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RESEARCH ARTICLE

Silicon-based plant defences, tooth wear and voles

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ABSTRACT

Plant–herbivore interactions are hypothesized to drive vole population cycles through the grazing-induced production of phytoliths in leaves. Phytoliths act as mechanical defences because they deter herbivory and lower growth rates in mammals. However, how phytoliths impair herbivore performance is still unknown. Here, we tested whether the amount of phytoliths changes tooth wear patterns. If confirmed, abrasion from phytoliths could play a role in population crashes. We applied dental microwear texture analysis (DMTA) to laboratory and wild voles. Lab voles were fed two pelleted diets with differing amounts of silicon, which produced similar dental textures. This was most probably due to the loss of food mechanical properties through pelletization and/or the small difference in silicon concentration between diets. Wild voles were trapped in Poland during spring and summer, and every year across a population cycle. In spring, voles feed on silica-rich monocotyledons, while in the summer they also include silica-depleted dicotyledons. This was reflected in the results; the amount of silica therefore leaves a traceable record in the dental microwear texture of voles. Furthermore, voles from different phases of population cycles have different microwear textures. We tentatively propose that these differences result from grazing-induced phytolith concentrations. We hypothesize that the high amount of phytoliths in response to intense grazing in peak years may result in malocclusion and other dental abnormalities, which would explain how these silicon-based plant defences help provoke population crashes. DMTA could then be used to reconstruct vole population dynamics using teeth from pellets or palaeontological material.

KEY WORDS: Arvicolinae, Dental microwear texture analysis, Feeding experiments, Phytoliths, Plant–herbivore interactions, Population cycles

INTRODUCTION

Multi-annual population fluctuations in small mammals have been known for decades, but the mechanisms that drive them are still a matter of debate. Predator–prey (Gilg et al., 2003; Hanski et al., 1991; Klemola et al., 2003; Korpela et al., 2014) and plant–herbivore (Hansson, 1971; Huitu et al., 2007; Massey et al., 2008; Reynolds et al., 2012) interactions are the leading hypotheses, but competition (Hansen et al., 1999a,b), climate (Kausrud et al., 2008) and population senescence (Tkadlec and Zejda, 1998) have also been proposed. In the plant–herbivore interaction scenario, plants would need defences induced by herbivory. Beside chemical defences (Carmona et al., 2011), deposition of silica bodies known as phytoliths could represent a powerful mechanical defence (Massey et al., 2009; Vicari and Bazely, 1993), especially in monocotyledons (grasses and sedges), which deposit more silica phytoliths than dicotyledons do (Hodson et al., 2005).

It has been shown that grazing induces an increased concentration of silicon (Si) in leaves (Hartley et al., 2015; Massey et al., 2007a; McNaughton and Tarrants, 1983; McNaughton et al., 1985; Reynolds et al., 2012), which reduces palatability and digestibility (Gali-Muhtasib et al., 1992; Massey et al., 2007b), and in turn lowers body mass and growth rate of voles (Massey and Hartley, 2006; Massey et al., 2008). Very recently, Wieczorek et al. (2015b) demonstrated in the first field-based study that (1) silicon concentrations in leaves are determined by the vole population density, and thus by the grazing intensity, of the previous year, and (2) overwintering success in voles is correlated with leaf silicon levels in autumn. However, one question remains: 'how does silicon have its effect on herbivore performance?' (Hartley, 2015, p. 152).

Three complementary factors may be at play. First, silicon has been found to induce intestinal abrasion, leading to a decrease in body mass and in whole-body resting metabolic rate (Wieczorek et al., 2015a). Second, the presence of phytoliths lining the cell walls may prevent the crushing of cells, so the herbivores extract less energy from silicon-rich monocotyledons (Hunt et al., 2008). And third, abrasive phytoliths (Massey et al., 2007b) could wear vole teeth (Hartley, 2015; Hartley et al., 2015). While some studies have found that phytoliths are softer than enamel (Sanson et al., 2007; Lucas et al., 2013; Erickson, 2014; but see Rabenold and Pearson, 2014), a recent study has demonstrated that materials softer than enamel, such as silica phytoliths, can and do wear enamel (Xia et al., 2015).

Here, we tested the hypothesis that the amount of silica in plants has a detrimental effect on voles through dental wear. For this purpose, lab voles were fed pellets with differing amounts of silicon, and wild voles were trapped at different seasons and during different phases of a population cycle. This is the first step towards addressing the question whether variations in tooth wear, due to fluctuations in plant silica levels, are a driver of vole population cycles. Dental microwear texture analysis (DMTA) quantifies tooth surface texture at microscopic scales (e.g. Calandra et al., 2012; Merceron et al., 2010; Scott et al., 2005; Ungar et al., 2003). DMTA does not directly relate to wear rates, but it is expected that different regimes of abrasiveness from different silica levels result in different microwear textures, which can be discriminated by DMTA. More specifically, an increase in dietary abrasiveness is expected to result in higher texture anisotropy (epLsar) and lower complexity (Asfc). Indeed, previous studies on wild, modern ungulates have shown that grazers have lower complexity and higher anisotropy than mixed feeders and browsers (Merceron et al., 2014; Schulz et al., 2013b; Scott, 2012; Souron et al., 2015; Ungar et al., 2007). 2D microwear analyses of herbivorous and omnivorous muroid rodents point to the same patterns (e.g. Firmat et al., 2011; Gomes Rodrigues et al., 2012).

Here, we addressed three aspects of the silica-wear hypothesis. First, laboratory voles raised in experimental conditions were analysed to assess whether varying silicon levels alone could

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produce different textures. Second, wild populations trapped in spring, when voles feed almost exclusively on silicon-rich monocotyledons, were compared with populations trapped in summer, which also incorporate silica-depleted dicotyledons in their diets (Tast, 1974; Batzli and Henttonen, 1990; Soininen et al., 2009, 2013; Calandra et al., 2015). Wild voles were then sampled during consecutive years of a population cycle to test whether fluctuations in plant silicon levels caused by vole population cycles induce different dental microwear textures.

MATERIALS AND METHODS

A total of 57 individuals of root vole, *Microtus oeconomus* (Pallas 1776) (Fig. 1A), were considered in this study. Ten individuals were raised in laboratory conditions on two different pelleted diets: the S-diet and the C-diet (see tables S1 and S2 in Wieczorek et al., 2015a for details). One of the root voles (Table S1) was removed from the analysis because of potential surface alteration. The S-diet was composed of sedges (62.16%), Poaceae grasses (25%), sunflower (10%) and additives (2.84%). The C-diet contained Poaceae grasses (85.8%), sunflower (8%) and additives (6.2%). This allowed testing for the effect of medium (C-diet: 1.04% Si) versus high (S-diet: 1.87% Si) silicon content on the dental microwear texture.

Additionally, wild individuals were trapped in Poland (Fig. 1B; Table S1). All these individuals were adults, or sub-adults feeding on the same resources as adults. While subtle age-related changes in the morphology of voles due to wear have been measured (Ledevin et al., 2010), DMTA should be insensitive to these inter-age changes and reflect only the mechanical properties of the food ingested. One dataset was composed of 34 individuals from the Biebrza Marshes to test for differences between seasons (spring and summer, in Olszowa Droga) and localities (Olszowa Droga and Barwik, in spring). The second dataset included individuals trapped in the Białowieża Primeval Forest once a year in autumn during one population cycle (Zub et al., 2012): in 1998 (increase phase, N=4), 1999 (peak, N=4) and 2001 (recovery phase, N=5); no voles could be trapped in 2000 (crash).

The anterior enamel band on the third triangle of the right second upper molar of each individual (Fig. 2A) was scanned with the surface profilometer Leica DCM8 (Leica Microsystems Inc., Wetzlar, Germany) with a 100× objective (Leica Microsystems, numerical aperture 0.90, working distance 0.9 mm) and white light source at the iPHEP lab (CNRS and University of Poitiers, France). Four 15×15 μm^2 sub-surfaces were extracted from lingual to buccal (Fig. 2B). Each sub-surface was then levelled (least square subtraction) and a scale-sensitive fractal analysis (SSFA; Scott et al., 2006) was run on them to quantify their complexity (Asfc) and anisotropy (epLsar). Complexity has been linked to food hardness, while anisotropy relates to toughness and abrasiveness (see review by Ungar, 2015). The median of the four sub-surfaces was computed for each individual prior to subsequent analyses. The heterogeneity of complexity (HAsfc) was adapted from Scott et al. (2006) and calculated as follows:

$$\mathrm{HAsfc} = \frac{\mathrm{MAD}}{\tilde{X}} = \frac{\mathrm{median}(|X_i - \tilde{X}|)}{\tilde{X}}, \qquad (1)$$

where X_i is the Asfc value for the sub-surface i, $\tilde{X}$ is the median of Asfc values for all four sub-surfaces, and MAD is the median absolute deviation of Asfc. This parameter has been proposed to be an indicator of dietary breadth (Scott et al., 2012; Souron et al., 2015; but see Schulz et al., 2013a).

Two outliers were identified (outside of 1.5 times the interquartile range) in summer in Olszowa Droga. These specimens had values for Asfc and HAsfc (3.265 and 0.530, respectively) and epLsar (0.009874) much higher than those of any other wild individual (Table S1). Lab vole MRI-PAS-8 was also identified as an outlier for Asfc (3.763). As it cannot be known whether they are legitimate, analyses were run with and without these outliers, and then compared and discussed.

With all individuals, some variables were not normally distributed (Shapiro–Wilk tests: Table S2), so Mann–Whitney U or Kruskal–Wallis tests were run, depending on the number of groups for the independent variable. The independent variables 'season' (spring versus summer in Olszowa Droga), 'locality' (Olszowa Droga versus Barwik in spring) and 'silica' (S-diet versus C-diet) contained two groups, while the variable cycle 'phase' included three groups (increase, peak and recovery, in Białowieża). When outliers were removed, all parameters were normally distributed (Table S2), allowing the application of parametric Welch t-tests or F-tests (two or three groups, respectively). Because each parameter characterizes a single aspect of the dental microwear texture, linear discriminant analyses (LDA) were performed to combine all three parameters (Asfc, epLsar and HAsfc) into one (two groups) or two (three groups) axes in order to maximize the between-group differences. Non-parametric (Mann–Whitney U or Kruskal–Wallis) and parametric (Welch t-tests or F-tests) tests (with and without outliers) were also run on the LDA coordinates. Because of sample sizes, only the statistics on the wild specimens of the Biebrza Marshes (Olszowa Droga summer N=12 and spring N=12, and Barwik spring N=10) can be considered robust. Levene's tests were also performed similarly to test for differences in variances between groups. Adjustment of P-values for multiple comparisons was not performed to balance type I and type II errors (Cabin and Mitchell, 2000; Nakagawa, 2004), and because only a few significant differences were found (see Results).

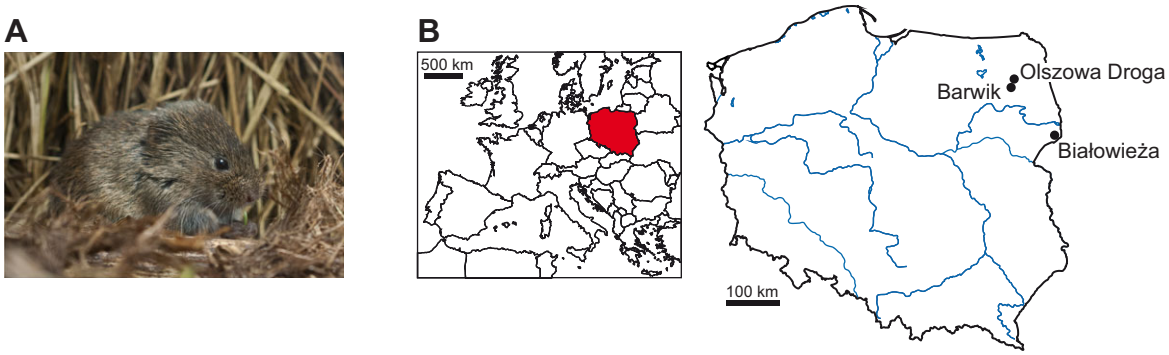

Fig. 1. The study species. (A) *Microtus oeconomus*. (B) Trapping localities of wild voles in Poland.

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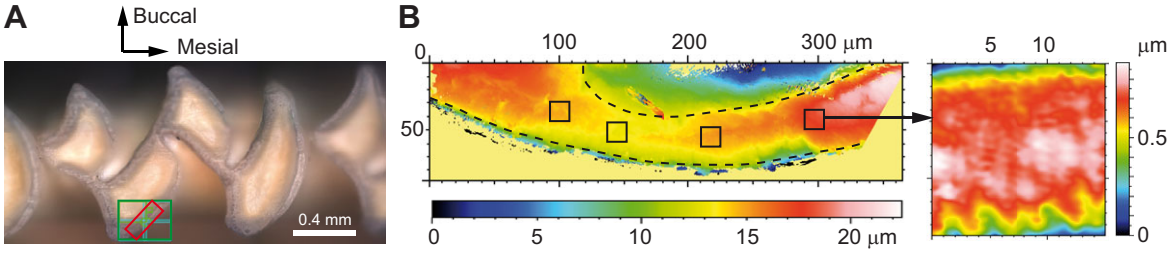

Fig. 2. Dental microwear texture analysis. (A) Right second upper molar (M2) highlighting the area scanned (green rectangle) and analysed (red rectangle). The mesial part of the M3 is visible on the left of the M2, and the distal part of the M1 on its right. The yellowish surfaces are dentine, and the white–grey bands represent enamel. (B) 3D model of the enamel band, rotated about 45 deg clockwise relative to A, with the four 15×15 μm^2 sub-surfaces extracted from lingual (left) to buccal (right). The dashed lines delimitate approximately the enamel.

The open-source software R (R Core Team, 2015) was used with the following packages: car (Fox and Weisberg, 2011), doBy (Højsgaard et al., 2014), R.utils (Bengtsson, 2015), readxl (Wickham, 2015), RSvgDevice (Luciani et al., 2014) and xlsx (Dragulescu, 2014).

RESULTS

The dental texture of lab voles fed with the S-diet showed somewhat higher anisotropy (epLsar) and heterogeneity (HAsfc) than voles fed the C-diet (Fig. S1A, Tables S3, S4A). The complexity (Asfc) seemed to be lower in the S-diet group, but the outlier in the C-diet blurred the signal. The S-diet was significantly more variable in anisotropy (Table S4B). Significant differences were found on the first axis (LD1) of the LDA only when the outlier was removed (Fig. 3A).

There was no significant difference in wild voles from the Biebrza Marshes (comparison of the two localities in the spring and of the two seasons in Olszowa Droga) for any of the parameters taken in isolation (Table S4A). However, summer individuals from Olszowa Droga had significantly higher values on LD1 than spring voles from the same locality, both with (Fig. S1B) and without outliers (Fig. 3B). The significant difference between the localities in spring was underscored only by the Mann–Whitney test on LD1 (Fig. 3C; Fig. S1B).

Voles from Białowieża did not show clear differences in dental texture during different phases of their population cycle between 1998 and 2001. However, a trend of decreasing anisotropy and heterogeneity (increasing LD1), and of increasing variance in complexity (Asfc), from increase to recovery phases was apparent (Fig. 3D; Fig. S1C, Tables S3, S4). There was no apparent trend on LD2.

DISCUSSION

This study is, to our knowledge, the first application of DMTA to rodents. We tested whether grazing-induced phytolith concentrations in leaves have an effect on vole population cycles through tooth wear using three approaches: (1) feeding laboratory voles with diets of different silicon concentration, (2) analysing seasonal and geographical variations in wild animals and (3) analysing variations across phases of a population cycle.

Feeding experiments

This study is the first to characterize the effect of silicon alone on dental microwear textures of voles in feeding experiments. Our aim was to quantify the microtextures resulting from different silicon concentrations. It was expected that the differences in the silicon content of the two pelleted diets would produce more contrasting textures, despite small sample sizes. Three factors could explain the

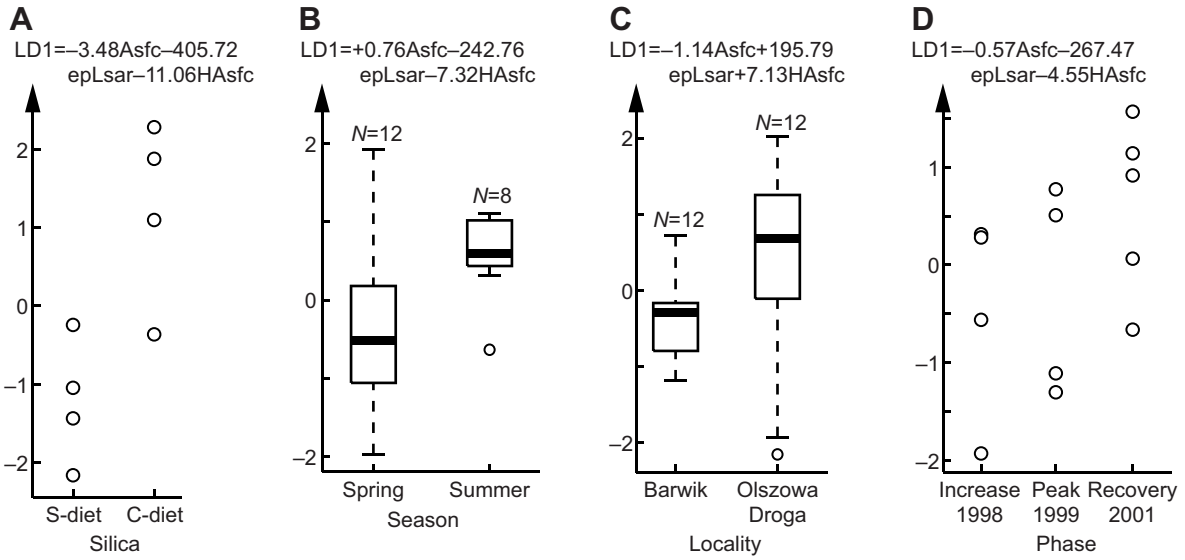

Fig. 3. Plots of the first axis of the linear discriminant analysis. (A) S-diet versus C-diet for laboratory voles. (B) Seasonal variation in wild voles in Olszowa Droga. (C) Geographical variation in wild voles in spring in the Biebrza Marshes. (D) Wild vole population phases in Białowieża. In B and C, the thick horizontal lines mark the median; the boxes enclose the first (25%) and third (75%) quartiles, i.e. the interquartile range (IQR); the whiskers (dashed lines) extend to 1.5 IQR; open circles represent outliers. Outliers for Olszowa Droga summer (B) and C-diet (A) were removed; for plots with outliers, see Fig. S1.

lack of differences. First, one of the voles fed the C-diet seems to be an outlier regarding complexity (Asfc). When this vole was removed, significant differences appeared on LD1 (Table S4A). However, outliers could not be identified with any certitude with this sample size (N=5). Second, while it can be advantageous that pellets homogenize food properties across diets, hardness, toughness and other structural properties of plants (lost during the production of pellets) are important in microwear texture formation. These properties dictate how the food will be processed and, thus, how abrasive particles will interact with tooth surfaces (Hua et al., 2015). Additionally, the S-pellets were harder than the C-pellets: some individuals had problems eating the S-pellets so that it was necessary to crumble the pellets with a hammer. This greater hardness might have led to more crushing and less shearing than usual for tough grass, and this could explain the observed lack of difference in anisotropy. This would also explain the larger variability in anisotropy for the S-diet (Table S4B). Third, both pelleted diets analysed here are within the grazing range, as they are mainly composed of grasses and sedges. Their silicon levels are not very different (1.04% versus 1.87%) and correspond to the levels measured in wild plants (mean Si concentration in grasses 1.58%, and in sedges 1.39%; Hodson et al., 2005). It seems that sedge and grass have similar abrasiveness levels in regard to vole teeth. In sum, such variations in silicon concentration alone are not sufficient to affect microwear textures; larger variations and/or other food properties seem to be critical. Ideally, therefore, further studies should be conducted using both pellets and natural forage with manipulated Si concentrations.

Seasonal and geographical variation

LDA, which combine all three parameters (Asfc, epLsar and HAsfc), yielded significant differences between spring and summer in Olszowa Droga, both with and without outliers (Table S4). This implies that the dietary differences are not large, but are still present. Higher anisotropy (epLsar) and heterogeneity (HAsfc), and lower complexity (Asfc) seem to characterize spring voles. Spring individuals therefore have a diet richer in tough foods like grass, and poorer in hard items like seeds, than specimens trapped in summer. This textural signal is consistent with the expected seasonal variation in the dietary phytolith content. Studies conducted in the Arctic also show that in spring, *M. oeconomus* is forced to feed on silicon-rich sedges (genus *Carex*, mean Si=1.31% of dry matter mass; Hodson et al., 2005; although there is a wide inter-annual variation in the Biebrza Marshes; Wieczorek et al., 2015b) because other food sources are scarce. In summer, the dietary spectrum is wider and includes less abrasive dicotyledons (mean Si=0.43%; Hodson et al., 2005) as well (Tast, 1974; Batzli and Henttonen, 1990; Soininen et al., 2009, 2013). This seasonal shift in food sources is also reflected in the stable isotopic compositions of teeth from Arctic voles (Calandra et al., 2015), and in the present microwear texture data on Polish animals. This implies that DMTA can track variations in the dietary abrasiveness of wild populations.

It should be noted that the variation in Si between *Carex* sedges and dicotyledons (Hodson et al., 2005) is of the same order as that between the two pelleted diets. It is therefore surprising that differences were detected in wild specimens but not in feeding experiments (but see above). It could be that plants from the trapping area show more variation in Si content than average, but this has not been tested. Alternatively, the homogenization of plants through pellet production might not be suitable for this type of analysis, as detailed above.

Olszowa Droga and Barwik localities both belong to the Biebrza Marshes region. These localities are very close geographically, around 10 km apart (Fig. 1B), and are very similar in terms of environment. It is therefore not surprising that the voles inhabiting these localities show indistinguishable microwear textures in spring, as demonstrated by the Welch t-tests (Table S4A). However, with the same data, the Mann–Whitney test found significant differences between the localities on LD1. Although the sites are very similar, Barwik is located closer to the mineral edges of a river valley and is characterized by a higher influx of silica, whereas Olszowa Droga is separated from the mineral part of the valley by alder forests on organic soils, and is thus most probably characterized by a lower Si concentration (Struyf et al., 2009). These differences in soil and water Si concentrations may lead to different Si uptake by plants (Hartley et al., 2015; McNaughton et al., 1985; Quigley and Anderson, 2014; Wieczorek et al., 2015b). More soil Si may also imply more grit that would wear the teeth (e.g. Lucas et al., 2013). Nevertheless, grit is unlikely to play a role in this case, as voles are selective feeders that avoid ingesting soil particles together with plant material (e.g. Tast, 1974, where dust accounted for <0.1% of the stomach contents). So, phytoliths seem to be the main wearing agents of these wild voles.

Variation across population phases

Plants respond to grazing intensity of the previous year by adjusting the Si content (by 0.6–1 percentage point; Wieczorek et al., 2015b) in their leaves (see also Massey et al., 2008; Reynolds et al., 2012), and voles are in turn negatively affected by increased phytolith levels (Massey and Hartley, 2006; Massey et al., 2008). But how plant phytoliths affect voles is still unclear. Here, we tested whether the known variations in leaf Si concentration (in response to vole density) can be detected through DMTA. The data presented here on the dental microwear textures across phases of population cycles do not allow definitive conclusions to be drawn. Nevertheless, these preliminary results should help to orientate future studies.

No clear impact of the known variations in leaf Si was found on enamel surface textures. Yet, textures reflect apparent dietary trends across phases, and we addressed whether these trends could be the result of grazing-induced variations in phytolith content. There was a slight increase in complexity (Asfc) and a decrease in heterogeneity (HAsfc) from the increase to peak phases, but there was no change in anisotropy (epLsar) (Fig. S1C). Repeated and significant damage to leaves (at least 20% of leaves eaten) is required to induce a response in plants (Hartley et al., 2015; Massey et al., 2007a; Wieczorek et al., 2015b). So, vole density in the increase phase is therefore probably not high enough to induce a significant increase in leaf Si that would result in more abrasive foods during the peak year. Unfortunately, no vole could be trapped during the following crash year because of low vole density. The hypothesis would be supported if these voles showed a strong texture signal for increased abrasiveness (higher epLsar, lower Asfc). High abrasiveness, which would reduce the ability to process food efficiently, could be one of the causes of the population crash. Nevertheless, data for the following recovery year lend some support to the hypothesis. Plants would not suffer intense grazing during crash years, so they should build up fewer phytoliths in leaves during the vole recovery phase. In turn, anisotropy values should be lower, and complexity values higher, in vole teeth from the recovery phase. This is what we observed. However, two additional factors that could explain the lack of signal across population phases should be considered. First, voles

were trapped at different sites in Białowieża, where the population dynamics is dissimilar between locations (Zub et al., 2012). This could dampen the cyclic signal. Second, voles feed mainly on dicotyledons in autumn (Tast, 1974; Batzli and Henttonen, 1990; Soininen et al., 2009, 2013; see also Calandra et al., 2015), so the increased abrasiveness in monocotyledons rejected by voles would not impact tooth textures during this season.

The Si concentrations in vole faeces could not be measured in these individuals, but data are available for voles trapped in the same area in November between 2008 and 2010 (Wieczorek et al., 2015b). However, these voles were eventually released for mortality estimations, so DMTA could not be conducted on this material. After a low-density year in 2007, vole density peaked in 2008, dropping again through 2009 and 2010. The Si concentration in vole faeces follows the same pattern but shifted by 1 year (Fig. S2, Table S5), as in leaves (Wieczorek et al., 2015b). Si in faeces therefore results from the ingestion of Si from leaves, which is dependent on vole density in the previous year. Thus, these data support our hypothesis.

Intra-specific competition should also be considered. It is expected that an increase in vole density would result in more intra-specific competition, whereby individuals would feed on a larger spectrum of food resources. As less-abrasive dicotyledons are preferred (because they are consumed when available), expanding the dietary spectrum in the summer/autumn would result in the ingestion of more abrasive monocotyledons. In this case, an observed increase in dietary abrasiveness would not result from an increase in the Si content of monocotyledons, but from an increase in the proportion of Si-rich plants in the diet. As DMTA is a proxy for diet (and, in this case, dietary abrasiveness), the Si content of monocotyledons and the proportion of Si-rich plants in the diet could produce the same DMTA results. Our preliminary study cannot differentiate between these two effects, although the observed similar degree of variability in DMTA would support the Si-content alternative (but see Schulz et al., 2013a). So, future studies should combine DMTA (for abrasiveness reconstruction) with stomach/faeces content analyses (for both dietary reconstruction and Si content). It should be noted that DMTA and stomach content analyses record diet at different time scales (Merceron et al., 2010).

Tooth wear and population cycles

Whatever the cause, DMTA does detect variations in dietary abrasiveness across the phases of a population cycle. Nevertheless, these correlative results require a mechanism by which higher abrasiveness could help provoke population crashes. Tooth wear rates might seem unimportant in voles as their molars are ever-growing (Hillson, 2005). Moreover, in rabbits, wear of the continuously growing cheek teeth is partly compensated for by growth (Müller et al., 2014). Yet, less effective occlusion, and mastication, could play a role in provoking population crashes. Increased wear can also lead to malocclusion (Capello, 2008) and other dental abnormalities in the ever-growing cheek teeth of rabbits (Müller et al., 2014), which could prevent efficient mastication and energy uptake, eventually leading to population crashes. Alternatively, as plants become more abrasive and less palatable following a vole peak year, voles would avoid these plants (Gali-Muhtasib et al., 1992; Massey et al., 2007b), so would not have access to enough palatable food, causing their health condition to deteriorate, leading to a population crash. Their teeth would in turn undergo less wear. Another aspect is that occurrences of malocclusion increase with ageing (e.g. in rats; Dontas et al., 2010), which supports the senescence hypothesis (Tkadlec and Zejda, 1998). Malocclusion is unlikely to play a role here, but other dental abnormalities could be present. However, no significant dental abnormalities were observed in the specimens studied; a more in-depth investigation of this aspect is nevertheless warranted. This should be combined with an assessment of the health condition (e.g. body mass and fat) of the voles, in order to examine whether malocclusion indeed decreases health condition in wild voles.

The present study is, to our knowledge, the first to analyse dental microwear textures in voles. Our study points to important issues, which should be taken into account when planning studies of plant–herbivore interactions. To detect the impact of annual changes in plant abrasiveness, future studies should be run across all phases of vole population cycles, although it may be difficult to trap enough specimens during crash years. It is also important to monitor abiotic factors like water level or Si content in soil because they can influence the Si concentration in leaves.

Tooth wear alone does not seem to be a major driving factor in population cycles, as the known variations in leaf Si were only marginally recorded in DMTA. Instead, intestinal damage (Wieczorek et al., 2015a) and resistance to cell crushing (Hunt et al., 2008) are more likely to be responsible for the cyclic mortality of vole populations due to plant defences. Yet, DMTA has been proven to indicate dietary abrasiveness in a wide range of mammals, so, if confirmed by future studies on voles, it could be used as a proxy of leaf Si concentration. It would then be a tool that can be applied to teeth from pellets or palaeontological material, in order to reconstruct vole population dynamics, mediated by the delayed effects of grazing on plant phytolith production.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

K.Z., P.A.S., A.Z. and G.M. designed the study. P.A.S. performed the feeding experiments. K.Z. and A.Z. prepared the specimens. I.C. and G.M. performed DMTA, analysed the data and wrote the manuscript. K.Z., P.A.S. and A.Z. helped draft the manuscript and gave final approval for publication.

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Supplementary information

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