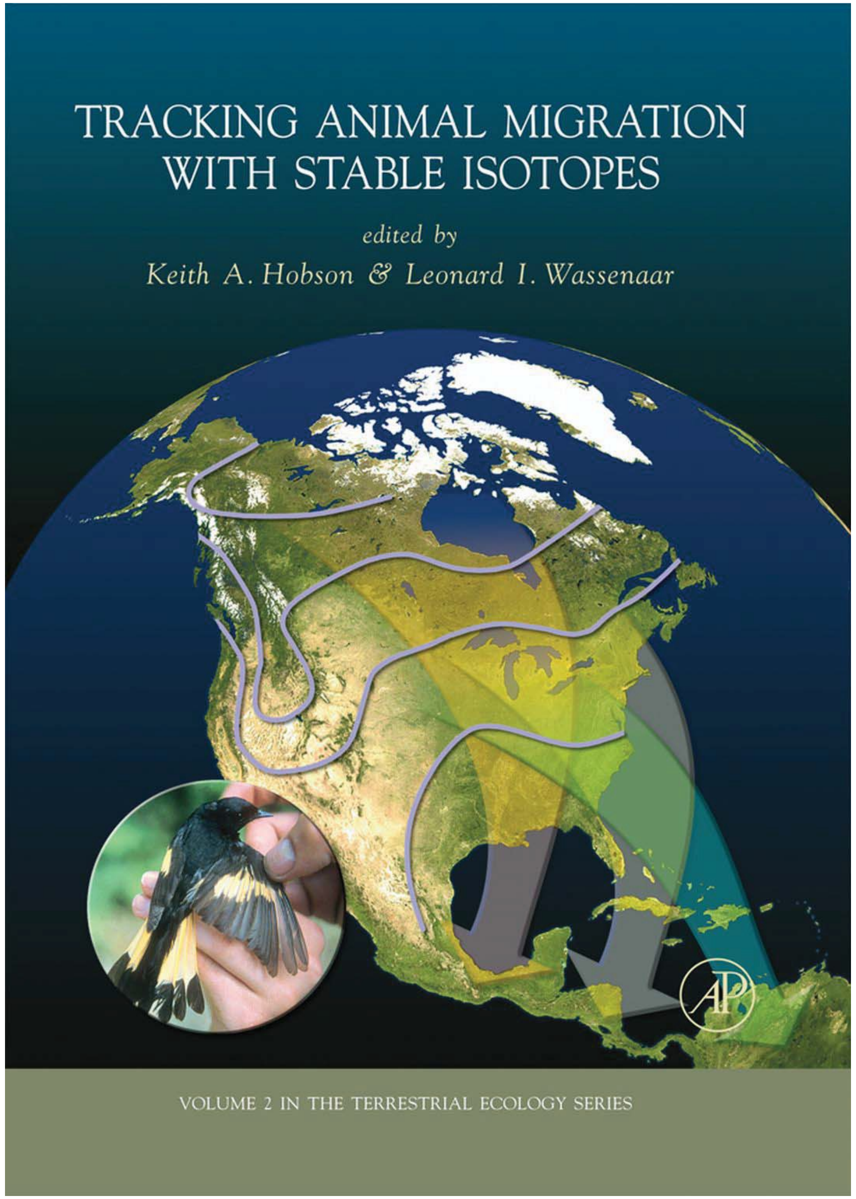
Sharing is encouraged

Karagandy, 2025

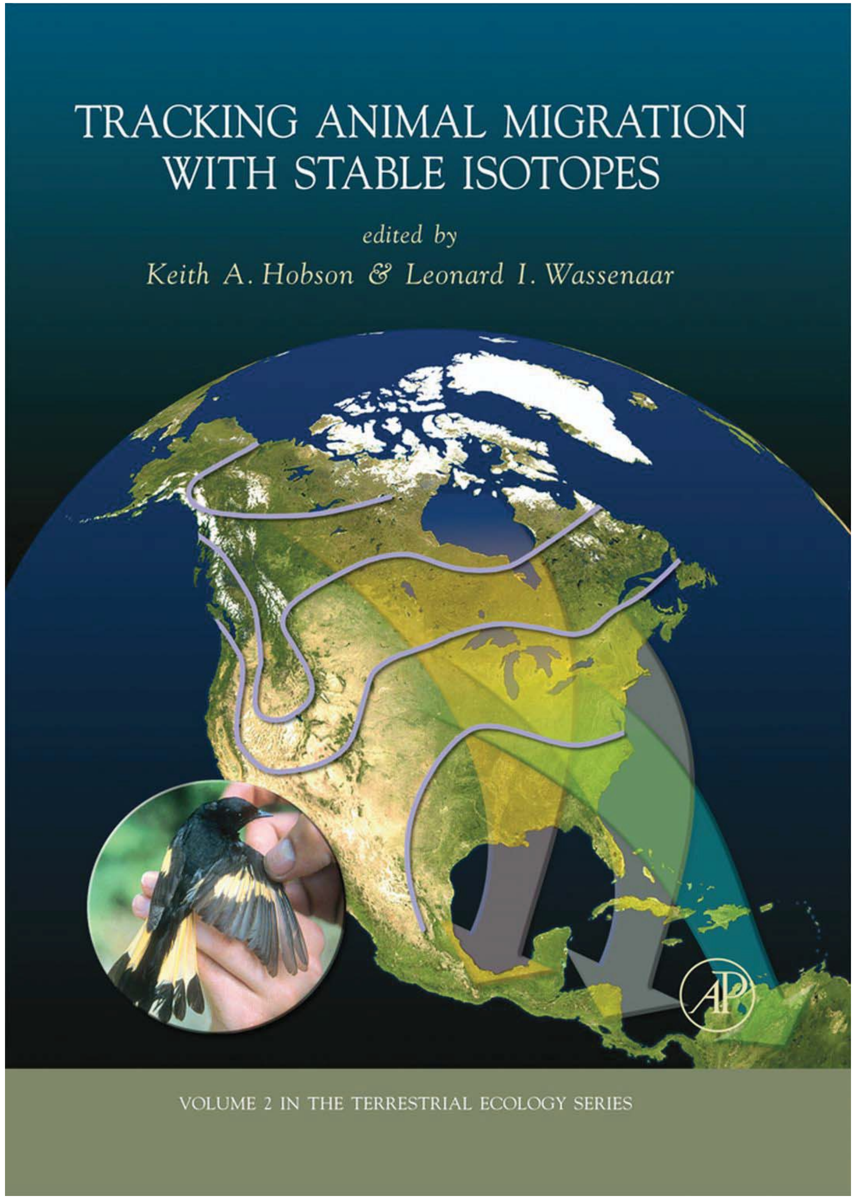
Introduction



Prof. Keith Hobson
(1954-2024)

Dr. Leonard Wassenaar,
University of Ottawa

Introduction



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Prof. Dr. Christian Voigt,
Leibniz-Institut für Zoo- und Wildtierforschung (IZW)

Dr. Alexandre Courtiol,
Leibniz-Institut für Zoo- und Wildtierforschung (IZW)

Introduction

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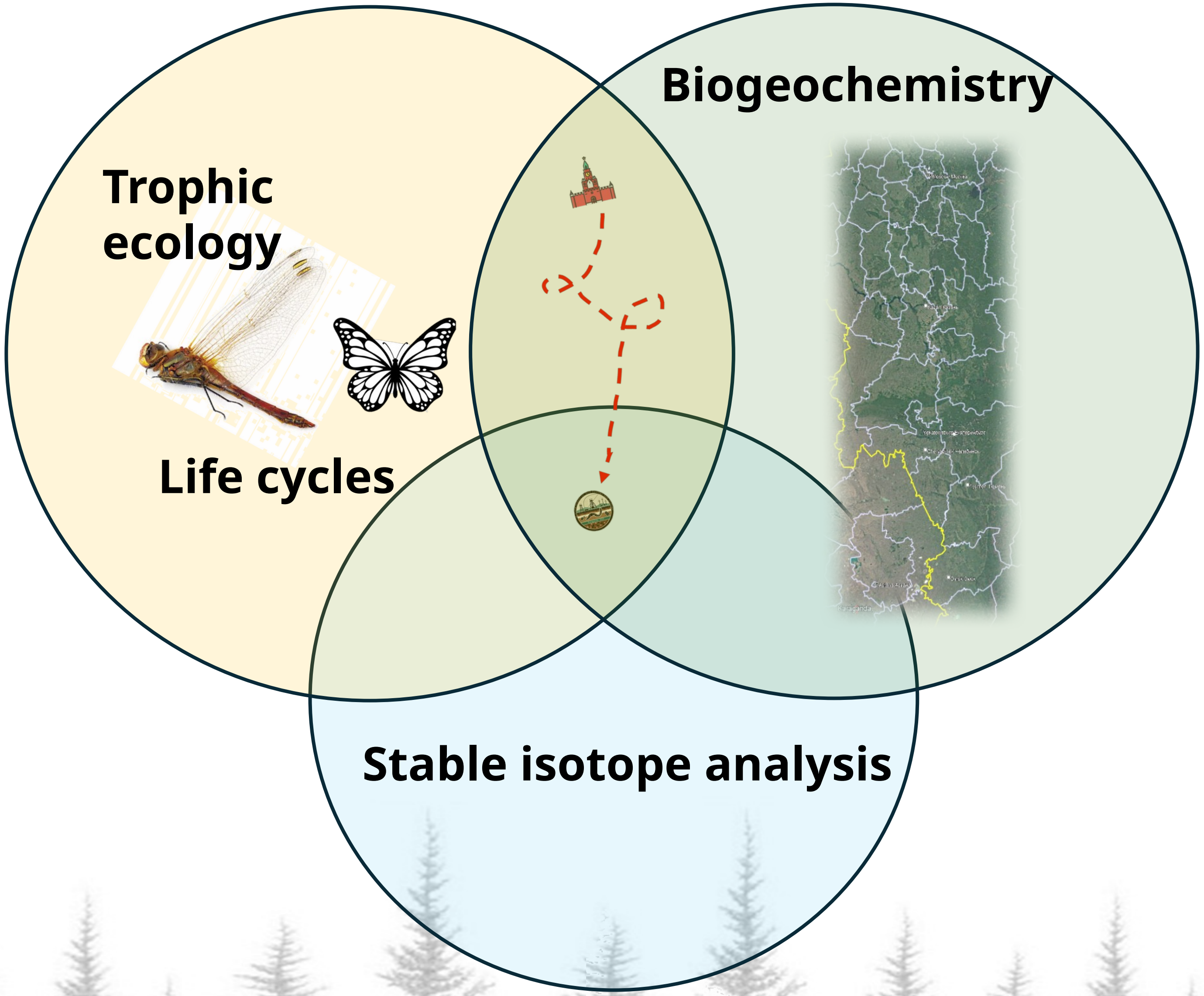


Stable isotope analysis

Stable isotope analysis of solids: technical aspects

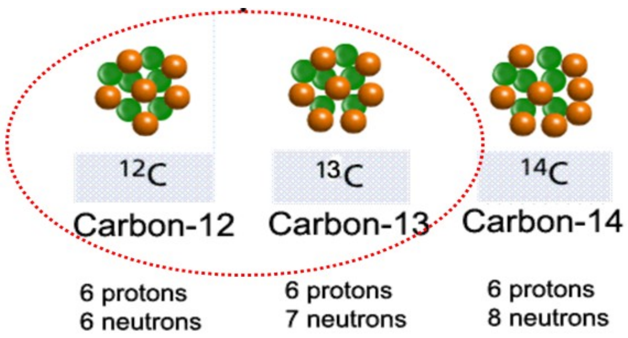
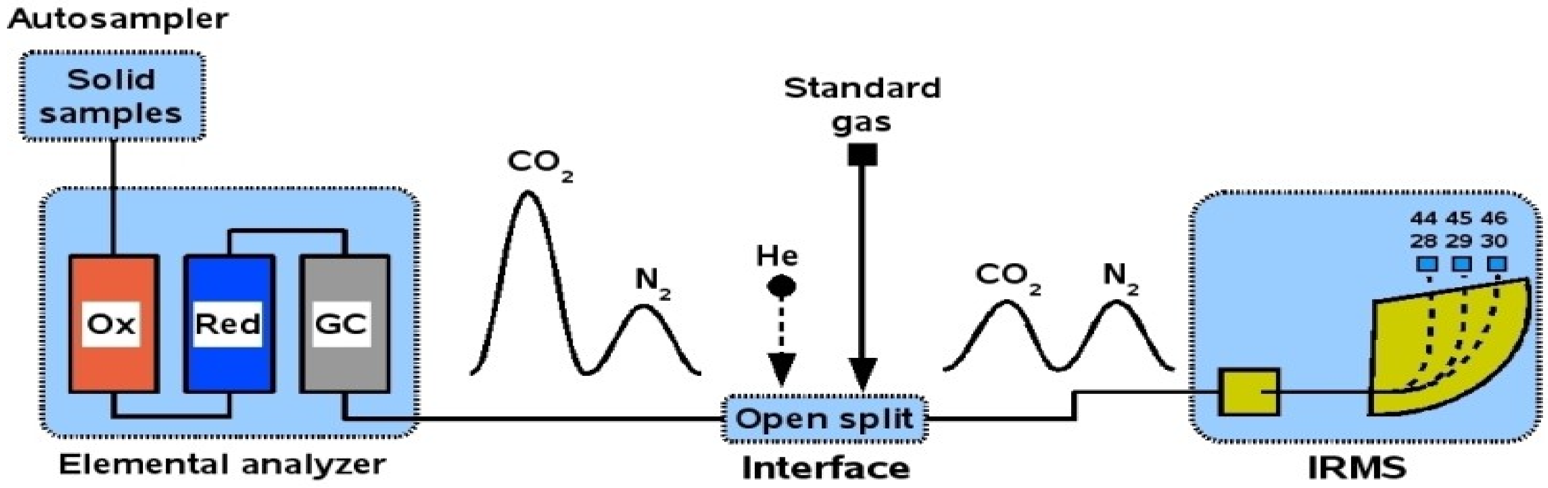


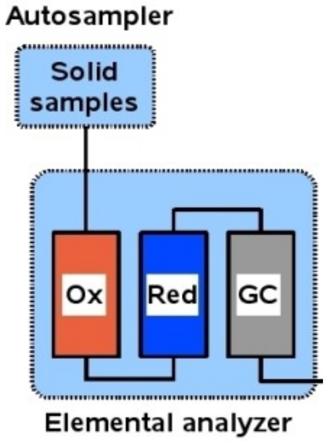
TABLE 2.1 Approximate Elemental Abundances as (dry) Mass Fraction in wt. %, the Stable Isotope Ratios of Interest, and Expected Stable Isotopic Ranges for Bulk Tissues (e.g., α- or β-Keratins Like Hair or Feathers) Commonly Used in Migratory Research

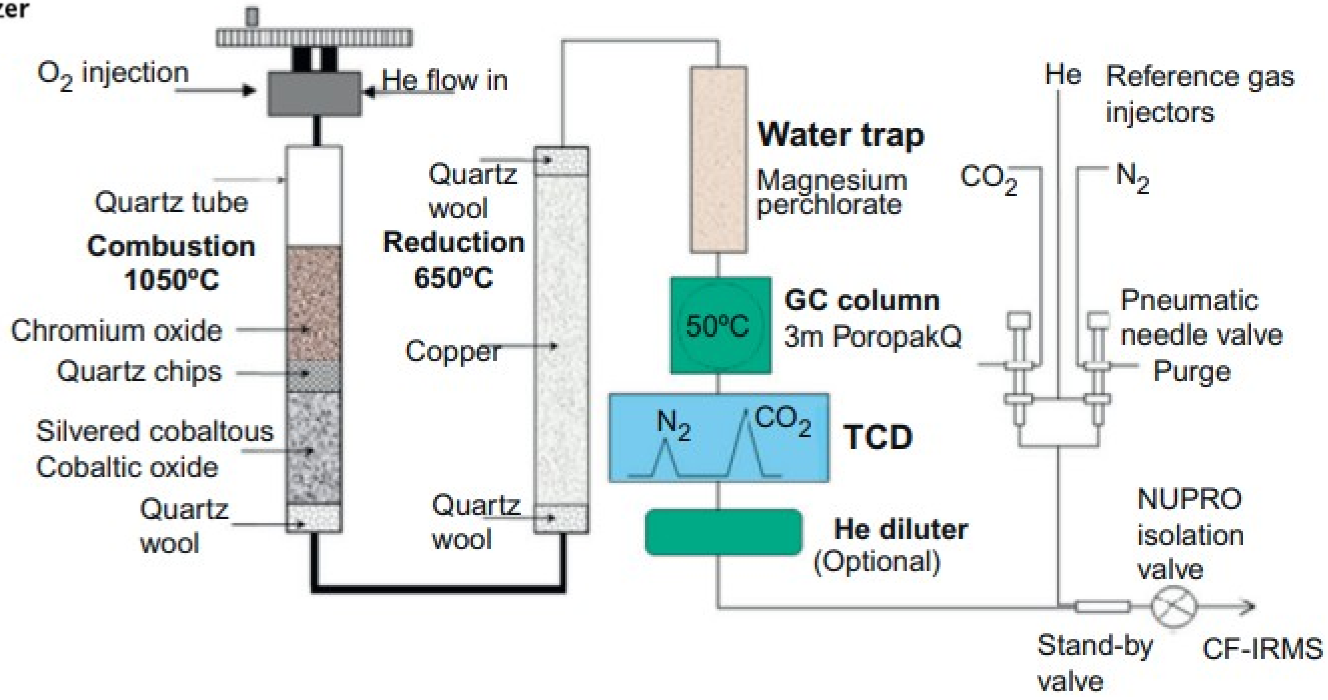
Element	Wt. %	Isotope Ratios	δ-Range	Mass Required
LIGHT ISOTOPES				
Carbon[a]	30–40 wt. %	$^{13}C/^{12}C$	−5 to −65‰ (PDB)	0.2–1.5 mg
Oxygen[b]	27–40 wt. %	$^{18}O/^{16}O$	+10 to +30‰ (VSMOW)	0.2–0.5 mg
Nitrogen[a]	12–19 wt. %	$^{15}N/^{14}N$	−2 to +25‰ (Air)	0.5–1.5 mg
Hydrogen[b]	6–8 wt. %	$^{2}H/^{1}H$	−250 to +90‰ (VSMOW)	0.1–0.4 mg
Sulfur	5–20 wt. %	$^{34}S/^{32}S$	−20 to +30‰ (CDT)	1–2 mg
HEAVY ISOTOPES				
Strontium	<100 − x ppb	$^{87}Sr/^{86}Sr$	Absolute ratios	2–30 mg[c]

(Wassenaar, 2019)



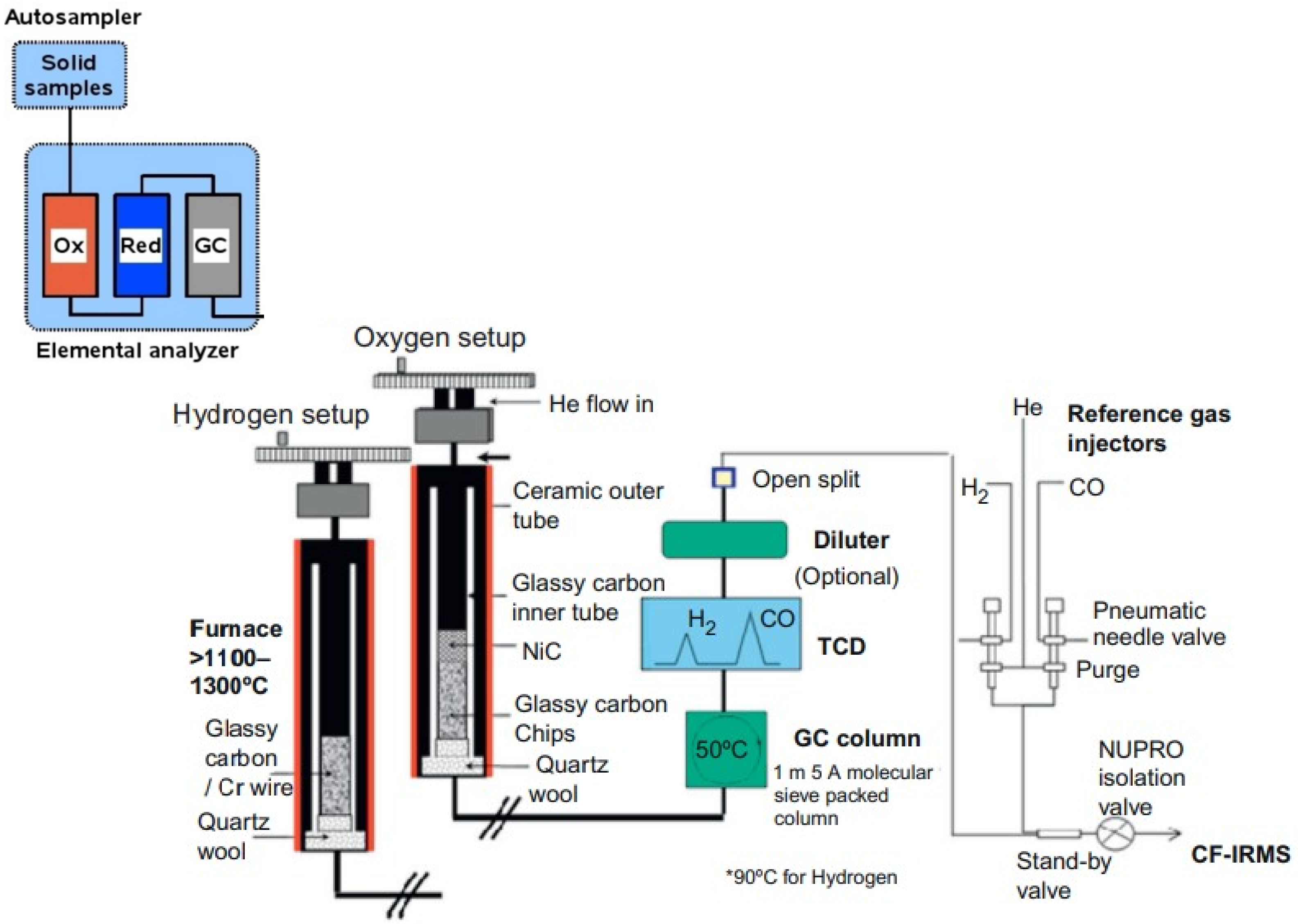
Stable isotope analysis of solids: technical aspects (for CN setup)





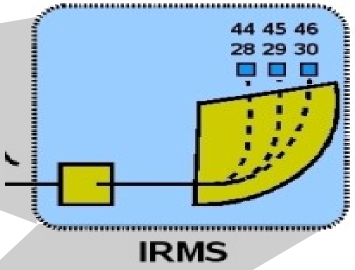
(Wassenaar, 2019)

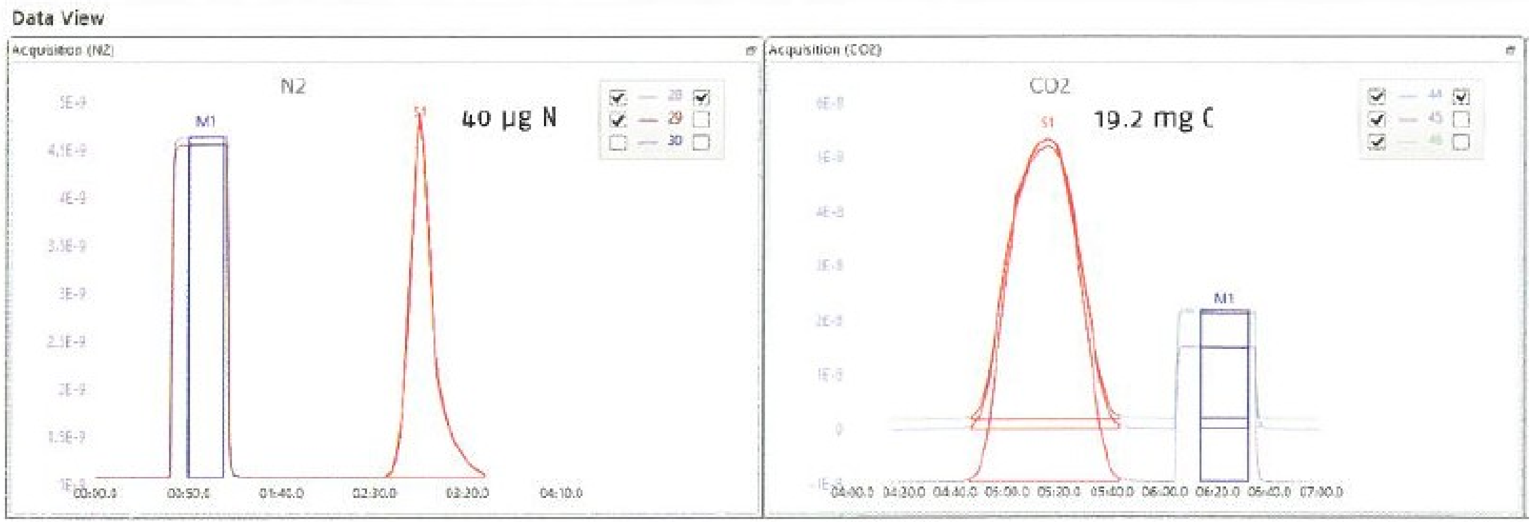
Stable isotope analysis of solids: technical aspects (for HO setup)



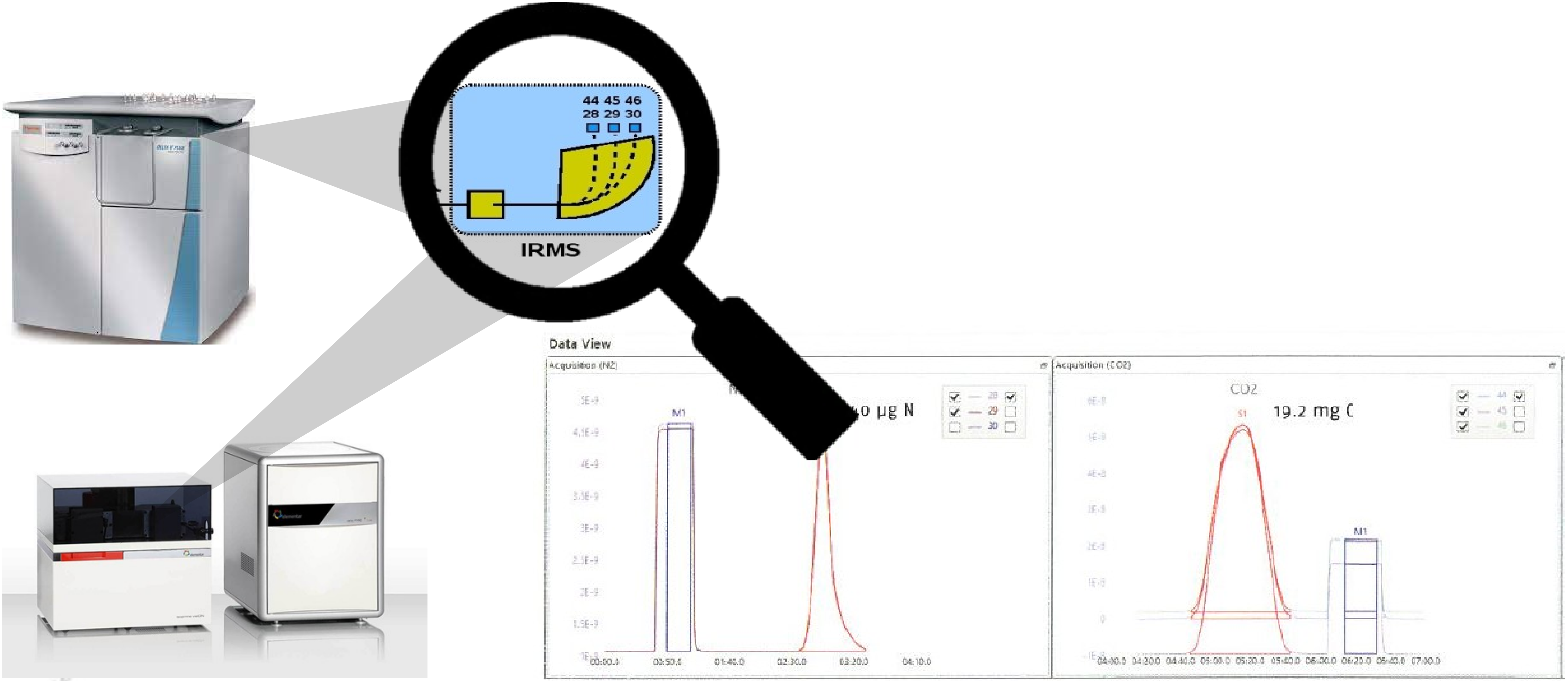
(Wassenaar, 2019)

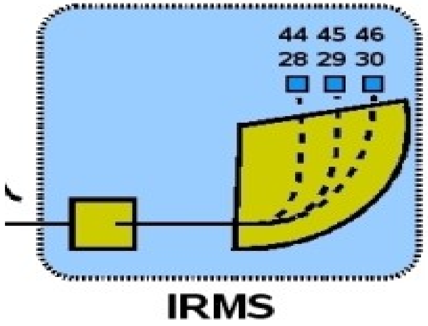
Stable isotope analysis of solids: technical aspects

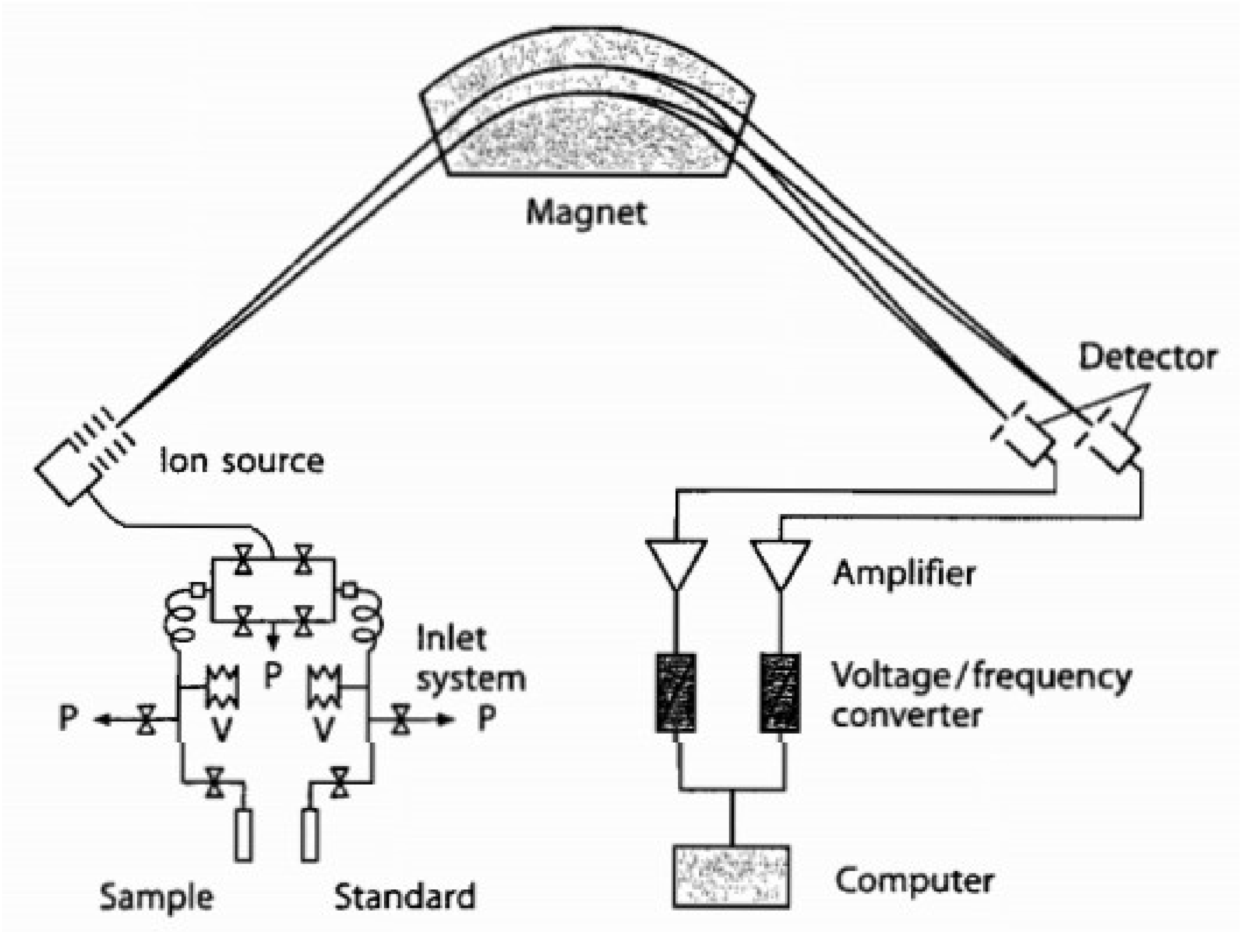




Stable isotope analysis of solids: technical aspects




44 45 46
28 29 30
IRMS


Magnet
Detector
Ion source
Amplifier
Inlet system
P
V
V
P
P
Voltage/frequency converter
Sample
Standard
Computer

Stable isotope analysis: isotopes of biogenic elements

Element	Stable isotopes	Natural frequency (%)
H	^{1}H, ^{2}H (D)	99.958; 0.0155
O	^{16}O, ^{17}O, ^{18}O	99.759; 0.037; 0.204
C	^{12}C, ^{13}C	98.892; 1.108
N	^{14}N, ^{15}N	99.635; 0.365

1. Mass-dependent fractionation of isotopes during chemical reactions and physical processes.

2. Mass-independent fractionation of isotopes.

- Processes in which the degree of isotope fractionation depends on the difference in their mass in a non-linear manner.
- This phenomenon is much less common than mass-dependent fractionation and has been studied much less extensively.

Stable isotope analysis: standards

$$\delta(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} * 1000$$

where R – is the isotopic ratio of less abundant (heavy) isotope to the more abundant (light) isotope in sample (e.g. $^{13}C/^{12}C$)

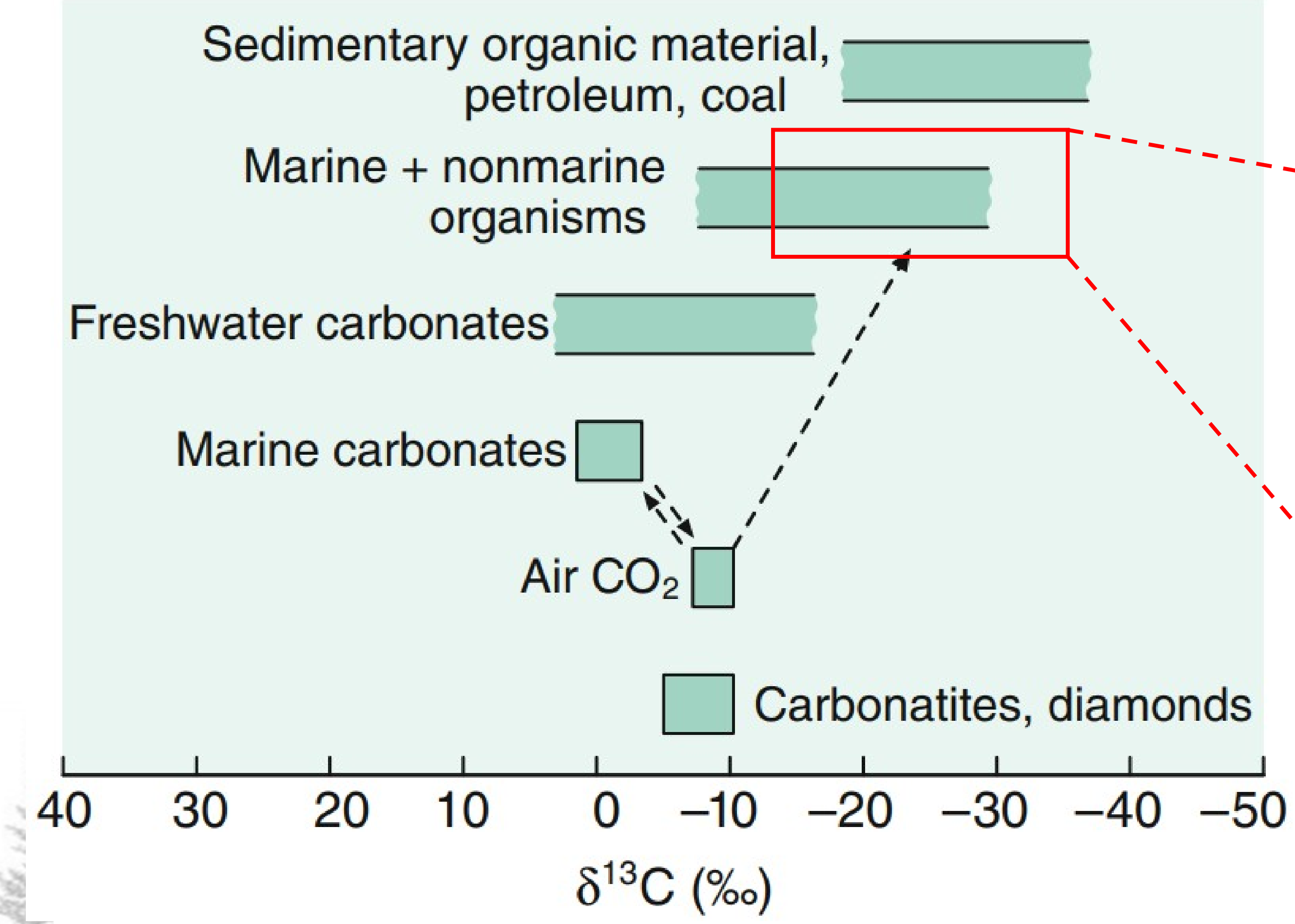
Element	Standard	Ratios
H	(V)SMOW – standard mean ocean water	$D/H = 1{,}5576 \cdot 10^{-4}$
O	(V)SMOW (in water), (V)PDB (in carbonates)	$^{18}O/^{16}O_{VSMOW} = 2{,}0052 \cdot 10^{-3}$ $^{18}O/^{16}O_{VPDB} = 2{,}0672 \cdot 10^{-3}$
C	(V)PDB – carbonate shells of Belemnitella americana from the PeeDee Formation in South Carolina (Cretaceous period)	$^{13}C/^{12}C = 1{,}1237 \cdot 10^{-2}$
N	Atmospheric nitrogen	$^{15}N/^{14}N = 3{,}6764 \cdot 10^{-3}$

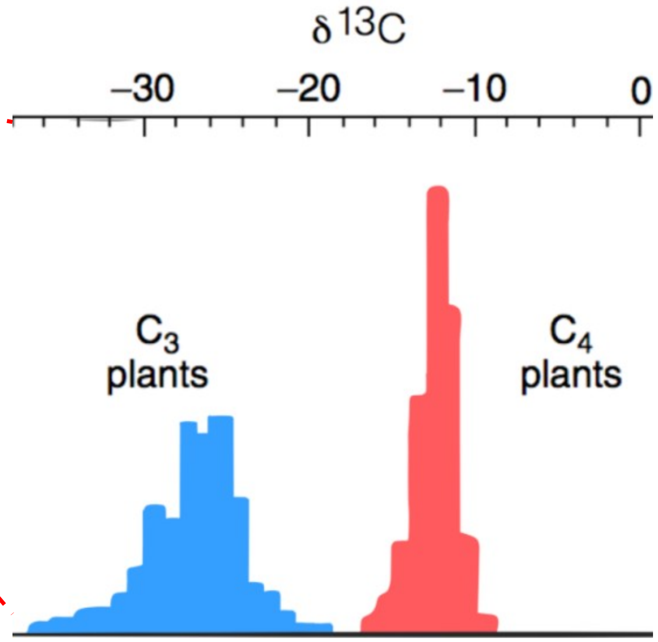
Stable isotopes distribution: reasons and isoscapes

Stable isotopes of carbon: natural range of values

Carbonates are the most **enriched** in ^{13}C ($\delta^{13}C$ values up to 20‰)

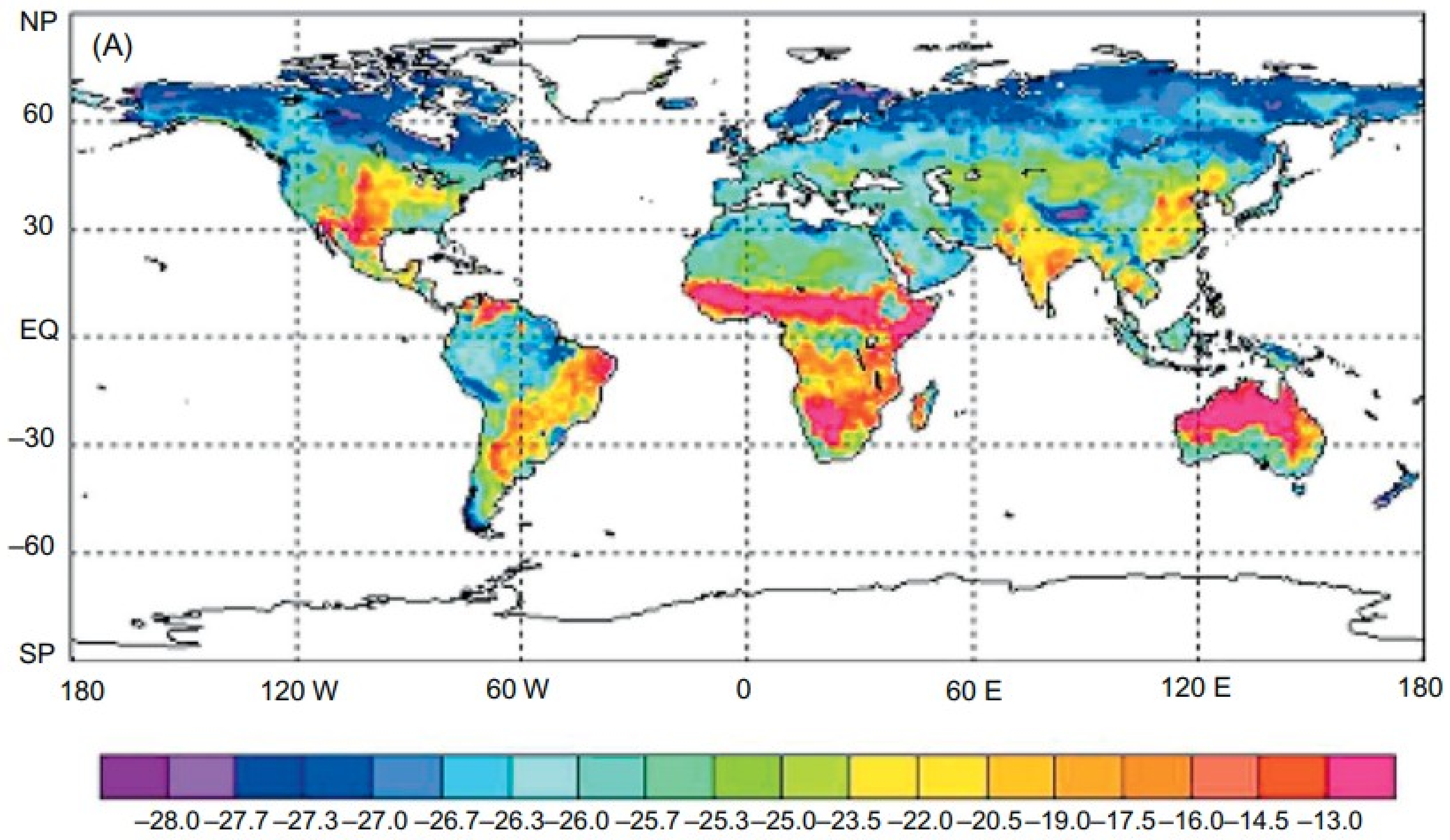
Biogenic methane is the most **depleted** in ^{13}C ($\delta^{13}C$ values up to -110‰)





(Hoefs, 2015)

Stable isotope composition of carbon: global distribution of values in plants



(Bowen and West, 2019)

Stable isotope composition of carbon: fractionation in living organisms

- ✓ Proteins and nucleic acids are slightly depleted in ^{13}C compared to carbohydrates ($\Delta \approx -1‰$)

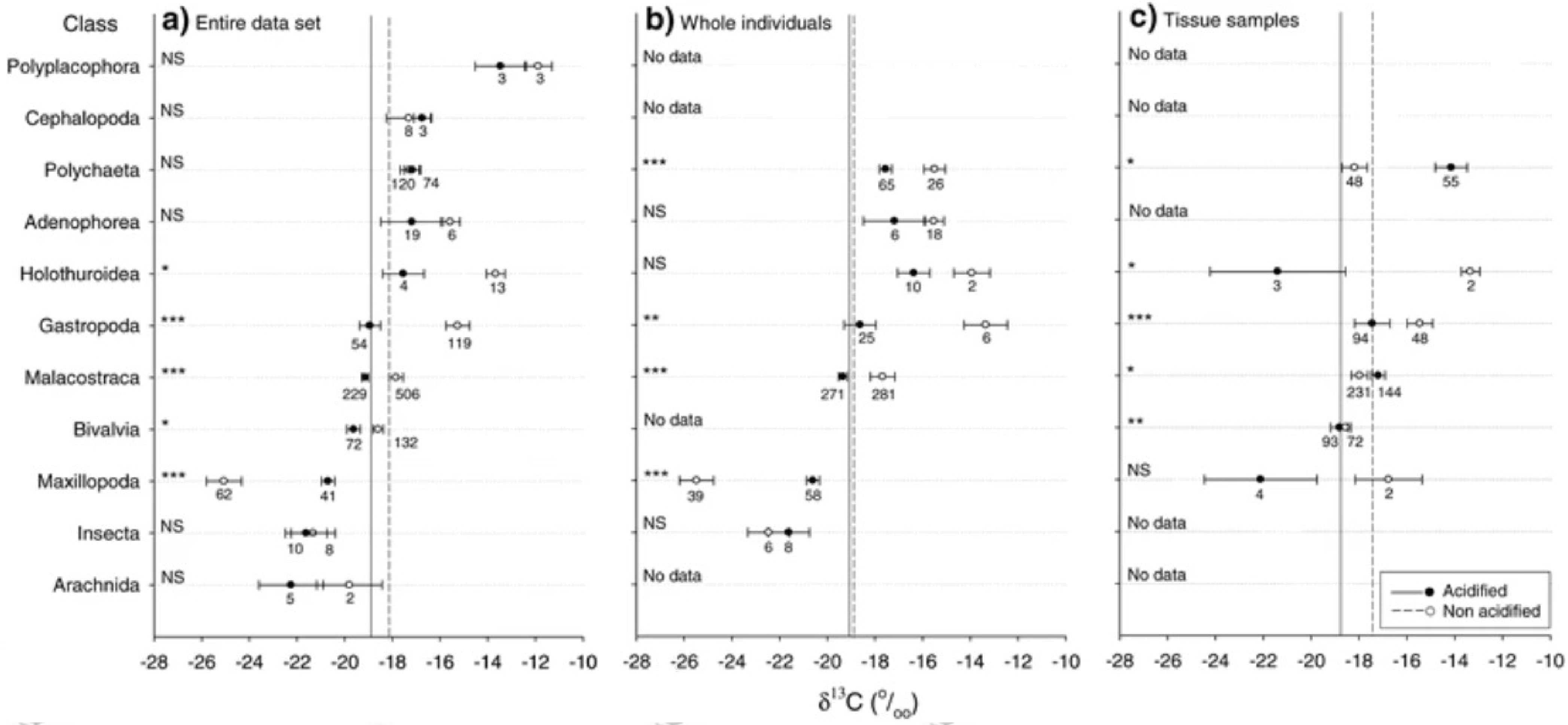
- ✓ Lipids are depleted in ^{13}C compared to carbohydrates ($\Delta \approx -5$ to $-8‰$).

~ Carbon in positions where it is bonded to hydrogen is depleted in ^{13}C; in positions where it is bonded to oxygen, it is enriched in ^{13}C.

«You are what you eat (plus a few per mil)»

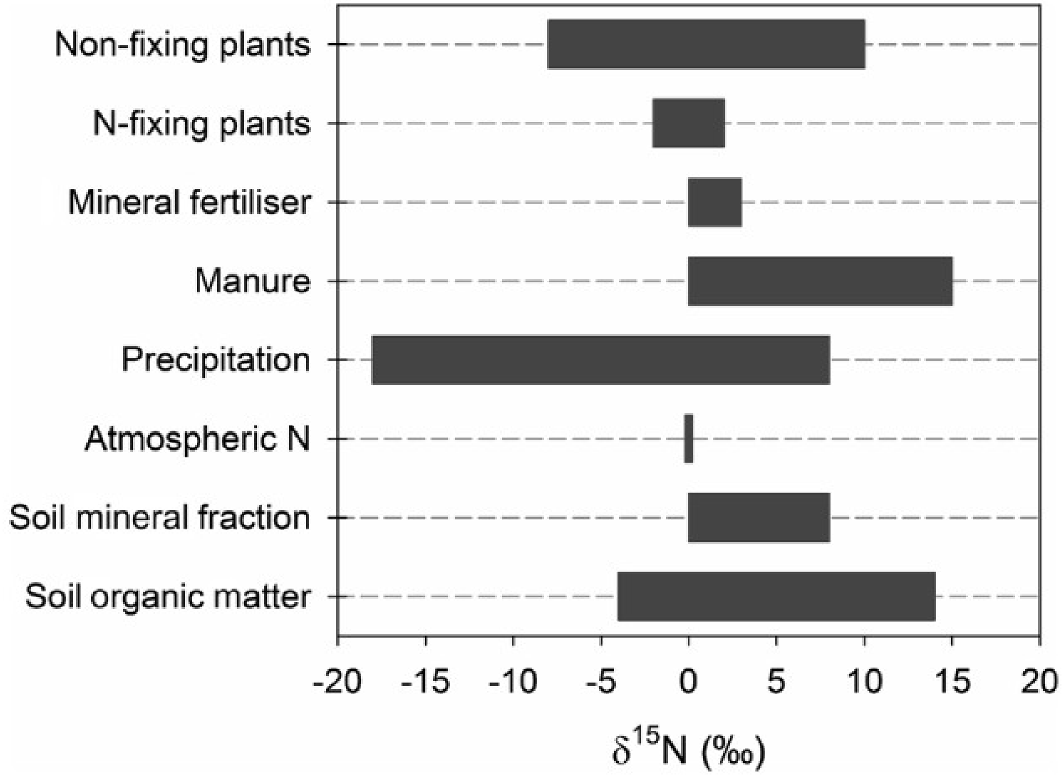
(*DeNiro and Epstein*, 1976)

Stable isotope composition of carbon: decarbonization of samples

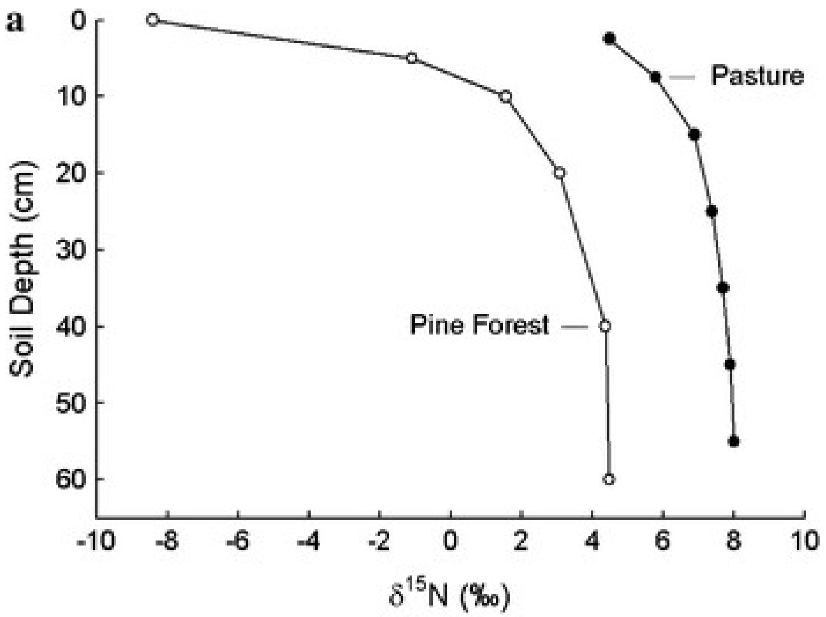


(*Mateo et al., 2008*)

N_2 in the atmosphere is in equilibrium with the dissolved N_2 in the ocean – together, these two pools account for up to 99% of the total nitrogen on the Earth.

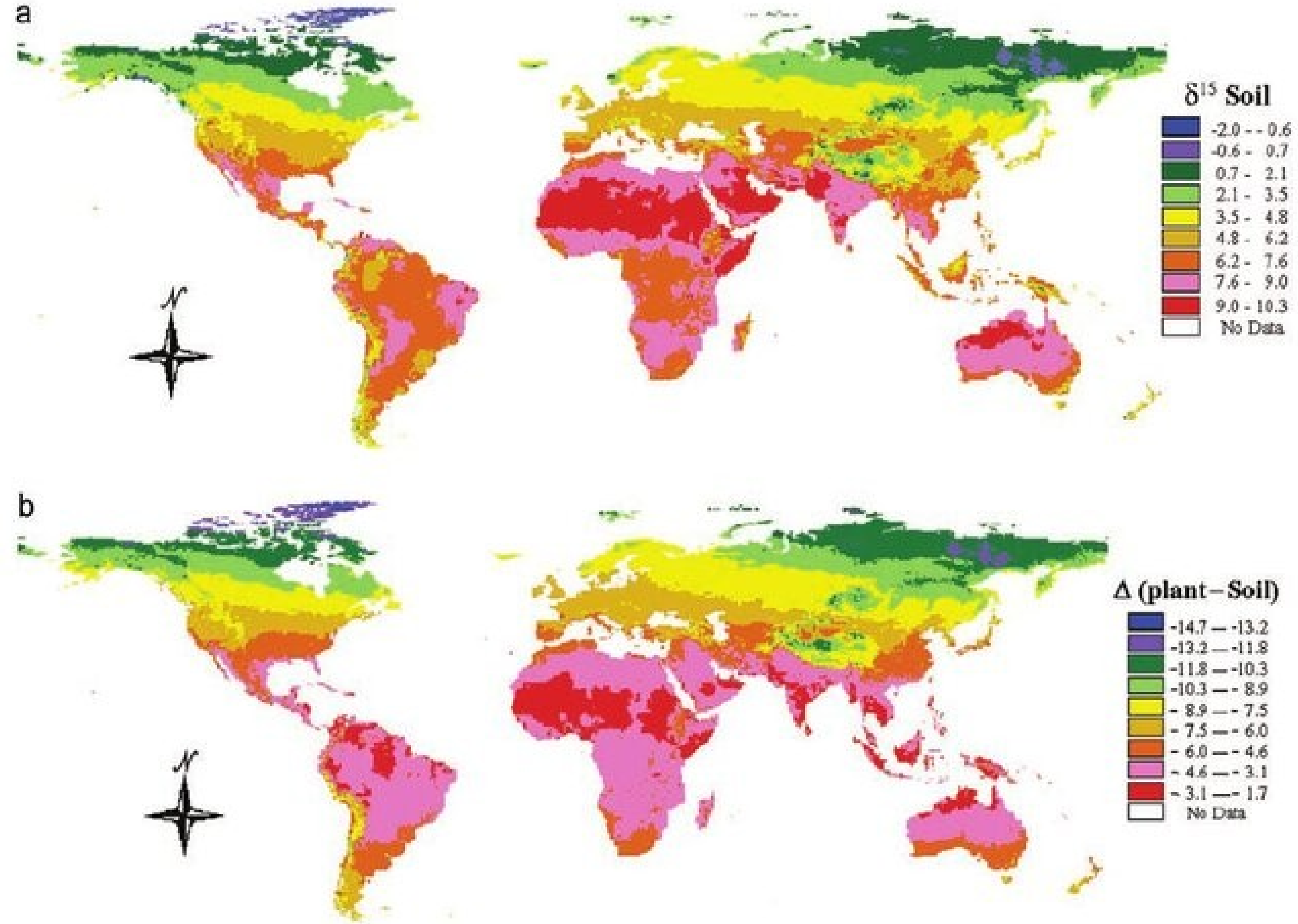


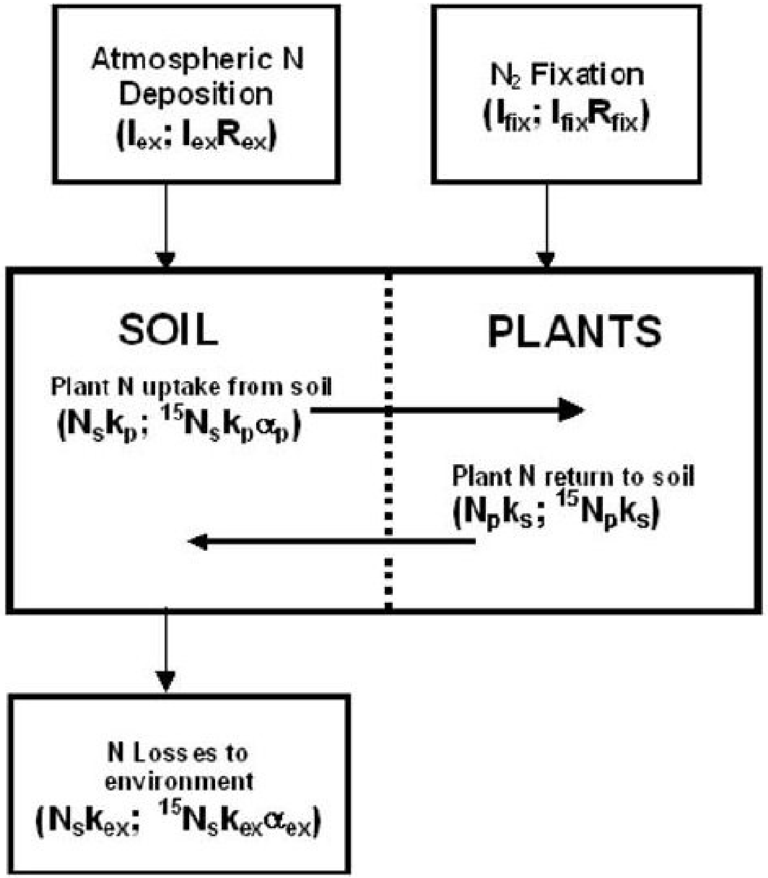
(*Fiorentino et al., 2014*)



(*Hobbie and Ouimette, 2009*)

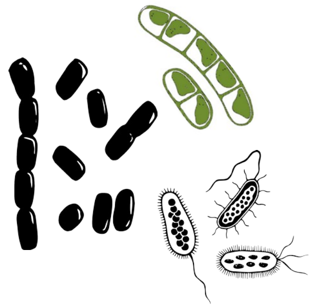
Stable isotope composition of nitrogen: global distribution of values





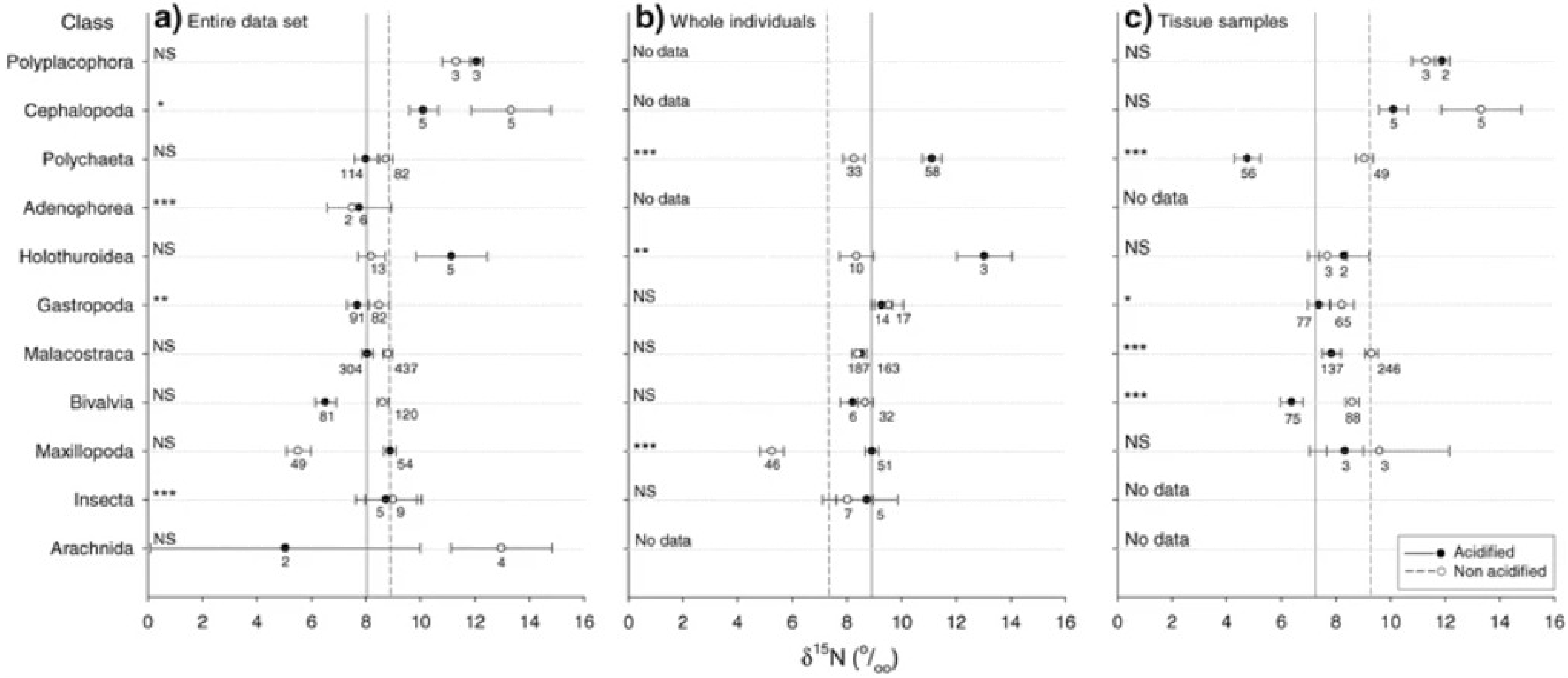
(Amundson et al., 2003)

Introduction



Processes		Δ, ‰
Nitrogen biogenic fixation	N2 → Norg	-3 to +2
NH_4^+ assimilation	NH_4^+ → Norg	-9 to -27
NO_3^- assimilation	NO_3^- → Norg	0 to -19
Ammonification	Norg → NH4+	0 to -5

Stable isotope composition of nitrogen: decarbonization of samples



(*Mateo et al., 2008*)

Trophic fractionation of ^{13}C and ^{15}N in soil food webs

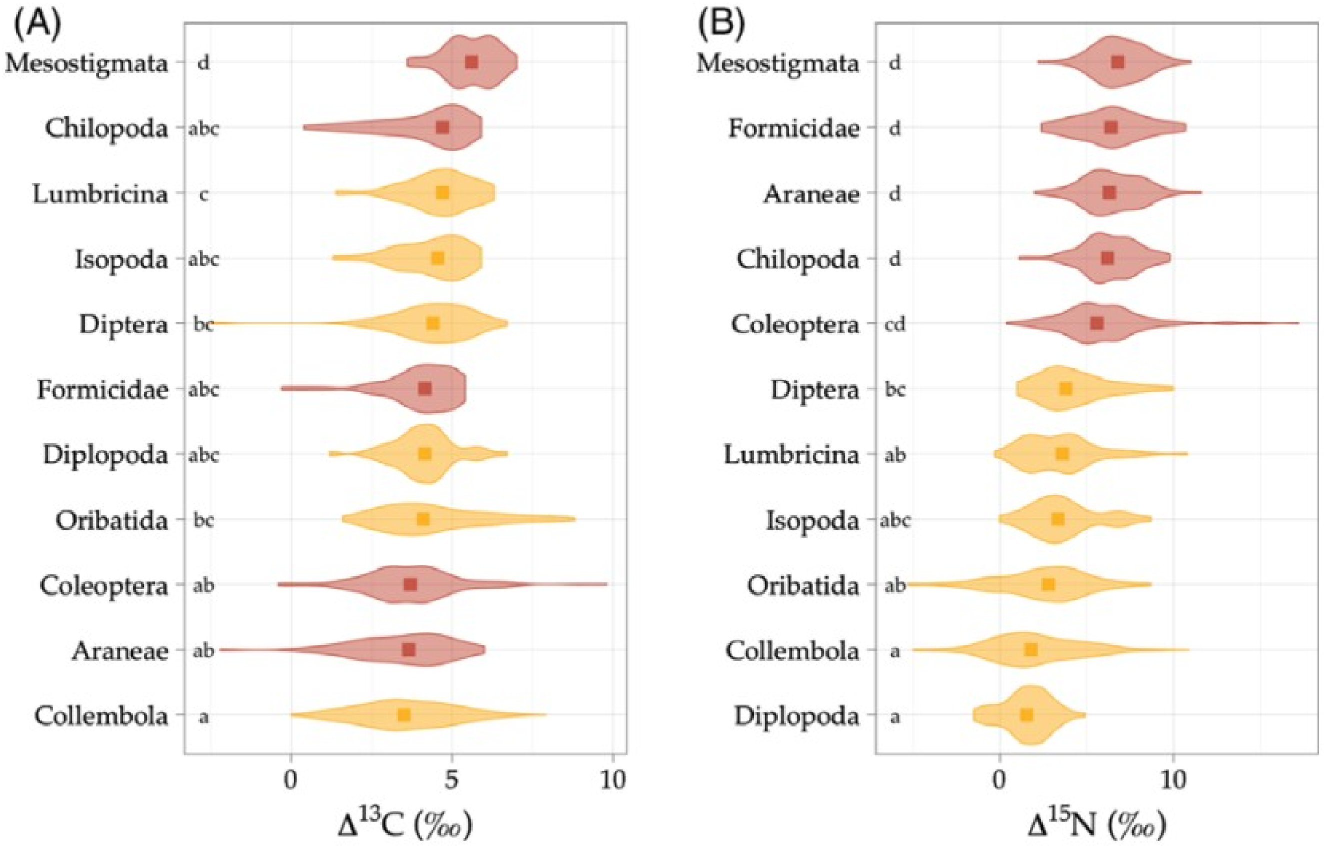
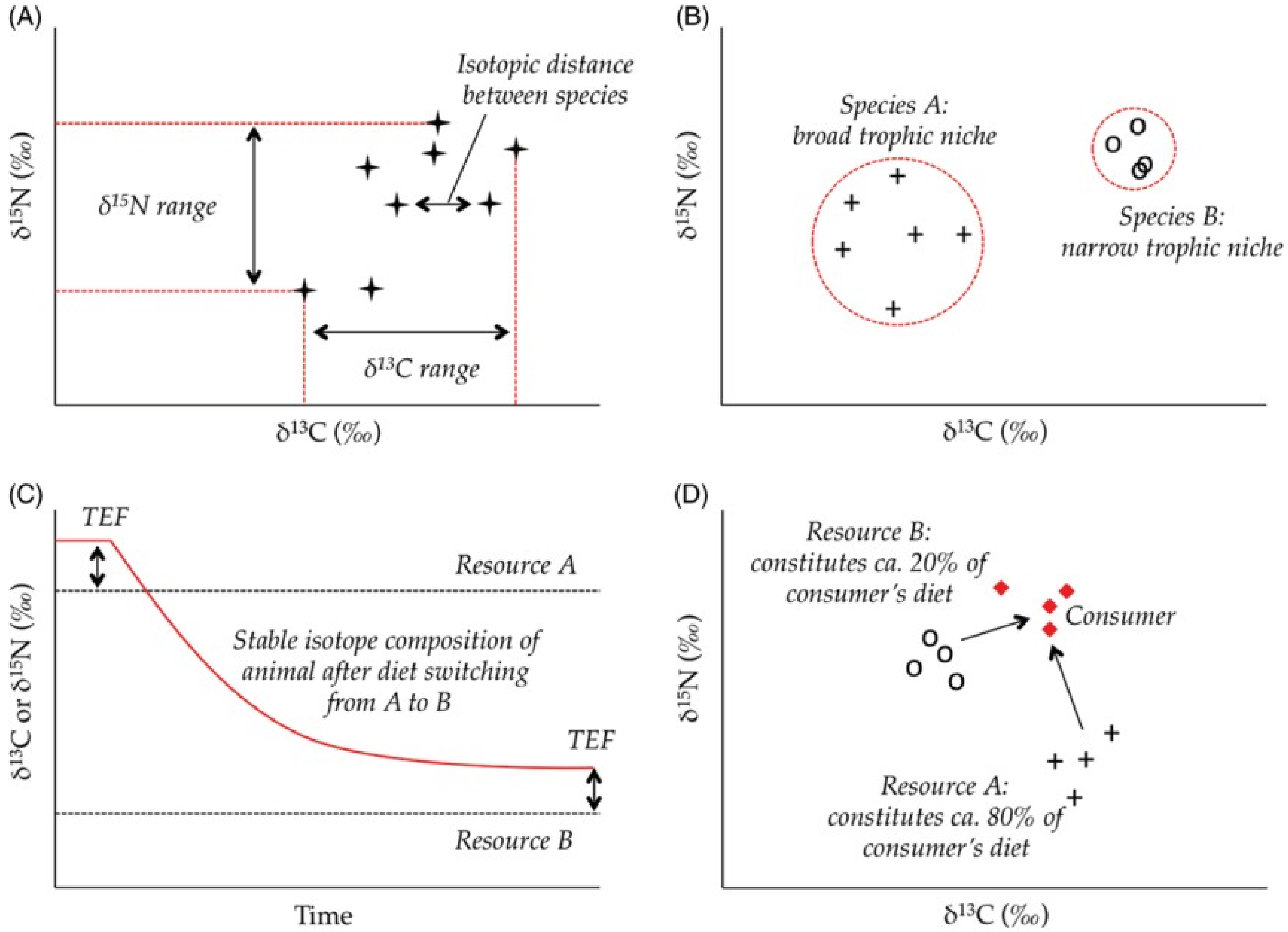


Fig. 7. Ranges of stable isotope values of soil animal groups of high taxonomic rank in temperate forests. Litter-normalized $\Delta^{13}C$ (A) and $\Delta^{15}N$ values (B) are presented as violin plots (mirrored kernel density estimation) and medians. Only groups represented by more than 30 data records are shown. Mean $\Delta^{13}C$ and $\Delta^{15}N$ values for the groups sharing the same letter are not significantly different (pairwise comparisons using Nemenyi-test with Chi-squared approximation performed in the *PMCMR* package in R). Groups were *a priori* assigned to decomposers (yellow) or predators (brown).

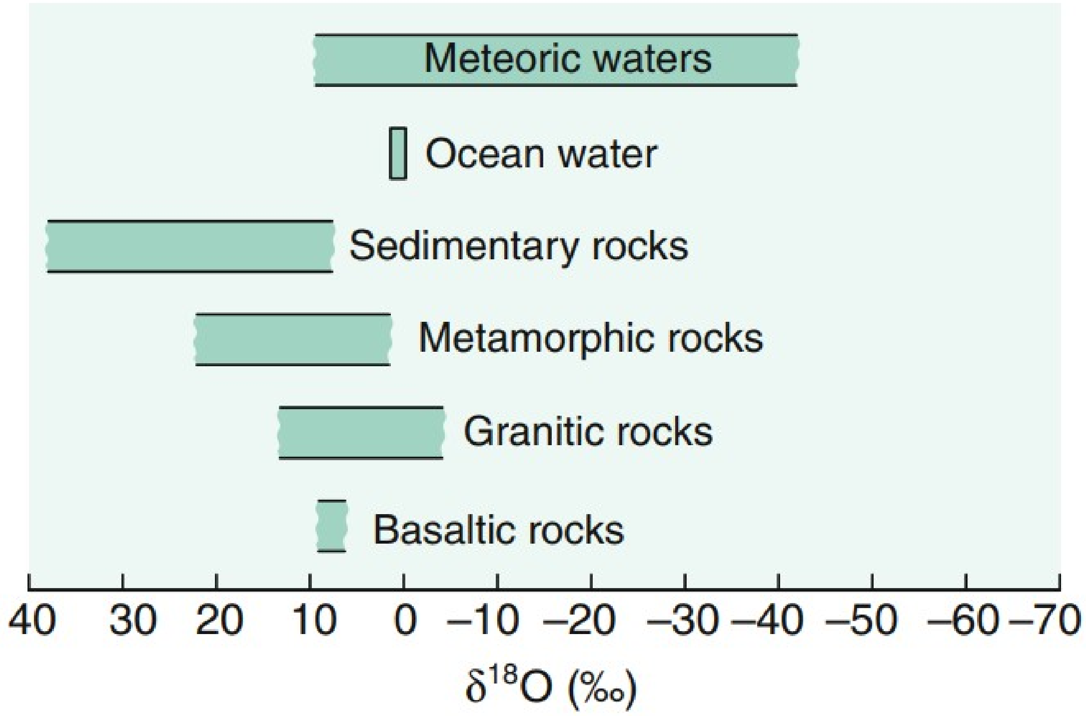
(Potapov, Tiunov and Scheu, 2019)

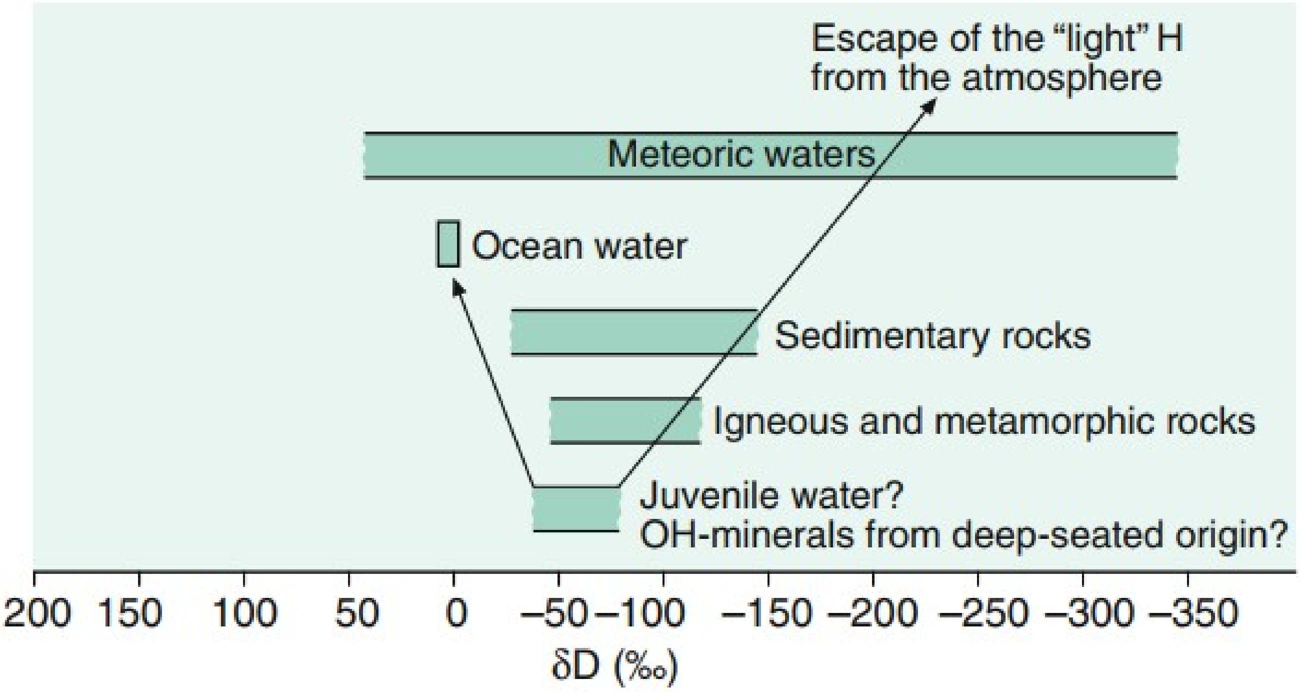
Basic principles for interpreting stable isotope ($\delta^{13}C$, $\delta^{15}N$) bi-plots



(Potapov, Tiunov and Scheu, 2019)

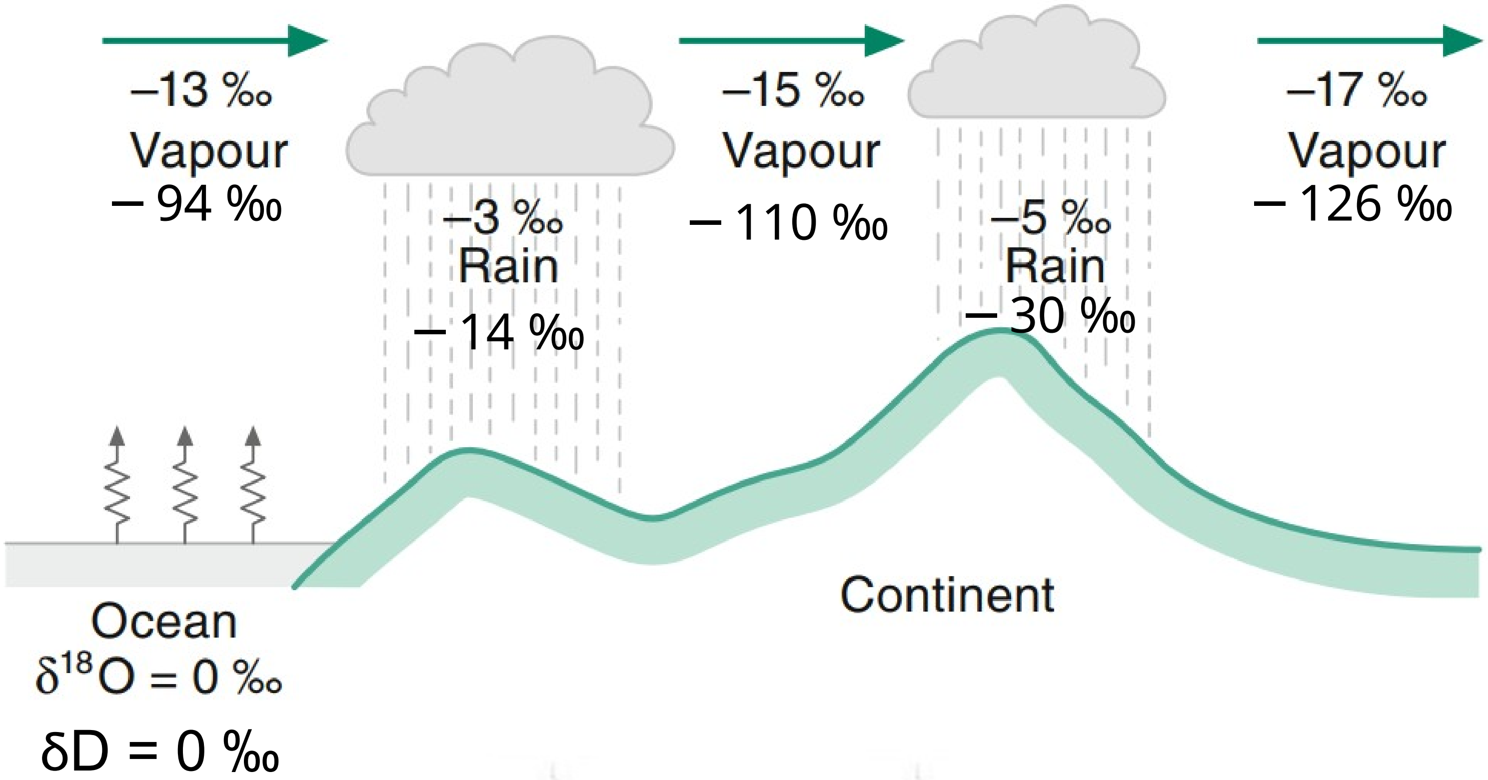
- ✓ ^{18}O is more common — studies mainly use the $\delta^{18}O$ value
- ✓ Ocean water has more ^{2}H (D) than most objects on Earth, so the δD values are commonly negative





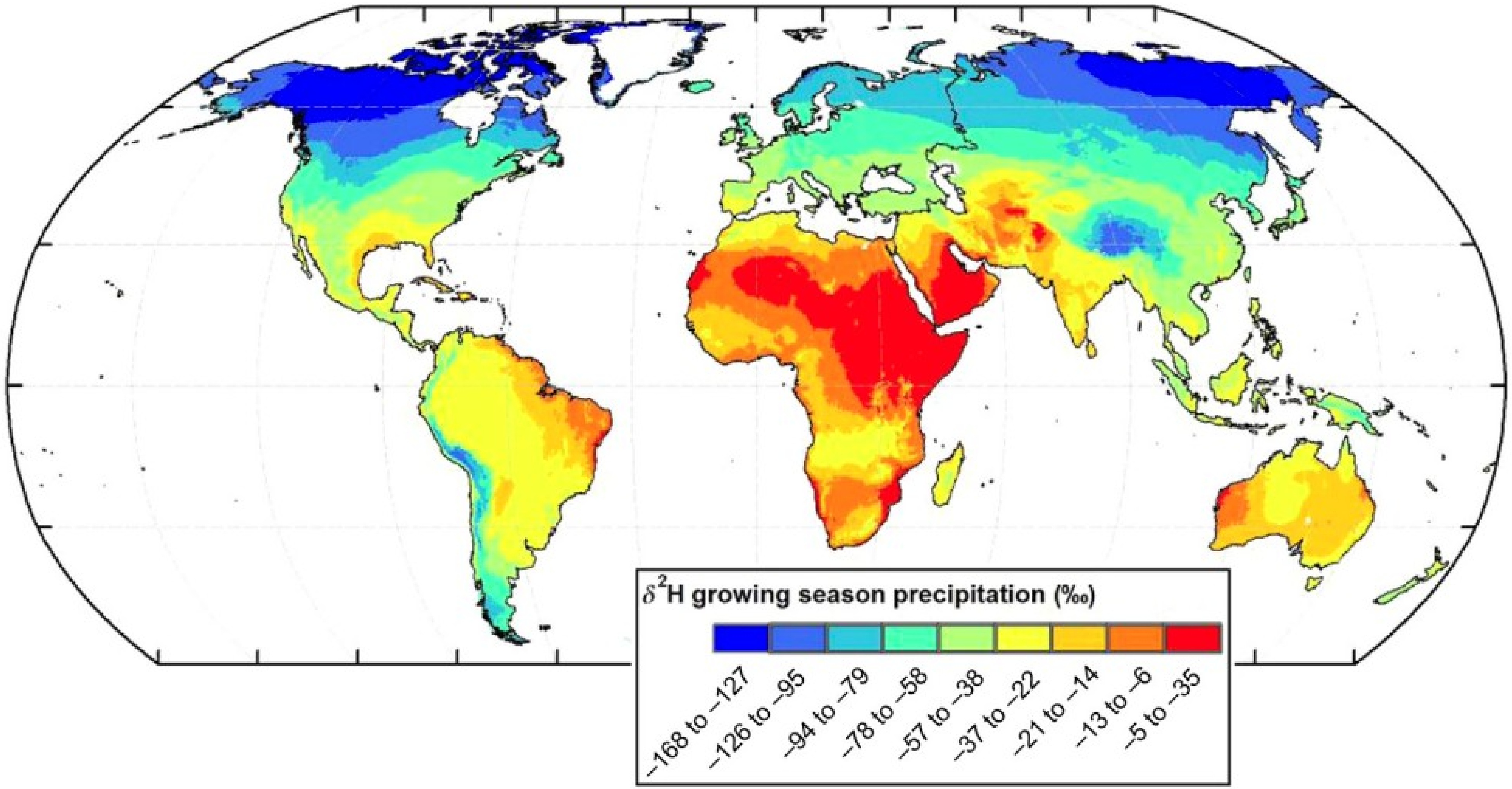
(Hoefs, 2015)

Stable isotopes of hydrogen and oxygen: rainfall-fractionation



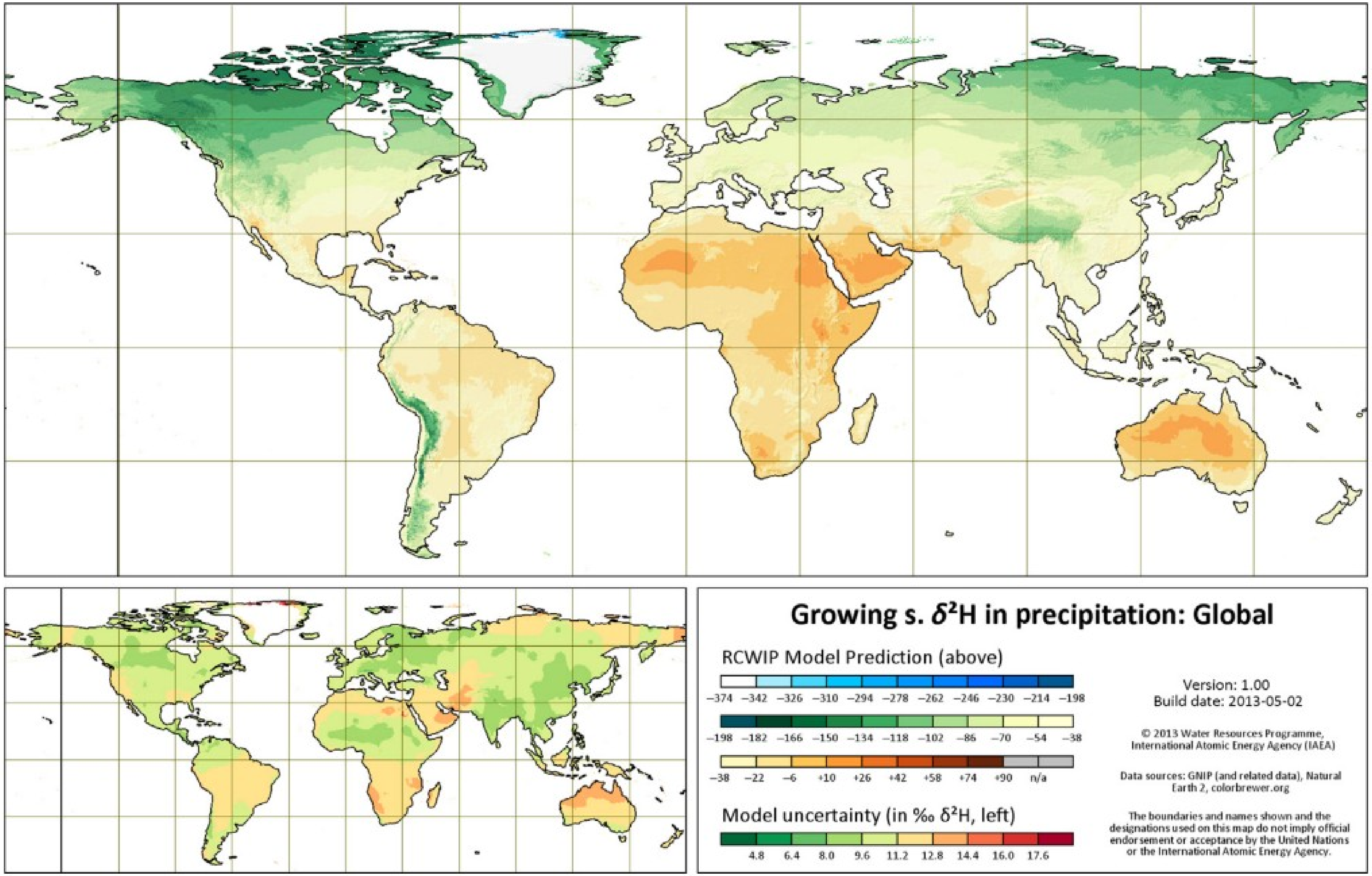
(foll. Hoefs, 2015)

Stable isotopes of hydrogen: global water isoscapes



(Bowen and West, 2019)

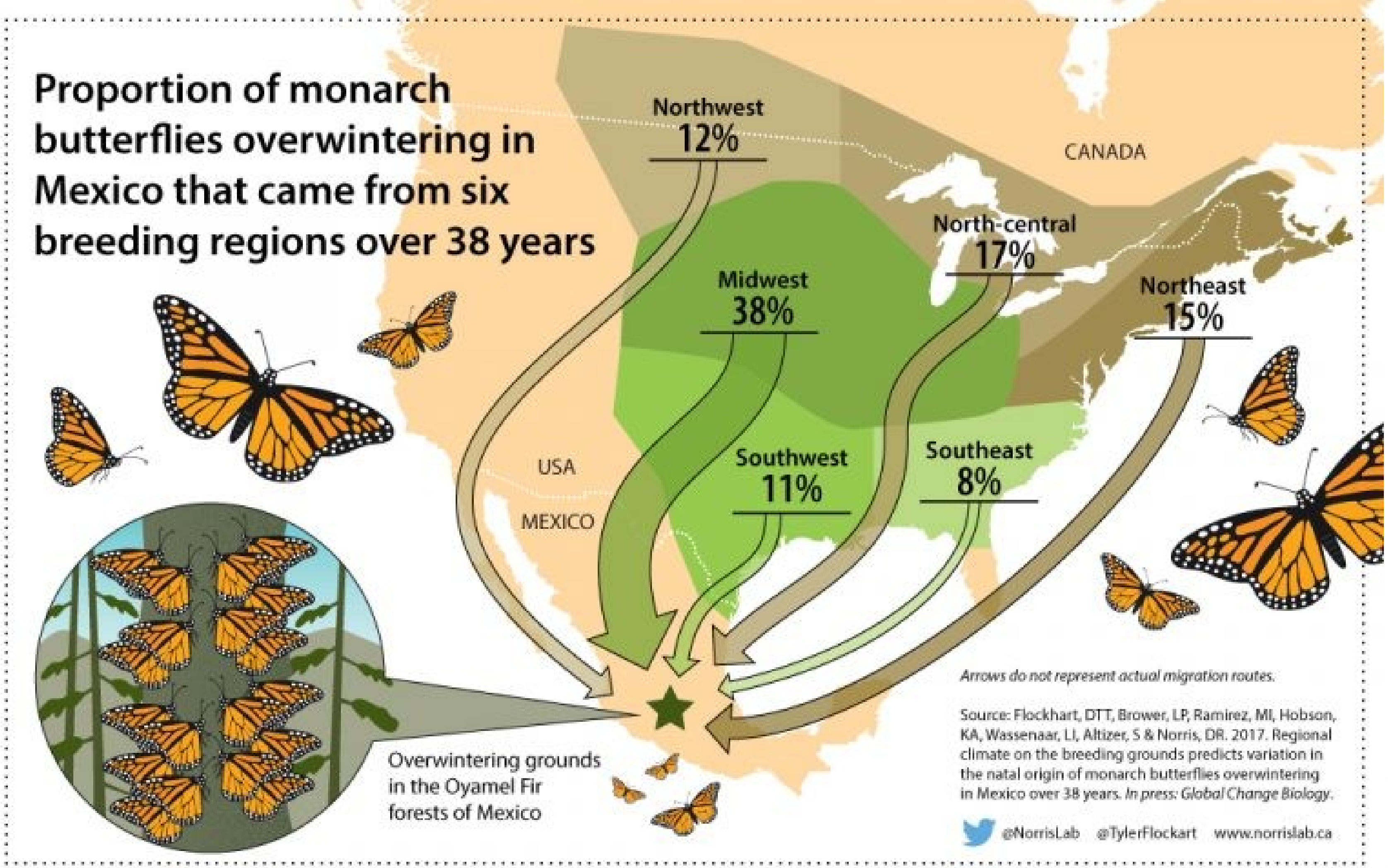
Stable isotopes of hydrogen: global changes in δ^2H of precipitation



(Bowen and West, 2019)

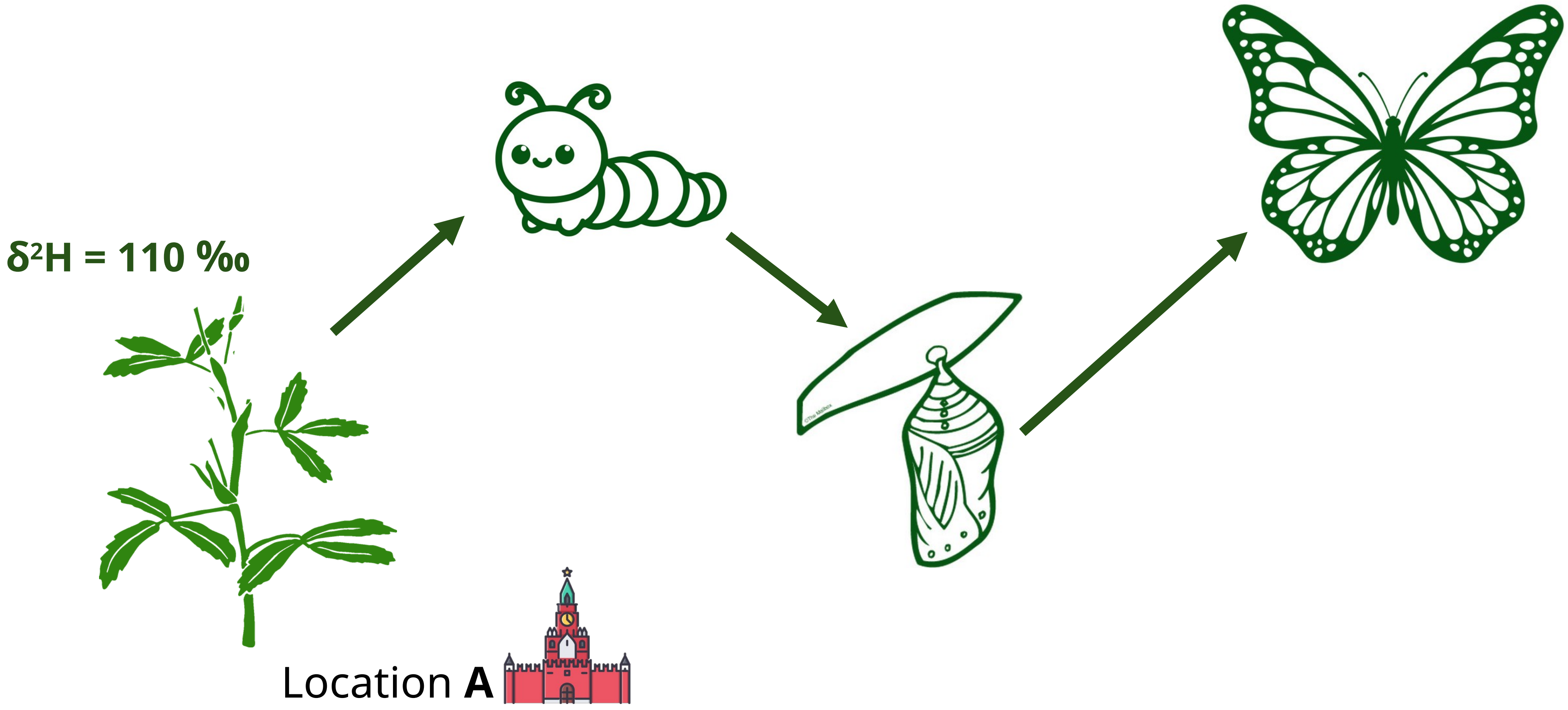
Stable isotopes as predictors of invertebrates' origin

Origin and migration tracking: monarch butterflies studies



(*Flockhart et al., 2017*)

Origin and migration tracking: origins of origins



(foll. Miller, 1994; Schimmelmann et al., 1993; Hobson et al., 1999)

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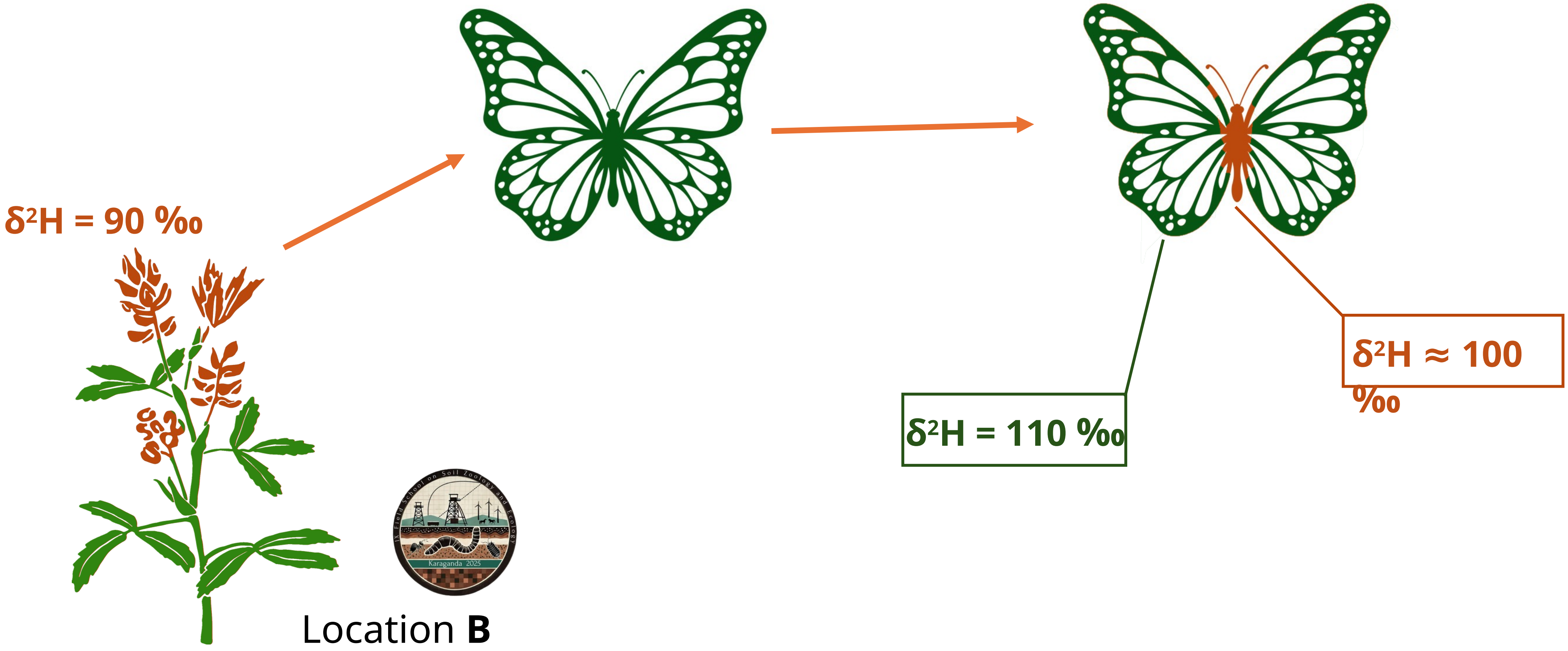

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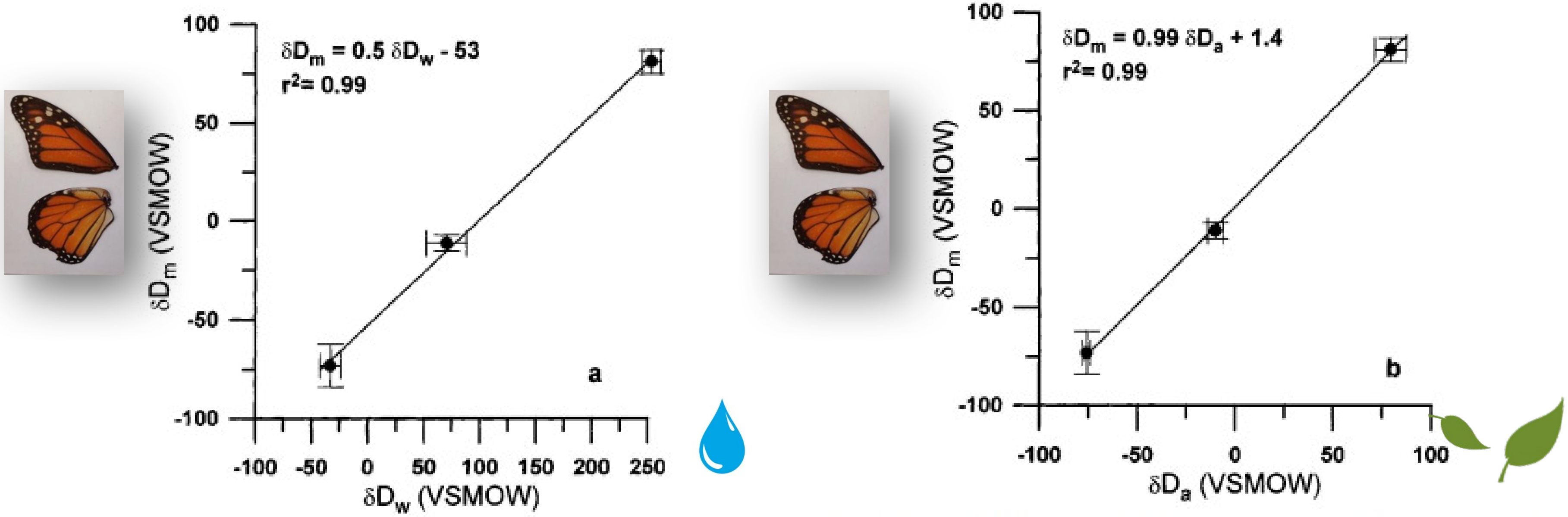
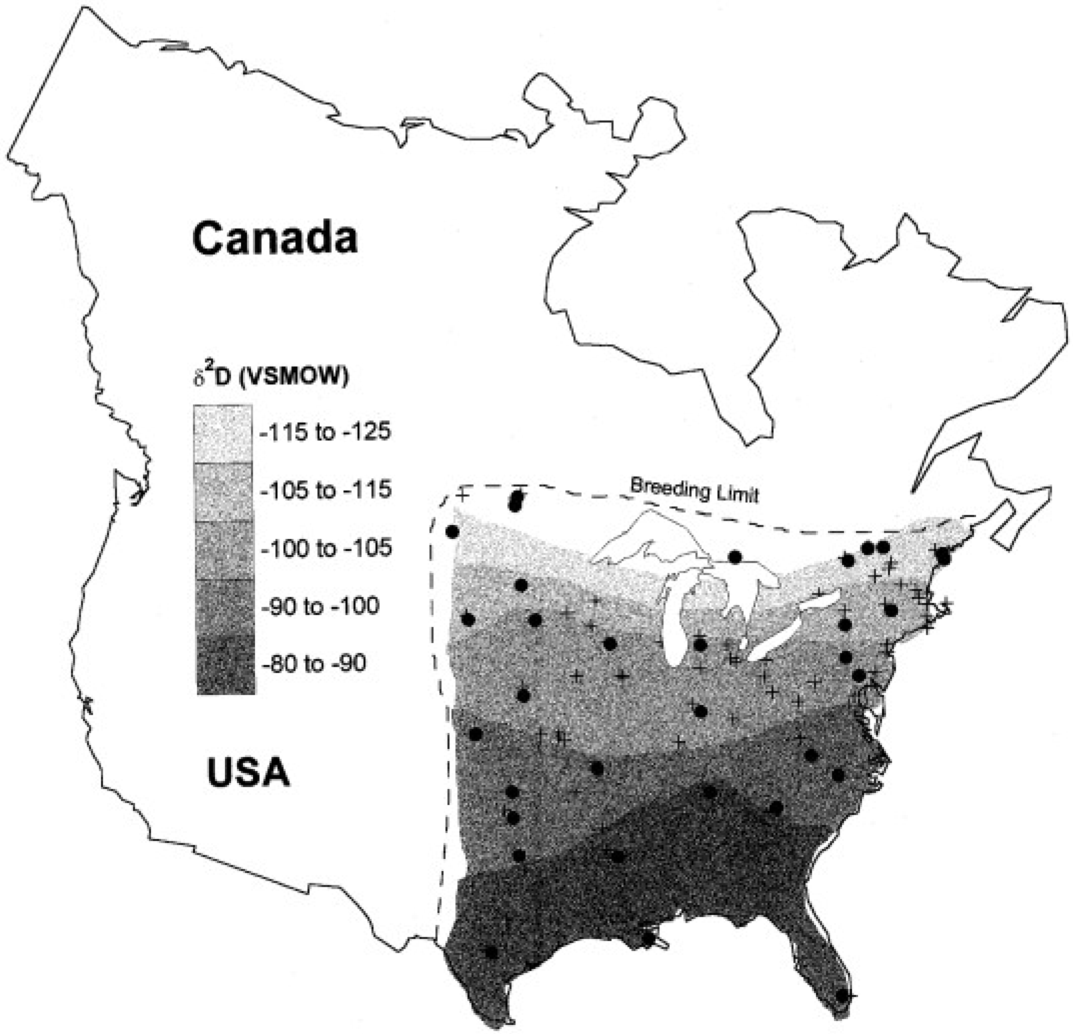
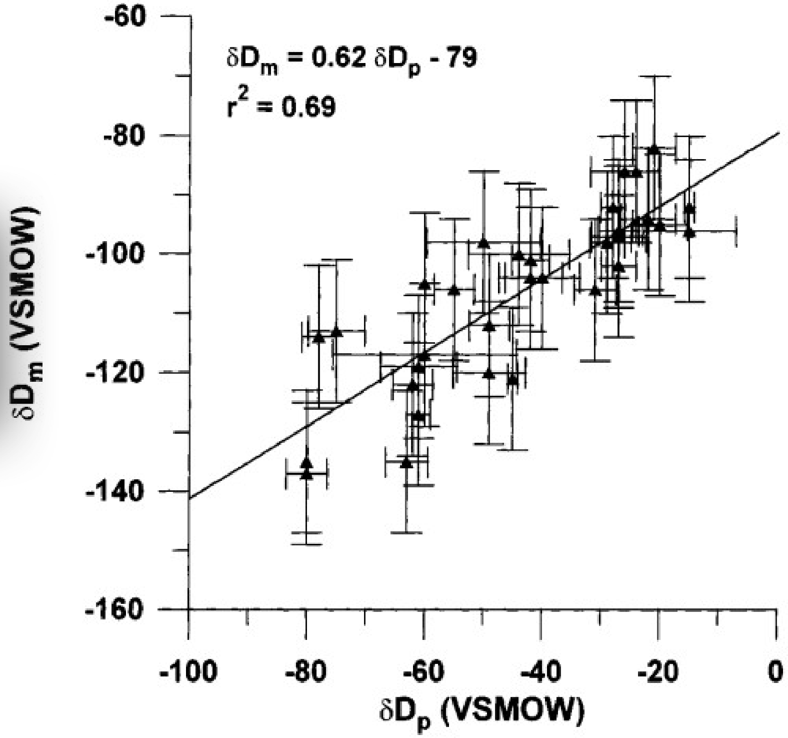


Fig. 1 Relationship between δD values of wings of laboratory-reared monarchs (δD_m) and those of **a** growth water (δD_w) used to raise the larval host plant (*A. curassavica*), and **b** the larval host plant (δD_a). Data from Table 1

(Hobson et al., 1999, 2017)

Origin and migration tracking: monarch butterflies studies





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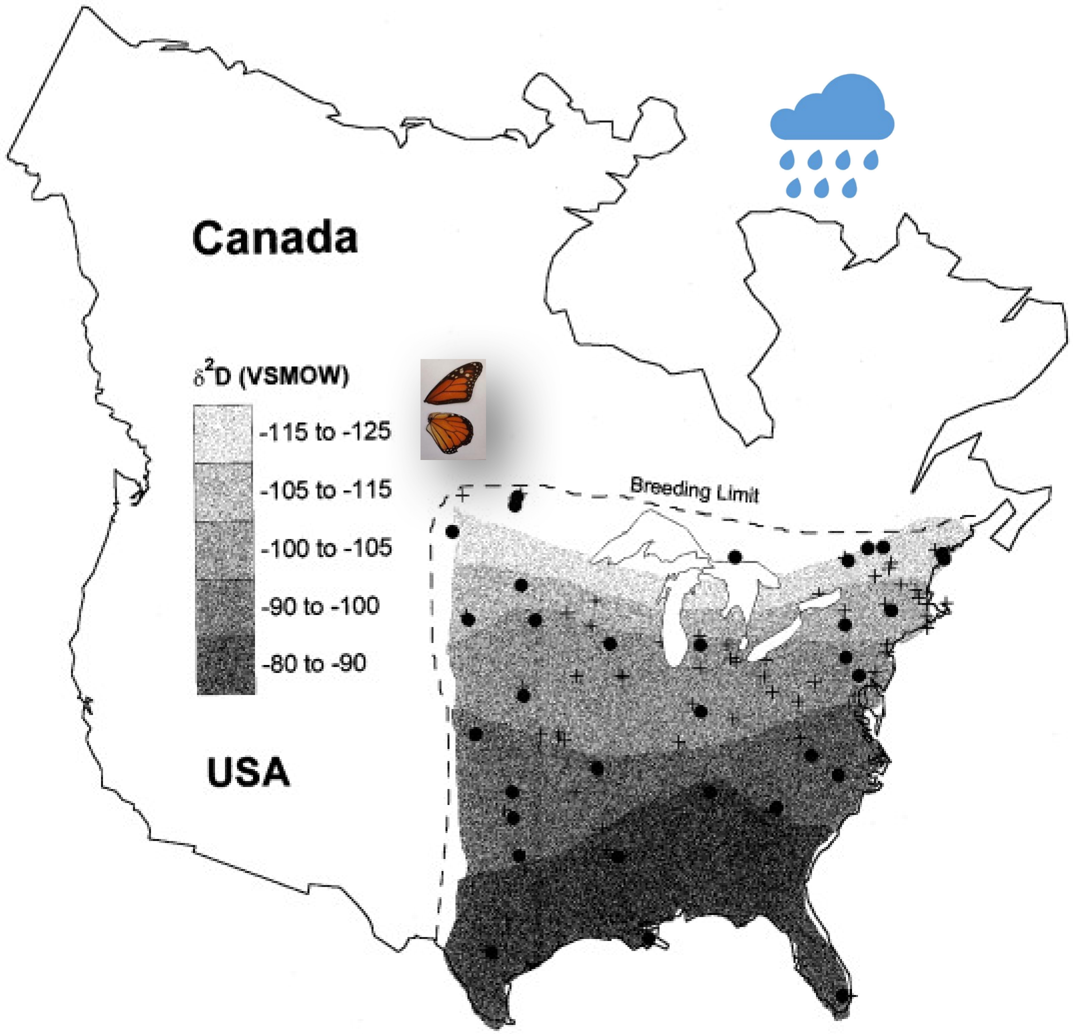
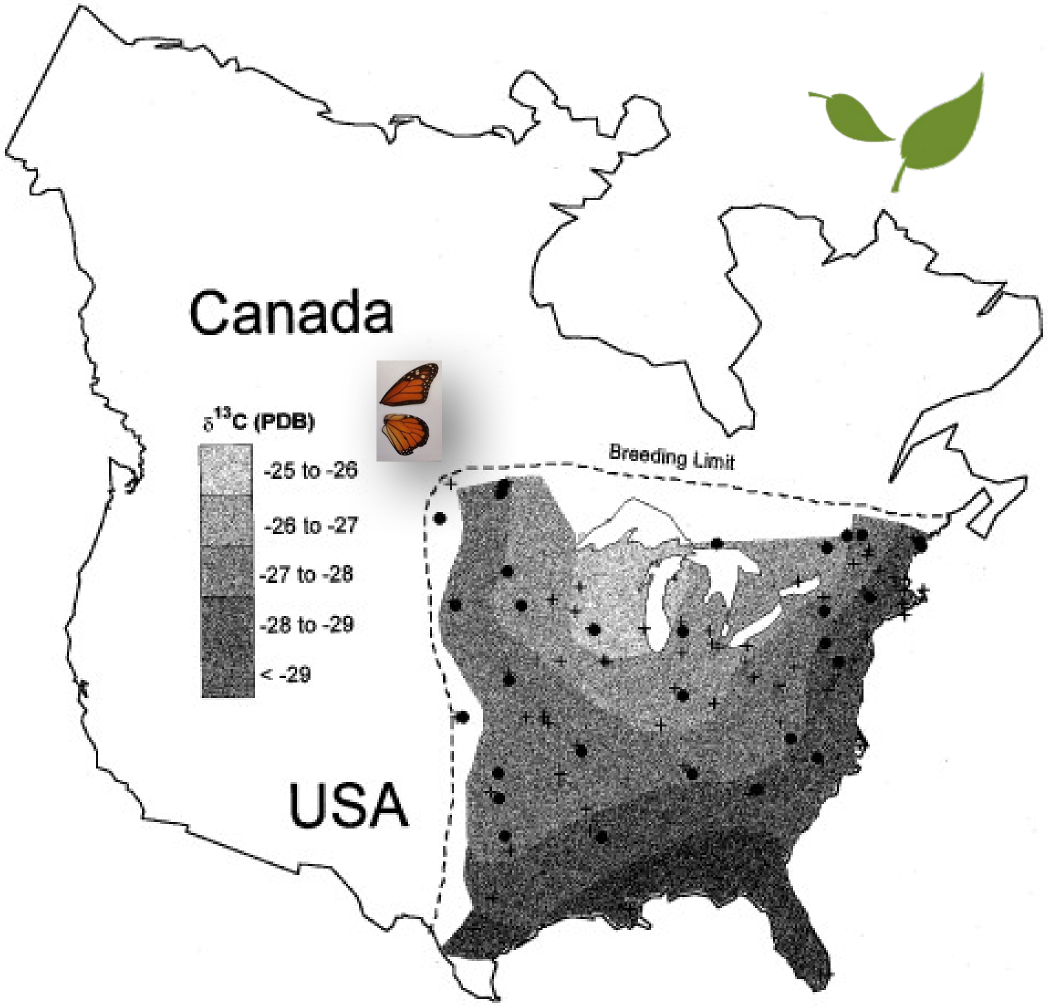
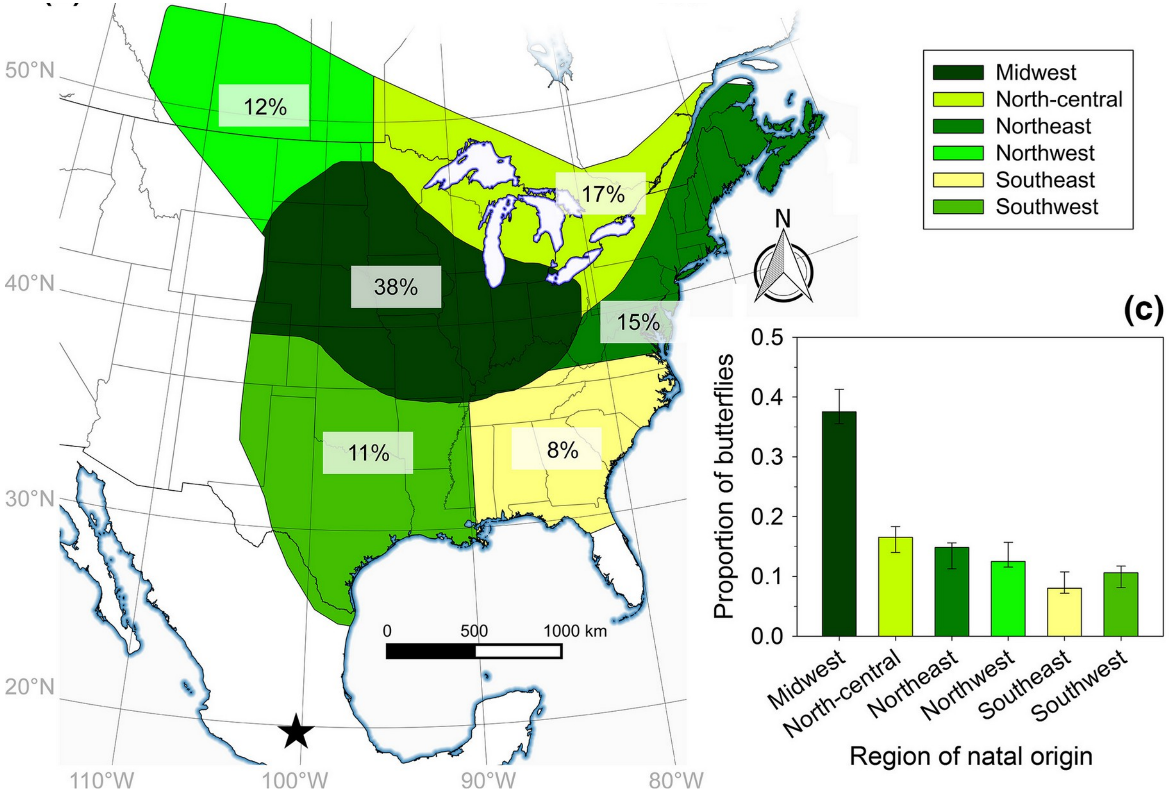




(Hobson et al., 1999)

Combination of stable isotope tracing with invertebrate morphology



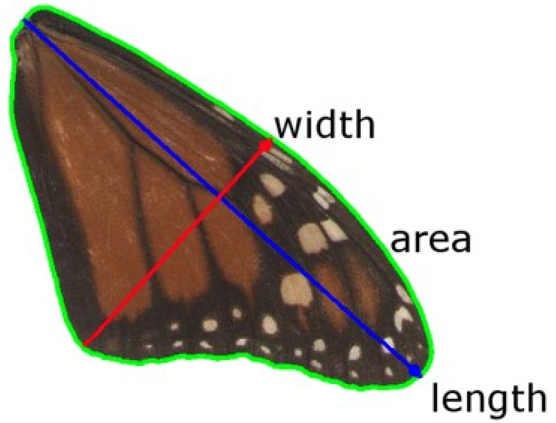


Table 1 Results of likelihood ratio tests used to test the effect of migration distance on wing morphology using the high point method

Response variable	χ^2	df	p	Distance parameter estimate	95% confidence interval
Wing Area	5.62	1	0.02	0.0124	0.0022, 0.0226
Wing Length	5.20	1	0.02	3.966e-4	5.577e-5, 7.374e-4
Roundness	1.99	1	0.159	3.084e-6	−1.21e-6, 7.38e-6
Aspect Ratio	0.19	1	0.659	1.89e-6	−6.52e-6, 1.03e-5

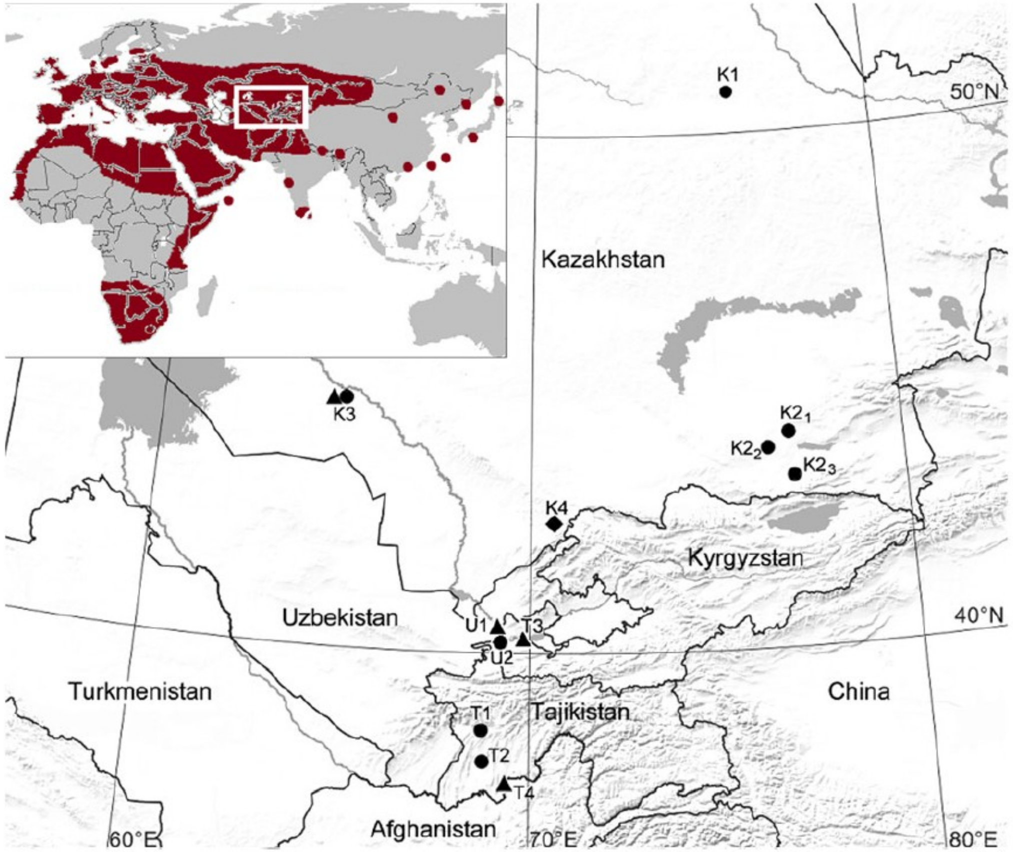
For each wing morphology response variable, a global linear mixed effects model was constructed with sex, mean maximum daily temperature and migration distance as fixed effects, and year as a random effect. Distance was then removed from each model and compared to the global model using a likelihood ratio test. The χ^2 test statistic was used to calculate the p-value for each likelihood ratio test

(Flockhart et al., 2017a,b)

Multi-biogenic isotopic approach

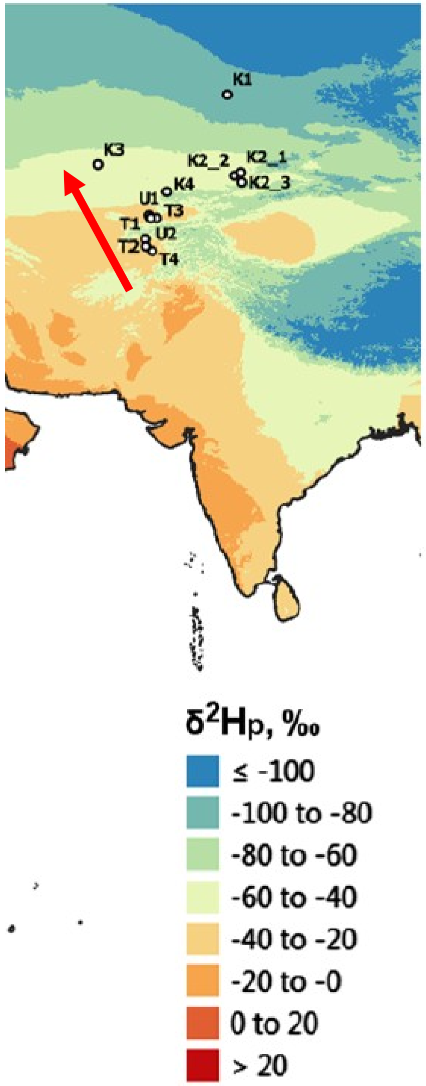
Origin and migration tracking: multi-isotope approaches

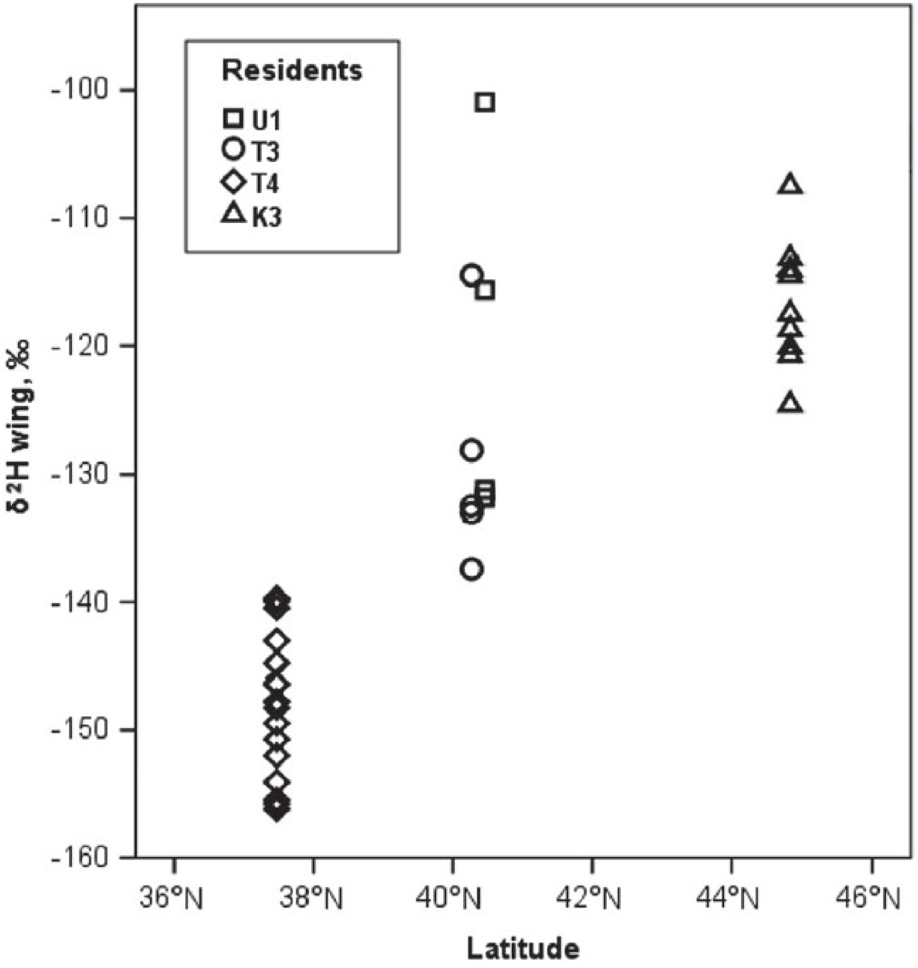
Fig 2. Specimens of *Sympetrum fonscolombii* of different cohorts. Immigrants are at the top, and residents are at the bottom (males are on the left, and females are on the right). [Colour figure can be viewed at wileyonlinelibrary.com].



(Borisov et al., 2020)

Origin and migration tracking: multi-isotope approaches





(*Borisov et al., 2020*)

Origin and migration tracking: multi-isotope approaches

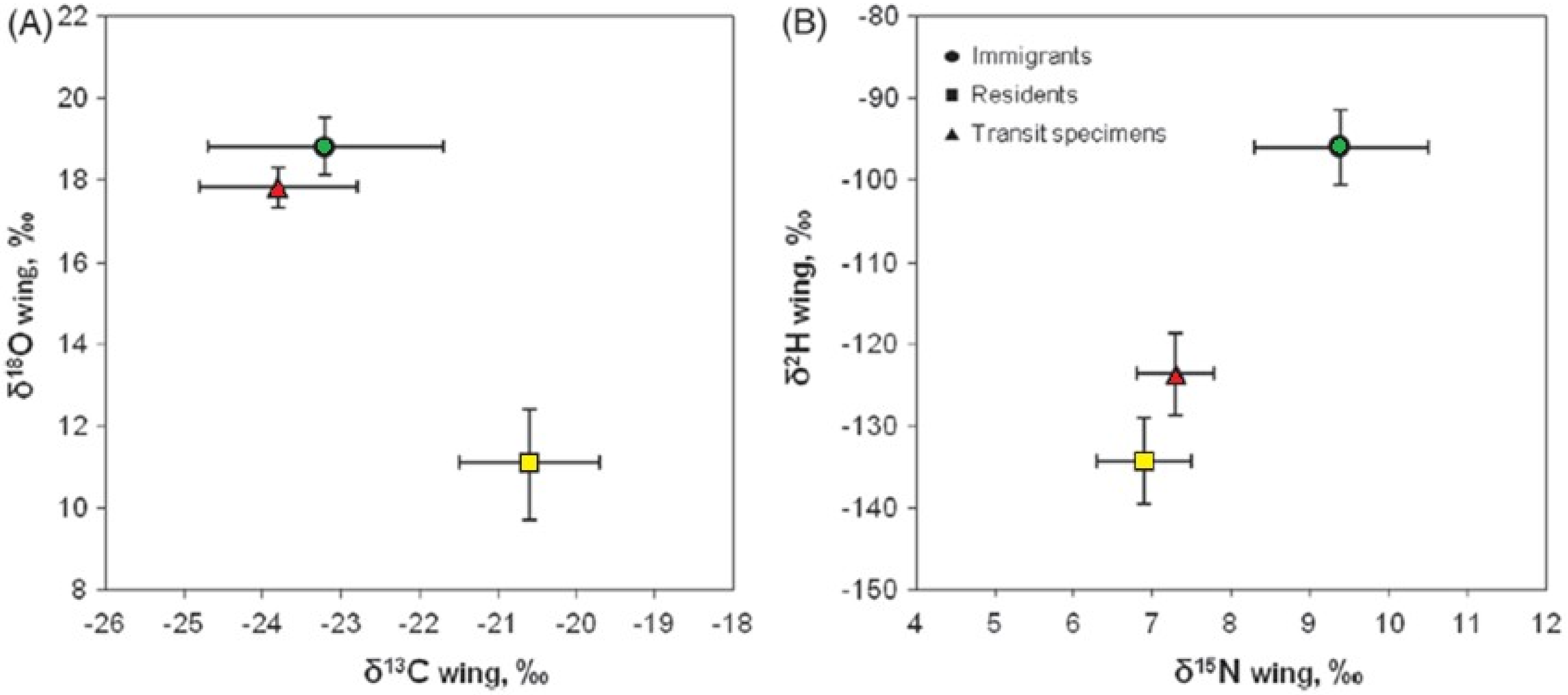
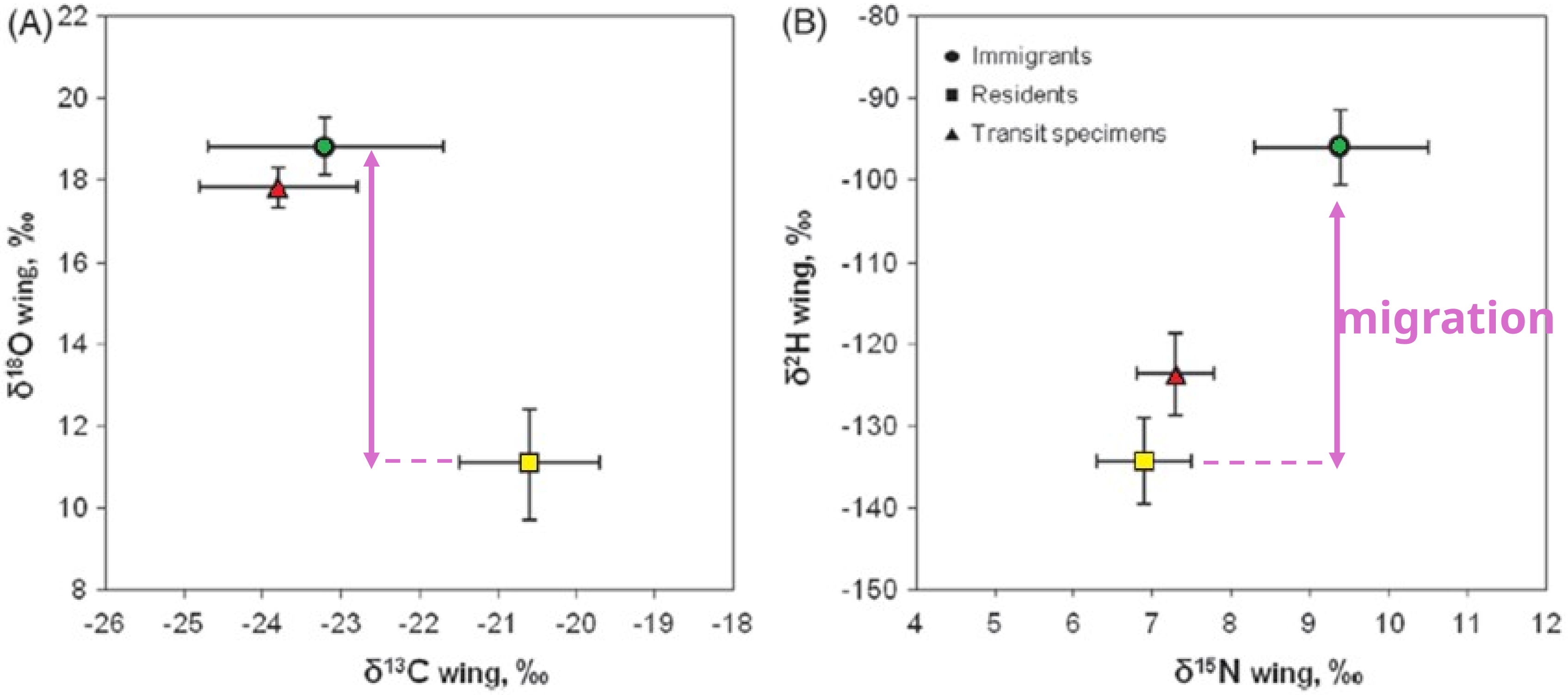


Fig 8. Multi-isotope signatures of *Sympetrum fonscolombii* cohorts: $\delta^{18}O$, $\delta^{13}C$ (a), $\delta^{2}H$, and $\delta^{15}N$ (b) values for wings. Means and 95% confidence intervals are shown.

(Borisov et al., 2020)

Origin and migration tracking: multi-isotope approaches



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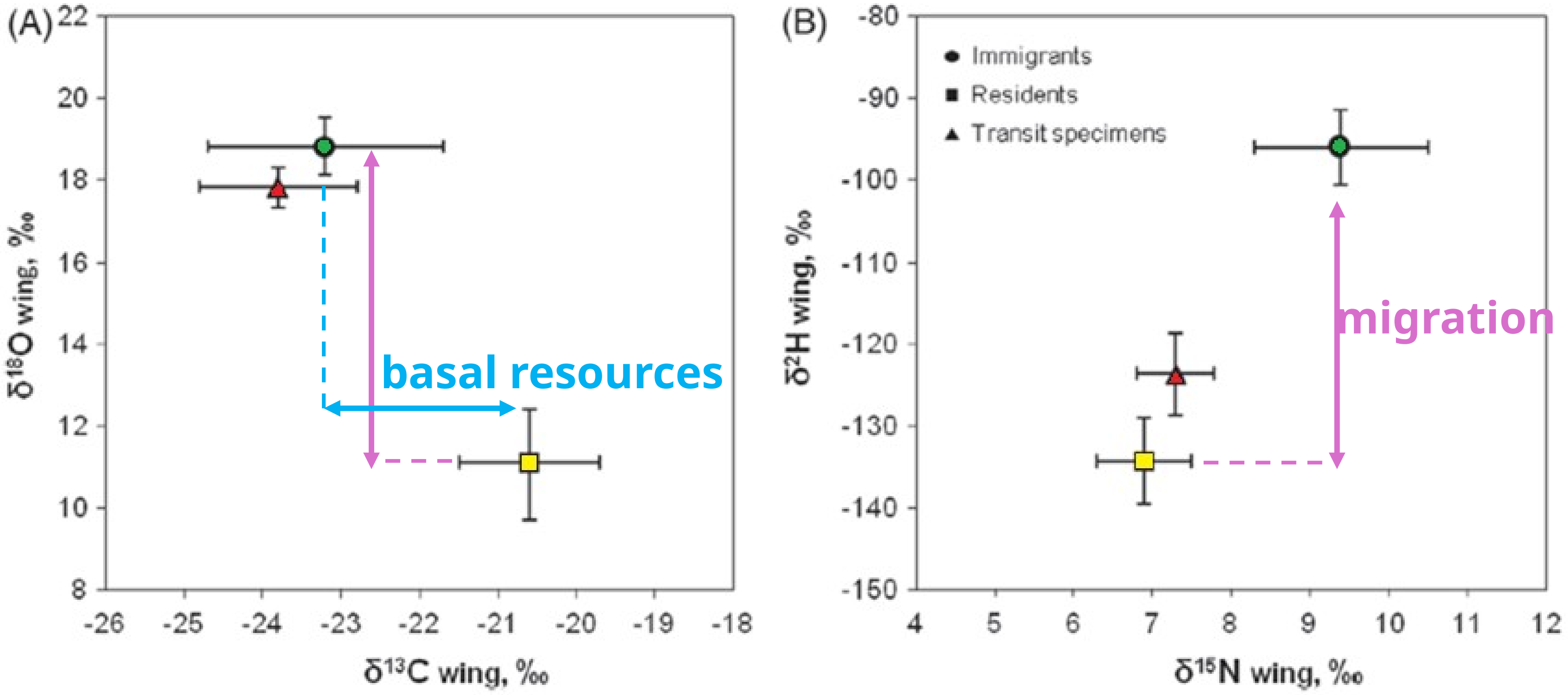

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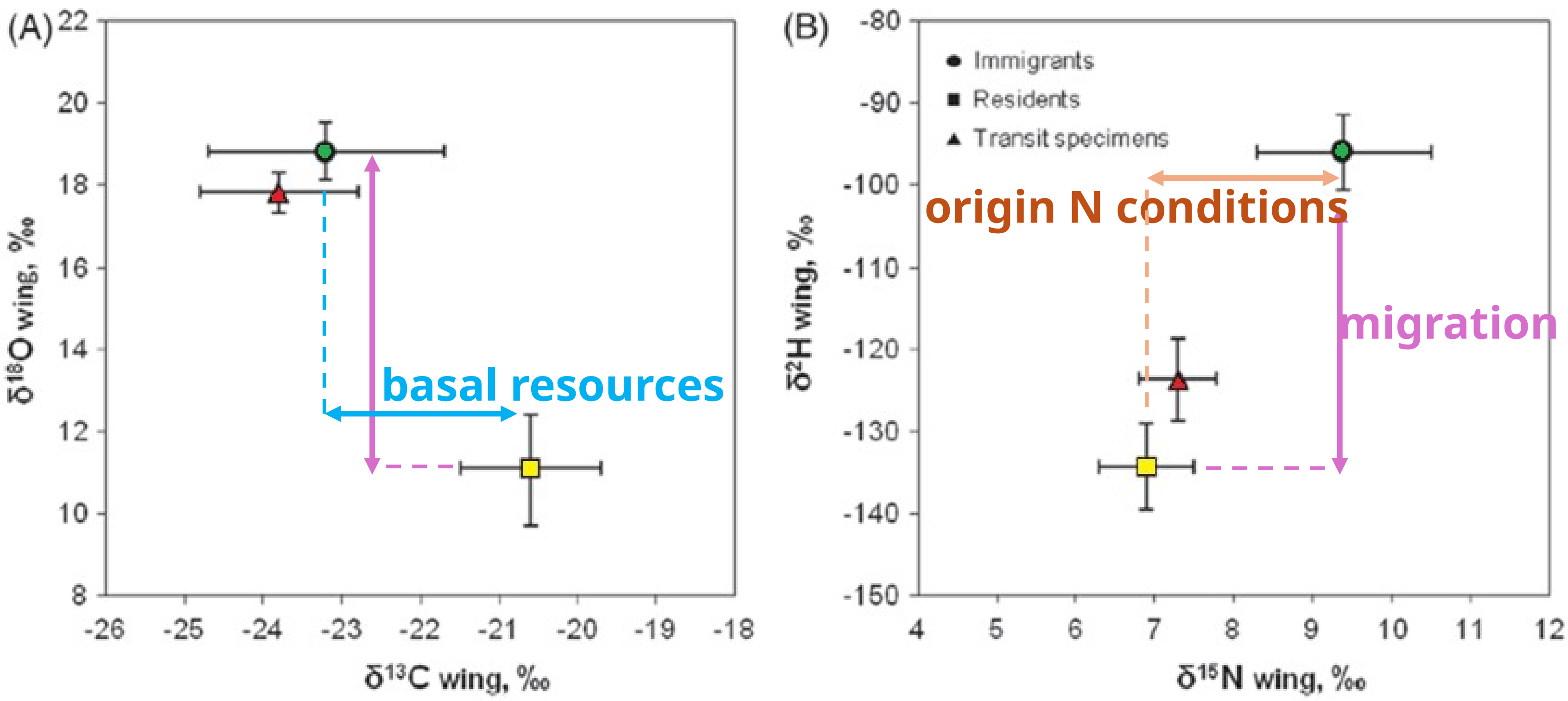

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(Borisov et al., 2020)

Multi-isoscapes of invertebrates: a proxy snapshots of ecosystems

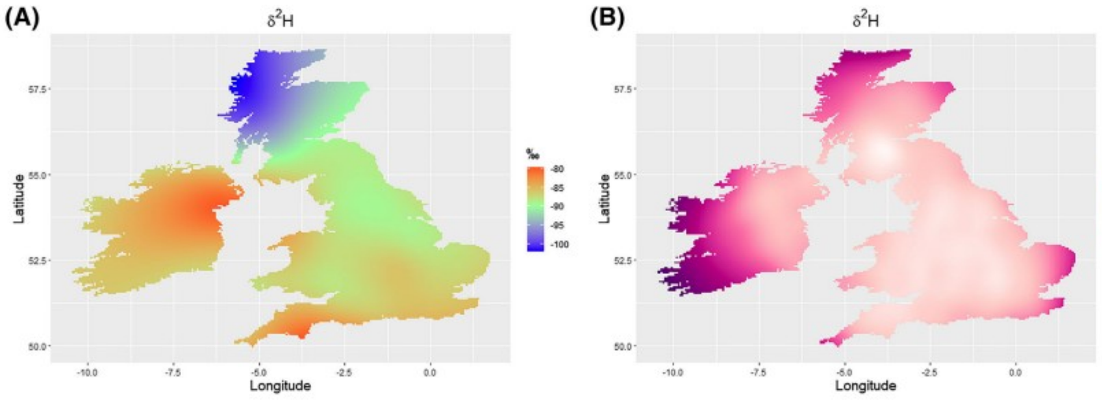


FIGURE 2 A, Generated UK/Ireland isoscape for δ^2H and B, standard deviation

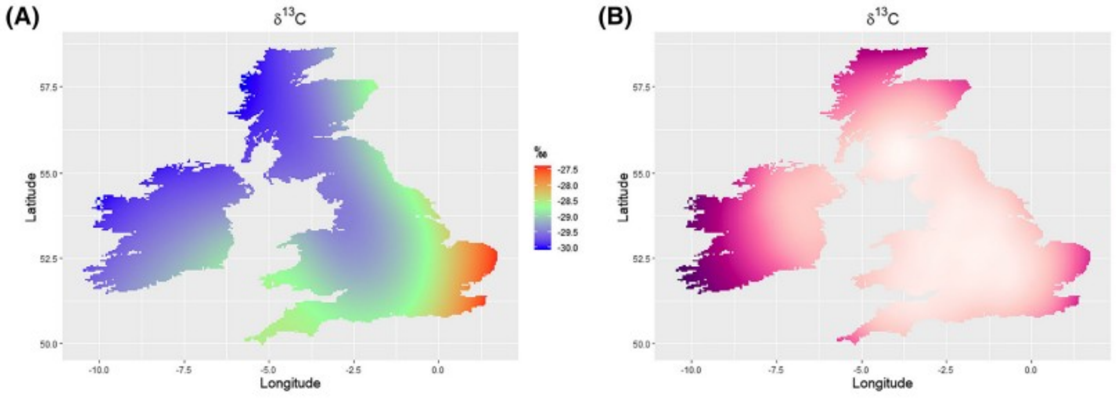
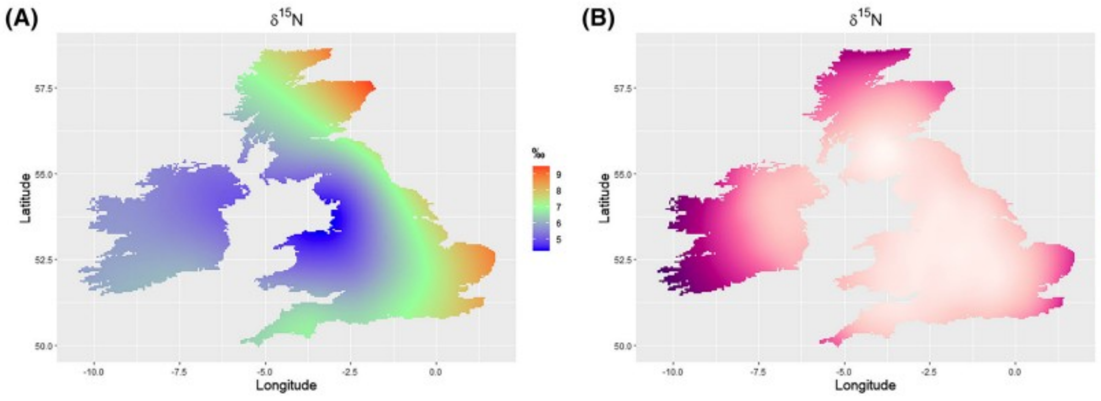


FIGURE 4 A, Generated UK/Ireland isoscape for δ^{13}C and B, standard deviation



- Migration intensity
- Foodweb complexity
- Agricultural load
- Climate and geology

(foll. Newton, 2021)

Combination with “heavier” abiogenic isotopes

From geology to ecology: strontium isotopes

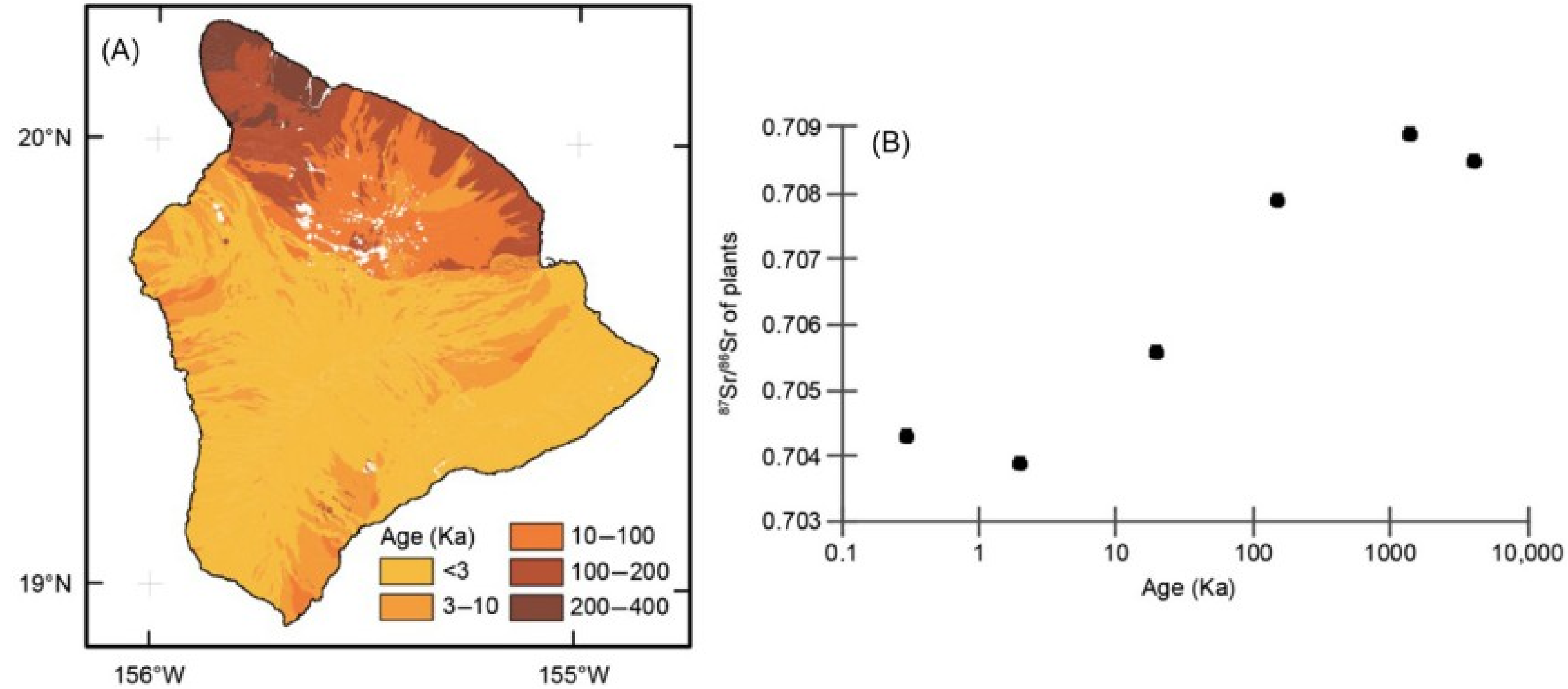


FIGURE 3.1 Spatial patterning of an environmental property underlying variation in ecosystem Sr isotope ratios on the Big Island of Hawai'i. (A) The spatial distribution of approximate landscape surface ages (after Trusdell, Wolfe, & Morris, 2006) on the Big Island of Hawai'i. (B) A strong relationship exists between surface age and plant $^{87}Sr/^{86}Sr$, reflecting the decreased availability of basalt-derived Sr and increased accumulation of Sr from sea-salt aerosols with greater surface age (Kennedy et al., 1998).

(Bowen and West, 2019)

From geology to ecology: strontium isotopes

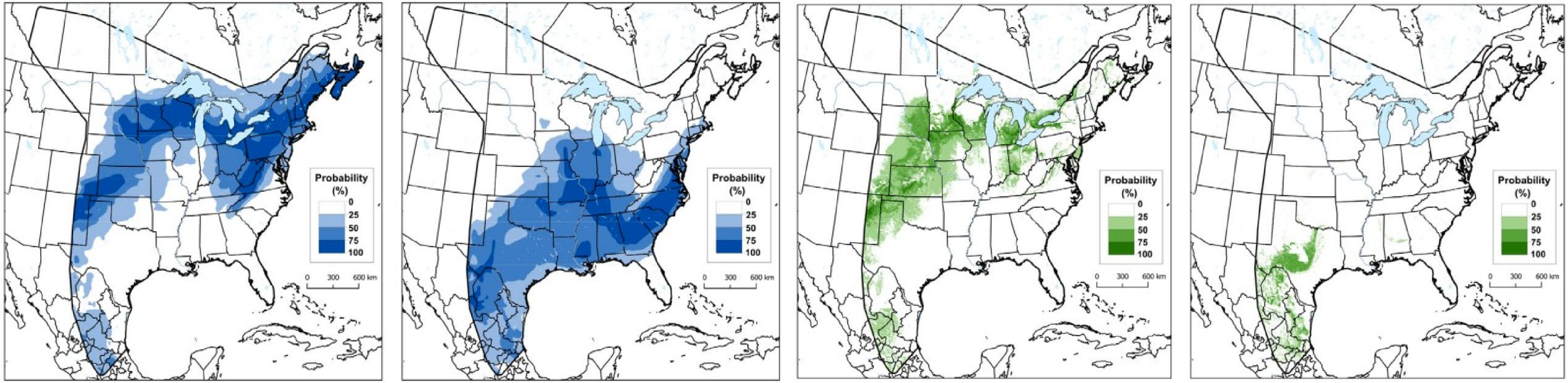
Table 1. Description and summary statistics including main bedrock, soil type, and the $^{87}Sr/^{86}Sr$ value of leachable Sr from soil minerals ('Mineral'), organic soil ('Organic'), milkweed plants ('Milkweed'), and mean value of monarch butterfly wing tissue ('Monarch') from seven locations across Canada. The standard deviation and sample size are included because multiple butterflies were raised on plants grown in soil from each location.

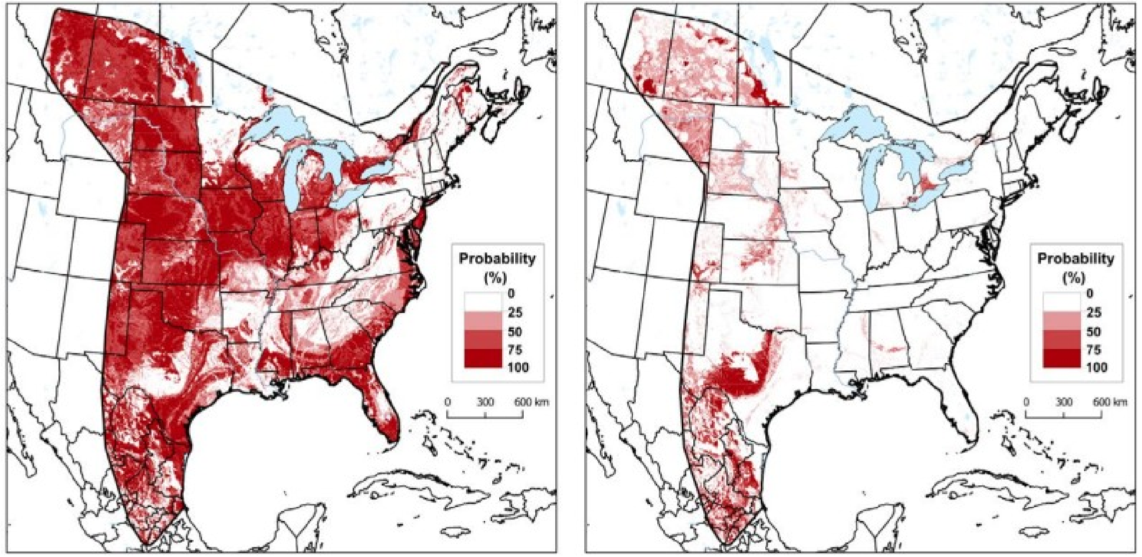
Location	Coordinates	Main bedrock [30]	Soil type [31]	Mineral	Organic	Milkweed	Monarch (SD)
Coquitlam, BC[a]	49.3°N, 122.8°W	Mesozoic intrusive	Ferro-humic podzolic	0.70817	0.70789	0.70808	0.70836 (0.00012) $n = 4$
St. Albert, AB	53.6°N, 113.6°W	Mesozoic sedimentary	Black chernozemic	0.70945	0.70956	0.70959	0.70969 (0.00042) $n = 8$
Saskatoon, SK	52.1°N, 106.7°W	Mesozoic sedimentary	Dark brown chernozemic	0.70837	0.70870	0.70869	0.70872 (0.00012) $n = 8$
Thunder Bay, ON	48.4°N, 89.3°W	Paleoproterozoic sedimentary	Dystric brunisolic	0.71010	0.70931	0.70945	0.70958 (0.00031) $n = 12$
Guelph, ON	43.5°N, 80.2°W	Paleozoic sedimentary	Gray brown luvisolic	0.70860	0.70866	0.70868	0.70913 (0.00021) $n = 4$
Kingston, ON	44.2°N, 76.5°W	Paleozoic sedimentary	Gray luvisolic	0.70882	0.70890	0.70896	0.70923 (0.00050) $n = 6$
St. John's, NL	47.6°N, 52.7°W	Neoproterozoic sedimentary	Humo-ferric podzolic	0.70864	0.70864	0.70878	0.70877 (0.00004) $n = 8$

[a]Lime was added to this soil to improve plant growth and therefore Sr values may not represent those of natural soils found at this location.

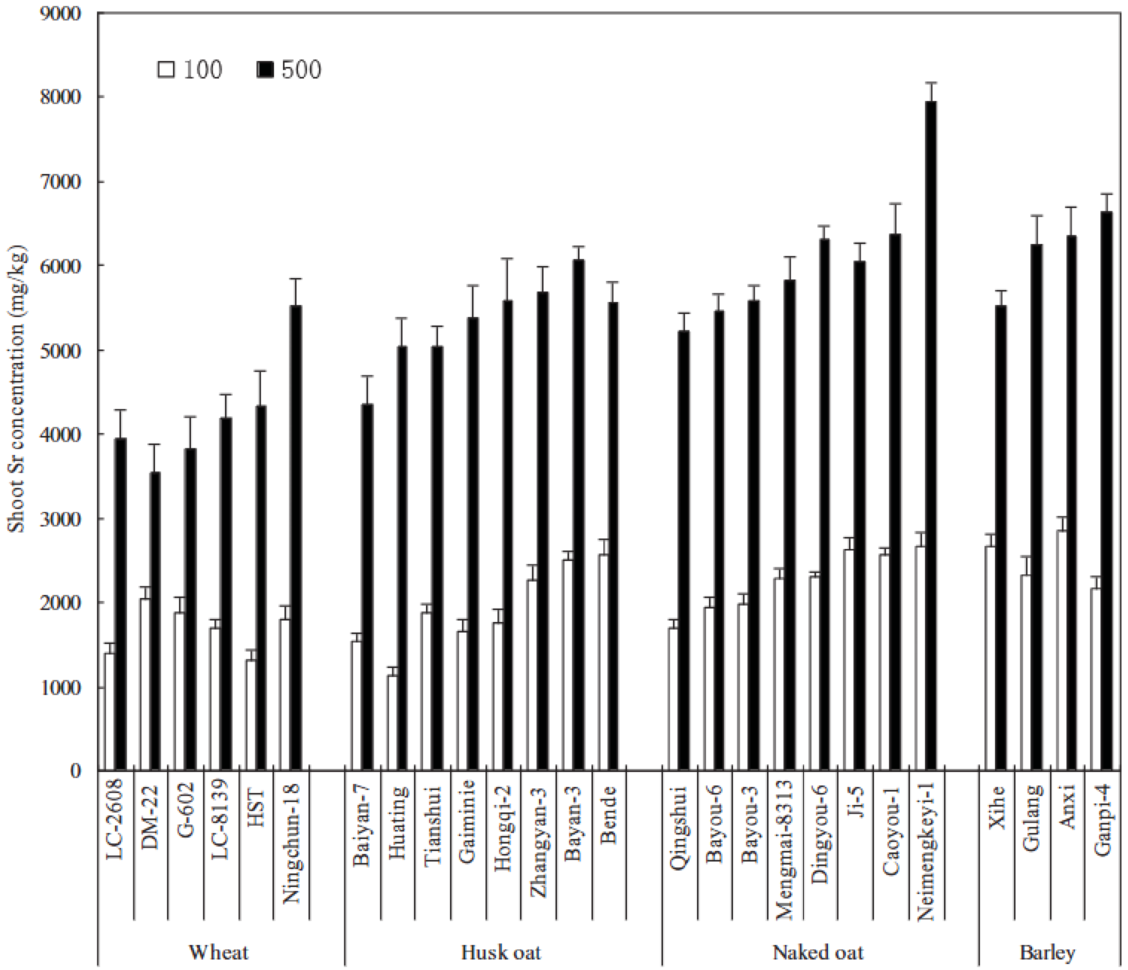
(Flockhart et al., 2015)

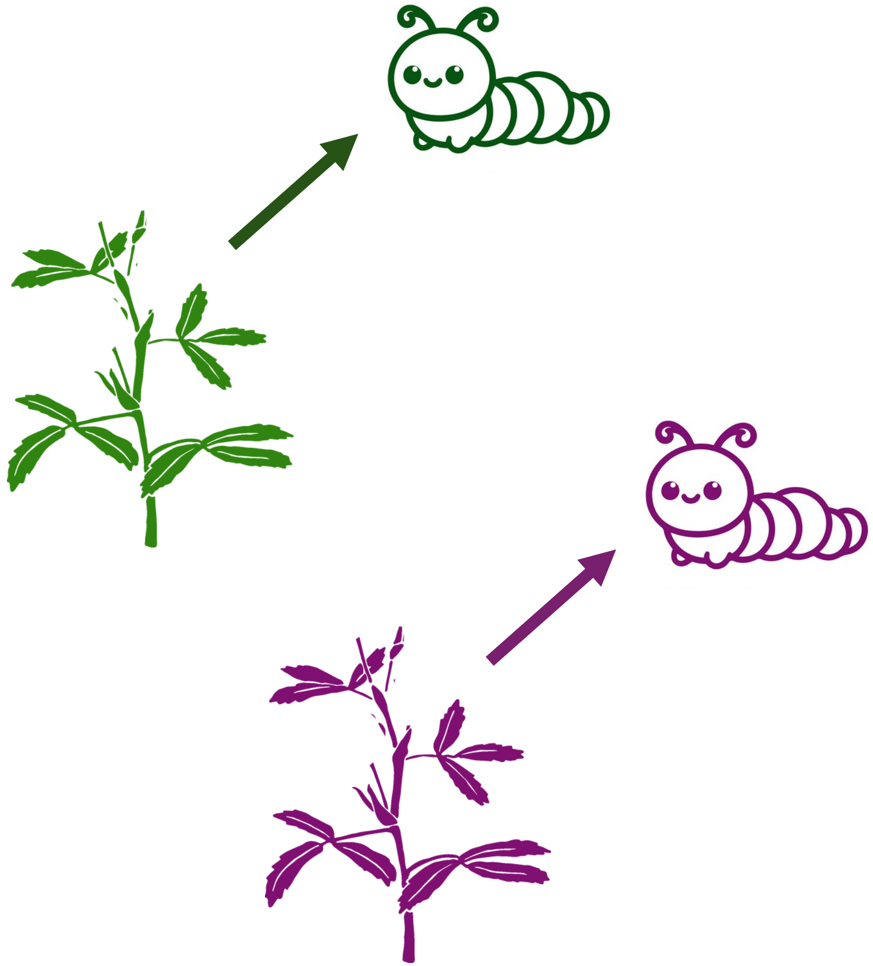
From geology to ecology: strontium isotopes





(Reich et al., 2021)


100
500
Shoot Sr concentration (mg/kg)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
LC-2608
DM-22
G-602
LC-8139
HST
Ningchun-18
Baiyan-7
Huating
Tianshui
Gaiminie
Hongqi-2
Zhangyan-3
Bayan-3
Bende
Qingshui
Bayou-6
Bayou-3
Mengmai-8313
Dingyou-6
Ji-5
Caoyou-1
Neimengkeyi-1
Xihe
Gulang
Anxi
Ganpi-4
Wheat
Husk oat
Naked oat
Barley

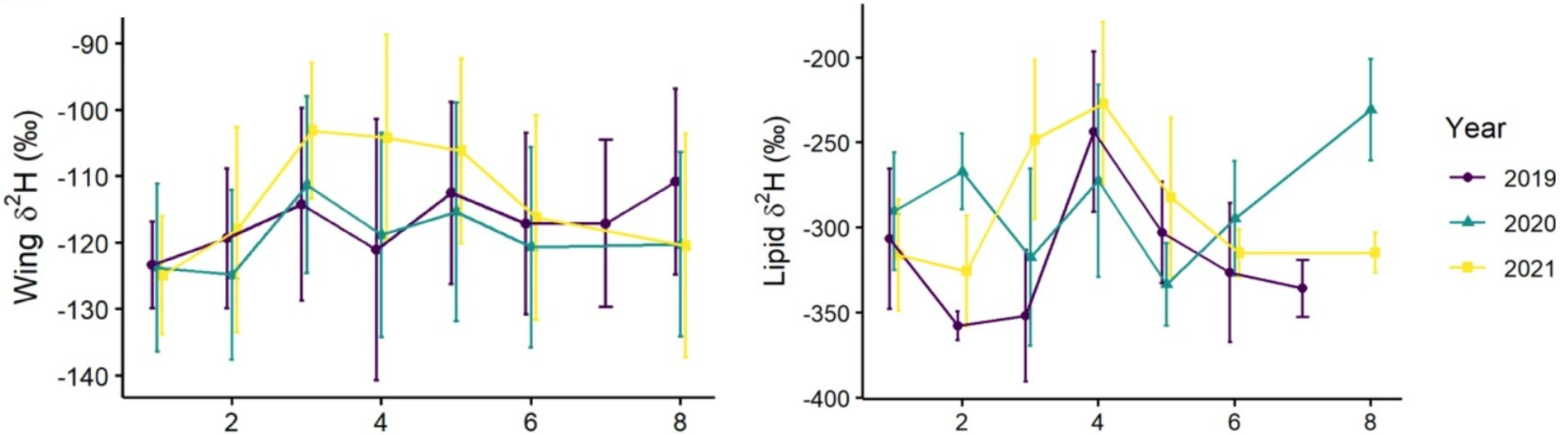


(Qi et al., 2015)

Not only wings:
insights in feeding origins

Stable isotope composition of hydrogen: monarch wings vs lipids

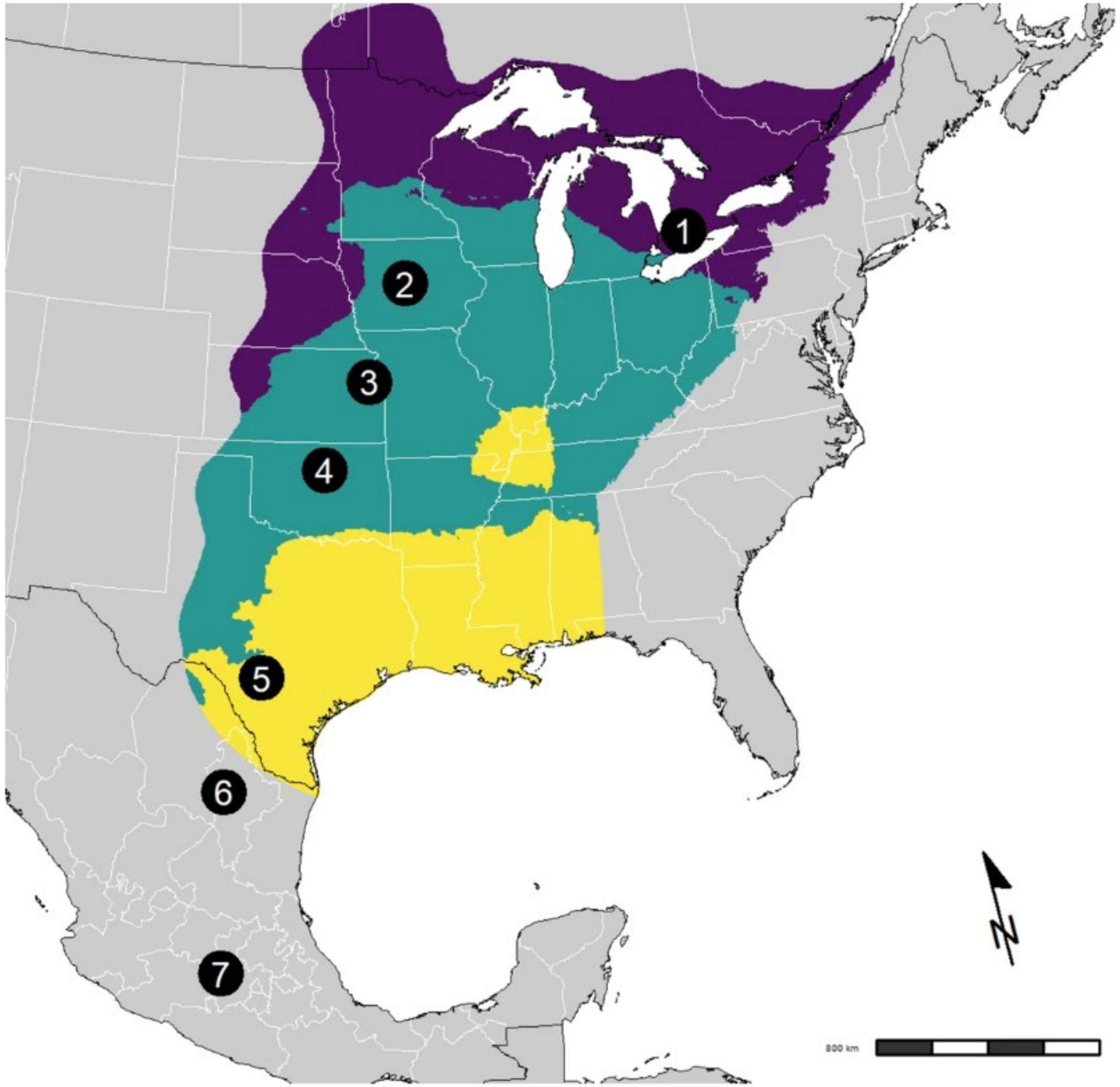
g. 4

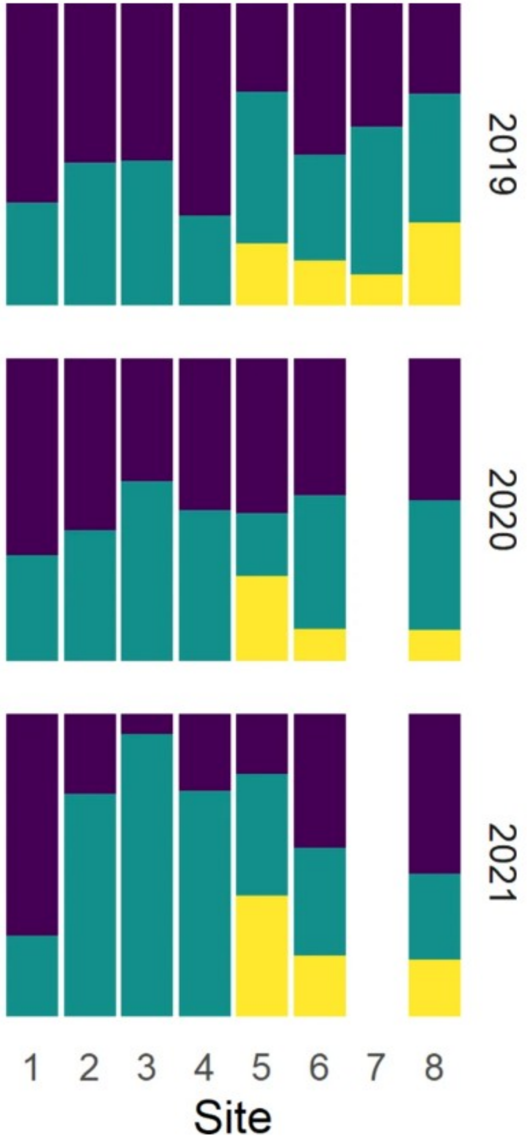


Stable-hydrogen isotope (δ^2H) abundance in (top) wings and (bottom) bulk lipids of migrating (sites 1–7) and overwintering (site 8) monarchs (*Danaus plexippus*, 2019–2021). Years are depicted separately (2019—purple; 2020—green; 2021—yellow). Sites correspond to those depicted in Fig. 5.

(Hobson et al., 2025)

Stable isotope composition of hydrogen in lipids reveal nectaring origins





(*Hobson et al., 2025*)

Values to mapping: IsoriX package in R

IsoriX

an R package for isoscape computation and inference of spatial origins using mixed models

www.github.com/courtiol/IsoriX

courtiol/**IsoriX**

This is the GitHub repository dedicated to the development of the R package IsoriX

Benefits:
- Reproducible methods & results
- Relatively simple
- Rach step of the analysis can be thoroughly studied
- Statistical methods accounting for many sources of uncertainty
- Compatible with Geographic Information System within and without R
- Works on Windows, MacOS, Linux, Unix; localy or remotely; with or without Internet
- Free & open source (anyone can use and improve IsoriX)

Limits:
- R knowledge required
- Some stable isotope knowledge required
- Not multivariate (1 isotope only, but isoscapes or assignment maps can be combined)
- Not Bayesian (prior information not considered)

(foll. Courtiol, 2024)

`www.github.com/courtiol/IsoriX`

IsoriX package overview

An interface between **R** packages (without help files: only ca. 1400 lines of codes):

```
tools::package_dependencies("IsoriX") ## note: list dependencies, not suggested packages
## $IsoriX
##  [1] "graphics"     "grDevices"    "grid"         "lattice"      "latticeExtra" "methods"      "numDeriv"
##  [8] "rasterVis"    "spaMM"        "stats"        "terra"        "tools"        "utils"        "viridisLite"
```

- **spaMM**, numDeriv, stats (for statistical computation)
- **terra** (for GIS)
- **rasterVis**, **lattice**, latticeExtra, viridisLite, graphics, grDevices, grid (for plotting)
- methods, tools, utils (for small geeky details)

(foll. Courtiol, 2024)

www.github.com/courtiol/IsoriX

IsoriX package overview

A collection of data to try things out and to make plots nicer:

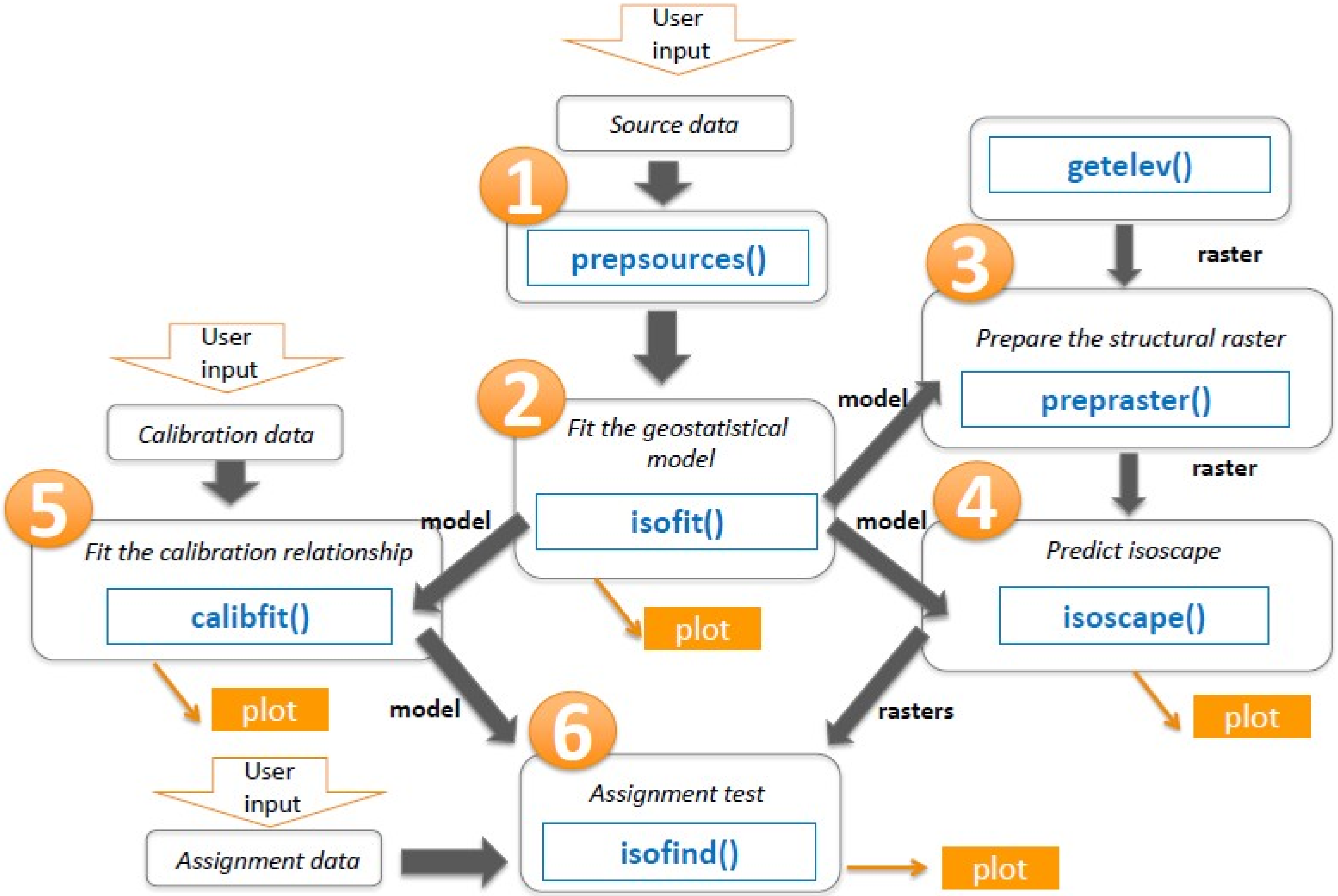
```
data(package = "IsoriX")$results[, c("Item", "Title")]
##       Item                 Title
##  [1,] "AssignDataAlien"    "Simulated assignment dataset"
##  [2,] "AssignDataBat"      "Assignment datasets for bat species"
##  [3,] "AssignDataBat2"     "Assignment datasets for bat species"
##  [4,] "AssignDataBat2Rev"  "Assignment datasets for bat species"
##  [5,] "AssignDataBatRev"   "Assignment datasets for bat species"
##  [6,] "CalibDataAlien"     "Simulated calibration dataset"
##  [7,] "CalibDataBat"       "Calibration datasets for bat species"
##  [8,] "CalibDataBat2"      "Calibration datasets for bat species"
##  [9,] "CalibDataBat2Rev"   "Calibration datasets for bat species"
## [10,] "CalibDataBatRev"    "Calibration datasets for bat species"
## [11,] "GNIPDataALLagg"     "Hydrogen delta values in precipitation water (aggregated per location)"
## [12,] "GNIPDataDE"         "Hydrogen delta values in precipitation water, Germany"
## [13,] "GNIPDataEUagg"      "Hydrogen delta values in precipitation water (aggregated per location)"
## [14,] "isopalette1"        "Colour palettes for plotting"
## [15,] "isopalette2"        "Colour palettes for plotting"
```

\+ CountryBorders, OceanMask, ElevRasterDE, PrecipBrickDE which are spatial objects and thus pre-loaded.

(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

Standard workflow

- Step 1. Prepare the source data
- Step 2. Fit the isoscape
- Step 3. Prepare the structural raster
- Step 4. Predict the isoscape
- Step 5. Fit the calibration function
- Step 6. Perform the assignment
- Summary



(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

Where does the bat Nnoc_15 comes from?

An entire workflow can take under 10 lines of code:

```
GNIPDataDEagg <- prepsources(data = GNIPDataDE)

GermanFit     <- isofit(data = GNIPDataDEagg, mean_model_fix = list(elev = TRUE, lat_abs = TRUE))

getelev()
ElevDE        <- rast("~/elevation_world_z5.tif")
ElevRasterDE  <- prepraster(ElevDE, isofit = GermanFit, aggregation_factor = 2)

GermanScape   <- isoscape(raster = ElevRasterDE, isofit = GermanFit)

CalibBats     <- calibfit(data = CalibDataBatRev, isofit = GermanFit)

Assigned15    <- isofind(data = subset(AssignDataBatRev, sample_ID == "Nnoc_15"),
                         isoscape = GermanScape, calibfit = CalibBats)
```

(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

IsoriX package overview: CatTien δ15N mapping example

~/R/archive/IZW_2024_School/Isotope course - Thursday afternoon/Vietnam_mapping_trial.xlsx

Data Preview:

Plot (character)	mean_d13C (double)	mean_d15N (double)	disp_d13C (double)	disp_d15N (double)	N_reps (double)	lat (double)	long (double)	elev (double)
A	-28.76915	2.68155779	2.37530554	2.125530551	23	11.42780	107.4256	138
DFL	-29.67366	0.02393874	17.14770200	1.711049984	100	11.44675	107.4406	129
DST	-30.42991	0.69214351	0.31987481	0.114692316	4	11.42603	107.4252	130
F	-28.65782	1.24496806	1.14764175	0.312767732	14	11.43503	107.4239	135
L	-29.72015	1.65426958	20.64305077	3.187432836	95	11.42905	107.4273	136
Trans_B-S-1	-29.00490	7.78607479	0.24181285	0.007036463	2	11.45072	107.3645	160
Trans_B-S-2	-29.28090	6.58662935	0.14182273	0.714848578	2	11.44972	107.3668	137
Trans_B-S-3	-30.56929	6.37642123	0.08315505	0.321362935	2	11.44978	107.3669	141
Trans_B-S-4	-30.73943	7.79260865	2.42527070	0.373937721	2	11.44897	107.3688	136
Trans_B-S-5	-29.32171	6.85520994	0.86420037	0.225912265	2	11.44831	107.3700	141
Trans_B-S-6	-30.64658	5.45567627	0.72441826	0.271446963	2	11.44700	107.3729	145
Trans_B-S-7	-32.42038	4.73828132	0.73258072	0.841359873	2	11.44519	107.3766	145
Trans_B-S-8	-30.91798	4.73463149	0.34486390	0.143762771	2	11.44503	107.3771	149
Trans_B-S-9	-31.73423	2.45599763	0.74890563	0.258840996	2	11.44186	107.3847	137
Trans_B-S-10	-30.28335	2.06357389	1.48352696	0.864475846	2	11.44136	107.3854	142
Trans_B-S-11	-31.66536	2.14635010	0.13876181	0.872521350	2	11.44075	107.3864	124

Previewing first 50 entries.

IsoriX package overview: CatTien δ15N mapping example

```
# we need specific column names for IsoriX
# separating dataframes for two isotopes
```

	source_ID	mean_source_value	var_source_value	n_source_value	lat	long	elev
1	A	2.68155779	2.125530551	23	11.42780	107.4256	138
2	DFL	0.02393874	1.711049984	100	11.44675	107.4406	129
3	DST	0.69214351	0.114692316	4	11.42603	107.4252	130
4	F	1.24496806	0.312767732	14	11.43503	107.4239	135
5	L	1.65426958	3.187432836	95	11.42905	107.4273	136
6	Trans_B-S-1	7.78607479	0.007036463	2	11.45072	107.3645	160
7	Trans_B-S-2	6.58662935	0.714848578	2	11.44972	107.3668	137
8	Trans_B-S-3	6.37642123	0.321362935	2	11.44978	107.3669	141
9	Trans_B-S-4	7.79260865	0.373937721	2	11.44897	107.3688	136
0	Trans_B-S-5	6.85520994	0.225912265	2	11.44831	107.3700	141
1	Trans_B-S-6	5.45567627	0.271446963	2	11.44700	107.3729	145

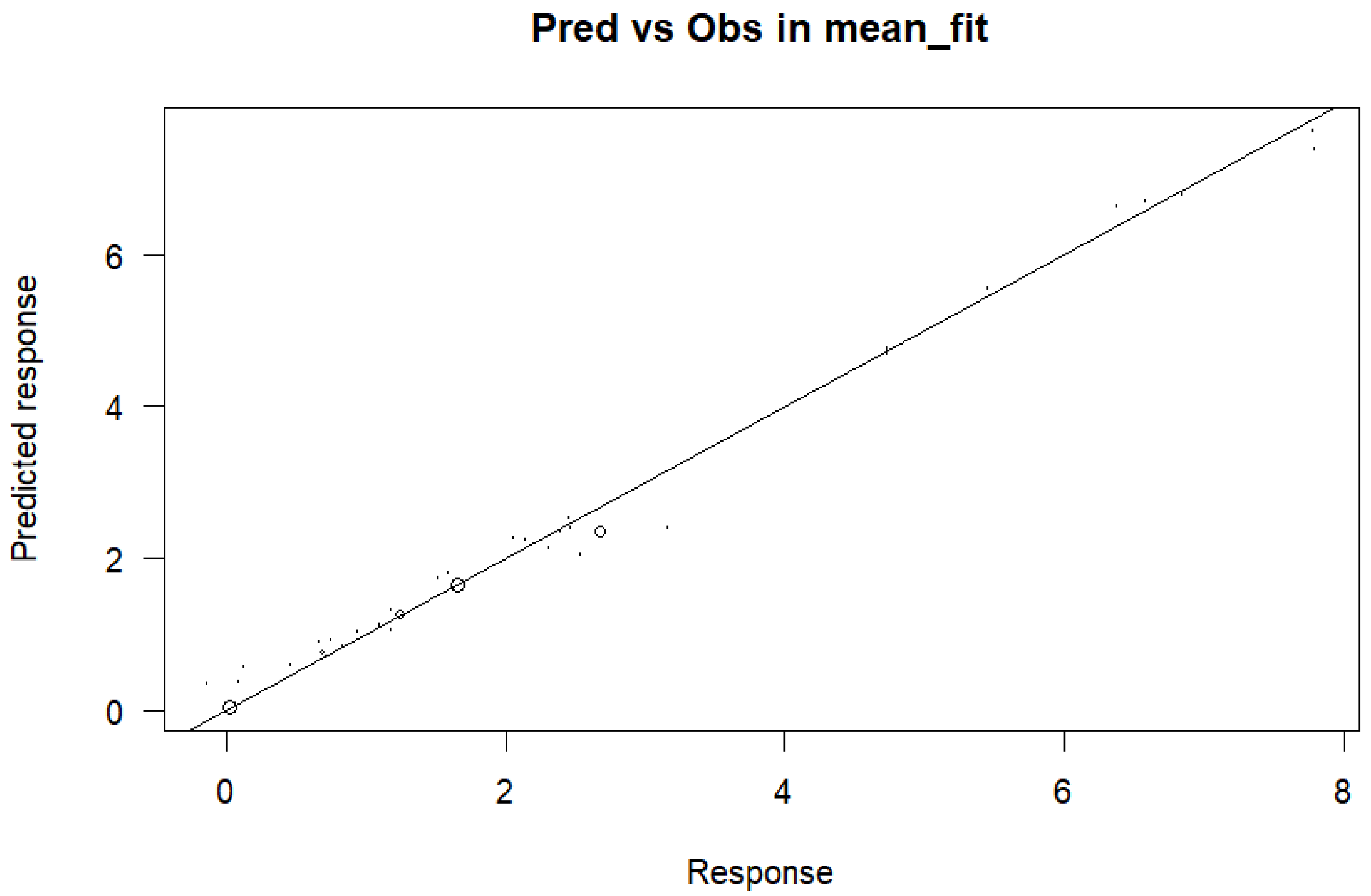
IsoriX package overview: CatTien $\delta^{15}N$ mapping example

```
# modelling nitrogen
```

```
> fitN <- isofit(data = d_V_N,
+                mean_model_fix = list(elev = FALSE, lat_abs = TRUE, lat_2 = FALSE,
+                                      long = TRUE, long_2 = FALSE))
[1] Fitting the following residual dispersion model using spaMM  :
[1] "var_source_value ~ 1 + (1|source_ID) + Matern(1|long + lat)"
[1] (it may take a while...)
[1] Fitting the following mean model using spaMM  :
[1] "mean_source_value ~ 1 + lat_abs + long + (1|source_ID) + Matern(1|long + lat)"
[1] (it may take a while...)
[1] Done!
[1] "Models were fitted in 7s."
> AIC(fitN$mean_fit)  # Try all TRUE one by one and compare AICs
        criterion     value   method    short name
      marginal AIC: 113.40763            mAIC
   conditional AIC:  63.95772 (plug-in)  cAIC
    dispersion AIC:  83.46132            dAIC
      effective df:  10.41788            GoFdf
```

IsoriX package overview: CatTien δ15N mapping example

```
plot(fitN)
```

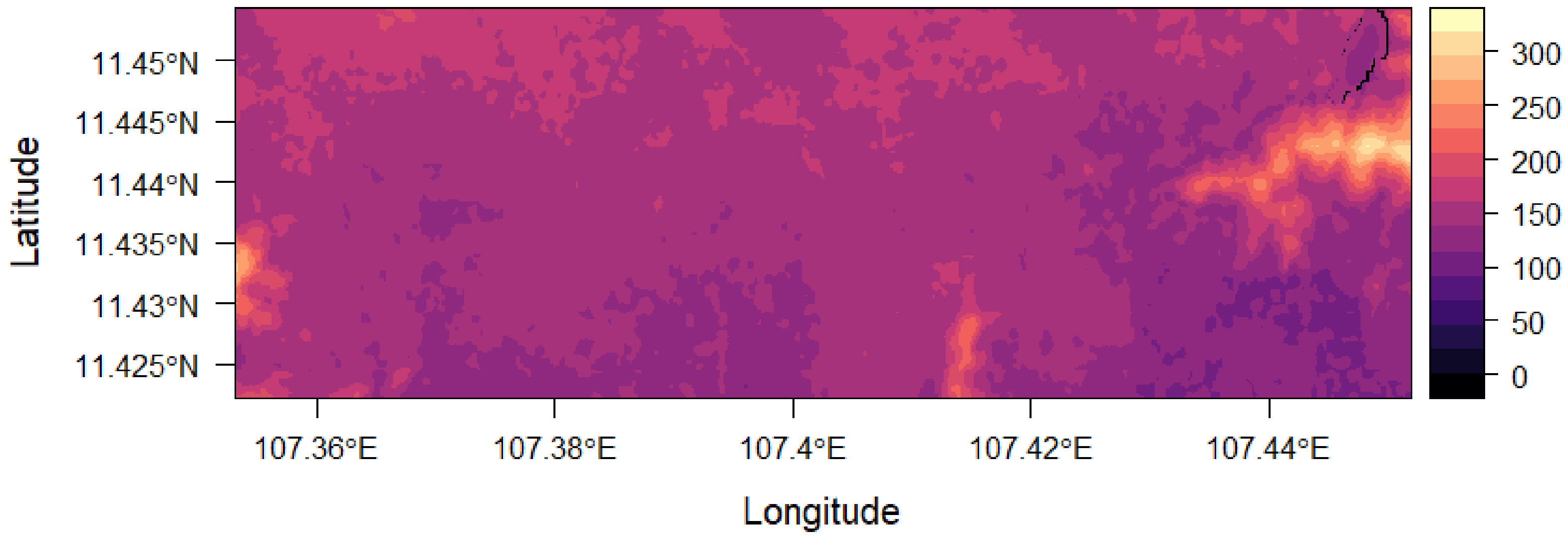


IsoriX package overview: CatTien δ15N mapping example

```
# prepare structural raster
?getelev # check z-values for the desired raster size - square meters per polygon

## X <- terra::vect(.kmz)

#install.packages('elevatr')

getelev(file = "elev_vietnam_rect_z14.tif", z = 14,
       long_min = min(data_viet$long), #terra::xmin
       long_max = max(data_viet$long),
       lat_min = min(data_viet$lat), #retta::ymin
       lat_max = max(data_viet$lat),
       margin_pct = 15)

elev_raster <- terra::rast("elev_vietnam_z14.tif")
elev_raster_rect <- terra::rast("elev_vietnam_rect_z14.tif")

str_raster <- prepraster(raster = elev_raster,
                         aggregation_factor = 4)
str_raster_rect <- prepraster(raster = elev_raster_rect,
                         aggregation_factor = 4)
```

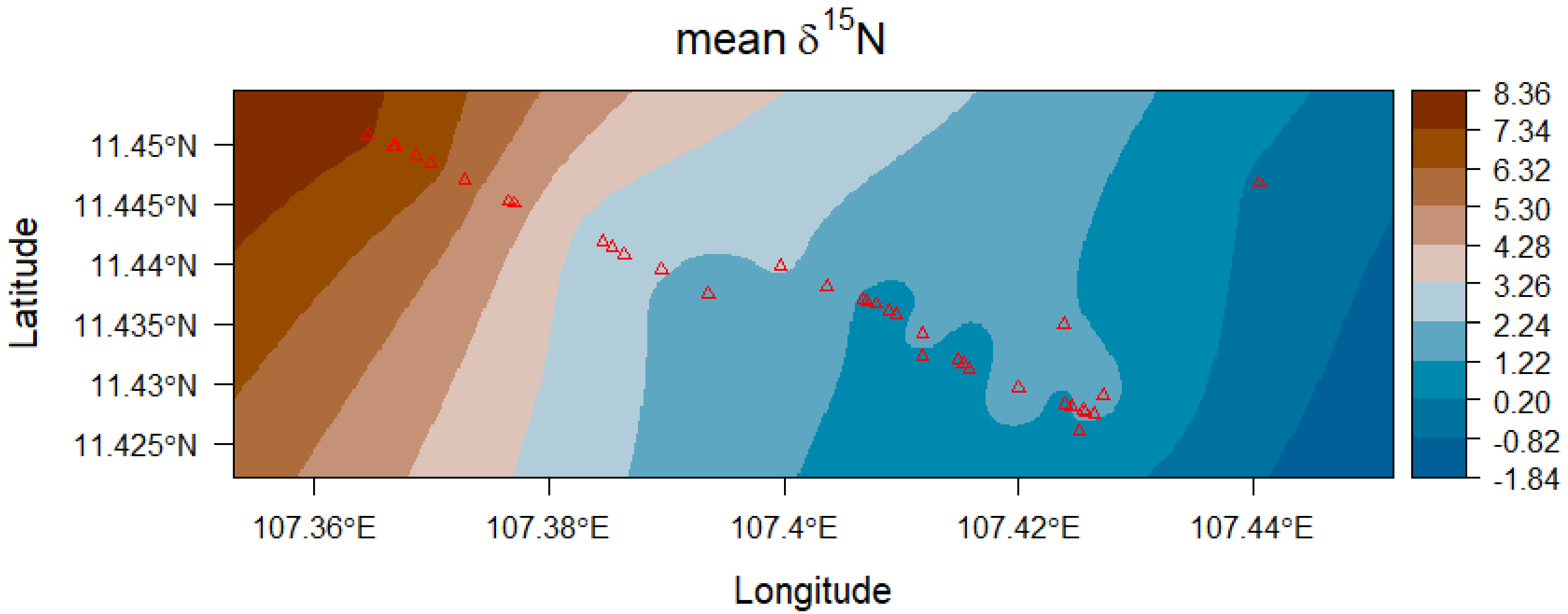
IsoriX package overview: CatTien $\delta^{15}N$ mapping example

```r
# prepare structural raster
?getelev # check z-values for the desired raster size - square meters per polygon

## X <- terra::vect(.kmz)

#install.packages('elevatr')

getelev(file = "elev_vietnam_rect_z14.tif", z = 14,
       long_min = min(data_viet$long), #terra::xmin
       long_max = max(data_viet$long),
       lat_min = min(data_viet$lat), #retta::ymin
       lat_max = max(data_viet$lat),
       margin_pct = 15)

elev_raster <- terra::rast("elev_vietnam_z14.tif")
elev_raster_rect <- terra::rast("elev_vietnam_rect_z14.tif")

str_raster <- prepraster(raster = elev_raster,
                         aggregation_factor = 4)
str_raster_rect <- prepraster(raster = elev_raster_rect,
                         aggregation_factor = 4)
```

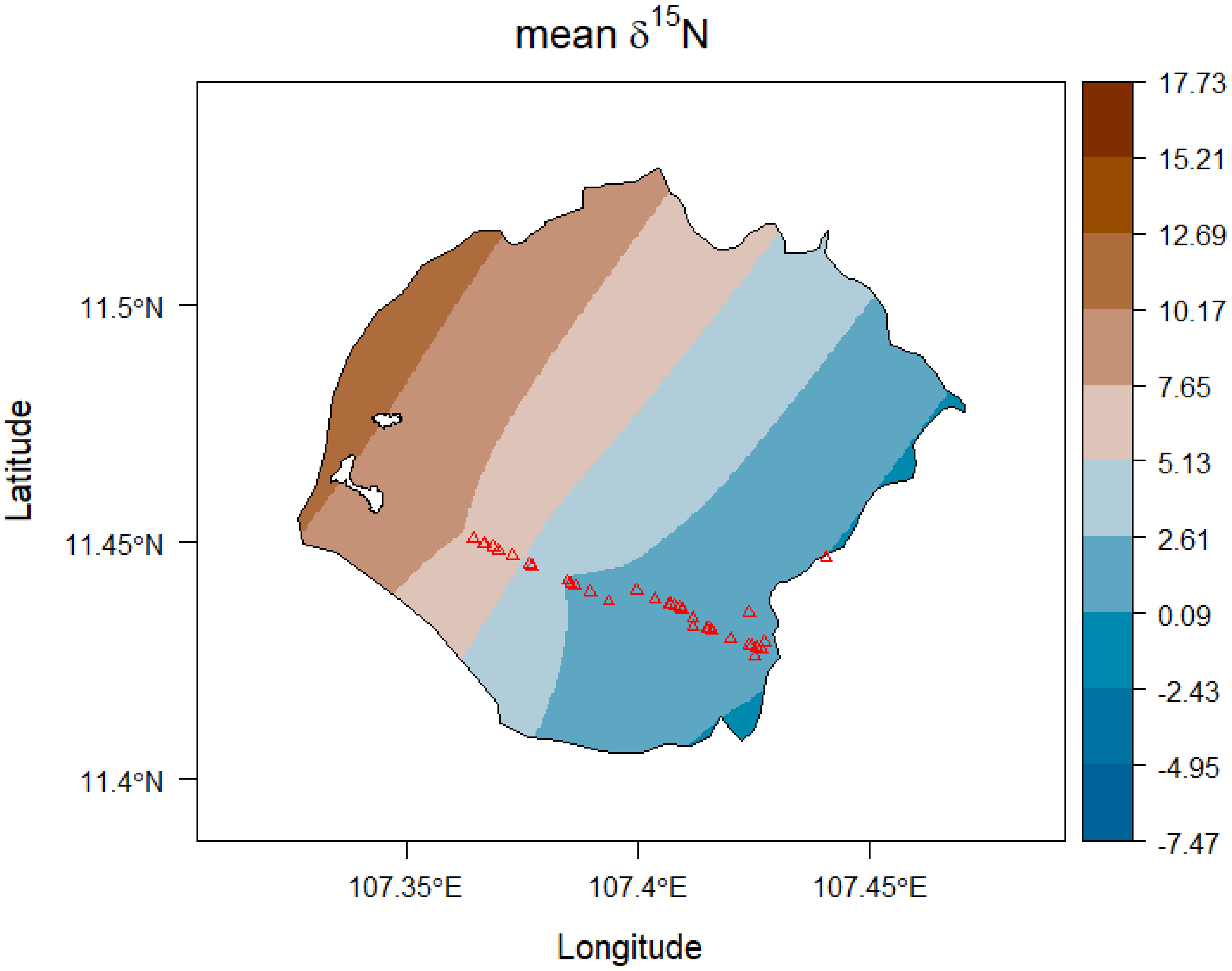


IsoriX package overview: CatTien δ15N mapping example

```
# isoscape

isoN <- isoscape(raster = str_raster, isofit = fitN)
plot(isoN, y_title = list(which = TRUE, title = bquote(delta^15*N)))
```



IsoriX package overview: CatTien δ15N mapping example

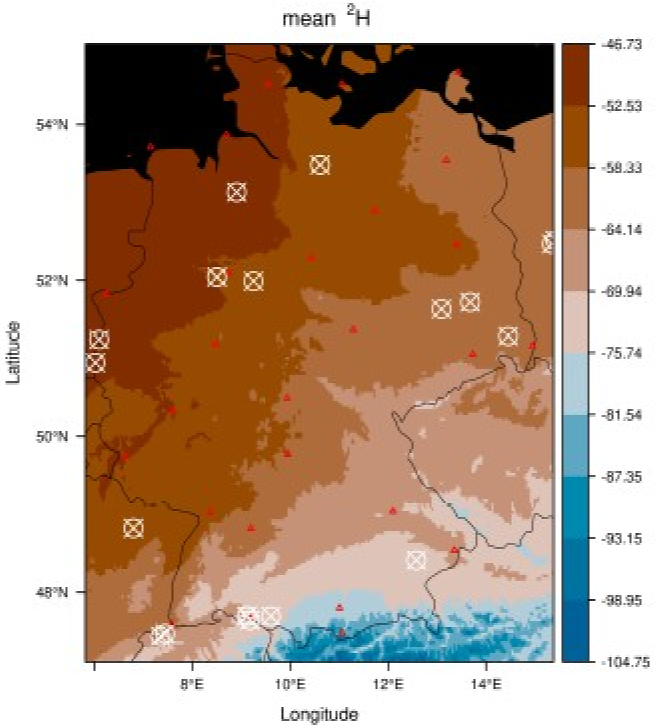


IsoriX package overview

```
## I have not enough data on termites with known coordinates,
## so the tracing is the original example on BATS from Germany
```

5b. Check your calibration data:

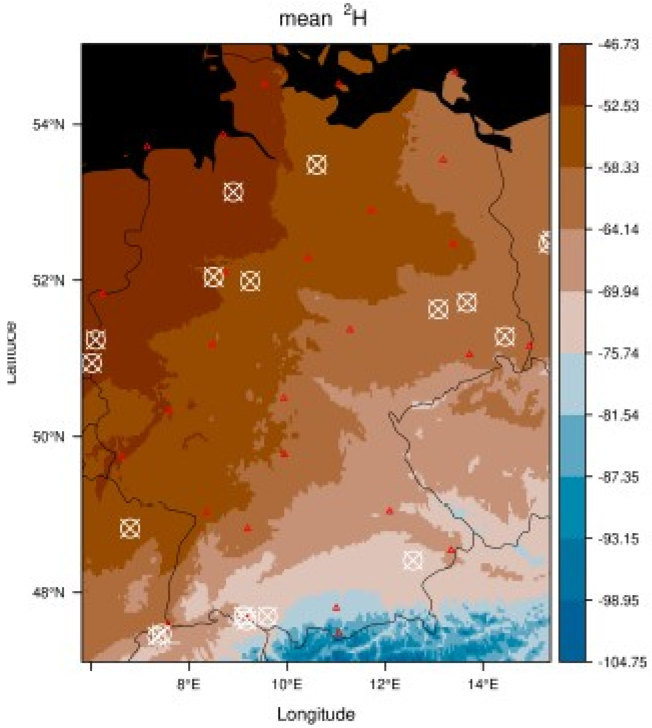
```
plot(GermanScape, plot = FALSE) + ## plot = FALSE to avoid premature plotting
  xyplot(lat ~ long, data = CalibDataBatRev, pch = 13, col = "white", cex = 2, lwd = 2)
```



(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

6a. Prepare your assignment data:

```
AssignDataBatRev
##     sample_ID      lat     long sample_value
## 1      Nnoc_1 51.72323 13.67630    -43.40550
## 2      Nnoc_2 51.72323 13.67630    -49.59752
## 3      Nnoc_3 51.72323 13.67630    -45.19298
## 4      Nnoc_4 51.69520 14.64945    -41.95605
## 5      Nnoc_5 51.70366 14.63915    -58.19690
## 6      Nnoc_7 51.69520 14.64945    -42.56502
## 7      Nnoc_8 51.70536 14.63289    -46.26581
## 8      Nnoc_9 51.70536 14.63289    -48.47353
## 9     Nnoc_10 51.89348 13.76488    -42.53986
## 10    Nnoc_11 51.84883 13.64525    -43.79554
## 11    Nnoc_12 51.60535 11.92700    -45.72407
## 12    Nnoc_13 51.96695 12.08462    -57.72157
## 13    Nnoc_15 51.96695 12.08462    -72.43155
## 14    Nnoc_16 51.45457 11.50689    -53.39763
```



(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

Step 6. Perform the assignment

Some important facts about the assignment test:

- The test is performed at the level of each sample and each cell defined by the structural raster.
- The test statistic is the difference between the predicted isoscape value at the origin location (deduced from the calibration fit) and the predicted isoscape value at the location under consideration (read from the isoscape).
- The null hypothesis of the test is that the sample comes from the cell being examined.
- A large p-value (i.e. non significant) indicates support for the null hypothesis.
- A small p-value (i.e. significant) indicates the rejection of the null hypothesis.

(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

IsoriX package overview

You can easily extract the outcome of the assignment test in the cell corresponding to a given location

Example 1: may the first bat (Nnoc_1) come from where it has been found?

```
extract(AssignedBats$sample$stat[[1]], cbind(AssignDataBat$long[1], AssignDataBat$lat[1]))
##       Nnoc_1
## 1 0.8424342
extract(AssignedBats$sample$pv[[1]], cbind(AssignDataBat$long[1], AssignDataBat$lat[1]))
##       Nnoc_1
## 1 0.9398607
```

Answer: it is possible (we cannot reject the proposition).

Example 2: may the bat 13 (Nnoc_15) come from where it has been found?

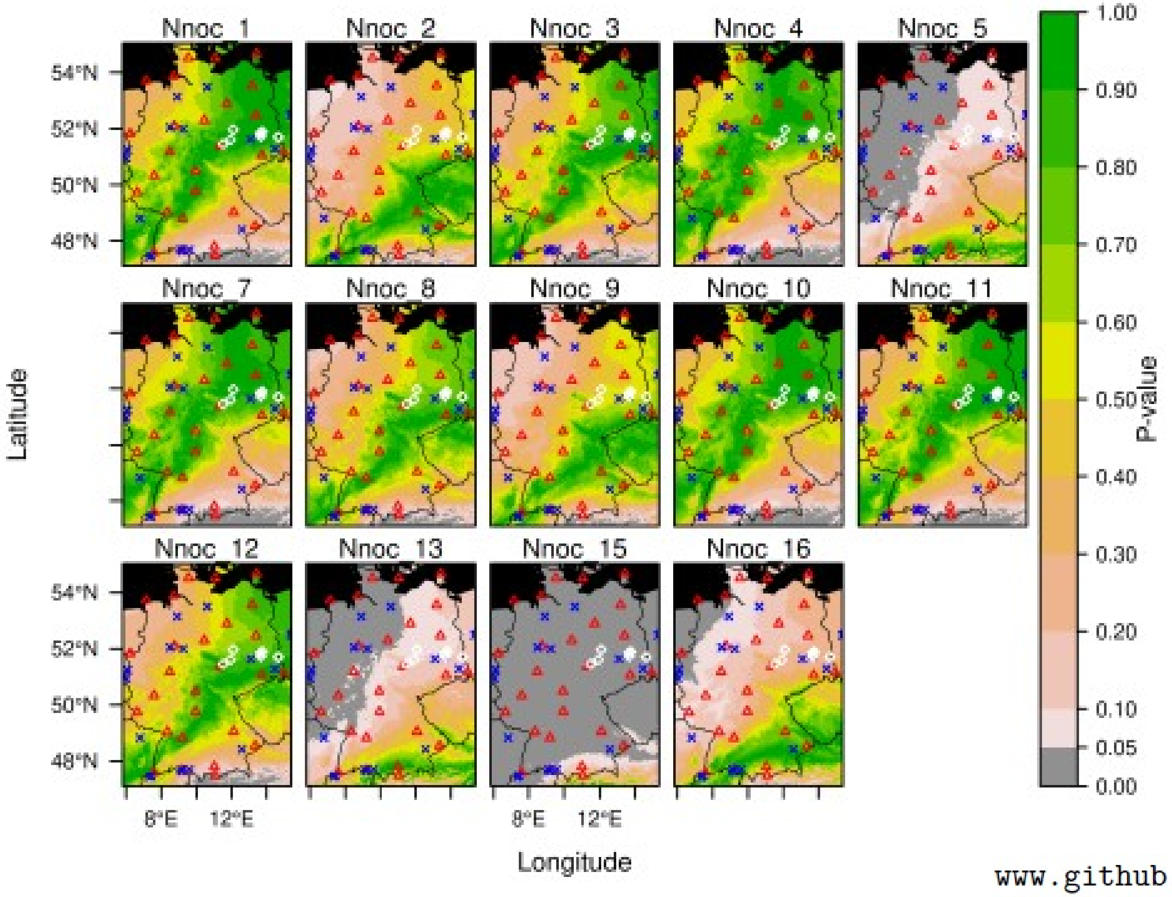
```
extract(AssignedBats$sample$stat[[13]], cbind(AssignDataBat$long[13], AssignDataBat$lat[13]))
##      Nnoc_15
## 1 -41.01754
extract(AssignedBats$sample$pv[[13]], cbind(AssignDataBat$long[13], AssignDataBat$lat[13]))
##        Nnoc_15
## 1 0.001996747
```

Answer: it is very unlikely (we can reject the proposition).

(foll. Courtiol, 2024)
www.github.com/courtiol/IsoriX

6c. Plot the assignment tests with `plot()`:

```
plot(AssignedBats, who = 1:14)
```



(foll. Courtiol, 2024)
`www.github.com/courtiol/IsoriX`

Acknowledgements

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Thanks for your attention!