THE FUNCTIONAL ROLE OF SILICON IN PLANT BIOLOGY

The ecology of herbivore-induced silicon defences in grasses

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Summary

1. Silicon as a defence against herbivory in grasses has gained increasing recognition and has now been studied in a wide range of species, at scales from individual plants in pots to plant communities in the field. The impacts of these defences have been assessed on herbivores ranging from insects to rodents to ungulates. Here, we review current knowledge of silicon mediation of plant–herbivore interactions in an ecological context.

2. The production of silicon defences by grasses is affected by both abiotic and biotic factors and by their interactions. Climate, soil type and water availability all influence levels of silicon uptake, as does plant phenology and previous herbivory. The type of defoliation matters and artificial clipping does not appear to have the same impact on silicon defence induction as herbivory which includes the presence of saliva. Induction of silicon defences has been demonstrated to require a threshold level of damage, both in the laboratory and in the field. In recent studies of vole–plant interactions, the patterns of induction were found to be quantitatively similar in glasshouse compared with field experiments, in terms of both the threshold required for induction and timing of the induction response.

3. The impacts of silicon defences differ between different classes of herbivore, possibly reflecting differences in body size, feeding behaviour and digestive physiology. General patterns are hard to discern however, and a greater number of studies on wild mammalian herbivores are required to elucidate these, particularly with an inclusion of major groups for which there are currently no data, one such example being marsupials.

4. We highlight new research areas to address what still remains unclear about the role of silicon as a plant defence, particularly in relation to plant–herbivore interactions in the field, where the effects of grazing on defence induction are harder to measure. We discuss the obstacles inherent in scaling up laboratory work to landscape-scale studies, the most ecologically relevant but most difficult to carry out, which is the next challenge in silicon ecology.

Key-words: defence induction, herbivory, insect, landscape scale, mammal, physical defences, plant–herbivore interactions, silica

Introduction

Silicon is the second most abundant element in the Earth's crust and, in grasses at least, may be present in greater amounts than macronutrients, comprising up to 10% dry weight in some species (Epstein 1999). Several hypotheses for an ecological role for this extensive accumulation have been put forward over recent years (Raven 1983; Ma 2004; Massey, Ennos & Hartley 2007a; Cooke & Leishman

2011), with one of the earliest suggestions being that silicon was a defence against herbivory. In agricultural systems, it has long been known that silicon enhances the resistance of crop plants to insect pests (e.g. McColloch & Salmon 1923; Ponnaiya 1951; Sasamoto 1953; Keeping, Meyer & Sewpersad 2013) and that application of soluble silicon leads to decreased damage by insect herbivores (Goussain, Prado & Moraes 2005). The effects of silicon augmentation on crop–pest interactions have been the subject of previous reviews (Keeping & Reynolds 2009; Reynolds, Keeping & Meyer 2009); here, we focus specifically

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on ecological systems and on the biotic and abiotic factors which affect the natural induction of silicon-based defences.

In one of the first studies in natural ecosystems, McNaughton & Tarrants (1983) proposed grass leaf silicification as an 'inducible defence' against vertebrate herbivores following their findings that grasses from grazed areas in African savannas had higher silicon contents than those from ungrazed ones and that clipped plants accumulated more silicon than undamaged ones. However, some grasses had intrinsically higher silicon contents, even when ungrazed, so the authors concluded silicon was 'best viewed as a qualitatively constitutive trait that is, nevertheless, quantitatively inducible by grazing' (McNaughton & Tarrants 1983). This work, supported by other early ecological studies (e.g. McNaughton *et al.* 1985; Brizuela, Detling & Cid 1986; Cid *et al.* 1990), suggested that silicon provided wild grasses with an effective defence against herbivores that could be rapidly mobilized in response to attack (Karban & Baldwin 1997), contrasting with previous notions that grasses were relatively undefended (Vicari & Bazely 1993).

Silicon defences are usually deployed as phytoliths or other forms of amorphous silica (SiO_2) in the leaf epidermis, or deposited in spines, trichomes or hairs on the leaf surface (Currie & Perry 2007; Hartley *et al.* 2015; Strömberg, Di Stilio & Song 2016). These structures render leaves tough and abrasive and therefore physically deter herbivores from feeding (Massey & Hartley 2006, 2009). In addition, they have been shown to reduce the digestibility of grasses (Shewmaker *et al.* 1989), act as a structural inhibitor of microbial digestion in ruminants (Harbers & Thouvenelle 1980; Harbers, Raiten & Paulsen 1981) and stimulate other plant defence mechanisms (Goussain, Prado & Moraes 2005; Fauteux *et al.* 2006; Ye *et al.* 2013). Adverse effects of silicon on rates of herbivory and animal performance have now been demonstrated on a range of insect herbivores (Massey, Ennos & Hartley 2006; Massey & Hartley 2009; Reynolds, Keeping & Meyer 2009; Keeping, Miles & Sewpersad 2014), rodents and lagomorphs (Gali-Muhtasib, Smith & Higgins 1992; Massey & Hartley 2006; Cotterill *et al.* 2007; Huitu *et al.* 2014; Wieczorek *et al.* 2015a,b) and ruminants (Massey *et al.* 2009). Studies on wild mammalian herbivores remain relatively lacking, however, in marked contrast to the numbers of studies on the effects of silicon on agricultural insect pests (Massey, Ennos & Hartley 2006; Kvedaras *et al.* 2009; Reynolds, Keeping & Meyer 2009; Keeping, Miles & Sewpersad 2014).

More recent work has expanded our understanding of silicon induction, that is the increase in silicon accumulation that occurs in plants when they are damaged, and its similarities and contrasts with other inducible defences. In common with many types of inducible plant defences, induction of silicon is often greater in response to attack by herbivores than to artificial clipping (e.g. Massey, Ennos & Hartley 2007b; Quigley & Anderson 2014),

although in contrast to other types of defence (Tanentzap, Vicari & Bazely 2014), the role of herbivore saliva in the expression of silicon is unclear. It also appears to be non-linearly related to both the frequency and intensity of damage, requiring multiple damage events and a threshold amount of biomass to be removed (Massey, Ennos & Hartley 2007b; Reynolds *et al.* 2012). It appears that the response of plant silicon levels to damage, particularly in the case of clipping, varies with plant species, genotype and phenological stage, as well as damage intensity (Kindomihou, Sinsin & Meerts 2006; Soininen *et al.* 2013). Unlike many induced defences (but see Haukioja & Neuvonen 1985), silicon induction persists for several months (Reynolds *et al.* 2012), reflecting the recalcitrant nature of silicon phytoliths, which are not remobilized once formed (Piperno 2006; Strömberg, Di Stilio & Song 2016) and hence tend to accumulate as leaf tissue ages. This persistence has consequences for the impact of induced silicon defences on herbivores, particularly for small mammals where delayed density-dependent effects drive population dynamics (Lindroth & Batzli 1986; Ergon, Lambin & Stenseth 2001; Smith *et al.* 2006; Ergon *et al.* 2011). A time lag in defence induction, due to the requirement for persistent herbivory and the long 'decay time' of induced silicon levels, could provide a mechanism for such delayed plant–herbivore feedbacks (Massey *et al.* 2008). Despite many experimental demonstrations of the importance of silicon in plant–herbivore interactions, there are cases where no changes in plant silicon levels in response to herbivory are observed, as well as examples of herbivores unaffected by silicon-based induced defences (e.g. Banuelos & Obeso 2000; Redmond & Potter 2006; Damuth & Janis 2011).

Studies on silicon-mediated plant–herbivore interactions now encompass a wide range of natural grass species and include scales from individual plants in glasshouses to plant communities in the field (Massey, Ennos & Hartley 2007b; Reynolds, Keeping & Meyer 2009; Soininen *et al.* 2013), allowing us to ask whether consistent patterns are emerging in its accumulation and impact, as well as assess which aspects of silicon induction remain poorly understood. We aim to address the following questions in this review:

1 How do biotic (specifically herbivory) and abiotic factors influence the production of silicon defences by natural grasses?
2 How does silicon uptake by these grasses impact on different classes of vertebrates and invertebrate herbivores?
3 Do silicon defences provide a viable hypothesis for explaining population regulation of wild grazing herbivores?

We review our current state of knowledge around these specific questions and summarize gaps in our understanding of each of these questions. We also suggest possible approaches for scaling up laboratory work to landscape-scale studies, an exciting future challenge in the study of silicon-based defences that is essential for answering the

third of these questions. We focus on grasses as this plant family has been the most comprehensively studied in terms of ecological aspects of silicon-mediated interactions between plants and their herbivores, although there is evidence of silicon induction in other angiosperm groups (Hodson *et al.* 2005; Cooke & Leishman 2011; Katz 2015).

Impact of herbivory: silicon induction varies with the type, amount and timing of damage

One of the features of silicon-based defences which has been frequently demonstrated is that herbivory induces silicon accumulation to a greater extent than does artificial clipping (e.g. Massey, Ennos & Hartley 2007b; Quigley & Anderson 2014). This is particularly the case in studies of mammalian herbivores, with relatively few studies demonstrating this differential effect in the case of insect herbivory (but see Gomes *et al.* 2005; Massey, Ennos & Hartley 2007b). For example, in North American studies, grasses from areas that had been heavily grazed by prairie dogs showed elevated concentrations of silicon compared to more lightly grazed ones, but mechanical defoliation did not induce this response, with silicon levels in clipped leaves lower than those in unclipped ones (Brizuela, Detling & Cid 1986; Cid *et al.* 1989, 1990), whereas in other cases, clipping led to induction in some grass species, but not in others (e.g. McNaughton *et al.* 1985; Kindomihou, Sinsin & Meerts 2006; Quigley & Anderson 2014). A recent literature review demonstrated that silicon induction was highly variable between species and dependent on the frequency and intensity of damage (see below), but on average, induction was more than twice as great in response to herbivory than to manual defoliation across 34 species/study combinations (Quigley & Anderson 2014).

Natural herbivory elicits a greater induction of defences than mechanical wounding (e.g. Hartley & Lawton 1987, 1991; Valkama *et al.* 2005; Farmer 2014), mediated through herbivore-specific molecular and physiological plant responses (e.g. Korth & Dixon 1997; Reymond *et al.* 2000). Oral secretions provide herbivore-specific cues for defence induction in many insects (Hartley & Lawton 1991; Alborn *et al.* 1997; Bonaventure, VanDoorn & Baldwin 2011; Tian *et al.* 2012). Components of insect saliva, plant cell wall fragments and other cues create a signalling cascade which triggers a defence response, including the production of the so-called wound hormones [jasmonic acid (JA) and salicylic acid], changes in gene expression and increases in secondary metabolites (Heil & Ton 2008; Bonaventure, VanDoorn & Baldwin 2011; Stam *et al.* 2014). Equivalent research on induced defence responses to vertebrate herbivory is relatively lacking (Walters 2010), although Tanentzap, Vicari & Bazely (2014) recently provided a breakthrough by demonstrating that moose and reindeer saliva could counter alkaloid defences produced as a result of a grass–endophyte mutualism. In the case of silicon defences, there has not yet been any test of whether the application of herbivore saliva induces uptake to the same extent as actual herbivory.

Nevertheless, it is apparent that silicon addition can lead to increased expression of a large spectrum of inducible defence responses and amplifies the JA-mediated induced defence response by serving as a priming agent for the JA pathway, while JA promotes Si accumulation (Fauteux *et al.* 2006; Ye *et al.* 2013). A better understanding of the mechanisms underlying silicon induction, the impacts of silicon uptake on other defence pathways in plants and the reasons for any observed differences in induction in response to clipping, insect and vertebrate herbivory would enable us to answer important questions about the ecological role of silicon. For example, we may gain insights into whether silicon defences can explain the higher levels of dietary specialization among insect herbivores and tight pairwise co-evolution between insects and their host plants, which is generally less common among mammals, particularly grazers.

There are other differences between clipping and herbivory relating to the various ways herbivores feed. Lepidoptera usually feed by shearing off plant material with their incisors, graminivorous orthopterans rely on the molar regions of their mandibles to mechanically disrupt the cell wall, while phloem-feeding insects such as aphids use a piercing and sucking mechanism (Bonaventure 2012). Each of these actions is likely to damage plant cells in a different way and to a greater extent than would mechanical snipping, which results in a cleaner cut and less disruption to the plant cells; hence, we might expect differences in the effects of herbivory between different guilds of insects and mammalian herbivores.

In fact, we still have surprisingly little data on the relative magnitude of silicon induction by different types of herbivore (but see Quigley & Anderson 2014). It is possible that herbivory by some species of mammalian herbivores might not result in the induction of chemical or physical defences, because the speed, pattern and amount of leaf removal might negate the signal for the plant to respond (Walters 2010). Some small mammals, such as voles, selectively remove the basal meristems of grasses and may disrupt the cell walls, whereas larger herbivores, such as ungulates, remove large portions of the above-ground biomass in a single bite, a very different type of tissue wounding. There are few studies addressing this, though Massey, Ennos & Hartley (2007b) compared silicon induction in response to mechanical damage and herbivory by locusts and voles. They demonstrated that although both types of herbivory induced silicon defences more than clipping, there was no difference between the impacts of the two herbivores on two different natural grasses.

Despite the tendency for insect and mammalian herbivores to elicit induction of silicon defences, this pattern is not universal; some studies have found that herbivory did not cause a measureable induction of silicon defences (e.g. Soininen *et al.* 2013; Quigley & Anderson 2014). These

examples tend to be field-based studies comparing silicon levels in grasses in grazed and ungrazed areas, where the levels of herbivory are unknown and may be of insufficient duration and/or intensity to elicit induction (see below), and where other site-based factors, for example local climate, soil type or previous grazing history, may influence induction (e.g. Georgiadis & McNaughton 1990; Fenner, Lee & Duncan 1993; Soininen *et al.* 2013). Laboratory studies may provide an explanation as some have demonstrated that silicon induction may require a threshold of damage, either in terms of amount of biomass removed or in terms of frequency of damage (Massey, Ennos & Hartley 2007b; Reynolds *et al.* 2012). These studies suggest a single instance of damage does not lead to induction, nor do damage levels of less than around 20% of total leaf area removed.

CASE STUDY: THE EFFECTS OF GRAZING BY VOLES ON SILICON INDUCTION IN THE FIELD

The complexity of the relationship between induction and damage intensity has been difficult to resolve given the lack of studies in the field; clear thresholds of herbivore damage required to induce elevated silicon accumulation have only been demonstrated in laboratory studies. Recently, we conducted field experiments using specially constructed grazing enclosures which exposed *Deschampsia caespitosa* plants to varying intensities of grazing by field voles (*Microtus agrestis*) to test the effects of grazing intensity and season on silicon induction (J. DeGabriel, S. Hartley, F. Massey, S. Reidinger and X. Lambin, unpublished data). We compared our field results to the laboratory results of Reynolds *et al.* (2012), which used the same study system.

Materials and methods

EXPERIMENTAL DESIGN

We erected a 36 × 36 m grazing enclosure, consisting of eighty-one 4 × 4 m cells in an area of natural clear-cut grassland in Kielder Forest in northern England that is habitat for populations of field voles. The enclosures were constructed from vole-proof wire mesh, which was sunken 30 cm below-ground and was at least 50 cm high, topped with a roll-top, which prevented voles from moving into neighbouring cells. The dominant plant species in each of the experimental cells was *D. caespitosa*, which is a major dietary component of field voles and their main overwinter food source. The enclosures were exposed to natural levels of vole grazing in previous years, but we trapped and removed all voles from the enclosures in the winter before commencing our experiment in spring.

From March 2009, we live-trapped wild voles in surrounding grassland using Ugglan traps (Grahnab, Marieholm, Sweden) and immediately introduced a single vole into each of 12 cells (giving a density of 50 voles ha^{-1}) and 6 voles into each of another 12 cells (giving a density of 300 voles ha^{-1}). The sex and body mass of each vole were recorded. Voles were allowed to graze freely in the cells for 3–4 days, before we retrapped and released them outside the enclosures. We repeated this grazing treatment roughly every 6–7 weeks between March and November 2009, as well as in

January, February and April 2010. Ability to access field sites over winter was restricted due to heavy snow.

We collected samples from a single *D. caespitosa* tussock in each enclosure approximately 1 month after each grazing treatment. Within each cell, we randomly chose 3 tussocks on each sampling occasion and took 5 tillers each from the centre and edge of those tussocks. We pooled the leaves from the three plants in plastic bags and stored them frozen at −20 °C for analysis. The leaves chosen were the youngest fully expanded and undamaged adult leaf blades available that were green and not contaminated with fungus, which we considered to be the most palatable to voles. Thus, at different times of year, the leaf samples were not exactly the same, as we deliberately did not collect new or young leaves that had not fully matured. We prepared and analysed the silicon content of the leaf samples using portable X-ray fluorescence (Reidinger, Ramsey & Hartley 2012).

In September 2009, we estimated the average grazing damage levels on *D. caespitosa*. We randomly selected a single tussock in each cell and haphazardly chose 100 leaves on the outside of the plant (covering the entire circumference of the tussock) and 100 leaves on the interior. We visually recorded how many of these leaves had been damaged by vole grazing and averaged the proportion of leaves damaged across the plant.

Results

EFFECTS OF GRAZING INTENSITY ON SILICON INDUCTION

We found that on average, approximately 5% of leaves were damaged in the 50 voles ha^{-1} treatment and 23·5% of leaves were damaged in the 300 voles ha^{-1} treatment. This was roughly equivalent to the 'low' (5% of leaves removed) and 'high' (20% of leaves removed) grazing treatments imposed in the laboratory study by Reynolds *et al.* (2012). We found remarkably similar patterns in the rates of silicon induction under the high and low grazing pressures in the field (Fig. 1a) to those reported by Reynolds *et al.* (2012). In both the laboratory and the field, silicon induction only occurred under the high grazing intensity treatment, but not the low. Furthermore, induction was delayed for 2 months after initiation of grazing, before an approximate doubling of silicon concentrations in the high, relative to the low grazing treatment by 5 months after the start of damage.

EFFECTS OF SEASON ON SILICON INDUCTION

We found that silicon concentrations increased in *D. caespitosa* in response to vole grazing during the summer and autumn, reaching a peak in winter, presumably as a result of accumulation in old leaves from the previous growing season. Concentrations of silicon then decreased rapidly in the spring, again presumably as a result of flushes of new leaves that had not taken up silicon (Fig. 1b).

Our results demonstrate that both threshold effects and seasonality are important in silicon uptake, and these factors have been found to influence induction in other studies. For example, in a study of *Agrostis tenuis*, Banuelos & Obeso (2000) found that silicon content of
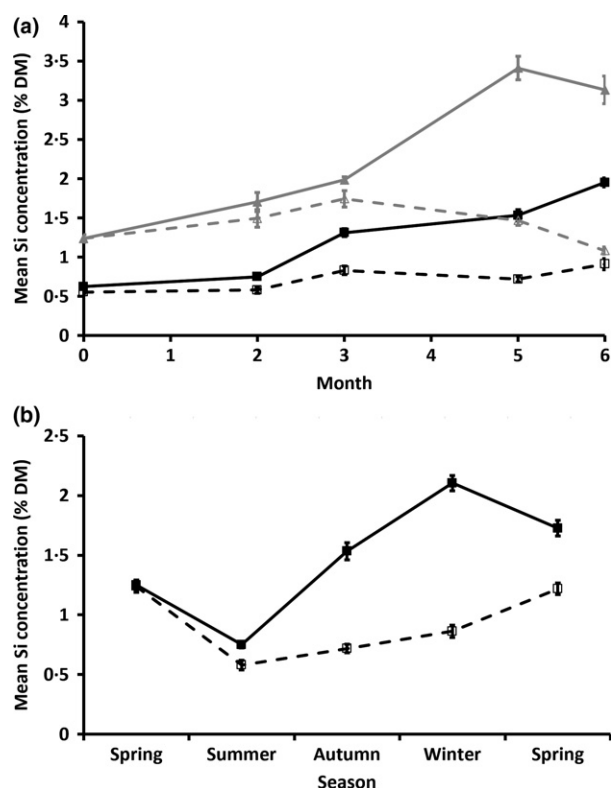
Fig. 1. Induction of silicon defences in *Deschampsia caespitosa* exposed to high (*c.* 20% of leaves damaged) and low (*c.* 5% of leaves damaged) levels of grazing by field voles (*Microtus agrestis*). Solid lines denote high grazing intensity, and broken lines denote low grazing intensity. (a) Comparison of silicon induction in *D. caespitosa* grown in glasshouse and grazed in the laboratory (grey lines, triangular symbols) (reproduced from Reynolds *et al.* 2012) and under field conditions (black lines, square symbols) in open grazing enclosures in northern England from May to November 2009 with a grazing intensity of 300 voles ha^{-1} and 50 voles ha^{-1} (J. DeGabriel, S. Hartley, F. Massey, S. Reidinger and X. Lambin, unpublished data). (b) Seasonal variation in silicon concentrations in *D. caespitosa* in field grazing enclosures under high (300 voles ha^{-1}) and low (50 voles ha^{-1}) grazing treatments from March 2009 to April 2010. Error bars represent standard error.

plants was higher in heavily grazed areas than within experimental exclosures during the summer, but no such differences were apparent in winter. This was in contrast to the results from our experimental field enclosures in northern England (Fig. 1b). There are similar phenological effects in plant responses to clipping: in a study of five tropical grass species, silicon content generally increased over time, although this effect varied with species, and for some species, the effect of clipping on leaf sheath silicon content differed between dates (Kindomihou, Sinsin & Meerts 2006). Similarly, the effect of mowing on the silicon levels of prairie foliage differed between July, when there was no effect, and October, when there was an increase (Seastedt, Ramundo & Hayes 1989).

There is also evidence that phenological variation in silicon content may differ between grass species growing in

different locations. For example, in North American prairies, shoot silicon concentrations increase throughout the growing season (Brizuela, Detling & Cid 1986; Seastedt, Ramundo & Hayes 1989), and the same trend was found in savanna grasses in Kenya (Georgiadis & McNaughton 1990). In contrast, in another African study, grasses in the Serengeti in Tanzania had higher silicon levels early in the growing season (McNaughton *et al.* 1985). This variation is more likely related to broader ecosystem differences across latitudes than effects of season *per se*. Clearly induction of silicon defences, whether in response to artificial damage or natural herbivory, is highly variable and its magnitude is contingent on a number of factors, including damage type, damage intensity, timing of damage, plant species and even tissue age (see below, Banuelos & Obeso 2000; Kindomihou, Sinsin & Meerts 2006).

Impact of herbivory: induction of silicon defences varies with plant species and genotype

The ability of different non-agricultural grass species to increase their silicon uptake in response to experimental removal of leaves by herbivores has been measured across a relatively narrow range of species under controlled conditions (Massey, Ennos & Hartley 2006, 2007b). In contrast, many studies have assessed such variability in relation to clipping and have demonstrated clear between-species differences in silicon uptake in response (e.g. McNaughton *et al.* 1985; Kindomihou, Sinsin & Meerts 2006; Soininen *et al.* 2013). Between-species variation in the magnitude of the differences in silicon levels in wild plants collected from naturally grazed vs. ungrazed areas is also well documented (e.g. McNaughton & Tarrants 1983; Brizuela, Detling & Cid 1986; Soininen *et al.* 2013). Such differences have also been demonstrated within species, which has led to the suggestion that herbivory drives the selection of ecotypes with increased ability to take up silicon (Detling & Painter 1983; McNaughton & Tarrants 1983; Banuelos & Obeso 2000). While that idea remains somewhat speculative for field populations, the existence of intraspecific genotypic differences in silicon induction in response to clipping is clear in laboratory experiments. For example, the silicon content of some genotypes of *A. tenuis* increased after clipping, whereas it declined in others (Banuelos & Obeso 2000). Similarly, Soininen *et al.* (2013) found that four different grass species showed marked within-species differences in silicon content following clipping, in addition to extensive between-species variation. Similarly, three grass species from the same genus, *Festuca*, showed very different patterns of silicon uptake and deposition in defensive structures (spines and phytoliths) in response to artificial damage and manipulation of silicon supply, as did two genotypes of one of these species, *F. arundinacea* (Hartley *et al.* 2015).

We do not know why there are such large differences in silicon content in quite closely related species and even

between genotypes of the same species. This is because we have a very limited understanding of silicon uptake at the physiological, biochemical and molecular level for most non-crop species (Hartley *et al.* 2015; though see Deshmukh & Belanger 2016). In crop species, particularly rice, many of the transporters responsible for silicon uptake and distribution within the plant have been identified and their role characterized (Ma *et al.* 2006; Ma & Yamaji 2006; Ma 2009; Ma & Yamaji 2015; Yamaji *et al.* 2015), but we still have limited understanding of how the impact of damage on silicon uptake and deposition interacts with abiotic factors. In addition, grasses have an array of different types of defences, which is a complicating factor with respect to disentangling silicon dynamics. Thus far, few studies (but see below Quigley & Anderson 2014; Wieczorek *et al.* 2015b) have attempted to simultaneously quantify experimentally the relative importance of biotic factors, such as grazing or other grass defences, and abiotic factors, such as water availability, on silicon uptake, particularly in the field.

Abiotic factors: induction of silicon defences in response to herbivory varies with soil type, water availability and climate

Abiotic factors influence silicon levels and can impact silicon defences (Soininen *et al.* 2013), although in many studies, it is hard to disentangle abiotic from biotic influences, particularly grazing levels. For example, it is unclear whether higher levels of silicon observed in plants from grazed sites in the North American prairies (Brizuela, Detling & Cid 1986) or the Serengeti (McNaughton *et al.* 1985) are due to a direct response to herbivory (i.e. induction), or to other abiotic differences between the sites. However, it is clear that plants from more heavily grazed sites could accumulate more silicon in leaves than those from ungrazed ones in the laboratory (Detling & Painter 1983; McNaughton & Tarrants 1983), suggesting some role for biotic drivers, regardless of abiotic conditions. However, uptake ultimately depends on availability of silicon, itself dependent on soil type and soil pH (Beckwith & Reeve 1964; Ehrlich *et al.* 2010) and, because silicon enters the plants in soluble form through the transpiration stream, it may also depend on water availability and climatic factors which influence transpiration, such as temperature (e.g. Raven 1983; Sangster, Hodson & Tubb 2001; Kindomihou, Sinsin & Meerts 2006; Faisal *et al.* 2012). However, the extent to which silicon uptake depends on transpiration rate remains a subject of debate (Hartley 2015).

A recent study by Wieczorek *et al.* (2015b) attempted to disentangle the relative importance of abiotic and biotic factors in silicon accumulation in a natural wetland system, where the dynamic hydrology might be predicted to have as large an impact as herbivory on the silicon content of foliage. The authors demonstrated the importance of abiotic factors in silicon accumulation in grazed systems,

with temperature and snow cover influencing silicon uptake in both leaves and rhizomes of a tussock sedge, while the level of winter flooding affected uptake in the rhizomes, but not in the leaves. However, although both herbivory and abiotic conditions influenced the uptake of soil available silicon by plants in this study, grazing appeared to be a more important driver than hydrology for foliar tissue silicon content (Wieczorek *et al.* 2015b). This contrasts with the study by Quigley & Anderson (2014), which found water availability had a greater impact on natural silicon levels than defoliation in one of the two species tested, although this study used clipping rather than natural herbivory.

Abiotic and biotic factors may interact in determining both the levels of silicon-based defences and their impact on herbivores. For example, the effectiveness of silicon-based plant defences against locusts has been shown to differ between plant species according to soil silicon availability. Under low soil silicon availability, the herbivores removed more leaf biomass from *L. perenne* than from *P. annua*, whereas under high-silicon availability the reverse was true. Consequently, herbivory shifted the competitive balance between the two grass species, with the outcome depending on the availability of soil silicon (Garbuzov, Reidinger & Hartley 2011). Overall, we see evidence that abiotic factors influence silicon-based responses to damage in plants, but we currently lack comprehensive experimental evidence of these influences, particularly in the case of field studies involving herbivores. Interactions between environmental drivers such as soil silicon and water availability and induction of silicon uptake in response to damage appear to be complex (Kindomihou, Sinsin & Meerts 2006; Soininen *et al.* 2013; Quigley & Anderson 2014).

Impacts of silicon defences on herbivores vary with herbivore type

Ecological studies with invertebrates feeding on natural grasses have demonstrated strong negative effects of plant silicon uptake on rates of herbivory and larval growth rates in a range of species across various feeding guilds (Massey, Ennos & Hartley 2006, 2007b; Massey & Hartley 2009). However, to date, many studies with invertebrates have been in crop species (e.g. Goussain, Prado & Moraes 2005; Kvedaras & Keeping 2007; Kvedaras *et al.* 2007, 2009; Griffin, Hogan & Schmidt 2015) and some have involved measuring effects when silicon has been sprayed on the plant surface, rather than being taken up and deposited naturally by the plant, which is likely to impact on herbivore responses (Moraes *et al.* 2004; Eswaran & Manivannan 2007). Ecological studies on the impacts of silicon on herbivores below-ground are particularly lacking. In one of the very few studies on this topic, silicon addition had no effect on root herbivores (masked chafer grubs), despite causing an increase in both root and leaf silicon content (Redmond & Potter 2006).

Similarly, only a relatively small number of studies have investigated the impacts of silicon defences on the food preferences and performance of mammals, but there is some evidence emerging which suggests silicon has a greater impact on the feeding behaviour of smaller herbivores, compared to larger species. For example, laboratory studies with captive animals have convincingly demonstrated that field voles, prairie voles (*Microtus ochrogaster*) and rabbits (*Oryctolagus cuniculus*) consistently reduce their consumption of grass species containing high concentrations of silicon (Gali-Muhtasib, Smith & Higgins 1992; Massey & Hartley 2006; Cotterill *et al.* 2007). Furthermore, field voles fed diets containing higher concentrations of silicon exhibited slower growth rates and higher mortality under controlled conditions (Massey & Hartley 2006; Huitu *et al.* 2014). In contrast, Massey *et al.* (2009) found that sheep were less impacted by silicon defences than were smaller herbivores, although more studies on larger grazers are required to confirm the consistency of this pattern.

One possible reason for observed differences in effects of silicon in grasses on insects and larger mammalian herbivores may be attributed to the differential impacts of the wearing of teeth and mouthparts (reviewed by Strömberg, Di Stilio & Song 2016). Silicon phytoliths have been clearly shown to cause significant and irreversible mandibular wear in the lepidopteran *Spodoptera* (Massey & Hartley 2009; Reynolds, Keeping & Meyer 2009); the extent of wear correlated with a reduction in digestive efficiency of the caterpillars, suggesting that such wear could contribute to diet selection and the impact of silicon on herbivore growth rates (Massey & Hartley 2009). In addition, the extent and nature of deposition of silicon at the leaf surface has been shown to influence the abrasiveness of natural grass species and hence potentially their vulnerability to herbivores (Hartley *et al.* 2015). In contrast, recent studies have demonstrated that silicon phytoliths in many grass species are softer than tooth enamel of mammal groups including ungulates, macropods and primates (Sanson, Kerr & Gross 2007; Rabenold & Pearson 2011; Erickson 2014; Lucas *et al.* 2014; Rivals *et al.* 2014). However, there is evidence that some grass species contain phytoliths that are harder than tooth enamel (Erickson 2014), although whether these are selected by herbivores is unclear. Furthermore, Calandra *et al.* (2016) found effects of silicon on microwear patterns in the teeth of voles and have proposed this as a mechanism by which silicon may contribute to population crashes. Hummel *et al.* (2011) provide a compelling argument for a role of silicon in the evolution of high-crowned teeth, showing a strong positive correlation between faecal silicon levels and hypsodonty across a range of large African herbivores with differing diets and digestive systems. McArthur (2014) points out that teeth and chewing are an often neglected, but crucial component of understanding herbivore diet selection, especially given the importance of food

processing time on digestion. In support of this idea, high-silicon levels have been shown to reduce the bite rate of sheep (Massey *et al.* 2009), with impacts on processing time and digestive efficiency likely to explain why the sheep preferred to feed on grasses low in silicon.

It has been suggested that while phytoliths may not wear down mammalian teeth, they may reduce animals' access to cell contents by preventing cell walls being broken apart (Massey & Hartley 2006). Consequently, variation in age, body size and digestive physiology may play a role in determining differential effects of silicon. Variation in bite size and offtake rate among different size classes of herbivores may impact the induction of silicon defences, while the greater amount of biomass ingested by large herbivores could potentially dilute the potency of silicon defence. Negative relationships between herbivore body size and diet quality as a result of increased digestive efficiency have been well described (Bell 1970; Jarman 1974), although a recent paper by Steuer *et al.* (2014) challenges this paradigm. Research to date has generally focussed on the positive aspects of animal nutrition, but an understanding of the interactive role of plant defences on the digestibility of plants for different size and age classes of herbivores is missing. Silicon defences in grasses are an excellent system to test such nutritional hypotheses.

Most grazers have developed the ability to digest a lot of fibre in grasses, but not silicon. Thus, it may act as an effective bulking agent and prevent fibre (structural carbohydrates) and, ultimately, dry matter digestibility (Shewmaker *et al.* 1989). As epidermal silicon can prevent enzyme-aided infiltration by fungal hyphae (Fauteux *et al.* 2006), it seems likely that it can protect some of the fibre fractions from degradation by cellulases. Watling *et al.* (2011) found that carbon occluded in phytoliths includes cellulose, lignin and carboxylic acids, which suggests that there could be some chemical interaction between these fractions. In addition, silicon is likely to impact on nitrogen absorption by preventing the leaf cell walls being broken apart (Massey & Hartley 2006; Hunt *et al.* 2008), which is presumably one way silicon reduces growth rates and fecundity of voles and insects. The impact is predicted to be more marked in small, hindgut-fermenting herbivores, such as voles which are more likely to be N limited, compared to the larger ruminants which can utilize endogenous sources of N, or lagomorphs which practice caecotrophy to avoid N limitation. Nevertheless, silicon has been shown to inhibit microbial digestion in ruminants (Harbers, Raiten & Paulsen 1981), so further studies are required to validate this hypothesis. Wieczorek *et al.* (2015a) elucidated the physiological mechanisms underpinning the negative effects of an abrasive plant diet on the performance of root voles (*Microtus oeconomus*). Voles fed a diet of sedges containing silicon and high concentrations of fibre had reduced absorptive efficiency in the small intestine, with shorter villi and more mucus

cells, compared to controls. Consequently, these animals had reduced body mass and lower resting metabolic rate, which they suggested was because voles were unable to increase food intake sufficiently to compensate for the impacts of abrasiveness on the gut. Further studies on the physiological impact of silicon abrasiveness on vertebrate guts would be intriguing.

The impacts of silicon on herbivore growth rates and reproduction are predicted to be more significant for herbivores that exhibit population cycles, such as voles (Reynolds *et al.* 2012), because the negative feedback from delayed density dependence of silicon induction in relation to herbivore density provides a nutritional mechanism to explain population regulation (Massey & Hartley 2006; Massey *et al.* 2008; Wieczorek *et al.* 2015b). Conversely, feedback between herbivore population density and grazing pressure means that cyclic herbivore species are more likely to drive patterns of silicon induction, compared to non-cyclic herbivores (Wieczorek *et al.* 2015b). Theoretical models have provided support for this hypothesis, indicating that a threshold level of herbivore damage is required to initiate sufficient silicon induction to elicit population cycles (Reynolds *et al.* 2012). Recently, Wieczorek *et al.* (2015b) demonstrated that grazing by voles at a spatial scale relevant to their home ranges resulted in significant induction of silicon defences in sedges in Poland, while Massey *et al.* (2008) found correlations between silicon levels in *D. caespitosa* and *M. agrestis* densities in northern England. However, as yet there have been no empirical studies in natural grasslands at the landscape scale relevant to animal populations which convincingly demonstrate that vole grazing pressure is sufficient to induce silicon defences to the level required to affect herbivore population dynamics (Hartley 2015). Nevertheless, the work in Polish and English grassland systems, including the advances in understanding the effects of eating high-silicon diets on animals' digestive physiology (Wieczorek *et al.* 2015a), gives some support to the hypothesis that silicon defences may drive vole population cycles. The next step is to expand these studies to understand how local effects of silicon on vole meta-populations drive population cycles at a landscape scale.
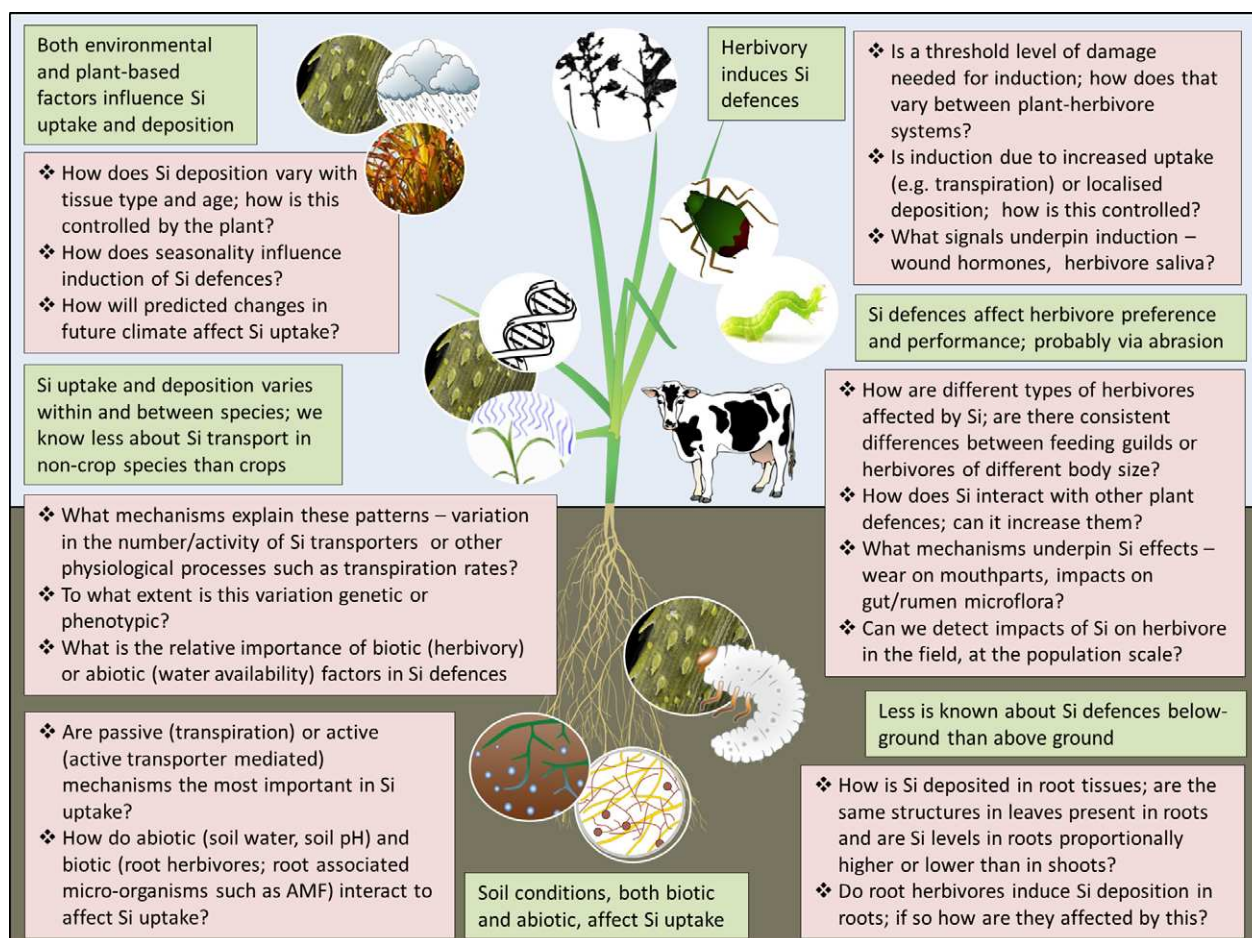

Fig. 2. A summary of research needs for silicon-mediated ecological interactions between plants and herbivores. Green boxes summarize established knowledge, while the pink boxes suggest key knowledge gaps and potential research questions for future work, as depicted by the graphics in circles.

Landscape-scale studies of wild herbivore populations

Linking plant defence to the regulation of wild herbivore populations is inherently difficult (Bazely *et al.* 1997; Foley, Iason & Makkar 2007; DeGabriel *et al.* 2014). Two studies have successfully demonstrated relationships between N availability and reproductive success in mammal populations mediated by constitutive tannin concentrations (DeGabriel *et al.* 2009; McArt *et al.* 2009), but no such relationships have as yet been demonstrated for induced defence systems. Attempts to link silicon defences to mammal population cycles are hampered by the complexity of the diets of wild herbivores in natural grasslands, which may result in insufficient grazing pressure on a single plant species to induce silicon to levels comparable to those producing antiherbivore effects in no-choice laboratory studies. Secondly, spatial variation in silicon concentrations as a result of the biotic and abiotic factors described above means that averaged values for a site may under- or overestimate the extreme values that animals ingest within their home ranges. Finally, at certain time points, for example during the 'crash phase' of a cycle, natural grazing intensity may be insufficient to elicit high levels of silicon induction. These effects are evident from Fig. 1a as, despite the similarities in patterns of silicon induction between the laboratory and field studies, the absolute concentrations of silicon in the plants grown in the glasshouse were significantly higher than the plants from the field. Given the complexities of the field environment, to reveal relationships between induction of silicon defences and herbivore population dynamics in natural grasslands, we need to first obtain quantitative data on the intensity of grazing on individual grass species in order to have confidence that herbivores are eating silicon-accumulating plants. We also need to design sampling strategies with sufficient numbers of samples collected across an appropriate spatial scale to capture the variation in silicon concentrations in field environments. We need to select places and times where herbivore densities are high enough to elicit sufficient grazing pressure to exceed the threshold required to cause induction of silicon defences. Finally, we need to be aware of abiotic factors that may impact on silicon induction, as described above, and use this information to inform our selection of sites and the timing of our experimental manipulations and sample collection.

Conclusion

Much is now known about silicon-based defences in grasses and their impact on herbivores (Fig. 2), though it is also clear that silicon defences in natural grasses exhibit enormous variability, both within and between species. Induction of silicon defences is affected by abiotic factors such as soil silicon availability, by variation in biological process such as transpiration rates, and by plant genotype, as well as by the amount and type of damage a plant receives (Fig. 2). However, much of this current understanding has been derived from studies in the laboratory and glasshouse, which is in large part due to difficulties inherent in field studies, where multiple, interacting factors may simultaneously impact on the uptake and use of silicon for defence. Although relationships between silicon concentration and animal feeding preferences and performance can be demonstrated in the laboratory (Massey & Hartley 2006; Massey, Ennos & Hartley 2007b), as we increase spatial scale the effects of grazing on silica induction become harder to demonstrate, particularly at a landscape scale (Soininen *et al.* 2013; Huitu *et al.* 2014; Wieczorek *et al.* 2015b). Only a handful of large-scale studies have been conducted so far, but encouragingly, the patterns of induction, in terms of threshold damage levels required, time for it to occur and its magnitude seem similar in the glasshouse and in enclosures (Fig. 1). Crucially though, we still lack a landscape-scale demonstration of the impact of herbivores on silicon induction and vice versa. This does not indicate that silicon defences do not have any functional relevance in real ecosystems. Rather, ecologists need to overcome the difficulties inherent in observing effects at landscape scales where there is a need to tease apart the confounding factors that could impact silicon induction and its effect on herbivores. There are a number of other such key knowledge gaps which currently prevent us having a full understanding of the ecological role of silicon-based defences against herbivores. We highlight some of them in Fig. 2 and suggest them as potential future research areas to provide novel insights into the mechanisms by which silicon can underpin plant–herbivore interactions in grasses.

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Data accessibility

Data for this paper have been deposited in the Dryad Digital repository http://dx.doi.org/10.5061/dryad.kd5s9 (Hartley & DeGabriel 2016).

References

Alborn, H.T., Turlings, T.C.J., Jones, T.H., Stenhagen, G., Loughrin, J.H. & Tumlinson, J.H. (1997) An elicitor of plant volatiles from beet armyworm oral secretion. *Science*, **276**, 945–949.

Banuelos, M.J. & Obeso, J.R. (2000) Effect of grazing history, experimental defoliation, and genotype on patterns of silicification in *Agrostis tenuis* Sibth. *Ecoscience*, **7**, 45–50.

Bazely, D.R., Vicari, M., Emmerich, S., Filip, L., Lin, D. & Inman, A. (1997) Interactions between herbivores and endophyte-infected *Festuca rubra* from the Scottish islands of St. Kilda, Benbecula and Rum. *Journal of Applied Ecology*, **34**, 847–860.

Beckwith, R.S. & Reeve, R. (1964) Studies on soluble silica in soils. II. The release of monosilicic acid from soils. *Australian Journal of Soil Research*, **2**, 157–168.

Bell, R.H.V. (1970) The use of the herb layer by grazing ungulates in the Serengeti. *Animal Populations and Relations to their Food Resources* (ed. A. Watson), pp. 111–124. Blackwell, Oxford, UK.

Bonaventure, G. (2012) Perception of insect feeding by plants. *Plant Biology*, **14**, 872–880.

Bonaventure, G., VanDoorn, A. & Baldwin, I.T. (2011) Herbivore-associated elicitors: FAC signaling and metabolism. *Trends in Plant Science*, **16**, 294–299.

Brizuela, M.A., Detling, J.K. & Cid, M.S. (1986) Silicon concentration of grasses growing in sites with different grazing histories. *Ecology*, **67**, 1098–1101.

Calandra, I., Zub, K., Szafranska, P.A., Zalewski, A. & Merceron, G. (2016) Silicon-based plant defences, tooth wear and voles. *Journal of Experimental Biology*, **219**, 501–507.

Cid, M.S., Detling, J.K., Brizuela, M.A. & Whicker, A.D. (1989) Patterns in grass silicification: response to grazing history and defoliation. *Oecologia*, **80**, 268–271.

Cid, M.S., Detling, J.K., Whicker, A.D. & Brizuela, M.A. (1990) Silicon uptake and distribution in *Agropyron smithii* as related to grazing history and defoliation. *Journal of Range Management*, **43**, 344–346.

Cooke, J. & Leishman, M.R. (2011) Is plant ecology more siliceous than we realise? *Trends in Plant Science*, **16**, 61–68.

Cotterill, J.V., Watkins, R.W., Brennon, C.B. & Cowan, D.P. (2007) Boosting silica levels in wheat leaves reduces grazing by rabbits. *Pest Management Science*, **63**, 247–253.

Currie, H.A. & Perry, C.C. (2007) Silica in plants: Biological, biochemical and chemical studies. *Annals of Botany*, **100**, 1383–1389.

Damuth, J. & Janis, C.M. (2011) On the relationship between hypsodonty and feeding ecology in ungulate mammals, and its utility in palaeoecology. *Biological Reviews*, **86**, 733–758.

DeGabriel, J.L., Moore, B.D., Foley, W.J. & Johnson, C.N. (2009) The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. *Ecology*, **90**, 711–719.

DeGabriel, J.L., Moore, B.D., Felton, A.M., Ganzhorn, J.U., Stolter, C., Wallis, I.R. *et al.* (2014) Translating nutritional ecology from the laboratory to the field: milestones in linking plant chemistry to population regulation in mammalian browsers. *Oikos*, **123**, 298–308.

Deshmukh, R.K. & Belanger, R.R. (2016) Molecular evolution of aquaporins and silicon influx in plants. *Functional Ecology*, **30**, 1277–1285.

Detling, J.K. & Painter, E.L. (1983) Defoliation responses of western wheatgrass populations with diverse histories of prairie dog grazing. *Oecologia*, **57**, 65–71.

Ehrlich, H., Demadis, K.D., Pokrovsky, O.S. & Koutsoukos, P.G. (2010) Modern views on desilicification: biosilica and abiotic silica dissolution in natural and artificial environments. *Chemical Reviews*, **110**, 4656–4689.

Epstein, E. (1999) Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 641–664.

Ergon, T., Lambin, X. & Stenseth, N.C. (2001) Life-history traits of voles in a fluctuating population respond to the immediate environment. *Nature*, **411**, 1043–1045.

Ergon, T., Ergon, R., Begon, M., Telfer, S. & Lambin, X. (2011) Delayed density-dependent onset of spring reproduction in a fluctuating population of field voles. *Oikos*, **120**, 934–940.

Erickson, K.L. (2014) Prairie grass phytolith hardness and the evolution of ungulate hypsodonty. *Historical Biology*, **26**, 737–744.

Eswaran, A. & Manivannan, K. (2007) Effect of foliar application of lignite fly ash on the management of papaya leaf curl disease. *Acta Horticulturae*, **740**, 271–275.

Faisal, S., Callis, K.L., Slot, M. & Kitajima, K. (2012) Transpiration-dependent passive silica accumulation in cucumber (*Cucumis sativus*) under varying soil silicon availability. *Botany-Botanique*, **90**, 1058–1064.

Farmer, E. (2014) *Leaf Defence*. Oxford University Press, Oxford, UK.

Fauteux, F., Chain, F., Belzile, F., Menzies, J.G. & Belanger, R.R. (2006) The protective role of silicon in the *Arabidopsis*-powdery mildew pathosystem. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 17554–17559.

Fenner, M., Lee, W.G. & Duncan, S.J. (1993) Chemical features of *Chionochloa* species in relation to grazing by ruminants in South Island, New Zealand. *New Zealand Journal of Ecology*, **17**, 35–40.

Foley, W., Iason, G. & Makkar, H. (2007) Transdisciplinary studies of plant secondary metabolites: Lessons from ecology for animal science and vice versa. *Proceedings of the 7th International Symposium on the Nutrition of Herbivores* (eds Q.X. Meng, J.X. Liu & W.Y. Zhu). Beijing.

Gali-Muhtasib, H.U., Smith, C.C. & Higgins, J.J. (1992) The effect of silica in grasses on the feeding behavior of the prairie vole *Microtus ochrogaster*. *Ecology*, **73**, 1724–1729.

Garbuzov, M., Reidinger, S. & Hartley, S.E. (2011) Interactive effects of plant-available soil silicon and herbivory on competition between two grass species. *Annals of Botany*, **108**, 1355–1363.

Georgiadis, N.J. & McNaughton, S.J. (1990) Elemental and fiber contents of savanna grasses: variation with grazing, soil type, season and species. *Journal of Applied Ecology*, **27**, 623–634.

Gomes, F.B., de Moraes, J.C., dos Santos, C.D. & Goussain, M.M. (2005) Resistance induction in wheat plants by silicon and aphids. *Scientia Agricola*, **62**, 547–551.

Goussain, M.M., Prado, E. & Moraes, J.C. (2005) Effect of silicon applied to wheat plants on the biology and probing behaviour of the greenbug *Schizaphis graminum* (Rond.) (Hemiptera : Aphididae). *Neotropical Entomology*, **34**, 807–813.

Griffin, M., Hogan, B. & Schmidt, O. (2015) Silicon reduces slug feeding on wheat seedlings. *Journal of Pest Science*, **88**, 17–24.

Harbers, L.H., Raiten, D.J. & Paulsen, G.M. (1981) The role of plant epidermal silica as a structural inhibitor of rumen microbial digestion in steers. *Nutrition Reports International*, **24**, 1057–1066.

Harbers, L.H. & Thouvenelle, M.L. (1980) Digestion of corn and sorghum silage observed by scanning electron microscopy. *Journal of Animal Science*, **50**, 514–526.

Hartley, S.E. (2015) Round and round in cycles? Silicon-based plant defences and vole population dynamics. *Functional Ecology*, **29**, 151–153.

Hartley, S.E. & DeGabriel, J.L. (2016) Data from: The ecology of herbivore-induced silicon defences in grasses. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.kd5s9.

Hartley, S.E. & Lawton, J.H. (1987) Effects of different types of damage on the chemistry of birch foliage, and the responses of birch feeding insects. *Oecologia*, **74**, 432–437.

Hartley, S.E. & Lawton, J.H. (1991) Biochemical aspects and significance of the rapidly induced accumulation of phenolics in birch foliage. *Phytochemical Induction by Herbivores* (eds D.W. Tallamy & M.J. Raupp), pp. 105–132. Wiley, New York, NY.

Hartley, S.E., Fitt, R.N., McLamon, E.L. & Wade, R.N. (2015) Defending the leaf surface: intra- and inter-specific differences in silicon deposition in grasses in response to damage and silicon supply. *Frontiers in Plant Science*, **6**, 35.

Haukioja, E. & Neuvonen, S. (1985) Induced long-term resistance of birch foliage against defoliators – defensive or incidental? *Ecology*, **66**, 1303–1308.

Heil, M. & Ton, J. (2008) Long-distance signalling in plant defence. *Trends in Plant Science*, **13**, 264–272.

Hodson, M.J., White, P.J., Mead, A. & Broadley, M.R. (2005) Phylogenetic variation in the silicon composition of plants. *Annals of Botany*, **96**, 1027–1046.

Huitu, O., Forbes, K.M., Helander, M., Julkunen-Tiitto, R., Lambin, X., Saikkonen, K. *et al.* (2014) Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores. *Frontiers in Plant Science*, **5**, 478.

Hummel, J., Findeisen, E., Suedekum, K.-H., Ruf, I., Kaiser, T.M., Bucher, M. *et al.* (2011) Another one bites the dust: faecal silica levels in large herbivores correlate with high-crowned teeth. *Proceedings of the Royal Society B-Biological Sciences*, **278**, 1742–1747.

Hunt, J.W., Dean, A.P., Webster, R.E., Johnson, G.N. & Ennos, A.R. (2008) A novel mechanism by which silica defends grasses against herbivory. *Annals of Botany*, **102**, 653–656.

Jarman, P.J. (1974) Social organization of antelope in relation to their ecology. *Behaviour*, **48**, 215–267.

Karban, R. & Baldwin, I.T. (1997) *Induced Responses to Herbivory*. The University of Chicago Press, Chicago, IL, USA.

Katz, O. (2015) Silica phytoliths in angiosperms: phylogeny and early evolutionary history. *New Phytologist*, **208**, 642–646.

Keeping, M.G., Meyer, J.H. & Sewpersad, C. (2013) Soil silicon amendments increase resistance of sugarcane to stalk borer *Eldana saccharina* Walker (Lepidoptera: Pyralidae) under field conditions. *Plant and Soil*, **363**, 297–318.

Keeping, M.G., Miles, N. & Sewpersad, C. (2014) Silicon reduces impact of plant nitrogen in promoting stalk borer (*Eldana saccharina*) but not sugarcane thrips (*Fulmekiola serrata*) infestations in sugarcane. *Frontiers in Plant Science*, **5**, 289.

Keeping, M.G. & Reynolds, O.L. (2009) Silicon in agriculture: new insights, new significance and growing application. *Annals of Applied Biology*, **155**, 153–154.

Kindomihou, V., Sinsin, B. & Meerts, P. (2006) Effect of defoliation on silica accumulation in five tropical fodder grass species in Benin. *Belgian Journal of Botany*, **139**, 87–102.

Korth, K.L. & Dixon, R.A. (1997) Evidence for chewing insect-specific molecular events distinct from a general wound response in leaves. *Plant Physiology*, **115**, 1299–1305.

Kvedaras, O.L. & Keeping, M.G. (2007) Silicon impedes stalk penetration by the borer *Eldana saccharina* in sugarcane. *Entomologia Experimentalis Et Applicata*, **125**, 103–110.

Kvedaras, O.L., Keeping, M.G., Goebel, F.R. & Byrne, M.J. (2007) Larval performance of the pyralid borer *Eldana saccharina* Walker and stalk damage in sugarcane: Influence of plant silicon, cultivar and feeding site. *International Journal of Pest Management*, **53**, 183–194.

Kvedaras, O.L., Byrne, M.J., Coombes, N.E. & Keeping, M.G. (2009) Influence of plant silicon and sugarcane cultivar on mandibular wear in the stalk borer *Eldana saccharina*. *Agricultural and Forest Entomology*, **11**, 301–306.

Lindroth, R.L. & Batzli, G.O. (1986) Inducible plant chemical defences – a cause of vole population cycles. *Journal of Animal Ecology*, **55**, 431–449.

Lucas, P.W., van Casteren, A., Al-Fadhalah, K., Almusallam, A.S., Henry, A.G., Michael, S. *et al.* (2014) The role of dust, grit and phytoliths in tooth wear. *Annales Zoologici Fennici*, **51**, 143–152.

Ma, J.F. (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, **50**, 11–18.

Ma, J.F. (2009) Silicon uptake and translocation in plants. *The Proceedings of the International Plant Nutrition Colloquium XVI*. Department of Plant Sciences, UC Davis, Davis, CA, USA.

Ma, J.F. & Yamaji, N. (2006) Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, **11**, 392–397.

Ma, J.F. & Yamaji, N. (2015) A cooperative system of silicon transport in plants. *Trends in Plant Science*, **20**, 435–442.

Ma, J.F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M. *et al.* (2006) A silicon transporter in rice. *Nature*, **440**, 688–691.

Massey, F.P., Ennos, A.R. & Hartley, S.E. (2006) Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *Journal of Animal Ecology*, **75**, 595–603.

Massey, F.P., Ennos, A.R. & Hartley, S.E. (2007a) Grasses and the resource availability hypothesis: the importance of silica-based defences. *Journal of Ecology*, **95**, 414–424.

Massey, F.P., Ennos, A.R. & Hartley, S.E. (2007b) Herbivore specific induction of silica-based plant defences. *Oecologia*, **152**, 677–683.

Massey, F.P. & Hartley, S.E. (2006) Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 2299–2304.

Massey, F.P. & Hartley, S.E. (2009) Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *Journal of Animal Ecology*, **78**, 281–291.

Massey, F.P., Smith, M.J., Lambin, X. & Hartley, S.E. (2008) Are silica defences in grasses driving vole population cycles? *Biology Letters*, **4**, 419–422.

Massey, F.P., Massey, K., Ennos, A.R. & Hartley, S.E. (2009) Impacts of silica-based defences in grasses on the feeding preferences of sheep. *Basic and Applied Ecology*, **10**, 622–630.

McArt, S.H., Spalinger, D.E., Collins, W.B., Schoen, E.R., Stevenson, T. & Bucho, M. (2009) Summer dietary nitrogen availability as a potential bottom-up constraint on moose in south-central Alaska. *Ecology*, **90**, 1400–1411.

McArthur, C. (2014) Do we ditch digestive physiology in explaining the classic relationship between herbivore body size diet and diet quality? *Functional Ecology*, **28**, 1059–1060.

McColloch, J.W. & Salmon, S.C. (1923) The resistance of wheat to the Hessian Fly – a progress report. *Journal of Economic Entomology*, **16**, 293–298.

McNaughton, S.J. & Tarrants, J.L. (1983) Grass leaf silicification – natural selection for an inducible defense against herbivores. *Proceedings of the National Academy of Sciences of the United States of America*, **80**, 790–791.

McNaughton, S.J., Tarrants, J.L., McNaughton, M.M. & Davis, R.H. (1985) Silica as a defense against herbivory and a growth promotor in African grasses. *Ecology*, **66**, 528–535.

Moraes, J.C., Goussain, M.M., Basagli, M.A.B., Carvalho, G.A., Ecole, C.C. & Sampaio, M.V. (2004) Silicon influence on the tritrophic interaction: Wheat plants, the greenbug *Schizaphis graminum* (Rondani) (Hemiptera : Aphididae), and its natural enemies, *Chrysoperla externa* (Hagen) (Neuroptera : Chrysopidae) and *Aphidius colemani viereck* (Hymenoptera : Aphidiidae). *Neotropical Entomology*, **33**, 619–624.

Piperno, D.R. (2006) *Pytoliths: A comprehensive Guide for Archaeologists and Palaeoecologists*. AltaMira Press, Oxford, UK.

Ponnaiya, B. (1951) Studies on the genus *Sorghum*. II The cause of resistance in sorghum to the insect pest *Atherigona indica* M. *Madras University Journal*, **21**, 203–217.

Quigley, K.M. & Anderson, T.M. (2014) Leaf silica concentration in Serengeti grasses increases with watering but not clipping: insights from a common garden study and literature review. *Frontiers in Plant Science*, **5**, 568.

Rabenold, D. & Pearson, O.M. (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.

Raven, J.A. (1983) The transport and function of silicon in plants. *Biological Reviews of the Cambridge Philosophical Society*, **58**, 179–207.

Redmond, C.T. & Potter, D.A. (2006) Silicon fertilization does not enhance creeping bentgrass resistance to cutworms and white grubs. *Applied Turfgrass Science* [Online], **6**, 1–7.

Reidinger, S., Ramsey, M.H. & Hartley, S.E. (2012) Rapid and accurate analyses of silicon and phosphorus in plants using a portable X-ray fluorescence spectrometer. *New Phytologist*, **195**, 699–706.

Reymond, P., Weber, H., Damond, M. & Farmer, E.E. (2000) Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell*, **12**, 707–719.

Reynolds, O.L., Keeping, M.G. & Meyer, J.H. (2009) Silicon-augmented resistance of plants to herbivorous insects: a review. *The Annals of Applied Biology*, **155**, 171–186.

Reynolds, J.J.H., Lambin, X., Massey, F.P., Reidinger, S., Sherratt, J.A., Smith, M.J. *et al.* (2012) Delayed induced silica defences in grasses and their potential for destabilising herbivore population dynamics. *Oecologia*, **170**, 445–456.

Rivals, F., Takatsuki, S., Maria Albert, R. & Macia, L. (2014) Bamboo feeding and tooth wear of three sika deer (*Cervus nippon*) populations from northern Japan. *Journal of Mammalogy*, **95**, 1043–1053.

Sangster, A.G., Hodson, M.J. & Tubb, H.J. (2001) Silicon deposition in higher plants. *Silicon in Agriculture* (eds L.E. Datnoff, G.H. Snyder & G.H. Korndorfer), pp. 85–113. Elsevier Science, Amsterdam, the Netherlands.

Sanson, G.D., Kerr, S.A. & Gross, K.A. (2007) Do silica phytoliths really wear mammalian teeth? *Journal of Archaeological Science*, **34**, 526–531.

Sasamoto, K. (1953) Studies on the relation between insect pests and silica content in rice plant (II). On the injury of the second generation larvae of rice stem borer. *Oyo Kontyu*, **9**, 108–110.

Seastedt, T.R., Ramundo, R.A. & Hayes, D.C. (1989) Silica, nitrogen and phosphorus dynamics of tallgrass prairie. *11th North American Prairie Conference*, pp. 205–210. University of Nebraska Press, Lincoln, NE, USA.

Shewmaker, G.E., Mayland, H.F., Rosenau, R.C. & Asay, K.H. (1989) Silicon in C$_3$ grasses – effects on forage quality and sheep preference. *Journal of Range Management*, **42**, 122–127.

Smith, M.J., White, A., Lambin, X., Sherratt, J.A. & Begon, M. (2006) Delayed density-dependent season length alone can lead to rodent population cycles. *The American Naturalist*, **167**, 695–704.

Soininen, E.M., Brathen, K.A., Jusdado, J.G.H., Reidinger, S. & Hartley, S.E. (2013) More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. *Oikos*, **122**, 30–41.

Stam, J.M., Kroes, A., Li, Y., Gols, R., van Loon, J.J.A., Poelman, E.H. *et al.* (2014) Plant interactions with multiple insect herbivores: From community to genes. *Annual Review of Plant Biology*, **65**, 689–713.

Steuer, P., Suedekum, K.-H., Tuetken, T., Mueller, D.W.H., Kaandorp, J., Bucher, M. *et al.* (2014) Does body mass convey a digestive advantage for large herbivores? *Functional Ecology*, **28**, 1127–1134.

Strömberg, C., Di Stilio, V. & Song, Z. (2016) Functions of phytoliths in vascular plants: an evolutionary perspective. *Functional Ecology*, **30**, 1286–1297.

Tanentzap, A.J., Vicari, M. & Bazely, D.R. (2014) Ungulate saliva inhibits a grass-endophyte mutualism. *Biology Letters*, **10**, 20140460.

Tian, D., Peiffer, M., Shoemaker, E., Tooker, J., Haubruge, E., Francis, F. *et al.* (2012) Salivary glucose oxidase from caterpillars mediates the

induction of rapid and delayed-induced defenses in the tomato plant. *PLoS ONE*, **7**, e36168.

Valkama, E., Koricheva, J., Ossipov, V., Ossipova, S., Haukioja, E. & Pihlaja, K. (2005) Delayed induced responses of birch glandular trichomes and leaf surface lipophilic compounds to mechanical defoliation and simulated winter browsing. *Oecologia*, **146**, 385–393.

Vicari, M. & Bazely, D.R. (1993) Do grasses fight back? The case for anti-herbivore defences. *Trends in Ecology & Evolution*, **8**, 137–141.

Walters, D. (2010) *Plant Defense: Warding off Attack by Pathogens, Herbivores and Parasitic Plants*, Wiley-Blackwell, Chichester, UK.

Watling, K.M., Parr, J.F., Rintoul, L., Brown, C.L. & Sullivan, L.A. (2011) Raman, infrared and XPS study of bamboo phytoliths after chemical digestion. *Spectrochimica Acta. Part A, Molecular and Biomolecular Spectroscopy*, **80**, 106–111.

Wieczorek, M., Szafranska, P.A., Labecka, A.M., Lazaro, J. & Konarzewski, M. (2015a) Effect of the abrasive properties of sedges on the intestinal absorptive surface and resting metabolic rate of root voles. *Journal of Experimental Biology*, **218**, 309–315.

Wieczorek, M., Zub, K., Szafranska, P.A., Ksiazek, A. & Konarzewski, M. (2015b) Plant-herbivore interactions: silicon concentration in tussock sedges and population dynamics of root voles. *Functional Ecology*, **29**, 187–194.

Yamaji, N., Sakurai, G., Mitani-Ueno, N. & Ma, J.F. (2015) Orchestration of three transporters and distinct vascular structures in node for inter-vascular transfer of silicon in rice. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 11401–11406.

Ye, M., Song, Y., Long, J., Wang, R., Baerson, S.R., Pan, Z. *et al.* (2013) Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, E3631–E3639.