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Silica and Nitrogen Modulate Physical Defense Against Chewing Insect Herbivores in Bioenergy Crops *Miscanthus* × *giganteus* and *Panicum virgatum* (Poaceae)

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ABSTRACT Feedstock crops selected for bioenergy production to date are almost exclusively perennial grasses because of favorable physiological traits that enhance growth, water use, and nutrient assimilation efficiency. Grasses, however, tend to rely primarily on physical defenses, such as silica, to deter herbivores. Silica impedes processing of feedstocks and introduces a trade-off between managing for cost efficiency (i.e., yield) and plant defenses. To test how silica modulates herbivory in two of the most preferred feedstock crops for production across the central United States, miscanthus (*Miscanthus* × *giganteus* Greef and Deuter ex Hodkinson and Renvoize) and switchgrass (*Panicum virgatum* L.), we examined the performance of two immature generalist insect herbivores, fall armyworm (*Spodoptera frugiperda* (J.E. Smith)) and the American grasshopper [*Schistocerca americana* (Drury)], on grasses grown under silica and nitrogen amendment. Both miscanthus and switchgrass assimilated nitrogen and silica when grown in amended soil that altered the consumption and conversion efficiency of herbivores consuming leaf tissue. The magnitude of nutrient assimilation, however, depended on intrinsic plant traits. Nitrogen increased conversion efficiency for both fall armyworm and American grasshopper but increased consumption rate only for fall armyworm. Silica reduced conversion efficiency and increased consumption rate only for the American grasshopper. Because of this variability, management strategies that reduce silica or increase nitrogen content in feedstock crops to enhance yields may directly influence the ability of bioenergy grasses to deter certain generalist herbivores.

KEY WORDS *Spodoptera frugiperda*, *Schistocerca americana*, biofuel feedstock, fertilization, herbivory

To meet growing bioenergy demands and government mandates for sustainable fuels, alternative feedstock crops for conversion of lignocellulose to fuel (ethanol) and direct combustion of biomass are being explored. Most of these plant species are grasses (Carroll and Somerville 2009, Somerville et al. 2010); perennial grasses such as switchgrass (*Panicum virgatum* L.) and miscanthus (*Miscanthus* × *giganteus* Greef and Deuter ex Hodkinson and Renvoize) are more sustainable than are most dicot crops because of physiological traits that enhance photosynthesis, water use, and nitrogen (N) efficiency (Somerville et al. 2010). A potential problem with these grasses as feedstock crops is that they rely on multiple physical traits that reduce herbivory (Onoda et al. 2011). Resistant traits include leaf toughness, usually conferred by a combination of cell walls and cell thickness, and influ-

enced primarily by lignin and specialized uptake and storage of silica (Si) (Read and Stokes 2006, and references therein). Breeding practices to increase yield and enhance biomass conversion into fuel minimize these physical traits to enhance degradation of cellulose bound in recalcitrant lignin matrices (Li et al. 2010) and to reduce Si-induced slag (glass-like silicates and oxides) and corrosive alkali sulfates produced during thermochemical combustion (Jenkins et al. 1998). Management practices to reduce Si content of plants selected as feedstocks may introduce a tradeoff between cultivation for biofuel production and resistance to herbivores.

Whereas lignin concentration positively correlates with the performance of some arthropod herbivores (Diawara et al. 1991), other generalist insect herbivores respond variably and in concert with other plant metabolites (Timonen et al. 2005, Brodeur-Campbell et al. 2006). By contrast, Si particles physically prevent the destruction of chlorenchyma cells (Hunt et al. 2008) and reduce growth rate and nutrient retention of generalist arthropod herbivores (Massey et al. 2006, 2007; Keeping and Kvedaras 2008).

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Miscanthus and switchgrass are two preferred feedstocks under consideration for large-scale cultivation in the central United States. Both grasses vary in Si content with temperature and precipitation (Woli et al. 2011) and experience minimal arthropod herbivory in established field plots (Semere and Slater 2007, Parrish and Fike 2009). However, recent research demonstrated the potential for common maize pests to consume foliage of these species in biofuel crop trial plots (Spencer and Raghu 2009, Nabity et al. 2011). In field and greenhouse trials, both the western corn rootworm (*Diabrotica virgifera virgifera* LeConte) and the fall armyworm [*Spodoptera frugiperda* (J.E. Smith)] were able to consume leaf tissue but experienced reduced survivorship, lower pupal and adult weight, and decreased assimilation of plant N on diets of miscanthus and switchgrass in comparison with maize (*Zea mays* L.; Prasifka et al. 2009, 2011; Spencer and Raghu 2009; Nabity et al. 2011). Decreased leaf toughness seemed to account, in part, for enhanced herbivore assimilation of plant N and overall arthropod performance on maize (Nabity et al. 2011), but the mechanisms underlying the reduced suitability of miscanthus and switchgrass for this herbivore remain unknown.

Because leaf toughness is a multifaceted trait, we selected one component, Si content, to test how biomechanical defenses in biofuel feedstock grasses modulate herbivore performance. We selected generalist herbivores because they display maximum behavioral and physiological flexibility when encountering sub-optimal nutrient levels (Behmer 2009). In addition, because resource conditions influence defense capacity in plants (e.g., Coley et al. 1985) we tested the inducible nature of Si (Massey et al. 2007) across a range of resource conditions. We predicted that 1) herbivore performance will decrease as Si-based mechanical defenses are enhanced and 2) enhanced resource conditions (+N) will reduce Si-based defense capacity. Understanding the implications of nutrient manipulation for plant biomechanical defense against herbivores will be essential for managing the trade-offs associated with breeding feedstock crops for optimal biofuel processing.

Materials and Methods

Plants and Insects. To determine how Si and N alter the suitability of biofuel feedstock crops to herbivores, we conducted a greenhouse experiment to control soil nutrient conditions for three biofuel feedstock crop species. We planted 1-yr-old miscanthus rhizomes and switchgrass seeds (Prairie Moon Nursery, Winona, MN) and maize (Dekalb DKC-61-72) in 2-liter pots in potting mix (Sunshine LCI, Sun Gro Horticulture, Bellevue, WA), and watered as needed with one of four treatments: 300 mg liter⁻¹ soluble Si in the form NaSiO₃ · 9H₂O (1 mM in solution; -N +Si), ≈400 mg liter⁻¹ N (2.6 g 15-5-15 N-P-K liter⁻¹; +N -Si), combined 300 mg liter⁻¹ soluble Si and 400 mg liter⁻¹ N (+N +Si), and unamended water (-N -Si).

Species were blocked within nutrient treatments and rotated within block and as blocks every 3 d to prevent environmentally induced bias within the greenhouse. All plants were grown at full sunlight with supplemental lighting (photoperiod of 16:8 [L:D] h) at 28 ± 2°C for the duration of the experiment (June–August). Each grass species under each nutrient treatment was grown for >3 wk before harvesting foliage to feed to insects.

To test suitability of grasses to generalist herbivores, we obtained cohorts of the corn strain of the fall armyworm from French Agricultural Research, Lambertson, MN, and the American grasshopper [*Schistocerca americana* (Drury)] from the USDA-ARS-NPARRL, Sidney, MT. Fall armyworm larvae were reared from eggs to the fifth instar on artificial diet (Waldbauer et al. 1984), whereas grasshoppers were obtained at the second instar and reared until the fifth instar on prepared diet (iceberg lettuce + 1:1 wheat germ:bran). Both insect colonies were maintained in an insectary set at 25 ± 2°C with a photoperiod 16:8 (L:D) h until individuals were used in performance experiments.

Insect Performance Experiments. Before performance experiments, larvae and nymphs were starved for 24 h, weighed, and then allowed to feed. Leaves of each grass species and nutrient treatment were clipped from greenhouse plants and pooled by species × nutrient treatment with cut edges submerged in water to prevent additional desiccation during the time required for experimental setup. Then, ad libitum plant tissue was randomly selected, weighed (two- to three-fold more tissue by weight than insect mass), and placed in near-airtight plastic containers with one insect apiece. After 24 h, uneaten tissue was collected and dried overnight in an oven at 70°C, and insects were starved for 24 h. After the 24-h starvation period, insects were weighed, frass was collected, and all insects and frass were dried overnight at 70°C. One trial was conducted for *S. frugiperda* larvae ($n = 15$ per species-nutrient combination) whereas two trials ($n = 5$) were conducted for *Sc. americana* nymphs. The insect performance experiments were conducted consecutively through time with separate cohorts of plants grown under the conditions described above. To control for changes in nutrient content during the 24-h feeding experiment, leaves ($n = 10$) of each species × nutrient combination were prepared and weighed as described but were used for elemental analysis directly. Efficiency of conversion of ingested food to body mass (ECI) was calculated by $ECI = \text{weight gained (g)} / \text{food ingested (g)} \times 100$.

Elemental Analyses. To determine the assimilation of plant nutrients into insect mass, carbon (C), N, and percent Si (% Si) of dry mass were measured for consumed tissue and resulting frass. For total C and N content per unit dry mass (and C:N), leaf tissue was dried at 70°C, ground to a fine powder, and analyzed using an Elemental Combustion System (model 4010, Costech Analytical Technologies, Valencia, CA). Si content per unit dry mass was assessed for each plant and frass sample following Woli et al. (2011). In brief,

Table 1. Elemental composition (mean ± SE) of plant tissue fed to fall armyworm larvae and American grasshopper nymphs for each plant species and nutrient treatment

	<i>n</i>	% N	% Si
Maize	35	3.3 ± 0.2a	1.0 ± 0.1a
Miscanthus	36	2.0 ± 0.1b	1.7 ± 0.2b
Switchgrass	35	2.7 ± 0.1c	2.0 ± 0.2c
-N -Si	25	1.9 ± 0.1a	0.9 ± 0.1a
+N -Si	25	3.2 ± 0.2b	0.6 ± 0.1a
-N +Si	28	2.2 ± 0.1c	2.8 ± 0.2b
+N +Si	28	3.2 ± 0.1b	1.9 ± 0.2c

Different lowercase letters indicate significant ($P \leq 0.05$) main effects among values within a column.

≈25 mg of tissue was dry-ashed at 550°C for 5 h in a muffle furnace and then allowed to cool overnight. Samples were then fused with 2 g of NaOH over a Bunsen burner for 15 min, covered in deionized water, and cooled overnight. The remaining solution was mixed with 2 ml of H₂SO₄, brought up to 100 ml in total volume with water, and injected into a Lachat QuikChem 8000 flow injection analyzer (Lachat Instruments/Hach Company, Loveland, CO) at 820 nm absorbance. All leaf and insect samples were compared with soil samples of known Si content prepared under the methods described above to serve as a positive control.

Statistical Analysis. We analyzed elemental content of frass and plant tissue by first log transforming all percentage data and then applying a mixed model analysis of variance (ANOVA) in Proc Mixed 9.1 (SAS Institute 2004). Plant species, nutrient amendment, and insect species were included as fixed effects and, when the initial ANOVA yielded a significant difference at $P = 0.05$, post hoc comparisons were made between least squares means (LSmeans). To test the degree to which growth relates to Si or N content, we used regression analysis to determine the association between growth and elemental content for each insect species (Proc Reg 9.1, SAS Institute 2004). Consumption rate (grams of tissue consumed per day) and ECI were analyzed using analysis of covariance, with initial mass covarying with consumption and consumption covarying with ECI (Proc GLM 9.1, SAS Institute 2004).

Results

Nutrient amendment increased plant N and Si content, but the degree of enhancement depended on the

Table 2. Elemental composition (mean ± SE) of plant tissue fed to fall armyworm larvae and American grasshopper nymphs for the resulting species × nutrient interactions

Interaction	Maize			Miscanthus			Switchgrass		
	<i>n</i>	% N	% Si	<i>n</i>	% N	% Si	<i>n</i>	% N	% Si
-N -Si	8	1.9 ± 0.1A	0.6 ± 0.0a	9	1.5 ± 0.1a	0.9 ± 0.1a	8	2.5 ± 0.2a	1.1 ± 0.1a
+N -Si	8	4.3 ± 0.2B	0.3 ± 0.2a	8	2.4 ± 0.1b	0.5 ± 0.0a	9	2.9 ± 0.1b	0.9 ± 0.2a
-N +Si	9	2.7 ± 0.2C	2.5 ± 0.2b	10	1.6 ± 0.1a	3.5 ± 0.5b	9	2.2 ± 0.3a	2.8 ± 0.2b
+N +Si	10	4.0 ± 0.1D	1.3 ± 0.1c	9	2.5 ± 0.2b	1.5 ± 0.1a	9	3.2 ± 0.1b	2.9 ± 0.3b

Different lowercase letters indicate significant ($P \leq 0.05$) main effects among values within a column.

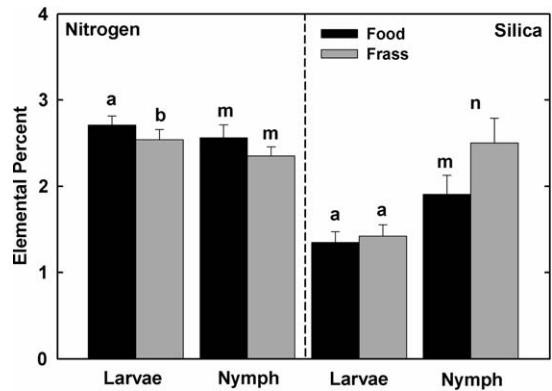


Fig. 1. Mean (±SE) composition of nitrogen (left panel) and silica (right panel) in foliage fed to insects and the resulting frass across all nutrient treatments and species (top panel). Differences among values are separated by insect and depicted by lowercase letters at $P < 0.05$.

species (Tables 1 and 2). All plant species increased N ($F = 84.96$, $df = 2$, $P < 0.0001$) or Si ($F = 35.54$, $df = 2$, $P < 0.0001$) when amended separately but only switchgrass, and maize assimilated more N and Si when both nutrients were applied together. Maize assimilated more N but lower Si than the other grasses.

Both fall armyworms and American grasshoppers assimilated N from food, but armyworms ($F = 4.75$, $df = 1$, $P = 0.03$) were more effective at assimilating N from food when ingesting higher Si diets than grasshoppers ($F = 0.88$, $df = 1$, $P = 0.3$; Figs. 1 and 2). ECI of foliage depended on species ($F = 4.37$, $df = 2$, $P = 0.01$) and nutrient amendment ($F = 5.4$, $df = 3$, $P = 0.002$) as did consumption (species: $F = 41.31$, $df = 2$, $P < 0.0001$; nutrient: $F = 8.0$, $df = 3$, $P < 0.0001$). N amendment greatly enriched N content of maize (Table 1), and this enrichment resulted in greater consumption by fall armyworms and greater ECI relative to performance on other less suitable grasses (Table 3). Across grasses, N amendment increased ECI for fall armyworms. Consumption of maize and switchgrass did not depend on nutrient treatment; however, consumption of miscanthus depended on N content for fall armyworms (Fig. 3). In contrast, grasshoppers consumed more miscanthus yet with lower ECI than on other grasses. Although N amendment increased ECI by grasshoppers, there was no corresponding increase in consumption. There was a negative relationship between N and Si content across plant spe-

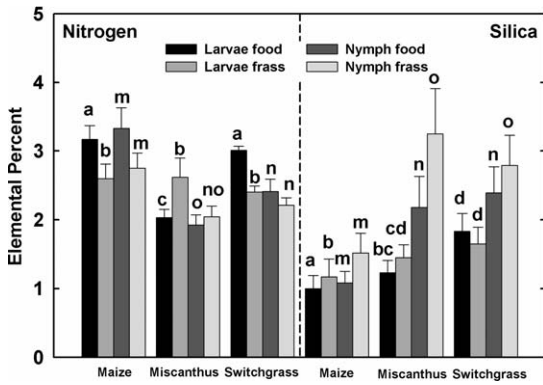


Fig. 2. Mean (\pm SE) composition of nitrogen (left panel) and silica (right panel) in foliage fed to insects and the resulting frass for each plant species for each insect. Differences among values are separated by insect and depicted by lowercase letters at $P < 0.05$.

cies. Although percentage of foliar N and Si explained a low percentage of the variance, fall armyworm growth increased as the percentage of foliar N increased, but the growth response of grasshoppers was not significantly associated with the percentage of foliar N (Table 4).

Si amendment differentially altered consumption patterns with respect to insect species. Miscanthus assimilated more Si than maize or switchgrass (Table 1), and this resulted in greater consumption by grasshoppers yet lower ECI relative to other grasses (Fig. 3; Table 3). Si amendment also increased consumption of all grasses by grasshoppers relative to N-amended grasses but reduced ECI; overall ECI on grasses was higher for grasshoppers than fall armyworms. This increased ECI occurred concomitant with a greater concentration of Si in frass for grasshoppers relative to fall armyworms (Figs. 1 and 2). Fall armyworm growth

Table 3. Consumption and efficiency of conversion (mean \pm SE) of ingested tissue (ECI) of fall armyworm and American grasshopper feeding on maize, miscanthus, and switchgrass grown under four nutrient treatments

	n	Consumption (g)	ECI
Fall armyworm			
Maize	40	0.083 \pm 0.003a	16.5 \pm 2.4a
Miscanthus	40	0.055 \pm 0.003b	10.1 \pm 0.8b
Switchgrass	40	0.047 \pm 0.003c*	13.9 \pm 1.1b
-N -Si	30	0.056 \pm 0.004ac	10.2 \pm 1.8a
+N -Si	30	0.075 \pm 0.003b	18.8 \pm 1.8b
-N +Si	30	0.064 \pm 0.003a	9.5 \pm 1.7a
+N +Si	30	0.051 \pm 0.004c	15.5 \pm 1.8b
American grasshopper			
Maize	62	0.053 \pm 0.003a	37.8 \pm 4.4a
Miscanthus	60	0.100 \pm 0.010b	24.3 \pm 3.0b
Switchgrass	55	0.035 \pm 0.004c*	36.0 \pm 5.9b
-N -Si	44	0.065 \pm 0.007ab	30.7 \pm 5.0a
+N -Si	47	0.058 \pm 0.007a	38.0 \pm 4.4ab
-N +Si	43	0.080 \pm 0.014b	19.1 \pm 3.6c
+N +Si	43	0.049 \pm 0.007a	43.9 \pm 6.2b

Different lowercase letters indicate significant ($P \leq 0.05$) main effects among values within a column.
*, $P = 0.06$.

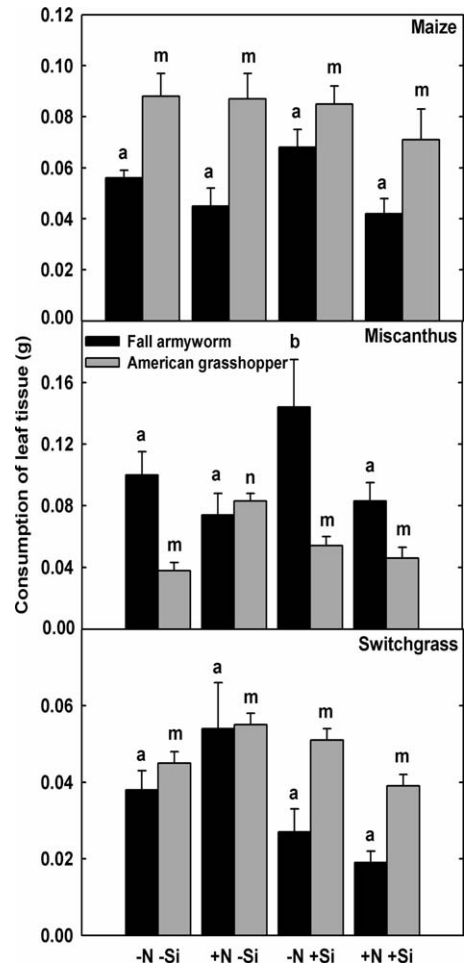


Fig. 3. Mean (\pm SE) consumption of maize (top), miscanthus (middle), and switchgrass (bottom) foliage by fall armyworm and American grasshopper. Differences among nutrient treatments are depicted by lowercase letters at $P < 0.05$.

declined as the percentage of foliar Si content increased but no change in growth of grasshoppers occurred with increasing Si content (Table 4).

Discussion

The bioenergy feedstock crops miscanthus and switchgrass assimilated additional N and Si when grown in amended soil and, as a result, produced foliage that altered the consumption and ECI of two generalist chewing insect herbivores. Given that most plants respond to enhanced nutrient conditions by reducing defenses against herbivores (see Coley et al. 1985), and N content is generally negatively correlated with Si content among grasses (Table 3; Massey et al. 2007), the interplay between these common soil components will be important in determining nutrient management for bioenergy crop cultivation.

N enhancement of grasses differentially altered consumption by herbivores according to taxon. Fall

Table 4. Regression analysis for the relationship of % N to % Si in grasses and the corresponding relationships of growth of fall armyworm and American grasshopper relative to % N and % Si of foliage

	<i>n</i>	Function	<i>R</i> ²	<i>P</i>
% N vs % Si	102	$Y = 0.2774 - 0.4983\log(x)$	0.052	0.02
Fall armyworm				
% N vs growth (g)	48	$Y = 0.0009 + 0.0204\log(x)$	0.088	0.04
% Si vs growth (g)	48	$Y = -0.0096 - 0.0085\log(x)$	0.099	0.03
American grasshopper				
% N vs growth (g)	55	$Y = 0.0241 + 0.0059\log(x)$	0.002	0.75
% Si vs growth (g)	53	$Y = -0.0267 - 0.006\log(x)$	0.009	0.51

armyworms consumed more miscanthus, switchgrass, and maize foliage when plants were fertilized, probably because of its greater N content. This finding is consistent with previous research indicating that lepidopterans preferentially consume more protein (N) relative to carbohydrates (Simpson et al. 1988) and that grasshoppers consume protein and carbohydrates in equal ratios. For both insect species, N enhancement increased conversion efficiency. In the field, switchgrass and miscanthus respond variably to N amendment largely because of a lack of consistency in prior land use practices (i.e., preestablishment soil quality), harvest dates (to account for seasonal nutrient cycling), and optimal (multiple) harvest regimes (Heaton et al. 2009, Parrish and Fike 2009). Because herbivore consumption may be stimulated by N fertilization, designing N management strategies to reduce the susceptibility of field-cultivated bioenergy crops to herbivores may be necessary for sustainable biofuel feedstock production.

Herbivore consumption depended on the amount of Si sequestered by the plant and on the tolerance of the herbivore to high Si diets. Si amendment increased Si content in all grasses, but miscanthus accumulated more relative to unamended plants. This variability in Si assimilation probably resulted from intrinsic qualities (i.e., Si transporters) that drive Si uptake (Ma and Yamaji 2006). Once assimilated, Si differentially altered consumption and ECI by fall armyworms and grasshoppers: Si enhancement did not influence fall armyworm consumption rates but increased consumption by grasshoppers. This compensatory feeding resulted from the indigestibility of Si (Barbehenn 1993) and suggests Si may play a stronger role in physical defense against herbivory for miscanthus relative to maize and switchgrass grown under similar conditions. Moreover, fall armyworm conversion efficiencies were lower on all grass species compared with grasshopper conversion efficiencies. Although the corn strain fall armyworm has a more limited host range than the rice strain, it is not uncommon for insect herbivores to maintain conversion efficiencies between 10 and 30% on their typical host plants (Mattson 1990). Therefore, the conversion efficiencies observed in this experiment do not suggest a strong host effect on fall armyworm. Rather, the reduced ability to digest grasses by armyworms relative to grasshoppers suggests that, in the presence of tough, high-Si diets, grasshoppers convert more ingested food into body mass compared with fall armyworms

and subsequently may have more potential to overcome Si-based defenses. Field-grown cultivars of switchgrass have higher baseline Si content compared with miscanthus (Woli et al. 2011); however, our data suggest miscanthus may have a greater capacity to assimilate Si when it is available. Thus, mobile Si in soil in combination with intrinsic properties of bioenergy cultivars will determine leaf-level Si and its ability to deter herbivory.

Although we did not test the degree with which these grasses preferentially absorb one element over the other, the observation that insects from two orders consume species differentially both with and without nutrient enhancement suggests plant nutrient content will, in part, influence selective pressure for pest development and host switching. Current strategies indicate that growing biofuels in marginal lands will provide an alternative energy source while simultaneously offsetting carbon emissions (Fargione et al. 2008). Although marginal lands are conventionally classified relative to their agroeconomic benefit, new strategies take into account soil quality, current land use, and environmental degradation (Gopalakrishnan et al. 2011). This approach may direct feedstock cultivation toward lands with depleted nutrients, enhanced weathering (e.g., erosion), or enhanced nutrients if nitrate contamination is considered. As Si mobilization and uptake increase under weathering, and use of N-deficient lands or nitrate-enhanced groundwater for irrigation leads to N application to crops, proposed land use management strategies may directly influence the ability of bioenergy grasses to remain resistant to herbivores.

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