



Forage silica and water content control dental surface texture in guinea pigs and provide implications for dietary reconstruction

Daniela E. Winkler^{a,1}, Ellen Schulz-Kornas^b, Thomas M. Kaiser^c, Annelies De Cuyper^d, Marcus Claus^e, and Thomas Tütken^a

^aApplied and Analytical Paleontology, Institute of Geosciences, Johannes Gutenberg University, 55128 Mainz, Germany; ^bMax Planck Weizmann Center for Integrative Archaeology and Anthropology, Max Planck Institute for Evolutionary Anthropology, 04103 Leipzig, Germany; ^cCenter of Natural History (CeNak), Universität Hamburg, 20146 Hamburg, Germany; ^dLaboratory of Animal Nutrition, Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium; and ^eClinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

Edited by Dolores R. Piperno, Smithsonian Institution, Washington, DC, and approved December 6, 2018 (received for review August 15, 2018)

Recent studies have shown that phytoliths are softer than dental enamel but still act as abrasive agents. Thus, phytolith content should be reflected in dental wear. Because native phytoliths show lower indentation hardness than phytoliths extracted by dry ashing, we propose that the hydration state of plant tissue will also affect dental abrasion. To assess this, we performed a controlled feeding experiment with 36 adult guinea pigs, fed exclusively with three different natural forages: lucerne, timothy grass, and bamboo with distinct phytolith/silica contents (lucerne < grass < bamboo). Each forage was fed in fresh or dried state for 3 weeks. We then performed 3D surface texture analysis (3DST) on the upper fourth premolar. Generally, enamel surface roughness increased with higher forage phytolith/silica content. Additionally, fresh and dry grass feeders displayed differences in wear patterns, with those of fresh grass feeders being similar to fresh and dry lucerne (phytolith-poor) feeders, supporting previous reports that “fresh grass grazers” show less abrasion than unspecialized grazers. Our results demonstrate that not only phytolith content but also properties such as water content can significantly affect plant abrasiveness, even to such an extent that wear patterns characteristic for dietary traits (browser–grazer differences) become indistinguishable.

surface texture | tooth wear | microtexture | grazing | phytoliths

The interaction between ingesta and the occlusal surfaces of teeth during mastication leads to abrasive wear. Wear features on tooth enamel such as pits and scratches have been recognized as indicators of diet ever since Baker et al.’s (1) original microwear study on sheep. Since then, many studies have been published on tooth wear analyses at different spatial scales [mesowear, microwear, dental microwear texture analysis (DMTA)] for a large variety of animals, and characteristic wear patterns related to their assumed natural diets have been described (2–7). Dental wear patterns derived from these methods have been employed for dietary reconstruction in extant and extinct mammals whose diet could not be observed by other means, as well as to assess environmental and climatic conditions of past ecosystems (8–13).

One way to corroborate the interpretation of tooth wear patterns is to combine different tooth wear methods and other dietary proxies (e.g., stable isotopes, stomach content analysis) on the teeth of the same individuals (14–19). However, several studies found a discrepancy between the mesowear pattern and carbon isotope compositions (15–17) or dental microwear texture and rumen contents of the same animals (18, 19), suggesting that both proxies may provide different dietary information. Regardless, controlled feeding experiments wherein animals receive a well-characterized diet for a defined period of time prior to dental wear analysis remain the gold standard for the interpretation of causal relationships between diet characteristics and dental wear patterns (20–22) in extant animals.

A major focus of diet reconstruction via dental wear has been on the browser–grazer dichotomy in large ungulates. The browser–grazer dichotomy is well differentiated in mesowear, microwear, and DMTA. Browsers are characterized by a lower amount of abrasive tooth wear, which is reflected in sharper mesowear profiles (3, 23) and wear facet microwear dominated by pits (24). In contrast, grazers show rounded to blunt tooth cusps in mesowear and scratch-dominated microwear. A recent approach (25) extended the mesowear method to small mammals and found cusp shape to reflect the expected gradient corresponding to dietary abrasion. DMTA is currently represented by two methods, scale-sensitive fractal analysis (SSFA) (26) and 3D surface texture analysis (3DST) (5, 27). Both approaches employ optical profilometry to obtain 3D representations of the enamel surface at submicron resolution and evaluate the overall distribution and 3D geometry of topographic features. In SSFA, surface features are described by four parameters partially derived from industrial application (*Asfc*, *HAsfc*, *epLsa*, *Tfv*), for example, showing high complexity and low anisotropy values for browsers, and the opposite pattern exhibited by grazers (28, 29). 3DST uses 46 standardized parameters

Significance

Ingesta leave characteristic wear features on the tooth surface, which enable us to reconstruct the diet of extant and fossil vertebrates. However, whether dental wear is caused by internal (phytoliths) or external (mineral dust) silicate abrasives is controversially debated in paleoanthropology and biology. To assess this, we fed guinea pigs plant forages of increasing silica content (lucerne < grass < bamboo) without any external abrasives, both in fresh and dried state. Abrasiveness and enamel surface wear increased with higher forage phytolith content. Additionally, water loss altered plant material properties. Dental wear of fresh grass feeding was similar to lucerne browsing, while dried grass caused more grazer-like wear. Fresh grass grazing could be confounded with browsing, being a major pitfall for paleodietary reconstructions.

Author contributions: M.C. and T.T. designed research; D.E.W. and M.C. performed research; D.E.W. and A.D.C. analyzed data; D.E.W., E.S.-K., T.M.K., M.C., and T.T. wrote the paper; E.S.-K. and T.M.K. gave advice on data interpretation; and A.D.C. analyzed nutrient composition of plant forages.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

This open access article is distributed under [Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 \(CC BY-NC-ND\)](https://creativecommons.org/licenses/by-nc-nd/4.0/).

¹To whom correspondence should be addressed. Email: daniela.winkler@uni-mainz.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1814081116/-DCSupplemental.

Published online January 3, 2019.

belonging to six different sets of analyses from engineering application (ISO 12781, ISO 25175, motif, furrow, direction, and isotropy) to characterize wear features. Using this method, browser teeth are characterized by overall flat surfaces with low peaks, while grazers are characterized by greater surface roughness, more and higher peaks, and deeper dales, but overall a generally less variable pattern (20, 27).

Regardless of the method applied, the question of which inherent mechanical properties of the diet are responsible for this difference between grazers and browsers is still under debate. On the one hand, internal abrasives, namely opal phytoliths [amorphous silica bodies with complex ultrastructural patterns (30)], have long been suggested as the causal agents of the scratch-dominated tooth wear characteristic of grazers (31–33). Recent studies, however, produced equivocal results on whether phytoliths can actually scratch enamel (34–37). On the other hand, grazing is associated with feeding close to the ground and often also with seasonally arid and hence dusty grassland environments. Therefore, airborne mineral dust and soil particles adherent to vegetation have been suggested as the other agents causing tooth wear (31, 38–40). In a controlled feeding experiment with sheep, Merceron et al. (21) tested the effect of feeding clover and grass with and without addition of less than ~0.8% silt/fine sand-sized (<100 μm), quartz-rich mineral dust as an external abrasive to sheep. They found no significant influence of the added mineral dust on dental microwear texture and argued that phytolith content and mechanical properties of the forage are the main causes of dental abrasion. In contrast, in controlled feeding experiments with rabbits and guinea pigs, both an increase in phytolith contents as well as in external abrasives led to more pronounced molar wear when measured by computer tomography (22, 41). However, the forages used in the latter experiments consisted of pelleted diets and dental microwear texture was not evaluated; therefore, results are not directly comparable.

A viable approach to further investigate the effect of phytoliths is a controlled feeding experiment with plants of low (browse) and high (grass) phytolith/silica content. We tested whether, in the absence of adherent soil particles or external mineral dust in general, enamel surface texture varies between a browsing (dicot: lucerne), a grazing (monocot: grass), and a phytolith-rich grazing (monocot: bamboo) diet in a small mammal model, the guinea pig (*Cavia porcellus*). Domestic guinea pigs are hindgut fermenters and derived from the wild Brazilian guinea pig (*Cavia aperea*). Their wild ancestors are generalist grazers and primarily feed on Poaceae (42). Applying 3DST, we expected enamel surface roughness on wear facets to increase with phytolith content. We further predicted that the moisture content of the forage would also have an effect on tooth wear and therefore fed each of the three forages both in a fresh state and—from the same batch—in a dried “hay” state. It is expected that water functions as a plasticizer, whereby in wet conditions high moisture content of cells leads to an increase in modulus of elasticity and reduces the probability of brittle fracture (43). For grass, it is known that small changes in the volume fraction of the sclerenchyma fibers will produce large changes in the stiffness and strength of the leaf (44), with increasing stiffness or toughness in drier material (45, 46). Furthermore, a recent study (37) showed that native phytoliths have lower indentation hardness than phytoliths extracted by dry ashing; therefore, the hydration state of plant tissue could also affect dental abrasion. We thus expected to find differences between fresh and dry forages (smaller volume fraction) that would elucidate the empirical observation that so-called “fresh grass grazers” do not follow the same dental wear patterns as “dry grass grazers” (3).

Results

There was a significant difference in acid detergent-insoluble ash content in the feces of the six guinea pig groups [general linear model, $F_{(3,32)} = 663.628$, $P < 0.001$], with a significant effect of

forage [$F_{(2,32)} = 988.795$, $P < 0.001$; lucerne < grass < bamboo, all post hoc tests, $P < 0.001$] as well as a significantly higher content in feces from animals feeding on fresh forages [$F_{(1,32)} = 13.298$, $P = 0.001$; Fig. 1]. The X-ray diffraction (XRD) analysis showed no detectable contamination with external abrasives of siliciclastic mineral dust (SI Appendix, Fig. S1).

Descriptive statistics of all 3DST parameters and results of statistical tests are given in SI Appendix, Tables S3 and S4, and selected parameters are displayed in Fig. 2 and in SI Appendix, Fig. S2. There was a distinct trend for increasing surface roughness from the two lucerne groups and the fresh grass group to the dry grass group and both bamboo groups. Surface textures of both the dry and fresh lucerne groups were characterized by low roughness (Sa , Sq), a low number of dales of medium width and volume (Sda , Sdv), rounded peaks (Spc), and an overall medium depth and density of furrows ($metf$, $medf$). The group fed dry lucerne showed no significant differences to the group fed fresh lucerne, but had slightly larger dales, a higher density of peaks (Spd), and a more isotropic orientation of features (IsT).

Surface textures of the group fed fresh grass had the highest density of peaks of all feeding groups but were almost indistinguishable from both fresh and dry lucerne in height and volume parameters. It showed no significant differences to the group fed fresh lucerne, but slightly lower volume (Vmc , Vv), more pronounced flatness ($FLTq$, $FLTl$), and smaller hills (Shv , Sha). Dry grass was significantly different from fresh grass in 34 out of 44 parameters as signified by Lincon test; however, only three parameters (IsT , meh , $S5v$) were confirmed by Cliff's method. The surface texture profiles of the dry grass group were characterized by large and deep dales, larger and higher peaks, deep furrows, and a greater overall surface roughness than fresh grass and both the fresh and dry lucerne groups.

Both bamboo groups were well distinguishable from all other feeding groups and showed significant differences in 41 3DST parameters; 13 were confirmed by Cliff's method. The group fed fresh bamboo showed surface textures with very large, deep dales combined with a low number of sharp, wide, and large peaks. Furrows were very deep but few in density, and anisotropic. Surface roughness was highest in this group. The dry bamboo group did not differ significantly in any surface texture parameters from the fresh bamboo group.

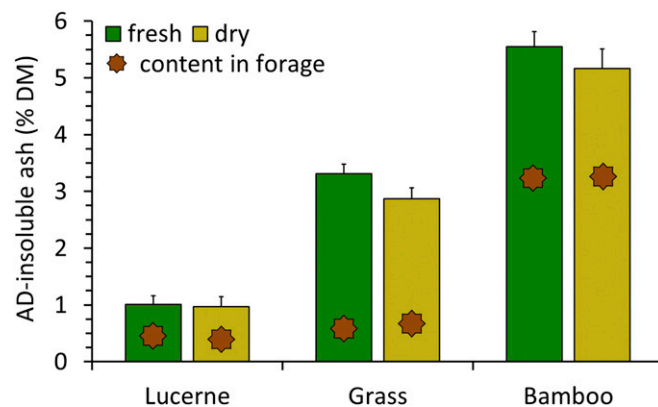


Fig. 1. Mean (+SD) acid detergent-insoluble ash (ADIA) (a proxy for silica content) of feces of guinea pigs (*Cavia porcellus*) fed lucerne (*Medicago sativa*), timothy grass (*Phleum pratense*), or bamboo (*Phyllostachys aureosulcata* f. *spectabilis*) in fresh and dried state ($n = 6$ per group). The ADIA values for milled plant forages are indicated by the star (data from SI Appendix, Table S1). Note that variation in the difference of ADIA content of diet and feces is due to different digestibilities of the three forages (which was highest for the grass diets and was higher for fresh than for dried diets).

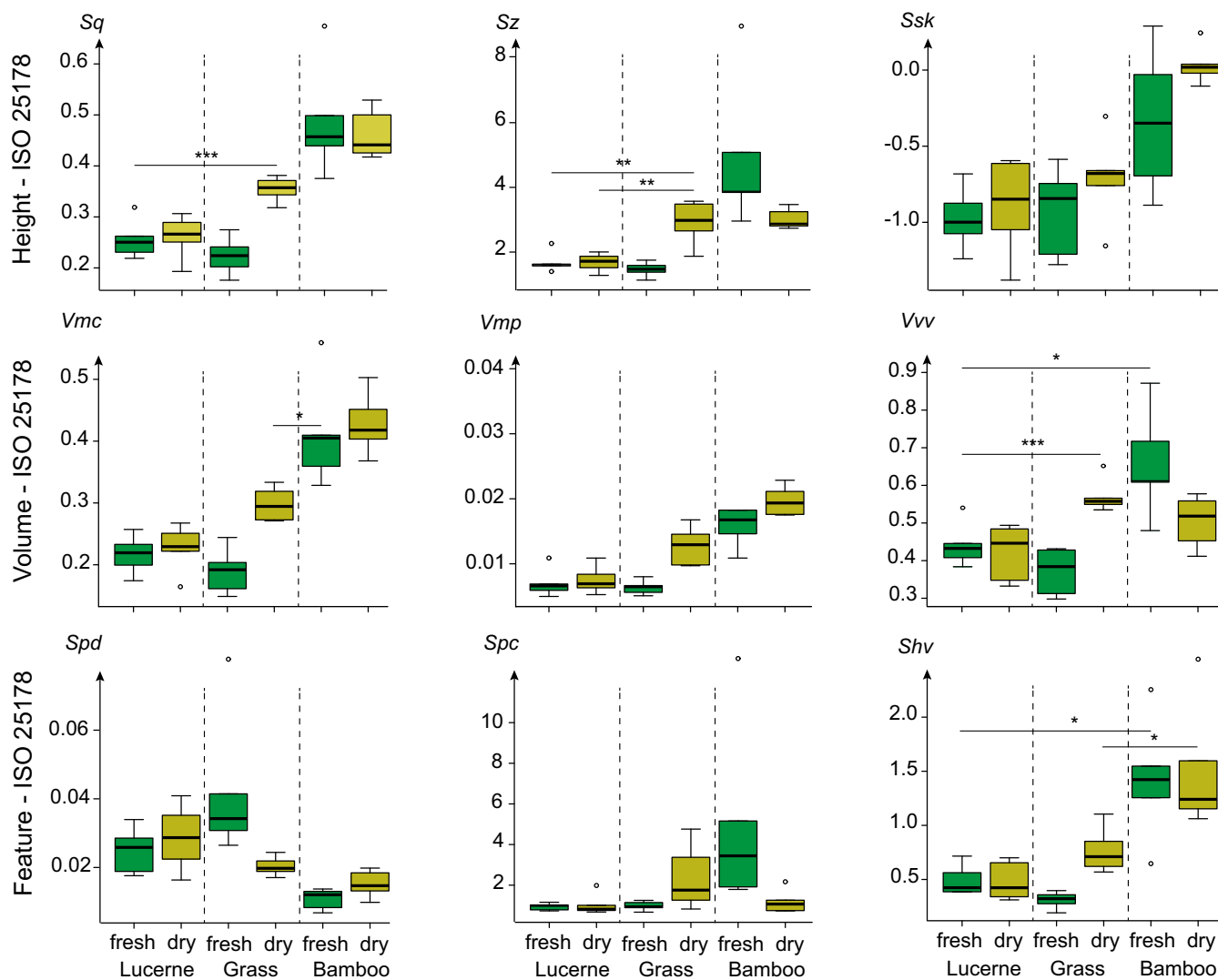


Fig. 2. Selection of 3DST parameters with best discrimination between feeding groups. Significance levels from Lincon test: *0.05, **0.01, and ***0.001, confirmed by Cliff's method. The thick horizontal bar represents the median; the box encloses the first (25%) and third (75%) quartiles; the whiskers extend to 1.5 times the length of the box (the interquartile range); the unfilled dots represent outliers. *Shv*, closed hill volume; *Spc*, arithmetic mean peak curvature; *Spd*, density of peaks; *Sq*, SD of the height distribution (or RMS surface roughness); *Ssk*, skewness of the height distribution; *Sz*, maximum height, height between the highest peak and the deepest valley; *Vmc*, material volume of the core at given material ratio ($p = 10\%$, $q = 80\%$); *Vmp*, material volume of peaks ($p = 10\%$); *Vv*, void volume at a given material ratio ($p = 10\%$). Additional parameters are displayed in *SI Appendix*, Fig. S2.

Discussion

As intended by the experimental design, the forages differed in silica content, the animals actually ingested these different forages, and forages did not contain external abrasives. In plants, the main accumulation of silica is found in the form of phytoliths; thus, silica content is an adequate estimate for phytolith content (47, 48). The natural diet with the highest silica content in this experiment, bamboo, produced a clearly distinguishable surface texture signal. At the same time, our results suggest that, at least in the fresh state, the difference in plant anatomy between browse and grass, with the notable parallel venation in the latter (49, 50), has little effect on the surface texture formation.

We observed ambiguous effects of forage water content. Theoretically, the propagation of cracks should require less force in a more brittle plant material, as it has less resistance to plastic deformation compared to a well-hydrated leaf (49). The mechanics of crack propagation, elastic deformation, and tough and brittle fracture are highly complex, especially in biological composite materials (49, 51). Nevertheless, Schulz et al. (27) suggested that

soft, dicotyledonous leaves (browse) as in lucerne do not present high degrees of mechanical resistance to fracture, and thus antagonistic teeth are prone to a large amount of tooth-to-tooth contact (attrition). High levels of attrition can lead to a sharpening effect in teeth as observed in sharp mesowear profiles of large ungulates (3). On a micrometer scale, however, the contact of antagonistic teeth without a bulk of food in between is thought to reduce surface roughness on chewing facets and abrade peaks, leading to flat profiles with rounded peaks (27, 52).

This is exactly the condition we observe in both lucerne feeding groups and the fresh grass group. The larger density of peaks and not significantly higher overall surface roughness in the dry lucerne group might relate to the fact that guinea pigs consumed dried lucerne in slightly larger bulk, because they could ingest more material of a dried forage with one "mouthful." We hypothesize that dry material can be packed more densely than fresh material. In accordance with this concept, the instantaneous intake rate of dried lucerne or grass was higher than that of the same forages in a fresh state in cattle (53, 54). Additionally, salivation does not vary

substantially between fresh and dry forages per unit dry matter intake (DMI) in cattle (55). If this would also hold true for guinea pigs, the drier material would have been less hydrated and lubricated. An oral cavity more densely packed with material of lower hydration status would then lead to less attrition and preservation of more distinct surface texture features such as peaks. Comparing bite size and mouthful on the same forages in fresh and dry state would be required to ultimately corroborate this interpretation.

Previous studies have found that ungulate grazers show greater surface roughness, more voluminous surfaces, and a high density of peaks compared to browsers (5, 8, 27). In the present study, the fresh grass group showed lowest surface roughness and volume, highest density of peaks and rounded peaks, all attributes typically associated with browsing and also present in the lucerne feeding groups. We note that timothy grass has a moderate silica content compared to other Poaceae (56), and yet gave either a rougher, grazer-like texture (in the dried state) or a texture resembling that for browsers (in the fresh state). The resemblance between fresh-grass grazers and browsers has been noted before in mesowear (3) and also in the cranial morphology of ungulates (57), and fresh-grass grazer has long been recognized as a dietary category distinct from other grazers (57–59). The common explanation for discrepancies in tooth wear between fresh-grass grazers and general grazers is that the former forage in more humid environments, whereas the latter consume more soil material and grit or airborne mineral dust as they feed in more arid and hence dusty habitats (57, 59). Furthermore, dry grasses are often more mature and may have built up more phytoliths compared to the younger grasses that are consumed by fresh-grass grazers. Our results, however, show that such a distinction between fresh-grass grazing and dry-grass grazing can even be produced in absence of external abrasives and with fresh and dry grass of the same maturation stage.

In spite of a similar level of silica content in the forage, the dry grass group shows significantly higher surface roughness, more voluminous textures, deeper furrows (or scratches as termed in microwear), higher density of peaks, and sharper peaks than the fresh grass group. Thus, it was generally more similar in surface texture to the bamboo groups than the fresh grass group was. The observed difference in surface texture cannot be explained by a change in daily DMI in the dry grass group, as this did not differ from daily DMI in the fresh grass group (*SI Appendix, Fig. S4*). We thus conclude that drying has significantly changed the material properties of the grass as shown by Vincent (45) and Henry et al. (46). In contrast, such an effect is not seen in

lucerne. Thus, the inherent structural properties of the grass blades or their abrasiveness was changed by drying, even though they became more brittle and should fracture more easily. A recent study (37) suggests that phytolith characteristics depend on the extraction method, with native phytoliths showing lower indentation hardness than phytoliths extracted by dry ashing. Consequently, the dehydration of plant tissues could have altered the hardness of phytoliths and led to more abrasion in the dry grass group. Additionally, we hypothesize that grass hay is consumed in larger bulks per mouthful, which may prevent peaks from being worn by attrition, as mentioned above.

The influence of phytolith content on surface texture patterns is further corroborated by the bamboo groups. Bamboo resembles grass in the parallel arrangement of vascular bundles (49) and is the plant with the highest phytolith content (32 g/kg SiO₂ in dry matter) in our experiment. Guinea pigs that fed on bamboo show the highest enamel surface roughness of all groups. It could be assumed that bamboo has a lower nutritional value, and thus both bamboo groups showed a higher DMI compared to the other feeding groups, which in turn led to more abrasive tooth wear. This, however, is not the case. The bamboo groups did consume substantially more dry matter than the lucerne groups, but only slightly more than the grass groups (*SI Appendix, Fig. S4*). Hence, the high phytolith content is most likely responsible for the increased abrasiveness observed in bamboo feeding groups.

The effect of drying is less pronounced and not consistent in bamboo, with the dry bamboo group having more voluminous textures with deeper furrows, but similar roughness and flatness as the fresh bamboo group. We suggest that this is due to the smaller difference in water content between fresh and dry bamboo as compared to fresh and dry grass—bamboo showed a weight loss of 40–50% after drying, while grass (and lucerne) had a weight loss of 70–80%. Additionally, bamboo had a very high lignin content, which could contribute to maintaining the structural integrity of leaves after drying. Similar to bamboo, there were no significant differences confirmed by all statistical tests between fresh lucerne and dry lucerne, with the latter causing only slightly rougher and more voluminous textures with a higher density of peaks. We conclude that the mastication of lucerne hay leads to similar tooth wear as the mastication of fresh lucerne, indicating that variation in hydration state does not affect the material properties of lucerne, suggesting again that the higher lignification than that of grass might play a more important role.

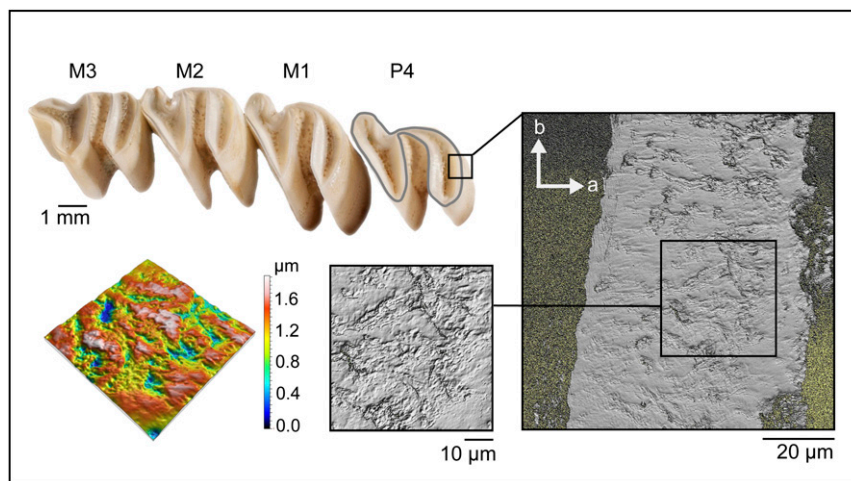


Fig. 3. Graphical representation of the measurement position on the chewing facet of the right upper fourth premolar (P4) of individual 2CC4R1 (lucerne dry); the gray line indicates the enamel band of P4. The original size of the 3D scan is 160 × 160 μm; subsequently, an area of 60 × 60 μm is cut out manually. a, anterior; b, buccal.

Based on our small sample of three forages, we thus hypothesize that both a high moisture content, and a low lignin content, will lead to more distinct changes in material properties of the plant matter due to drying and result in distinct wear effects of fresh and dry material. This hypothesis could be tested further by measuring the effect of the same batch of grass, harvested at different stages of lignification, fed in both fresh and dry state to a model organism. To which degree surface texture is directly linked to the concentration of abrasive silica could be tested by feeding a standardized diet of different silica levels.

Conclusion

In absence of external abrasives, phytolith and water content of the plant forages are important factors leading to distinctly different enamel surface textures, whereas the change in material properties due to drying is possibly mitigated by the degree of lignification. Grazing on phytolith-rich plants (fresh/dry bamboo) is well characterized by rough, voluminous 3DST and can be distinguished from general grazing (dry grass) and browsing (fresh/dry lucerne). In our experiment, fresh-grass grazing, however, is indistinguishable by the enamel 3DST from browsing in guinea pigs. This highlights that mechanical material properties of the organic plant matter may be as important as its phytolith content or environmental factors (e.g. dustiness) and need to be taken into account when applying dental wear proxies for dietary reconstruction of extant and extinct herbivores.

Materials and Methods

The controlled feeding experiment was approved of by the Cantonal Veterinary Office in Zurich, Switzerland (license no. ZH135/16) and performed at the University of Zurich in September/October 2017.

Animals, Housing, and Diets. Adult female Dunkin Hartley (HsdDhl:DH) guinea pigs ($n = 36$; initial body mass, 263 ± 14 g) were housed in groups of six in sheltered outside stables (2.25 m^2 each), each protected from direct sunlight and rain, equipped with an infrared lamp, a ground covered in a thick layer of sawdust, two large wooden shelters, and two plastic tubes, two open dishes, and two nipple drinkers. Stables were sheltered to the sides by canvas to prevent airborne dust from contaminating the forages. No extra gnawing material was provided. Moreover, gnawing activities typically involve only the incisors, and as we assess 3D surface textures on premolars (which are formed during food mastication), the influence of non-diet-related gnawing activities is unlikely.

Water (supplemented with vitamin C at 200 mg/L) and food were provided for ad libitum consumption. Fresh forages were harvested daily during September/October 2017 from two monoculture fields of either lucerne (*Medicago sativa*) or timothy grass (*Phleum pratense*), or from pots of a single species of bamboo (*Phyllostachys aureosulcata* f. *spectabilis*) purchased from a local tree nursery in Zurich, and provided several times a day. Timothy grass was up to 40 cm tall with seed heads bearing seeds. Bamboo plants were between 150 and 200 cm tall, and only leaves (with petioles) and thin stems were provided as forage. From each batch of grass and bamboo, a proportion was oven-dried at 50 °C (at least 8 h for the grass and at least 3 h for the bamboo to yield similar material of a typical hay consistency). Because of space limitations for growing lucerne, lucerne hay had been prepared prior to the experiment by sun curing of material harvested from the same field at the same stage of maturity. Careful handling of lucerne plants resulted in lucerne hay whose stems and leaves remained connected. The lucerne and bamboo were watered daily and sprinkled to remove adherent mineral dust. Grass was very moist after harvesting (which occurred at dawn) and was subsequently sprinkled to keep it fresh and remove mineral dust and grit. We checked for the presence of any adhering external mineral dust by performing XRD analysis on ball-milled forage samples of all three fodder plants using a Seifert XRD 3000 TT diffractometer (60). Forage samples of 50- to 100-g fresh weight were collected on 3 consecutive days, pooled, and homogenized to achieve a representative sample. One-gram aliquots of lucerne powder were additionally mixed with 0.5, 1, 2, and 4 wt% of quartz meal (4 μm , 99.5% SiO_2), respectively, to empirically determine the threshold value for the detection of external crystalline SiO_2 contamination (*SI Appendix, Fig. S1*). The nutrient composition, including the acid detergent-insoluble ash content (a proxy for silica) (61), of the fresh and dried forages is given in *SI Appendix, Table S1*. The silica content of the three plant

forages was low in lucerne (3.9–4.7 g/kg dry matter), slightly higher in timothy grass (5.8–6.7 g/kg dry matter), and high in bamboo (32.1–32.5 g/kg dry matter), which agrees well with literature data for these plant species (56, 62). The moisture loss due to oven drying at 50 °C displays the inverse pattern with $82 \pm 4\%$ in lucerne, $75 \pm 4\%$ in grass, and $46 \pm 8\%$ in bamboo, with a significant difference (ANOVA, $df = 2$, $P < 0.001$) between bamboo and the other two forages (Sidak post hoc test, $P < 0.001$ in both cases). As intended, the forages differed in silica (and hence phytolith) content, and as reported in the literature, bamboo contained a very high level of lignin, both when expressed as absolute concentration, and as a proportion of dietary fiber (*SI Appendix, Table S1*). On a dry-matter basis, nutrient composition was similar between fresh and dried forages of a plant species (*SI Appendix, Table S1*). For visualization of phytoliths, we also performed plasma etching of grass and bamboo leaves for up to 9 h using a K1050x RF-plasma etcher (Quorum Technologies) and took SEM images on a Phenom Pro (Thermo Fisher) (*SI Appendix, Fig. S3*).

All animals were provided with their designated diet from the first day onward; the pelleted compound diet provided by the animal breeder was phased out during a 5-d acclimatization period, after which the animals were maintained solely on their designated diets for 21 d. During this time, feces were collected from each individual animal for a 4-d period in a metabolic cage. A pooled sample of these 4-d feces was subsequently analyzed for their silica content (Fig. 1). During the metabolic cage phase, daily forage intake was measured and is given as dry-matter intake in *SI Appendix, Fig. S4*. Although all groups gained weight during the 3 wk of the experiment, the weight gain in the bamboo groups was considered too low for a normal development of growing guinea pigs. At the end of the experiment, the animals were euthanized with carbon dioxide. The skulls were dissected and stored frozen at -20 °C until maceration.

Skull Preparation and 3D Surface Texture Measurements. Skulls were macerated in an enzyme-containing warm water bath at 55 °C for 3 d at the Center of Natural History at University of Hamburg (Hamburg, Germany). Surface texture measurements were acquired using the high-resolution confocal disc-scanning surface measuring system μsurf Custom (Nanofocus AG) with a blue LED (470 nm) and high-speed progressive-scan digital camera (984×984 pixel), set to a 100 \times long-distance objective (numerical aperture, 0.8; resolution in x , $y = 0.16 \mu\text{m}$; step size in $z = 0.06 \mu\text{m}$), and processed with MountainsMap Premium, version 7.4.8676, Software (DigitalSurf; www.digitalsurf.com). We measured the anterior enamel band on the right upper fourth premolar (P4) (Fig. 3). As the default scanning area ($160 \times 160 \mu\text{m}$) exceeds the width of the enamel bands in *C. porcellus*, surface scans were cropped manually in MountainsMap Premium to a size of $60 \times 60 \mu\text{m}$. We followed the suggested filtering procedure of Schulz et al. (20) and took four nonoverlapping scans per individual facet (in a few individuals, only three scans could be acquired, as enamel bands were too thin or showed damages). In a comparison of filtering protocols, Kubo et al. (63) recently found this routine to be the best suited to detect dietary differences. Forty-four surface texture parameters were quantified using the ISO 25178 (roughness), motif, furrow, isotropy, and ISO 12871 (flatness) analysis (*SI Appendix, Table S2*). We excluded one individual from the bamboo fresh group because the enamel on both upper fourth premolars was damaged.

Statistical Analyses. Fecal silica concentrations were compared between the groups using a general linear model in SPSS 23.0 (IBM) with forage (lucerne/grass/bamboo) and state (fresh/dry) as cofactors (the interaction was not significant), confirming the normal distribution of residuals with a Kolmogorov–Smirnov test, and comparing forages by Sidak post hoc test. Statistical analyses of texture results were performed using the software R, version 3.3.1 (64) with the packages *xlsx* (65), *rJava* (66), *doBy* (67), *R.utils* (68), and *WRS*, version 0.12.1 (69). Surface texture data are generally non-normally distributed. Therefore, we used the procedure of Wilcox (70), applying the robust heteroscedastic Welch–Yuen omnibus test (71, 72) coupled with a heteroscedastic pairwise comparison test (ref. 73: “Lincon test,” analogous to Dunnett’s T3 test). Significances were confirmed using the robust heteroscedastic rank-based test according to Cliff (74). For a detailed description of the statistical procedure, see Calandra et al. (52) and Schulz et al. (27). The significance level was set to 0.05.

ACKNOWLEDGMENTS. We thank Louise Martin, Nicole Schmid, and Kathrin Zbinden for support in animal husbandry; Victor Haus, Jan Hediger, and Daryl Codron for support in plant management; Jennifer Leichter for correction of English language and for XRD sample preparation; Ralf Meffert for XRD analyses; and Michael Maus for technical support in plasma etching and SEM imaging. This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 Research and Innovation Programme (Grant Agreement 681450) (ERC Consolidator Grant Agreement to T.T.).

1. Baker G, Jones LHP, Wardrop ID (1959) Cause of wear in sheep's teeth. *Nature* 184: 1583–1584.
2. Fraser D, Theodor JM (2011) Comparing ungulate dietary proxies using discriminant function analysis. *J Morphol* 272:1513–1526.
3. Fortelius M, Solounias N (2000) Functional characterization of ungulate molars using the abrasion-attrition wear gradient: A new method for reconstructing palaeodiets. *Am Mus Novit* 3301:1–36.
4. Calandra I, Merceron G (2016) Dental microwear texture analysis in mammalian ecology. *Mammal Rev* 46:215–228.
5. Schulz E, Calandra I, Kaiser TM (2010) Applying tribology to teeth of hoofed mammals. *Scanning* 32:162–182.
6. DeSantis LR, et al. (2013) Direct comparisons of 2D and 3D dental microwear proxies in extant herbivorous and carnivorous mammals. *PLoS One* 8:e71428.
7. Purnell MA, Crumpton N, Gill PG, Jones G, Rayfield EJ (2013) Within-guild dietary discrimination from 3-D textural analysis of tooth microwear in insectivorous mammals. *J Zool* (1987) 291:249–257.
8. Winkler DE, et al. (2013) Indications for a dietary change in the extinct bovid genus *Myotragus* (Plio-Holocene, Mallorca, Spain). *Geobios* 46:143–150.
9. Solounias N, Teaford M, Walker A (1988) Interpreting the diet of extinct ruminants: The case of a non-browsing giraffid. *Palaeobiology* 14:287–300.
10. Scott RS, et al. (2005) Dental microwear texture analysis shows within-species diet variability in fossil hominins. *Nature* 436:693–695.
11. Merceron G, Schulz E, Kordos L, Kaiser TM (2007) Palaeoenvironment of *Dryopithecus brancoi* at Rudabánya, Hungary: Evidence from dental meso- and micro-wear analyses of large vegetarian mammals. *J Hum Evol* 53:331–349.
12. Semprebon G, Rivals F (2007) Was grass more prevalent in the pronghorn past? An assessment of the dietary adaptations of Miocene to recent Antilocapridae. *Palaeogeogr Palaeoclimatol Palaeoecol* 253:332–347.
13. Rivals F, et al. (2018) Dietary traits of the ungulates from the HWK EE site at Olduvai Gorge (Tanzania): Diachronic changes and seasonality. *J Hum Evol* 120:203–214.
14. Louys J, Ditchfield P, Meloro C, Elton S, Bishop LC (2012) Stable isotopes provide independent support for the use of mesowear variables for inferring diets in African antelopes. *Proc Biol Sci* 279:4441–4446.
15. Jones DB, Desantis LR (2017) Dietary ecology of ungulates from the La Brea tar pits in southern California: A multi-proxy approach. *Palaeogeogr Palaeoclimatol Palaeoecol* 466:110–127.
16. Loffredo LF, DeSantis LR (2014) Cautionary lessons from assessing dental mesowear observer variability and integrating paleoecological proxies of an extreme generalist *Cormohipparion emslii*. *Palaeogeogr Palaeoclimatol Palaeoecol* 395:42–52.
17. Tütken T, Kaiser TM, Vennemann T, Merceron G (2013) Opportunistic feeding strategy for the earliest Old World hypsodont equids: Evidence from stable isotope and dental wear proxies. *PLoS One* 8:e74463.
18. Merceron G, Escarguel G, Angibault JM, Verheyden-Tixier H (2010) Can dental microwear textures record inter-individual dietary variations? *PLoS One* 5:e9542.
19. Berlioz E, Azorit C, Blondel C, Tellado Ruiz MS, Merceron G (2017) Deer in an arid habitat: Dental microwear textures track feeding adaptability. *Hystrix Ital J Mammal* 28:222–230.
20. Schulz E, et al. (2013) Dietary abrasiveness is associated with variability of microwear and dental surface texture in rabbits. *PLoS One* 8:e56167.
21. Merceron G, et al. (2016) Untangling the environmental from the dietary: Dust does not matter. *Proc Biol Sci* 283:20161032.
22. Müller J, et al. (2014) Growth and wear of incisor and cheek teeth in domestic rabbits (*Oryctolagus cuniculus*) fed diets of different abrasiveness. *J Exp Zool A Ecol Genet Physiol* 321:283–298.
23. Kaiser TM, Solounias N, Fortelius M, Bernor RL, Schrenk F (2000) Tooth mesowear analysis on *Hippotherium primigenium* from the Vallesian Dinotheriensande (Germany)—a blind test study. *Carolinea* 58:103–114.
24. Walker A, Hoek HN, Perez L (1978) Microwear of mammalian teeth as an indicator of diet. *Science* 201:908–910.
25. Ulbricht A, Maul LC, Schulz E (2015) Can mesowear analysis be applied to small mammals? A pilot-study on leporines and murines. *Mamm Biol* 80:14–20.
26. Scott RS, et al. (2006) Dental microwear texture analysis: Technical considerations. *J Hum Evol* 51:339–349.
27. Schulz E, Calandra I, Kaiser TM (2013) Feeding ecology and chewing mechanics in hoofed mammals: 3D tribology of enamel wear. *Wear* 300:169–179.
28. Scott JR (2012) Dental microwear texture analysis of extant African Bovidae. *Mammalia* 6:157–174.
29. Ungar PS, et al. (2012) Early neogene environments in East Africa: Evidence from dental microwear of tragulids. *Palaeogeogr Palaeoclimatol Palaeoecol* 342–343:84–96.
30. Schulz-Kornas E, Braune C, Winkler DE, Kaiser TM (2017) Does silica concentration and phytolith ultrastructure relate to phytolith hardness? *Biosurf Biotribol* 3:135–143.
31. Damuth J, Janis CM (2011) On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. *Biol Rev Camb Philos Soc* 86:733–758.
32. Rabenold D, Pearson OM (2011) Abrasive, silica phytoliths and the evolution of thick molar enamel in primates, with implications for the diet of *Paranthropus boisei*. *PLoS One* 6:e28379.
33. Rabenold D, Pearson OM (2014) Scratching the surface: A critique of Lucas et al. (2013)'s conclusion that phytoliths do not abrade enamel. *J Hum Evol* 74:130–133.
34. Lucas PW, et al. (2013) Mechanisms and causes of wear in tooth enamel: Implications for hominin diets. *J R Soc Interface* 10:20120923.
35. Sanson GD, Kerr SA, Gross KA (2007) Do silica phytoliths really wear mammalian teeth? *J Archaeol Sci* 34:526–531.
36. Xia J, et al. (2015) New model to explain tooth wear with implications for microwear formation and diet reconstruction. *Proc Natl Acad Sci USA* 112:10669–10672.
37. Kaiser TM, Braune C, Kalinka G, Schulz-Kornas E (2018) Nano-indentation of native phytoliths and dental tissues: Implication for herbivore-plant combat and dental wear proxies. *Evol Syst* 2:55–63.
38. Kovalevsky VO (1874) Monographie der Gattung *Anthracotherium* und Versuch einer natürlichen Classification der fossilen Huftiere. *Palaeontographica* 22:210–228.
39. Lucas PW, et al. (2014) The role of dust, grit and phytoliths in tooth wear. *Ann Zool Fenn* 51:143–152.
40. Mayland HF, Shewmaker GE, Bull RC (1977) Soil ingestion by cattle grazing crested wheatgrass. *J Range Manage* 30:264–265.
41. Müller J, et al. (2015) Tooth length and incisal wear and growth in guinea pigs (*Cavia porcellus*) fed diets of different abrasiveness. *J Anim Physiol Anim Nutr (Berl)* 99: 591–604.
42. Guichón ML, Cassini MH (1998) Role of diet selection in the use of habitat by pampas cavies *Cavia aperea pamparum* (Mammalia, Rodentia). *Mammalia* 62:23–36.
43. Blahovec J (2007) Role of water content in food and product texture. *Int Agrophys* 21: 209–215.
44. Vincent JFV (1982) The mechanical design of grass. *J Mater Sci* 17:856–860.
45. Vincent JFV (1983) The influence of water-content on the stiffness and fracture properties of grass leaves. *Grass Forage Sci* 38:107–114.
46. Henry DA, Simpson RJ, Macmillan RH (2000) Seasonal changes and the effect of temperature and leaf moisture content on intrinsic shear strength of leaves of pasture grasses. *Aust J Agric Res* 51:823–831.
47. Piperno D (1988) *Phytolith Analysis: An Archeological and Geological Perspective* (Academic, San Diego).
48. Prychid CJ, Rudall PJ, Gregory M (2004) Systematics and biology of silica bodies in monocotyledons. *Bot Rev* 69:377–440.
49. Sanson G (2006) The biomechanics of browsing and grazing. *Am J Bot* 93:1531–1545.
50. Wright W, Vincent JFV (1996) Herbivory and the mechanics of fracture in plants. *Biol Rev Camb Philos Soc* 71:401–413.
51. Lucas PW (2004) *Dental Functional Morphology: How Teeth Work* (Cambridge Univ Press, Cambridge, UK).
52. Calandra I, Schulz E, Pinnow M, Krohn S, Kaiser TM (2012) Teasing apart the contributions of hard dietary items on 3D dental microtextures in primates. *J Hum Evol* 63:85–98.
53. Gallij JR, Cangiano CA, Demment MW, Laca EA (2006) Acoustic monitoring of chewing and intake of fresh and dry forages in steers. *Anim Feed Sci Technol* 128:14–30.
54. Estrada JC, Delagarde R, Faverdin P, Peyraud JL (2004) Dry matter intake and eating rate of grass by dairy cows is restricted by internal, but not external water. *Anim Feed Sci Technol* 114:59–74.
55. Meyer RM, Bartley EE, Morrill JL, Stewart WE (1964) Salivation in cattle. I. Feed and animal factors affecting salivation and its relation to bloat. *J Dairy Sci* 47:1339–1345.
56. Hodson MJ, White PJ, Mead A, Broadley MR (2005) Phylogenetic variation in the silicon composition of plants. *Ann Bot* 96:1027–1046.
57. Mendoza M, Janis CM, Palmqvist P (2002) Characterizing complex craniodental patterns related to feeding behaviour in ungulates: A multivariate approach. *J Zool (Lond)* 258:223–246.
58. Hofmann RR, Stewart DRM (1972) Grazer or browser: A classification based on the stomach-structure and feeding habit of East African ruminants. *Mammalia* 36:226–240.
59. Janis CM (1988) An estimation of tooth volume and hypsodonty indices in ungulate mammals and the correlation of these factors with dietary preferences. *Teeth Revisited. Proceedings of the Vllth International Symposium on Dental Morphology. Mémoires du Muséum National d'Histoire Naturelle, Paris (Serie C) 53*, eds Russell DE, Santoro J-P, Signouneau-Russell D (Muséum National d'Histoire Naturelle, Paris), pp 367–387.
60. Sonibare OO, Haeger T, Foley SF (2010) Structural characterization of Nigerian coals by X-ray diffraction, Raman and FTIR spectroscopy. *Energy* 35:5347–5353.
61. Hummel J, et al. (2011) Another one bites the dust: Faecal silica levels in large herbivores correlate with high-crowned teeth. *Proc Biol Sci* 278:1742–1747.
62. Li B, et al. (2014) Phylogenetic variation of phytolith carbon sequestration in bamboos. *Sci Rep* 4:4710.
63. Kubo MO, Yamada E, Kubo T, Kohno N (2017) Dental microwear texture analysis of extant sika deer with considerations on inter-microscope variability and surface preparation protocols. *Biosurf Biotribol* 3:155–165.
64. R Core Team (2016) R: A Language and Environment for Statistical Computing, version 3.3.1 (R Foundation for Statistical Computing, Vienna).
65. Dragulescu AA (2014) xlsx: Read, write, format Excel 2007 and Excel 97/2000/XP/2003 files. R package, version 0.5.7. Available at <https://CRAN.R-project.org/package=xlsx>. Accessed November 16 2018.
66. Urbanek S (2016) rJava: Low-level R to Java interface. R package, version 0.9-8. Available at <https://CRAN.R-project.org/package=rJava>. Accessed November 16 2018.
67. Hojsgaard S, Halekoh U (2016) doBy: Groupwise statistics, LMeans, linear contrasts, utilities. R package, version 4.5-15. Available at <https://CRAN.R-project.org/package=doBy>. Accessed November 16 2018.
68. Bengtsson H (2016) R.utils: Various programming utilities. R package, version 2.4.0. Available at <https://CRAN.Rproject.org/package=R.utils>. Accessed November 16 2018.
69. Wilcox RR, Schönbrodt FD (2010) The WRS package for robust statistics. R package, version 0.12.1. Available at r-forge.r-project.org/projects/wrs. Accessed November 16 2018.
70. Wilcox RR (2012) *Introduction to Robust Estimation and Hypothesis Testing* (Elsevier Academic, London).
71. Welch BL (1938) The significance of the difference between two means when the population variances are unequal. *Biometrika* 29:350–362.
72. Yuen KK (1974) The two-sample trimmed *t* for unequal population variances. *Biometrika* 61:165–170.
73. Dunnett CW (1980) Pairwise multiple comparisons in the unequal variance case. *J Am Stat Assoc* 75:796–800.
74. Cliff N (1996) *Ordinal Methods for Behavioral Data Analysis* (Erlbaum, Mahwah, NJ).