

Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates

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1 **Experimental demonstration of the antiherbivore effects of silica in grasses:**
2 **impacts on foliage digestibility and vole growth rates**

3

4 Running title: silica and vole performance

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1 **SUMMARY**

2 The impacts of plant-based factors on the population dynamics of mammalian herbivores has
3 been the subject of much debate in ecology, but the role of antiherbivore defences in grasses has
4 received relatively little attention. Silica has been proposed as the primary defence in grasses and
5 is thought to lead to increased abrasiveness of foliage so deterring feeding, as well as reducing
6 foliage digestibility and herbivore performance. However, at present there is little direct
7 experimental evidence to support these ideas. In this study, we tested the effects of manipulating
8 silica levels on the abrasiveness of grasses and on the feeding preference and growth performance
9 of field voles, specialist grass-feeding herbivores. Elevated silica levels did increase the
10 abrasiveness of grasses and deterred feeding by voles. We also demonstrated, for the first time,
11 that silica reduced the growth rates of both juvenile and mature female voles by reducing the
12 nitrogen they could absorb from the foliage. Furthermore, we found that vole feeding leads to
13 increased levels of silica in leaves suggesting a dynamic feedback between grasses and their
14 herbivores. We propose that silica induction due to vole grazing reduces vole performance and
15 hence could contribute to cyclic dynamics in vole populations.

16

17 **Keywords:**

18 plant defence, population cycles, *Microtus agrestis*, feeding preference, herbivore performance,
19 induction

20

1 1. INTRODUCTION

2 Grasses dominate many terrestrial ecosystems and are of great ecological and economic
3 importance, as are the grazing mammals associated with them. Although co-evolution between
4 grasses and grazers has received a great deal of attention (Stebbins 1981; McNaughton 1984;
5 Jernvall & Fortelius 2002; Prasad *et al.* 2005), the ecological mechanisms by which grasses
6 defend themselves against herbivores remain poorly understood (O'Regan & Mentis 1989;
7 Vicari & Bazely 1993). The silica content of grasses can be considerably higher than in many
8 other plants and has been proposed as an antiherbivore defence strategy (McNaughton *et al.*
9 1985; Gali-Muhtasib *et al.* 1992; Vicari & Bazely 1993). Silica is deposited as phytoliths in
10 leaves. These are harder than tooth enamel (Baker *et al.* 1959), so they could potentially increase
11 the abrasiveness of leaves and deter feeding (Vicari & Bazely 1993). However, currently the
12 evidence supporting the effects of silica on the palatability of grasses to vertebrate herbivores is
13 limited: there have been relatively few experimental studies and none which manipulate silica
14 content and measure the subsequent changes in abrasiveness of leaves. For example, Gali-
15 Muhtasib *et al.* (1992) found prairie voles (*Microtus ochrogaster*) preferred leaves of grasses
16 with lower silica content when comparing cut leaves of two *Bromus* species, but the effect of
17 silica on abrasiveness was not tested. It has been also proposed that phytoliths may disrupt
18 microbial action in the gut of herbivores, reducing the digestibility of grasses (Vicari & Bazely
19 1993), but almost nothing is known about the effects of silica on either grass leaf digestibility or
20 herbivore performance.

21

22 Many small mammalian herbivores show strong selectivity in feeding preference, both within and
23 between plant species. In voles, these preferences have previously been shown to relate to both
24 the nutrient content (e.g. nitrogen) of their food plants and the levels of physical and chemical
25 antiherbivore defences (Batzli & Cole 1979; Marquis & Batzli 1989; Hartley *et al.* 1995; Hjalten

1 *et al.* 1996). Despite this evidence of the importance of food quality for vole behaviour, the
2 effects of silica, the principle defence in grasses have not been fully investigated.

3
4 For small, fast growing and short lived mammalian herbivores such as voles, food quality is
5 particularly significant because of their relatively high metabolic rates (Demment & Van Soest
6 1985), and because they have a limited capacity to increase rates of food consumption to
7 compensate for poor quality diets (Zynel & Wunder 2002). In addition, the growth rates early in
8 development are highly dependant upon nutrient intake. Therefore in voles, food quality has the
9 potential to dictate the time taken to reach sexual maturity and the onset of breeding each year
10 (Krebs & Myers 1974; Ergon *et al.* 2001). There has been considerable interest in the factors
11 influencing the growth rates and reproductive output in voles because of the impact these
12 parameters have on individual fitness and potentially on vole population dynamics (Argell *et al.*
13 1995; Klemola *et al.* 2000a; Ergon *et al.* 2001; Turchin & Batzli 2001; Ergon *et al.* 2004).
14 Changes in food quality, if they have significant effects on vole growth and reproduction, may
15 play a role in these population processes.

16
17 Here, for the first time, we test both the proposed mechanisms by which silica acts as a defence,
18 namely its ability to increase abrasion and its effect on foliage digestibility and herbivore
19 performance. We measure the effects of silica on the feeding preference and growth rates of field
20 voles (*Microtus agrestis* L.). We focus on the effects of silica on the growth of female field voles
21 as their reproductive performance, which is linked to their growth, is crucial to population growth
22 and they are more likely to be affected by changes in food quality than males (Ostfeld 1985;
23 Agrell *et al.* 1995). By manipulating the levels of silica in two species of grass we tested whether
24 silica is an effective defence against voles. We hypothesized that silica will: i) increase the
25 abrasiveness of grass leaves reducing feeding by of voles; ii) reduce the digestibility of grasses to
26 voles resulting in reduced growth rates of both juvenile and adult voles. We also tested whether

1 vole feeding would induce silica defences by measuring the effect of vole damage on silica
2 levels.

3

4 **2. METHODS**

5 *Study species and plant growth conditions*

6 Short-tailed field voles (*Microtus agrestis* L.) are abundant in grasslands across Europe (Corbet
7 & Harris 1991) and feed selectively on a range of grass species. We selected two grass species
8 (*Festuca ovina* L. and *Lolium perenne* L.) found abundantly in European grasslands, which vary
9 in their natural levels of foliar silica, nutrients and palatability to voles (table 1). Leaf samples of
10 each grass species were collected from 10 replicate plants in 10 sites around East Sussex in
11 August 2005, selected to represent a range of soil types and habitats, to confirm that our
12 manipulated silica levels were within the range occurring in natural communities (table 1).

13

14 Grasses were grown in inert growth media (perlite), under glasshouse conditions for 12–28 weeks
15 (15–25 °C, 16/8 h L/D). Plants for preference trials were grown individually into 5×5×5 cm
16 plugs, while plants for vole growth performance trials and leaf digestibility experiments were
17 grown in sward trays (20×30×5 cm). Grasses were watered every 3 days with 25 ml (individual
18 plants) or 300 ml (sward trays) of Hoagland's solution. To half of the plants, 150mg l⁻¹ of soluble
19 silica was added as NaSiO₃·9H₂O to the Hoagland's solution (Cid *et al.* 1990).

20

21 *Feeding preference*

22 Voles used in feeding trials were all non-lactating adults from a captive bred colony fed on a
23 standard dried diet (N = 2.97%, C:N = 16.4, SiO₂ = 2.64% dry matter: rabbit maintenance diet,
24 B&K Feeds Universal) and fresh leaves of *Triticum avenae* daily. Voles were limited to water and
25 a dried diet for 24 h prior to the trials.

26

1 Intraspecific feeding preference trials ($n = 10$) with captive-bred, non-lactating adult voles used
2 three individual grass plants (12 weeks old) per silica treatment per trial (*F. ovina* and *L.*
3 *perenne*). The leaf area of each plant was measured before and after each trial (AM-200 leaf area
4 meter, ADC BioScientific). Plants were randomly arranged in a 2×3 grid, placed in a vole cage
5 with sawdust to the base of grasses. A single vole was added to each cage until approximately
6 50% leaf area was eaten.

7

8 ***Growth of juvenile voles***

9 Twenty-four newly weaned (between 7 – 11 g), captive-bred, female voles were randomly
10 assigned to a specific diet treatment (*F. ovina* or *L. perenne*; low or high silica). Voles were
11 caged individually in glass tanks (45×30×30 cm, containing sawdust, cotton wool bedding, water
12 and a standard dried diet) and 12 g of fresh grass from the particular treatment added daily. Voles
13 were weighed every 3 – 4 days over a 2 month period. Faecal samples were collected after 1
14 month, dried and analyzed for total nitrogen content (see below).

15

16 ***Growth of mature voles***

17 Ten adult female voles (between 20 – 24 g) were caught from a field site close to the University
18 of Sussex and kept for 2 months prior to digestion efficiency trials. Voles were kept individually
19 (as above) and assigned sequentially to each diet treatment (*F. ovina* or *L. perenne*; low or high
20 silica) for 7 days in a random order. Voles were given 15 g of fresh grass leaves daily. At the end
21 of each diet treatment, voles were weighed and samples of faeces removed from each cage, dried
22 and analyzed for nitrogen content (see below).

23

24 ***Silica induction***

25 *F. ovina* and *L. perenne* plants were grown in compost under standard greenhouse conditions for
26 3 months. Plants were then randomly assigned to damaged or undamaged treatments ($n = 10$). For

1 the vole damage treatment, plants were placed in a cage with a single vole until they were eaten
2 to approximately 5 cm above ground level which took approximately 5 hours. The damage
3 treatment was repeated every 3 weeks for 12 months. Plants were then left to regrow for 6 weeks
4 before collecting leaf samples for silica analysis.

5

6 ***Chemical and physical analyses***

7 Foliar silica content ($n = 10$) was determined by fusing oven dried leaf samples (approximately
8 0.2 g) in sodium hydroxide followed by analysis using the colorimetric silicomolybdate technique
9 (Allen 1989). Total foliar phenolic content of dried leaf samples ($n = 10$) was determined using
10 the Folin-Ciocalteu method (Massey *et al.* 2005), using a tannic acid standard. Foliar and faecal
11 nitrogen ($n = 10$) was analyzed using flash combustion of dried leaf samples (approximately 2.5
12 mg) followed by gas chromatographic separation (Elemental Combustion System; Costech
13 Instruments) calibrated against a standard of composition $C_{26}H_{26}N_2O_2S$. Specific leaf area (= dry
14 leaf mass per unit area) and leaf water content was measured for each species and silica treatment
15 ($n = 10$). Abrasiveness of grass samples ($n = 10$) was determined using a development of the
16 method described by Hammond and Ennos (2000). Fixed areas of grass leaves were ground
17 against a Perspex plate using a modified Martindale abrasion and pilling tester (Model 404,
18 James H. Heal, Halifax). The degree of abrasion by phytoliths on the Perspex was quantified
19 using a laser perthometer to measure the depth of grooves scratched in the surface.

20

21 ***Statistical analysis***

22 Grass leaf characteristics (leaf abrasiveness, silica, nitrogen and phenolic contents) were
23 compared between species and silica treatments using two-way ANOVAs. Feeding preference
24 was calculated as the mean leaf area removed per treatment as a proportion of the total leaf area
25 removed per trial. These data were then transformed using arcsine square-root to achieve
26 normality, before being compared across silica treatments using paired t-tests. Growth rates of

1 juvenile voles (derived from the slope of linear regressions lines: $r^2 = 73.9 - 96.8 \%$) were
2 compared between grass species and silica treatments using 2-way ANCOVA, with initial vole
3 weight as covariate. The proportion of nitrogen absorbed from grass (grass N – fecal N/ grass N)
4 was compared between species and silica treatments using 2-way ANOVA. For adult voles,
5 growth rates relative to initial weight and the proportion of nitrogen absorbed from grass were
6 compared between species and silica treatments using 2-way ANOVAs with vole as a random
7 blocking term. For the induction experiment, the silica content of grasses after 16 months were
8 compared between species and damage treatments using a 2-way ANOVA.

9

10 **3. RESULTS**

11 *Leaf abrasiveness and feeding preference*

12 The presence of silica in grass leaves led to increases of 29% and 42% in leaf abrasiveness in *F.*
13 *ovina* and *L. perenne* (figure 1a), resulting in a reduction of 75% and 63% in feeding by field
14 voles on the two grass species respectively (figure 1b).

15

16 *Vole growth performance and leaf digestibility*

17 Juvenile female voles reared for two months on leaves of *F. ovina* and *L. perenne* containing high
18 silica levels had 41% and 35% lower growth rates respectively than voles reared on low silica
19 leaves (figure 2a, ANCOVA: initial weight (cov) $F_{1,19} = 0.03$ and $p = 0.872$, species $F_{1,19} = 11.07$
20 and $p = 0.004$, silica $F_{1,19} = 35.42$ and $p < 0.001$, and species \times silica $F_{1,19} = 0.05$ and $p = 0.831$).

21 The same trend was evident in mature female voles, where silica reduced their growth rate by
22 over 50% during a period of only seven days of exposure (figure 2b, ANOVA: vole $F_{9,27} = 0.41$
23 and $p = 0.917$, silica $F_{1,27} = 4.51$ and $p = 0.043$). Over the 7 day period there was no difference in
24 the response on different grass species (vole $F_{9,27} = 0.41$ and $p = 0.917$, species $F_{1,27} = 1.68$ and p
25 $= 0.206$).

26

1 Comparisons of foliar and faecal nitrogen levels between silica treatments revealed that juvenile
2 voles reared on high silica diets absorbed proportionally less nitrogen than voles reared on low
3 silica diets (figure 2c ANOVA: species $F_{1,20} = 125.43$ and $p < 0.001$, silica $F_{1,20} = 8.64$ and $p =$
4 0.008 , species \times silica $F_{1,20} = 0.24$ and $p = 0.63$). For mature voles, high silica diets resulted in
5 38% and 19% less nitrogen absorbed from *F. ovina* and *L. perenne* leaves respectively (figure 2d
6 ANOVA: vole $F_{9,27} = 6.83$ and $p < 0.001$, species $F_{1,27} = 67.41$ and $p < 0.001$, silica $F_{1,27} = 52.97$
7 and $p < 0.001$, species \times silica $F_{9,27} = 4.36$ and $p = 0.046$). As the silica treatments had no
8 detectable effects on either leaf nitrogen or phenolic contents (table 1), these results suggest that
9 it was the presence of silica that led to the reduced digestibility of grass leaves.

10

11 ***Silica induction***

12 Feeding by voles led to an increase in foliar silica content of over 400% in both *F. ovina* and *L.*
13 *perenne* compared with undamaged plants (figure 3, ANOVA: species $F_{1,36} = 12.88$ and $p <$
14 0.001 , damage $F_{1,36} = 134.47$ and $p < 0.001$, species \times damage $F_{1,36} = 5.22$ and $p = 0.028$).

15

16 **4. DISCUSSION**

17 We have demonstrated that higher silica levels in grass leaves resulted in both increased
18 abrasiveness of leaves and reduced feeding by voles. These results suggest that the elevated
19 abrasiveness due to silica is an effective feeding deterrent to vertebrate herbivores and support the
20 suggestion that silica may have played a significant role in driving the evolution of dental
21 adaptations found in mammalian grazers (Janis & Fortelius 1988; Jernvall & Fortelius 2002;
22 Prasad *et al.* 2005). Silica levels in natural grassland systems display intraspecific spatio-temporal
23 variation, reflecting variation in biotic factors, such as grazing levels (McNaughton & Tarrants
24 1983; Brizuela *et al.* 1986), and abiotic factors, such as pH and soil type (O'Reagain & Mentis
25 1989). Hence, in addition to interspecific variation in silica content, there is potentially a large
26 degree of intraspecific variation in silica between environments. We propose that voles can detect

1 and respond to these variations in silica content between plants and, in combination with the
2 highly selective nature of vole feeding, this could ultimately result in changes in plant species
3 abundance and hence the composition of plant communities (Moen *et al.* 1993; Howe & Brown
4 1999; Clay 2001; Howe & Lane 2004).

5
6 We have also demonstrated, for the first time, that silica in grass leaves can reduce the growth
7 performance of voles, and our results strongly suggest that the mechanism by which this occurs is
8 a reduction in the amount of nitrogen voles can absorb from grasses. Despite the nitrogen levels,
9 phenolic concentration and water content of grass species being unaffected by the silica
10 treatments, mature voles absorbed 38% and 17% less nitrogen when fed on high silica plants of
11 *F. ovina* and *L. perenne* respectively. The presence of silica reduced the nitrogen available to
12 voles in food where nitrogen is already limiting for a herbivore with a small body mass, a high
13 metabolic rate and a low capacity to increase consumption in the face of declining food quality
14 (Zynel & Wunder 2002).

15
16 The substantial reductions in the growth rates of juvenile voles we observed could have
17 implications for the time taken to reach sexual maturity, and hence for population growth.
18 Similarly, despite the relatively short time scale of the feeding trials with mature voles, we still
19 found evidence that silica in grasses significantly affected body mass and hence, potentially
20 reproductive output. It is well established that female body mass and growth rate are prime
21 determinants of both the number of litters and their size in voles as well as adult survival (Krebs
22 & Myers 1974; Cole & Batzli 1979; Ergon *et al.* 2001; Ylonen & Eccard 2004). For example,
23 when studying the factors affecting body mass of voles in a reciprocal transplant experiment
24 between populations of varying body masses, Ergon *et al.* (2001) found that the immediate
25 environment was the major predictor of vole condition. The factors in the immediate environment

1 responsible were not identified, but we have shown that food quality, specifically the silica
2 content of grasses, is worthy of further investigation as a potential mechanism for these effects.
3

4 Vole populations, as with many small mammals, are characterised by cyclical shifts in local
5 population densities (Agrell *et al.* 1995; Klemola *et al.* 2000a), but the mechanisms underpinning
6 these fluctuations are still a topic of debate (Agrell *et al.* 1995; Jaing & Shao 2003; Klemola *et al.*
7 2000b, Stenseth *et al.* 2003; Hornfeldt *et al.* 2005). Previously, many studies have focused on
8 predator-vole interactions as the prime mechanism determining vole cycles (Hansson 1987;
9 Henttonen *et al.* 1987; Hanski *et al.* 1993; Klemola *et al.* 2000a). However, more recent evidence
10 has suggested that changes in environment, including reductions in food quality, may also play a
11 significant role (Agrell *et al.* 1995; Ergon *et al.* 2001; Turchin & Batzli 2001). Some aspects of
12 food quality have been measured with respect to vole population growth (Agrell *et al.* 1995;
13 Klemola *et al.* 2000a), but no studies have examined the effects of silica, the principle defence in
14 their food plants. Although previous studies have found positive correlations between grazing
15 levels and grass silica content (McNaughton & Tarrants 1983, McNaughton *et al.* 1985; Brizuela
16 *et al.* 1986; Banuelos & Obeso 2000), we provide the first experimental demonstration that
17 feeding by voles leads directly to dramatic increases in the levels of silica in grass leaves. Hence,
18 this study highlights how silica induction due to grazing could contribute to cyclic dynamics in
19 voles: in years of high population densities, high grazing pressure will lead to induction of silica
20 defences and a subsequent reduction in vole body mass, reproductive outputs and survival.
21

22 The role of defences in the dynamic feedbacks between woody plants and mammalian herbivores
23 has been well studied (Bryant *et al.* 1991), but to date, no such plant-based mediation has been
24 demonstrated for grasses and their herbivores. Further, no previous studies have investigated the
25 effects of silica on herbivore performance, despite it being the principle defence in grasses. Our
26 study demonstrates that silica is induced by 400% in response to vole grazing, and that vole

1 feeding is reduced by 75% and growth rates by 40% on diets containing silica. Effects of this
2 magnitude seem likely to have significant ecological impacts on vole populations if they occur in
3 the field. Silica-based defences thus have the potential to impact on both grassland community
4 composition and on the population dynamics of mammalian herbivores.

5

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12

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1 Table 1 (i) The chemical composition of grass leaves under high and low silica treatments (%
 2 dry mass; mean values \pm s.e.m.) and (ii) the leaf silica content of study species from field
 3 sites (minimum – maximum values in plants collected from 10 different natural plant
 4 communities).

	(i) Silica treatment	Leaf silica content	Leaf nitrogen content	Total phenolic content	(ii) Field silica levels
<i>Festuca ovina</i>	Low	0.52 \pm 0.04	2.18 \pm 0.18	1.27 \pm 0.05	0.65 – 3.54
	High	2.44 \pm 0.17	2.18 \pm 0.18	1.25 \pm 0.11	
<i>Lolium perenne</i>	Low	0.54 \pm 0.10	3.10 \pm 0.10	1.39 \pm 0.05	0.58 – 4.52
	High	4.68 \pm 0.34	3.12 \pm 0.10	1.38 \pm 0.05	
ANOVA [†]	species	***	***	n.s.	
	silica	***	n.s.	n.s.	
	species \times silica	***	n.s.	n.s.	

5 [†] ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

6

1 **FIGURE LEGENDS**

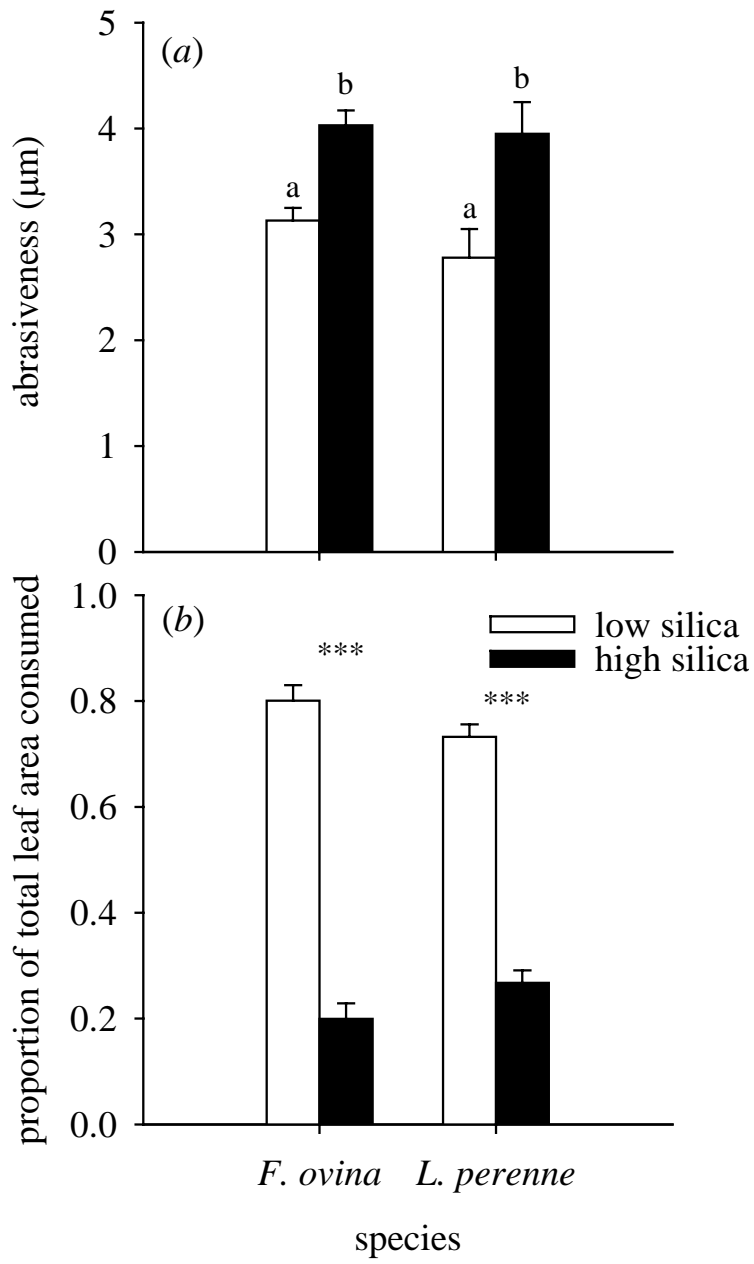
2 Figure 1. (a) Leaf abrasiveness (\pm SE) of *F. ovina* and *L. perenne* from high and low silica
3 treatments. Letters above bars denote significant differences between treatments (Tukey; $p < 0.05$).
4 (b) Vole feeding preference comparing grasses from high and low silica treatments based on trial
5 means (\pm SE), paired t-test: *** $p < 0.001$.

6
7 Figure 2. Growth rates (\pm SE) of (a) juvenile voles over two months and (b) mature voles over seven
8 days on high and low silica diets of *F. ovina* and *L. perenne*. Proportion of nitrogen absorbed from
9 grass leaves by (c) juvenile and (d) mature voles (nitrogen absorbed = N in grass – N in faeces/ N in
10 grass). Letters above bars denote significant differences between treatments (Tukey; $p < 0.05$).

11
12 Figure 3. Levels of silica in vole grazed and undamaged control plants of *F. ovina* and *L. perenne*.
13 Values are means (\pm SE), bars not sharing a common letter differ significantly (Tukey's Test $p <$
14 0.05).

15

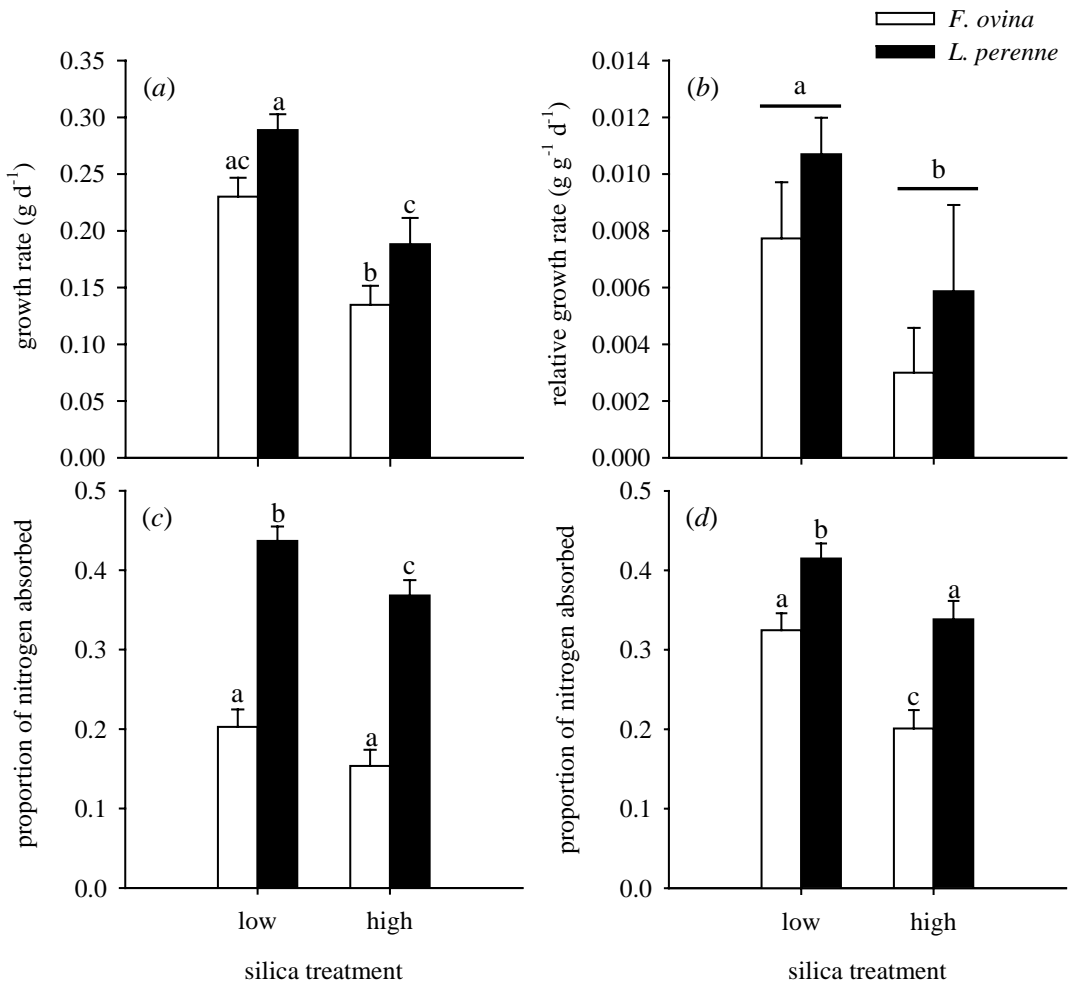
1 Figure 1



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1 Figure 2



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1 Figure 3

