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# $^{13}\text{C}$ analysis of cow tail hair and farm slurry can be used to implicitly distinguish between different dairy production systems

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## Abstract

**Background** Public interest in the way food is produced on the farm and processed along the food-production chain is increasing. The analysis of isotopic signatures ( $^{13}\text{C}$ ) in cow tail hair provides a method to reconstruct the dietary proportion of maize in cow diets. Based on this, we further investigated whether there is a relationship between isotopic signatures in cow tail hair or farm slurry and the proportion of maize of the total utilized agricultural area per farm [%]. We did an on-farm survey on 17 dairy farms in coastal Northwest Germany and collected cow tail hair from dairy cows and slurry samples on each farm. The farms differed in their feeding regime ( $\text{C}_3$  vs.  $\text{C}_4$  plants), their site conditions (sandy soil = 'Geestland'; organic soil = 'Peatland'; clayey soil = 'Marshland'), and in the area cultivated with maize as a proportion of the total utilized agricultural area per farm.

**Results** We found a positive relationship between  $\delta^{13}\text{C}$  values in both cow tail hair and slurry and the annual dietary proportion of maize ( $R^2 = 0.67$ ; and  $R^2 = 0.63$ ). Furthermore, we confirmed that there was a relationship between  $\delta^{13}\text{C}$  values in cow tail hair and area of maize as a proportion of the total utilized agricultural area per farm ( $R^2 = 0.69$ ).

**Conclusion** Our findings suggest a general applicability of using isotopic signatures ( $^{13}\text{C}$ ) along a wide gradient of site conditions and productions systems in practice.

**Keywords** Stable isotopes, Natural  $^{13}\text{C}$  abundance, Environmental indicator, Maize, Dietary composition, Traceability

## Background

Public interest in the agricultural and food-producing sectors, in particular in the way food is produced on the farm and processed along the food-production chain, is increasing (Gregory 2000; Marzia Ingrassia et al. 2017). In this context, understanding material cycles and source–sink systems of nutrient elements is becoming increasingly important. For the understanding of such cycles and pathways the evaluation and further development of indicator methods is essential (Bockstaller

et al. 2008; Girardin et al. 1999). A common method for tracing elements is the analysis of the isotopic signature (Camin et al. 2016; Zhao et al. 2013). By investigating the natural abundance of carbon and nitrogen isotopes, the metabolic, conversion and uptake processes can be traced without the use of artificial labelling techniques (Camin et al. 2016; Pillonel et al. 2005).

In food production, the method of isotopic signature analysis has been applied in various forms: besides the analysis of products like meat or milk (Kornelx et al. 1997; Rossmann et al. 2000), the analysis of animal tissues (for example hair) is also widespread. The isotopic signature of cow tail switch hairs has been found particularly suitable for tracing differences in animal feeding regimes among agricultural production systems over a period of time (Hammes et al. 2017; Schwertl et al. 2005). Cow tail switch hairs have a high potential to serve as an isotopic

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archive as they have continuous growth, allow non-invasive sampling and as a dead tissue do not change composition post hoc (Nakamura et al. 1982; White et al. 1999).

The economic and environmental performance of dairy farming is directly linked to the forage production (Reijs et al. 2013). There is an interest to make milk production independent of arable land, use more grassland and thus to prevent feed–food competition (Knaus 2013). Maize as a forage crop is also cultivated on arable land. Maize is a  $C_4$  plant and differs from  $C_3$  plants in photosynthetic mechanism which is directly reflected in the  $^{13}C$  signatures (Nakamura et al. 1982; Smith and Epstein 1971).  $C_3$  plants (e.g. grasses, legumes, cereals, soy) discriminate much more strongly against the heavy  $^{13}C$ , whereas  $C_4$  plants (e.g. maize, sugar cane, sorghum) discriminate less against  $^{13}C$  and have correspondingly higher  $\delta^{13}C$  values (Knobbe et al. 2006; Smith and Epstein 1971). A correlation between the proportion of maize in the feeding ration and the  $\delta^{13}C$  content of the tail switch hairs has been demonstrated in some studies (Hammes et al. 2017; Schwertl et al. 2005, 2003; Wittmer et al. 2010). The previous studies used the isotopic signature ( $\delta^{13}C$  values) mainly on farms on sandy and organic soils, and so far, information for its use on other soil, e.g. clayey marshland soils, is lacking.

Lower Saxony has the second highest dairy stock size in Germany and has shown a strong intensification of dairy production in recent years in the northwest region along the North Sea coast. In Lower Saxony in particular the study region (Landkreis Wesermarsch) has the highest dairy cow stock per agricultural area (> 80 cows per 100 ha agricultural area) and the highest proportion of permanent grassland (> 70% of total utilized agricultural area) (Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Verbraucherschutz 2022). Clayey marshland soils are common along the North Sea coast and along major rivers and are important sites for grassland-based dairy farming. These highly clayey marshland soils are limited in their use for arable farming and often cultivation of maize is not possible.

In relation to the main soil conditions, the management and production systems on the farms can also differ, which also has a direct influence on the feeding regime. An analysis of a wider range of soil landscapes, related feeding regimes, animal tissues, and production systems would be needed to prove the robustness of  $\delta^{13}C$  isotopic signatures as a tool to retrospectively estimate the annual dietary proportion of maize without analysing the feed components (Hammes et al. 2017).

Slurry from dairy cows also is a product of metabolism processes and can function as an archive as it is usually produced and stored over a period of several months. Sampling of slurry is comparatively easy and slurry is

readily available while cattle tail hair is often sheared for hygienic reasons and thus kept too short making for the purposes of isotopic analysis. By including slurry in our study, we wanted to test and develop a broader application of the isotopic signature method in practice. For example, Bol et al. (2004) and Glaser et al. (2001) used the relationship between animal feeding and isotopic composition of slurry to systematically produce  $^{13}C$  enriched or depleted slurry.

On-farm research is always dependent on the information provided by the farm managers. There is always a certain residual uncertainty in the data obtained on farm management, dairy production and feeding regime (Piepho et al. 2011). Therefore, we included fact-based land use data (proportion of maize area of total utilized agricultural area of the farms) and tested its relationship to the  $\delta^{13}C$  isotopic signature of cow tail hair and slurry. To our knowledge, information on the relationship between land use and isotopic analyses of cow tail hair and slurry is scarce.

We did on-farm research on 17 farms in the North Sea region of Germany which differed in regional soil and site conditions (soil landscapes), production systems and feeding regimes. For the purpose of this study, the term ‘soil landscapes’ refers not only to soil properties and soil chemical mechanisms, but also includes the respective production systems as well (Frame 1992). We obtained data on farm structure and management and took samples of cow tail hair and slurry on the 17 farms.

We hypothesize that (i) the method of tracing back the diet of cows by using the  $\delta^{13}C$  signature of cow tail switch hair is a robust tool that works on several soil landscapes and under varying farming and production systems and that (ii) conclusions from the  $\delta^{13}C$  isotopic signatures in cow hair can be drawn concerning land use data and (iii) the  $\delta^{13}C$  isotopic signature of slurry is related to the feeding regime and land use.

## Methods

### Study region and farms

The study region was located between 53°11' to 53°24' North and 8°01' to 8°22' East in north-western Germany and is part of the watershed area of the Jade river. The region comprises three different soil landscapes: Geestland, Peatland and Marshland. The Geestland (moraine-based sandy upland) is characterized by a soil texture with a high proportion of sand (73% sand, 21% silt, and 6% clay) and is located in the southwest of the study region. On the Marshland farms in the north-east of the region, soils with a high clay content predominate (5% sand, 45% silt, and 50% clay). The Peatland farm soils have a proportion of 30% sand, 38% silt, and 32% clay on average and are situated between the Geestland and

Marshland region. Dairy farming and forage production based on grassland, especially on the Marshland and Peatland landscapes, are the most prominent forms of land use and characterize the region.

### Farms

Of the 17 farms investigated, nine can be allocated to the Geestland, five to the Peatland, and three to the Marshland region. The uneven distribution of farms among the soil landscapes is a result of requirements within a collaborative research project on nutrient inputs from dairy farms into drainage ditches. Nevertheless, the farms reflect the variation of farm types that are typical for this North Sea region (Tables 1, 2): from smaller dairy farms with about 70 dairy cows to bigger farms with more than 500 dairy cows. The grassland proportion of the utilized agricultural area varies from 37 to 100% (Geestland: 37–77%; Peatland: 75–100%; Marshland: 94–100%) and utilization of grassland ranges from cutting-only systems (all-year housing of cows) to combinations of cutting with summer grazing. The dietary proportion of maize differs strongly between farms from 0 to 46% based on dry matter. Grazing farms either supplement the cows' diet during summer with maize ( $C_4$  plant) or only use grass ( $C_3$  plants).

The description of the feeding regime and the annual dietary proportions follows information acquired in face-to-face interviews with the farmers in the years 2020 and 2021. In the 'all-year housing' regime, cows were kept in stables throughout the year and were fed a diet that always contained maize. In the 'summer grazing with maize' regime, cows were on pasture for a minimum of 120 days per year and a minimum of 6 h per day. During nights, for milking and in winter cows were kept in stable and received additional feeds like maize silage and concentrates. Farms assigned to the 'summer grazing without maize' regime kept their cows on pasture for a minimum of 120 days per year and a minimum of 6 h per day and in the stable during nights and for milking; during wintertime feeding was like in the 'summer grazing with maize' regime. However, these cows did not receive any maize ( $C_4$  plant) while they were in the stable and are fed by  $C_3$  plants year-round.

### Sampling design and preparation

#### Hair sampling and preparation

Sampling was done according to the protocol of Hammes et al. (2017) and Schwertl et al. (2005, 2003). On two consecutive days in early March 2021 tail switch hairs were plucked by the farmers themselves from two adult dairy cows that had lived on the farm for at least 2 years at that time. The hair samples were stored in an airtight bag and frozen until further processing. We prepared the hair

samples for the stable isotope analysis as follows (Hammes et al. 2017; Schwertl et al. 2005, 2003): to remove contaminants, like traces of faeces, the hairs were soaked and washed by ultra-sonication with deionized water, dried (40 °C, 48 h), soaked in a 2:1 mixture of methanol/chloroform (approximately 2 h), rinsed with deionized water, soaked in deionized water for another 30 min, and rinsed again; finally, the hairs were dried again (40 °C, 48 h).

Ten hairs per farm (five per cow) were randomly chosen and further processed. A minimum hair length of 12 cm was necessary in order to retrospectively assign sections of hair to certain time periods in the past: in this case, to periods of summer and winter feeding to reflect an annual diet. We assumed a medium growth rate of 0.8 mm/day and a time shift of 80 days for a changed diet to be detectable in the hair and followed the procedure described in Hammes et al. (2017) to separate hair sections for isotopic analyses: 1–3.5 cm of hair (0 cm would be the hair root) representing the November period (winter feeding, 2020) and 8.5–11 cm for the August period (summer feeding, 2020). The sampling periods were chosen in accordance with Hammes et al. (2017) to ensure comparability. Each segment was cut into ~1 mm pieces and put into a 4 × 6 mm tin capsule. Ten samples per farm (five replications for each time period, that is summer and winter feeding) with ~1 mg weight of material were analysed for  $\delta^{13}\text{C}$  isotopic signature.

#### Sampling and preparation of slurry

Samples of slurry of each farm ( $n=17$ ) were collected in late winter/early spring 2020 and 2021, before the first slurry application to the fields, and frozen for storage until analysis. To prepare for isotopic analysis, samples were freeze dried, ground to powder (Fritsch Pulverisette 7; for 2.5 min, 400 r/min) and <1 mg of material weighed into tin capsules.

#### Isotopic analyses

All  $^{13}\text{C}$  analyses were conducted at the Centre for Stable Isotope Research and Analysis, University of Göttingen. The  $\delta^{13}\text{C}$  values of all samples were determined by combustion in an elemental analyzer NA1110 (CE-Instruments, Rodano, Milano, Italy) interfaced with a ConFlo III-Interface (Thermo Electron, Bremen, Germany) to an isotope ratio mass spectrometer Delta XP (Thermo Electron, Bremen, Germany). Following Mariotti (1984), the  $\delta^{13}\text{C}$  value is expressed in parts per thousand [‰] and defined as:

$$\delta^{13}\text{C}[\text{‰}] = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] * 1000,$$

where  $R$  represents the ratio of  $^{12}\text{C}/^{13}\text{C}$  isotopes of the sample and the standard. All samples were measured

**Table 1** Characterization of the production systems of the farms in the study. Data obtained during on-farm survey

ID	Soil landscape	Agricultural area [ha]	Grassland [%]	Maize land [%]	Milking cows	Milk yield (ECM) per cow [kg]	Feeding regime	Maize [% DM]	Grass [% DM]	Concentrates [% DM]
A	Geest	145	49	41	103	9922	All-year housing	30	37	33
B	Peatland	145	75	25	138	10,521	All-year housing	27	40	33
C	Geest	96	65	32	122	10,365	Summer grazing with maize	11	51	38
D	Marshland	63	100	0	98	9520	Summer grazing with maize	14	51	35
E	Peatland	224	100	0	133	5280	Summer grazing without maize	0	85	15
F	Peatland	182	96	4	199	7911	Summer grazing with maize	19	43	38
G	Geest	241	56	41	326	9834	Summer grazing with maize	30	37	33
H	Geest	158	48	38	241	9840	Summer grazing with maize	41	27	33
I	Geest	201	56	34	201	8625	Summer grazing with maize	27	49	24
J	Geest	244	77	23	222	8749	All-year housing	30	37	33
K	Geest	313	75	25	523	8286	Summer grazing with maize	24	44	32
L	Peatland	103	92	8	202	8219	Summer grazing with maize	16	38	46
M	Marshland	76	100	0	72	10,061	Summer grazing without maize	0	60	40
N	Marshland	168	94	3	148	8878	Summer grazing with maize	15	42	43
O	Peatland	102	92	6	94	6146	Summer grazing with maize	14	50	36
P	Geest	242	37	53	374	9068	All-year housing	46	24	30
Q	Geest	48	72	19	71	9621	Summer grazing with maize	17	41	43

**Table 2** Characterization of farm size and production system in the three soil landscapes

	Geestland (n = 9)	Peatland (n = 5)	Marshland (n = 3)	All soil landscapes (n = 17)
	Data on production system			
Agricultural area [ha]	188 (48–313)	151 (102–224)	102 (62–168)	162 (48–313)
Grassland [%]	59 (37–77)	91 (75–100)	98 (94–100)	76 (37–100)
Maize land [%]	34 (19–53)	9 (0–25)	1 (0–3)	21 (0–53)
Milking cows	242 (71–523)	153 (94–202)	106 (98–148)	192 (71–523)
Milk yield (ECM) per cow [kg]	9368 (8286–10,365)	7615 (5279–10,521)	9486 (8878–10,061)	8873 (5279–10,521)
Maize [% DM]	28 (11–46)	15 (0–27)	10 (0–15)	21 (0–46)
Grass [% DM]	38 (24–51)	51 (38–85)	51 (42–60)	44 (24–85)
Concentrates [% DM]	33 (24–43)	34 (15–46)	39 (35–43)	34 (15–46)
	$\delta^{13}\text{C}$ [‰]			P-value
Cow tail hair	– 20.30 ( $\pm 0.57$ ) b	– 22.50 ( $\pm 0.76$ ) ab	– 24.20 ( $\pm 0.98$ ) a	< 0.01
Slurry	– 23.28 ( $\pm 0.53$ ) b	– 25.62 ( $\pm 0.71$ ) a	– 28.06 ( $\pm 0.92$ ) a	< 0.01

Means with minimum and maximum values in brackets.  $\delta^{13}\text{C}$  values for cow tail hair and slurry in 2020. One-way ANOVA with soil landscapes as main factor; with post hoc Tukey test. Values in brackets show standard errors. Means with different letters in one row differ significantly ( $\alpha = 0.05$ )

against a laboratory working standard (acetanilid) which was calibrated against an international standard v-PDB beforehand.

### Statistics

Statistical analysis was carried out using R Development Core Team 2020 (Version 4.0.2). In the first step, we investigated the relationship between the annual dietary proportion of maize and the  $\delta^{13}\text{C}$  values in cow switch tail hair and slurry by linear regression and plotted the data using the package ggplot2. In the second step, the same procedure was applied to explore the relationship between the  $\delta^{13}\text{C}$  content of cow switch tail hair and slurry and the proportion of maize land of the total utilized agricultural area [%]. Effects of the soil landscape (Geestland, Peatland, Marshland) on  $\delta^{13}\text{C}$  values in cow tail hair and slurry were analysed by conducting a one-way analysis of variance (ANOVA). Both ANOVAs were followed by a Tukey post hoc test ( $\alpha = 0.05$ ) to compare means of soil landscapes.

### Results

#### Relationship between the annual dietary proportion of maize and the $\delta^{13}\text{C}$ values in cow tail hair

The proportion of maize in the dairy cow diet of the 17 farms in our investigation varied from 0 to 46% (Table 2). Accordingly, we found  $\delta^{13}\text{C}$  values in cow tail hair in a range from – 26.10 to – 18.73 ‰. Four farms kept their cows in an all-year housing system with no grazing at all, while 13 farms did summer grazing: 11 with additional maize feeding and two with no maize in the cow diet.

We found that 67% of the variation in annual  $\delta^{13}\text{C}$  values of cow tail hair (average of the summer and winter

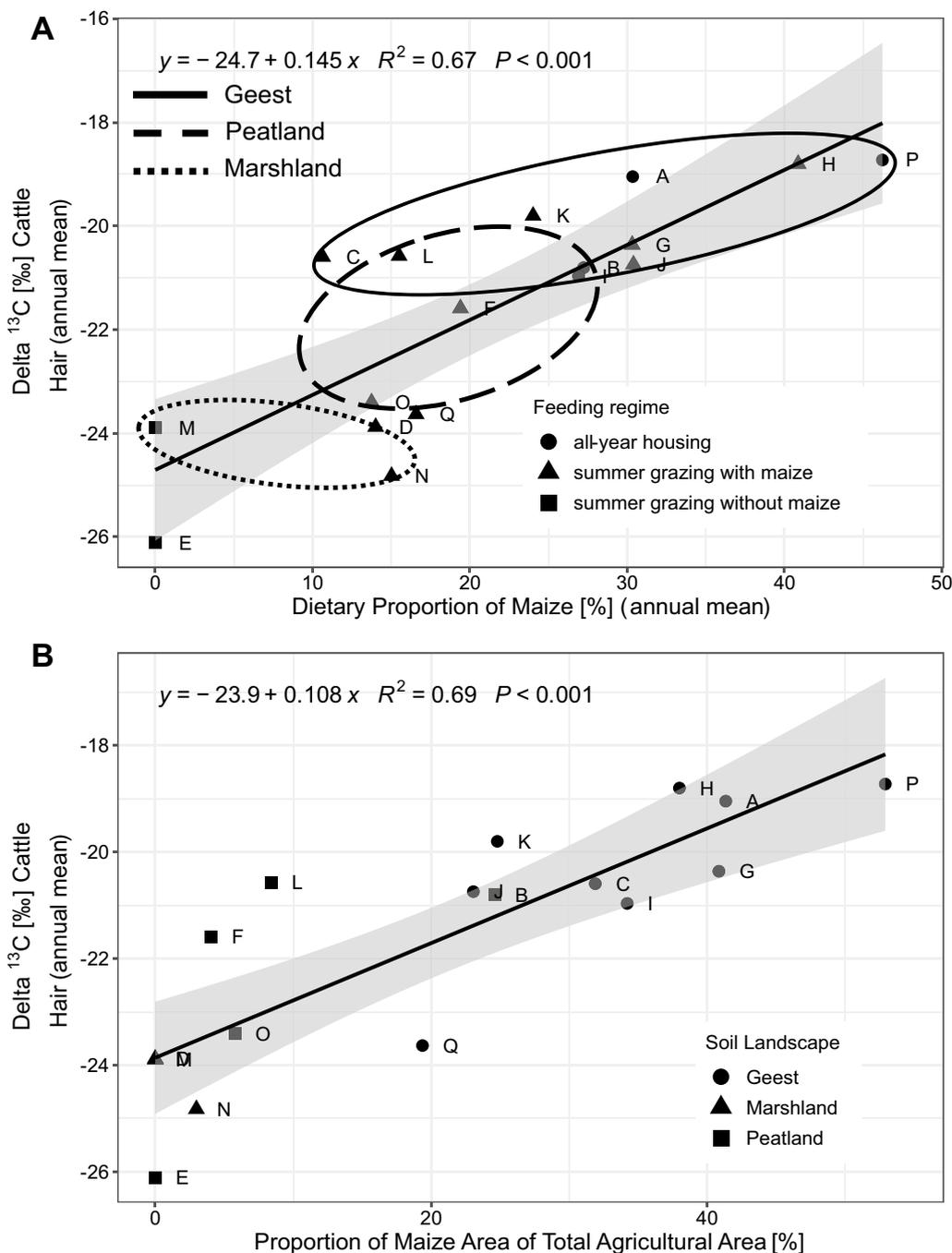
feeding time periods) can be explained by the annual dietary proportion of maize (Fig. 1A). This is somewhat less than the  $R^2$  of 0.89 reported by Hammes et al. (2017). The linear model indicates a  $\delta^{13}\text{C}$  value of – 24.7‰ (intercept) with no maize in the diet, and less negative values with increasing proportions of maize in the diet. This value is close to the intercept of – 25.3 ‰ for a similar on-farm study as reported by Hammes et al. (2017). The 95% confidence interval (CI) for mean dietary proportion of maize (21%) is – 21.67‰ (– 22.35, – 20.98).

#### The effect of feeding regime and soil landscape on the $\delta^{13}\text{C}$ values in cow tail hair

The  $\delta^{13}\text{C}$  values of cow hair differed significantly among the feeding regimes (summer grazing with maize, summer grazing with no maize, no grazing/all-year housing;  $P < 0.05$ ; not shown) and between soil landscapes (Geestland, Peatland, Marshland;  $P < 0.01$ ; Table 2). Lower  $\delta^{13}\text{C}$  values in the cow hair could be attributed to the Marshland; all farms there did grazing (Fig. 1A, dotted circle; Table 2). Distinctly higher  $\delta^{13}\text{C}$  values in cow tail hair ( $P < 0.01$ ) were found in the Geestland; feeding regimes there always included higher proportions of maize in the diet (Fig. 1, solid circle; Table 2). On Peatland farms, cow tail hair showed a wider range of  $\delta^{13}\text{C}$  values and all three feeding regimes were represented (Fig. 1A, dashed line, Table 2).

#### Relationship between $\delta^{13}\text{C}$ values in cow tail hair and the proportion of maize of total agricultural area

A positive relationship between  $\delta^{13}\text{C}$  values in cow tail hair and the proportion of maize area of total agricultural area can be confirmed ( $P < 0.001$ ; Fig. 1B): 69%



**Fig. 1** **A** Relationship between  $\delta^{13}\text{C}$  (annual mean from summer and winter feeding) values in cattle tail hair and the annual dietary proportion of maize (DM). **B** Relationship of the area under maize [% of total agricultural area per farm] to  $\delta^{13}\text{C}$  values in cattle tail hair (annual mean). Each point refers to one farm (A–Q) and symbols indicate feeding regime. The solid line includes Geestland farms, the dashed circle Peatland farms, and the dotted line Marshland farms.  $R^2$  show coefficient of determination and grey bands show 95% confidence interval

of the variation in annual  $\delta^{13}\text{C}$  values of cow tail hair can be explained by the area cultivated with maize on the farm and confidence interval for mean proportion of maize area of total agricultural area is  $-21.60\%$

( $-22.26, -20.94$ ). This value is in a range of the coefficient of determination found for the relationship of  $\delta^{13}\text{C}$  values in cow tail and the annual dietary proportion of maize ( $R^2 = 0.67$ ; Fig. 1A). The linear model indicates a

$\delta^{13}\text{C}$  value of  $-23.9\text{‰}$  (intercept) for a situation with no maize cultivated on the farm (total agricultural area).

#### **Analysis of $\delta^{13}\text{C}$ values in cow slurry and the relationship to the annual dietary proportion of maize**

Samples of slurry were taken on each farm. The factor 'soil landscape' had a significant effect on  $\delta^{13}\text{C}$  values of slurry ( $P < 0.01$ , Table 2). The  $\delta^{13}\text{C}$  values of slurry from Marshland farms were significantly lower than those of Geestland farms while  $\delta^{13}\text{C}$  values of slurry from Peatland were in a range between those of Marshland and Geestland farms but not significantly different from either of the two soil landscapes.

We found a positive relationship between  $\delta^{13}\text{C}$  values of slurry from the farms and the annual proportion of maize in the cow diet ( $R^2 = 0.63$ ,  $P < 0.001$ ; Fig. 2A): farms with lower  $\delta^{13}\text{C}$  values (more negative) in their slurry had a lower proportion of maize in their diet. The 95% confidence interval (CI) for mean dietary proportion of maize (21%) is  $-24.84\text{‰}$  ( $-25.63$ ,  $-24.06$ ).

#### **Relationship between $\delta^{13}\text{C}$ values in slurry and the proportion of maize cultivated on the farm of the total agricultural area of the farm**

We determined that there was a positive relationship between  $\delta^{13}\text{C}$  values in slurry and the proportion of maize cultivated on the farm of total agricultural area per farm ( $P < 0.001$ ; Fig. 2B): 51% of the variation in annual  $\delta^{13}\text{C}$  values of the slurry could be explained by the proportion of maize cultivated on the farm of the total agricultural area of the farm (CI for mean 21 ha =  $-24.78\text{‰}$  ( $-25.67$ ,  $-23.89$ )). The linear model indicated a  $\delta^{13}\text{C}$  value of  $-26.9\text{‰}$  (intercept) for a situation with no maize cultivated on the farm (total agricultural area).

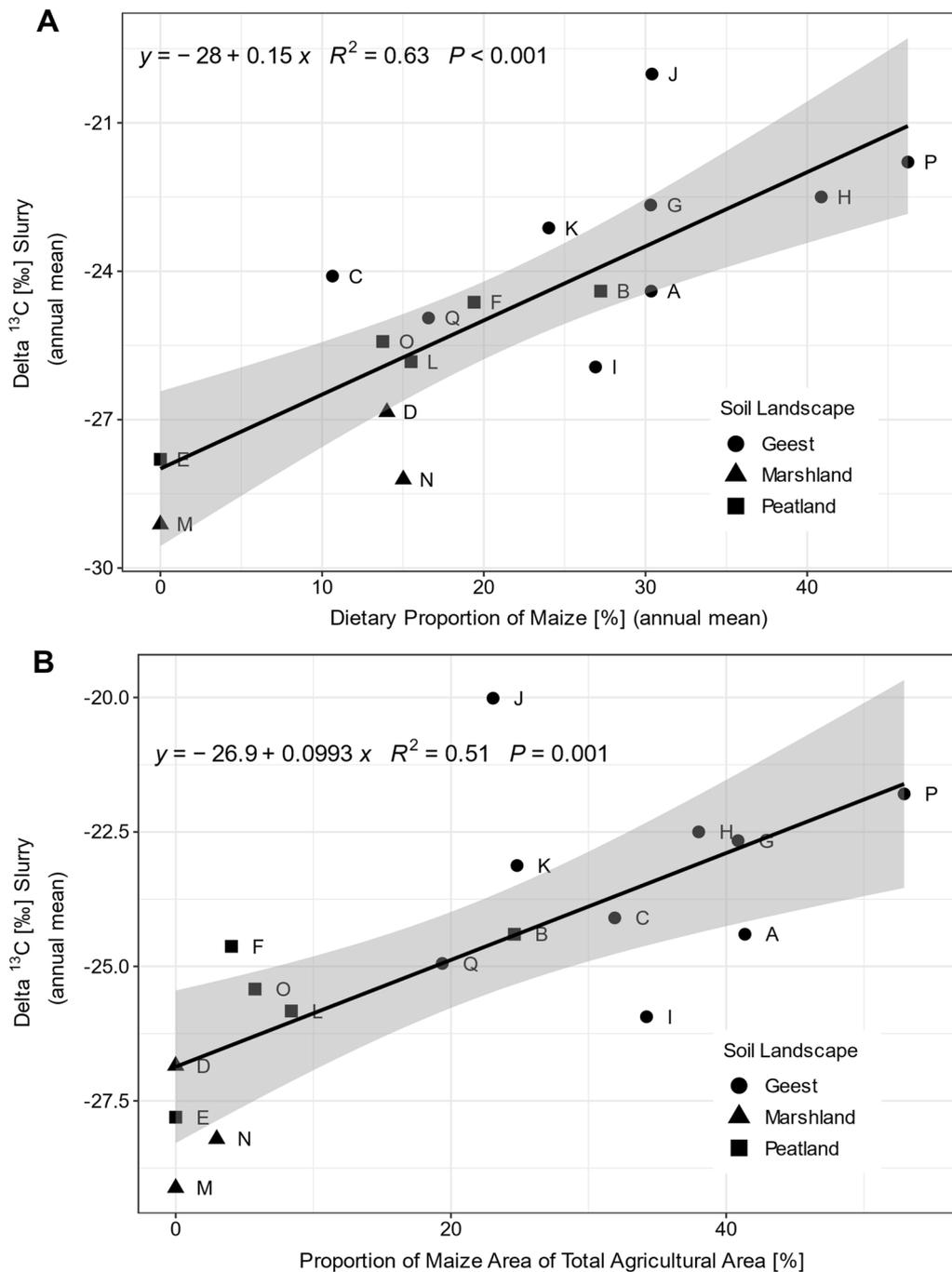
## **Discussion**

#### **Relationship between $\delta^{13}\text{C}$ values in cow tail hair and slurry and the proportion of maize in the cow diet**

Our results show that there is a relationship between the proportion of maize in the cow diet and the  $\delta^{13}\text{C}$  values of cow tail hair.  $C_4$  and  $C_3$  plants differ in terms of their photosynthetic metabolism and therefore in their discrimination of  $^{13}\text{C}$  (Smith and Epstein 1971). This is reflected in the isotopic composition of the cow tail hair (Nakamura et al. 1982). In our study we found that a higher proportion of  $C_4$  plants (maize) in the diet led to less negative  $\delta^{13}\text{C}$  values in the cow tail hair. This finding confirms results of previous studies (Hammes et al. 2017; Schwertl et al. 2003). In addition, we widened the approach of the previous studies by including marshland as a region of dairy production. We also determined  $\delta^{13}\text{C}$  values in slurry of all farms

in the study. Our aim was to show that the relationship between maize in the cow diet and  $\delta^{13}\text{C}$  values in cow tail hair and slurry is valid for a wider range of environmental conditions like different soil landscapes and related production systems.

We found a relationship between  $\delta^{13}\text{C}$  values of slurry and the dietary proportion of maize (Fig. 2A). More maize ( $C_4$ ) in the diet will also affect the isotopic signature of slurry at the farm level (Amelung et al. 1999; Glaser et al. 2001). As slurry is usually produced and stored over several months it also has an archive function for  $\delta^{13}\text{C}$  values and reflects the feeding regime over that time period. Accordingly, Geestland farms in our study with the highest proportion of maize of total utilized land ( $P < 0.001$ ; Table 3) and the significantly highest dietary proportion of maize ( $P < 0.05$ ) had the highest  $\delta^{13}\text{C}$  values in slurry ( $P < 0.01$ ; Table 2). This relationship has been used to systematically produce isotopically enriched or depleted slurry for experimental set-ups (Angers et al. 2007; Bol et al. 2004). In practice, slurry samples are more readily available than samples from cow tail hair. Cow tail hair is cut in regular intervals for hygienic reasons and is often too short for the intended purposes. The relationship between  $\delta^{13}\text{C}$  values in slurry and annual dietary proportion of maize is similar to that of cow tail hair and proportion of maize ( $R^2 = 0.63$  and  $R^2 = 0.67$ , respectively). From our results we conclude that an isotopic analysis of cow slurry can be used as a reasonable proxy for the estimation of feed ratios ( $C_3$  vs.  $C_4$  plants). However, in order to fully explain the relationship of  $\delta^{13}\text{C}$  values in slurry and annual dietary proportion of maize more information on the slurry management of the farm is needed, such as the origin of the slurry (from cows alone or also from heifers or bulls) or the type and duration of slurry storage. Additionally, the dietary composition varies throughout the year depending on forage availability and forage quality. In order to improve the relationships and models, especially the composition of the concentrates should be identified. It is important to know if  $C_4$  plants, usually maize, are used as concentrate feed additionally to the feeding of silage maize. In contrast to Hammes et al. (2017), six farms (A, G, H, I, K, L) fed maize additionally as concentrate feed in single components, while 11 farms fed conventional multicomponent concentrates where proportions of single ingredients are unidentified. Data points of those farms with  $C_4$  in concentrates lie above or on the regression line (Fig. 1A). Including the proportion of maize concentrates of those six farms into overall dietary proportion of maize, the  $R^2$  of the relationship  $^{13}\text{C}$  hair ~ dietary proportion of maize improves to 0.77. Thus, the supplemental and not accounted for feeding of  $C_4$  plants in concentrates may explain part of the variation. Systematic research on the topic is scarce.



**Fig. 2** **A** Relationship between the  $\delta^{13}\text{C}$  values of slurry and the annual dietary proportion of maize (DM). **B** Relationship of the  $\delta^{13}\text{C}$  values in cattle slurry (annual mean) and the area cultivated with maize [as a proportion of the total agricultural area per farm]. Each point refers to one farm (A–Q) and symbols indicate soil landscapes.  $R^2$  shows coefficients of determination and grey bands show 95% confidence interval

**$\delta^{13}\text{C}$  values in cow tail hair and slurry in different soil landscapes and proportion of maize of the farmland**

Dairy production and feeding systems are closely related to soil landscapes. The area has three major soil landscapes: Geestland (sandy), Peatland (organic) and

Marshland (clayey). The method of isotopic measurements of cow tail hair has been applied on a range of site conditions before: Hammes et al. (2017) worked on predominantly sandy or coarse textured soils on a west–east gradient in the North German Plain; Schnyder et al.

**Table 3** Proportion of maize of total utilized agricultural area per farm [%] for different soil landscapes in 2020

Soil landscape	Maize / total utilized agricultural area [%]
Geestland	34.05 b
Peatland	8.56 a
Marshland	0.99 a

(2006) and Schwertl et al. (2005) both worked in Southern Germany on drained peat soils and mineral soils (Luvisols and Cambisols), respectively; and Wittmer et al. (2010) studied semi-arid grassland in Inner Mongolia, China. Our study introduces clayey Marshland soils as a production site for dairy cattle and grassland farming in addition to sandy and peat soils in the same region. Clayey marsh soils in coastal regions are often difficult to use as arable land, but can be very productive grassland for dairy farming (Reijs et al. 2013). This combination of soil landscapes in a region is not uncommon along the North Sea coast in Germany and also in the Netherlands dairy production and grassland can be found on those three soil types (van den Pol-van Dasselaar et al. 2020a).

The extent of use of maize in the dairy production system is mainly affected by constraints to arable farming like a high water-table and soil conditions that make tillage difficult and costly. Maize cultivation is thus often limited in Peatland and Marshland (Stirm and St-Pierre 2003). In the region of our study, cultivation of maize is possible in the whole of the Geestland region, but only partly on Marshland or Peatland soils.

The use of grazing on a farm is often independent of soil landscapes and depends, however, a great deal on the mindset of the farmer (Becker et al. 2018; van den Pol-van Dasselaar et al. 2020b); but also on constraints like herd size, availability of suitable grazing fields close to the farm, labour availability, and forage storage facilities (Charlton and Rutter 2017; Frame 1992; Ketelaar-de Lauwere et al. 2000; Schaak and Musshoff 2018).

Soil landscapes and production systems are related and there is no Geestland farm where no maize is used in the diet for dairy cows and conversely, no Marshland farm in our study that keeps its cows indoors all-year round. However, these combinations represent the real conditions in dairy farming in north-west Germany.

Farms in the three soil landscapes differed significantly in the area cultivated with maize as a proportion of the total utilized agricultural area ( $P < 0.001$ ; Table 3) and in the proportion of maize in the feed diet for dairy cows ( $P < 0.05$ ; not shown).

On average, the proportion of maize of the total utilized agricultural area was 34% for Geestland farms, 9%

for Peatland farms, and only 1% for Marshland farms. The comparatively high proportion of maize in the cow diet on Peatland farms can be explained by the fact that farms designated to one soil landscape can also have fields in an adjacent soil landscape—farms can be situated on the border of two landscapes or a farm might have taken over a smaller farm in another soil landscape. In our study, farms were categorized to that soil landscape where they had the highest amount of farmland (ranging from a proportion of 33.3% in each soil landscape to 100% in one soil landscape).

As the proportion of maize in the diet is related to the extent of cultivation of maize on the farms, our results allow us to draw conclusions from the isotopic signature of cow hair not only on the proportion of maize in the feed diet ( $R^2 = 0.67$ ; Fig. 1A), but also to the proportion of maize area of the total agricultural area ( $R^2 = 0.69$ ; Fig. 1B). There is also a relationship between the isotopic signature of slurry and the proportion of maize area of total agricultural area ( $R^2 = 0.51$ ; Fig. 2B). Among the factors that may contribute to the unexplained variance is the purchase of additional forage for the dairy production, often maize silage, when supply from own resources of the farm is not sufficient. Of the 17 study farms, six purchased additional maize silage in 2020 (0.33–3.10 t cow<sup>-1</sup>).

The relationship of the isotopic signatures in cow tail hair and slurry with the proportion of cultivation of maize of the total agricultural area widens the scope of application of the method and enables a more fact-based analysis independent of questionnaire data (on feeding, grazing and composition of the diet). Data on farm size and cultivation of different crops are readily available and correct. The use of data from questionnaires and interviews is indispensable but these instruments unavoidably introduce some degree of uncertainty (Becker et al. 2015). Data from on-farm research have the potential to reflect farming reality and can help to validate indicator methods for use under practice conditions, demonstrating prospects and limitations (Crofoot 2010; Kyveryga 2019; Nielsen 2010). The uncertainties could at least partly be avoided when, as we did in our survey, fact-based land use data can be used. At the farm scale, the relationship between isotopic signatures ( $\delta^{13}\text{C}$  values) of cow tail hair and of slurry with dietary proportion of maize and with the proportion of maize of the total utilized agricultural land proved to be fairly robust among a range of production systems and soil landscapes. Generally, the composition of concentrate feeds should be considered in detail as well. Results suggest that an application of using isotopic signatures ( $\delta^{13}\text{C}$  values) is feasible under practice conditions.

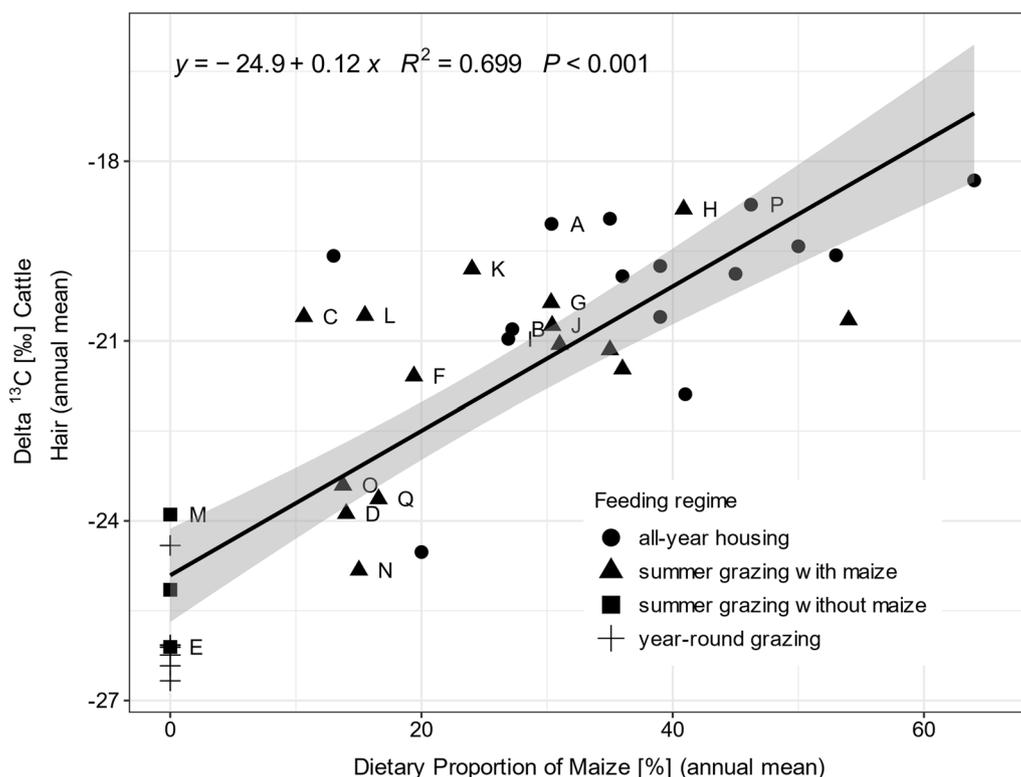
### Extending the application of isotopic signatures in cow tail hair with data from a previous study

The regression model on the relationship between the annual dietary proportion of maize and  $\delta^{13}\text{C}$  values in cow tail hair (Fig. 1A) in our study is very similar to that of Hammes et al. (2017):  $y = -24.70 + 0.145x$ ,  $R^2 = 0.67$ ,  $P < 0.001$ , and  $y = -25.74 + 0.124x$ ,  $R^2 = 0.89$ ,  $P < 0.001$  for our study and for Hammes et al. (2017), respectively. The lower intercept and slightly steeper slope in the model of Hammes et al. (2017) and the higher  $R^2$  might be explained by a greater sample size (six more farms) and the inclusion of data from suckler cow farms in Hammes et al. (2017). Including suckler cow farms implies that there were more farms with no maize in the diet as maize is often not available and too costly for use in suckler cow production (Steinwider and Häusler 2004).

To further extend and generalize our findings, we merged our data with those from Hammes et al. (2017) (Fig. 3). In doing this, we also extended our own data, which ranged from moderate to intensive dairy production systems, with the extensive feeding regime of suckler cows. The regression model for this extended data set on the relationship between the annual dietary proportion

of maize and  $\delta^{13}\text{C}$  values in cow tail hair had an improved  $R^2$  of 0.70 and a narrower confidence interval for mean dietary proportion of maize (24%):  $-22.02\%$  ( $-22.49$ ,  $-21.55$ ). This implies that 70% of the variation in annual  $\delta^{13}\text{C}$  values of cow tail hair could be explained by the variation in annual dietary proportion of maize. A larger sample size usually implies a higher variation in production systems and thus in feeding regimes (Clymo 2014; Webster and Oliver 1990). Thus, we would have expected a higher coefficient of determination of the merged data. In both studies, most farms had between 10 and 50% of maize in the diet, which might vary throughout the year depending on forage availability and quality. There are also physiological limits to the use of maize and concentrates. In this respect the data reflect the practice of dairy farming in Germany. Additionally, all farms from the dataset of Hammes et al. (2017) were located on predominantly sandy soils in the North German Plain along an east–west gradient stretching almost the whole of Germany. The soils are very similar to the soils in our Geestland soil landscape category.

We can conclude that, firstly, a replication of the method of using isotopic signatures ( $\delta^{13}\text{C}$  values) in cow



**Fig. 3** Combination of data presented here with data from Hammes et al. (2017). Relationship between  $\delta^{13}\text{C}$  (annual mean) values in cow tail hair and annual dietary proportion of maize. Each point refers to one study farm (A–Q) and symbols indicate feeding regime. Grey bands show 95% confidence interval. Additional data points from Hammes et al. (2017) do not have letters as farm-IDs. Year-round grazing only in the Hammes et al. (2017) data set

tail hair as an indicator of the proportion of maize in the diet of dairy cows is feasible in practice and that, secondly, the inclusion of a wider range of differing production systems is possible and leads to higher accuracy.

## Conclusions

We have been able to confirm all three hypotheses as stated in the introduction. The use of  $\delta^{13}\text{C}$  isotopic measurements of cow tail hair and cow slurry as an indicator of the proportion of maize in dairy cow diets provides replicable results under a wide range of conditions in practice. This suggests a general applicability in practice. The method can also be applied to clayey marsh soils, which are an important site for dairy cattle and grassland farming. The isotopic analysis of the cow tail hair and slurry also allows us infer information on the proportion of maize of the total utilized agricultural area per farm.

## Abbreviations

ANOVA	Analysis of variance
C	Carbon
CI	Confidence interval (95%)
DM	Dry matter
ECM	Energy-corrected milk (4.0% fat, 3.4% protein)
N	Nitrogen

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## Author contributions

F.S. did the data acquisition, formal analysis and interpretation and wrote the manuscript. J.I. conceptualized the study and gave valuable input for interpretation. M.K. conceptualized and supervised the study and contributed to writing and editing. All authors read and approved the final manuscript.

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## Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

### Ethics approval and consent to participate

A statement of ethical clearance by the university animal welfare officer is attached as a PDF.

### Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## References

- Amelung W, Bol R, Friedrich C (1999) Natural  $^{13}\text{C}$  abundance: a tool to trace the incorporation of dung-derived carbon into soil particle-size fractions. *Rapid Commun Mass Spectrom* 13:1291–1294. [https://doi.org/10.1002/\(SICI\)1097-0231\(19990715\)13:13%3C1291::AID-RCM637%3E3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-0231(19990715)13:13%3C1291::AID-RCM637%3E3.0.CO;2-C)
- Angers DA, Rochette P, Chantigny MH, Lapierre H (2007) Use of  $^{13}\text{C}$  abundance to study short-term pig slurry decomposition in the field. *Soil Biol Biochem* 39:1234–1237. <https://doi.org/10.1016/j.soilbio.2006.12.028>
- Becker T, Blume L, Kayser M, Isselstein J (2015) Development of a validity test for survey data on milk-from-grass from German dairy farms. *Grassl Sci Eur* 20:84–86
- Becker T, Kayser M, Tonn B, Isselstein J (2018) How German dairy farmers perceive advantages and disadvantages of grazing and how it relates to their milk production systems. *Livest Sci* 214:112–119. <https://doi.org/10.1016/j.livsci.2018.05.018>
- Bockstaller C, Guichard L, Makowski D, Aveline A, Girardin P, Plantureux S (2008) Agri-environmental indicators to assess cropping and farming systems. A review. *Agron Sustain Dev* 28:139–149. <https://doi.org/10.1051/agro:2007052>
- Bol R, Moering J, Preeedy N, Glaser B (2004) Short-term sequestration of slurry-derived carbon into particle size fractions of a temperate grassland soil. *Isotopes Environ Health Stud* 40:81–87. <https://doi.org/10.1080/10256010310001605955>
- Camin F, Bontempo L, Perini M, Piasentier E (2016) Stable isotope ratio analysis for assessing the authenticity of food of animal origin. *Compr Rev Food Sci Food Saf* 15:868–877. <https://doi.org/10.1111/1541-4337.12219>
- Charlton GL, Rutter SM (2017) The behaviour of housed dairy cattle with and without pasture access: a review. *Appl Anim Behav Sci* 192:2–9. <https://doi.org/10.1016/j.applanim.2017.05.015>
- Clymo RS (2014) Reporting research: a biologist's guide to articles, talks and posters. Cambridge University Press, Cambridge, New York
- Crofoot A (2010) Advantages of on-farm research. In: Proceedings of the New Zealand Grassland Association. pp. 1–2
- Frame J (1992) Improved grassland management. Farming Press; Distributed in North America by Diamond Farm Enterprises, Ipswich, U.K.: Alexandria Bay, NY
- Girardin P, Bockstaller C, der Werf HV (1999) Indicators: tools to evaluate the environmental impacts of farming systems. *J Sustain Agric* 13:5–21. [https://doi.org/10.1300/J064v13n04\\_03](https://doi.org/10.1300/J064v13n04_03)
- Glaser B, Bol R, Preeedy N, McTiernan KB, Clark M, Amelung W (2001) Short-term sequestration of slurry-derived carbon and nitrogen in temperate grassland soil assessed by  $^{13}\text{C}$  and  $^{15}\text{N}$  natural abundance measurements. *J Plant Nutr Soil Sci* 164:467–474. [https://doi.org/10.1002/1522-2624\(200110\)164:5%3C467::AID-JPLN467%3E3.0.CO;2-Q](https://doi.org/10.1002/1522-2624(200110)164:5%3C467::AID-JPLN467%3E3.0.CO;2-Q)
- Gregory NG (2000) Consumer concerns about food. *Outlook Agric* 29:251–257
- Hammes V, Nüsse O, Isselstein J, Kayser M (2017) Using  $^{13}\text{C}$  in cattle hair to trace back the maize level in the feeding regime—a field test. *PLoS ONE* 12:e0188926. <https://doi.org/10.1371/journal.pone.0188926>
- Ingrassia M, Bacarella S, Columba P, Altamore L, Chironi S (2017) Traceability and labelling of food products from the consumer perspective. *Chem Eng Trans* 58:865–870. <https://doi.org/10.3303/CET1758145>
- Ketelaar-de Lauwere CC, Ipema AH, Lokhorst C, Metz JHM, Noordhuizen JPTM, Schouten WGP, Smits AC (2000) Effect of sward height and distance between pasture and barn on cows' visits to an automatic milking system and other behaviour. *Livest Prod Sci* 65:131–142. [https://doi.org/10.1016/S0301-6226\(99\)00175-X](https://doi.org/10.1016/S0301-6226(99)00175-X)
- Knaus W (2013) Re-thinking dairy cow feeding in light of food security. *AgroLife Sci J* 2:36–40
- Knobbe N, Vogl J, Pritzkow W, Panne U, Fry H, Lochotzke HM, Preiss-Weigert A (2006) C and N stable isotope variation in urine and milk of cattle depending on the diet. *Anal Bioanal Chem* 386:104–108. <https://doi.org/10.1007/s00216-006-0644-6>

- Kornel BE, Werner T, Roßmann A, Schmidt H-L (1997) Measurement of stable isotope abundances in milk and milk ingredients—a possible tool for origin assignment and quality control. *Z Für Leb -Forsch A* 205:19–24
- Kyveryga PM (2019) On-farm research: experimental approaches, analytical frameworks, case studies, and impact. *Agron J* 111:2633–2635. <https://doi.org/10.2134/agronj2019.11.0001>
- Mariotti A (1984) Natural  $^{15}\text{N}$  abundance measurements and atmospheric nitrogen standard calibration. *Nature* 311:251–252. <https://doi.org/10.1038/311251a0>
- Nakamura K, Schoeller DA, Winkler FJ, Schmidt H-L (1982) Geographical variations in the carbon isotope composition of the diet and hair in contemporary man. *Biomed Mass Spectrom* 9:390–394
- Niedersächsisches Ministerium für Ernährung, Landwirtschaft und Verbraucherschutz (2022) Die niedersächsische Landwirtschaft in Zahlen 44–45
- Nielsen RL (2010) A practical guide to on-farm research. *Agron. Dep. Purdue Univ West Lafayette IN*
- Piepho H-P, Richter C, Spilke J, Hartung K, Kunick A, Thöle H (2011) Statistical aspects of on-farm experimentation. *Crop Pasture Sci* 62:721. <https://doi.org/10.1071/CP11175>
- Pillonel L, Badertscher R, Casey M, Meyer J, Rossmann A, Schlichtherle-Cerny H, Tabacchi R, Bosset J-O (2005) Geographic origin of European Emmental cheese: characterisation and descriptive statistics. *Int Dairy J* 15:547–556
- Reijs JW, Daatselaar CHG, Helming JFM, Jager J, Beldman ACG (2013) Grazing dairy cows in North-West Europe: economic farm performance and future developments with emphasis on the Dutch situation. LEI Wageningen UR, The Hague
- Rossmann A, Haberhauer G, Hölzl S, Horn P, Pichlmayer F, Voerkelius S (2000) The potential of multielement stable isotope analysis for regional origin assignment of butter. *Eur Food Res Technol* 211:32–40
- Schaak H, Musshoff O (2018) Grazing adoption in dairy farming: a multivariate sample-selection approach. *J Agric Resour Econ* 43(2):292–305
- Schnyder H, Schwertl M, Auerswald K, Schäufele R (2006) Hair of grazing cattle provides an integrated measure of the effects of site conditions and interannual weather variability on  $\delta^{13}\text{C}$  of temperate humid grassland. *Glob Change Biol* 12:1315–1329. <https://doi.org/10.1111/j.1365-2486.2006.01169.x>
- Schwertl M, Auerswald K, Schnyder H (2003) Reconstruction of the isotopic history of animal diets by hair segmental analysis. *Rapid Commun Mass Spectrom* 17:1312–1318. <https://doi.org/10.1002/rcm.1042>
- Schwertl M, Auerswald K, Schäufele R, Schnyder H (2005) Carbon and nitrogen stable isotope composition of cattle hair: ecological fingerprints of production systems? *Agric Ecosyst Environ* 109:153–165. <https://doi.org/10.1016/j.agee.2005.01.015>
- Smith BN, Epstein S (1971) Two categories of  $^{13}\text{C}/^{12}\text{C}$  ratios for higher plants. *Plant Physiol* 47:380–384
- Steinwider A, Häusler J (2004) Anforderungen an die Fütterung im Mutterkuhbetrieb. *Bericht* 31:5–20
- Stirm JW, St-Pierre NR (2003) Identification and characterization of location decision factors for relocating dairy farms. *J Dairy Sci* 86:3473–3487
- van den Pol-van Dasselaar A, Bastiaansen-Aantjes L, Bogue F, O'Donovan M, Huyghe C (eds) (2020a) Grassland use in Europe: a syllabus for young farmers. Éditions Quae, Versailles
- van den Pol-van Dasselaar A, Hennessy D, Isselstein J (2020b) Grazing of dairy cows in Europe—an in-depth analysis based on the perception of grassland experts. *Sustainability* 12:1098. <https://doi.org/10.3390/su12031098>
- Webster R, Oliver MA (1990) Statistical methods in soil and land resource survey, Spatial information systems. Oxford University Press, Oxford [England]; New York
- White CD, Longstaffe FJ, Law KR (1999) Seasonal stability and variation in diet as reflected in human mummy tissues from the Kharga Oasis and the Nile Valley. *Palaeogeogr Palaeoclimatol Palaeoecol* 147:209–222
- Wittmer MHOM, Auerswald K, Schönbach P, Schäufele R, Müller K, Yang H, Bai YF, Susenbeth A, Taube F, Schnyder H (2010) Do grazer hair and faeces reflect the carbon isotope composition of semi-arid  $\text{C}_3/\text{C}_4$  grassland? *Basic Appl Ecol* 11:83–92. <https://doi.org/10.1016/j.baae.2009.10.007>
- Zhao Y, Zhang B, Chen G, Chen A, Yang S, Ye Z (2013) Tracing the geographic origin of beef in China on the basis of the combination of stable isotopes and multielement analysis. *J Agric Food Chem* 61:7055–7060

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