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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**

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4 Running title: silica and vole performance

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SUMMARY

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

Keywords:

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

1. INTRODUCTION

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

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

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

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

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

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

2. METHODS

Study species and plant growth conditions

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

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

Feeding preference

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

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

Growth of juvenile voles

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

Growth of mature voles

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

Silica induction

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

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

Chemical and physical analyses

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

Statistical analysis

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

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

3. RESULTS

Leaf abrasiveness and feeding preference

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

Vole growth performance and leaf digestibility

Juvenile female voles reared for two months on leaves of *F. ovina* and *L. perenne* containing high silica levels had 41% and 35% lower growth rates respectively than voles reared on low silica leaves (figure 2a, ANCOVA: initial weight (cov) $F_{1,19} = 0.03$ and $p = 0.872$, species $F_{1,19} = 11.07$ and $p = 0.004$, silica $F_{1,19} = 35.42$ and $p < 0.001$, and species \times silica $F_{1,19} = 0.05$ and $p = 0.831$). The same trend was evident in mature female voles, where silica reduced their growth rate by over 50% during a period of only seven days of exposure (figure 2b, ANOVA: vole $F_{9,27} = 0.41$ and $p = 0.917$, silica $F_{1,27} = 4.51$ and $p = 0.043$). Over the 7 day period there was no difference in the response on different grass species (vole $F_{9,27} = 0.41$ and $p = 0.917$, species $F_{1,27} = 1.68$ and $p = 0.206$).

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

Silica induction

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

4. DISCUSSION

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

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

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

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

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

1 **FIGURE LEGENDS**

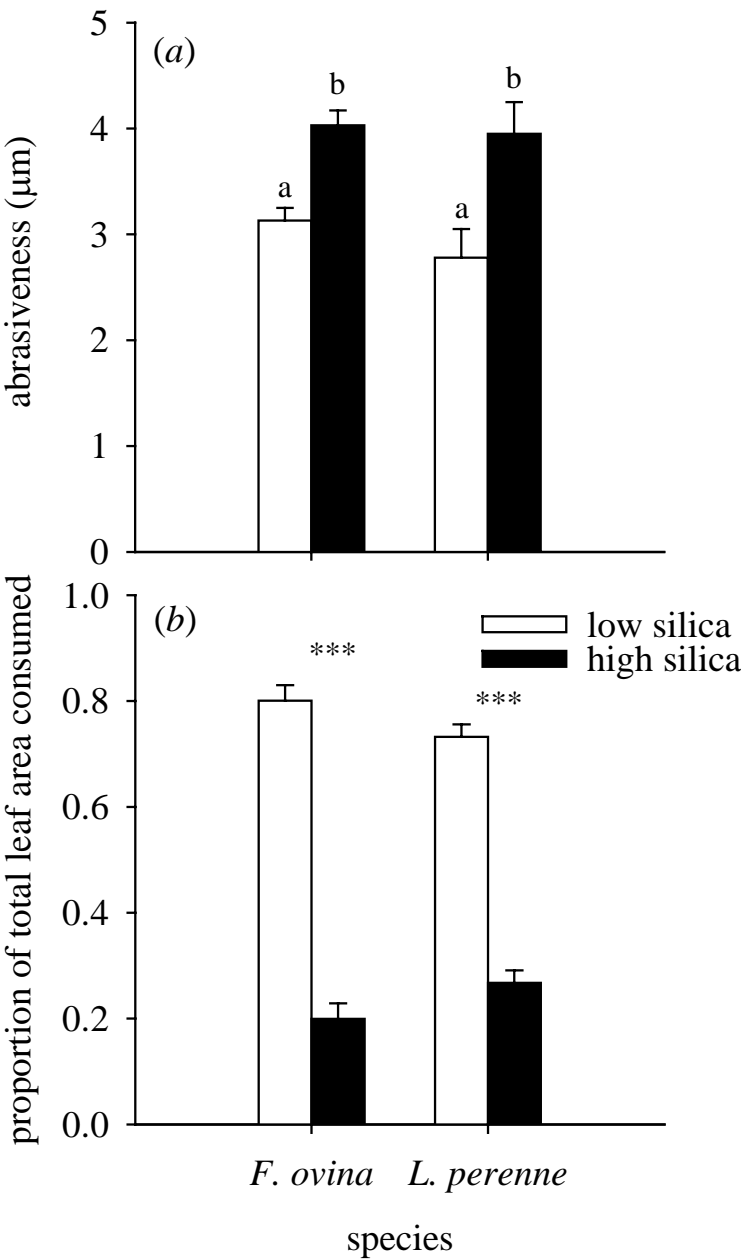
2 Figure 1. (a) Leaf abrasiveness (\pm SE) of *F. ovina* and *L. perenne* from high and low silica
3 treatments. Letters above bars denote significant differences between treatments (Tukey; $p < 0.05$).
4 (b) Vole feeding preference comparing grasses from high and low silica treatments based on trial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

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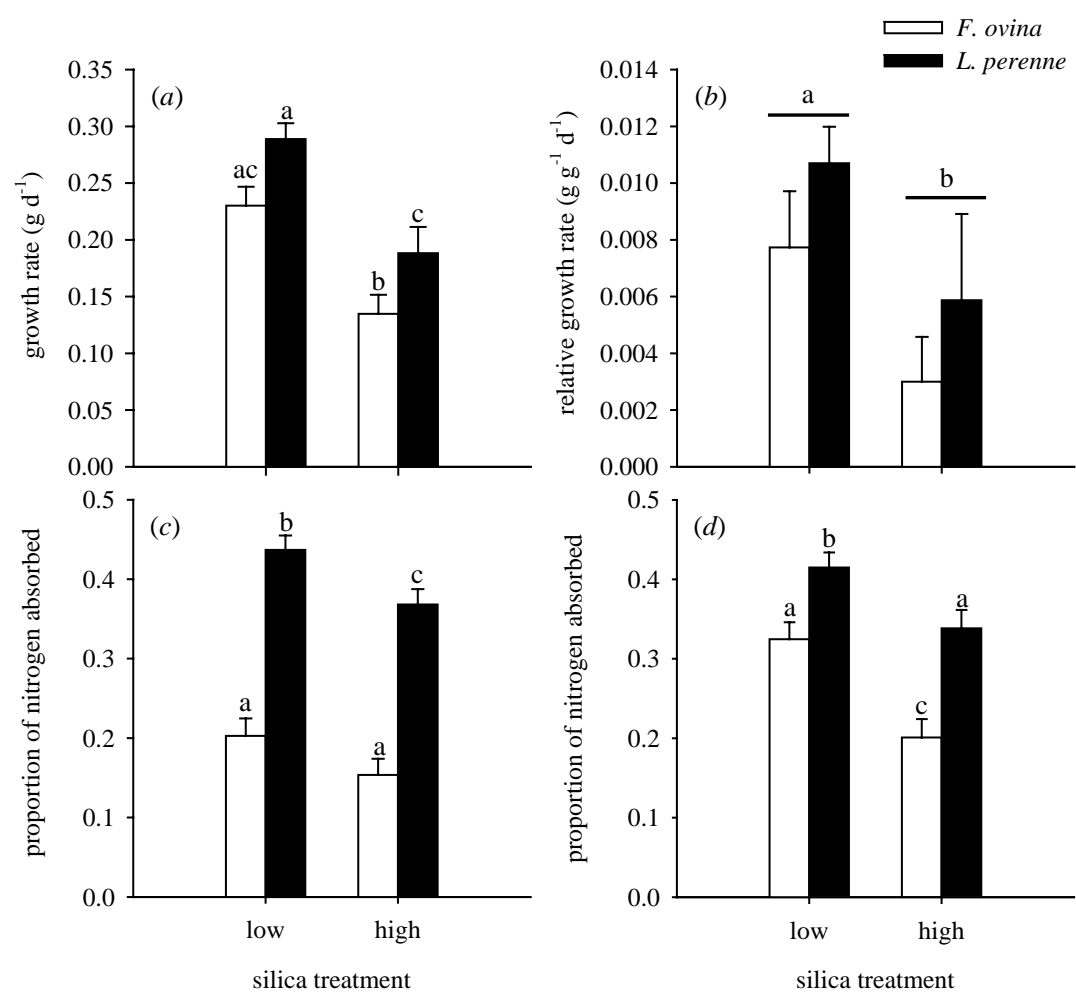
1 Figure 1



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1 Figure 2



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1 Figure 3

