


RESEARCH ARTICLE

Grazing high and low: Can we detect horse altitudinal mobility using high-resolution isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) time series in tail hair? A case study in the Mongolian Altai

Nicolas Lazzerini¹  | Aurélie Coulon^{2,3} | Laurent Simon⁴ | Charlotte Marchina⁵ | Bayarkhuu Noost⁶ | Sébastien Lepetz¹ | Antoine Zazzo¹

¹Archéozoologie, Archéobotanique: sociétés, pratiques et environnements (UMR 7209 AASPE), CNRS, Muséum national d'Histoire naturelle, CP 5655 rue Buffon, 75005, Paris, France

²Centre d'Ecologie et des Sciences de la Conservation (UMR 7204 CESCO), CNRS, Muséum national d'Histoire naturelle, CP 13557 rue Cuvier, 75005, Paris, France

³CEFE, CNRS, Univ. Montpellier, Univ. Paul Valéry Montpellier 3, EPHE, IRD, Montpellier, France

⁴Univ. Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR 5023 LEHNA, 69622 Villeurbanne, France

⁵Institut Français de Recherche sur l'Asie de l'Est (IFRAE), FRE 2025, Inalco/Université de Paris/CNRS, 2 rue de Lille, 75007 Paris, France

⁶Mongolian Academy of Sciences, Institute of History and Archaeology, Mongolia

Correspondence

N. Lazzerini and A. Zazzo, Archéozoologie, Archéobotanique: sociétés, pratiques et environnements (UMR 7209 AASPE), CNRS, Muséum national d'Histoire naturelle, CP 56, 55 rue Buffon-75005, 75005 Paris, France. Email: nicolas.lazzerini@edu.mnhn.fr; zazzo@mnhn.fr

Funding information

CNRS; French archaeological mission in Mongolia (MAEDI); Inalco Early Career Research Grant; LabEx BCDiv, Grant/Award Number: ANR-10-LABX-0003-BCDiv

Rationale: Carbon and nitrogen stable isotope time series performed in continuously growing tissues (hair, tooth enamel) are commonly used to reconstruct the dietary history of modern and ancient animals. Predicting the effects of altitudinal mobility on animal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values remains difficult as several variables such as temperature, water availability or soil type can contribute to the isotope composition. Modern references adapted to the region of interest are therefore essential.

Methods: Between June 2015 and July 2018, six free-ranging domestic horses living in the Mongolian Altai were fitted with GPS collars. Tail hairs were sampled each year, prepared for sequential C and N isotope analysis using EA-IRMS. Isotopic variations were compared with altitudinal mobility, and Generalized Additive Mixed (GAMMs) models were used to model the effect of geographic and environmental factors on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Results: Less than half of the pasture changes were linked with a significant isotopic shift while numerous isotopic shifts did not correspond to any altitudinal mobility. Similar patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variations were observed between the different horses, despite differences in mobility patterns. We propose that water availability as well as seasonal availability of N_2 -fixing type plants primarily controlled horse hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, overprinting the influence of altitude.

Conclusions: Our study shows that altitudinal mobility is not the main factor that drives the variations in horse tail hair $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and that seasonal change in the animal dietary preference also plays an important role. It is therefore risky to interpret variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of animal tissues in terms of altitudinal mobility alone, at least in C_3 -dominated environments.

1 | INTRODUCTION

Nomadic pastoralism is a husbandry practice which consists in moving livestock between different pastures in order to provide herd animals with a continuous source of fresh graze.¹ In mountainous regions,

exploitation of pastures located at different elevations is commonly observed in order to take advantage of seasonal variation in pasture productivity. In general, highland pastures are exploited during the summer months, when still easily accessible, and lowland pastures during the winter months. Comfort can also drive mobility patterns,

as herders can choose to move to valley bottoms during winter to protect themselves from the cold winds.² This basic model is of course flexible and can change over time and space under the influence of various ecological, environmental and socio-political factors such as graze availability, access rights, water availability or pasture stocking rate.^{3–5} Therefore, documenting modern and ancient mobility patterns of domestic animals is fundamental to understanding the evolution of herding practices.

Animal mobility can be tracked by telemetry, e.g. with GPS devices set on the animals, but this method remains expensive and restricted to modern animals. An alternative approach consists in the isotopic analysis of animal tissues. C and N isotopes are the most commonly used isotopic systems. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of plants vary widely and systematically across the globe in response to environmental and climatic differences. They are incorporated into animal tissues through diet, thus offering the possibility of tracing the provenance of animals in isotopically contrasting environments. When analysed sequentially in continuously growing tissues (i.e. hair, horn, teeth), stable isotope time series can provide a quantitative method to study the mobility of modern and ancient organisms both domestic and wild.^{6–9} Among these tissues, hair has probably the best potential because it grows rapidly and continuously and is biologically inactive once formed. Controlled feeding experiments have shown that it is possible to detect short-term (daily) changes in diet.^{8,10–12} Therefore, temporally resolved hair isotope records have been used to explore fine-scale spatial ecology of mammals.^{7,13,14} Under specific conditions, keratin, the protein constitutive of hair, horn and hooves, can be preserved over several thousands of years and be used for palaeoenvironmental and palaeocological reconstructions.¹⁵

Altitude has an important impact on climatic and soil conditions, and thus on primary production^{16,17} and plant degradation,^{18,19} and consequently influences the C- and N-isotopic composition of soils and plants.^{20,21} Below, we briefly review the extant literature regarding the effect of altitude on plant C- and N-isotopic ratios and its potential to reconstruct animal vertical mobility.

The major mechanism influencing the $\delta^{13}\text{C}$ values of terrestrial plants is the photosynthetic pathway used for fixing carbon: plants using the C_3 pathway have an average $\delta^{13}\text{C}$ value of -26‰ , while plants using the C_4 pathway have an average $\delta^{13}\text{C}$ value of -12‰ and plants using the CAM pathway vary between those values.^{22,23} While CAM plants are not of high importance in Mongolia, the vegetation is dominated by C_3 plants, followed by C_4 plants, especially in southern Mongolia.²⁴ C_4 plants dominate in arid environments characterized by high temperatures and brightness, thanks to a higher water use efficiency, while C_3 plants dominate in temperate, humid and shaded environments.²⁵ Those differences trigger an increase in the relative proportion of C_3 vs C_4 plants with altitude.^{26,27} This vertical stratification of vegetation has been exploited to determine altitudinal mobility of modern and past livestock through isotopic analyses of tooth enamel with ingestion of C_4 plants in lowlands during winter and C_3 plants in highlands during summer.^{26–29} It is noteworthy that in the case of domestic

herbivores, the increase in $\delta^{13}\text{C}$ values during winter can also be driven by the ingestion of ^{13}C -enriched fodder provided by the herder and is not necessarily caused by altitudinal mobility.^{30–32} In C_3 -dominated environments, the range of isotopic variations is more restricted. Nevertheless, several studies have documented an increase in the $\delta^{13}\text{C}$ values of C_3 plants with altitude.^{33–39} This pattern is related to high carboxylation rates relative to stomatal conductance at high altitudes resulting in lower ^{13}C discrimination.^{35,39} This positive correlation can be recorded in the $\delta^{13}\text{C}$ values of animal tissues, such as hair.¹³ An opposite pattern was reported in SW USA and in China.^{40,41} The higher plant $\delta^{13}\text{C}$ values measured at lower altitude were explained by increased drought stress at lower elevations in these arid and semi-arid regions. Finally, several authors proposed that local climatic factors could modulate the effects of water availability and hence locally complicate the relationship between $\delta^{13}\text{C}$ values and altitude.^{37,42}

Several studies have documented a decrease in plant and soil $\delta^{15}\text{N}$ values with altitude.^{39,42–44} This trend was attributed to the decrease in mineralization and lower net nitrification rates at higher altitudes induced by lower temperature and higher precipitation.^{42–46} However, more complex trends have also been observed. Indeed, Liu et al⁴⁷ showed that the correlation between plant $\delta^{15}\text{N}$ values and altitude is negative below 1350 m above sea level (asl) and positive above 1350 m asl. They explained this result by the fact that plant $\delta^{15}\text{N}$ values are controlled by precipitation below 1350 m asl, and by temperature above 1350 m asl. More recently, Szpak et al³⁹ showed that the negative relationship between $\delta^{15}\text{N}$ values and altitude observed in Andean plants disappears above 2000 m asl and when precipitation exceeds 400 mm per year. This complexity is explained by the fact that soil nitrogen is affected by plant physiological and biogeochemical processes, and not only by local microhabitat.²¹

This brief overview highlights the difficulty in predicting the effects of altitude on plant $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values as several variables such as temperature, water availability or soil type can play a role. Moreover, animal seasonal dietary preferences,^{48–50} or manipulation of the diet by the herder, can further obfuscate this relationship. In order to infer modern and past animal mobility based on isotopic variations measured in their tissues it is therefore important to build a modern reference for the region of interest. We investigated this question by analyzing the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variations in tail hair of domestic horses living in the Altai mountains of western Mongolia. We hypothesized that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variations in horse tail hair are correlated to mobility between pasture areas and altitude. The studied animals were fitted with GPS collars which allowed us to monitor their movements and to relate these observations to isotopic variations. The primary purposes of this study were (i) to test this hypothesis in a C_3 -dominated environment and (ii) decipher the effects of altitudinal mobility and other environmental and ecological factors on C and N stable isotope variations in tail hair, to better understand under which conditions these isotope tracers can give information on altitudinal mobility.

2 | EXPERIMENTAL

2.1 | Study area and sample collection

The study area is located at the northernmost part of the aimag (province) of Bayan-Ölgii in the sum (district) of Nogoonuur (Figure 1). Elevations range from 1500 to more than 4000 m asl. A north-south fault splits the landscapes in half with a lowland plain to the east and the Altai Mountains to the west. The climate is strongly continental with long, cold winters and short, hot summers. During the study period (June 2015 to July 2018), monthly temperatures averaged +18.0°C during summer (June–August) and −17.6°C during winter (December–February) with extremes ranging from +34 to −44°C. Average annual rainfall is 131 mm with a maximum of precipitation occurring during summer (meteorological station based at Nogoonuur village – Figure 1 – <http://fr.climate.org>). In the winter, there is very low snowfall and most of the time no snow cover at all. Open water is rare, and consists of few intermittent streams. The vegetation consists of semi-arid and alpine steppes and is mainly composed of grasses, with a few forbs and bushes exclusively near rivers in lowlands and valley exit. It is dominated by Asteraceae such as *Artemisia* spp., Fabaceae such as *Caragana* spp. and Poaceae, all of them using the C₃ photosynthetic pathway.

Six horses (5 males and 1 female) were equipped with Globalstar® GPS collars (Lotek Wireless Inc., Newmarket, ON, Canada) between June 2015 and July 2018 (Table 1). Collars were programmed to record their position every 13 h. They also provided altitude and temperature data. GPS monitoring lasted on average 580.5 d (min = 244, max = 896). Monitoring was interrupted for a few months for two individuals, due to technical problems (Figure S1, supporting information).

The horses belong to four different families of Kazakh-Mongolian nomadic pastoralists, with different mobility patterns. They moved freely and were only brought back to the camp occasionally, when needed. When palatable plants become scarce, horses move towards other pastures, either by themselves, or directed by the herder. Herders always keep the herd accessible in order to avoid theft and to milk the females during summer and fall. During winter, horses may be supplemented with fodder (usually harvested in August–September). It was not possible to plan in advance which animals would be supplemented. However, we recorded the periods where the surveyed animals were foddered through interviews conducted yearly with the herders. Three of the six animals were never complemented during the study period, while the other three were occasionally given some fodder for limited periods of time (Figure S1, supporting information). Tail hair from each horse was collected during immobilization operations on four different occasions (June 2015, September 2016, November 2017 and July 2018 – Table 1). Tail hairs were stored in plastic bags (MiniGrip®; Aphareta, GA, USA) until analysis.

2.1.1 | Hair sample preparation and stable isotope ratio analysis

Heavy and long tail hairs were selected and cleaned following the protocol published by O'Connell et al.⁵¹ for keratinous tissues. Each hair was sonicated twice for 10 min in a solution of methanol and chloroform (2:1, v/v), rinsed with distilled water and oven-dried overnight at 50°C. Individual hairs were serially sectioned into 7.5 mm samples in order to maximize temporal resolution while keeping enough material for C- and N-isotope analysis. The 610

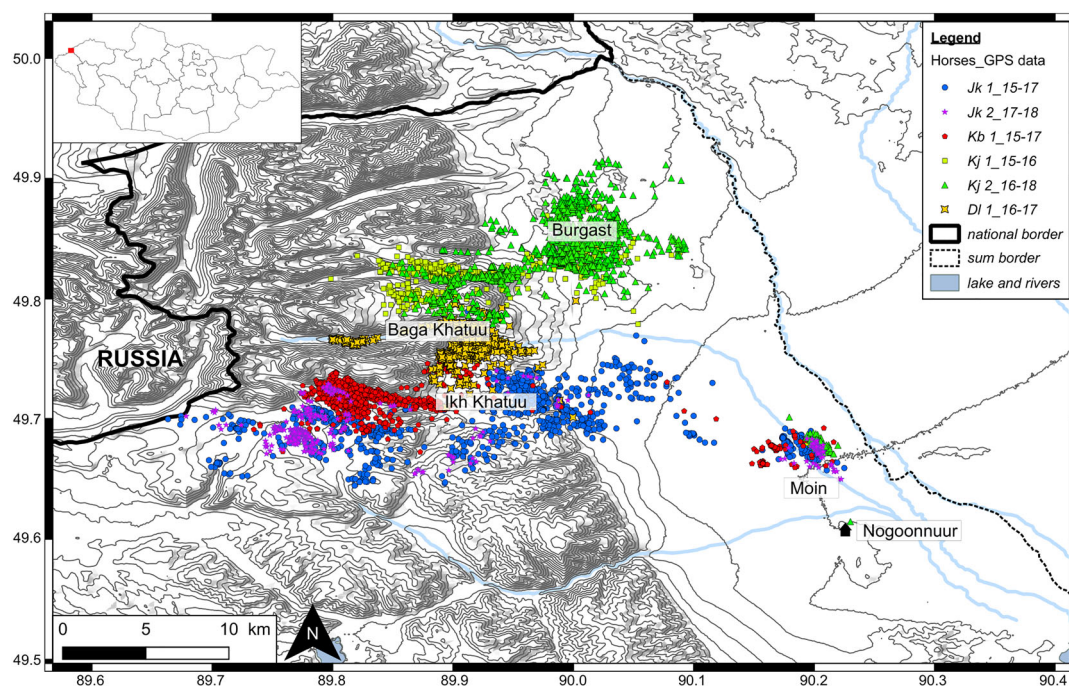


FIGURE 1 Map of the study area and its location in Mongolia (top left) with GPS fixes of the six monitored domestic horses and toponymy of interest [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Characteristics of the GPS and hair data of the six study horses with GPS-based mobility of six study horses. MCP area is the minimum convex polygon estimate of pasture area.¹ Sampled when a horse was not equipped with a GPS collar. One-way ANOVA with Tukey's post-hoc HSD was used to compare the means of altitude between horses. N = number. *** = the average altitude of the individual is significantly (p -value < 0.05) different from that of all other horses. Detailed mobility patterns are provided in Figure S1 (supporting information)

Horse id		Jk 1	Jk 2	Kb 1	Kj 1	Kj 2	DI 1
Year of birth		2010	2009–2010	2007	1998	2013	2003
Sex		M	F	M	M	M	M
Start GPS		16/06/2015	30/11/2017	17/06/2015	19/06/2015	08/09/2016	16/09/2016
End GPS		29/11/2017	31/07/2018	08/08/2017	08/09/2016	29/07/2018	26/11/2017
N days		896	244	781	446	689	427
N GPS data		1299	421	1395	771	1256	775
Hair sampling date		16/06/2015 05/09/2016 30/11/2017	30/11/2017 30/07/2018	17/06/2015 18/09/2016 19/11/2017	19/06/2015 08/09/2016	08/09/2016 27/11/2017 30/07/2018	16/09/2016 17/11/2017 23/07/2018 ¹
Altitude (m.asl)	Mean \pm SD	2115 \pm 476***	2580 \pm 477	2433 \pm 432***	2278 \pm 459***	2014 \pm 356***	2366 \pm 138***
	Max	2916.2	3215.6	3156.6	3154.2	3103.1	2813.8
	Min	1449.5	1446.8	1205.2	1468.1	1428.6	1698.0
N pasture		6	5	4	4	5	3
N pasture changes		23	5	11	14	15	4
N pasture changes/year		9.4	7.5	5.1	11.5	8	3.4
Total MCP area used (km ²)		434	237	328	104	164	65
Mean MCP area used by pasture (km ²)		27.8 \pm 24.3	27.1 \pm 24.8	21.3 \pm 17.9	11.6 \pm 6.9	24.8 \pm 24.0	14.1 \pm 11.1
Average (\pm SD) nb days on pasture		36 \pm 26	54 \pm 58	59 \pm 78	31 \pm 18	43 \pm 39	85 \pm 80

individual hair segments generated were weighed in ultralight tin capsules (mass range 151–499 μ g) and combusted in an elemental analyzer (PyroCUBE; Elementar, Hanau, Germany) coupled in continuous flow with an isotope ratio mass spectrometer from Isoprime Ltd (Cheadle Hulme, UK) for C and N stable isotope analysis. In-house standards of casein calibrated against secondary international standards from the International Atomic Energy Agency, Vienna, Austria (IAEA-CH3, IAEA-CH6, IAEA-N1, IAEA-N2) were analyzed every twelve samples as quality control. The long-term precision (SD) for the internal laboratory standard was better than 0.20‰ (C) and 0.11‰ (N). Isotope data are presented in δ notation [$\delta = (R_{\text{sample}}/R_{\text{standard}}) - 1$], with R the isotope ratio ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$) of the sample or international standard (V-PDB, V-AIR).

2.2 | Data analysis

2.2.1 | GPS-based inference of mobility

GPS data were corrected for outliers and failed GPS readings. Horse mobility was detected using the Lavielle's method,^{52,53} a method of segmentation of time series of observations implemented in the R package adehabitatHR.⁵⁴ This method discriminates series of successive locations ("segments") during which movements happen at the same spatial scale but between which movements happen at different spatial scales. This allows the determination of changes in animal movement, and hence to discriminate between small-scale movements (within each pasture, in this study) and large-scale movements among pastures. Due to the nature of the landscape and after trials (where segmentation was based on x or y coordinates),

segmentation was based on altitude and each segment was considered as a pasture, after visual validation. Pasture area was estimated as the size of the 95% MCP (Minimum Convex Polygon) of the corresponding segment (i.e. area of the smallest polygon joining 95% of the innermost locations of a segment).

2.2.2 | Isotopically based inference of mobility

Hairs may grow at different rates within and between individual horses. In order to compare isotopic and GPS data for different individuals, it was first necessary to convert hair length measurements into time (i.e. calendar dates). Growth rates (mm.d^{-1}) were determined for each individual horse by looking at overlapping patterns of stable isotope variations in the hairs collected at the different sampling periods. We divided the length between the root and the match zones of the hairs by the period of time between collection dates.⁵⁵ The apical and basal boundaries of the match zone provided respectively maximum and minimum estimates of growth rates. We assumed that the growth rate was constant during the study period.^{10,55}

The isotope ratios of horse hair ($\delta^{15}\text{N}_{\text{hair}}$ and $\delta^{13}\text{C}_{\text{hair}}$ values) were converted into diet isotopic compositions ($\delta^{15}\text{N}_{\text{diet}}$ and $\delta^{13}\text{C}_{\text{diet}}$ values) using the isotope turnover model developed by Ayliffe et al.¹⁰ This model assumes that hair isotope ratios represent dietary inputs from three pools with different half-lives (0.5, 4 and 138 d) and fraction contributions (41, 15 and 44%). We used the same turnover parameters and a 3‰ diet-hair enrichment factor for both elements.^{10,56} Moreover, hair does not immediately record the isotope composition of the new diet once formed, but only after the new hair follicle reaches the skin surface.¹¹ This time lag can vary

from 6 to 15 d depending on the animal age and species.^{11,57} In the case of horses, as the duration of this time lag had not been documented, we subtracted 7 d following Zazzo et al.¹¹

We used one-way analysis of variance (ANOVA) with Tukey's post hoc HSD to compare the means of $\delta^{15}\text{N}_{\text{diet}}$, $\delta^{13}\text{C}_{\text{diet}}$ variations between horses, between seasons and between months separately.

We investigated animal mobility from isotopic temporal variations by calculating the first derivative of carbon and nitrogen isotope compositions, $d(\delta^{13}\text{C}_{\text{diet}})$ and $d(\delta^{15}\text{N}_{\text{diet}})$ in ‰/day (Figure S2, supporting information). We considered that values of $d(\delta^{13}\text{C}_{\text{diet}})$ or $d(\delta^{15}\text{N}_{\text{diet}})$ larger than 1σ (standard deviation, estimated over the whole time-series of $d(\delta^{13}\text{C}_{\text{diet}})$ and $d(\delta^{15}\text{N}_{\text{diet}})$) indicated a significant mobility.⁷ We compared those $d(\delta^{13}\text{C}_{\text{diet}})$ - and $d(\delta^{15}\text{N}_{\text{diet}})$ -inferred mobilities with the changes of pastures inferred from the analysis of the GPS data. We considered that isotope-inferred mobility matched GPS-inferred mobility when a significant shift was simultaneous with a change of pasture inferred from the GPS data. In the case of a significant isotopic shift which covers several samples, we only considered the first sample of the series.

2.2.3 | Testing the effects of mobility and the environment on isotopic ratios

We investigated the effect of geographic and environmental variables on isotopic variations. Based on GPS data, we calculated the means of different variables over each horsehair segment: altitude, latitude, longitude, temperature; we also estimated 10-day averaged values of NDVI (Normalized Difference Vegetation Index) as a proxy of photosynthetic activity.⁵⁸ The NDVI raster had a 300m resolution, and was freely generated by the land service of Copernicus, the Earth Observation program of the European Commission.⁵⁹ In each raw raster, all flag values (Missing, Cloud/Shadow, Snow/ice, Water) were replaced with NA values. A Pearson's correlation matrix was computed with R to assess dependency between variables. This test triggered the exclusion of longitude and temperature from the predictive variables. All the other correlations were lower than 0.23. Generalized Additive Mixed Models (GAMMs) were used to model the effect of geographic and environmental factors on the spatio-temporal variations of the stable isotope composition of diet using Gaussian distribution. Non-linear relationships were used to reveal cyclic seasonal variation of $\delta^{13}\text{C}_{\text{diet}}$ and $\delta^{15}\text{N}_{\text{diet}}$ values. GAMMs were implemented in R using the package *gamm4*.⁶⁰ Models explaining $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variations of diet were fitted with the following predictor variables, Julian day (using a cyclic spline), altitude, latitude, NDVI. To account for inter-individual variations, models included 'individual' identity as a random effect. We also included an autoregressive model for errors (argument *correlation* = *corARMA* to the *gamm* function (package: *mgcv*) to account for the presence of temporal autocorrelation in the isotopic data. We rescaled data because some predictor variables were on different scales. We checked the normality of our data. We proceeded to a backward model selection based on AIC (Akaike information criterion). Histograms and QQ plots were used to

inspect residuals for normality and independence. All data analyses were conducted in R (version 3.4.4).

3 | RESULTS

3.1 | Altitudinal mobility inferred by GPS

Lavielle's segmentation of GPS data indicated that the horses moved between 3.4 and 11.5 times per year during the study period (mean = 7.5, s.d. = 2.9). The study animals spent between 31 and 85 d on each pasture (mean = 51.3 d, s.d. = 19.6, median = 30 d), and used a mean area ranging from 65 to 434 km² (mean = 221.9 km², s.d. = 140.2) (Table 1). All the horses but one (DI 1) displayed an East-West spatial movement pattern (Figure 1) with sharp altitudinal mobility between the eastern depression (1205–1468 m asl) and the western mountain pastures (up to 2800–3215 m asl). Horse DI 1 remained at a high altitude of 2366 ± 138 m asl during the study period (see Figure S1, supporting information, for detailed data).

3.2 | Tail hair carbon and nitrogen isotope ratios

The tail hair growth rate varied between 0.50 and 0.85 mm.d⁻¹ with a mean of 0.69 ± 0.11 mm.d⁻¹ (see Table S1, supporting information). As a result, the 7.5 mm increments taken on each tail hair represented an average period of 11.1 ± 1.9 d (ranging from 8.8 to 15 d).

Summary statistics are given in Table S1 (supporting information). The $\delta^{13}\text{C}_{\text{diet}}$ values were not statistically different among horses (Tukey HSD p-value >0.05). The intra-individual variability ranged between 3.2‰ (DI 1) and 6.0‰ (Kb 1) (Table S1, supporting information). We observed a very good agreement in $\delta^{13}\text{C}_{\text{diet}}$ values between different hair from the same individual (Figure 2) and limited ($\leq 2\%$) differences between hair from different horses from the same herder (Jk 1 vs Jk 2; Kj 1 vs Kj 2). Overall, the annual variability in $\delta^{13}\text{C}_{\text{diet}}$ values was 6.0‰ (between -30.2‰ and -24.2‰, Table S1, supporting information). The summer $\delta^{13}\text{C}$ values were significantly lower than those in the other seasons ($-27.9 \pm 0.7\%$; p-value <0.001; $n = 149$; 21 June to 2 September), with a minimum value in August ($-28.3 \pm 0.7\%$, p-value <0.05, $n = 37$ – results not shown). On average, winter showed the highest $\delta^{13}\text{C}$ values ($-27.2 \pm 0.6\%$) but these values did not significantly differ from those in spring ($-27.3 \pm 1.0\%$) and autumn ($-27.4 \pm 0.8\%$).

There was no statistical difference in $\delta^{15}\text{N}_{\text{diet}}$ values between the different individuals (Tukey HSD; p-value >0.05). There was a good agreement in N-isotope ratios between hair from the same individuals (Jk 1, Kb 1; DI 1, Kj 2 – Figure 3) and also between different individuals of the same herd. Intra-individual variability of $\delta^{15}\text{N}_{\text{diet}}$ values varied between 4.6‰ (Jk 1) and 10.6‰ (DI 1). Overall, the annual variability in $\delta^{15}\text{N}$ values was 10.6‰ (between -2.1‰ and +8.5‰; Table S1, supporting information). The N-isotope composition was not statistically different between seasons. The lowest $\delta^{15}\text{N}_{\text{diet}}$ values were recorded in July ($+2.1 \pm 1.8\%$, p-value

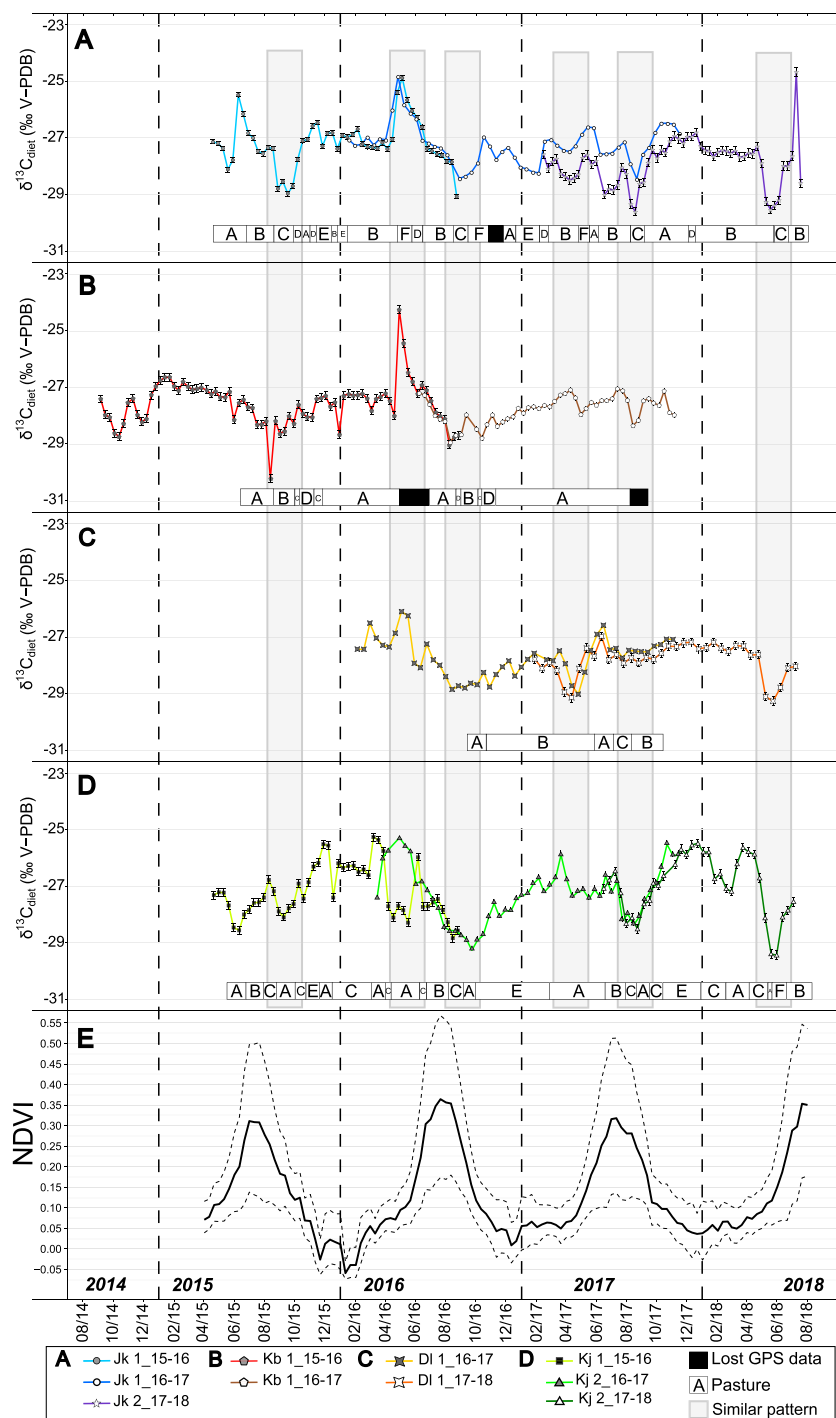


FIGURE 2 Diet carbon isotope profiles of tail hair of six GPS-monitored horses. Each panel represents a different herder (A–D), each color a different tail hair and each symbol a different horse. To ease comparison, pastures identified by Lavielles segmentation of altitudinal data are also reported, with letters at the bottom of each panel (one letter = one pasture). E, Mean NDVI (black line) temporal variations in the study area, with standard deviation (dashed line). Grey shadings represent similar patterns in several hair profiles [Color figure can be viewed at wileyonlinelibrary.com]

<0.05 , $n = 55$ – results not shown) and were surrounded by the highest $\delta^{15}\text{N}_{\text{diet}}$ values, measured in May and September.

3.3 | Correlation between isotopic shifts and altitudinal mobility

In total, GPS tracking allowed the detection of 80 altitudinal mobilities; 53 significant carbon isotopic shifts were identified for all horses (Table 2), including 18 that matched a change of pasture inferred

from the GPS data. The analysis of $d(\delta^{13}\text{C}_{\text{diet}})$ values hence allowed us to detect 22.5% of the 80 changes of pasture inferred from the GPS data for all the horses, ranging from 0% (Jk 2) to 34.8% (Jk 1) (Table 2). The analysis of $d(\delta^{15}\text{N}_{\text{diet}})$ values also identified 54 significant isotopic shifts for all horses; 16 (20%) shifts were linked with an altitudinal mobility inferred from the GPS data for all the horses, ranging from 0% (DI 1) to 38.5% (Kj 1). The C- and N-isotopic shifts were synchronous on 14 occasions. The combination of the $d(\delta^{13}\text{C}_{\text{diet}})$ and $d(\delta^{15}\text{N}_{\text{diet}})$ values allowed the detection of 38.8% of the 80 changes of pasture recorded in all individuals,

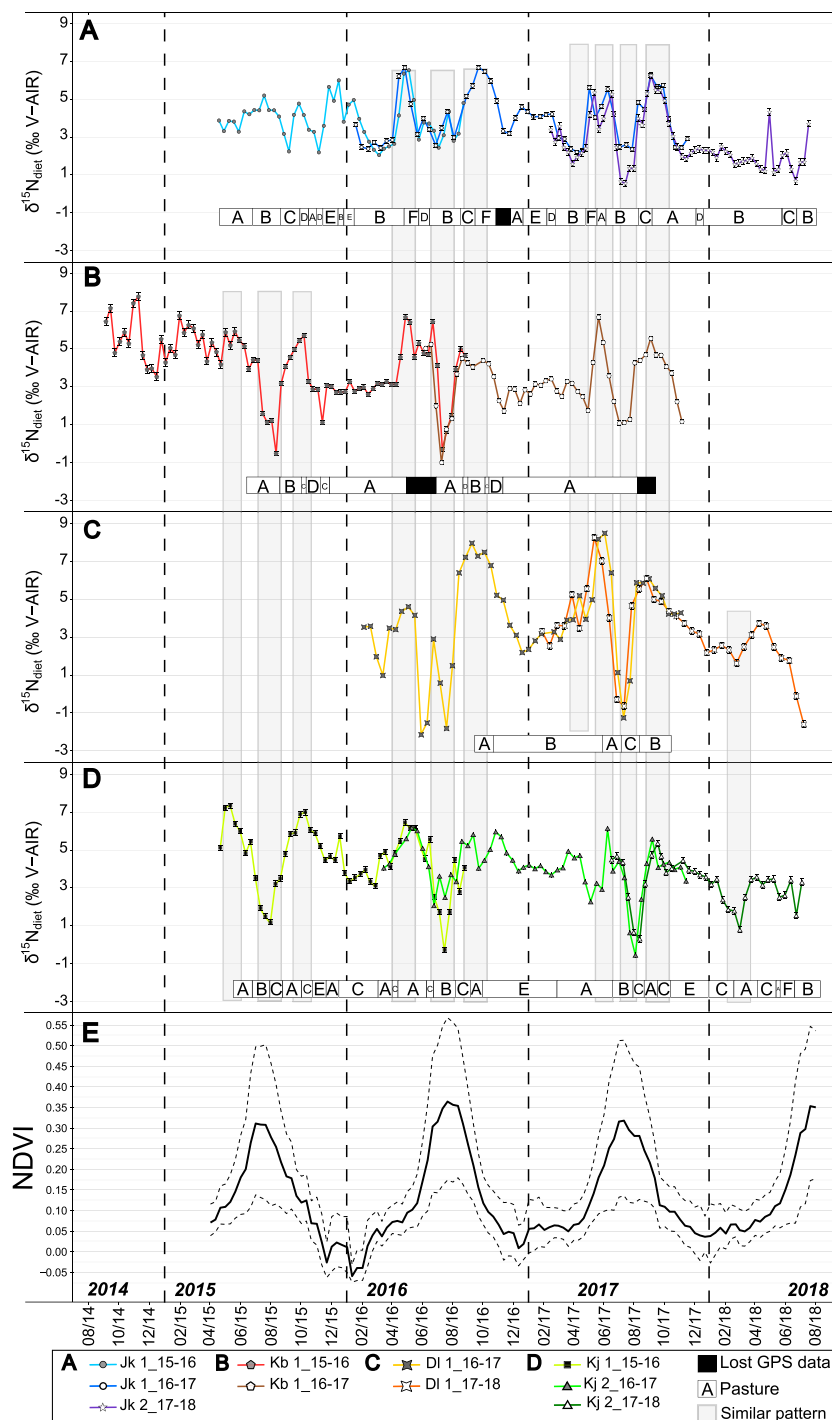


FIGURE 3 Diet nitrogen isotope profiles of tail hair of six GPS-monitored horses. Each panel represents a different herder (A–D), each color a different tail hair and each symbol a different horse. To ease comparison, pastures identified by Lavielles segmentation of altitudinal data are also reported, with letters at the bottom of each panel (one letter = one pasture). E, Mean NDVI (black line) temporal variations in the study area, with standard deviation (dashed line). Grey shadings represent similar patterns in several hair profiles [Color figure can be viewed at wileyonlinelibrary.com]

varying individually from 18.2% (Jk 2) to 69.2% (Kj 1). Among the 107 isotopic shifts detected by either C- or N-isotope ratios, 76 (71%) were not linked with an altitudinal mobility.

3.4 | Factors influencing carbon and nitrogen stable isotope ratios

The stable isotope ratios measured along tail hairs and their corresponding date, latitude, longitude, altitude, NDVI and

temperature are provided in Table S2 (supporting information). The AIC scores of the different GAMM models are provided in Table S3 (supporting information). The autoregressive model for temporal autocorrelation was not selected in the final models.

The best GAMM model investigating variation in $\delta^{13}\text{C}_{\text{diet}}$ values in tail hair included Julian day, Latitude and NDVI as the only predictors (Table 3). The cyclic smooth term on Julian day was significant (Figure 4A, Table 3). The predicted values of Julian day on the $\delta^{13}\text{C}_{\text{diet}}$ values showed a strong annual cycle (Figure 4A) with inter-annual variability. This seasonal cycle was characterized by a rapid

TABLE 2 Summary of isotopic vs GPS inferred mobility. Each ratio represents the number of mobilities inferred through a significant isotopic shift over the number of changes of pasture inferred by GPS tracking. The number of significant isotopic shifts not associated with a change of pasture is also given. The analysis was done for each horse and for each herder (who had more than one horse monitored). Detailed results are provided in Figure S2 (supporting information)

Horse	Significant shift of $\delta^{13}\text{C}$ values linked with mobility	Significant shift of $\delta^{15}\text{N}$ values linked with mobility	Significant shift of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values linked with mobility	Number of significant isotopic shift not linked with change of pasture	
				For $\delta^{13}\text{C}$ values	For $\delta^{15}\text{N}$ values
Jk 1	8/23 (34.8%)	3/23 (13.0%)	9/23 (39.1%)	4	13
Jk 2	0/11 (0%)	1/11 (9.1%)	1/11 (9.1%)	7	9
Kb 1	2/10 (20%)	3/10 (30%)	4/10 (36.4%)	6	4
DI 1	1/4 (25%)	0/4 (0%)	1/4 (25%)	2	3
Kj 1	4/13 (30.8%)	5/13 (38.5%)	9/13 (69.2%)	6	3
Kj 2	3/19 (15.8%)	4/19 (21.1%)	7/19 (36.8%)	10	6
Total	18/80 (22.5%)	16/80 (20%)	31/80 (38.8%)	35	38
Total without pastures < 11 days	17/71 (23.9%)	14/71 (19.7%)	29/71 (40.8%)	-	-
Herder					
Jk	8/29 (27.6%)	4/29 (13.8%)	10/29 (34.5%)	11	18
Kj	6/27 (22.2%)	9/27 (33.3%)	14/27 (51.9%)	16	9

TABLE 3 Summary of GAMMs investigating the effects of geographical, temporal and environmental variability in $\delta^{13}\text{C}_{\text{diet}}$ and $\delta^{15}\text{N}_{\text{diet}}$ values. The estimates and significance of terms are shown for the best model fit

Model terms	Parametric coefficients				Approximate significance of smooth terms				R ²	AIC
	Estimate	SE	t	p	Df	Rank	F	p		
δ ¹³ C _{diet}										
Intercept	−0.05	0.15	−0.33	0.74					0.47	708.4
Latitude	0.28	0.12	−2.41	0.02						
NDVI	−0.08	0.10	−0.83	0.41						
s (Julian date, k = 30)					19.47	19.47	10.02	<2 ^e -16		
δ ¹⁵ N _{diet}										
Intercept	−0.02	0.14	−0.18	0.86					0.45	702.18
Altitude	−0.19	0.06	−2.93	0.004						
NDVI	−0.02	0.10	−0.24	0.81						
s (Julian date)					27.64	27.64	5.11	5.7e-14		

summer (June–September) decrease followed by a slow autumn to spring increase in $\delta^{13}\text{C}_{\text{diet}}$ values, with the exception of winter 2017/2018 where the increase in $\delta^{13}\text{C}_{\text{diet}}$ values was rapid. Finally, $\delta^{13}\text{C}_{\text{diet}}$ values were positively correlated with latitude (Figure 4B) and negatively correlated with NDVI (Figure 4C).

The best GAMM model investigating variation of $\delta^{15}\text{N}_{\text{diet}}$ values in the tail hairs included 'Julian day', altitude and NDVI as predictors (Table 3). The cyclic smooth term on 'Julian day' was significant (Figure 5A, Table 3). The predicted values of $\delta^{15}\text{N}_{\text{diet}}$ showed an annual cycle (Figure 5A) with inter-annual variability. This annual cycle was characterized by important summer variations (amplitude ~2–3‰), with a rapid decrease from June to mid-summer and followed by a strong increase from mid-summer to September. Winter and autumn were mainly characterized by low $\delta^{15}\text{N}_{\text{diet}}$ values with little variations. The predicted effect of NDVI on $\delta^{15}\text{N}_{\text{diet}}$ values was weak (Figure 5C). Finally, $\delta^{15}\text{N}_{\text{diet}}$ values were negatively correlated with altitude (Figure 5B, Table 3).

4 | DISCUSSION

The C and N stable isotope ratios of tail hair recorded several significant variations over the study period. However, less than 40% of the animal altitudinal movements were correlated to either C- or N-isotopic shifts, and the majority (71%) of the significant isotopic shifts were not correlated with altitudinal mobility. A significant isotopic change is hence not necessarily related to altitudinal mobility. Moreover, despite differences in the horse mobility histories, we observed similarities in their pattern of C- and N-isotope variations (Figures 2 and 3), suggesting that factors other than altitude alone, such as weather events, influence plant isotopic composition. Despite this, a negative correlation was found between $\delta^{15}\text{N}_{\text{diet}}$ values and altitude, including the one horse (DI 1) with a reduced vertical mobility (<200 m) (Figure 5B). In the discussion below, we will first focus on the potential error on growth rate, which can affect the time assignment of our data. We will then

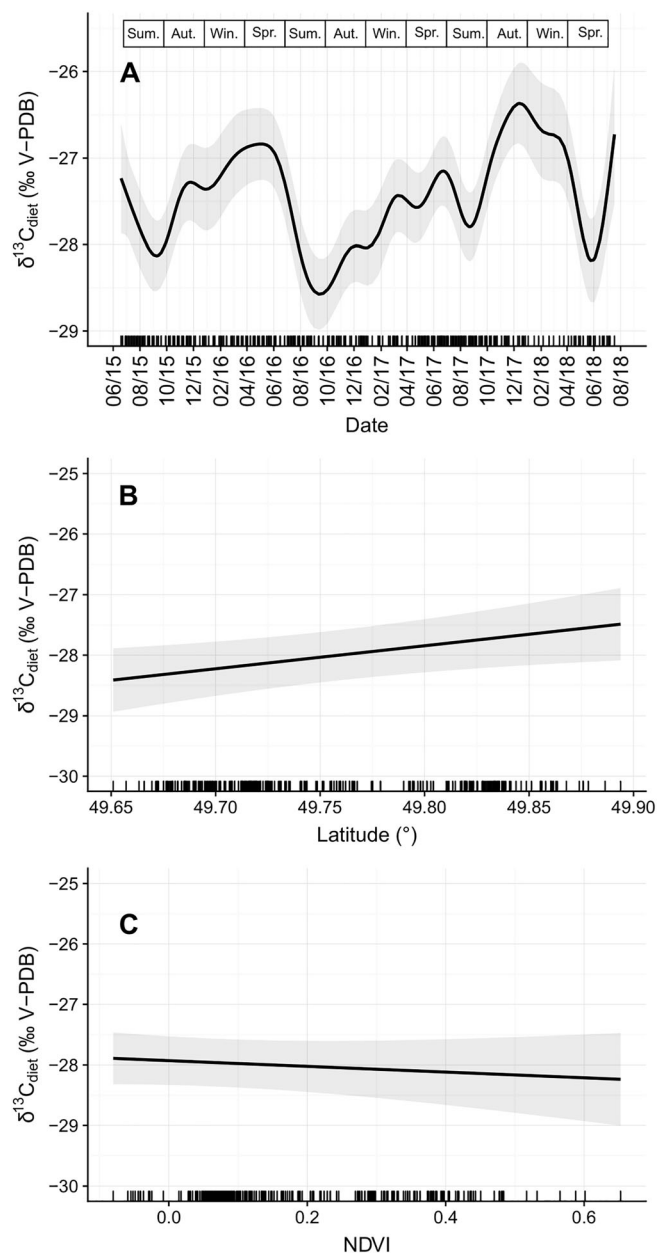


FIGURE 4 Smoother plots showing the relationships between predicted of $\delta^{13}\text{C}_{\text{diet}}$ values from the GAMM model and Julian day (A – Smooth term), latitude (B) and NDVI (C). Grey shadings represent the 95% confidence interval. Tick marks at the bottom of each graph indicate sample points

discuss the different factors driving change in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of tail hair.

4.1 | Time assignment and resolution of the isotopic record

We measured a mean tail hair growth rate of $0.69 \pm 0.11 \text{ mm.d}^{-1}$ (Table S2, supporting information). This range of values is similar to those previously reported in domestic horses or wild equids^{8,10,61–63}. Potential variability in the growth rate of a unique hair could reduce

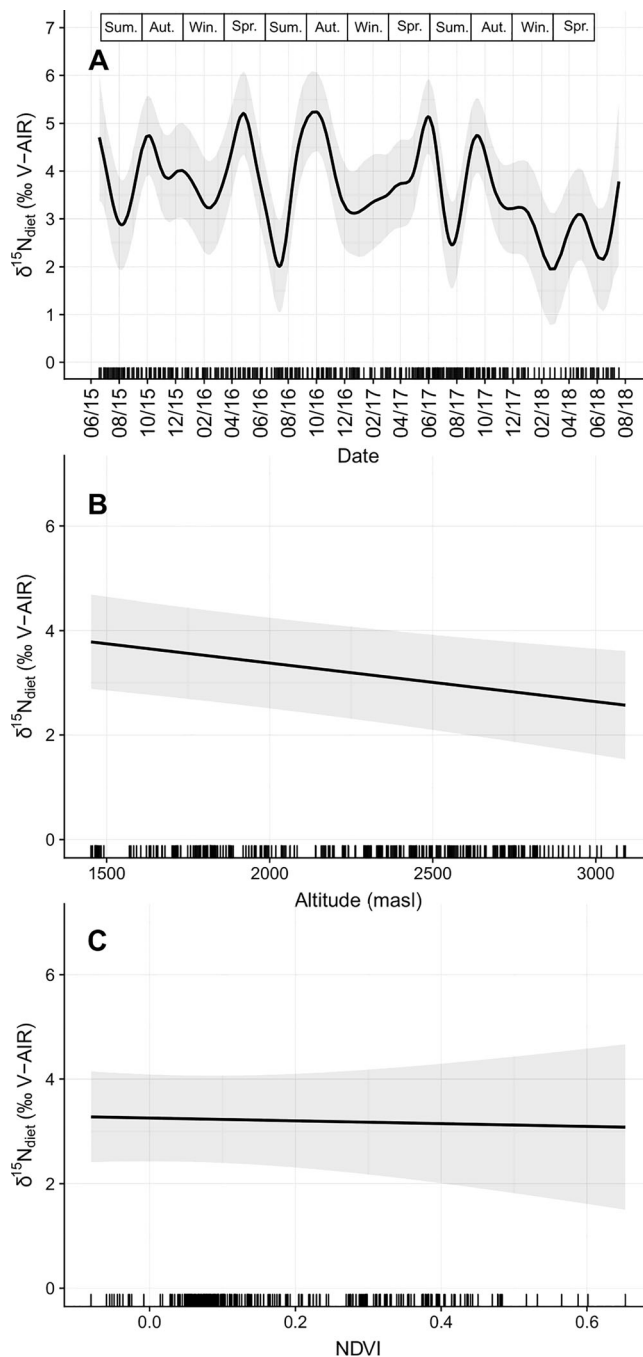


FIGURE 5 Smoother plots showing the relationships between predicted of $\delta^{15}\text{N}_{\text{diet}}$ values from the GAMM model and Julian day (A – Smooth term), altitude (B) and NDVI (C). Grey shadings represent the 95% confidence interval. Tick marks at the bottom of each graph indicate sample points

the temporal precision of chronologies, given the high frequency of mobility of some individuals, as well as the correlation between different hairs, dampening the peaks of existing seasonal patterns. Auerwald et al⁶⁴ showed that season and animal age had an impact on the tail hair growth of cattle. They found that the growth rate was slower in winter than in summer and decreased with increasing age. This is in contradiction to the results of other studies showing

that hair growth rate could be considered as constant over a year with no clear seasonal signal^{8,55,62}. In our study, growth rates were calculated independently for each hair. We observed an agreement between the isotopic profiles from different individuals from the same herd (and the same mobility pattern e.g. Jk 1 and Jk 2), but also for horses from different herds (Figures 2 and 3). This suggests that the assumption of a constant growth rate at the individual hair level is reasonable, regardless of the time of year or the age of the animal, and that this parameter has little impact on the temporal accuracy of our time line.

In addition to hair growth rate, the temporal resolution of our isotopic records (i.e. the timespan represented by each hair fragment) is mainly determined by the length of each fragment. Because a minimum sample mass is needed for isotopic analyses, the fragment length chosen was ~7.5 mm. The variability in growth rate between hairs of the same individuals⁸ precluded the combination of multiple hairs (which would have allowed a reduction of fragment length for the same mass). However, the duration of the stay in most pastures (median = 30 d) was larger than the isotopic resolution (11.1 ± 1.9 d) and only nine pastures were used for a duration shorter than or equal to 11 d. The removal of these short-term pastures only marginally increased the proportion of pasture changes identified by isotopic shifts (Table 2). We are therefore confident that the temporal resolution of our isotopic records was appropriate to detect pasture changes.

4.2 | Environmental control on variations in hair $\delta^{13}\text{C}$ values

We did not find any influence of altitude on the variation in $\delta^{13}\text{C}_{\text{diet}}$ values. This result is somewhat surprising because several studies have shown the existence of an altitudinal gradient in the carbon isotopic composition of plants.^{13,34,38,40-42} However, this is in good agreement with Burnik-Sturm et al.¹⁴ (Table S2, supporting information, and personal communication, May 13, 2019), who did not find any altitudinal effect on the $\delta^{13}\text{C}$ values of C_3 plants (*Artemisia* sp. and *Stipa* sp. – from 1600 to 2800 m. asl) in the Dzungarian Gobi in south-western Mongolia. In arid and semi-arid regions, drought stress is the most significant factor controlling plants $\delta^{13}\text{C}$ values, with higher drought stress in lowland than in high altitude, leading to a decrease in plant $\delta^{13}\text{C}$ values with altitude.^{40,41,65} We propose that animal feeding ecology could mask the relationship between plant $\delta^{13}\text{C}$ values and altitude. Indeed, the horse's diet is very selective and can vary seasonally.^{48,66} It is mainly composed of grasses, but can also include trees and shrubs. When domestic horses were grazing in lowland pastures, they remained preferentially near rivers or humid areas (i.e. Moin in Figure 1) and had access to grasses, trees and shrubs. On the contrary, when at high altitude they only had access to grasses. The relationship between plant $\delta^{13}\text{C}$ values and altitude seems less pronounced for grasses than for trees or forbs, and this could also explain our results.³³ Finally, the observed influence of latitude on $\delta^{13}\text{C}_{\text{diet}}$ values could also be explained by local differences

in water availability between the northern and southern ends of the lowland areas. Indeed higher $\delta^{13}\text{C}_{\text{diet}}$ values (mean = $-27.1 \pm 0.1\text{‰}$) were measured for the two horses (Kj 1 and Kj 2; mean latitude = $49.83 \pm 0.03^\circ$) which spent more time near Burgast, than for the other four horses (mean = $-27.6 \pm 0.3\text{‰}$ – Table S1, supporting information) which were located further south in Moin (mean latitude = $49.71 \pm 0.03^\circ$).

Horse tail hairs showed a cyclic seasonal pattern characterized by high winter and low summer $\delta^{13}\text{C}_{\text{diet}}$ values (Figure 4A). This pattern has been described elsewhere in the literature, and is often interpreted as reflecting a seasonal change in the relative proportion of C_3 vs C_4 plants in the diet, either through direct consumption of perennial C_4 plants during winter,^{14,29} C_4 winter foddering,^{30,67,68} or because of animal altitudinal mobility and the decrease in the relative abundance of C_4 taxa with increasing elevation.^{26,67,69,70} Here, the average $\delta^{13}\text{C}_{\text{diet}}$ values range between -30.2‰ and -24.2‰ , suggesting that the horses' diet was essentially composed of C_3 plants as expected for a mountainous steppe environment in central Asia.^{24,71} Thus, we cannot invoke the influence of C_4 plants in the diet to explain the seasonal pattern observed in the domestic horses of the Mongolian Altai. In a C_3 environment, several factors influence the carbon isotopic composition of plants. The C-isotope ratios of C_3 plants vary on a seasonal scale (1–2‰), with the highest values occurring during the warm/dry season and the lowest values occurring during the wet/cold season.⁷²⁻⁷⁴ In a semi-arid environment, like the Eurasian steppe, water availability is the main factor controlling plant $\delta^{13}\text{C}$ values, with more negative values associated with higher water availability.^{23,42,75-78} Indeed, during periods of water stress, C_3 plants limit water losses by closing their stomata involving a decrease in CO_2 fixation, leading to a ^{13}C enrichment of the leaves.⁷⁸⁻⁸¹ When water availability increases, water use efficiency decreases and this results in a decrease in leaf $\delta^{13}\text{C}$ values.^{23,79,80} In western Mongolia, most of the precipitations occur during summer and it is accompanied by snowmelt from high altitude, which begins in late spring at the same time as the growing season (NDVI – Figure 2E). This could explain the minimum $\delta^{13}\text{C}_{\text{diet}}$ values recorded in summer. Moreover, during periods of active growth, photosynthesis is more efficient at using the ^{13}C isotope which causes an increase in $\delta^{13}\text{C}$ values.⁷⁹ The increase observed in April/May, simultaneous with rapid increase of NDVI, before summer precipitation, could therefore be explained by this mechanism. The increase in $\delta^{13}\text{C}_{\text{diet}}$ values during winter cannot be explained by the lack of water availability, as there is no more photosynthesis (NDVI near 0 – Figure 2E). A strong decrease in photosynthetic capacity occurs during plant senescence. No difference in $\delta^{13}\text{C}$ values between senescent leaves and green leaves has been established so far; thus, plant senescence (observed in winter) cannot explain this pattern either.^{82,83} However, different parts of a plant may differ in their $\delta^{13}\text{C}$ values,^{84,85} with roots and stems being more ^{13}C -enriched than leaves.⁸⁵ During winter, horses cannot be as selective as they are during summer and possibly have to eat the stems by digging through snow with their sharp and strong hooves, hence potentially explaining the increase in $\delta^{13}\text{C}_{\text{diet}}$ values during this period.

We also observed inter-annual variability in $\delta^{13}\text{C}_{\text{diet}}$ values with a larger amplitude in 2016 than in 2017 (Figure 4A). If water availability is the main factor of variations in $\delta^{13}\text{C}$ values, these results should translate into inter-annual variations in the precipitation patterns with more precipitation in 2016 (larger ^{13}C depletion in 2016 than in 2017). The amount of rain at the Nogoonnur station was not larger in 2016 (118 and 169 mm in 2016 and 2017, respectively – available at: <http://www.infoclimat.fr>), but snow cover was much higher in March 2016 than in March 2017 (995 mm vs 5 mm, respectively). This difference in snowfalls could lead to higher water input into rivers and soils during summer 2016 and ultimately to lower plant $\delta^{13}\text{C}$ values not only during summer, but also in the following winter. Interviews with herders confirmed this inter-annual difference in climate and vegetation growth between the two years (data not shown). However, we are aware that the survey period is too short to build strong inferences and that more work would be needed to ascertain this conclusion.

4.3 | Environmental control on seasonal variation in hair $\delta^{15}\text{N}$ values

The large and synchronous variations measured in tail hair $\delta^{15}\text{N}$ values indicate that this tissue is able to record seasonal variation in plant isotopic composition at the regional scale. Plant $\delta^{15}\text{N}$ values are highly sensitive to variations in environmental factors and plant physiology, such as water and N availability, pathway of N assimilation, source of nitrogen and whether N is acquired directly from the soil or through symbiotic relationships with mycorrhiza. As a result, $\delta^{15}\text{N}$ values can vary drastically between different microhabitats.^{21,45,84,86} The water input from snowmelt occurs during late spring and early summer and is synchronous with the start of the growing season (NDVI – Figure 3E). Snowmelt may bring an important water supply in soil, increasing water at both valley bottoms and rich pastures soils at higher altitude. Increased water availability in soils increases soil denitrification and N loss, driving the overall ecosystem $\delta^{15}\text{N}$ values upwards. This could explain the increase in $\delta^{15}\text{N}$ values measured in spring, synchronous with the decrease in $\delta^{13}\text{C}_{\text{diet}}$ values. In turn, the general decrease in tail hair $\delta^{15}\text{N}$ values between October and December could be explained by soil mineralization and nitrification in relation with a decrease in water availability.^{77,87} It is noteworthy that the observed ^{15}N enrichment in late winter/early spring could also be explained by animal weight loss during this time period,^{88,89} but since we did not monitor this parameter it is not possible to test this hypothesis.

The sharp (ca 3–5 per mil) decrease in $\delta^{15}\text{N}$ values, observed for all animals in May–June (followed by an increase of the same magnitude in July–August), is somewhat more complicated to explain. In July, all the animals were grazing in high altitude pastures, and the low $\delta^{15}\text{N}$ values measured are responsible for the negative correlation between $\delta^{15}\text{N}_{\text{diet}}$ values and altitude (Figure 5B) in line with conclusions from other studies.^{13,39,42,90} However, this spike is

also recorded for the one horse (DI 1) with no or very limited vertical mobility (<200 m) and is rapidly (ca 1 month later) followed by a sharp increase in $\delta^{15}\text{N}_{\text{diet}}$ values that is not observed in $\delta^{13}\text{C}$ values.

Climatic factors are not likely to explain this phenomenon because it is too rapid and it would also affect plant $\delta^{13}\text{C}$ values. Therefore, this isotope spike, which occurs when pasture productivity is at its highest (Figure 3E), seems more related to the fact that all animals had access to a different type of plants for a limited period of time in June–July rather than to mobility between different pastures itself. In order to explain this abrupt decrease in diet $\delta^{15}\text{N}$ values, we propose that the horses had access to N_2 -fixing plants (e.g. such as Fabaceae) during early-summer as the $\delta^{15}\text{N}$ values of plants that rely on N_2 fixation are usually near 0‰ reflecting atmospheric isotopic ratios.²¹ Preferential consumption of plants of the Fabaceae family during summer has been observed in the Przewalski's horse living in eastern Europe.⁴⁸ It is noteworthy that several species belonging to the N_2 -fixing plant family Fabaceae, such as *Caragana spinosa*, *Caragana bungei* or *Caragana pygmae*, were identified in the study area (preliminary identification by Prof. Oyuntsetseg Batlai, personal communication). Isotopic analysis of four *Caragana* sp. samples taken during the June 2015 fieldtrip ($\delta^{15}\text{N} = 0.8 \pm 1.8\text{‰}$) confirms their N_2 -fixing status. Other Fabaceae, such as *Medicago* sp., *Thermopsis* sp., *Glycyrrhiza* sp. and *Oxytropis aciphylla*, are also consumed by horses in summer.^{91,92} *Medicago* sp. (clover) is abundantly consumed by horses, and can be found in the Mongolian Altai.^{91,92} N-isotope ratios close to zero are measured in every horse, and, thus, an important increase in the proportion of N_2 -fixing plants in the horse's diet during a short period of maximum productivity could explain the decrease observed in tail hair $\delta^{15}\text{N}$ values. Direct observation of the horse dietary preferences would be necessary to confirm this hypothesis.

5 | CONCLUSIONS

Despite the fact that Mongolian horse tail hair exhibits seasonal variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, few significant isotopic shifts matched with changes of pasture, showing that a significant isotopic change is not necessarily related to an altitudinal mobility in the strong continental climate and C_3 -dominated environment of western Mongolia. At first order, we propose that variations in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were influenced by seasonal change in soil moisture rather than altitudinal mobility or winter foddering as proposed in the literature. We also found a correlation between the $\delta^{15}\text{N}$ values of tail hair and altitude but because a similar pattern of low summer $\delta^{15}\text{N}$ values was found for a horse with no or very little altitudinal mobility, we propose that short-term diet switches occurring at high altitude rather than altitudinal mobility *per se* are responsible for the observed N-isotope shift. Hair has received increased interest as an archive of past animal life cycle. Because of its extensive management system, the Mongolian horse is a good animal model to

study past herding practices provided that the complexity of the factors driving the isotope record is not overlooked.

ACKNOWLEDGEMENTS

The authors are deeply indebted to all the herders who kindly participated in the study and accepted to host two of us (AZ and CM) during the 2017 winter and 2018 summer fieldtrips. They are thankful to Prof O. Batlai (Natl Univ Mongolia) for her help with plant identification. They would like to thank A. Pinhède (Direction de l'Archéologie de Chartres Métropole.) who created the map of the study area and Ts. Turbat (Institute of History and Archaeology, Mongolian Academy of Sciences) for the logistics and assistance during the fieldwork. They are grateful to K. Auerswald and two anonymous reviewers whose constructive comments helped to improve the manuscript. Fieldwork was supported by the French archaeological mission in Mongolia (CNRS, MNHN, MAEDI, director: SL) and by the CNRS. This research was funded by a Ph.D. grant to NL from the French National Research Agency LabEx ANR-10-LABX-0003-BCDiv, in the context of the "Investissements d'avenir" n°ANR-11-IDEX-0004-02 and an Inalco Early Career Research Grant 2017 to CM.

ORCID

Nicolas Lazzzerini  <https://orcid.org/0000-0003-3811-935X>

REFERENCES

- Wright, J, Makarewicz C. Perceptions of pasture: the role of skill and networks in maintaining stable pastoral nomadic systems in Inner Asia. In: Kerner S, Dann R, Jensen PB eds. *Climate and Ancient Societies*. Copenhagen: Mus.Tusculanum Press; 2015:267-288.
- Finke P. Le pastoralisme dans l'ouest de la Mongolie: contraintes, motivations et variations. *Cah D'Asie Cent*. 2004;(11/12):245-265.
- Kerven C, Robinson S, Behnke R, Kushenov K, Milner-Gulland EJ. Horseflies, wolves and wells: Biophysical and socio-economic factors influencing livestock distribution in Kazakhstan's rangelands. *Land Use Policy*. 2016;52:392-409. <https://doi.org/10.1016/j.landusepol.2015.12.030>
- Lkhagvadorj D, Hauck M, Dulamsuren C, Tsogtbaatar J. Pastoral nomadism in the forest-steppe of the Mongolian Altai under a changing economy and a warming climate. *J Arid Environments*. 2013;88:82-89. <https://doi.org/10.1016/j.jaridenv.2012.07.019>
- Marchina C. *Nomad's Land. Éleveurs. Animaux et Paysage Chez Les Peuples Mongols*. Bruxelles: Zones sensibles; 2019.
- Ventresca Miller AR, Makarewicz CA. Isotopic approaches to pastoralism in prehistory: Diet, mobility, and isotopic reference sets. In: *Isotopic Investigations of Pastoralism in Prehistory*. London: Routledge; 2017:9-22 <https://doi.org/10.4324/9781315143026-1>.
- Cerling TE, Wittemyer G, Ehleringer JR, Remien CH, Douglas-Hamilton I. History of animals using isotope records (HAIR): A 6-year dietary history of one family of African elephants. *Proc Natl Acad Sci*. 2009;106(20):8093-8100.
- West AG, Ayliffe LK, Cerling TE, et al. Short-term diet changes revealed using stable carbon isotopes in horse tail-hair. *Funct Ecol*. 2004;18(4):616-624.
- Hobson KA. Applying isotopic methods to tracking animal movements. In: Hobson KA, Wassenaar LJ eds. *Tracking Animal Migration with Stable Isotopes*. Vol. 2. Terrestrial Ecology. London, UK: Academic Press; 2008:45-78.
- Ayliffe LK, Cerling TE, Robinson T, et al. Turnover of carbon isotopes in tail hair and breath CO₂ of horses fed an isotopically varied diet. *Oecologia*. 2004;139(1):11-22.
- Zazzo A, Moloney AP, Monahan FJ, Scrimgeour CM, Schmidt O. Effect of age and food intake on dietary carbon turnover recorded in sheep wool. *Rapid Commun Mass Spectrom*. 2008;22(18):2937-2945. <https://doi.org/10.1002/rcm.3693>
- Zazzo A, Harrison SM, Bahar B, et al. Experimental determination of dietary carbon turnover in bovine hair and hoof. *Can J Zool*. 2007;85(12):1239-1248.
- Männel TT, Auerswald K, Schnyder H. Altitudinal gradients of grassland carbon and nitrogen isotope composition are recorded in the hair of grazers. *Glob Ecol Biogeogr*. 2007;16(5):583-592. <https://doi.org/10.1111/j.1466-8238.2007.00322.x>
- Burnik Šturm M, Ganbaatar O, Voigt CC, Kaczensky P. Sequential stable isotope analysis reveals differences in dietary history of three sympatric equid species in the Mongolian Gobi. *J Appl Ecol*. 2016;54(4):1110-1119. <https://doi.org/10.1111/1365-2664.12825>
- Iacumin P, Davanzo S, Nikolaev V. Spatial and temporal variations in the ¹³C/¹²C and ¹⁵N/¹⁴N ratios of mammoth hairs: Palaeodiet and palaeoclimatic implications. *Chem Geol*. 2006;231(1-2):16-25. <https://doi.org/10.1016/j.chemgeo.2005.12.007>
- Raich JW, Russell AE, Vitousek PM. Primary productivity and ecosystem development along an elevational gradient on Mauna Loa, Hawai'i. *Ecology*. 1997;78(3):707-721. [https://doi.org/10.1890/0012-9658\(1997\)078\[0707:PPAEDA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1997)078[0707:PPAEDA]2.0.CO;2)
- Zhang Y, Qi W, Zhou C, et al. Spatial and temporal variability in the net primary production of alpine grassland on the Tibetan plateau since 1982. *J Geogr Sci*. 2014;24(2):269-287. <https://doi.org/10.1007/s11442-014-1087-1>
- Drewnik M. The effect of environmental conditions on the decomposition rate of cellulose in mountain soils. *Geoderma*. 2006;132(1):116-130. <https://doi.org/10.1016/j.geoderma.2005.04.023>
- Coûteaux MM, Sarmiento L, Bottner P, Acevedo D, Thiéry JM. Decomposition of standard plant material along an altitudinal transect (65-3968m) in the tropical Andes. *Soil Biol Biochem*. 2002;34(1):69-78. [https://doi.org/10.1016/S0038-0717\(01\)00155-9](https://doi.org/10.1016/S0038-0717(01)00155-9)
- Wang G, Jia Y, Li W. Effects of environmental and biotic factors on carbon isotopic fractionation during decomposition of soil organic matter. *Sci Rep*. 2015;5(1):11043. <https://doi.org/10.1038/srep11043>
- Craine JM, Elmore AJ, Aida MPM, et al. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist*. 2009;183(4):980-992. <https://doi.org/10.1111/j.1469-8137.2009.02917.x>
- O'Leary MH. Carbon isotopes in photosynthesis. *Bioscience*. 1988;38(5):328-336. <https://doi.org/10.2307/1310735>
- Kohn MJ. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo) ecology and (paleo) climate. *Proc Natl Acad Sci*. 2010;107(46):19691-19695.
- Pyankov VI, Gunin PD, Tsoog S, Black CC. C4 plants in the vegetation of Mongolia: Their natural occurrence and geographical distribution in relation to climate. *Oecologia*. 2000;123(1):15-31.
- Ehleringer JR. The influence of atmospheric CO₂, temperature, and water on the abundance of C3/C4 taxa. In: *A History of Atmospheric CO₂ and Its Effects on Plants, Animals, and Ecosystems*. New York, NY: Springer; 2005:214-231.

26. Makarewicz CA, Arbuckle BS, Öztan A. Vertical transhumance of sheep and goats identified by intra-tooth sequential carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotopic analyses: Evidence from chalcolithic Köşk Höyük, Central Turkey. *J Archaeol Sci.* 2017;86:68-80. <https://doi.org/10.1016/j.jas.2017.01.003>
27. Tornero C, Balasse M, Bălăşescu A, Chataigner C, Gasparyan B, Montoya C. The altitudinal mobility of wild sheep at the Epigravettian site of Kalavan 1 (Lesser Caucasus, Armenia): Evidence from a sequential isotopic analysis in tooth enamel. *J Human Evol.* 2016;97:27-36. <https://doi.org/10.1016/j.jhevol.2016.05.001>
28. Balasse M, Ambrose SH. Mobilité altitudinale des pasteurs néolithiques dans la vallée du rift (Kenya): Premiers indices de l'analyse du $\delta^{13}\text{C}$ de l'émail dentaire du cheptel domestique. *Anthropozoologica.* 2005;40(1):147-166.
29. Henton E, Ruben I, Palmer C, et al. The seasonal mobility of prehistoric gazelle herds in the Azraq Basin, Jordan: Modelling alternative strategies using stable isotopes. *Environ Archaeol.* 2017;0(0):1-13. <https://doi.org/10.1080/14614103.2017.1316432>
30. Makarewicz CA, Pederzani S. Oxygen ($\delta^{18}\text{O}$) and carbon ($\delta^{13}\text{C}$) isotopic distinction in sequentially sampled tooth enamel of co-localized wild and domesticated caprines: Complications to establishing seasonality and mobility in herbivores. *Palaeogeogr Palaeoclimatol Palaeoecol.* 2017;485:1-15. <https://doi.org/10.1016/j.palaeo.2017.01.010>
31. de Winter NJ, Snoeck C, Claeys P. Seasonal Cyclicity in trace elements and stable isotopes of modern horse enamel. *PLoS ONE.* 2016;11(11):e0166678. <https://doi.org/10.1371/journal.pone.0166678>
32. Makarewicz CA. Winter pasturing practices and variable fodder provisioning detected in nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopes in sheep dentinal collagen. *J Archaeol Sci.* 2014;41:502-510. <https://doi.org/10.1016/j.jas.2013.09.016>
33. Körner C, Farquhar GD, Roksandic Z. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia.* 1988;74(4):623-632. <https://doi.org/10.1007/BF00380063>
34. Körner C, Farquhar GD, Wong SC. Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia.* 1991;88(1):30-40. <https://doi.org/10.1007/BF00328400>
35. Li M, Liu H, Li L, Yi X, Zhu X. Carbon isotope composition of plants along altitudinal gradient and its relationship to environmental factors on the Qinghai-Tibet plateau. *Polish J Ecol.* 2007;55(1):67-78.
36. Li C, Zhang X, Liu X, Luukkanen O, Berninger F. Leaf morphological and physiological responses of *Quercus aquifolioides* along an altitudinal gradient. *Silva Fenn.* 2006;40(1):5-13.
37. Morecroft MD, Woodward FI. Experimental investigations on the environmental determination of $\delta^{13}\text{C}$ at different altitudes. *J Exp Bot.* 1990;41(10):1303-1308. <https://doi.org/10.1093/jxb/41.10.1303>
38. Li J, Wang G, Liu X, Han J, Liu M, Liu X. Variations in carbon isotope ratios of C3 plants and distribution of C4 plants along an altitudinal transect on the eastern slope of mount Gongga. *Sci China Ser Earth Sci.* 2009;52(11):1714-1723. <https://doi.org/10.1007/s11430-009-0170-4>
39. Szpak P, White CD, Longstaffe FJ, Millaire J-F, Sánchez VFV. Carbon and nitrogen isotopic survey of northern Peruvian plants: Baselines for paleodietary and paleoecological studies. *PLoS ONE.* 2013;8(1):e53763. <https://doi.org/10.1371/journal.pone.0053763>
40. van de Water PK, Leavitt SW, Betancourt JL. Leaf $\delta^{13}\text{C}$ variability with elevation, slope aspect, and precipitation in the Southwest United States. *Oecologia.* 2002;132(3):332-343. <https://doi.org/10.1007/s00442-002-0973-x>
41. Liu X, Gao C, Su Q, Zhang Y, Song Y. Altitudinal trends in $\delta^{13}\text{C}$ value, stomatal density and nitrogen content of *Pinus tabulaeformis* needles on the southern slope of the middle Qinling Mountains, China. *J Mountain Sci.* 2016;13(6):1066-1077. <https://doi.org/10.1007/s11629-015-3532-8>
42. Liu X, Zhao L, Gasaw M, Gao D, Qin D, Ren J. Foliar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of C3 plants in the Ethiopia Rift Valley and their environmental controls. *Chin Sci Bull.* 2007;52(9):1265-1273. <https://doi.org/10.1007/s11434-007-0165-5>
43. Amundson R, Austin AT, Schuur EA, et al. Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochem Cycles.* 2003;17(1):1031. <https://doi.org/10.1029/2002GB001903>
44. Liu X, Wang G. Measurements of nitrogen isotope composition of plants and surface soils along the altitudinal transect of the eastern slope of Mount Gongga in Southwest China. *Rapid Commun Mass Spectrom.* 2010;24(20):3063-3071. <https://doi.org/10.1002/rcm.4735>
45. Handley LL, Austin AT, Stewart GR, et al. The ^{15}N natural abundance ($\delta^{15}\text{N}$) of ecosystem samples reflects measures of water availability. *Funct Plant Biol.* 1999;26(2):185-199. <https://doi.org/10.1071/pp98146>
46. Garten CT. Variation in foliar ^{15}N abundance and the availability of soil nitrogen on Walker branch watershed. *Ecology.* 1993;74(7):2098-2113. <https://doi.org/10.2307/1940855>
47. Liu X, Wang G, Li J, Wang Q. Nitrogen isotope composition characteristics of modern plants and their variations along an altitudinal gradient in Dongling Mountain in Beijing. *Sci China Ser Earth Sci.* 2010;53(1):128-140. <https://doi.org/10.1007/s11430-009-0175-z>
48. Slivinska K, Kopij G. Diet of the Przewalski's horse *Equus przewalskii* in the Chernobyl exclusion zone. *Polish J Ecol.* 2011;59(4):841-847.
49. Duncan P. *Horses and Grasses: The Nutritional Ecology of Equids and Their Impact on the Camargue.* New York: Springer-Verlag; 1992.
50. Putman RJ, Pratt RM, Ekins JR, Edwards PJ. Food and feeding behaviour of cattle and ponies in the New Forest, Hampshire. *J Appl Ecol.* 1987;24(2):369-380. <https://doi.org/10.2307/2403881>
51. O'Connell TC, Hedges REM, Healey MA, Simpson AHRW. Isotopic comparison of hair, nail and bone: Modern analyses. *J Archaeol Sci.* 2001;28(11):1247-1255. <https://doi.org/10.1006/jasc.2001.0698>
52. Lavielle M. Detection of multiple changes in a sequence of dependent variables. *Stochastic Processes Appl.* 1999;83(1):79-102. [https://doi.org/10.1016/S0304-4149\(99\)00023-X](https://doi.org/10.1016/S0304-4149(99)00023-X)
53. Lavielle M. Using penalized contrasts for the change-point problem. *Signal Processing.* 2005;85(8):1501-1510. <https://doi.org/10.1016/j.sigpro.2005.01.012>
54. Calenge C. The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling.* 2006;197(3):516-519. <https://doi.org/10.1016/j.ecolmodel.2006.03.017>
55. Wittemyer G, Cerling TE, Douglas-Hamilton I. Establishing chronologies from isotopic profiles in serially collected animal tissues: An example using tail hairs from African elephants. *Chem Geol.* 2009;267(1-2):3-11. <https://doi.org/10.1016/j.chemgeo.2008.08.010>
56. Sponheimer M, Robinson T, Ayliffe L, et al. Nitrogen isotopes in mammalian herbivores: Hair $\delta^{15}\text{N}$ values from a controlled feeding study. *Int J Osteoarchaeol.* 2003;13(1-2):80-87. <https://doi.org/10.1002/oa.655>
57. Jones RJ, Ludlow MM, Troughton JH, Blunt CG. Changes in the natural carbon isotope ratios of the hair from steers fed diets of C4, C3 and C4 species in sequence. *J Aust N Z Assoc Adv Sci.* 1981;12(3):85-87. Available at <http://agris.fao.org/agris-search/search.do?recordID=AU8100415>. Accessed February 5, 2019

58. Pettorelli N, Gaillard J-M, Mysterud A, et al. Using a proxy of plant productivity (NDVI) to find key periods for animal performance: The case of roe deer. *Oikos*. 2006;112(3):565-572. <https://doi.org/10.1111/j.0030-1299.2006.14447.x>
59. European Commission Copernicus Land Monitoring Service – Global Component. Available: <https://land.copernicus.eu/global.2018>.
60. Wood S, Scheipl F. gamm4: Generalized Additive Mixed Models using mgcv and lme4. R package version 0.2–5. 2014. Available: <https://CRAN.R-project.org/package=gamm4>.
61. Burnik Šturm M, Pukazhenth B, Reed D, et al. A protocol to correct for intra- and interspecific variation in tail hair growth to align isotope signatures of segmentally cut tail hair to a common time line. *Rapid Commun Mass Spectrom*. 2015;29(11):1047-1054. <https://doi.org/10.1002/rcm.7196>
62. Dunnett M. The diagnostic potential of equine hair: A comparative review of hair analysis for assessing nutritional status, environmental poisoning, and drug use and abuse. *Adv Equine Nutr-III*. 2005;85-106.
63. Sharp ZD, Atudorei V, Panarello HO, Fernández J, Douthitt C. Hydrogen isotope systematics of hair: Archeological and forensic applications. *J Archaeol Sci*. 2003;30(12):1709-1716. [https://doi.org/10.1016/S0305-4403\(03\)00071-2](https://doi.org/10.1016/S0305-4403(03)00071-2)
64. Auerswald K, Rossmann A, Schäufele R, Schwertl M, Monahan FJ, Schnyder H. Does natural weathering change the stable isotope composition (^2H , ^{13}C , ^{15}N , ^{18}O and ^{34}S) of cattle hair? *Rapid Commun Mass Spectrom*. 2011;25(24):3741-3748. <https://doi.org/10.1002/rcm.5284>
65. Wang C, Liu D, Luo W, et al. Variations in leaf carbon isotope composition along an arid and semi-arid grassland transect in northern China. *J Plant Ecol*. 2016;9(5):576-585. <https://doi.org/10.1093/jpe/rtw006>
66. Salter RE, Hudson RJ. Feeding ecology of feral horses in Western Alberta. *J Range Manag*. 1979;32(3):221-225. <https://doi.org/10.2307/3897127>
67. Makarewicz CA. Sequential $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses of early Holocene bovid tooth enamel: Resolving vertical transhumance in Neolithic domesticated sheep and goats. *Palaeogeogr Palaeoclimatol Palaeoecol*. 2017;485:16-29. <https://doi.org/10.1016/j.palaeo.2017.01.028>
68. Makarewicz CA, Winter-Schuh C, Byerly H, Houle J-L. Isotopic evidence for ceremonial provisioning of Late Bronze Age khirigsuurs with horses from diverse geographic locales. *Quater Int*. 2018;476:70-81. <https://doi.org/10.1016/j.quaint.2018.02.030>
69. Tornero C, Aguilera M, Ferrio JP, et al. Vertical sheep mobility along the altitudinal gradient through stable isotope analyses in tooth molar bioapatite, meteoric water and pastures: A reference from the Ebro valley to the Central Pyrenees. *Quater Int*. 2017;484:94-106. <https://doi.org/10.1016/j.quaint.2016.11.042>
70. Makarewicz C, Tuross N. Finding fodder and tracking transhumance: Isotopic detection of goat domestication processes in the near east. *Curr Anthropol*. 2012;53(4):495-505.
71. Still CJ, Berry JA, Collatz GJ, DeFries RS. Global distribution of C3 and C4 vegetation: Carbon cycle implications. *Global Biogeochem Cycles*. 2003;17(1):6-16-14. <https://doi.org/10.1029/2001GB001807>
72. Smedley MP, Dawson TE, Comstock JP, et al. Seasonal carbon isotope discrimination in a grassland community. *Oecologia*. 1991;85(3):314-320. <https://doi.org/10.1007/BF00320605>
73. Diefendorf AF, Mueller KE, Wing SL, Koch PL, Freeman KH. Global patterns in leaf ^{13}C discrimination and implications for studies of past and future climate. *Proc Natl Acad Sci*. 2010;107(13):5738-5743. <https://doi.org/10.1073/pnas.0910513107>
74. Fraser RA, Grün R, Privat K, Gagan MK. Stable-isotope microprofiling of wombat tooth enamel records seasonal changes in vegetation and environmental conditions in eastern Australia. *Palaeogeogr Palaeoclimatol Palaeoecol*. 2008;269(1):66-77. <https://doi.org/10.1016/j.palaeo.2008.08.004>
75. Murphy BP, Bowman DM. The carbon and nitrogen isotope composition of Australian grasses in relation to climate. *Funct Ecol*. 2009;23(6):1040-1049.
76. Flanagan LB, Farquhar GD. Variation in the carbon and oxygen isotope composition of plant biomass and its relationship to water-use efficiency at the leaf- and ecosystem-scales in a northern Great Plains grassland. *Plant Cell Environ*. 2014;37(2):425-438. <https://doi.org/10.1111/pce.12165>
77. Hartman G, Danin A. Isotopic values of plants in relation to water availability in the eastern Mediterranean region. *Oecologia*. 2010;162(4):837-852. <https://doi.org/10.1007/s00442-009-1514-7>
78. Ehleringer JR, Dawson TE. Water uptake by plants: Perspectives from stable isotope composition. *Plant Cell Environ*. 1992;15(9):1073-1082.
79. Farquhar GD, Ehleringer JR, Hubick KT. Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Biol*. 1989;40(1):503-537.
80. Farquhar GD, O'Leary MH, Berry JA. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Funct Plant Biol*. 1982;9(2):121-137. <https://doi.org/10.1071/pp9820121>
81. Cernusak LA, Barbour MM, Arndt SK, et al. Stable isotopes in leaf water of terrestrial plants. *Plant Cell Environ*. 2016;39(5):1087-1102.
82. Cloern JE, Canuel EA, Harris D. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. *Limnol Oceanogr*. 2002;47(3):713-729. <https://doi.org/10.4319/lo.2002.47.3.0713>
83. Wooller M, Smallwood B, Scharler U, Jacobson M, Fogel M. A taphonomic study of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in Rhizophora mangle leaves for a multi-proxy approach to mangrove palaeoecology. *Org Geochem*. 2003;34(9):1259-1275. [https://doi.org/10.1016/S0146-6380\(03\)00116-5](https://doi.org/10.1016/S0146-6380(03)00116-5)
84. Codron J, Codron D, Lee-Thorp JA, et al. Taxonomic, anatomical, and spatio-temporal variations in the stable carbon and nitrogen isotopic compositions of plants from an African savanna. *J Archaeol Sci*. 2005;32(12):1757-1772. <https://doi.org/10.1016/j.jas.2005.06.006>
85. Badeck F-W, Tcherkez G, Nogués S, Piel C, Ghashghaie J. Post-photosynthetic fractionation of stable carbon isotopes between plant organs – A widespread phenomenon. *Rapid Commun Mass Spectrom*. 2005;19(11):1381-1391. <https://doi.org/10.1002/rcm.1912>
86. Szpak P. Complexities of nitrogen isotope biogeochemistry in plant-soil systems: Implications for the study of ancient agricultural and animal management practices. *Frontiers Plant Sci*. 2014;5. <https://doi.org/10.3389/fpls.2014.00288>
87. Craine JM, Brookshire ENJ, Cramer MD, et al. Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant Soil*. 2015;396(1):1-26. <https://doi.org/10.1007/s11104-015-2542-1>
88. Rysava K, McGill RAR, Matthiopoulos J, Hopcraft JGC. Re-constructing nutritional history of Serengeti wildebeest from stable isotopes in tail hair: Seasonal starvation patterns in an obligate grazer. *Rapid Commun Mass Spectrom*. 2016;30(13):1461-1468. <https://doi.org/10.1002/rcm.7572>
89. Fuller BT, Fuller JL, Sage NE, Harris DA, O'Connell TC, Hedges REM. Nitrogen balance and $\delta^{15}\text{N}$: Why you're not what you eat during

- nutritional stress. *Rapid Commun Mass Spectrom.* 2005;19(18):2497-2506. <https://doi.org/10.1002/rcm.2090>
90. Samec CT, Yacobaccio HD, Panarello HO. Carbon and nitrogen isotope composition of natural pastures in the dry Puna of Argentina: A baseline for the study of prehistoric herd management strategies. *Archaeological Anthropological Sci.* 2017;9(2):153-163. <https://doi.org/10.1007/s12520-015-0263-2>
91. Sambuu J. *Mal Azu Aqai Deger-e-Ben Jagakizu Azillaqu Tuqai Arad-Du Ögkü Sanagulg-a Sural [Advice to Herders on How to Improve Their Husbandry Practices]*. Mongolia: Ulaanbataar; 1945.
92. Dash M. *Mongol Orny Bilcheeriin Mal Mallagaany Arga Turshlaga [Experience of Pastoralism in Mongolia]*. Ulsyn Khevelijn Khereg Erkhlekh Khoroo: Ulaanbataar, Mongolia; 1966.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Lazzerini N, Coulon A, Simon L, et al. Grazing high and low: Can we detect horse altitudinal mobility using high-resolution isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values) time series in tail hair? A case study in the Mongolian Altai. *Rapid Commun Mass Spectrom.* 2019;1–15. <https://doi.org/10.1002/rcm.8496>