

THE ECOLOGICAL DRIVERS AND CONSEQUENCES OF GRASS SILICON
ACCUMULATION

BY

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ABSTRACT

Silicon (Si) comprises more than a quarter of earth's crust by weight, and is an element accumulated in the leaf tissue of many early-diverging land plants, but relatively few angiosperms accumulate silicon. Grasses (Poaceae) are the only angiosperm family that incorporates vast (often > 5% of plant dry weight) amounts of silicon into their leaves as SiO₂ bodies called phytoliths, and this biomineralization affords grasses numerous benefits. Researchers have long presumed that phytolith accumulation evolved to deter herbivory, as modern studies show that phytoliths abrade grazer mouthparts and interfere with digestion. However, recent phylogenetic analysis and consideration of the fossil record has shed some doubt on the hypothesis that Si accumulation evolved in order to reduce herbivory. In my dissertation research, I sought to identify the primary drivers of variation in plant silicification, focusing specifically on variation in grassland ecosystems. I developed novel methods to quantify plant silicon (Appendix F), which I then applied to studies at the individual, landscape, and global scale. At the individual plant scale, I found that soil silicon supply positively influences a broad range of plant morphological and physiological traits which emphasizes an important role of silicon in plant fitness (Chapter 1). In a common garden study of Serengeti grass species, I found that water supply, but not defoliation, resulted in induced silicon uptake (Chapter 2). In Serengeti's highly heterogeneous soil landscape, I incorporated abiotic variation into a structural equation model, highlighting the importance of soil texture and plant-water relations in determining variation in grass leaf silicon (Chapter 3). Next, I highlighted the role of soil nutrients in driving leaf Si at grassland plots around the globe; importantly, I found no evidence of grazing-induced silicification at the global scale (Chapter 4). Finally, I performed a meta-analysis of previous plant Si studies, and I found that soil silicon supply and nitrogen availability were the only two stresses which consistently

elicited a signal in grass Si concentration (Chapter 5). These findings are important because they challenge the existing paradigm in plant silicon research: that grazing is the primary adaptive force behind grass Si accumulation. Instead, my research emphasizes a clear role of soil nutrients and plant water relations in driving silicon uptake, a pattern which emerged across multiple spatial scales from the individual plant to the global scale.

INTRODUCTION

Silicon & Life

Silicon is an element revered for its technological utility in the “Silicon Valley”, but not typically thought of as important for living organisms and the evolution of life. Silicon neighbors carbon on the periodic table, but unlike carbon its chemistry is dominated almost exclusively by highly stable Si-O bonds (Wainwright 1997). Researchers have yet to identify any organic molecules which contain or require silicon, but Si has been described as a bioinorganic essential element (Exley 1998) and has received a great deal of attention from biological researchers. Even Charles Darwin was intrigued by Si-rich biotic remains in dust samples, which he collected aboard the HMS Beagle and sent to German microscopist Christian Ehrenberg for analysis (Darwin 1846). In less than half a teaspoon of dust, Ehrenberg identified 67 types of *infusoria*, including 34 different types of plant silicon cells, which he termed *Phytolitharia*, or ‘plant stones’, and Ehrenberg is now considered the “father of phytolith studies” (Powers 1992).

Silicon is among the most prevalent elements in earth’s crust, second only to oxygen. It typically occurs as silicon dioxide (silica; SiO₂) which comprises 61.5% of earth’s continental crust (Wedepohl 1995). Certain geologic materials may contain much more or less silica; quartz, for instance is typically > 99 % SiO₂, while sedimentary rocks and extrusives of certain volcanic systems are relatively Si-depleted (Drees et al. 1989, Wedepohl 1995, Kervyn et al. 2010). Si is also common in the hydrosphere, where terrestrial silicate sources are dissolved into rivers and delivered to sea water as silicic acid (H₄SiO₄) at a rate of > 6 Tmol yr⁻¹ (Tréguer et al. 1995, Laruelle et al. 2009). As a common constituent of earth’s land surfaces and water, it is thus no surprise that living

organisms have adapted the ability to utilize silicon for an array of structural and functional purposes.

Living organisms absorb and polymerize dissolved Si (i.e. silicic acid) into hydrated amorphous Si ($n\text{H}_2\text{O} \cdot \text{SiO}_2$) in order to build a range of structural components; this biologically generated form of Si is commonly referred to as biogenic silica. Biogenic silica is necessary for frustule formation by diatoms, which on average consist of 60% biogenic silica (~28 % Si) in dry mass (Knoll 1975, Slicko-Goad 1984, Maldonado et al. 2010). For vertebrates, Si plays a vital role in collagen formation and maintenance of bones and cartilage (Carlisle 1988). Other organisms which utilize silicon include chrysophytes, radiolarians, sponges, and, of course, plants (Simpson & Volcani 1981).

Plant Silicon Accumulation

Plants are among the most heavily silicified living organisms. Takahashi, Ma, and Miyake (1990) created categorical groupings of Si of accumulation, ranging from non-accumulators (< 0.5 % Si) to passive (0.5 – 2 % Si) and active accumulators (> 2 % Si). Their rankings are based on assumptions of passive uptake from a 10 ppm Si soil solution and a transpiration coefficient of 500. Through the application of this classification scheme, authors identified grasses, sedges, clubmosses, and some liverworts, horsetails, and ferns as active Si-accumulators, while gymnosperms and dicotyledonous plants were consistently identified as non-accumulators (Ma & Takahashi 2002). Further phylogenetic analyses of plant silicification have suggested that Si accumulation did not become prevalent among angiosperms until the late Cretaceous, while early land plants generally accumulate high amounts of Si (Trembath-Reichert et al. 2015, Katz 2015). Among angiosperms, the Poales have been identified as the

highest Si accumulators, with some grasses and bamboos accumulating Si to above 10 % of plant dry weight (Hodson et al. 2005, Katz 2015). The collective range of plant Si values, from near 0 to > 10% plant dry weight, is more variable than that of any other plant nutrient, and may even exceed leaf nitrogen concentration (Epstein 2009).

Like all other mineral nutrients, plants obtain silicon from their soil environment. Silicic acid (H_4SiO_4), also referred to as dissolved or aqueous Si, is the plant-available form of Si; it is a relatively large solute (m.w. = 96.11 g/mol, diameter = 4.38Å) which is formed by the weathering of solid silicates, such as quartz, into the soil water (Fig 1). Silicic acid is transported across various root barriers (i.e. Casparian bands) by a group of modified aquaporins of the NOD26-like intrinsic protein subfamily (NIPs) which have evolved a unique selective filter permeable to silicic acid (Deshmukh & Belanger 2015, Trembath-Reichert et al. 2015). The genes LSi1 and LSi2 encode these transporters and have localized root positions which allow them to act in a coupled manner to transport Si from the external solution to roots and then from root cells to the apoplast (Ma & Yamaji 2015). Expression of LSi1 and LSi2 in *Xenopus* oocytes has confirmed that these proteins act exclusively in silicon influx/efflux, respectively (Ma et al. 2006, Ma et al. 2007). Strong selective pressure for adaptive evolution of NIP solute permeability suggests that functional divergence between monocot and dicot NIPs may provide some explanation for the differences observed in Si-accumulation between these two taxonomic groups (e.g. Liu & Zhu 2010).

After LSi1 and LSi2 load
silicic acid into the xylem,
transpiration delivers Si to
aboveground tissue. As water
evaporates from stomata, silicic

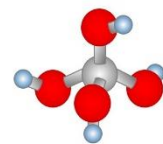
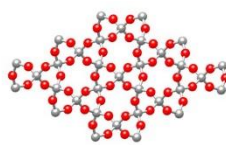


Figure 1. Chemical dissolution of a common silicate, quartz, into plant-available silicic acid. Quartz image licensed under Public domain via Wikimedia Commons.

acid polymerizes into solid, hydrated amorphous silica phytoliths ($n\text{H}_2\text{O} \cdot \text{SiO}_2$) (Ma & Yamai 2006, see Fig 2). Stomata, epidermal cells, and cells of the vascular system are most commonly silicified although large underlying sub-cuticular deposits may also form (Prychid et al. 2004). Interestingly, phytoliths provide leaf skeletons and hence are often used in archeological reconstructions of ancient vegetation (e.g. Rosen 1992). Individual subfamilies, tribes, and genera of grasses have diagnostic grass silica short cell (GSSC) phytolith morphotypes, and phytoliths are highly resistant to chemical weathering, resulting in a well-preserved phytolith fossil record (Piperno & Pearsall 1998).

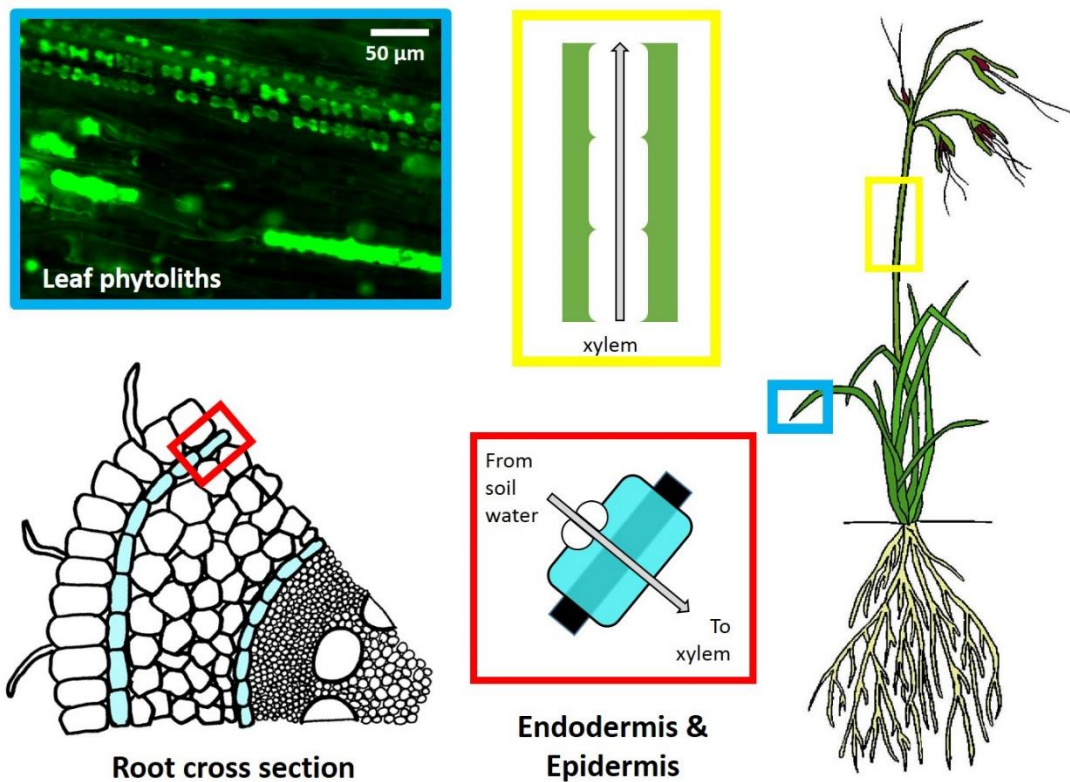


Figure 2. Silicon transport through the plant. Silicic acid crosses Casparian bands through specialized transport molecules at the root epidermis and endodermis (red panel). In the xylem, passive transpiration-driven water flow carries upward to aerial tissues (yellow panel). As water evaporates, Si polymerizes into amorphous SiO_2 phytoliths in leaves and stems (blue panel; image of autofluorescent phytoliths in *Sorghum bicolor* by K Quigley).

Soil is a complex matrix of living and non-living components which may each influence plant-available Si in a variety of ways. Silicon from phytoliths slowly weathers into the soil water, along with soil particles (sand, silt, clay) and parent material, supplying dissolved Si for plant uptake. Soils typically contain between 0.1 and 0.6 mM silicic acid (Epstein 1994), and plant uptake is directly influenced by Si supply. Numerous studies have shown that supplying grasses with Si-rich fertilizer results in Si-rich foliage (Street 1974, Massey et al. 2006, Massey et al. 2009). In natural systems, DSi concentration is influenced by the soil environment. For instance, high temperatures will speed up silicate dissolution (Drees et al. 1989), while organic material content can affect supply via the effects of organic acids on silicate weathering (Beckwith & Reeve 1964, Drees et al. 1989). Water influences DSi because it provides the solvent for mineral weathering, and soil moisture is affected by precipitation as well as soil particle size distribution (Monger & Kelly 2002). In summary, plant silicification exhibits wide variation according to phylogeny, and environmental conditions largely drive variation within taxonomic groups.

Benefits of Si accumulation

Grasses gain benefits from embedding abrasive, glass-like phytoliths into their leaves. From an evolutionary perspective, Si-accumulation is most commonly considered as an adaptation to the antagonistic arms race between grasses and grazing herbivores armed with diverse digestive strategies and mouthparts (Stromberg 2006, Stromberg et al. 2013). The expansion of abrasive, phytolith-rich grasses is thought to have been a major driver of hypsodonty, or high crowned cheek teeth, which is commonly observed among grazers (Williams & Kay 2001, Damuth & Janis 2011, Erickson 2013; see Fig 3). Some contention exists as to whether hypsodonty represents

a direct response to grass phytoliths or a more broad response to the expansion of open grasslands and associated ingestion of dietary grit (Jardine et al. 2012), but recent analysis has concluded that the presence of dust on food plays an insignificant role in driving the natural selection of novel dental strategies (Merceron et al. 2016).

Regardless, strong evidence exists that silica acts as a modern deterrent of herbivory in extant grasses by 1) abrading teeth and mouthparts (Massey & Hartley 2009, Erickson et al. 2012, Calandra et al. 2016), 2) reducing digestive efficiency (Massey & Hartley

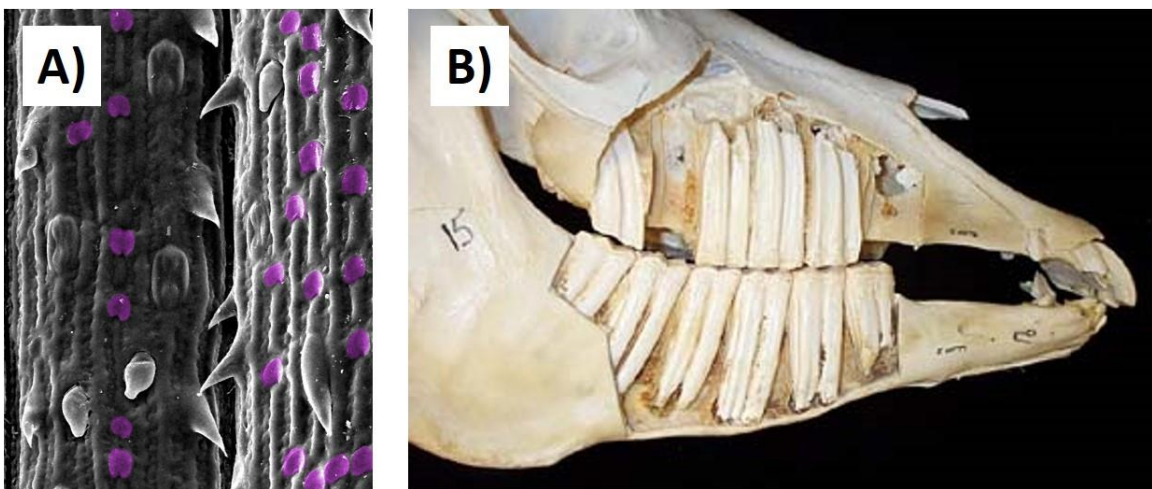


Figure 3. An arm's race between microscopic phytoliths (A) and grazer dentition (B)? Exposed jaw bones of a modern horse reveal extremely high-crowned (hypsodont) cheek teeth specialized for grinding grass leaf tissue which is rich in phytoliths (panel A, purple). Hypsodonty is common among extant grazing mammals. Panel B reprinted with permission from Dr. Richard Bowen, Colorado State University.

2006, Wieczorek et al. 2015), and 3) slowing herbivore growth rates (Massey & Hartley 2006, Massey & Hartley 2009).

Beyond the anti-herbivore benefits conferred by Si, it is well known for stress alleviation properties in agricultural literature (Ma 2004, Liang et al. 2007, Guntzer et al. 2012). Silicon can mediate plant tolerance to soil nutrients which are toxic in high doses; for instance, Si-treated plants show improved salt tolerance (Gong et al. 2006, Yin et al. 2015), and silicon can alleviate manganese (Li et al. 2015, Che et al. 2016), zinc (Cunha & Nascimento 2008), and cadmium toxicity (Nwugo & Huerta 2008, Vaculik et al. 2009).

Improved drought tolerance (Gong et al. 2005, Chen et al. 2011) and enhanced resistance to pathogenic fungi (Remus-Borel et al. 2005, Fauteux et al. 2006) also result when plants are supplied with silicon. Because of the myriad benefits conferred by silicification, silicon represents an important soil nutrient which, in adequate supply, has the capacity to provide broad-spectrum disease resistance in important food crops (Van Bockhaven et al. 2013).

Emerging Questions

In the past year, the topic of plant silicon accumulation has attracted much attention including the publication of two special journal features focusing specifically on the topic of plant silicon (Cooke & DeGabriel 2016, Cooke et al. 2016). However, many important questions remain unanswered, and researchers have yet to resolve the essentiality of silicon for plants (Raleigh 1939, Richmond & Sussman 2003, Struyf & Conley 2009). The ecology and ecophysiology of plant silicification are poorly understood and represent gaps in knowledge which could potentially contribute to our understanding of biogeochemical cycles (Conley 2002, Street-Perrott & Barker 2008, Struyf et al. 2010) and provide insight into the adaptive forces which have resulted in highly silicified extant grasses (Stromberg et al. 2016). Furthermore, our knowledge of induced plant Si accumulation is limited almost completely to laboratory studies. This observation, combined with the inconsistent responses of plants to defoliation, make the relative roles of grazing in relation to soil nutrients and water availability unclear.

Through the collective efforts of my dissertation research, I sought to address research gaps regarding the ecology and ecophysiology of silicon accumulation by grasses. I began by probing questions about the abiotic forces which might influence silicon uptake in a natural environment through two common garden studies: in **Chapter 1**, I present a common garden study on the effects of defoliation and water supply on

silica accumulation, and in **Chapter 2**, I investigated the effects of soils and silicon supply on plant physiological traits. From these controlled studies, I transitioned to an observational study of the natural drivers of grass leaf silicification along Serengeti's environmental gradients (**Chapter 3**). After investigating landscape scale patterns of plant Si accumulation, I transitioned to two global scale studies. In **Chapter 4**, I present a study of the relative roles of soil nutrients and herbivores in grass leaf silicification conducted at a network of grassland plots around the world. Finally, I conducted a meta-analysis to provide a disambiguous summary of previous plant silicon studies and to consider the potential adaptive drivers of grass Si accumulation (**Chapter 5**). Data analysis for several of these chapters required the development of novel methods for quantifying Si, based on ICP-OES validated NIRS calibrations, which is presented in **Appendix F**.

References

- Beckwith, R. S., & Reeve, R. (1964). Studies on soluble silica in soils. *Australian Journal of Soil Research*, 2, 33–45.
- 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(4), 501–507.
- Carlisle, E. M. (1988). Silicon as a trace nutrient. *The Science of the Total Environment*, 73, 95–106.
- Che, J., Yamaji, N., Shao, J. F., Ma, J. F., & Shen, R. F. (2016). Silicon decreases both uptake and root-to-shoot translocation of manganese in rice. *Journal of Experimental Botany*, In press.
- Chen, W., Yao, X., Cai, K., & Chen, J. (2011). Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biological Trace Element Research*, 142(1), 67–76.
- Conley, D. J. (2002). Terrestrial ecosystems and the global biogeochemical silica cycle. *Global Biogeochemical Cycles*, 16(4), 1–8.

- Cooke J., & DeGabriel, J.L. 2016. Editorial: Plant silicon interactions between organisms and the implications for ecosystems. *Frontiers in Plant Science* 7(1001): 1-3.
- Cooke, J., DeGabriel, J. L., & Hartley, S. E. (2016). The functional ecology of plant silicon: geoscience to genes. *Functional Ecology*, 30(8), 1270–1276.
- Cunha, K. P. V., & Nascimento, C. W. A. (2008). Silicon Effects on Metal Tolerance and Structural Changes in Maize (*Zea mays* L.) Grown on a Cadmium and Zinc Enriched Soil. *Water, Air, and Soil Pollution*, 197(1–4), 323–330.
- 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(3), 733–758.
- Darwin C. 1846. An account of the fine dust which often falls on vessels in the Atlantic Ocean. *Quarterly Journal of the Geological Society of London* 2: 26-30.
- Deshmukh, R., & Bélanger, R. R. (2015). Molecular evolution of aquaporins and silicon influx in plants. *Functional Ecology*, 30: 1277-1285.
- Drees, L. R., Wilding, L. P., Smeck, N. E., & Sankayi, A. L. (1989). Silica in soils: Quartz and disordered silica polymorphs. In *Minerals in Soil Environments* (pp. 913–974). Madison, WI: Soil Science Society of America.
- Epstein, E. (1994). The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences of the United States of America*, 91(1), 11–17.
- Epstein, E. (2009). Silicon: its manifold roles in plants. *Annals of Applied Biology*, 155(2), 155–160.
- Erickson, G. M., Krick, B. a, Hamilton, M., Bourne, G. R., Norell, M. a, Lilleodden, E., & Sawyer, W. G. (2012). Complex dental structure and wear biomechanics in hadrosaurid dinosaurs. *Science*, 338(6103), 98–101.
- Exley, C. (1998). Silicon in life: A bioinorganic solution to bioorganic essentiality. *Journal of Inorganic Biochemistry*, 69(3), 139–144.
- Fauteux, F., Chain, F., Belzile, F., Menzies, J. G., & Bélanger, 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(46), 17554–17559.
- Gong, H. J., Randall, D. P., & Flowers, T. J. (2006). Silicon deposition in the root reduces sodium uptake in rice (*Oryza sativa* L.) seedlings by reducing bypass flow. *Plant, Cell & Environment*, 29(10), 1970–9.
- Gong, H., Zhu, X., Chen, K., Wang, S., & Zhang, C. (2005). Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Science*, 169(2), 313–321.

- Guntzer, F., Keller, C., & Meunier, J. D. (2012). Benefits of plant silicon for crops: A review. *Agronomy for Sustainable Development*, 32(1), 201–213.
- Haynes, R. J. (2014). A contemporary overview of silicon availability in agricultural soils. *Journal of Plant Nutrition and Soil Science*, 177(6), 831–844.
- Hodson, M. J., White, P. J., Mead, a, & Broadley, M. R. (2005). Phylogenetic variation in the silicon composition of plants. *Annals of Botany*, 96(6), 1027–1046.
- Jardine, P. E., Janis, C. M., Sahney, S., & Benton, M. J. (2012). Grit not grass: Concordant patterns of early origin of hypsodonty in Great Plains ungulates and Glires. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 365–366, 1–10.
- Katz, O. (2015). Silica phytoliths in angiosperms : phylogeny and early evolutionary history. *New Phytologist*, 208(3), 642–646.
- Kervyn, M., Ernst, G. G. J., Keller, J., Vaughan, R. G., Klaudius, J., Pradal, E., ... Jacobs, P. (2010). Fundamental changes in the activity of the natrocarbonatite volcano Oldoinyo Lengai, Tanzania. *Bulletin of Volcanology*, 72(8), 913–931.
- Knoll, A. H. (1975). Silica, diatoms, and cenozoic radiolarian evolution. *Geology*, 3, 175–177.
- Laruelle, G. G., Roubex, V., Sferratore, a., Brodherr, B., Ciuffa, D., Conley, D. J., ... Van Cappellen, P. (2009). Anthropogenic perturbations of the silicon cycle at the global scale: Key role of the land-ocean transition. *Global Biogeochemical Cycles*, 23(4), 1–17.
- Li, P., Song, A., Li, Z., Fan, F., & Liang, Y. (2015). Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.). *Plant and Soil*, 354(1–2), 407–419.
- Liang, Y., Sun, W., Zhu, Y.-G., & Christie, P. (2007). Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environmental Pollution*, 147(2), 422–428.
- Liu, Q., & Zhu, Z. (2010). Functional divergence of the NIP III subgroup proteins involved altered selective constraints and positive selection. *BMC Plant Biology*, 10(1), 256.
- Ma, J. F. (2004). Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, 50(1), 11–18.
- Ma, J. F., & Takahashi, E. (2002). Appendix 3-A. In *Soil, Fertilizer, and Plant Silicon Research in Japan* (pp. 205–233). Amsterdam: Elsevier.
- Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., ... Yano, M. (2006). A silicon transporter in rice. *Nature*, 440(7084), 688–91.

- Ma, J. F., & Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, 11(8), 392–397.
- Ma, J. F., & Yamaji, N. (2015). A cooperative system of silicon transport in plants. *Trends in Plant Science*, 20(7), 435–442.
- Ma, J. F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T., ... Yano, M. (2007). An efflux transporter of silicon in rice. *Nature*, 448(7150), 209–212.
- Maldonado, M., Riesgo, A., Bucci, A., & Ru, K. (2010). Revisiting silicon budgets at a tropical continental shelf : Silica standing stocks in sponges surpass those in diatoms, 55(5), 2001–2010.
- 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(1), 281–291.
- Massey, F. P., Massey, K., Roland Ennos, a., & Hartley, S. E. (2009). Impacts of silica-based defences in grasses on the feeding preferences of sheep. *Basic and Applied Ecology*, 10(7), 622–630.
- 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(2), 595–603.
- 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.
- Merceron, G., Ramdarshan, A., Francisco, A., Gautier, D., Boisserie, J., Milhet, X., ... Pret, D. (2016). Untangling the environmental from the dietary : dust does not matter. *Proceedings of the Royal Society B: Biological Sciences*, 283, 20161032.
- Monger, H. C., & Kelly, E. F. (2002). Silica minerals. In J. B. Dixon & D. G. Schulze (Eds.), *Soil Mineralogy with Environmental Applications* (pp. 611–636). Madison, WI: Soil Science Society of America.
- Nwugo, C. C., & Huerta, A. J. (2008). Silicon-induced cadmium resistance in rice (*Oryza sativa*). *Journal of Plant Nutrition and Soil Science*, 171(6), 841–848.
- Piperno, D. R., & Pearsall, D. M. (1998). The Silica Bodies of Tropical American Grasses : Morphology , Taxonomy , and Implications for Grass Systematics and Fossil Phytolith Identification. *Smithsonian Contributions to Botany*, 85(85), 1–40.
- Powers AH. 1992. Great expectations: A short historical review of European phytolith systematics. In Mulholland & Rapp (Eds), *Phytolith Systematics: Emerging Issues*, Plenum Press: New York. 15-36.

- Prychid, C. J., Rudall, P. J., & Gregory, M. (2004). Systematics and Biology of Silica Bodies in Monocotyledons. *The Botanical Review*, 69(4), 377–440.
- Raleigh, G. (1939). Evidence for the essentiality of silicon for the beet plant. *Plant Physiology*, (1), 823–828.
- Rémus-Borel, W., Menzies, J. G., & Bélanger, R. R. (2005). Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiological and Molecular Plant Pathology*, 66(3), 108–115.
- Richmond, K. E., & Sussman, M. (2003). Got silicon? The non-essential beneficial plant nutrient. *Current Opinion in Plant Biology*, 6(3), 268–272.
- Rosen, A. M. (1992). Preliminary identification of silica skeletons from near eastern archaeological sites: an anatomical approach. In G. R. Jr. (Ed.), *Phytolith Systematics* (pp. 129–147). New York: Springer Science + Business Media.
- Sicko-Goad, L. M., Schelske, C. L., & Stoermer, E. F. (1984). Estimation of intracellular carbon and silica content of diatoms from natural assemblages using morphometric techniques, *vi*(6), 1170–1178.
- Simpson TL, BE Volcani. 1981. *Silicon and siliceous structures in biological systems*. Springer: New York.
- Street, J. R. (1974). *The influence of silica concentration on the chemical composition and decomposition rates of turfgrass tissue and water absorption rates among three turfgrass species*. PhD Dissertation. Ohio State University.
- Street-Perrott, F. A., & Barker, P. A. (2008). Biogenic silica: a neglected component of the coupled global continental biogeochemical cycles of carbon and silicon. *Earth Surface Processes and Landforms*, 33, 1436–1457.
- Strömberg, C. a E. (2006). Evolution of hypsodonty in equids: testing a hypothesis of adaptation. *Paleobiology*, 32(2), 236–258.
- Strömberg, C. a E., Dunn, R. E., Madden, R. H., Kohn, M. J., & Carlini, A. a. (2013). Decoupling the spread of grasslands from the evolution of grazer-type herbivores in South America. *Nature Communications*, 4, 1478.
- Stromberg, C. A., DiStilio, V. S., & Song, Z. (2016). Functions of phytoliths in vascular plants : an evolutionary perspective. *Functional Ecology*, 30, 1286–1297.
- Struyf, E., & Conley, D. J. (2009). Silica: an essential nutrient in wetland biogeochemistry. *Frontiers in Ecology and the Environment*, 7(2), 88–94.
- Struyf, E., Smis, A., van Damme, S., Meire, P., & Conley, D. J. (2010). The global biogeochemical silicon cycle. *Silicon*, 1(4), 207–213.

- Takahashi, E., Ma, J. F., & Miyake, Y. (1990). The possibility of silicon as an essential element for higher plants. *Comments on Agricultural & Food Chemistry*, 2(2), 99–122.
- Tréguer, P., Nelson, D. M., Van Bennekom, a J., Demaster, D. J., Leynaert, A., & Quéguiner, B. (1995). The silica balance in the world ocean: a reestimate. *Science*, 268(5209), 375–379.
- Trembath-Reichert, E., Wilson, J. P., McGlynn, S. E., & Fischer, W. W. (2015). Four hundred million years of silica biomineralization in land plants. *Proceedings of the National Academy of Sciences*, 112(17), 5449–5454.
- Vaculík, M., Lux, A., Luxová, M., Tanimoto, E., & Lichtscheidl, I. (2009). Silicon mitigates cadmium inhibitory effects in young maize plants. *Environmental and Experimental Botany*, 67(1), 52–58.
- Van Bockhaven, J., De Vleeschauwer, D., & Hofte, M. (2013). Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. *Journal of Experimental Botany*, 64(5), 1281–1293.
- Wainwright, M. (1997). The neglected microbiology of silicon - From the origin of life to an explanation for what Henry Charlton Bastian saw. *Quarterly Review in Microbiology*, 24(3), 83–85.
- Wedepohl, K. H. (1995). Composition of the Continental Crust. *Geochimica et Cosmochimica Acta*, 59(7), 1217–1232.
- Wieczorek, M., Szafranska, P. a., Labecka, a. M., Lazaro, J., & Konarzewski, M. (2015). Effect of the abrasive properties of sedges on the intestinal absorptive surface and resting metabolic rate of root voles. *Journal of Experimental Biology*, 218(2), 309–315.
- Williams, S. H., & Kay, R. F. (2001). A Comparative Test of Adaptive Explanations for Hypsodonty in Ungulates and Rodents. *Journal of Mammalian Evolution*, 8(3), 207–229.
- Yin, L., Wang, S., Tanaka, K., Fujihara, S., Itai, A., Den, X., & Zhang, S. (2015). Silicon-mediated changes in polyamines participate in silicon-induced salt tolerance in *Sorghum bicolor* L. *Plant, Cell & Environment*, 39, 245–258.

CHAPTER 1

SOILS MODERATE A COORDINATED MULTI-TRAIT SHIFT IN A MODEL C4 GRASS FOLLOWING SILICON FERTILIZATION

The following manuscript is formatted for and to be submitted to *Annals of Botany*.

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ABSTRACT

- *Background and Aims* Despite being considered a non-essential nutrient for plant growth, decades of research have touted the benefits of soil silicon amendment for plant nutrition. These benefits are especially apparent for important cereals of the plant family Poaceae, which are known to experience improved stress tolerance and increased yield when supplied with Si fertilizer. However, little is known about how the soil matrix moderates these responses. We sought to understand how soil Si addition affects a suite of plant traits, including stomatal density and conductance, across widely varying soils and to determine whether trait responses were coordinated at the whole-plant level.
- *Methods* We measured stomatal conductance and density, plant height and leaf length, leaf mass per area, and root and shoot biomass of an economically important model C4 grass, sorghum, grown in four different soil types with and without silicon fertilizer. Treatment responses were estimated for each trait using mixed models accounting for random effects of soil type, and whole-plant responses were visualized using principal components analysis.
- *Key Results* Soil silicon application resulted in increased maximum leaf length, plant height, root biomass, and shoot biomass, but we did not observe a significant LMA response. Si-treated plants also displayed a decrease in stomatal density on both leaf surfaces, while stomatal ratios remained constant between treatments. Despite the observed drop in stomatal density, +Si plants exhibited higher maximum sustained stomatal conductance than control plants. A principal components analysis (PCA) further indicated that traits respond to soil Si addition in a coordinated manner, along multi-trait axes of variation.

- *Conclusions* Collectively, these results highlight that individual traits respond in a coordinated manner to Si addition and that the soil matrix influences Si effects. When considering potential costs of leaf silicification as well relative competitive advantages that may be conferred by silicification, whole-plant responses should be considered, and soil properties must be taken into account.

Keywords: silicon, plant nutrition, conductance, stomatal density, Sorghum bicolor

INTRODUCTION

Plants absorb a range of mineral nutrients from their soil environment, many of which play a vital role in metabolism. In addition to the 17 micro- and macronutrients identified as essential for all plants (Epstein and Bloom 2005), researchers have pondered “the neglected microbiology of silicon” (Wainwright 1997) and have labeled silicon as “an anomaly” (Epstein 1994), “an enigmatic element in plant science” (Epstein 2001), and “quasi-essential” (Epstein 2002). Plants acquire silicon from their soil environment, where it occurs as the second most abundant element in earth’s lithosphere (Wedepohl 1995). Specialized Si-transport molecules allow plant roots to mobilize silicic acid (H_4SiO_4) in soil water across Casparian strips and into xylem (Ma and Yamaji 2006). Once in the xylem, transpiration mobilizes silicic acid to aerial tissues, where it polymerizes into solid bodies of opaline hydrated silica ($\text{SiO}_2 \cdot n \text{H}_2\text{O}$), referred to as phytoliths, in intracellular and extracellular spaces (Parry and Smithson 1964). Glass-like phytoliths have highly diagnostic shapes (i.e. Piperno and Pearsall 1998) and are very resistant to weathering, and are consequently well maintained in the fossil record. For example, researchers have discovered phytolith-rich dinosaur coprolites dating to the late Cretaceous (Prasad et al. 2005). Moderate leaf silicification is widespread throughout the plant kingdom (Trembath-Reichert et al. 2015), but certain groups occur as outliers. Clovers, for instance, have been shown to exclude monosilicic acid from the transpiration stream, most likely via a root barrier (Jones and Handreck 1969). In contrast, other plant groups are known to accumulate silicon to an extent which exceeds even leaf nitrogen concentration (Epstein 1994). Horsetails of the genus *Equisetum* are so highly abrasive due to phytolith content that they have been historically used to scour pots and pans and have received the nickname “scouring rush”.

The Poaceae (grasses) represent a taxonomically diverse and globally widespread group of silicon accumulators which includes important food crops (i.e. rice, wheat, corn, sugar cane). Early studies noted that graminaceous crops supplied with silicate fertilizer exhibited improvements in growth and yield relative to untreated plants (e.g. Clements 1963). Further studies have specifically noted that silicon improves crop tolerance to drought stress via changes in plant morphology, physiology, and gene expression. For instance, improved biomass yield of grasses grown under water deficit was observed following soil silicon application and was attributed to a corresponding increased uptake of essential macronutrients N, P, and K (Eneji et al. 2008). Si-induced alleviation of stress associated with phytotoxic soil elements is also known to improve plant vigor. Si application has been demonstrated to decrease Na uptake in wheat (Saquib et al. 2008) and Al in maize (Corrales et al. 1997). Improved aluminum tolerance of Si-treated maize has been attributed to increased production of beneficial flavonoids and root exudates (Kidd et al. 2001). Silicon fertilization is also known to improve growth parameters of Cd-stressed maize plants, despite a corresponding increase in cadmium uptake (Vaculik et al. 2009), while manganese toxicity is reduced in Si-treated rice plants via up-regulation of genes involved in photosynthetic enzyme production (Li et al. 2015). Likewise, silicon may promote accumulation of UV-B absorbing phenolics in leaf epidermal cells (Wen-bin et al. 2004) and production of antioxidant enzymes which reduce oxidative stress (Saquib et al. 2008). Finally, water use efficiency is improved by silicon fertilization. Rice plants provided with Si fertilizer sustained higher photosynthetic and transpiration rates and decreased uptake of nutrients K, Na, Ca, Mg, and Fe compared with drought stressed plants which did not receive Si (Chen et al. 2011), and improved CO₂ assimilation under drought stress has been observed in Si-treated wheat (Gong et al. 2005). Decreased shoot to root ratio has also been observed for *Sorghum* plants which received silicate

fertilizer, which corresponded with improved ability to extract water from dry soils and to maintain a higher stomatal conductance than untreated plants (Hattori et al. 2005).

When considering patterns of plant Si accumulation from a broader lens (i.e. landscape or ecosystem-scale), modeling plant silicification becomes complex. Beyond plant-available soil silicon, many additional environmental conditions may impact plant silicon uptake; herbivores, climate, and soil properties have all been implicated to induce or suppress grass leaf silicification. Only recently have studies begun to probe the ecological context of plant leaf silicification. Cooke and Leishman (2012) compared patterns of C and Si-based defensive compounds in Australian plant communities growing on two different soil types and found that, across a diverse group of paired plant species, plants growing at the site with greater soil silicon availability consistently tended toward Si over C-based defensive compounds. Building upon this pattern, Quigley et al. created a piecewise structural equation model describing relationships between soil and grass silicon pools along environmental gradients in Serengeti (2016). Their findings highlighted that soil texture, pH, and precipitation best predicted the plant-available pool of silicic acid, which was then highly correlated with grass leaf Si concentration. Another recent ecological study illustrated that patterns of subarctic grass silicification in response to removal of herbivores and local abiotic conditions are species-specific (Soininen et al. 2012).

While controlled laboratory studies of plant physiology emphasize a role of altered photosynthetic traits of Si-treated plants for improved stress tolerance, and field studies have demonstrated a role of soils in plant silicification, it remains unclear whether plants would respond consistently to Si addition in variable soil matrices. Moreover, since many leaf traits are tightly linked (i.e. Wright et al. 2007), it may be more appropriate to consider how a collective suite of traits change in response to a stimulus, rather than

how individual traits change. To address these gaps, we grew seeds of *Sorghum bicolor* in four different soils, with and without the addition of a silicate fertilizer, seeking to answer the following specific questions. (1) Do baseline plant traits of stomatal conductance, stomatal density, LMA, and various measures of growth vary for seeds grown in different soil types collected from adjacent parts of an ecosystem? (2) How does addition of a silicate fertilizer to various soils affect plant traits? (3) Are coordinated shifts in whole-plant morphology and physiology observed? Insight into these questions has important applications for widespread issues ranging from crop production of important C4 grasses (ex. corn, sugarcane, and switchgrass) and for improving models of the global biogeochemical silicon cycle (i.e. Struyf et al. 2010).

METHODS

Pot experiment

Soils from four sites with varying soil chemical and physical properties were collected from adjacent regions of Serengeti National Park for use in the pot experiment (Supplement S1). These soils represent four distinct World Reference Base (FAO 1998) soil subgroups: andosol, leptosol, phaeozem, and solonetz (Table 1). Sites vary in soil amorphous Si, plant-available dissolved Si (DSi), pH, organic matter, and texture, and have been previously integrated into an ecosystem model for grass leaf silicification in Serengeti (Quigley et al. 2016). Each soil was passed through a 2 mm sieve prior to planting seeds, in order to offset potentially confounding effects of soil compaction. Sorghum seeds were planted in plastic seed trays, with 3 seeds per 5cm wide x 5 cm deep cylindrical cell, and 5 replicate cells per each of the four soils in each treatment (120 seeds total, in 40 cells). Plants were grown under ambient conditions in an outdoor

common garden enclosure, located at the Tanzania Wildlife Research Institute (TAWIRI) in the center of Serengeti National Park (Supplement S1). The photoperiod during the experiment was approximately 12 hours, with temperatures ranging from a nighttime low of 11.4 °C and a daytime high of 30.2 °C (mean daily max = 27.6 °C; TM Anderson, unpublished weather station data). The experiment was performed during a local dry season, and light rains occurred on 16 of 45 experiment days; most rains occurred as evening showers lasting 30 minutes or less (K Quigley, personal observation), with a total of 40 mm of precipitation recorded (TM Anderson, unpublished weather station data). Seeds assigned to the + Si treatment received an aqueous solution of 500 ppm Si, as sodium metasilicate ($\text{Na}_2\text{O}_3\text{Si} \cdot 9\text{H}_2\text{O}$, Fisher Scientific) supplied to soil saturation once weekly. To control for sodium in the Si treatment, control plants received an equivalent rate of Na (23 mg/L) supplied once weekly to soil saturation. All plants were supplied with rain water once every other day to soil saturation, except on treatment application days and days with substantial precipitation.

Plant trait measurements

Germination success rate was recorded as the number of seeds (out of 15) that germinated within each soil * treatment group. After 45 days of growth, maximum plant height and maximum leaf length were recorded, prior to taking stomatal conductance readings. Plants were then watered, and stomatal conductance (g_s), as $\text{mmol m}^{-2} \text{s}^{-1}$, was measured for the adaxial surface of the youngest fully expanded leaf blade of one seedling in each of the replicate treatment x soil cells (i.e. 40 individual plants). This measurement was repeated at four 2 hour intervals using a Decagon SC-1 Leaf Porometer (Decagon Devices, Pullman, WA). We chose to measure adaxial g_s because this leaf surface typically receives direct photosynthetically active radiation. Immediately following Porometer measurements, plants were harvested. At the time of harvest,

plants were not yet root-bound to seed trays. Next, plants were dried in an oven at 60 °C for > 48 hours, and dry weight was recorded for roots and shoots of each whole plant and for individual leaves from which stomatal conductance was measured. Leaf mass per area (LMA) was calculated by dividing leaf dry weight by leaf area (calibrated against a 5 mm² square and calculated in ImageJ; Schneider et al. 2012). Abaxial and adaxial epidermal impressions ≥ 1 cm in length were created from the center of each leaf measured for g_s and LMA, using clear acrylic nail varnish. Peels were mounted on glass microscope slides, and stomata were counted at ten random sections of each peel under 200x magnification using a 3-D inverted microscope (Zeiss AxioObserver; Hamamatsu Orca C4742-80-12AG digital camera). Average stomatal density, as number of pores per mm², was then calculated from the 10 replicate counts for each surface of each leaf.

Data analysis

The three seeds planted within a single seed tray cell were considered pseudoreplicates and were either pooled or averaged prior to analysis. Thus, each site x treatment test group had five independent replicates, each representing 2-3 seedlings depending on mortality, except for g_s , which was only measured for one individual per cell.

After ensuring that each response variable had an approximately normal distribution, we investigated the effects of Si treatment on each individual plant trait by creating linear mixed models accounting for random site intercepts. Models were fit using the “lmer” function of the *lme4* package in the R Statistical programming environment, with the general syntax: TRAIT ~ TREATMENT + (1|SITE), where treatment had two factors (Si or Control), and site had four factors (Barafu, Lamai, Musabi, or Togoro) (R Development Core Team 2014, Bates et al. 2015). Each model was visually inspected for homoscedasticity of variance. To analyze variation in stomatal conductance (g_s), we averaged values obtained from time t=2 and t=4 hours as an

estimate of the maximum sustained g_s (full time series data shown in supplement S2). We then used this new g_s estimate to fit a model for fixed treatment effects and random site effects, similarly to other traits. Treatment contrasts were calculated using Tukey's test for multiple comparison in the "glht" function of the R package *multcomp* (Hothorn et al. 2008).

Finally, because trait-based shifts may be coordinated (e.g. Wright et al. 2007), we performed principal components analysis (PCA) to account for correlations among traits, collapse dimensionality along primary vectors of variation, and visualize multidimensional shifts in plant traits. Using the "princomp" function in base R, principal components and their loadings (eigen values) were calculated for a matrix of 10 traits (maximum height, maximum leaf length, LMA, abaxial stomatal density, adaxial SD, stomatal ratio, root biomass, shoot biomass, and R:S ratio). We also included sustained maximum g_s , calculated as g_s averaged for the 2 hour and 4 hour measurements, as a singular indicator of gas exchange rate. A two-dimensional plot of samples along the first two PC axes was then created using the R package *ggfortify* to visualize potential treatment and/or soil site * treatment clusters (Horikoshi and Tang 2016).

RESULTS

Germination success was generally very high; all seeds from both treatments grown in Barafu and Togoro soils and in +Si treatments for Lamai and Musabi soils germinated. Both Lamai and Musabi had an 86.6% germination success rate in untreated control soils. Seeds generally emerged sooner from Si treated soils than from untreated soils. Silicon-treated plants had a more compact and erect canopy structure, while many untreated plants (-Si soils) exhibited a yellow pigmentation with dark red spotting at the distal ends of leaves (Supplement S3). Control sorghum plants exhibited moderate differences in baseline plant traits, most of which were statistically non-significant. One

trait that did vary was shoot biomass, which was higher for plants grown in untreated Barafu soils than Lamai (diff = 6.7 mg, $t = -3.3$, $p = 0.01$), Musabi (diff = 7.4 mg, $t = -3.7$, $p < 0.01$), and Togoro soils (diff = 5.2 mg, $t = -2.6$, $p = 0.06$).

After accounting for a random effect of site, maximum leaf length, plant height, root and shoot biomass, and stomatal density were all significantly affected by soil Si addition. Maximum leaf length increased by 1.95 mm, on average, with the addition of Si (standard error = 0.32, $t = 6.14$), and maximum plant height increased by 1.91 mm (se = 0.41, $t = 4.62$) (Fig 1a-b). Root biomass and shoot biomass were also both significantly greater for plants grown in Si treated soils (by 8.94 ± 3.6 mg and 4.84 ± 1.2 mg; $t = 2.46$, $t = 4.08$, respectively) (Fig 1c-d). Finally, both adaxial stomatal density (diff = 30 pores * $\text{mm}^{-2} \pm 10$ se, $t = -2.94$) and abaxial stomatal density (diff = 29 pores * $\text{mm}^{-2} \pm 11$ se, $t = -2.59$) decreased significantly in the Si treatment group (Fig 2a-b). Stomatal densities for the two leaf surfaces shifted consistently for both upper and lower leaf surfaces, so stomatal ratio remained constant between treatments. LMA and root:shoot ratio were not significantly affected by Si addition (data not shown). A repeated measures ANOVA for g_s including random site effects (as above) indicated that stomatal conductance was significantly greater at $t = 2$ hr in the +Si group (Fig 2c; Si-treated $g_s = \text{Control } g_s + 30.0$; se = 7.6, $z = 3.96$, $p > |z| = 0.002$).

Principal components analysis indicated that the first five PC axes explained 88 % of cumulative variance, and 10 PCs were needed to provide 100 % variance explanation (full summary in supplement S4). PC 1 was dominantly driven by maximum leaf length and leaf height, followed by root biomass, then shoot biomass. PC2 represents strong variation in stomatal ratio, root:shoot biomass ratio, and abaxial stomatal density. Loading values of all traits for PCs 1-5 can be found in **Table 2**, and corresponding ordination diagrams PC1 vs. PC2 and PC3 are available in the

supplement (S5). Clustering analysis based Ward's algorithm of Euclidean distances indicated a moderate strength clustering along the first two PC axes (14/20 control and 14/20 Si plants clustered). Visualization of clustering for treatments along PC1 and PC 2 supported the notion that plant traits responded in a coordinated manner to Si treatment (Fig 3).

DISCUSSION

Silicon application has manifest benefits for plants, and in certain groups like grasses may improve plant fitness under stressful conditions. Despite the widespread use of silicate fertilizers in agriculture since the 1960s, the underlying changes in plant physiology which drive improved stress tolerance and yield remain poorly understood (Epstein 1999). In our study, we found that an increase in stomatal conductance associated with Si application was accompanied by increases in leaf length, plant height, and root and shoot biomass. Interestingly, Si-treated plants exhibited a stark decrease in stomatal density compared to control plants, indicating greater photosynthetic capacity per unit leaf area of plants grown in Si-amended soils despite no significant change in LMA. Our results suggest that the soil environment, through both direct supply of DSi and indirect properties which influence the rate of nutrient and water delivery to plants, have profound effects on overall plant growth.

While we expected to observe an increase in stomatal conductance following Si treatment, as has been previously recorded for sorghum (Hattori et al. 2007, Hattori et al. 2008, Sonobe et al. 2009), we were surprised by the novel finding that increased g_s was accompanied by a significant reduction in stomatal density relative to the control (-Si) group. Increased g_s has been attributed to Si-enhanced hydraulic conductance via an increase in leaf water potential under drought conditions due to decreased cuticular transpiration (Hattori et al. 2007). By improving hydraulic conductance, silicon may help

plants to take up greater amounts of water from dry soils with low water potential, without needing to shift resource investment toward roots. However, because of the observed decline in stomatal density and the lack of an LMA response to Si treatment, it is unclear exactly how plants with fewer stomata were able to sustain higher g_s , a pattern which opposes the positive relationship commonly observed between grass stomatal density and g_s (Muchow and Sinclair 1989, Xu and Zhou 2008). Although C4 grasses have generally high carbon assimilation efficiency due to benefits associated with Kranz anatomy, subcuticular silicification could further reduce excess water loss (i.e. Yoshida et al. 1962), and a leaf surface with fewer stomata could also reduce total water loss from the teichodes of guard cells at minimum aperture (Muchow and Sinclair 1989). These small additional gains in carbon assimilation relative to water loss could explain some of the observed improvements in growth and productivity.

Plant height, leaf length, and plant biomass all shifted similarly along the primary axis of variation, indicating an overall role of silicon in growth promotion. An early greenhouse study investigating the role of plant Si availability also noted increased leaf size, along with increased chlorophyll concentration and promotion of flowering in one species of Si-fed grasses (McNaughton et al. 1985), corroborating our results. Another previous study cited the role of silicon in improved water use efficiency via a stimulated shift toward investment in roots (Hattori et al. 2005); while we did observe increased root biomass of Si-treated plants, root to shoot biomass was not different between treatments, suggesting biomass increases in a balanced manner. Increases in plant size and biomass indicate that plant growth may be stimulated through both improved light capture aboveground and increased mining for water and nutrients belowground. For instance, stimulated uptake of macronutrients (N, P, K) has been observed following Si application (Eneji et al. 2008). Increased leaf length is likely due to increased production

of photosynthetic enzymes and chlorophyll which have been previously linked to Si supply (McNaughton et al. 1985, Li et al. 2015). Similarly, silica deposits in stems may substitute for lignin, resulting in plants that are taller (Okuda and Takahashi 1962), require less carbon for leaf construction (Cooke and Leishman 2011), and are more resistant to lodging and wind (Idris et al. 1975, Schoelynck and Struyf 2015).

Although soil properties beyond DSi supply are largely ignored when considering plant silicification, we observed that inclusion of soil collection site in our models as a random effect significantly improved overall predictive strength. Visual inspection of plant traits in response to different soils and consideration of specific soil properties provides additional insight into site-specific responses to Si addition. For instance, silicon application has been noted to reduce the uptake of cations (K, Na, Ca, Mg) of which soils from Barafu are especially enriched (Chen et al. 2011, DeWit 1978). Thus, the consistently very strong response of growth parameters (Fig 1) to Si addition could be due to alleviation of salt stress. Likewise, a consistently very strong response of plants grown in Togoro soils to Si addition could be due to the fact that this soil has extremely low baseline DSi availability. Finally, we noticed that in several instances, seeds sown in Lamai soils had different responses to Si treatment than the remaining 3 soils. For instance, Lamai plants were the only soil group which did not show a marked increase in leaf length and plant height (Fig 1) and the only plants which did not show a clear decline in stomatal density (Fig 2) with Si application. The soil we collected from Lamai was acidic with a high sand composition (Table 1), and high soil iron and manganese have been previously measured at Lamai (Jager 1982). These conditions, coupled with the observation that Lamai plants were the only soil group to decrease root:shoot biomass ratio under Si addition (data not shown), may indicate especially stressful soil conditions. In summary, while including soil as a random effect in modeling was optimal

in this study, there are likely cases when soil or collection site confers large differences in plant response to Si and should be treated as a fixed effect.

The relative costs and benefits of silicon application (and accumulation) are likely variable under different environmental conditions. For instance, drought studies often note a positive response of drought-stressed plants to Si addition, while plants with adequate water supply have greatly reduced or no response (Eneji et al. 2008, Chen et al. 2011). Eneji et al (2008) found that drought-stressed grasses provided with Si increased uptake of nitrogen, phosphorous, and potassium. Potassium uptake in particular was strongly correlated with Si uptake under conditions of water deficit. Although we did not measure potassium response in particular, we did notice that control plants exhibited chlorosis and possible signs of K-deficiency (Supplement S3) which have recently been revealed to be alleviated via antioxidant activity induced by Si supply (Chen et al. 2016). In contrast to Chen's results, however, we observed improved growth under adequate water supply, suggesting that Si supply is beneficial for plants even in the absence of drought stress. There is a possibility that control plants may have been Na-stressed, but this seems unlikely since the total dose was relatively low (23 ppm Na, applied 6x total with intermittent water supply), and since plants grown in Barafu soils, which are naturally saline, did not exhibit similar signs of stress (Jager 1982). Benefits conferred by Si-accumulation may also relate to plant age. In a previous study, researchers noted that silica content of third-year bamboo leaves was negatively associated with nutrient use efficiency, although first and second year NUE were unaffected (Motomura et al. 2008). Thus, silicification may only become detrimental once plants accumulate some threshold of silicon, perhaps resulting in silicification of particular cell types which may lose functionality when impregnated with silica (i.e. bulliform cells, Sangster and Perry 1969, Honaine and Osterrieth 2011). Recent in-situ

tracking of silicon deposition, using air-scanning electron microscopy, has revealed that cytoplasm shrinks as cells silicify, with negative consequences for cell viability (Kumar 2016). It is unclear whether this kind of cost would become apparent in short-lived plants like grasses, which experience frequent turnover due to grazing, burning, and seasonal senescence.

Finally, we note that plant silicification is also phylogenetically constrained. Wide variation exists ranging from plant species like clover, which completely excludes Si from xylem (Jones and Handreck 1969), to those which accumulate upwards of 10% dry weight silicon (Trembath-Reichert 2015). Grasses (Poaceae) are an especially interesting clade to consider, since they vary widely in not only extent of silicification, but also in other important physiological characters like photosynthetic strategy (C3 vs. C4) and stature. A tropical, towering bamboo may utilize silica in its stems to improve light competition and structural rigidity, while a halophytic marsh species like *Spartina* may accumulate Si in order to improve salt stress. Meanwhile rice, a commonly cited example of a Si-accumulating C3 grass from the Ehrartoideae subfamily, may gain the greatest benefit from improved water use efficiency. Given these vast differences in ecological demands among different members of the plant family Poaceae, further research is needed to disentangle the adaptive grounds for extreme variation in leaf silicification within this closely related group.

Conclusion

Silicon provides a suite of benefits to graminaceous plants, although many of the physiological changes underlying these benefits are poorly understood. Here we tested for soil-mediated effects of silicon fertilization in driving important metrics of plant growth for an important drought-tolerant C4 grass species. By showing that Si-treated Sorghum plants were able to maintain an increased stomatal conductance relative to control

plants, despite significantly lower stomatal densities, we provided evidence that silicon supply directly regulates plant traits vital for maintaining water balance. That differences in the effects of Si supply are observed for plants grown in different soils is of important consideration to ecologists because interactions between Si supply and the soil matrix may influence competition and/or species composition in natural environments. We conclude that it is vital for researchers to incorporate soil variation into future studies of Si-induced changes in plant physiology in order to better understand how plants respond to gradients of soil Si availability in natural environments.

SUPPLEMENTARY DATA

Supplementary data are available in a separate file and consist of Figure S1: Map of soil collection sites, Figure S2: Time series for stomatal conductance data, Table S3: Variance explanation of PCA components, and Figure S4: PCA ordination diagrams.

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LITERATURE CITED

Bates D, Machler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1, 1-48.

- Chen, D., Cao, B., Qi, L., Yin, L., Wang, S., Deng, X. (2016). Silicon-moderated K-deficiency-induced leaf chlorosis by decreasing putrescine accumulation in sorghum. *Annals of Botany*.
- Chen, W., Yao, X., Cai, K., Chen, J. (2011). Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biological Trace Element Research*, 142(1), 67–76.
- Clements, H. F. (1963). Interaction of factors affecting yield. *Annual Review of Plant Physiology*, 15, 409–442.
- Cooke, J., Leishman, M. R. (2011). Is plant ecology more siliceous than we realise? *Trends in Plant Science*, 16(2), 61–68.
- Cooke, J., Leishman, M. R. (2012). Tradeoffs between foliar silicon and carbon-based defences: evidence from vegetation communities of contrasting soil types. *Oikos*, 121(12), 2052–2060.
- Corrales, I., Poschenrieder, C., Barceló, J. (1997). Influence of silicon pretreatment on aluminium toxicity in maize roots. *Plant and Soil*, 190, 203–209.
- DeWit, H. A. (1978). *Soils and grassland types of the Serengeti Plain Tanzania*. Wageningen.
- Eneji, A. E., Inanaga, S., Muranaka, S., Li, J., Hattori, T., An, P., Tsuji, W. (2008). Growth and Nutrient Use in Four Grasses Under Drought Stress as Mediated by Silicon Fertilizers. *Journal of Plant Nutrition*, 31(2), 355–365.
- Epstein, E. (1994). The anomaly of silicon in plant biology. *Proceedings of the National Academy of Sciences of the United States of America*, 91(1), 11–17.
- Epstein, E. (1999). Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology*, 50, 641–664.
- Epstein E. 2001. Silicon in plants: Facts vs. concepts. In: *Silicon in Agriculture*. Datnoff LE, GH Snyder, GH Korndorfer, eds. Elsevier: New York. 1-15.
- Epstein E. 2002. Silicon in plant nutrition. In: *Second Silicon in Agriculture Conference*. Japanese Society of Soil Science and Plant Nutrition, Tsuruoka, Yamagata. 1-5.
- Epstein E, AJ Bloom. 2005. Inorganic components of plants. In: *Mineral Nutrition of Plants: Principles and Perspectives*. Sinauer: Sunderland, MA. 41-66.
- FAO. 1998. International Soil Reference and Information Center (ISRIC) and International Society of Soil Science (ISSSS). World Reference Base for Soil Resources. FAO, Rome.
- Gong, H., Zhu, X., Chen, K., Wang, S., Zhang, C. (2005). Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Science*, 169(2), 313–321.

- Hattori, T., Inanaga, S., Araki, H., An, P., Morita, S., Luxova, M., Lux, A. (2005). Application of silicon enhanced drought tolerance in Sorghum bicolor. *Physiologia Plantarum*, 123(4), 459–466. <http://doi.org/10.1111/j.1399-3054.2005.00481.x>
- Hattori, T., Sonobe, K., Inanaga, S. (2008). Effects of Silicon on Photosynthesis of Young Cucumber Seedlings Under Osmotic Stress. *Effects of Silicon on Photosynthesis of Young Cucumber Seedlings under Osmotic Stress*, 31(6), 1046–1058.
- Hattori, T., Sonobe, K., Inanaga, S., An, P., Tsuji, W., Araki, H., Morita, S. (2007). Short term stomatal responses to light intensity changes and osmotic stress in sorghum seedlings raised with and without silicon. *Environmental and Experimental Botany*, 60(2), 177–182.
- Horikoshi M, Y Tang. 2016. ggfortify: Data visualization tools for statistical analysis results. <https://CRAN.R-project.org/package=ggfortify>.
- Hothorn T, F Bretz, P Westfall. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50 (3): 346-363.
- Idris, M. D., Hossain, M. M., Choudhury, F. A. (1975). The effect of silicon on lodging of rice in presence of added nitrogen. *Plant and Soil*, 43, 691–695.
- Jager, T. J. (1982). *Soils of the Serengeti Woodlands*. Wageningen.
- Jones, L. H. P., Handreck, K. A. (1969). Uptake of silica by Trifolium incarnatum in relation to the concentration in the external solution and to transpiration. *Plant and Soil*, 1, 71–80.
- Kidd, P. S., Llugany, M., Poschenrieder, C., Gunsé, B., Barceló, J. (2001). The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). *Journal of Experimental Botany*, 52(359), 1339–52.
- Kumar, S., Milstein, Y., Brami, Y., Elbaum, M., Rivka, E. (2016). Mechanism of silica deposition in sorghum silica cells. *New Phytologist*, in press.
- Li, P., Song, A., Li, Z., Fan, F., Liang, Y. (2015). Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.). *Plant and Soil*, 354(1–2), 407–419.
- Ma, J. F., Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, 11(8), 392–397.
- 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(2), 528–535.

- Motomura, H., Hikosaka, K., Suzuki, M. (2008). Relationships between photosynthetic activity and silica accumulation with ages of leaf in *Sasa veitchii* (Poaceae, Bambusoideae). *Annals of Botany*, 101(3), 463–468.
- Muchow, R. C., Sinclair, T. R. (1989). Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench. *Plant, Cell Environment*, 12(4), 425–431.
- Okuda A, Takahashi E. 1962. Studies on the physiological role of Si in crop plants VIII: Specificity of Si uptake by rice plants. *J Sci Soil Manure* 33, 217-221.
- Parry, D. W., Smithson, F. (1964). Types of Opaline Silica Depositions in the Leaves of British Grasses. *Annals of Botany*, 28(109), 169–185.
- Piperno, D. R., Pearsall, D. M. (1998). The Silica Bodies of Tropical American Grasses : Morphology, Taxonomy, and Implications for Grass Systematics and Fossil Phytolith Identification. *Smithsonian Contributions to Botany*, 85(85), 1–40.
- Prasad, V., Strömberg, C. a E., Alimohammadian, H., Sahni, A. (2005). Dinosaur coprolites and the early evolution of grasses and grazers. *Science*, 310(5751), 1177–80.
- Quigley, K. M., Donati, G. L., Anderson, T. M. (2016). Variation in the soil “silicon landscape” explains plant silica accumulation across environmental gradients in Serengeti. *Plant and Soil*.
- Sangster, A. G., Perry, D. W. (1969). Some factors in relation to bulliform cell silicification in the grass leaf. *Annals of Botany*, 33(2), 315–323.
- Saqib, M., Zorb, C., Schubert, S. (2008). Silicon-mediated improvement in the salt resistance of wheat (*Triticum aestivum*) results from increased sodium exclusion and resistance to oxidative stress. *Functional Plant Biology*, 35(7), 633–639.
- Schneider CA, WS Rasband, KW Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9, 671-675.
- Schoelynck, J., Struyf, E. (2015). Silicon in aquatic vegetation. *Functional Ecology*, 30, 1323–1330.
- Soininen, E. M., Bråthen, K. A., Jurdado, J. G. H., Reidinger, S., Hartley, S. E. (2012). More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. *Oikos*, 122(1), 30–41.
- Sonobe, K., Hattori, T., An, P., Tsuji, W., Eneji, E., Tanaka, K., Inanaga, S. (2009). Diurnal Variations in Photosynthesis, Stomatal Conductance and Leaf Water Relation in *Sorghum* Grown with or without Silicon under Water Stress, (January 2008), 433–442.

- Struyf, E., Smis, A., van Damme, S., Meire, P., Conley, D. J. (2010). The global biogeochemical silicon cycle. *Silicon*, 1(4), 207–213.
- Trembath-Reichert, E., Wilson, J. P., McGlynn, S. E., Fischer, W. W. (2015). Four hundred million years of silica biomineralization in land plants. *Proceedings of the National Academy of Sciences*, 112(17), 5449–5454.
- Vaculík, M., Lux, A., Luxová, M., Tanimoto, E., Lichtscheidl, I. (2009). Silicon mitigates cadmium inhibitory effects in young maize plants. *Environmental and Experimental Botany*, 67(1), 52–58.
- Wainwright, M. (1997). The neglected microbiology of silicon - From the origin fo life to an explanation for what Henry Charlton Bastian saw. *Quarterly Review in Microbiology*, 24(3), 83–85.
- Wedepohl, K. H. (1995). Composition of the Continental Crust. *Geochimica et Cosmochimica Acta*, 59(7), 1217–1232.
- Wen-bin, L. I., Xin-hui, S. H. I., He, W., Fu-suo, Z. (2004). Effects of Silicon on Rice Leaves Resistance to Ultraviolet-B. *Acta Botanica Sinica*, 46(6), 691–697.
- Wright, I. J., Ackerly, D. D., Bongers, F., Harms, K. E., Ibarra-Manriquez, G., Martinez-Ramos, M., Wright, S. J. (2007). Relationships among ecologically important dimensions of plant trait variation in seven neotropical forests. *Annals of Botany*, 99(5), 1003–1015.
- Xu, Z., Zhou, G. (2008). Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *Journal of Experimental Botany*, 59(12), 3317–25.
- Yoshida, S., Ohnishi, Y., Kitagishi, K. (1962). Chemical forms, mobility and deposition of silicon in rice plant. *Journal of Soil Science and Plant Nutrition*, 8(3), 15–21.

Tables and Figures

Table 1. Soil properties of the four soils used in the pot study. Properties were measured from a bulk sample pooled from 4 soil cores taken at each site. Coordinates are provided in Universal Transverse Mercator (UTM) units for the 36S grid cell, and “WRB subgroup” represents an international taxonomic soil classification provided by the World Reference Base. ASi = amorphous Si (easily weathered pool), and DSi = dissolved Si (plant-available pool).

Site name	X coord	Y coord	WRB subgroup	ASi	DSi	pH	Sand	Clay	Organic matter
Barafu	742081	9710247	Andosol	14.88	0.114	7.7	32.98	12.54	5.04
Lamai	712279	9828882	Leptosol	6.37	0.081	5.86	59.65	20.25	5.15
Musabi	671463	9748430	Solonetz	4.59	0.088	7.23	32.66	26.20	5.02
Togoro	730299	9765640	Phaeozem	3.92	0.039	6.2	74.14	11.28	2.82

Table 2. Loadings (Eigen values) of PCs 1-5 for the ten traits included in the principal components analysis. Values < 0.10 are not shown. *SD* = stomatal density, *R:S ratio* = root:shoot ratio, and *Max. sus. g_s* = maximum sustained stomatal conductance (described in text).

Trait	PRINCIPAL COMPONENT AXIS				
	1	2	3	4	5
Max. height	-0.48				-0.22
Max. length	-0.49		-0.13	-0.14	
LMA	-0.11		-0.59	-0.53	-0.38
Abaxial SD	0.29	0.42			-0.38
Adaxial SD	0.27	-0.30	0.40	-0.28	-0.50
Ab:Ad SD ratio		-0.58	0.34	-0.14	-0.11
Root biomass	-0.39	0.27	0.36	0.17	-0.28
Shoot biomass	-0.33	-0.27		0.52	-0.30
R:S ratio	-0.15	0.49	0.43	-0.26	
Max. sus. g _s	-0.27	-0.13	0.18	-0.47	0.47

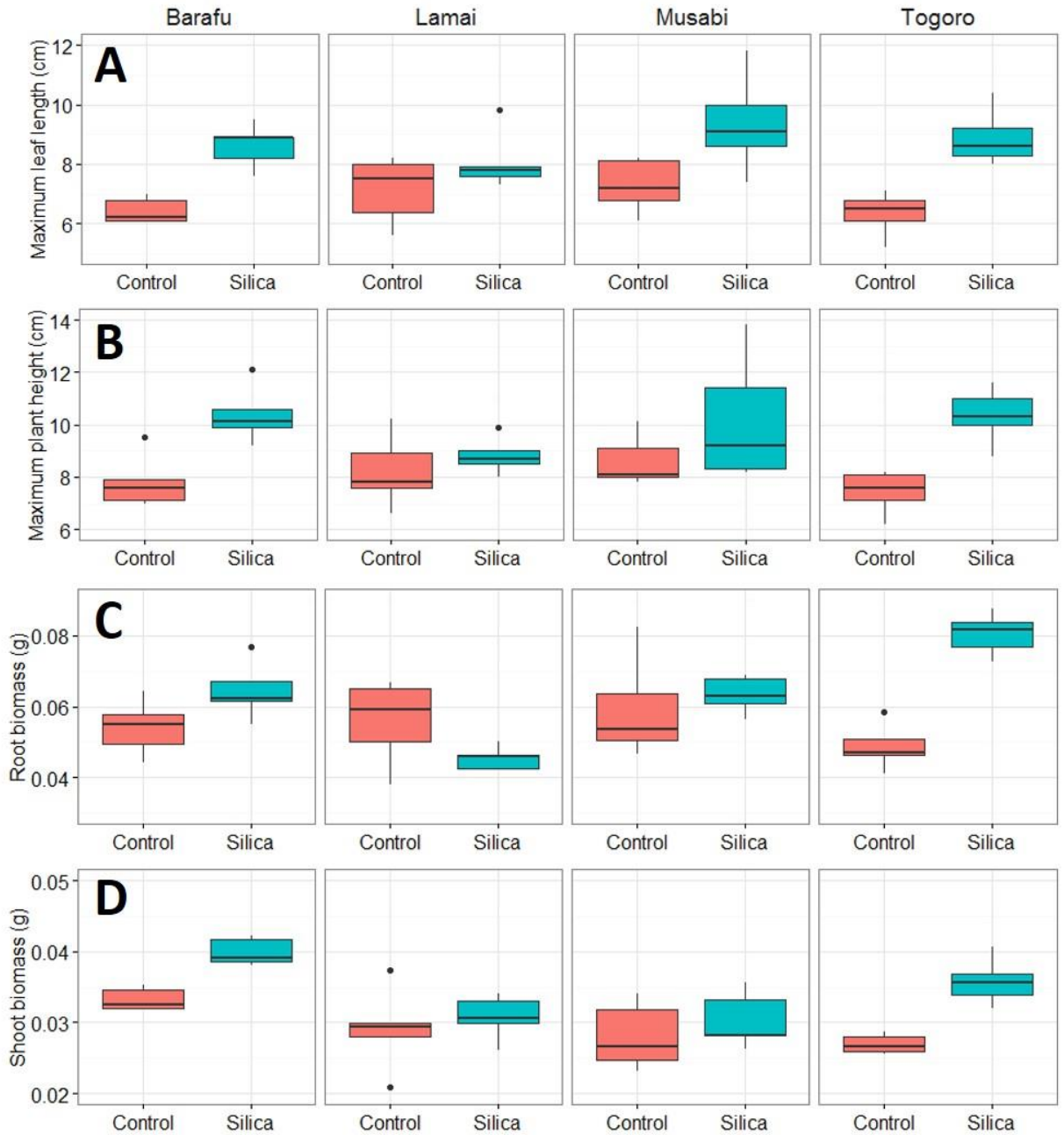


Figure 1. Response of growth parameters to silicon application: A) maximum leaf length, B) maximum plant height, C) root biomass, and D) shoot biomass. Soils are arranged alphabetically from left to right by collection site: Barafu, Lamai, Musabi, Togoro.

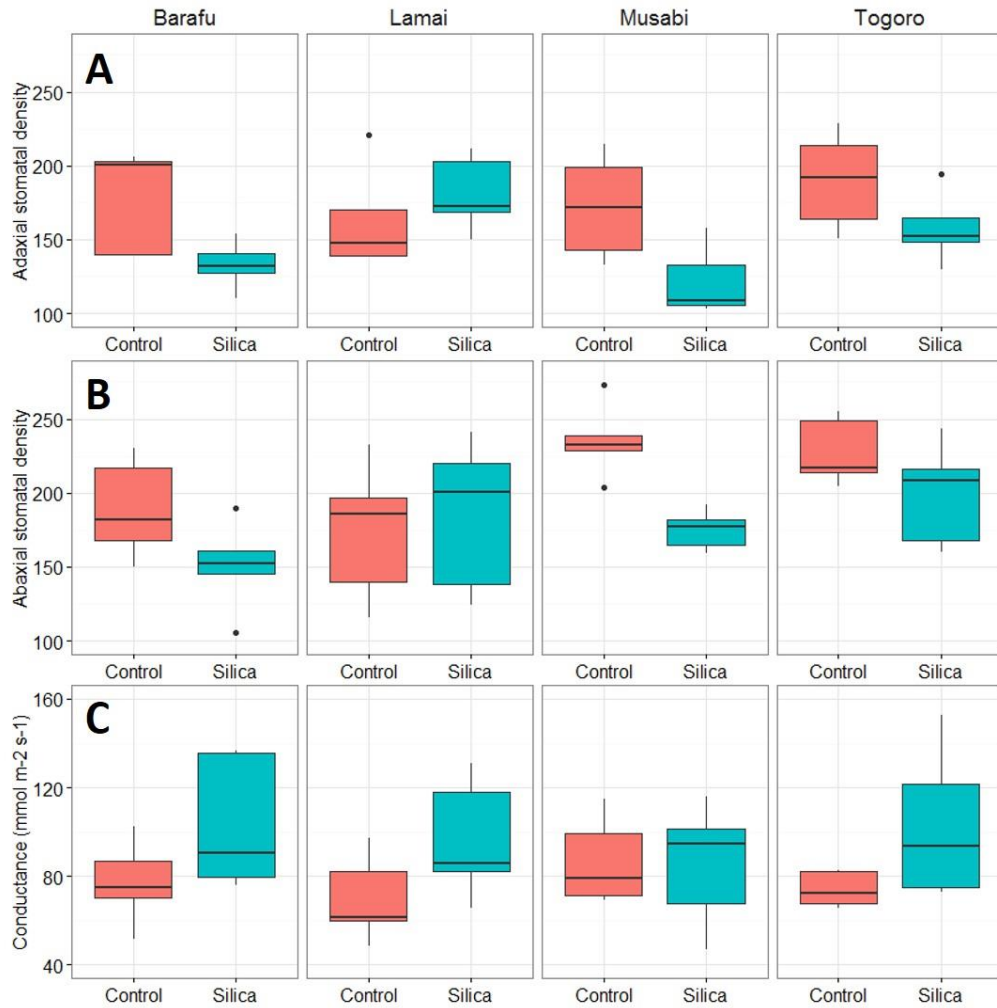


Figure 2. Stomatal densities (as n pores · mm⁻²) of adaxial (A) and abaxial (B) leaf surfaces of sorghum grown with and without the addition of silicate fertilizer. Each soil type had five replicates per treatment group, and each replicate was the pooled response of 3 pseudoreplicates grown in the same seed tray cell. Panel C shows stomatal conductance (averaged from time $t=2$ and time $t=4$; see supplement S2) in response to treatments. Mixed models indicated that these three traits were all significantly affected by Si treatment when allowing for a random effect of soil (see text).

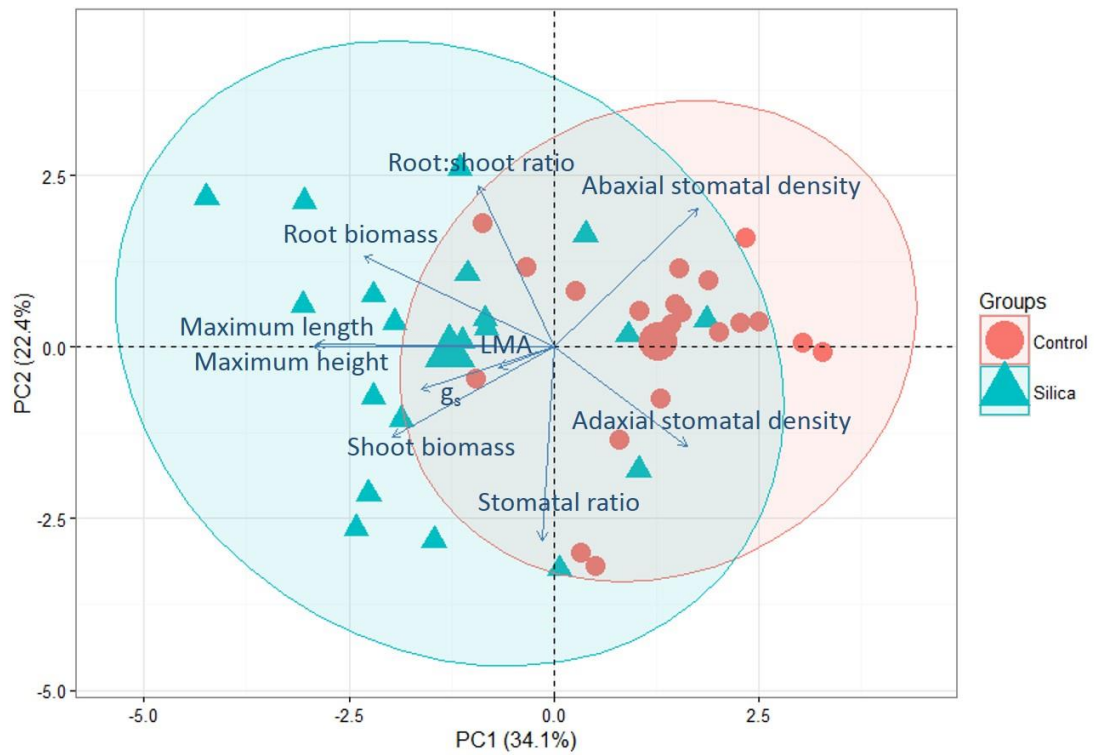


Figure 3. Clustering of control and +Si treatments along the first two principal components axes. Large symbols represent the centroid of each ellipse. LMA = leaf mass per area, and g_s = sustained maximum stomatal conductance. For the loadings of individual traits along these axes, see Table 2.

CHAPTER 2

LEAF SILICA CONCENTRATION IN SERENGETI GRASSES INCREASES WITH WATERING BUT NOT CLIPPING: INSIGHTS FROM A COMMON GARDEN STUDY AND LITERATURE REVIEW

The following manuscript was published in *Frontiers in Plant Science* (2014), and is reprinted with permission from Frontiers Media publishing. Stylistic variations are due to the requirements of the journal.

ABSTRACT

Grasses (Poaceae) lack the complex biochemical pathways and structural defenses employed by other plant families; instead they deposit microscopic silica (SiO₂) granules in their leaf blades (i.e. phytoliths) as a putative defense strategy. Silica accumulation in grasses has generally been considered an inducible defense; other research suggests silica accumulation occurs by passive diffusion and should therefore be closely coupled with whole plant transpiration. We tested the hypothesis that grasses increase leaf silica concentration in response to artificial defoliation in a common garden study in the Serengeti ecosystem of East Africa. Additionally, a watering treatment tested the alternative hypothesis that leaf silica was largely driven by plant water status. Leaf silica content of two dominant C4 Serengeti grass species, *Themeda triandra* and *Digitaria macroblephara*, was quantified after a 10-month clipping x water experiment in which defoliation occurred approximately every two months and supplementary water was added every two weeks. *Themeda* had greater silica content than *Digitaria*, and *Themeda* also varied in foliar silica content according to collection site. Clipping had no significant effect on leaf silica in either species and watering significantly increased silica content of the dominant tall grass species, *Themeda*, but not the lawn species, *Digitaria*. Our data, and those collected as part of a supplementary literature review, suggest that silicon induction responses are contingent upon a combination of plant identity (i.e., species, genotype, life history limitations) and environmental factors (i.e., precipitation, soil nutrients, grazing intensity). Specifically, we propose that an interaction between plant functional type and water balance plays an especially important role in determining silica uptake and accumulation.

Keywords: grass, grazing, silica, defoliation, induced defense, herbivory, phytoliths

INTRODUCTION

Plants have two general and non-mutually exclusive adaptive strategies to cope with herbivory: tolerance and resistance (Mauricio *et al.* 1997). Tolerance implies the capability to survive and regrow following damage by herbivores. In contrast, resistance strategies serve to prevent or reduce damage by herbivores and may entail direct resistance via production of toxic or distasteful secondary metabolites or indirect resistance by avoiding herbivores altogether through reduced apparency (Feeney 1976). From a resource allocation standpoint, defensive structures (i.e. spines, thorns) and phytochemicals (i.e. tannins) associated with direct herbivore resistance are costly because they require energy and nutrient resources that could otherwise be invested in growth or reproduction. Thus, while some defenses are constitutively expressed, many other plant defenses are induced only after damage is experienced as a way to reduce their costs (Agrawal 1998, Ariumura *et al.* 2000, Massey *et al.* 2007).

Some plant groups face relatively intense or frequent herbivory and utilize both tolerance and direct resistance strategies. For example, many species of grasses (family *Poaceae*) experience herbivory in the form of defoliation by large-bodied mammalian grazers, resulting in frequent and significant tissue loss (Gibson 2009). Due to rapid regrowth from a basal intercalary meristem following defoliation, grasses are highly resilient to grazing stress and are typically considered grazing “tolerators” (Stebbins 1972; Oyarzabal & Osterheld 2009; McNaughton 1979). On the other hand, grasses also utilize both secondary chemicals, e.g. phenolics (Schaller *et al.* 2012), and structural components, e.g. microscopic deposits of solid silica termed phytoliths (Ma & Yamaji 2006), to deter herbivores. Evidence suggests that phytoliths have been present in grasses since their early evolution, as long ago as the Late Cretaceous (Prasad *et al.* 2005, Stromberg 2011). Accumulation of silica phytoliths has been considered the main

defensive strategy of grasses (Coughenour 1985), as they can amass relatively large amounts of silica and lack chemical defenses as compared to dicotyledonous plants. However, other than grasses, silica accumulation occurs primarily in ancient plant groups such as mosses, ferns, and horsetail (Hodson et al 2005), and this fact, along with the observation that tooth enamel is considerably harder than phytoliths (Sanson et al. 2007) raises questions about the efficacy of silica as a deterrent of large-bodied mammalian grazers. Among angiosperms, Poales (the group containing grasses) are the principal silica accumulators, with wetland Gramineae accumulating up to 15% dry weight silica (Epstein 1999). Biogenic silica is assimilated when roots absorb silicic acid ($\text{Si}(\text{OH})_4$) from soil water, and solid amorphous silica (SiO_2) bodies precipitate in target cells of the epidermis as transpiration occurs (see Rudall et al 2014). Phytoliths leave behind a three-dimensional impression of the cells that they inhabit and, because of their diagnostic nature, are often used in paleoecological reconstruction (Piperno & Pearsall 1998).

Both active and passive mechanisms for silica uptake have been documented within the plant kingdom; active exclusion has also been observed in dicotyledonous angiosperms (Jones & Handreck 1969). Passive uptake allows grass roots to absorb silicon in its aqueous form, silicic acid ($\text{Si}(\text{OH})_4$), from the soil solution and implies limited control over silica accumulation, with stomatal conductance largely determining foliar silica content (Sangster *et al.* 2001). While early researchers assumed that passive uptake, in which tissue silica concentration increases as a function of transpirational water loss (Sangster & Parry 1970, Street 1974), was the predominant mechanism for silica accumulation, several lines of evidence have since suggested that active uptake is also often involved. For example, specialized silicon efflux transporters have been identified in rice (Ma et al. 2006, 2007), maize, and barley (Mitani et al. 2009) amongst other species, and gene

expression of these transporters is positively correlated with Si absorption from the soil solution, implying an energetic cost associated with silicon transport in these high-silicon accumulators. Grass foliar silica content exhibits high intra- and interspecific variation (Hodson et al. 2005), often in ways that are correlated with disturbance regimes such as grazing and fire frequency (Melzer et al. 2009). These lines of evidence suggest that active Si transport may be an important, and prevalent, mechanism for silica accumulation within Poaceae. An active mechanism could prove especially beneficial if plants are able to respond to herbivory by increasing silica uptake and silica is an effective defense mechanism, as several studies have suggested. For example, high silica content in plant tissues interferes with digestion (Massey & Hartley 2009), is unpalatable as forage (Gali-Muhtasib et al. 1992, Cotterill et al. 2007) and reduces growth rates of small-bodied mammals (Massey & Hartley 2006). Consistent with the putative effects of silica on extant herbivores, paleontological research suggests that grazing Hadrosaurid dinosaurs evolved the most complex dentition known to date, at least in part due to a high silica diet (Erikson et al. 2012).

Inducible uptake of silica, via an active metabolic mechanism, may also prove advantageous for coping with several types of abiotic stress. In addition to its effects as a documented anti-herbivore compound, biogenic silica is known to alleviate plant stressors such as heavy metals (Galvez et al 1987), pathogenic pests (Fauteux et al 2006; Heine et al 2007), salinity (Zhu et al 2004), high temperatures and drought (Agarie et al. 1998). Thus, it is logical that uptake, if active, may also be inducible under particular environmental conditions. Water stress affects plants ubiquitously, and it appears that silica accumulation alleviates the deleterious effects of drought for grasses. Agricultural studies indicate that soil Si fertilization decreases stomatal conductance, and

thus transpirational water loss (Street 1974, Gao 2006), and enhances the stability of rice cell membranes exposed to drought (Agarie et al. 1998).

The beneficial effects of plant biogenic silica, together with the apparently low cost of assimilating and using silica as a defense have left some to conclude that silica has largely been overlooked as a vital element for stress tolerance (Cooke and Leishman 2011). Our study had two goals relating to the ecological significance of plant silica. First, we aimed to quantify the response of leaf silica accumulation to interactions between water availability and defoliation in two African C4 grass species, one (*Themeda triandra*) a caespitose “bunch” grass and the other (*Digitaria macroblephera*) a short “lawn” grass. To achieve this goal we conducted a common garden study in Serengeti National Park, a grazer-dominated ecosystem in East Africa, in which we manipulated defoliation and water-availability over a ten-month growing period and quantified the silica responses of our two focal species. Previous research on similar grasses from the Serengeti demonstrated that grasses are capable of up-regulated silica concentrations in response to herbivory (McNaughton 1983). Consequently, we hypothesized that defoliation would induce silica uptake in our two study species. In a different study of African savanna grasses, defoliated bunch grasses exhibited greater stomatal conductance and transpiration rates than defoliated lawn grasses (Anderson et al. 2013). Following these observations, we proposed a second prediction that, if silica uptake is directly linked to transpiration rate as previously reported, the bunch grass *T. triandra* would exhibit a greater silica induction following defoliation than would the lawn grass, *D. macroblephera*. Moreover, if silica accumulation is tightly coupled with transpiration rates, we expected to observe increased silica accumulation under conditions of higher soil moisture. Alternatively, if no relationship between soil moisture and silica concentration was observed, this suggests that active, energy-dependent

silica uptake mechanisms may predominate in these species. Finally, we explored the possibility that the silica content of grasses was driven, at least in part, by their growing environment (soils, climate, herbivory, etc.). Thus, we also tested whether the site of collection was significantly related to silica variation among grasses in our common garden study.

Our second aim was to understand the results of our study in relation to a literature review of all studies reporting defoliation effects on leaf silica concentration in grasses. Our goal here was to search for a broad ecological consensus of graminoid responses to leaf defoliation and if possible, establish generalities about grass-grazer interactions and the induction of silica plant defense.

METHODS

COMMON GARDEN STUDY

Study system

Our study was conducted in the Serengeti ecosystem in northern Tanzania (2° 19' 51" S, 34° 50' 0" E). Serengeti is characterized by *Acacia-Commiphora* dominated savanna vegetation in the north and west of the ecosystem and edaphic grassland on volcanic soils in the Serengeti plains in the southeast (White 1983). A rain-shadow created by the volcanic highlands to the south and east of Serengeti creates a relatively strong precipitation gradient, in which rainfall decreases from >1100 mm yr⁻¹ in the northwest near the shores of Lake Victoria to ~600 mm yr⁻¹ in the Serengeti plains at the base of the Ngorongoro crater in the southeast (**Fig 1**). Rainfall is highly seasonal and typically falls in two characteristic phases: the short rains, occurring from November-December, and the long rains, occurring from February-May (Anderson et al. 2008). The soils of the

plains are heavily influenced by recent (~20,000-1,500 ya) volcanic activity and are highly porous with an underlying calcium carbonate hardpan, resulting in highly saline and alkaline soils with poor moisture retention (Anderson & Talbot 1965, DeWit 1978). Interestingly, recent eruptions of nearby volcano Oldoinyo Lengai, which continue to enrich soils in the plains with ash, are of natrocarbonatite origin, meaning that, in addition to being highly enriched in potassium and sodium carbonates, they are extremely depleted of silica (Gittins 1998, Keller et al. 2010). In contrast, the woodlands to the north are derived from granitic and quartzite parent material, while the western regions of the park are characterized by red clays (Udisols) and black cotton soils (Vertisols) resulting from alluvial processes associated with Lake Victoria (Jager 1982).

Serengeti is a “model” grazing ecosystem: massive herds of migratory zebra (*Equus quagga boehmi*) and wildebeest (*Connochaetes taurinus*) exert strong defoliation effects on the grasses that dominate the herbaceous layer. However, the frequency and intensity of herbivory changes along the rainfall gradient, with the most frequent and intense herbivory occurring in the Serengeti plains and decreasing with rainfall. Other significant herbivore species in Serengeti include Thomson’s gazelle (*Eudorcas thomsonii*), which also migrate, and several non-migratory herbivores such as Grant’s gazelle (*Nanger granti*), hartebeest (*Alcelaphus buselaphus*) and topi (*Damaliscus korrigum*). Running counter to the gradient in herbivory is an opposing gradient in fire frequency and intensity created by the tall, highly flammable, bunch grasses in the north and the procumbent, grazing tolerant grasses in the plains to the southeast that rarely burn due to their low aboveground biomass (Anderson et al. 2012). The two most dominant herbaceous species in Serengeti, in terms of both biomass and frequency, are the grasses *Themeda triandra*, a tall, caespitose bunch grass, and *Digitaria macroblephara*, a short-stature, grazing tolerant lawn grass.

Experimental design

In of June 2008, multiple individuals of *T. triandra* and *D. macroblephera*, were collected from 4 grassland sites spanning the ecosystem's soil and rainfall gradients and transplanted into a common garden constructed at the Serengeti Wildlife Research Centre (SWRC; **Fig 1**). *D. macroblephera* was collected from Barafu (BRS) in the southeastern plains, *T. triandra* was collected from Klein's camp (KCW) in the northern corridor, and both species were collected from the Togoro plains (TOG) and Musabi plains (MSB). Grasses were planted in three replicate blocks; each block consisted of a 6 x 9 m fenced area cleared of all vegetation and included six 2 x 2 m equally spaced plots. Three randomly selected individuals of *T. triandra* and *D. macroblephera* (one from each collection site) were planted in each plot. Mean \pm SE tiller number of initial *D. macroblephera* individuals was 13.59 ± 0.67 , and *T. triandra* transplants had a mean \pm sd = 9.08 ± 0.74 tillers. Plots were randomly assigned to one of the six combinations of clipping (two levels: clipped and unclipped) and watering (three levels: high, ambient and low). Water was manipulated by diverting rainfall with five 25 cm strips of clear plastic roof material placed across the plots at ~6cm height and slanted slightly to promote water runoff; the treatments were designed to intercept approximately 50% of ambient rainfall. Grasses were planted between the roof strips and at least 45 cm from the nearest grass to provide room for growth and to prevent light competition. To control for any potential effects of the roofing material, all plots were overlaid by the roofing material but holes were drilled in the high and ambient treatments so that no water was diverted and all rainwater reached the plot. Rainfall was collected, and the high water treatment was augmented with an amount that totaled ~150% of ambient every two weeks. Rainfall at SWRC is approximately 700 mm yr⁻¹ and the high water treatment was augmented to approximately 1000 mm yr⁻¹. Soil moisture at a depth of 12 cm was estimated via time-

domain reflectometry monthly in each plot with a FieldScout TDR 100 (Spectrum Inc.; Aurora, IL). In the clipping treatment, plants were defoliated to approximately 50% of their aboveground biomass every two months. Clipped biomass was dried, weighed and summed with harvested biomass to arrive at a final biomass for clipped plants. Unclipped plants were not defoliated throughout the entire duration of the experiment. Plants were grown in the common garden for 10 months, until they were harvested in April 2009. All plants still alive at the harvest were separated into above- and belowground fractions; the aboveground fraction was further separated into leaf and stem. All plant material was dried and weighed in the lab at SWRC; leaf fractions were transported to the US, ground in a Cyclone sample mill (UDY corp.; Fort Collins, CO) and stored until they were analyzed for leaf silica at Wake Forest University.

Biogenic silica quantification

Silicon (Si) content of leaf tissue was quantified by plasma spectroscopy (ICP-OES) after autoclave-induced digestion (AID) following the methods of Kraska and Breitenbeck 2010 (modified from Elliot & Snyder 1991). Briefly, 100mg of dried, ground sample was wetted with 1-octanol in a 50ml vortex tube. Next, 2mL of 50% H₂O₂ and 3.5mL of 50% NaOH were added. Samples were vortexed several times, until the reaction ceased, and autoclaved loosely capped at 121°C (20psi) for one hour. Deionized water was added to 50mL. Samples were brought to acidic pH using concentrated HCl, and diluted 1:10 in deionized water before analyzing by ICP-OES. Silicon content of samples was calculated by fitting peak intensity at 251nm to a standard curve (0.1ppm-10ppm Si; $r^2 \geq 0.998$). The standard curve was validated with a reference material of *Schizachyrium scoparium* (14g/kg Si). Silicon values were converted to silica (SiO₂) content by dividing by a conversion factor of 0.4674, since this more commonly reported value represents biogenic silica (phytolith) content of plant tissue.

Statistical analysis

A major goal of our statistical analysis was to understand how defoliation, water addition and their interaction, influenced leaf silica concentration in these two dominant Serengeti grasses. Therefore, we used linear mixed effect (LME) models to test the effects of species, clipping, and water on foliar silica accumulation. The model included species, clipping and water as fixed factors, while block was included as a random effect to account for the spatial design. Analysis was performed using the `lmer` function in *lme4* package of R statistical environment version 2.11.1 (Bates et al. 2008, R Development Core Team), with the original model structure: `lmer(SiO2 ~ species * clip * water + (1|block))`, where 'species*clip*water' represents the three-way fixed interaction effect and '(1|block)' represents the random intercepts that are estimated for each block. The function 'step' from the R package *lmerTest* was used to simplify the model so that only significant fixed effects remained in the model, and pairwise contrasts for individual treatment effects were subsequently calculated using the 'ghlt' command and a Tukey's test from the R package *multcomp* (Hothorn et al. 2008).

A second model was employed to explore the extent to which collection site could explain variation in foliar silica content for both species. Because we did not have sufficient replication of individual grasses at the site level, we could not explore the effects of site in a full model crossed with our other treatments. Consequently, we used a reduced statistical model to explore the main effects of site on leaf silica for each individual species averaged over all levels of water and defoliation. The model we employed for each species separately was: `lmer(SiO2 ~ site + (1|block))`. Model simplification was again conducted via the 'step' function in R.

Finally, soil moisture was statistically compared across treatments using a model with water as a fixed effect and time and block as random effects using the `lmer` function in

lme4 package of R as described above; the 'ghlt' command from the R package *multcomp* was used to conduct a Tukey's posthoc comparison of means.

LITERATURE REVIEW

In order to identify general trends in plant response to defoliation, we conducted an extensive search of primary literature sources to identify studies which provided data on silica content of Poales under both control and defoliation treatments. We used Google Scholar and Web of Science to identify appropriate primary research articles by using combinations of search terms such as 'clipping', 'grazing', 'defoliation', 'silica', and 'phytolith'. Within the order Poales, defoliation studies were only identified for grasses, so the final literature review is limited to Poaceae. We restricted our search to studies containing species-specific silica values in order to avoid the potential confounding effects of differing community composition on bulk, plot level silica content. These studies included laboratory studies and field studies in which grazing was experimentally prevented by herbivore exclosures. Grazing studies encompassed insect, small mammal, and large mammal herbivory, and the intensity of defoliation varied within and among studies (see discussion). Species-specific silicon values were converted to SiO₂ when necessary, assigned a unique identifier, and plotted as the log ratio normalized difference between defoliated and non-defoliated plants at the species level. Studies were then organized by defoliation method and grazer type and assigned to a general defoliation response category to facilitate interpretation of the silica response. Those cases with a $\geq 20\%$ increase in silica following defoliation were assigned a "+", those which decreased $\geq 20\%$ were assigned a "-", and those exhibiting less than a 20% relative change in silica content were assigned a "0".

RESULTS

SERENGETI COMMON GARDEN

Mean \pm SE soil moisture values were $11.1 \pm 1.6\%$ for the high, $9.8 \pm 1.5\%$ for the ambient and $9.7 \pm 1.5\%$ for the low watering treatment. Posthoc statistical comparisons with a Tukey's test demonstrated that the low and ambient water treatments were not statistically different from one another (difference = -0.1, $z = -0.26$, $p = 0.96$), but that the high water treatment was statistically greater than the low (difference = 1.4, $z = -2.9$, $p = 0.007$) and ambient (difference = 1.3, $z = 2.73$, $p = 0.01$) treatments. Therefore, the ambient and low water treatments are combined for the remainder of the manuscript (referred to as 'ambient' from here onward) and compared to the high water treatment in all analyses of watering effects.

Both the bunch-grass *T. triandra* and lawn-grass *D. macroblephara* were relatively high Si-accumulators: *T. triandra* had a mean \pm SE foliar SiO₂ content of $3.7 \pm 0.25\%$ dw ($n=20$), while *D. macroblephara* had a mean \pm SE SiO₂ of $2.7 \pm 0.10\%$ ($n=41$). Neither species showed a significant response to clipping as a main effect ($p=0.976$) or as an interaction effect when clipping was crossed with watering level ($p=0.453$; **Fig 2**). However, there was a significant species by watering interaction effect ($p<0.05$, **Table 1**) indicating that leaf silica concentrations of the two species differed in response to the watering treatment. This result arose because individuals of *T. triandra* that were watered had a higher leaf silica concentration ($4.4 \pm 0.3\%$) compared to those *T. triandra* plants that were maintained at ambient soil moisture levels ($3.3 \pm 0.3\%$). In contrast, no significant change in SiO₂ content was observed between watering treatments for *D. macroblephara* (**Fig 2**).

In the second analysis, in which the statistical model included the main effects of plant collection site for each species separately, *D. macroblephera* exhibited considerable within-site variability and consequently no statistically significant differences across sites. In contrast, the SiO₂ content of *T. triandra* plants varied significantly among sites (**Fig 3**; see **Fig 1** for sites): Silica was lowest for plants collected from TOG (mean \pm SE = 2.67 \pm 0.63%), intermediate for plants from MSB (mean \pm SE = 3.97 \pm 0.55%), and highest for plants from KCW (mean \pm SE = 4.18 \pm 0.53%). Independent contrasts indicated that grasses from the low rainfall site TOG accumulated significantly less silica than those collected from MSB (difference = -1.30, z = -2.822, p = 0.005) and KCW (difference = -1.51, z = -2.408, p = 0.016).

LITERATURE REVIEW

We identified 11 studies conducted from 1974-present which recorded species-specific silica induction under control and defoliation treatments. These studies represented 34 “cases” of potential silica induction for 15 different grass species. (**Table 2**). Due to the limited number of studies conducted and lack of species-specific replication, we could not employ meta-analytical statistical approaches; instead, general responses were considered and interpreted. Eleven of fifteen species surveyed are C3, and all are perennials, except for *Poa annua*. Silica content of surveyed species ranged from < 0.5% dry weight (non-defoliated *Anthoxanthum odoratum*) to >7% (grazed *Pascopyrum smithii*, *Deschampsia caespitosa*, and *Eustachys paspaloides*). To avoid pseudoreplication, we reported silica values for seasonal or site-level maxima for the three studies which reported multiple species-specific responses to the same defoliation method (Brizuela & Detling 1986, Cid et al. 1990, Banuelos & Obeso 2000). The majority of studies surveyed suggest that plants are able to respond to defoliation by altering their silica uptake, and indeed we observed an overall trend of silica induction following

defoliation (**Fig 4**). Despite this general trend, several studies found no significant change in silica content of defoliated grasses, defined here as less than a 20% change in foliar silica content in either direction. The literature review revealed substantial variation in the silica responses of the different species studied. For example, the C3 grass *Festuca* increased silica content more than 450% in response to herbivory by voles (Massey 2007), while silica content of *Pascopyrum* decreased by approximately 50% following clipping (Cid 1990). In general, the magnitude of induced silica uptake was greater under natural defoliation (*i.e.*, grazing) than for mechanical clipping. Further, the magnitude of silica increase when defoliation stimulated uptake much greater than the decrease in silica levels observed in the two studies in which clipping triggered down regulation of silica levels.

DISCUSSION

VARIATION IN SILICA BETWEEN *THEMEDA* AND *DIGITARIA*

Both species documented in this study were within the typical silica range for dryland grasses, but *Themeda* had significantly greater silica content than *Digitaria*. These two species have different suites of anatomical and physiological traits and may be considered distinct graminoid functional types (Coughenour 1985, Diaz & Cabido 1997). Lawn grasses, such as *Digitaria*, exhibit clonal, stoloniferous growth; their prostrate growth form allows them to reduce tissue loss to herbivores while quickly spreading horizontally to form lawns under high soil fertility (Cromsigt & Olff 2008). In contrast, the higher lignin content of tall, dense, and slow-growing bunch grass species, like *Themeda*, makes them less palatable to herbivores than lawn grasses (Sinclair & Norton-Griffiths 1979). Thus, as a consequence of their different life history strategies, it

is not surprising that these two species differ in the degree to which silica accumulates in their leaves.

As a documented growth promoter (Isa et al. 2010, McNaughton et al. 1985) and metabolically “cheap” structural substitute for carbon-based compounds such as lignin (Raven 1983, Cooke & Leishman 2011), silica deposition may provide an alternative mechanism for accelerated growth which would prove especially beneficial for bunch grasses. The increased growth rates that result from silica accumulation may substantially improve light interception of slow-growing species which primarily compete for sunlight (Ando et al. 2002). This idea is further supported by a positive correlation between leaf length and silica content observed in *Spartina* (Querne et al. 2012), again supporting the idea that silica allows improved growth without requiring significant carbon investment (Cooke & Leishman 2012). Further, slow-growing bunch grasses display greater leaf mass per area, LMA, (Fynn et al. 2011) and leaf dry matter content, LDMC, (Anderson et al. 2013) than fast-growing lawn species, indicating a greater relative investment in dry matter. The worldwide leaf economic spectrum (Wright et al. 2004) predicts a strong positive correlation between dry matter investment and leaf longevity, suggesting that, based on their higher LMA, bunch-grass species likely also have leaves of greater longevity than lawn-grass species. Thus, it may be beneficial for relatively long-lived bunch grasses to invest in immobile phytoliths (Endara & Coley 2011) which allow for rapid augmentation of cell structure of C-limited species under intense light competition. On the other hand, recent research revealed a negative correlation between leaf lifespan and silicon concentration when considering a broad range of plant functional types (Cooke & Leishman 2011). The reasons for differential responses to defoliation and high interspecific variation in this qualitatively ubiquitous defense within Poaceae remain unclear. Here we focused on two species representing

extremes of each of the two grass growth forms (bunch, lawn); greater sampling from along the gradient of short to tall grasses is needed to better understand the influence of these contrasting physiological forms on silica accumulation.

DEFOLIATION RESPONSE

Contrary to our initial predictions, clipping did not result in silica induction for either the lawn or bunch grass species, with nearly identical mean silica content observed for defoliated and non-defoliated individuals. There are several possible explanations for the lack of silica induction observed in our Serengeti common garden study. First, the frequency and/or intensity of defoliation may not have been great enough to elicit a response. For example, in the Serengeti plains, more than 1 million migratory ungulates are present in dense herds during the wet season, and localized grazing “hotspots” support dense spatially and temporally stable grazer communities that inflict especially frequent and intense herbivory on plant communities (Holdo et al. 2009, Anderson et al. 2010). Our literature review further corroborates this notion. For example, a laboratory study by Massey and colleagues revealed that a single defoliation event did not induce silica uptake, while repeated defoliation by herbivores (16 events total) significantly increased silica content of ryegrass and fescue, revealing the importance of both frequency and duration of defoliation (Massey *et al.* 2007). Extent of tissue removal (5% vs. 25%) is also proven to affect both induction and relaxation of silica response, demonstrating a threshold effect required for induction (Reynolds 2012). In our study, grasses were defoliated on a bi-monthly basis, four times total. For at least certain areas of Serengeti, this may be lower than levels of natural grazing intensity due to Serengeti’s rich herbivore fauna.

In addition, manual defoliation (i.e. clipping) likely does not elicit the same response as natural grazing under laboratory or field conditions, a well-known effect with respect to

induced defenses. Our literature review revealed that clipping and grazing both resulted in silica induction in ~50% of the studies (5 of 11 clipping cases, 12 of 23 grazing cases), but the magnitude of this response was much greater under grazing defoliation, as illustrated in **Figure 4**. Among grazing studies, silica induction was observed in response to insect, small mammal, and large mammal grazing. We propose that clipping may not be sufficient to represent both the direct and indirect effects of grazing on silica dynamics in grasses; this may be especially true for grass species growing in the Serengeti plains, which have a long co-evolutionary history with herbivores. Large-bodied grazers compact soils, resulting in increased bulk density (e.g., Bakker et al. 2004, Holdo and Mack 2014, Veldhuis et al. 2014) and matric water potential and, thus, alter the ability of roots to absorb water. While manual defoliation studies yield information about certain physiological responses, the literature review presented here suggests that this approach may not properly mimic the complex physiological responses associated with natural grazing, some of which may result from indirect effects associated with modification of soil water (see below). In summary, plants perceive herbivore effects through a suite of signals, some of which, such as changes in the abiotic environment, are not elicited directly by the removal of tissues.

WATER AND COLLECTION SITE EFFECTS

Themeda individuals in the high water treatment accumulated significantly greater foliar silica than those in the ambient water group (**Fig 2**), but this trend was not observed for *Digitaria*. This suggests either differential silica uptake mechanisms of the two species or different generalized physiological responses to drought which indirectly influence silica accumulation. Since *Themeda* responded to changes in water availability, we hypothesize that active uptake mechanisms are present in this species which allow it to increase uptake of silicic acid from the soil solution when water is abundant. If true, this

then raises the question, why would *Digitaria* not also increase uptake of a ubiquitously beneficial element under similar conditions? One possible explanation is that the different life histories of these two species, coupled with the proposed costs of silica accumulation (Cooke & Leishman 2012) have resulted in selection for differential silica uptake mechanisms, in which tall, bunch species but not short, lawn species, have incorporated active silicon uptake mechanisms such as the use of specialized energy-dependent transport molecules. *Digitaria* has highly flexible leaves which are capable of curling under drought conditions to prevent transpiration; this drought tolerance mechanism would likely be hindered due to the loss of bulliform cell function associated with leaf tissue silicification (Honaine & Osterrieth 2011). Conversely, the benefit of improved drought tolerance associated with silica accumulation (Gao et al. 2006, Eneji et al. 2008) is likely of greater relative importance for bunch grass species, such as *Themeda*, which experience intense light competition and a significant seasonal water deficit. Increased silica uptake under high water availability may serve to buffer bunch grasses against future drought events while simultaneously producing an erect canopy structure (see Isa 2010) for light competition and offsetting carbon costs. Under this proposed hypothesis, that bunch grasses benefit more than lawn grasses due to more intense light competition, any anti-herbivore benefits are secondary to the direct adaptive significance of silica accumulation, which is related to water and/or light limitation

Consistent with the observed effects of watering treatments, the silica differences among collection sites also indicate that water availability may be an important determinant of grass foliar silica content. *Themeda* individuals collected from high rainfall sites KCW and MSB, where light competition is expected to be more intense, exhibited significantly greater silica content than individuals collected from the low rainfall site TOG, where light competition is expected to be less intense. In contrast, the leaf silica content of *Digitaria*

individuals was not influenced by collection site (**Fig 3**). This observation reinforces the notion that water strongly influences foliar silica content of grasses and implies a pattern of increasing silica content of tall bunch grass species, like *Themeda*, with increasing distance from the plains and increasing light competition.

Other possible explanations exist for the observed site-level variation in silica content of *T. triandra*. Numerous fertilization studies have indicated that plant silica content is strongly correlated with availability of silicic acid in the soil medium (Jones & Handreck 1969, Fox et al. 1969, Van Der Vorm 1980, Gali & Smith 1992), and more recently, ecological studies have demonstrated a similar relationship between soils and plants in natural systems. Biogenic silica content of grasses in a South African savanna was higher for plants collected from basaltic soils than for those collected from granitic soils, reflecting differences in weatherability and dissolved silica (DSi) content of these contrasting parent materials (Melzer et al. 2011). Cooke and Leishman compared foliar silica content of plant communities from Hawkesbury sandstone and a nearby diatrema forest with nearly three times as much plant available soil silicon; though not statistically significant, they observed a general trend of higher foliar silica content for plants from the diatrema site, including a five-fold difference in leaf silica observed for the sedge *Schoenus melanostachys* (2012). The unique nature of the silica-depleted carbonatite ash that enriches the Serengeti plains may contribute to a gradient in available soil silicon, in which available silicon increases toward the northern and western corridors of the park. However, more work is needed to document how soil silicon availability varies within SNP and how it may interact with gradients in soil moisture, pH, and other important soil nutrients known to vary across the landscape (DeWit 1978, Jager 1982). Finally, we caution that, due to geographic separation, plant genotype should also vary

among sites (Soininen 2013), and we cannot rule out genotypic variation as an important influence on silica accumulation patterns.

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

The results of our literature review suggest that induced silica response to defoliation is dynamic and dependent upon interactions with additional biotic and abiotic factors. For example, defoliation by burning resulted in more than a 1/3 decrease in silica content of *Aristida* grass (Dufek et al. in press). Moreover, seasonal (Carey 2013, Georgiadis 1990, Georgiadis 1988, Struyf 2005) and inter-annual (Morton 1974, Pakkala 2000) variations in field measurements of grass silica content indicate that leaf tissue silicification is highly plastic. While seasonal fluctuations in grass silica content are well-documented, it remains unclear whether dynamic silica values measured in the field occur in response to changes in grazing intensity, temperature, precipitation, or, most likely, a complex interaction among these stochastic variables.

Silica accumulation is considered a central axis of grass-grazer coevolution throughout the literature, often cited as a driving force behind the evolution of hypsodonty and the coupled taxonomic radiation of grasses and grazers (Mendoza & Palmqvist 2008, Hummel et al. 2010). The evolution of large-bodied mammalian grazers is thought to have selected for specialized traits of the Poaceae such as meristematic growth from a well-protected crown, rapid growth rate, and high levels of silicon accumulation (McNaughton 1979, Coughenour 1985). Likewise, these plant traits are thought to have influenced herbivore dentition, digestion, and behavior (McNaughton 1984; Massey and Hartley 2006; Erickson et al. 2012). Our study and literature review, however, suggest that the evolutionary significance of silica accumulation may be a remnant of tradeoffs associated with the leaf economic spectrum that resulted in the evolution of two contrasting grass functional types. Silica accumulation may have first served to improve

drought tolerance with herbivore deterrence as a secondary role. Fossil data support this notion: grass-dominated habitats preceded the appearance of North American and Mediterranean grazing specialists (Stromberg 2006, Stromberg et al. 2007), and an investigation of the Gran Barranca fossil record indicates that open grass habitats were not necessary pre-conditions for favoring early hypsodont mammals in earth's earliest grass-dominated systems (Stromberg et al. 2013).

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REFERENCES

- Agarie, S., N. Hanaoka, O. Ueno, A. Miyazaki, F. Kubota, W. Agata, and P.B. Kaufman. 1998. "Effects of Silicon on Tolerance to Water Deficit and Heat Stress in Rice Plants, Monitored by Electrolyte Leakage." *Plant Production Science* 1 (2): 96–103.
- Agrawal, Anurag A, and Matthew T Rutter. 1998. "Dynamic Anti-Herbivore Defense in Ant-Plants: The Role of Induced Responses." *Oikos* 83: 227–236.
- Anderson, G.D, and L M Talbot. 1965. "Soil Factors Affecting the Distribution of the Grassland Types and Their Utilization by Wild Animals on the Serengeti Plains, Tanganyika." *Journal of Ecology* 53 (1): 33–56.

- Anderson, T Michael, J Grant C Hopcraft, Stephanie Eby, Mark Ritchie, James B Grace, and Han Olf. 2010. "Landscape-Scale Analyses Suggest Both Nutrient and Antipredator Advantages to Serengeti Herbivore Hotspots." *Ecology* 91 (5) (May): 1519–29. <http://www.ncbi.nlm.nih.gov/pubmed/20503883>.
- Anderson, T Michael, Mark E Ritchie, and Samuel J McNaughton. 2007. "Rainfall and Soils Modify Plant Community Response to Grazing in Serengeti National Park." *Ecology* 88 (5) (May): 1191–201. <http://www.ncbi.nlm.nih.gov/pubmed/17536405>.
- Anderson, T. Michael, Yan Dong, and Samuel J. McNaughton. 2006. "Nutrient Acquisition and Physiological Responses of Dominant Serengeti Grasses to Variation in Soil Texture and Grazing." *Journal of Ecology* 94 (6) (November): 1164–1175. doi:10.1111/j.1365-2745.2006.01148.x.
- Anderson, T. Michael, Joey Shaw, and Han Olf. 2011. "Ecology's Cruel Dilemma, Phylogenetic Trait Evolution and the Assembly of Serengeti Plant Communities." *Journal of Ecology* 99 (3) (May 1): 797–806. doi:10.1111/j.1365-2745.2011.01795.x. <http://doi.wiley.com/10.1111/j.1365-2745.2011.01795.x>.
- Anderson, T.M., B.B. Kumordzi, Wimke Fokkema, H.V. Fox, and Han Olf. 2013. "Distinct Physiological Responses Underlie Defoliation Tolerance in African Lawn and Bunch Grasses." *International Journal of Plant Sciences* 174 (5): 769–778.
- Ando, Ho, Ken-ichi Kakuda, Hiroshi Fujii, Katsuya Suzuki, and Tatsuya Ajiki. 2002. "Growth and Canopy Structure of Rice Plants Grown under Field Conditions as Affected by Si Application Growth and Canopy Structure of Rice Plants Grown under Field Conditions as Affected by Si Application." *Soil Science and Plant Nutrition* 48 (3): 429–432.
- Arimura, G, R Ozawa, T Shimoda, T Nishioka, W Boland, and J Takabayashi. 2000. "Herbivory-Induced Volatiles Elicit Defence Genes in Lima Bean Leaves." *Nature* 406 (6795) (August 3): 512–5. doi:10.1038/35020072. <http://www.ncbi.nlm.nih.gov/pubmed/10952311>.
- Bakker, E S, H Olf, M Boekhoff, J M Gleichman, and F Berendse. 2004. "Impact of Herbivores on Nitrogen Cycling: Contrasting Effects of Small and Large Species." *Oecologia* 138 (1) (January): 91–101. doi:10.1007/s00442-003-1402-5. <http://www.ncbi.nlm.nih.gov/pubmed/14566555>.
- Banuelos, M.J., and J.R. Obeso. 2000. "Effect of Grazing History, Experimental Defoliation, and Genotype on Patterns of Silicification in *Agrostis Tenuis* Sibth." *Ecoscience* 7 (1): 45–50.
- Bezeau, L.M., A. Johnston, and S. Smoliak. 1966. "Silica and Protein Content of Mixed Prairie and Fescue Grassland Vegetation and Its Relationship to the Incidence of Silica Urolithiasis." *Canadian Journal of Plant Science* 46: 625–631.
- Brizuela, Miguel A, and James K Detling. 1986. "Silicon Concentration of Grasses Growing in Sites With Different Grazing Histories." *Ecology* 67 (4): 1098–1101.

- Carey, J C, and R W Fulweiler. 2013. "Nitrogen Enrichment Increases Net Silica Accumulation in a Temperate Salt Marsh." *Limnology and Oceanography* 58 (1): 99–111. doi:10.4319/lo.2013.58.1.0099.
- Cid, M S, J K Detling, a D Whicker, and M A Brizuela. 1990. "Silicon Uptake and Distribution in *Agropyron Smithii* as Related to Grazing History and Defoliation." *Journal Of Range Management* 43 (4): 344–346. <http://www.jstor.org/stable/3898929>.
- Coley, P.D., Bryant, J.P., Chapin, F.S. 1985. "Resource Availability and Plant Antiherbivore Defense." *Science* 230: 895–899.
- Cooke, Julia, and Michelle R Leishman. 2011a. "Is Plant Ecology More Siliceous than We Realise?" *Trends in Plant Science* 16 (2) (February): 61–68. doi:10.1016/j.tplants.2010.10.003. <http://www.ncbi.nlm.nih.gov/pubmed/21087891>.
- . 2012. "Tradeoffs between Foliar Silicon and Carbon-Based Defences: Evidence from Vegetation Communities of Contrasting Soil Types." *Oikos* 121 (February) (December 10): no–no. doi:10.1111/j.1600-0706.2012.20057.x. <http://doi.wiley.com/10.1111/j.1600-0706.2012.20057.x>.
- Cooke, Julia, and Michelle R. Leishman. 2011b. "Silicon Concentration and Leaf Longevity: Is Silicon a Player in the Leaf Dry Mass Spectrum?" *Functional Ecology* 25 (6) (December 29): 1181–1188. doi:10.1111/j.1365-2435.2011.01880.x. <http://doi.wiley.com/10.1111/j.1365-2435.2011.01880.x>.
- Cotterill, Jane V, Richard W Watkins, Clare B Brennon, and David P Cowan. 2007. "Boosting Silica Levels in Wheat Leaves Reduces Grazing by Rabbits." *Pest Management Science* 63 (3): 247–253. doi:10.1002/ps. <http://www.ingentaconnect.com/search/expand?pub=infobike://jws/ps/2007/00000063/00000003/art00007>.
- Coughenour, Michael B. 1985. "Graminoid Responses to Grazing by Large Herbivores: Adaptations, Exaptations, and Interacting Processes." *Annals of the Missouri Botanical Garden* 72 (4): 852–863.
- Cromsigt, Joris P G M, and Han Olff. 2008. "Dynamics of Grazing Lawn Formation : An Experimental Test of the Role of Scale-Dependent Processes." *Oikos* 117: 1444–1452. doi:10.1111/j.2008.0030-1299.16651.x.
- DeWit, H.A. 1978. "Soils and Grassland Types of the Serengeti Plain Tanzania". Wageningen.
- Diaz, Sandra, and Marcelo Cabido. 1997. "Plant Functional Types and Ecosystem Function in Relation to Global Change." *Journal of Vegetation Science* 8: 463–474.
- Dufek, Nickolas A., Lance T. Vermeire, Richard C. Waterman, and Amy C. Ganguli. 2014. "Fire and Nitrogen Addition Increase Forage Quality of *Aristida Purpurea*." *Rangeland Ecology and Management* 67(3):298-306).

- Elliott, C L, and George H Snyder. 1991. "Autoclave-Induced Digestion for the Colorimetric Determination of Silicon in Rice Straw." *Journal of Agricultural Food Chemistry* 39: 1118–1119.
- Endara, María-José, and Phyllis D. Coley. 2011. "The Resource Availability Hypothesis Revisited: A Meta-Analysis." *Functional Ecology* 25 (2) (April 19): 389–398. doi:10.1111/j.1365-2435.2010.01803.x. <http://doi.wiley.com/10.1111/j.1365-2435.2010.01803.x>.
- Eneji, A. Egrinya, S. Inanaga, S. Muranaka, J. Li, T. Hattori, P. An, and W. Tsuji. 2008. "Growth and Nutrient Use in Four Grasses Under Drought Stress as Mediated by Silicon Fertilizers." *Journal of Plant Nutrition* 31 (2) (February 18): 355–365. doi:10.1080/01904160801894913. <http://www.tandfonline.com/doi/abs/10.1080/01904160801894913>.
- Epstein, Emanuel. 1999. "Silicon." *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 641–664.
- Erickson, Gregory M, Brandon a Krick, Matthew Hamilton, Gerald R Bourne, Mark a Norell, Erica Lilleodden, and W Gregory Sawyer. 2012. "Complex Dental Structure and Wear Biomechanics in Hadrosaurid Dinosaurs." *Science* 338 (6103) (October 5): 98–101. doi:10.1126/science.1224495. <http://www.ncbi.nlm.nih.gov/pubmed/23042891>.
- Euliss, K.W., B.L. Dorsey, K.C. Benke, M.K. Banks, and a.P. Schwab. 2005. "The Use of Plant Tissue Silica Content for Estimating Transpiration." *Ecological Engineering* 25 (4) (November): 343–348. doi:10.1016/j.ecoleng.2005.06.003. <http://linkinghub.elsevier.com/retrieve/pii/S0925857405001412>.
- Fauteux, François, Florian Chain, François Belzile, James G Menzies, and Richard R Bélanger. 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 (46): 17554–17559. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1859967&tool=pmcentrez&rendertype=abstract>.
- Feeny, Paul. 1976. "Plant Apparency and Chemical Defense." In *Biochemical Interaction Between Plants and Insects*, edited by J.W. Wallace, 1–40. New York: Plenum Press.
- Fynn, Richard, Craig Morris, David Ward, and Kevin Kirkman. 2011. "Trait-Environment Relations for Dominant Grasses in South African Mesic Grassland Support a General Leaf Economic Model." *Journal of Vegetation Science* 22 (3) (June 4): 528–540. doi:10.1111/j.1654-1103.2011.01268.x. <http://doi.wiley.com/10.1111/j.1654-1103.2011.01268.x>.
- Gali, Hala U, and Christopher C Smith. 1992. "Effect of Silicon Supply on Growth, Fertility, and Mineral Composition of an Annual Brome, *Bromus Secalinus* L. (Gramineae)." *American Journal of Botany* 79 (11): 1259–1263.

- Gali-Muhtasib, H.U., C.C. Smith, and J.J. Higgins. 1992. "The Effect of Silica in Grasses on the Feeding Behavior of the Prairie Vole , *Microtus Ochrogaster* Author." *Ecology* 73 (5): 1724–1729.
- Galvez, L., R.B. Clark, L.M. Gourley, and J.W. Maranville. 1987. "Silicon Interactions with Manganese and Aluminum Toxicity in Sorghum." *Journal of Plant Nutrition* 10: 1139–1147.
- Gao, Xiaopeng, Chunqin Zou, Lijun Wang, and Fusuo Zhang. 2006. "Silicon Decreases Transpiration Rate and Conductance from Stomata of Maize Plants." *Journal of Plant Nutrition* 29 (9) (September): 1637–1647. doi:10.1080/01904160600851494. <http://www.tandfonline.com/doi/abs/10.1080/01904160600851494>.
- Georgiadis, Nicholas J, and Samuel J McNaughton. 1988. "Interactions between Grazers and a Cyanogenic Grass, *Cynodon Plectostachyus*." *Oikos* 51: 343–350.
- . 1990. "Elemental and Fibre Contents of Savanna Grasses: Variation with Grazing, Soil Type, Season, and Species." *Journal of Applied Ecology* 27 (2): 623–634.
- Gibson D. J. *Grasses and Grassland Ecology* Oxford Univ. Press (2009).
- Gittins, J. 1998. "Differentiation of Natrocarbonatite Magma at Oldoinyo Lengai Volcano , Tanzania." *Mineralogical Magazine* 62 (6): 759–768.
- Heine, Gregor, George Tikum, and Walter J Horst. 2007. "The Effect of Silicon on the Infection by and Spread of *Pythium Aphanidermatum* in Single Roots of Tomato and Bitter Gourd." *Journal of Experimental Botany* 58 (3) (January): 569–577. doi:10.1093/jxb/erl232. <http://www.ncbi.nlm.nih.gov/pubmed/17158106>.
- Hodson, M J, P J White, a Mead, and M R Broadley. 2005. "Phylogenetic Variation in the Silicon Composition of Plants." *Annals of Botany* 96 (6) (November): 1027–1046. doi:10.1093/aob/mci255. <http://dx.doi.org/10.1093/aob/mci255>.
- Holdo, Ricardo M, Robert D Holt, and John M Fryxell. 2009. "Opposing Rainfall and Plant Nutritional Gradients Best Explain the Wildebeest Migration in the Serengeti." *The American Naturalist* 173 (4) (April): 431–45. doi:10.1086/597229. <http://www.ncbi.nlm.nih.gov/pubmed/19243258>.
- Holdo, R. M., Mack, M. C. 2014. "Functional attributes of savanna soils: contrasting effects of tree canopies and herbivores on bulk density, nutrients and moisture dynamics." *Journal of Ecology*, 102: 1171–1182. doi: 10.1111/1365-2745.12290.
- Honaine, M. Fernandez, and M L Osterrieth. 2011. "Silicification of the Adaxial Epidermis of Leaves of a Panicoid Grass in Relation to Leaf Position and Section and Environmental Conditions." *Plant Biology (Stuttgart, Germany)* 14 (4) (July): 596–604. doi:10.1111/j.1438-8677.2011.00530.x. <http://www.ncbi.nlm.nih.gov/pubmed/22188340>.

- Hothorn, Torsten, Frank Bretz, and Peter Westfall. 2008. "Simultaneous Inference in General Parametric Models." *Biometrical Journal. Biometrische Zeitschrift* 50 (3) (June): 346–63. doi:10.1002/bimj.200810425. <http://www.ncbi.nlm.nih.gov/pubmed/18481363>.
- Hummel, Jürgen, Eva Findeisen, Karl-Heinz Südekum, Irina Ruf, Thomas M Kaiser, Martin Bucher, Marcus Clauss, and Daryl Codron. 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 (1712) (June 7): 1742–1747. doi:10.1098/rspb.2010.1939..
- Isa, Mami, Shuqin Bai, Takushi Yokoyama, Jian Feng Ma, Yushi Ishibashi, Takashi Yuasa, and Mari Iwaya-Inoue. 2010. "Silicon Enhances Growth Independent of Silica Deposition in a Low-Silica Rice Mutant, *Isi1*." *Plant and Soil* 331 (1-2) (January 19): 361–375. doi:10.1007/s11104-009-0258-9. <http://link.springer.com/10.1007/s11104-009-0258-9>.
- Jager, T.J. 1982. "Soils of the Serengeti Woodlands". Wageningen.
- Johnston, A., M. Bezeau, and S. Smoliak. 1967. "Variation in Silica Content of Range Grasses." *Canadian Journal of Plant Science* 47: 65–71.
- Jones, L.H.P., and K.A. Handreck. 1969. "Uptake of Silica by *Trifolium Incarnatum* in Relation to the Concentration in the External Solution and to Transpiration." *Plant and Soil* 1: 71–80.
- Jones, L.H.P., A.A. Milne, and S.M. Wadham. 1963. "Studies of Silica in the Oat Plant." *Plant and Soil* (3): 358–371.
- Keller, Jörg, Jurgis Klaudius, Matthieu Kervyn, Gerald G. J. Ernst, and Hannes B. Mattsson. 2010. "Fundamental Changes in the Activity of the Natrocarbonatite Volcano Oldoinyo Lengai, Tanzania." *Bulletin of Volcanology* 72 (8) (May 25): 893–912. doi:10.1007/s00445-010-0371-x. <http://link.springer.com/10.1007/s00445-010-0371-x>.
- Kraska, Joseph E., and Gary a. Breitenbeck. 2010. "Simple, Robust Method for Quantifying Silicon in Plant Tissue." *Communications in Soil Science and Plant Analysis* 41 (17) (August 31): 2075–2085. doi:10.1080/00103624.2010.498537. <http://www.tandfonline.com/doi/abs/10.1080/00103624.2010.498537>.
- Ma, Jian Feng, Kazunori Tamai, Naoki Yamaji, Namiki Mitani, Saeko Konishi, Maki Katsuhara, Masaji Ishiguro, Yoshiko Murata, and Masahiro Yano. 2006. "A Silicon Transporter in Rice." *Nature* 440 (7084) (March 30): 688–91. doi:10.1038/nature04590. <http://www.ncbi.nlm.nih.gov/pubmed/16572174>.
- Ma, Jian Feng, and Naoki Yamaji. 2006. "Silicon Uptake and Accumulation in Higher Plants." *Trends in Plant Science* 11 (8) (August): 392–397. doi:10.1016/j.tplants.2006.06.007. <http://www.ncbi.nlm.nih.gov/pubmed/16839801>.

- Ma, Jian Feng, Naoki Yamaji, Namiki Mitani, Kazunori Tamai, Saeko Konishi, Toru Fujiwara, Maki Katsuhara, and Masahiro Yano. 2007. "An Efflux Transporter of Silicon in Rice." *Nature* 448 (7150) (July 12): 209–212. doi:10.1038/nature05964. <http://www.ncbi.nlm.nih.gov/pubmed/17625566>.
- Massey, Fergus P, a Roland Ennos, and Sue E Hartley. 2006. "Silica in Grasses as a Defence against Insect Herbivores: Contrasting Effects on Folivores and a Phloem Feeder." *Journal of Animal Ecology* 75 (2) (March): 595–603. doi:10.1111/j.1365-2656.2006.01082.x. <http://www.ncbi.nlm.nih.gov/pubmed/16638012>.
- . 2007. "Herbivore Specific Induction of Silica-Based Plant Defences." *Oecologia* 152 (4) (July): 677–683. doi:10.1007/s00442-007-0703-5. <http://sro.sussex.ac.uk/22901/>.
- Massey, Fergus P, and Sue E Hartley. 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 (September 22): 2299–2304. doi:10.1098/rspb.2006.3586. <http://rspb.royalsocietypublishing.org/content/273/1599/2299>.
- Mateos-Naranjo, Enrique, Luis Andrades-Moreno, and Anthony J Davy. 2013. "Silicon Alleviates Deleterious Effects of High Salinity on the Halophytic Grass *Spartina densiflora*." *Plant Physiology and Biochemistry* 63 (February): 115–21. doi:10.1016/j.plaphy.2012.11.015. <http://www.ncbi.nlm.nih.gov/pubmed/23257076>.
- Mauricio, R., M.D. Rausher, and D.S. Burdick. 1997. "VARIATION IN THE DEFENSE STRATEGIES OF PLANTS : ARE RESISTANCE AND TOLERANCE MUTUALLY EXCLUSIVE ?" *Ecology* 78 (5): 1301–1311.
- McNaughton, S.J. 1979. "Grazing as an Optimization Process : Grass-Ungulate Relationships in the Serengeti." *The American Naturalist* 113 (5): 691–703.
- McNaughton, S.J. 1984. "Grazing Lawns: Animals in Herds, Plant Form, and Coevolution." *The American Naturalist* 124 (6): 863–886.
- McNaughton, S.J., J.L. Tarrants, M.M. McNaughton, and R.H. Davis. 1985. "Silica as a Defense against Herbivory and a Growth Promotor in African Grasses." *Ecology* 66 (2): 528–535.
- Melzer, Susan E, Alan K Knapp, Kevin P Kirkman, Melinda D Smith, John M Blair, and Eugene F Kelly. 2009. "Fire and Grazing Impacts on Silica Production and Storage in Grass Dominated Ecosystems." *Biogeochemistry* 97 (2-3) (September 15): 263–278. doi:10.1007/s10533-009-9371-3. <http://www.springerlink.com/index/10.1007/s10533-009-9371-3>.
- Melzer, Susan E., Oliver a. Chadwick, Anthony S. Hartshorn, Lesego M. Khomo, Alan K. Knapp, and Eugene F. Kelly. 2011. "Lithologic Controls on Biogenic Silica Cycling in South African Savanna Ecosystems." *Biogeochemistry* 108 (1-3) (April 8): 317–334. doi:10.1007/s10533-011-9602-2. <http://link.springer.com/10.1007/s10533-011-9602-2>.

- Mendoza, M, and P Palmqvist. 2008. "Hypsodonty in Ungulates: An Adaptation for Grass Consumption or for Foraging in Open Habitat?" *Journal of Zoology* 274 (2) (February): 134–142. doi:10.1111/j.1469-7998.2007.00365.x. <http://doi.wiley.com/10.1111/j.1469-7998.2007.00365.x>.
- Mitani, Namiki, Naoki Yamaji, and Jian Feng Ma. 2009. "Identification of Maize Silicon Influx Transporters." *Plant & Cell Physiology* 50 (1) (January): 5–12. doi:10.1093/pcp/pcn110. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2638714&tool=pmcentrez&rendertype=abstract>.
- Morton, B.B., and M.W. Jutras. 1974. "Silica Concentrations in Grazed and Ungrazed Forage Species." *Agronomy Journal* 66: 10–12.
- Oyarzabal, Mariano, and Martín Oesterheld. 2009. "Phosphorus Reserves Increase Grass Regrowth after Defoliation." *Oecologia* 159 (4) (April): 717–24. doi:10.1007/s00442-008-1263-z. <http://www.ncbi.nlm.nih.gov/pubmed/19132398>.
- Pahkala, K, and M Pihala. 2000. "Different Plant Parts as Raw Material for Fuel and Pulp Production." *Industrial Crops and Products* 11 (2-3) (March): 119–128. doi:10.1016/S0926-6690(99)00050-3. <http://linkinghub.elsevier.com/retrieve/pii/S0926669099000503>.
- Piperno, Dolores R, and Deborah M Pearsall. 1998. "The Silica Bodies of Tropical American Grasses : Morphology , Taxonomy , and Implications for Grass Systematics and Fossil Phytolith Identification." *Library* 85 (85): 1–40. <http://si-pddr.si.edu/dspace/handle/10088/7002>.
- Prasad, Vandana, Caroline a E Strömberg, Habib Alimohammadian, and Ashok Sahni. 2005. "Dinosaur Coprolites and the Early Evolution of Grasses and Grazers." *Science* 310 (5751) (November 18): 1177–80. doi:10.1126/science.1118806. <http://www.ncbi.nlm.nih.gov/pubmed/16293759>.
- Querné, Jérémy, Olivier Ragueneau, and Nathalie Poupart. 2012. "In Situ Biogenic Silica Variations in the Invasive Salt Marsh Plant, *Spartina Alterniflora*: A Possible Link with Environmental Stress." *Plant and Soil* 352 (1-2) (October 1): 157–171. doi:10.1007/s11104-011-0986-5. <http://link.springer.com/10.1007/s11104-011-0986-5>.
- Raven, J.A. 1983. "The Transport and Function of Silicon in Plants." *Biological Reviews of the Cambridge Philisophical Society* 58 (2): 179–207.
- Rudall, Paula J., Christina J. Prychid, and Thomas Gregory. 2014. "Epidermal Patterning and Silica Phytoliths in Grasses: An Evolutionary History." *The Botanical Review* 80 (1) (March 6): 59–71. doi:10.1007/s12229-014-9133-3. <http://link.springer.com/10.1007/s12229-014-9133-3>.
- Sangster, A.G., M.J. Hodson, and H.J. Tubb. 2001. "Silicon Deposition in Higher Plants." In *Silicon in Agriculture*, 85–113. New York: Elsevier.

- Sangster, A.G., and D.W. Parry. 1970. "Silica Deposition in the Grass Leaf in Relation to Transpiration and the Effect of Dinitrophenol." *Annals of Botany* 35 (3): 667–677.
- Sanson, Gordon D., Stuart a. Kerr, and Karlis a. Gross. 2007. "Do Silica Phytoliths Really Wear Mammalian Teeth?" *Journal of Archaeological Science* 34 (4) (April): 526–531. doi:10.1016/j.jas.2006.06.009.
<http://linkinghub.elsevier.com/retrieve/pii/S0305440306001245>.
- Schaller, Jörg, Carsten Brackhage, and E Gert Dudel. 2012. "Silicon Availability Changes Structural Carbon Ratio and Phenol Content of Grasses." *Environmental and Experimental Botany* 77 (April): 283–287.
 doi:10.1016/j.envexpbot.2011.12.009.
<http://linkinghub.elsevier.com/retrieve/pii/S0098847211003169>.
- Sinclair, A.R.E. and M. Norton-Griffiths. 1979. *Serengeti: dynamics of an ecosystem*. University of Chicago Press, Chicago, Illinois, USA.
- Soininen, Eeva M., Kari Anne Bråthen, Juan German Herranz Jusgado, Stefan Reidinger, and Susan E. Hartley. 2013. "More than Herbivory: Levels of Silica-Based Defences in Grasses Vary with Plant Species, Genotype and Location." *Oikos* 122 (1) (January 25): 30–41. doi:10.1111/j.1600-0706.2012.20689.x.
<http://doi.wiley.com/10.1111/j.1600-0706.2012.20689.x>.
- Stebbins, G. L. 1972. The evolution of the grass family. Chapter 1 in V. B. Youngner & C. M. McKell (eds), *The Biology and Utilization of Grasses*. Academic Press, New York.
- Street, John R. 1974. "The Influence of Silica Concentration on the Chemical Composition and Decomposition Rates of Turfgrass Tissue and Water Absorption Rates among Three Turfgrass Species". Ohio State University.
- Strömberg, Caroline a E. 2006. "Evolution of Hypsodonty in Equids: Testing a Hypothesis of Adaptation." *Paleobiology* 32 (2): 236–258.
[http://www.bioone.org/doi/abs/10.1666/0094-8373\(2006\)32\[236:EOHIET\]2.0.CO;2](http://www.bioone.org/doi/abs/10.1666/0094-8373(2006)32[236:EOHIET]2.0.CO;2).
- Strömberg, Caroline a E, Regan E Dunn, Richard H Madden, Matthew J Kohn, and Alfredo a Carlini. 2013. "Decoupling the Spread of Grasslands from the Evolution of Grazer-Type Herbivores in South America." *Nature Communications* 4 (January): 1478. doi:10.1038/ncomms2508. <http://www.ncbi.nlm.nih.gov/pubmed/23403579>.
- Strömberg, Caroline a.E. 2011. "Evolution of Grasses and Grassland Ecosystems." *Annual Review of Earth and Planetary Sciences* 39 (1) (May 30): 517–544.
 doi:10.1146/annurev-earth-040809-152402.
<http://www.annualreviews.org/doi/abs/10.1146/annurev-earth-040809-152402>.
- Strömberg, Caroline a.E., Lars Werdelin, Else Marie Friis, and Gerçek Saraç. 2007. "The Spread of Grass-Dominated Habitats in Turkey and Surrounding Areas during the Cenozoic: Phytolith Evidence." *Palaeogeography, Palaeoclimatology, Palaeoecology* 250 (1-4) (June): 18–49. doi:10.1016/j.palaeo.2007.02.012.
<http://linkinghub.elsevier.com/retrieve/pii/S0031018207001009>.

- Struyf, Eric, Stefan Van Damme, Britta Gribsholt, and Patrick Meire. 2005. "Freshwater Marshes as Dissolved Silica Recyclers in an Estuarine Environment (Schelde Estuary, Belgium)." *Hydrobiologia* 540 (1-3) (May): 69–77. doi:10.1007/s10750-004-7104-0. <http://link.springer.com/10.1007/s10750-004-7104-0>.
- Twiss, P.C. 1969. "Morphological Classification of Grass Phytoliths." *Soil Science of America Proceedings* 33 (1): 109–115.
- Veldhuis, M. P., Howison, R. A., Fokkema, R. W., Tielens, E., Olf, H. 2014. "A novel mechanism for grazing lawn formation: large herbivore-induced modification of the plant–soil water balance." *Journal of Ecology*. doi: 10.1111/1365-2745.12322
- Wright, Ian J, Peter B Reich, Mark Westoby, David D Ackerly, Zdravko Baruch, Frans Bongers, Jeannine Cavender-Bares, et al. 2004. "The Worldwide Leaf Economics Spectrum." *Nature* 428 (6985) (April 22): 821–7. doi:10.1038/nature02403. <http://www.ncbi.nlm.nih.gov/pubmed/21196061>.
- Zhu, Zhujun, Guoqiang Wei, Juan Li, Qiongqiu Qian, and Jingquan Yu. 2004. "Silicon Alleviates Salt Stress and Increases Antioxidant Enzymes Activity in Leaves of Salt-Stressed Cucumber (*Cucumis Sativus* L.)." *Plant Science* 167 (3) (September): 527–533. doi:10.1016/j.plantsci.2004.04.020. <http://linkinghub.elsevier.com/retrieve/pii/S0168945204001797>.

FIGURES

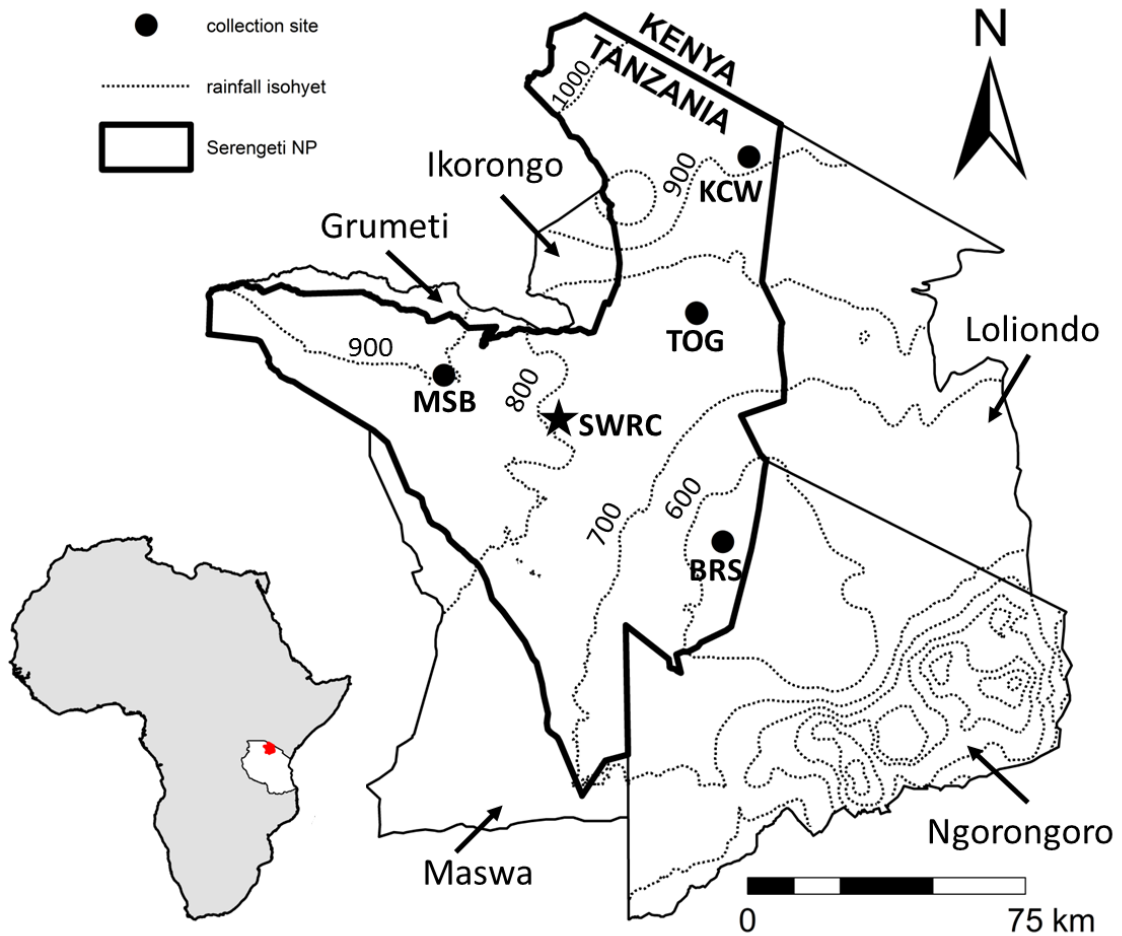


FIGURE 1. Map showing the locations of the four collection sites of *Digitaria* and *Themeda* in Serengeti National Park; surrounding protected areas are directly labeled. Inset of Africa shows the location of this region in northern Tanzania in red. The common garden was constructed at the central Serengeti Wildlife Research Centre (SWRC). Mean annual precipitation is approximately 498mm/yr at BRS, 676mm/yr at TOG, 766mm/yr at KCW, and 891mm/yr at MSB (values from Anderson *et al.* 2007).

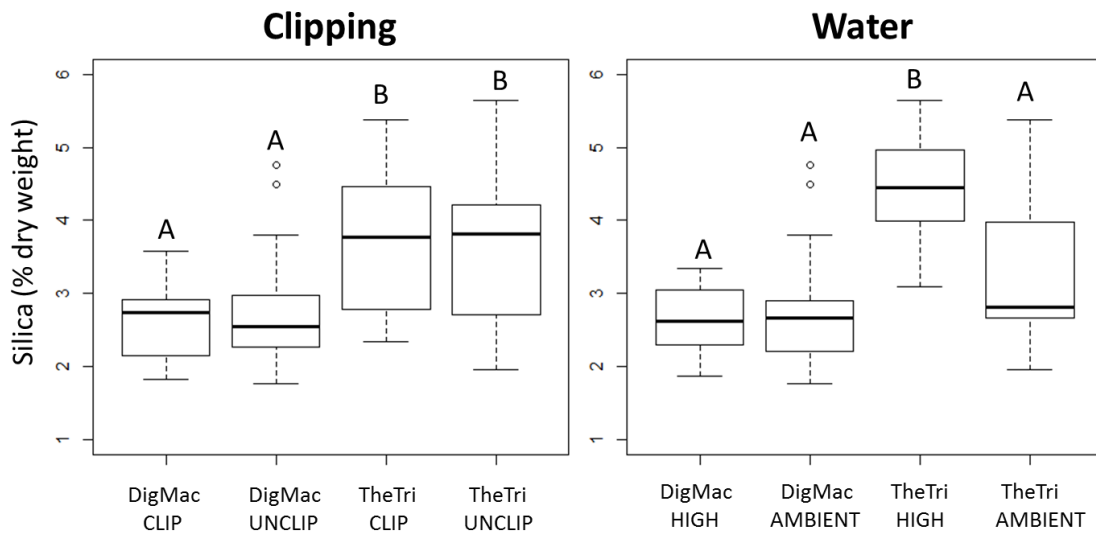


FIGURE 2. Silica content of *Digitaria macroblephera* (DigMac) and *Themeda triandra* (TheTri) leaves as affected by defoliation and watering treatments. Defoliated individuals were clipped to 50% of maximum height bi-monthly a total of 4 times. High water treatment individuals received approximately 150% of ambient rainfall, and ambient treatment represents low and ambient water groups pooled (see methods). Groups which differ significantly are indicated by a letter change.

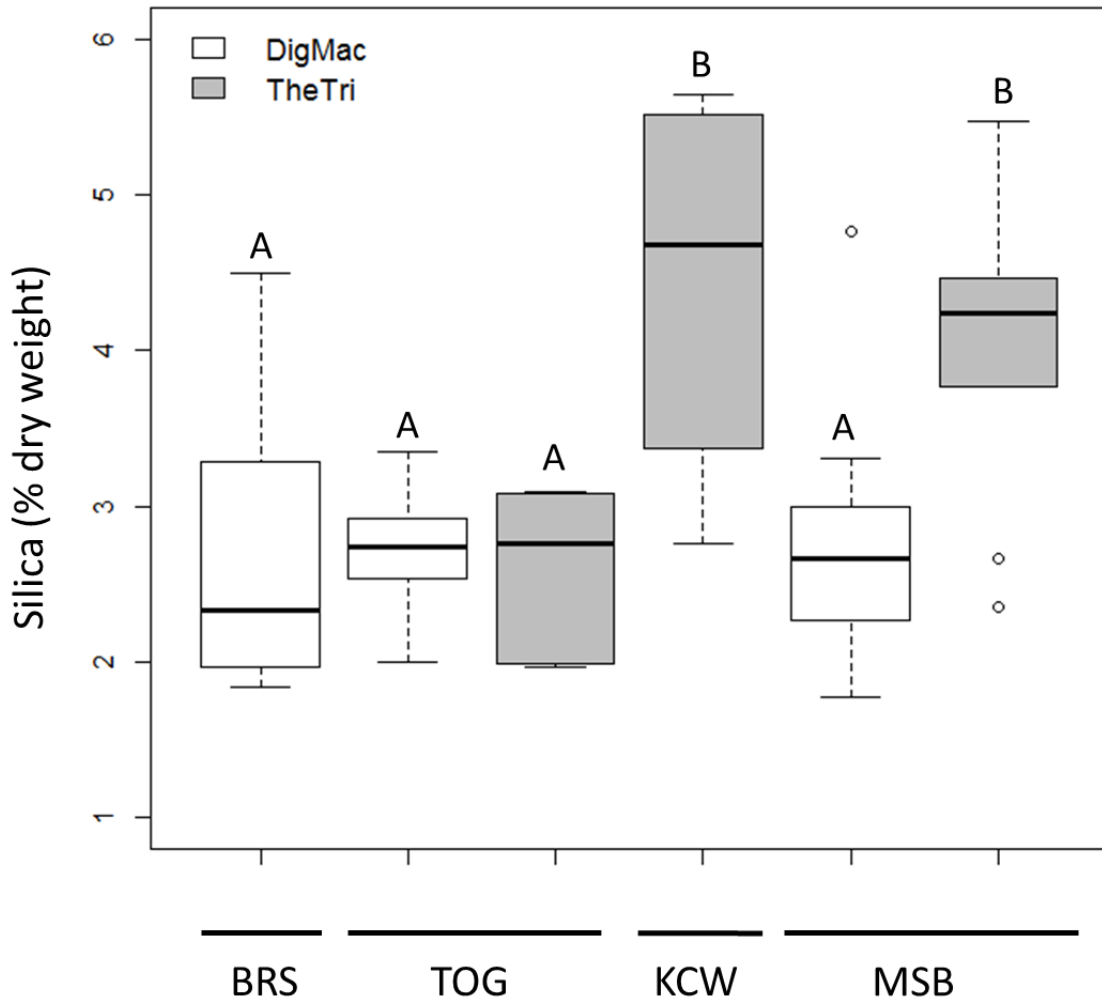


FIGURE 3. Foliar silica content of each species in relation to plant collection sites. Both species were collected at the intermediate sites, MSB and TOG. *Themeda* (TheTri) does not occur at the shortgrass plains site BRS, and *Digitaria* (DigMac) does not occur at KCW in the northern woodlands. Sites are arranged in order of increasing mean annual precipitation: BRS = 498mm/yr , TOG= 676mm/yr, KCW= 766mm/yr, MSB= 891mm/yr.

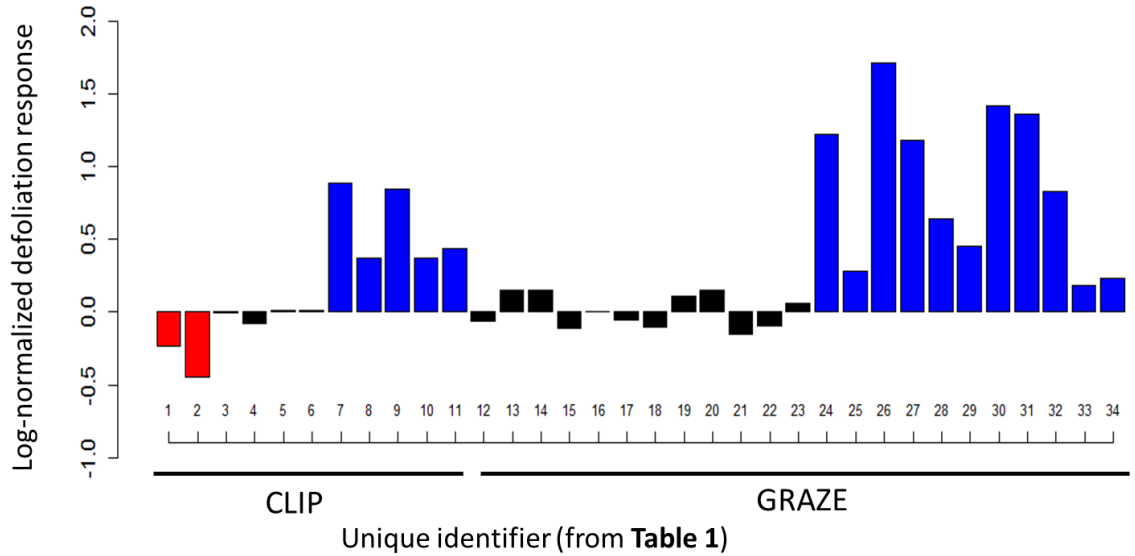


FIGURE 4. Log-normal change in foliar silica content of defoliated grasses identified in the literature review. Blue bars indicate a $\geq 20\%$ increase in silica following defoliation, red bars indicate a $\geq 20\%$ decrease, and black bars indicate $< 20\%$ change in either direction. Individual responses are arranged according to unique identifiers from Table 1; Unique identifiers 1-11 represent clipping studies, and 12-34 represent grazing studies.

CHAPTER 3

VARIATION IN THE SOIL 'SILICON LANDSCAPE' EXPLAINS PLANT SILICA ACCUMULATION ACROSS ENVIRONMENTAL GRADIENTS IN SERENGETI

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ABSTRACT

Aims: Beneficial aspects of plant silicon (Si) have been identified, but little is known about how multiple abiotic conditions interact in a natural environment to influence plant Si-accumulation. Consequently, we identified abiotic correlates of soil and plant Si pools in a pristine African savanna and quantified relationships among precipitation, soil properties, and grass silicon concentration along environmental gradients.

Methods: Soil properties were measured at 63 sites spanning soil and precipitation gradients in the Serengeti ecosystem of northern Tanzania. Bulk and species-specific grass samples were collected at 18 of those sites and combined with soil and climate data into a structural equation model to describe landscape drivers of foliar silicon concentration.

Results: Soil amorphous silicon (ASi) was negatively related to precipitation but was unrelated to soil dissolved silicon (DSi). The final structural equation model included three predictor variables, each negatively associated with soil DSi: precipitation, soil percent sand, and soil pH, which, together, explained ~74% of variation in soil DSi. Finally, soil DSi was positively related to grass Si concentration, explaining ~60% of variation.

Conclusions: While evidence exists for biotic induction of silicon accumulation (e.g. herbivory), our results indicate that natural abiotic variation, which has largely been ignored, is equally important and may determine the extent to which inducibility is possible. Environmental heterogeneity, by constraining or promoting Si dissolution and availability, plays a significant role in silicon accumulation by grasses.

Key words: silicon; biogenic silica; savanna; grassland; phytoliths; Serengeti National Park

INTRODUCTION

The element silicon (Si) represents an ecological conundrum for plant ecologists: it is abundant in the earth's crust (>28% by weight, Wedepohl 1995), yet its status as an essential, beneficial, or non-essential element for plant growth remains uncertain. For example, some plants, such as horsetails of the genus *Equisetum*, require silicon as an essential nutrient (Chen & Lewin 1969), while silicon appears to play little role in leaf structure and function for most angiosperms. However, for important food crops, silicon accumulation results in improved tolerance of stress, including heat (Agarie *et al.* 1998), drought (Hattori *et al.* 2005), salt (Yeo *et al.* 1999), heavy metals (Corrales *et al.* 1997), disease (Rémus-Borel *et al.* 2005), and herbivory (Cotterill *et al.* 2007).

Despite the abundance, availability, and beneficial effects of silicon, few angiosperms and few recently-evolved plant taxa are considered Si-accumulators, defined by Takahashi (1990) as plants with more silicon than expected by passive transpiration at standard conditions (~ 0.5% plant dry weight at 10 mg kg⁻¹ soil Si and transpiration coefficient of 500 g H₂O g dry wt⁻¹). Hodson and colleagues attributed 67% of variation in Si concentration of 735 plant species to high-level phylogenetic relationships (group and order) and found that Si-accumulators were most common among early-diverging land plants, such as liverworts, mosses, and horsetail (Hodson *et al.* 2005). One of few exceptions among angiosperms is the more recently evolved grasses (Poaceae) (Takahashi 1990, Hodson *et al.* 2005, Trembath-Reichert *et al.* 2015). Silica biomineralization by plants requires that soil silicates, including amorphous Si (ASi), be weathered into dissolved Si (silicic acid [H₄SiO₄], hereafter DSi), absorbed

by plant roots, transported via the transpiration stream, and incorporated into leaf tissue as solid amorphous phytoliths ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Barber & Shone 1966; summarized in Fig 1).

Because plants are able to absorb and transport large amounts of Si, recent studies have sought to better integrate Si-accumulating plants into the biogeochemical Si-cycle (Conley 2002, Gerard et al. 2008, Street-Perrott & Barker 2008, Struyf & Conley 2009). Phytoliths contribute substantially to non-crystalline or “amorphous” Si (ASi) in topsoil, especially in biomes dominated by Si-accumulating vegetation such as grasslands (Blecker et al. 2006). This substantial pool of ASi is then weathered by precipitation and becomes available to plants as DSi. As the ultimate source of Si, parent material may also be weathered into DSi, although Si derived from parent material is far less labile than phytolith Si. For instance, at ambient temperature and neutral pH the equilibrium reaction between quartz and soluble Si occurs at a rate approximately 1/20 that of amorphous Si and soluble Si (Lindsay 1979). The exact mineral dissolution and chemical equilibrium rates, however, are dependent upon underlying mineralogy (Cornelis et al. 2011), such as the type of metals present (McKeague & Cline 1962, Beckwith & Reeve 1963). Soil organic matter may also correlate with soil ASi, since organic matter serves as a proxy for the amount of plant litter entering soil, while organic molecules are known to enhance dissolution of silicates (Drees et al. 1989) and retard monosilicic acid adsorption by soils (Beckwith and Reeve 1964), thus affecting DSi availability. Soil pH, another aspect of mineralogy, may also influence phytolith solubility. High pH is known to facilitate breaking of siloxane bonds, favor silicate adsorption by Fe and Al, and decrease mineral weathering, but the overall effect of soil pH on ASi dissolution remains unclear (Haynes 2014). Mineral dissolution rates are also heavily influenced by climate: soluble silica concentrations nearly double

with a temperature increase from 5 to 25 °C (e.g., Drees et al. 1989), and precipitation influences both Si weathering and leaching (Gerard et al. 2002, Monger & Kelly 2002). Through its effects on pore space, particle size distribution (i.e. soil texture) has well-known effects on soil water potential and leaching (water is more tightly bound in clay-rich soils) which effects plant-available Si.

Clearly, many individual drivers of silicon dissolution at the soil-plant interface are understood, but these relationships have yet to be statistically linked in a whole ecosystem context. In grassland systems, phytoliths play an especially important role in the Si cycle because they comprise the majority of the soil ASi pool (Saccone et al. 2007) and act as both a source (highly labile) and sink (grassland soils contain 10,000x more biogenic Si than aboveground biomass in grasslands; Blecker et al. 2006) of Si which may be weathered into the soil water. Experimental addition of soluble Si to soils suggests that soil Si availability is positively correlated with plant uptake (Garbuzov et al. 2011), although subsequent research has failed to link soil Si availability and Si accumulation by vegetation *in situ* (Cooke & Leishman 2012). Likewise, little is known about how the direct source of plant Si (DSi) varies along environmental gradients, like precipitation. In one of the few studies of its kind, Blecker et al. (2006) showed that, across a bioclimatic sequence in Great Plains grasslands, primary productivity (ANPP) was positively correlated with rainfall, and soil biogenic Si (e.g. phytoliths) decreased with precipitation. This trend was attributed to faster soil phytolith turnover rates at high rainfall, tall grass sites, but plant-available soil Si (DSi) was not quantified. By quantifying pools of silicon at the plant-soil interface and modeling the relationships among abiotic conditions, soil ASi and DSi, and plant Si, we sought to address a major gap in the field of plant silicon research.

The first aim of our study was to quantify variation in what we termed the “silicon landscape” of Serengeti National Park. The silicon landscape consisted of three important Si pools at the soil-plant interface: 1) soil amorphous Si [ASi], 2) soil dissolved Si [DSi]), and 3) grass foliar silicon concentration. This first aim served to quantify spatial patterns of Si pools across the Serengeti landscape. Our second aim was to identify the most important environmental correlates of each major pool of silicon by utilizing Serengeti’s natural environmental gradients. In order to achieve this aim, we quantified the relationships between each of the 3 Si pools and naturally occurring gradients in precipitation and soil properties (soil pH, soil texture, soil organic matter) with general predictions following those previously described. After quantifying individual Si pools and the environmental drivers of each, our final aim was to combine environmental variables, the two soil Si pools, and plant Si concentration into a novel system-level analysis using structural equation modeling. Broadly speaking, soil ASi, soil DSi, and plant Si were predicted to all relate in a positive manner due to known relationships between supply, dissolution, and plant uptake.

METHODS

Study site

The Serengeti ecosystem covers an area of ~ 25,000 km² in northern Tanzania and southern Kenya, with boundaries defined by the annual migration of some 1.2 million ungulate herbivores across a savanna landscape. Serengeti National Park (SNP) lies at the center of the ecosystem, and is surrounded by a buffer zone of additional protected areas in both countries. Natural gradients in climate, soils, and regional geology make SNP an ideal system to approach our study questions about abiotic controls on plant Si

accumulations. Seasonal rains are influenced by the Intertropical Convergence Zone (ITCZ) and average 600 to >1000 mm yr⁻¹ along a gradient from the Serengeti plains in the southeast to Lake Victoria located northwest of SNP (Fig 2). The southeastern one-third of the ecosystem is edaphically-determined treeless plains underlain by soils derived from volcanic ash (White 1983) and classified as vitric andosols under the World Reference Base system (FAO 1998). These soils are rich in sodium, calcium, and potassium (Dawson 1963, Anderson & Talbot 1965), which have leached over long periods of time to form a shallow, impenetrable carbonate layer (*caliche*) resulting in a sodic and alkaline topsoil with poor moisture retention (de Wit 1978, Sinclair 1979). Most nearby volcanoes have gone extinct, but the southeastern plains continue to be enriched by silicon-depleted aeolian ash of the sole active volcano, Ol'Doinyo Lengai. The northern and western two-thirds of the ecosystem are classified as *Acacia-Commiphora* deciduous woodland vegetation type (White 1983), which sit atop soils of granitic or gneissic origin in the north (phaeozems) and solonetz in the west mixed with vertisols that vary along catenal gradients (Jager 1982). Shallow, rocky leptosols occur in patches throughout the north and west (Fig 2). We chose to focus our vegetation analysis on grasses due to their role as Si accumulators and their ecological importance in this system. Grasses in SNP account for approximately 85% of herbaceous primary productivity, providing the base of the food web for both resident and migratory grazing herbivores (Anderson, Metzger & McNaughton 2007).

Data collection

We measured a range of properties for 63 soils collected throughout Serengeti between February 2014 and June 2015. Collection sites varied in regional precipitation, soil parent material and topographic position along a catenal gradient. Plant samples were simultaneously collected within a one meter radius of soil samples at 18 of these sites,

which span the entire range of soil samples (Fig 2, Table 1). At each site, a soil sample was collected to a depth of 20 cm using a soil auger. A bulk aboveground grass sample was collected at each of 18 plant sites to reflect the local community; the dominant species present in each bulk grass sample, hereafter referred to as “community” samples, are provided in Table S1. In addition, separate samples of the two most dominant species in the ecosystem, *Themeda triandra* and *Digitaria macroblephera*, were collected in order to test for species-specific patterns of Si-accumulation and determine whether individual species reflect patterns observed at the community level (e.g. Blecker et al. 2006). While these two species often co-occur, *D. macroblephera* is a shorter in stature and often dominant in dry and heavily grazed areas of Serengeti, while *T. triandra* is a taller-statured species and tends to dominate in mesic, fire-prone regions of Serengeti. In total, *D. macroblephera* was present at 14 of 18 sites, and *T. triandra* at 13 of 18 sites, resulting in 45 plant samples in total (14 DM + 13 TT + 18 bulk; see Table 1). The near infrared difference vegetation index (NDVI) was determined for each site by averaging 16 day composite imagery acquired by the Moderate Resolution Imaging Spectroradiometer (MOD13Q1) between the years 2000 and 2009 (LPDAAC 2013; <http://lpdaac.usgs.gov>). These values are typically used as an estimate of aboveground biomass production. Compound topographic index (CTI), a quantitative index of topographic wetness, was calculated as a function of the contributing upslope area, flow direction, and slope angle (e.g. Gessler et al. 1995) from a hydrologically conditioned digital elevation model obtained from the World Wildlife Fund (Lehner et al. 2006). Mean annual precipitation (MAP) was interpolated using ~40 years of rain gauge data from 58 locations throughout Serengeti (see Anderson et al. 2007).

Plant and soil analysis

After collection, plant and soil samples were transported to Wake Forest University for chemical analysis. Soil pH and texture (particle size distribution) were calculated using standard laboratory methods outlined in Robertson *et al.* (1999). Organic matter was estimated by loss on ignition (LOI) after ashing 5 g of each soil sample in a muffle furnace at 400 °C for 16 hours. We chose to separately quantify two different forms of soil silicon: amorphous silicon (ASi) and easily-soluble silica, an estimate of dissolved silicon (DSi). ASi consists of both pedogenic and biogenic (i.e. phytolith) sources of silicon, but excludes crystalline mineral sources of silicon (i.e. quartz). It represents the total soil silicon pool available for biological cycling on short time scales (Sommer *et al.* 2006). In contrast, easily soluble silica estimates equilibrium Si concentration in soil pore water (Zysset *et al.* 1999) and provides an estimate of DSi available for plants (Haysom and Chapman 1975). We followed methods of Clymans *et al.* for extraction of both ASi and DSi (2011). Briefly, ASi was extracted by incubating 30 mg of dried soil (<2 mm) in a weak alkalizing agent, 0.1 M Na₂CO₃, at 80° C for 4 hours. DSi was extracted by shaking 2mg of dried soil (<2 mm) for 16 hours in 0.01 M CaCl₂, is a weak extracting agent assumed to extract only readily soluble Si (Berthelsen *et al.* 2001), followed by centrifugation at 4000 rpm for 30 minutes. Both ASi and DSi soil extracts were filtered through 0.45 um pore size filters (Lab Safety Supply™ 14A842) and brought to acidic pH using HCl (Certified ACS Plus) and distilled deionized water (18.2 MΩ cm, Milli-Q, Millipore, Bedford, MA, USA) prior to inductively coupled plasma optical emission spectrometry (ICP OES) analysis. Plant samples were divided into leaf and stem portions, and leaves were dried, ground, and analyzed for carbon, nitrogen, and phosphorus at the Kansas State University Soil Testing Laboratory. Leaf Si was extracted from all 41 plant samples by autoclave induced digestion as described elsewhere (Quigley and Anderson 2014). We chose to focus specifically on Si concentration of leaves which form the bulk of plant biomass for Serengeti grasses and

are the preferred, high-protein portion of plants; stems are usually left behind by grazing ungulates since they are rich in insoluble lignin and cellulose, and hence, of low food value (Jarman and Sinclair 1979). Soil and plant extracts were analyzed using a Prodigy ICP OES system (Teledyne Leeman Labs, Hudson, NH, USA) by comparing the Si atomic emission intensity at 251.611 nm to a standard reference solution (1000 mg/L Si, High Purity Standards, Charleston, SC, USA) diluted to 0.1 – 10 ppm Si in a matrix-matched medium, and all calibration curves had a validation $r^2 \geq 0.998$. Samples were analyzed in triplicate in axial view mode at radio-frequency power of 1.3 kW, a plasma flow rate of 18 L/min, nebulizer pressure of 30 psi, sample flow rate of 0.6 mL/min, and a 15 second integration time. A certified reference material (BCR 129, Institute for Reference Materials and Measurements, Geel, Belgium) was analyzed to estimate recovery, and the limit of detection was calculated according to IUPAC's recommendations as 3 times the standard deviation of a blank solution, divided by the slope of the calibration curve.

Hypotheses, candidate models, and statistical analyses

All statistical analyses were conducted in the R statistical environment version 3.1.2 (R Development Core Team). Our first approach was to compare mean categorical differences in soil ASi, soil DSi, and plant Si among major soil types (WRB subgroups) using one-way analysis of variance correcting for unequal variance. Specific contrasts among soil groups were conducted with Tukey HSD post-hoc comparisons. Next we explored linear relationships among soil Si pools (ASi and DSi) and additional landscape properties, such as rainfall, topography, and soil chemical and physical properties using ordinary least squares linear regression. Preliminary analysis identified four abiotic variables, which were the focus of subsequent models: precipitation, soil pH, soil texture, and soil organic matter.

Linear models were combined into a single system-level analysis using structural equation modeling (SEM; e.g. Shipley 2009) in the R package *lavaan* (Rosseel 2012). SEM is conceptually similar to path analysis but includes advanced techniques for evaluating the goodness-of-fit of between a hypothesized model and the observed data. We began with an *a priori* model (Figure 3) which allowed us to simultaneously determine the indirect influence of abiotic conditions on plant Si, as mediated by their direct influence on soil ASi and DSi.

Because plants and soil were not sampled at all sites, the model was evaluated in two parts: one for abiotic effect on soils ($n = 63$) and one for DSi effects on plant Si ($n = 45$ from 18 sites). For the soil model, site level variation in precipitation, soil pH, soil texture, and soil organic matter were included as predictors and soil Si fractions (ASi and DSi) at the 63 soil collection sites were included as response variables. Because we also sought to test whether the size of the ASi pool influences DSi, we included a path between ASi and DSi in the *a priori* model. For the second model, the 45 leaf Si samples (*Digitaria*: $n = 14$; *Themeda*: $n = 13$; bulk: $n = 18$) were included as response variables with soil DSi at the respective sites as a predictor. Because of the nested sampling, this model was analyzed with a mixed effects model using the R package *lme4* (Bates et al. 2014) in which soil DSi was included as a fixed effect, site and species were included as random effects. To arrive a final model, we utilized a model simplification procedure in which insignificant paths ($p > 0.05$) were removed from the full model in a stepwise fashion. Significant ($p < 0.05$) covariances among predictors were allowed to stay in the final model. In the final model, individual regression coefficients are presented as standardized path strengths and the coefficient of determination is presented for each response variable (i.e., r^2). As random site effects were included in the final model, we

report both marginal (exclusive) and conditional (inclusive) r^2 values for plant Si as described in Nakagawa & Schielzeth (2013).

Finally, we used ordinary least squares linear regression to explore relationships between leaf Si and other important leaf constituents including leaf % C, % N and % P. Previous studies indicate that strong univariate relationships with leaf Si may be indicative of life history tradeoffs associated with foliar Si accumulation and provide an indication of costs of this defensive strategy.

RESULTS

Variation in soil and plant silicon pools

The Si content measured for the reference material was not statistically different from the reference value as indicated by a t-test at the 95% confidence level, and a limit of detection well below any of the measured soil or plant Si values (10 $\mu\text{g/L}$) was calculated collectively indicating high recovery and instrument sensitivity. Replicate analyses of a single sample ($n = 12$) further validated methodological accuracy. Soil ASi ranged from $<2 \text{ mg g}^{-1}$, predominantly at sites near the center of the system, to $>14 \text{ mg g}^{-1}$ at one of the shortgrass plains sites (Supplementary Fig S2, Table 1). Soils of the Serengeti plains soils had significantly higher ASi pools than those of the surrounding savanna (mean \pm SE plains: $7.30 \pm 1.06 \text{ mg g}^{-1}$, savanna: $4.83 \pm 0.26 \text{ mg g}^{-1}$; $t_{13.5} = 2.26$, $p = 0.04$), but soil ASi did not differ systemically among WRB soil subgroups ($F_{4,58} = 1.31$, $p = 0.31$; Fig 3a). Variation in soil ASi across sites was best explained by a negative relationship with precipitation (Fig 4b; $F_{1,61} = 15.1$, adjusted $r^2 = 0.19$, $p < 0.01$). Although several of the sites with a large ASi pool had a soil pH > 8 , allowing for an interaction between precipitation and soil pH did not improve the predictive power of the linear regression.

Soil DSi ranged from 0.015 mg g⁻¹ to 0.149 mg g⁻¹ and was lowest in central Serengeti (Fig S1, Table 1). Mean DSi in the plains (0.092 ± 0.008 mg g⁻¹) and in the savanna-woodland sites (0.078 ± 0.005 mg g⁻¹) were not significantly different ($t_{21.9} = 1.49$, $p = 0.15$). Soil DSi did, however, vary significantly across the five WRB soil types ($F_{4,58} = 9.03$, $p < 0.001$): DSi was lower in leptosols than andosols (diff = 0.05 mg g⁻¹, $p < 0.01$) and solonetz (diff = 0.04 mg g⁻¹, $p = 0.02$), while andosols had greater DSi than phaeozems (diff = 0.05 mg g⁻¹, $p < 0.01$; Fig 3b). Soil DSi displayed negative linear relationships with soil % sand (Fig 4d; $F_{1,61} = 112.2$, adj. $r^2 = 0.64$, $p < 0.01$) and precipitation (Fig 4c; $F_{1,61} = 7.78$, adj. $r^2 = 0.10$, $p < 0.01$), while soil pH provided additional explanation of the residual variance. Neither topography (CTI) nor NDVI were statistically related to either of the soil Si pools.

Si values for the community grass samples varied more than two-fold across the landscape (range = 2.1 – 5.3% Si dry weight), with highest leaf silicon values recorded in the southern plains where DSi availability was also greatest. In general, leaf Si concentration was strongly related to soil silicon availability, which explained 61% of the variance in leaf Si when site was included as a random effect in a linear mixed model (Fig 4a). *Digitaria*, *Themeda*, and bulk samples exhibited similar leaf Si concentration, each ranging from about 2 - 5% dry weight (mean ± SE % dw *Digitaria*: 3.27 ± 0.17, *Themeda*: 3.69 ± 0.22, bulk: 3.42 ± 0.17). The bulk community samples strongly reflected species-specific values for *Digitaria* leaf silica (Pearson's $r = 0.89$, $n = 14$, $p < 0.01$) but only weakly reflected *Themeda* leaf silica values (Pearson's $r = 0.50$, $n = 13$, $p = 0.08$); *Digitaria* and *Themeda* leaf Si were strongly correlated at the 9 sites where they coexisted (Pearson's $r = 0.69$, $p = 0.04$). Notably, neither of the individual plant species nor the bulk sample demonstrated a linear relationship between leaf Si concentration and precipitation ($p > 0.15$ for all regressions, Table 1).

Structural equation model results

When sand, silt, and clay were tested for their fit in SEM, % sand provided the best variance explanation and, hence, was used as the sole soil texture variable in subsequent models. Our *a priori* model, which included all possible paths from precipitation, soil organic matter, soil pH, and soil % sand, explained 72.3% of variation in DSi and 22.5% of variation in ASi, several non-significant ($p > 0.05$) abiotic predictor variables indicated that this model was over-fit. After elimination of insignificant paths and allowing covariation between precipitation and % sand and precipitation and soil pH, our final model explained 19% and 74% of the variation in soil ASi and soil DSi, respectively (Fig 5). In the mixed model predicting leaf Si, including plant species as a random effect did not improve model fit, as indicated by a lack of increased variance explanation; species was excluded from all subsequent models. Our plant Si prediction was, however, improved by including site as a random effect, as is observed by comparing the marginal and conditional correlation coefficients in Figure 5. In terms of their standardized indirect effects, soil texture (% sand) had the greatest influence on plant Si (-0.41), followed by pH (-0.18) and precipitation (-0.11).

Leaf-level correlations

Additional measurements of leaf chemistry revealed that leaf % C was negatively related to leaf % Si, with the greatest negative slope being for *Themeda triandra* (estimate = -2.12 ± 0.53 SE, adj. $r^2 = 0.56$, $F_{11} = 16.29$, $p < 0.01$; Figure S1). Slopes for *Digitaria macroblephera* and the bulk grass sample were slightly less negative (*Digitaria* = -1.24 ± 0.21 , adj. $r^2 = 0.71$, $F_{12} = 33.5$, $p < 0.01$; bulk = -0.79 ± 0.24 , adj. $r^2 = 0.36$, $F_{16} = 10.68$, $p < 0.01$; Figure S1). Neither leaf phosphorus, nor leaf nitrogen exhibited a linear relationship with leaf Si concentration (data not shown).

DISCUSSION

We observed clear spatial patterns in the soil Si landscape of Serengeti. Soil ASi was highest in the southeastern part of the system, where recent volcanic ash deposits from Ol'Ddoinyo Lengai are of natrocarbonatite origin (e.g., Bell 1989). Soil ASi generally declined to the north and west, where precipitation is highest, and the lowest soil ASi occurred near the center of the ecosystem at the fringe of the Serengeti plains (Table 1, Fig S2). The localized region of very low ASi in central Serengeti does not appear to correspond with a specific soil group (Fig 3), although a general gradient of increasing ASi was observed with increasing proximity to the Ngorongoro highlands. These high ASi concentrations were surprising at first, but can be explained upon further consideration of the region's geological history. While recent eruptions of Ol'Doinyo Lengai in 1940, 1960, and 2008 were of natrocarbonatite origin, and the Rift Valley contains one of the highest concentrations of carbonatite volcanoes globally, we do not know the Si content of extrusives from the surrounding, and now long extinct, volcanoes which have historically influenced the region's soil (Bell 1989, Keller *et al.* 2010). Thus, despite low input of Si by recent volcanic ash, grasslands plains in existence since the Pleistocene may compensate for low source input by supplying a large and readily soluble pool of phytoliths (e.g., Kanno and Arimura 1958). Furthermore, ash deposits in the plains have leached to form a caliche layer which affects the chemistry, mineralogy, and hydrology of topsoils, and hence, dissolution rate of the ASi pool.

Soil DSi exhibited a striking north-to-south pattern (Table 1, Supplementary Fig 2), whereby values increased by about 3-fold from the northernmost to southernmost study sites. We were surprised to observe a negative relationship between soil pH and soil DSi, and while soil pH clearly influences Si dissolution (Liang *et al.* 2015 and references within), the relative strength and direction of this relationship appear to be

dependent upon underlying mineralogy and the range of soil pH represented in a given study. For instance, divalent and trivalent cations, such as Fe and Al, greatly reduce ASi solubility when adsorbed to its surface (Haynes 2014). Furthermore, because other abiotic gradients profoundly influenced soil DSi in our study area, pH explained relatively little of the variance in soil DSi (see below). Our structural equation model highlighted the importance of precipitation and texture in driving soil DSi variation. Precipitation has offsetting effects in grasslands: greater productivity, and thus higher silica uptake, on the one hand, and acceleration of phytolith dissolution on the other (Blecker *et al.* 2006). Our results suggest that the negative effects of precipitation on DSi, likely due to dilution and/or leaching, outweigh those expected as a result of increased productivity and phytolith production. Precipitation also results in weathering, which may lead to a greater fraction of soil Si which is adsorbed to metals and resistant to dissolution (Haynes 2014). Similar to previous findings (Blecker *et al.* 2006), we observed an overall negative trend between precipitation and soil ASi, which may indicate that increased phytolith production is offset by the effects of precipitation on leaching of DSi through the topsoil. Variation in soil texture, however, proved to be the strongest predictor of plant-available soil Si (DSi), overshadowing the observed effects of both precipitation and soil pH. We present three hypotheses to explain this strong statistical relationship. First, soil texture is known to explain patterns in primary productivity after accounting for precipitation (e.g. Epstein *et al.* 1997). Thus, soil texture (here, % sand) may be driving plant Si through its effects on biomass production. We explored this hypothesis by testing for a linear relationship between satellite-derived NDVI (see methods) and soil texture but found no support for this explanation. Second, the relationship between % sand and DSi may be an indirect consequence of plant community composition (e.g., McNaughton 1983). However, species composition alone is unable to explain observed differences in plant Si: where *Cynodon dactylon* was one of the two most dominant grass species (n = 7),

sand ranged from 19% – 77%, and mean DSi was 0.09 ± 0.03 mg/g SE, as compared to 0.09 ± 0.01 mg/g SE across all sites (Table 1; Supplementary Table 1). Third, texture may influence DSi through its effects on soil hydrology and leaching. Sand allows for translocation of small (~ 0.20 μm) phytoliths through the topsoil and below the grass rooting horizon and rapid percolation of DSi to ground water (Fishkis et al. 2009). Of the possible mechanisms which explain the strong link between soil texture and Si availability, the one proposing that sandy soils experience greater leaching seems most plausible.

Herbivory and fire are also important in savanna ecosystems, and both have been implicated as additional factors related to grass Si accumulation (Massey *et al.* 2007a, Reynolds *et al.* 2012, Melzer et al. 2009, Alexandre et al. 2011). Grazing intensity in SNP is famously high and may have resulted in especially strong selection for Si accumulation in Serengeti grasses ($\sim 3.5\%$ dry weight Si, on average) relative to more commonly studied species, such as *Lolium* and *Festuca*, which average closer to 1-2% foliar Si. High leaf Si concentration of shortgrass plains grasses is consistent with increased uptake of Si associated with grazing, as these samples were collected during a seasonal peak in grazing associated with the migration. Of further interest is the potential role of migratory grazers in putative transport of Si across the landscape. In Serengeti, grazers contribute to Si availability by consuming large amounts of Si-rich grass and returning undigested material and easily-weathered grass phytoliths to the soil in dung. Dung from grazers has been referred to as a silica “dissolution hotspot” due to accelerated weathering of phytoliths following digestion by cattle (Vandevenne *et al.* 2013). The rapid influx of ~ 1.2 million large-bodied grazers into the Serengeti plains could result in a pulse of soil ASi, which is slow to solubilize into DSi due to local abiotic conditions (i.e. low rainfall, high pH). This mechanism could contribute to high observed

soil phytolith (ASi) content in the plains and offset the low-Si parent material there (i.e. volcanic ash). Thus, the migration has the potential to act as a mechanism of nutrient translocation, similar to the export of nitrogen out of the Serengeti plains (e.g., Holdo et al. 2006), but in the opposite direction. Fire may affect the Si cycle by reducing ASi input to soils due to export/loss of ash (Alexandre et al. 2011), and by altering the amount of Si taken up by vegetation (Melzer et al 2009, Dufek et al. 2014).

We were surprised to find no statistically significant relationship between soil ASi and plant-available soil DSi. Large reservoirs of soil biogenic Si corresponded with high Si export from soils in other systems (Ronchi et al 2015), albeit for different soil (pH < 4) and vegetation community (forest). The modern savanna grasslands of Serengeti were generated in the Pleistocene, nearly 1 mya (Cerling 1992). In light of the high productivity and stability of these grasslands, the soil ASi pool seems to have become saturated by phytolith input (Kelly et al. 1998) to the point that it no longer limits the size of the DSi pool and has reached an equilibrium state characterized by a large and slowly growing pool of ASi (e.g., Alexandre et al. 2011). In summary, we suspect that soils throughout Serengeti have a capacity, or solid phase Si reserve supply, which is non-limiting; instead, factors which influence dissolution now limit the plant-available DSi pool. While a Si budget does not yet exist for grasslands (Conley 2002), the observed buildup of soil ASi in Serengeti, combined with the globally widespread occurrence and abundance of grasses (> 40% of earth's land surface; White et al. 2000) emphasizes the source/sink role that grasses play in the silicon biogeochemical cycle.

Finally, our phytochemical analysis corroborates previous studies suggesting a tradeoff between silicon and carbon-based structural molecules. Schaller *et al.* (2012) concluded that observed tradeoffs among silicon, cellulose, and phenol concentration in *Phragmites* leaves indicate a tradeoff between productivity and stability/defense. Plant

silica accumulation is also negatively correlated with lignin and cellulose in aquatic macrophytes (Schoelynck et al. 2010), and with leaf carbon, total phenols, and tannins of Australian eucalypt communities (Cooke & Leishman 2012). McNaughton et al. (1985) noted growth promotion and increased chlorophyll concentration in silica-fed Serengeti grasses, indicating that Si is an effective, and perhaps even beneficial, substitute for carbon, while Massey et al.'s measurements of 18 grass species provide strong evidence of a multivariate growth-defense tradeoff (2007b). While compelling, we acknowledge that the negative relationship between leaf % C and leaf % Si is likely the result of more complex multivariate tradeoffs occurring in leaf phytochemistry (Agrawal 2007).

Conclusions

We found that variation in soil DSi explains a significant amount of the observed variation in grass Si content across a highly heterogeneous savanna landscape. While soil DSi did not appear to be limited by the total pool of soil ASi, abiotic conditions were tightly linked to soil Si dissolution. Our results indicate an especially strong role for soil texture in determining soil DSi, likely through its influence on soil hydrology, and highlight that heterogeneity in Si-accumulation by grasses is related to soil DSi availability. Because soil ASi tends to be saturated in grasslands, and leaf Si concentration is strongly correlated with soil DSi, abiotic conditions that affect dissolution of the ASi pool may be implicated in plant growth and defense strategies and, perhaps, even plant community composition along environmental gradients. In Serengeti, the soil ASi pool appears to act as a major Si sink, and our study warrants further research into biome-specific variation in the biogeochemical Si cycle and the potential role of grazing herbivores in Si transport and mobilization.

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LITERATURE CITED

- Agarie S, Hanaoka N, Ueno O, Miyazaki A, Kubota F, Agata W, Kaufman PB. 1998. Effects of silicon on tolerance to water deficit and heat stress in rice plants, monitored by electrolyte leakage. *Plant Production Science* 1 (2): 96–103.
- Agrawal, AA. 2007. Macroevolution of plant defense strategies. *Trends in Ecology & Evolution* 22 (2): 103–9.
- Anderson, TM, Metzger KL, McNaughton SJ. 2007. Multi-scale analysis of plant species richness in Serengeti Grasslands. *Journal of Biogeography* 34 (2): 313–323.
- Alexandre A, Bouvet M, Abbadie L. 2011. The role of savannas in the terrestrial Si cycle: A case-study from Lamto, Ivory Coast. *Global and Planetary Change* 78 (3-4): 162–169.
- Anderson GD, Talbot LM. 1965. Soil factors affecting the distribution of the grassland types and their utilization by wild animals on the Serengeti plains, Tanganyika. *Journal of Ecology* 53 (1): 33–56.
- Anderson TM, Metzger KL, McNaughton SJ. 2007. Multi-scale analysis of plant species richness in Serengeti grasslands. *Journal of Biogeography* 34 (2): 313-323.
- Barber DA, Shone MGT. 1966. The absorption of silica from aqueous solutions by plants. *Journal of Experimental Botany* 17 (3): 569–578.
- Bates D, Maechler M, Bolker B and Walker S. 2014. lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1-7, <http://CRAN.R-project.org/package=lme4>.

- Beckwith RS, Reeve R. 1964. Studies on soluble silica in soils. *Australian Journal of Soil Research* 2: 33–45.
- Bell K. 1989. *Carbonatites: Genesis and Evolution*. K Bell (ed). Unwin Hyman, London.
- Blecker SW, McCulley RL, Chadwick OA, Kelly EF. 2006. Biologic cycling of silica across a grassland bioclimate sequence. *Global Biogeochemical Cycles* 20 (3): 1–11.
- Berthelsen S, Kordorfer GH. 2005. Methods for Silicon Analysis in Soil, Plant, and Fertilizers. *Proceedings of the Third International Conference on Silicon in Agriculture* 85–91. Uberlandia, Brazil.
- Beusen AHW, AF Bouwman, HH Dürr, ALM Dekkers, J Hartmann. 2009. Global patterns of dissolved silica export to the coastal zone: Results from a spatially explicit global model. *Global Biogeochemical Cycles* 23 (4): 1–13.
- Cerling TE. 1992. Development of grasslands and savannas in East Africa during the Neogene. *Global and Planetary Change* 5(3): 241–247.
- Chen C, Lewin J. 1969. Silicon as a nutrient element for *Equisetum arvense*. *Canadian Journal of Botany* 47 (1): 125–131.
- Clymans W, Struyf E, Govers G, Vandevenne F, Conley DJ. 2011. Anthropogenic impact on amorphous silica pools in temperate soils. *Biogeosciences* 8: 2281–2293.
- Conley DJ. 2002. Terrestrial ecosystems and the global biogeochemical silica cycle. *Global Biogeochemical Cycles* 16 (4): 1–8.
- Cooke J, Leishman MR. 2012. Tradeoffs between foliar silicon and carbon-based defences: evidence from vegetation communities of contrasting soil types. *Oikos* 121 (12): 2052–2060.
- Cornelis JT, B Delvaux, RB Georg, Y Lucas, J Ranger, S Opfergelt. 2011. Tracing the Origin of Dissolved Silicon Transferred from Various Soil-Plant Systems towards Rivers: A Review. *Biogeosciences* 8 (1): 89–112.
- Corrales I, Poschenrieder C, Barceló J. 1997. Influence of silicon pretreatment on aluminium toxicity in maize roots. *Plant and Soil* 190 (2): 203–209.
- Cotterill JV, RW Watkins, CB Brennon, DP Cowan. 2007. Boosting Silica Levels in Wheat Leaves Reduces Grazing by Rabbits. *Pest Management Science* 63 (3): 247–53.
- Dawson JB. 1963. Carbonatitic volcanic ashes in northern Tanganyika. *Bulletin Volcanologique* 27: 1–11.

- Drees, LR, Wilding LP, Smeck NE, and Sankayi AL. 1989. Silica in soils: quartz and disordered silica polymorphs. In: Dixon, Weed, and Dinauer (eds) Minerals in soil environments, 913–974. Madison, WI: Soil Science Society of America.
- DeWit HA. 1978. Soils and grassland types of the Serengeti plain Tanzania. Wageningen.
- Dufek NA, Vermeire LT, Waterman RC, Ganguli AC. 2014. Fire and Nitrogen Addition Increase Forage Quality of *Aristida Purpurea*. *Rangeland Ecology & Management* 67 (3): 298–306.
- Epstein HE, WK Lauenroth, IC Burke. 1997. Effects of temperature and soil texture on ANPP in the U.S. Great Plains. *Ecology* 78 (8): 2628–31.
- FAO. 1998. International Soil Reference and Information Center (ISRIC) and International Society of Soil Science (ISSS). World Reference Base for Soil Resources. FAO, Rome.
- Fishkis O, Ingwersen J, Streck T. 2009. Phytolith transport in sandy sediment: experiments and modeling. *Geoderma* 151 (3-4): 168–178.
- Garbuzov M, Reidinger S, Hartley SE. 2011. Interactive effects of plant-available soil silicon and herbivory on competition between two grass species. *Annals of Botany* 108 (7): 1355–1363.
- Gérard F, François M, Ranger J. 2002. Processes Controlling Silica Concentration in Leaching and Capillary Soil Solutions of an Acidic Brown Forest Soil (Rhône, France). *Geoderma* 107: 197-226.
- Gérard F, Mayer KU, Hodson MJ, Ranger J. 2008. Modelling the Biogeochemical Cycle of Silicon in Soils: Application to a Temperate Forest Ecosystem. *Geochimica et Cosmochimica Acta* 72: 741–58.
- Gessler PE, Moore ID, McKenzie NJ, Ryan PJ. 1995. Soil-landscape modelling and spatial prediction of soil attributes. *International Journal of Geographical Information Systems* 9 (4): 421–432.
- Hattori T, Inanaga S, Araki H, An P, Morita S, Luxova M, Lux A. 2005. Application of silicon enhanced drought tolerance in *Sorghum bicolor*. *Physiologia Plantarum* 123 (4): 459–466.
- Haynes RJ. 2014. A contemporary overview of silicon availability in agricultural soils. *Journal of Plant Nutrition and Soil Science* 177 (6): 831–44.
- Haysom M, Chapman L. 1975. Some Aspects of the Calcium Silicate Trial at Mackay. In *Proceedings of the Queensland Society of Sugar Cane Technologists* 42:117–22.
- Hodson MJ, White PJ, Mead A, Broadley MR. 2005. Phylogenetic variation in the silicon composition of plants. *Annals of Botany* 96 (6): 1027–1046.

- Holdo RM, Holt RD, Coughenour MB, Ritchie ME. 2006. Plant productivity and soil nitrogen as a function of grazing, migration and fire in an African savanna. *Journal of Ecology* 95 (1): 115–128.
- Jager TJ. 1982. Soils of the Serengeti woodlands, Tanzania. Agricultural research report 912 / Serengeti Research Institute Publication no. 301. Pudoc, Wageningen, the Netherlands.
- Jarman, PJ, Sinclair ARE. 1979. Feeding strategy and the pattern of resource partitioning in ungulates. In: ARE Sinclair and M Norton-Griffiths (eds.), *Serengeti: dynamics of an ecosystem*. University of Chicago Press, Chicago, pp 131-163.
- Kanno, I. and A. Shizuoki. 1958. Plant opal in Japanese soils. *Soil Science and Plant Nutrition* 4(2): 62-67.
- Keller J, Klaudius J, Kervyn M, Ernst GGJ, Mattsson HB. 2010. Fundamental Changes in the Activity of the Natrocarbonatite Volcano Oldoinyo Lengai, Tanzania. *Bulletin of Volcanology* 72 (8): 893–912.
- Kelly EF, Chadwick OA, Hilinski TE. 1998. The effect of plants on mineral weathering. *Biogeochemistry* 42: 21–53.
- Lehner B, Verdin K, Jarvis A. 2006. HydroSHEDS technical documentation. World Wildlife Fund US. Washington, DC. Available at <http://hydrosheds.cr.usgs.gov>
- Liang Y, Hua H, Zhu YG, Zhang J, Cheng C, Römheld V. 2006. Importance of plant species and external silicon concentration to active silicon uptake and transport. *New Phytologist*, 172 (1): 63–72.
- Liang Y, Nicolik M, Belanger R, Gong H, Song A. 2015. Silicon in Agriculture. In: *Silicon in agriculture: from theory to practice*. Springer, pp 45–68.
- Lindsay, WL. 1979. Silica. In: *Chemical Equilibria in Soils*, Wiley, New York, pp 51-55.
- LPDAAC (2013) Land processes Distributed Active Archive Center, Datapool @ LP DAAC
- Massey FP, Ennos RA, Hartley SE. 2007. Grasses and the resource availability hypothesis: the importance of silica-based defences. *Journal of Ecology* 95 (3): 414–424.
- . 2007b. Herbivore specific induction of silica-based plant defences. *Oecologia* 152 (4): 677–683.
- McKeague JA, Cline MG. 1962. The Adsorption of Monosilicic Acid by Soil and Other Substances. *Canadian Journal of Soil Science* 43: 83–96.

- McNaughton SJ. 1983. Serengeti grassland ecology : The role of composite environmental factors and contingency in community organization. *Ecological Monographs*. 53 (3): 291–320.
- McNaughton SJ, Tarrants JL. 1983. Grass leaf silicification : natural selection for an inducible defense against herbivores. *Proceedings of the National Academy of Sciences* 80 (3): 790–791.
- McNaughton, SJ, Tarrants JL, McNaughton MM, Davis RH. 1985. Silica as a defense against herbivory and a growth promotor in African grasses. *Ecology* 66 (2): 528–535.
- Melzer SE, Knapp AK, Kirkman KP, Smith MD, Blair JM, Kelly EF. 2009. Fire and Grazing Impacts on Silica Production and Storage in Grass Dominated Ecosystems. *Biogeochemistry* 97 (2-3): 263–78.
- Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R² from generalized linear mixed-effects models. *Methods in Ecology and Evolution* 4 (2): 133–142.
- Quigley KM, Anderson TM. 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: 1–10.
- R Development Core Team. 2014. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna.
- Rémus-Borel W, Menzies JG, Bélanger RR. 2005. Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiological and Molecular Plant Pathology* 66 (3): 108–115.
- Reynolds, JJH, Lambin X, Massey FP, Reidinger S, Sherratt JA, Smith MJ, White A, Hartley SE. 2012. Delayed induced silica defences in grasses and their potential for destabilising herbivore population dynamics. *Oecologia* 170 (2): 445–56.
- Robertson GP, Coleman DC, Bledsoe CS, Sollins P (eds). 1999. *Standard soil methods for long-term ecological research*. Oxford University Press: New York.
- Ronchi B, Barão L, Clymans W, Vandevenne F, Batelaan O, Govers G, Struyf E, Dassargues E. 2015. Factors controlling Si export from soils: a soil column approach. *Catena* 133: 85–96.
- Rosseel Y. 2012. Lavaan: an R package for structural equation modeling. *Journal of Statistical Software* 48(2): 1-36.
- Saccone L, Conley DJ, Koning E, Sauer D, Sommer M, Kaczorek D, Blecker SW, Kelly EF. 2007. Assessing the extraction and quantification of amorphous silica in soils

- of forest and grassland ecosystems. *European Journal of Soil Science* 58(6): 1446-1459.
- Schaller J, Brackhage C, Dudel EG. 2012. Silicon availability changes structural carbon ratio and phenol content of grasses. *Environmental and Experimental Botany* 77: 283–287.
- Schoelynck J, Bal K, Backx H, Okruszko T, Meire P, Struyf E. 2010. Silica uptake in aquatic and wetland macrophytes: a strategic choice between silica, lignin and cellulose? *New Phytologist* 186 (2): 385–391.
- Shipley B. 2009. Confirmatory path analysis in a generalized multilevel context. *Ecology* 90 (2): 363–368.
- Sinclair ARE. 1979. Dynamics of the Serengeti ecosystem: process and pattern. In: ARE Sinclair and M. Norton-Griffiths (eds.) *Serengeti: dynamics of an ecosystem*. University of Chicago Press, Chicago, pp 1-30.
- Sommer M, Kaczorek D, Kuzyakov Y, Breuer J. 2006. Silicon pools and fluxes in soils and landscapes—a review. *Journal of Plant Nutrition and Soil Science* 169: 310-329.
- Street-Perrott FA and Barker PA. 2008. Biogenic Silica: A Neglected Component of the Coupled Global Continental Biogeochemical Cycles of Carbon and Silicon. *Earth Surface Processes and Landforms* 33: 1436–1457.
- Struyf E and Conley DJ. 2009. Silica: An Essential Nutrient in Wetland Biogeochemistry. *Frontiers in Ecology and the Environment* 7 (2): 88–94.
- Takahashi E. 1990. The possibility of silicon as an essential element for higher plants. *Comments on Agricultural & Food Chemistry* 2 (2): 99–122.
- Trembath-Reichert E, Wilson JP, McGlynn SE, Fischer WW. 2015. Four hundred million years of silica biomineralization in land plants. *Proceedings of the National Academy of Sciences* 112(17): 5449-5454.
- Vandevenne FI, Barão AL, Schoelynck J, Smis A, Ryken N, VanDamme S, Meire P, Struyf E. 2013. Grazers : biocatalysts of terrestrial silica cycling. *Proceedings of the Royal Society B* 280: 1–9.
- Wedepohl KH. 1995. Composition of the continental crust. *Geochimica et Cosmochimica Acta* 59 (7): 1217–1232.
- White F. 1983. *The Vegetation of Africa*. Paris: UNESCO.
- White R, Murray S, Rohweder M. 2000. Pilot analysis of global ecosystems: grassland ecosystems technical report. World Resources Institute, Washington, DC.

Yeo AR, Flowers SA, Rao G, Welfare K, Senanayake N, Flowers TJ. 1999. Silicon reduces sodium uptake in rice (*Oryza sativa* L.) in saline conditions and this is accounted for by a reduction in the transpirational bypass flow. *Plant, Cell and Environment* 22: 559–565.

Zysset M, Blaser P, Luster J, Gehring AU. 1999. Aluminum solubility control in different horizons of a podzol. *Soil Science Society of America* 63: 1106-1115.

Figures and Tables

Table 1. Site level variation in abiotic conditions and associated soil and plant Si measurements. Leaf Si may be converted to biogenic silica (SiO₂) concentration by dividing by a conversion factor of 0.4674. Sites are arranged in order of increasing precipitation. For a list of species present in the bulk grass sample at each site, see supplementary table 1. For plant collection site locations and splined surfaces of soil ASi and DSi see Supplementary Materials.

SITE	MAP	Soil type	Soil factors			Leaf Si (%)		
	mm * yr ⁻¹		pH	ASi (mg/g)	DSi (mg/g)	bulk	Digitaria	Themeda
1	615	andosol	8.03	14.05	0.07	4.46	3.76	—
2	620	andosol	8.17	10.51	0.05	3.54	3.55	—
3	622	andosol	8.04	11.47	0.09	3.78	3.77	—
4	634	andosol	6.76	5.8	0.11	2.38	3.07	—
5	685	andosol	6.26	6.06	0.15	5.05	5.33	4.70
6	703	andosol	6.47	5.39	0.12	4.41	3.89	3.58
7	703	andosol	6.65	3.5	0.08	2.98	2.48	—
8	721	andosol	7.78	3.11	0.06	2.29	2.09	3.32
9	749	solonetz	5.92	4.24	0.13	3.01	—	4.67
10	749	andosol	5.94	5.08	0.14	3.80	—	4.18
11	757	andosol	6.11	3.47	0.11	3.32	—	5.09
12	760	phaeozem	6.14	5.49	0.12	3.59	3.39	3.94
13	789	solonetz	6.07	4.96	0.08	2.80	2.54	2.26
14	790	solonetz	5.7	3.94	0.09	3.49	3.03	3.48
15	805	phaeozem	6.21	3.48	0.06	3.49	3.17	2.90
16	889	vertisol	5.53	5	0.1	3.06	3.18	3.60
17	910	vertisol	6.41	2.54	0.05	3.29	2.55	3.18
18	924	phaeozem	5.52	3.96	0.06	2.76	—	3.13

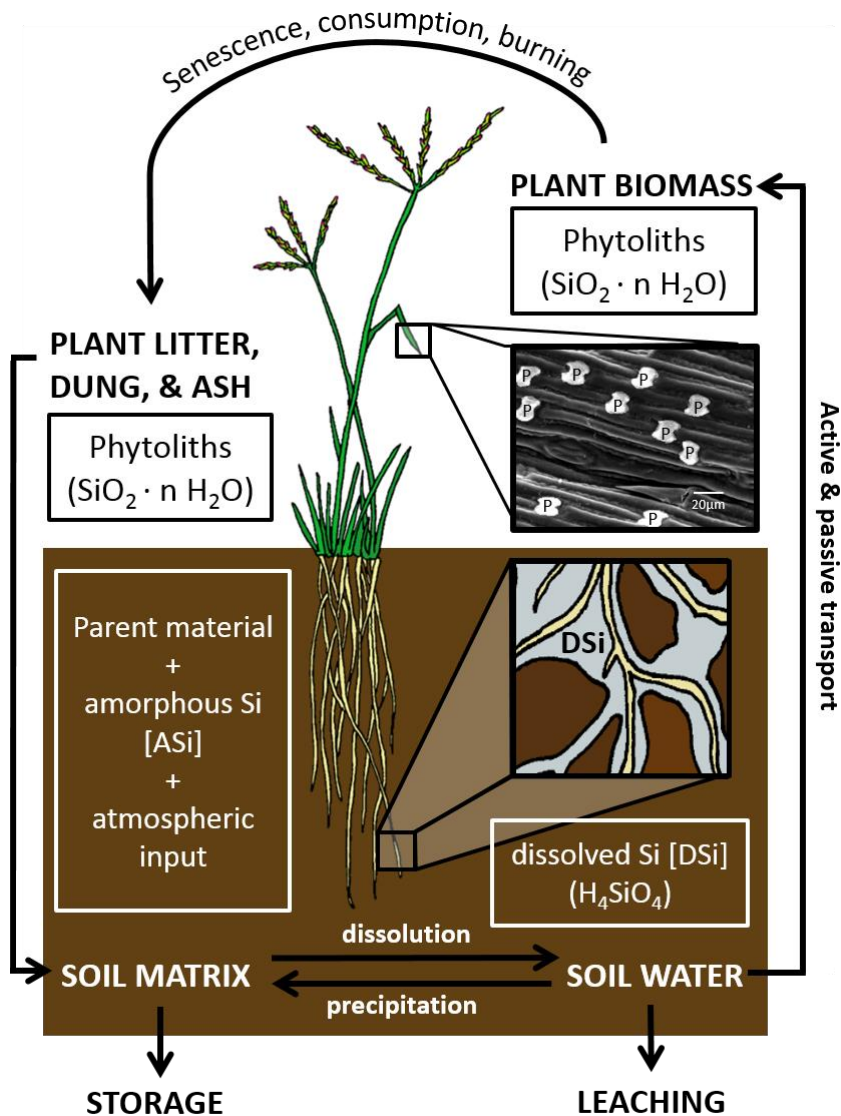


Figure 1. Generalized conceptual model of Si flux in grasslands, emphasizing the plant-soil interface. Silicon exists in crystalline and amorphous (ASi) forms in soil. The amorphous Si pool consists of pedogenic and biogenic sources, but phytoliths (“P” in upper right image), a biogenic source, dominate. ASi is readily weathered into the soil water, where it occurs as silicic acid (H_4SiO_4) or dissolved silicon (DSi). DSi is absorbed by plant roots and transported to shoots via the transpiration stream. As water is lost through stomates, solid SiO_2 precipitates in specialized cells. These solid silica deposits, known as phytoliths, are returned to soil via plant litter, ash, and animal dung.

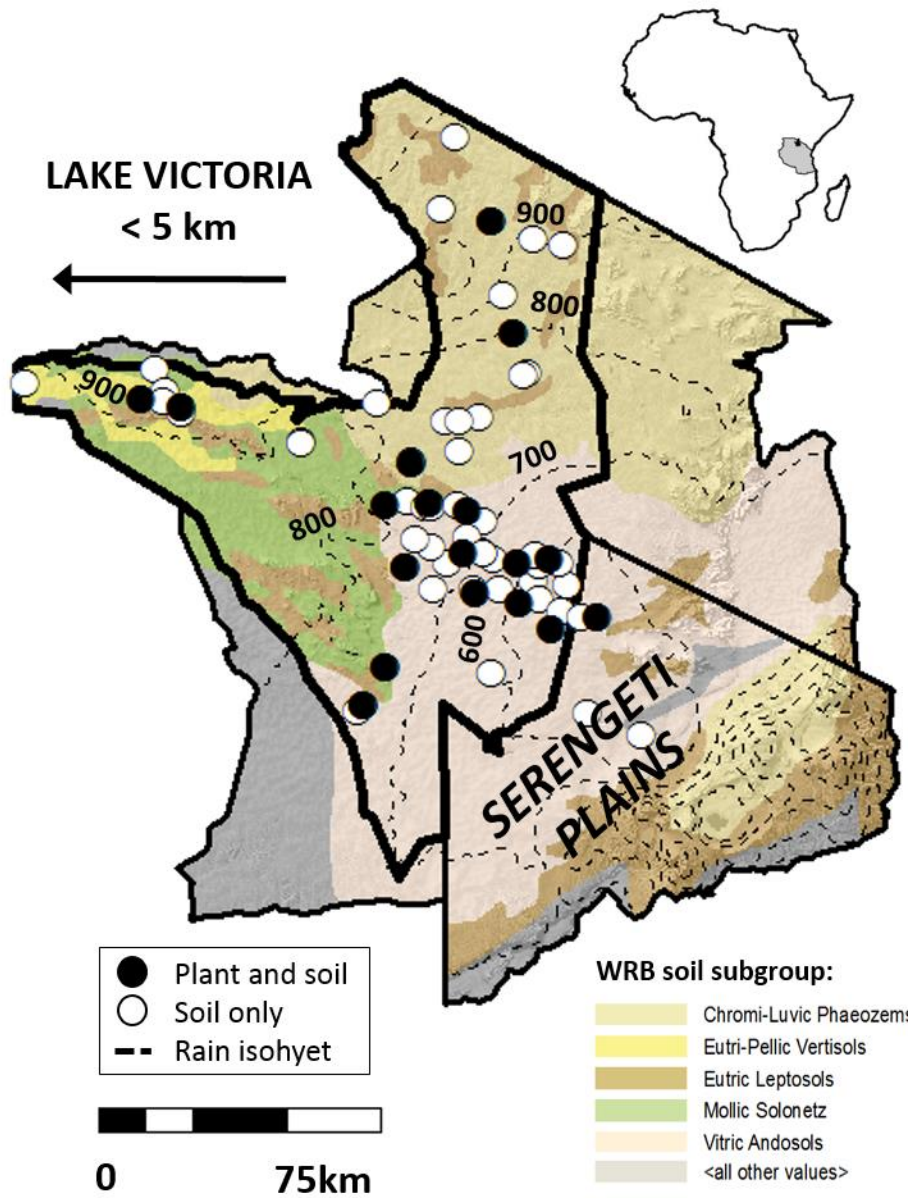


Figure 2. Major soil types of Serengeti National Park (SNP, bold outline) and surrounding protected areas. Soil types from which < 3 samples were collected are labeled as “other”. Numbers along isohyet bands represent precipitation in mm yr⁻¹. Soil subgroup classifications are from the World Reference Base (FAO 1998).

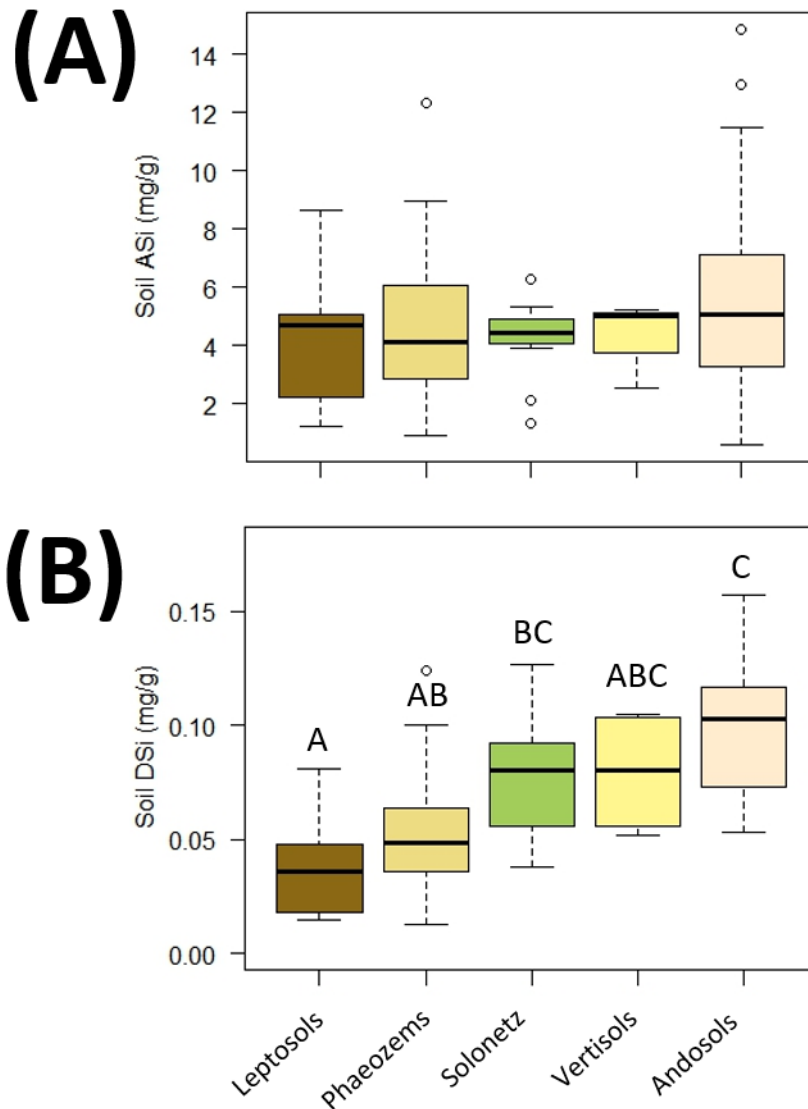


Figure 3. Variation in soil amorphous silicon (a) and soil dissolved silicon (b) pools for the major World Reference Base soil types of Serengeti. Colors correspond with Fig 2, and letters above boxes indicate significantly different groups. Soil ASi did not vary significantly among WRB soil subgroups. In contrast, soil DSi did vary with soil type: leptosols had lower DSi than both solonetz and andosols, while andosols had significantly greater DSi than phaeozems.

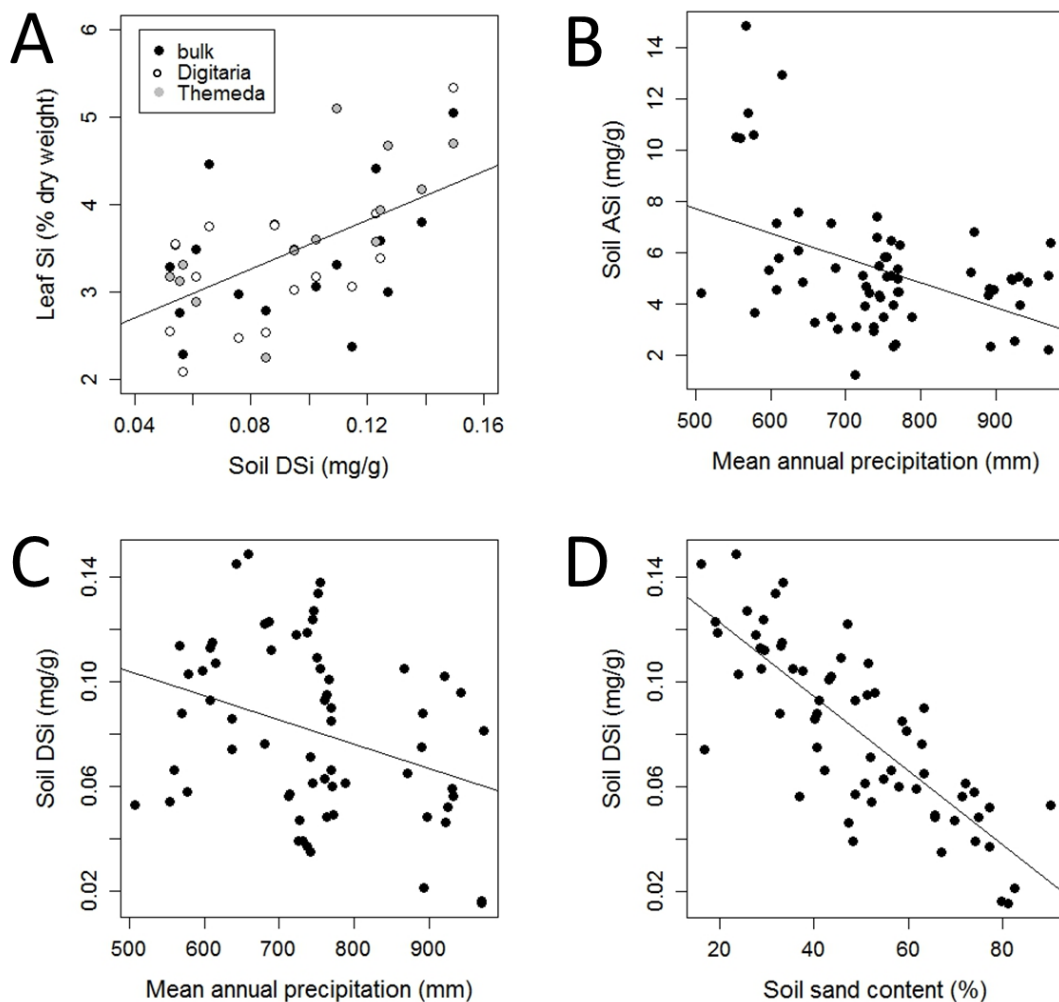


Figure 4. Univariate relationships between plant-available soil silicon (DSi) and leaf Si concentration (Panel A) and between abiotic conditions and soil silicon pools (Panels B-D). Leaf Si concentration was strongly correlated with soil dissolved Si, and inclusion of site as a random factor improved predictive strength (Panel A; line of best fit represents linear regression between all plant samples pooled and soil DSi; see text for species-specific fits). Soil ASi and soil DSi exhibited negative correlations with precipitation (Panels B, C). Soil DSi also exhibited a strong negative correlation with soil percent sand (Panel D). Regression statistics are provided in text.

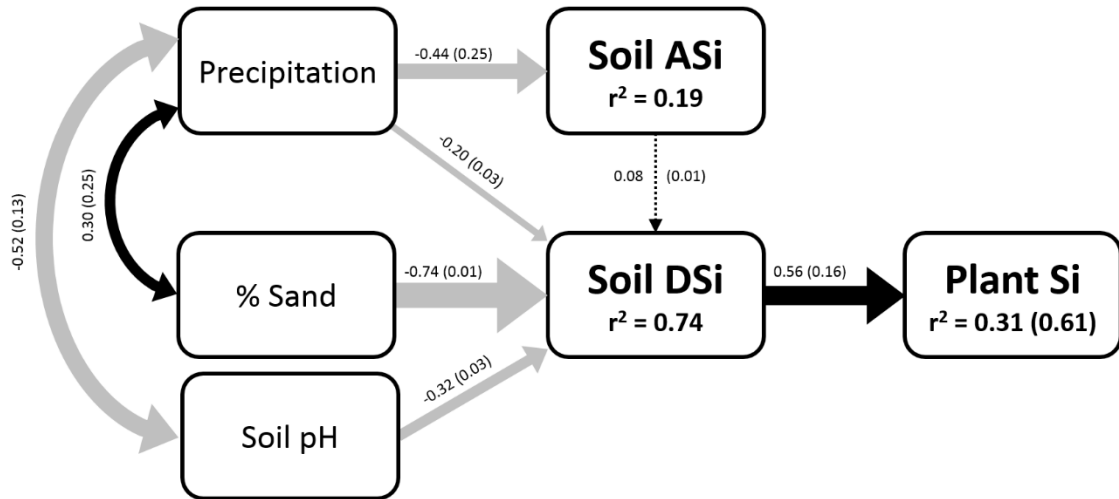


Figure 5. Final model for predicting Si concentration of grasses. Gray arrows represent negative relationships and black arrows represent positive relationships; arrow thickness corresponds with relationship strength. Numbers along arrows represent the standardized path coefficient estimates based on observed variables and covariance between variables (see text), with standard errors in parentheses. Precipitation was a strong predictor of both soil ASi and soil DSi, while percent sand and soil pH provided additional variance explanation of the soil DSi pool. For plant Si, marginal r^2 is provided with conditional r^2 in parentheses. All paths are significant, except that between soil ASi and soil DSi, which is represented by a dashed arrow ($p = 0.27$).

CHAPTER 4

SOIL NUTRIENTS AND PRECIPITATION, NOT HERBIVORY, DRIVE GLOBAL PATTERNS OF GRASS LEAF SILICIFICATION

The following manuscript is formatted for and to be submitted to *Ecology Letters*.

Stylistic variations are due to the requirements of the journal.

ABSTRACT

Plants vary remarkably in the silicon (Si) contents of their leaves, yet the drivers of leaf silicification remain contentious. Grasses accumulate high levels of leaf Si, with potential benefits including herbivore defense, improved water balance and reduced leaf construction costs. We analyzed grasses from 17 sites ranging in temperature (MAT: 0.3 – 21.1 °C) and precipitation (MAP: 365 – 1898 mm yr⁻¹) and in which nutrient availability and grazing were experimentally manipulated. Grass leaf silicon concentration showed no response to grazer exclusion, but leaf Si concentration decreased with NPK fertilizer across all sites. NPK addition was consistently the best correlate of leaf Si, while soil carbon emerged as an important site-level influence of leaf silica. Furthermore, grasses demonstrated a negative correlation between leaf C and leaf Si, a pattern which was strongest at dry and weakest at mesic sites. Global-scale relationships suggest that soil quality and water availability, rather than defoliation, are the primary drivers of global patterns of grass leaf silicification in grasses.

Keywords: Leaf silica, plant nutrition, climate, stoichiometry, grazing, soil nutrients, grass

INTRODUCTION:

Plant lineages silicify their tissues to vastly different extents, yet the ecological and evolutionary drivers of phytogenic silica are unclear. For example, leaf Si concentrations are near-zero in legumes (e.g. Jones & Handreck 1969, Parry & Winslow 1977) and most gymnosperms, intermediate in many terrestrial angiosperms (typically 0.5 - 1% dry weight; Trembath-Reichert et al. 2015, Ma & Takahashi 2002), and may reach 5-10 % of plant dry weight in the “Si accumulators”, such as grasses and early diverging land plants (e.g., bryophytes and pteridophytes). A family of transmembrane proteins, modified from aquaporins (Ma et al. 2006, Mitani & Ma 2005, Liu & Zhu 2010, Gregoire et al. 2012), allows plants to mobilize silicic acid (H_4SiO_4) from the soil water to aboveground tissue, where it polymerizes as silica (SiO_2) within and between cells (Piperno 2006). Beyond plant species identity, a plant’s abiotic and biotic environment also influence the extent of leaf silicification, and soil properties represent one such source of variation. For instance, soil particle size distribution was found to influence plant-available soil dissolved Si (DSi) along an environmental gradient (Quigley et al. 2016b). Soil DSi supply strongly correlated with leaf Si, a pattern which is consistently supported by experimental Si fertilization (e.g. Van der Vorm 1980, Gali & Smith 1992, Massey et al. 2007).

Previous studies have identified a range of benefits of plant Si-accumulation for abiotic stress tolerance, including salinity (Liang 1998, Liang 1999, Yin et al. 2015), drought (Hattori et al. 2005), metal toxicity (Corrales et al. 1997, Cunha & Nascimento 2008, Li et al. 2012, Vaculik et al. 2012, Che et al. 2016), and solar radiation (Yao et al. 2011, Fang et al. 2011). Plants may actively regulate Si uptake in response to external stimuli— a widespread phenomenon known as an induced response (Karban & Myers 1989). Natural enemies, ranging from fungal pathogens (e.g. Rémus-Borel et al. 2005)

to insect and mammalian herbivores, present an often substantial source of biotic stress which may be alleviated by Si-accumulation and/or induce Si uptake. Following speculation that hypsodonty likely evolved in response to the expansion of high-silica grasses (Stebbins 1981) and early research emphasizing the role of Si as an inducible defense against herbivory (e.g., McNaughton & Tarrants 1983, McNaughton et al. 1985), the role of herbivores in leaf silification continues to be a compelling hypothesis. Under some circumstances large mammals (e.g., sheep) prefer low silica grasses (Massey et al. 2009), grazed plants induce Si uptake after defoliation (Massey et al. 2007, Reynolds et al. 2012), and herbivores, both insects and small mammals, may experience negative fitness consequences of high silicon diets (Massey & Hartley 2006, Massey & Hartley 2009). However, recent synthesis has questioned the generality of herbivore-silica interactions by demonstrating that plant damage does not always induce uptake (Quigley and Anderson 2014), and that species identity (Kindomihou et al. 2006, Garbuzov et al. 2011, Soininen et al. 2012, Hartley et al. 2015) and extent of damage (e.g. Reynolds et al. 2012) influence the extent to which inducibility is observed. Analysis of the fossil record and Cenozoic grass-grazer coevolution has shed further doubt on the hypothesis that grass Si-accumulation evolved as an adaptation to increased grazing pressure by large-bodied herbivores (Stromberg et al. 2016).

Silicon appears to have a role in leaf economics (e.g., Wright et al. 2004). Across a suite of plant species, a negative correlation was observed between Si concentration and leaf longevity (Cook & Leishman 2011). Authors attributed this pattern to short-lived leaves utilizing Si as a cheap substitute for carbon. Phytoliths deposited in and around cell walls provide an inorganic alternative for structural rigidity (Kaufman et al. 1999) and, as discussed previously, may also provide an inorganic form of defense against herbivores. Furthermore, silica is 27-fold less metabolically expensive (i.e. ATP cost) than

lignin on a per-weight basis and 20-fold less costly on a per volume basis (Raven 1983). However, unlike many other plant nutrients which may be re-mobilized and transported in phloem, Si deposition is irreversible which presents a potential cost (Loneragan et al. 1976). It has been suggested that plants must make a “strategic choice” as to the structural compound in which they invest, and negative correlations have been observed between silica and carbon-based compounds such as lignin, cellulose, phenols and, more broadly, between silica and total leaf carbon (Schoelynck et al. 2010, Cooke & Leishman 2012). Due to the ubiquitous nature of Si in soils and its cheap metabolic cost of acquisition, it seems likely that plants growing in stressful (i.e. nutrient-poor) environments may shift toward a Si-accumulation strategy to conserve the amount of carbon allocated to structural or defensive components. Similarly, water availability may influence relationships between leaf C and Si. Since transpirational water loss tied to carbon fixation becomes more costly when precipitation is scarce, it may be beneficial for plants growing in arid sites to utilize Si for growth and defense.

The fact that Si is energetically cheap to obtain relative to carbon (Raven 1983) combined with the wide-spread putative benefits of Si accumulation, raises the compelling question of why so few plant taxa are Si accumulators. Similarly, for taxonomic groups which accumulate Si (e.g. the Poaceae), we lack a broader understanding of what drives the impressive variation observed in leaf Si (Quigley and Anderson 2014). To date, studies have focused on how Si effects the growth of agricultural plants, small landscape-scale patterns of foliar Si concentration, (e.g., Cooke and Leishman 2012, Soininen et al. 2012, Quigley et al. 2016b), and phylogenetic patterns of Si accumulation (e.g. Hodson et al. 2005, Trembath-Reichert et al. 2015, Stromberg et al. 2016), but have yet to examine the drivers of silicification across climatic gradients or in a stoichiometric context within a single clade which accumulates

Si. Furthermore, questions regarding the role of herbivores in natural systems have been largely limited to insects and small mammals, and abiotic conditions like soils are rarely taken into account when modeling plant Si. The goals of this study were to (1) test the relative importance of herbivores and soil nutrients as driver of grass silicification, (2) determine if silicification is contingent on climatic conditions, and (3) test for leaf-level trade-offs between grass leaf Si and C. To quantify the relative effects of soil nutrients, herbivory, and climate, we analyzed leaf samples collected from 17 Nutrient Network (NutNet) grassland sites across 5 continents where nutrient addition (NPK) and herbivory were manipulated. We first tested for main effects of experimental treatments with the expectations that 1) leaf Si would be higher outside of exclosures (where herbivores were allowed to graze) relative to within exclosures if Si uptake is induced by herbivory (e.g., McNaughton et al. 1983, McNaughton et al. 1985), and 2) leaf Si concentration would be reduced in plots which had NPK fertilizer added, if soil nutrient availability shifts plant resource allocation strategy. Significant variation in climate, soil properties, and relative grazing intensity among sites allowed us to address the relative importance of each in determining leaf Si concentration. In particular, we expected grasses collected from arid sites, where photosynthetic water loss is most costly, to have greater leaf Si concentration than those collected from mesic sites.

MATERIAL AND METHODS:

Study sites and experimental design

Data were collected from 17 sites within the Nutrient Network, a coordinated research network in which the same nutrient addition x fertilization experiment has been carried out since 2007 using a standardized protocol (e.g., Borer et al. 2014;

<http://nutnet.umn.edu/>). Sites represented a broad range of climate and biome space (mean annual precipitation (MAP): 365 – 1898 mm yr⁻¹; mean annual temperature (MAT): 0.3 – 22.1 °C; see supplementary methods) spanning five continents (Figure 1; Supplementary Table 1). At each participating site, ten 5 x 5 m plots were established in a block design within a footprint of > 1000 m² and randomly assigned to one of ten treatments that were combinations of fertilization and grazer removal by fencing (see O'Halloran et al. 2013). Treatments included a control (no fence, no nutrients added), 8 nutrient plots consisting of nitrogen, phosphorous, and potassium addition (plus pairwise and 3-way combinations of these), fenced plots, and fence + NPK plots. Nutrient treatments were applied yearly at 10 g m⁻² y⁻¹ prior to the growing season, and an initial 100 g m⁻² micronutrient mix (Fe, S, Mg, Mn, Cu, Zn, B, Mo) was applied the first year only. Fences were > 2 m high and consisted of t-posts at four corners, surrounded by wire mesh to 1 m height. At most sites, the edges of the mesh were secured to the ground forming a 30 cm skirt around the enclosure which served to exclude burrowing animals (see supplementary methods for full description). A grazing index (range: 0 – 29; Supplementary Table 1), which served to weight the relative intensity of grazing among sites, was calculated for each site by integrating grazer biomass and categorical relative abundance as reported by each site principal investigator. Within each plot, aboveground standing biomass was collected ≥ 2 years post treatment from each plot by clipping to the ground and pooling two, 10 cm wide x 1 m long strips. Samples were subdivided to 6 major groups (litter, bryophytes, graminoids, legumes, forbs, and woody plants) by local principal investigators, weighed, dried and sent to Wake Forest University for analysis of leaf chemistry. Because all sites were dominated by graminoids, and legumes and forbs had consistently very low Si concentrations near the limit of detection, we chose to focus subsequent analysis on graminoids only.

Measurement of leaf Si and C

Leaf Si and C concentrations were estimated using Near Infrared Spectroscopy (NIRS). All plant samples were ground using a cyclone mill (UDY Corp., Fort Collins, CO), dried at 60 °C for a minimum of 48 hours, then scanned in triplicate on a Bruker infrared multi-purpose analyzer (Bruker Optics Inc., Billerica, MA). The Kennard-Stone algorithm (Kennard & Stone 1969), which identifies maximum variation among the NIRS spectra, was used to select a subset of leaf samples (~20% of samples; n = 345) for wet lab chemical analysis and subsequent entry into calibration models. Wet lab leaf silicon was estimated by plasma spectroscopy (ICP-OES) following autoclave digestion (Quigley et al. 2016a), and leaf C was estimated by combustion on a CN 2000 combustion analyzer (LECO, Saint Joseph, MI) at the Kansas State University soil testing laboratory. Standard qualitative metrics confirm that the ICP-OES reference method provided within-sample accuracy (standard error of 12 replicate samples = 0.019%) and a low limit of detection (10 ug/L). A Partial Least Squares (PLS) regression model was fit to the training data and it performed well in external validation (83% R²; See supplementary methods).

Leaf stoichiometry: NIRS model construction and estimation

The resulting calibration samples were further subdivided into calibration (model development) and validation (test set; 20%) subsets using a modified Kennard-Stone algorithm that incorporates variation in both the spectra and the Si values (“SPXY”; Snee, 1977; Sapiro *et al.*, 2012). Using the “leaf.spec” R package (Griffith and Anderson, *in review*) we developed an optimal Partial Least Squares (PLS) regression model with the calibration dataset. We considered multiple calibration models subset to different continents, subset to plant functional types, and using a variety of spectral

preprocessing steps and specific spectral regions. Leaf C was estimated in a similar manner as described in detail in Anderson & Griffith et al. (*in review*).

Data analysis

We focused our data analysis on graminoids collected from plots which constitute the fully factorial NPK fertilizer x grazer exclusion experiment: control, enclosure, NPK and NPK + enclosure. All statistical analyses were conducted in the R statistical programming environment (version 3.3.0, R Development Core Team, 2012). The response variable, leaf Si, exhibited a significant right skew (Anderson-Darling test $A = 4.13$, $p = 2.66e^{-10}$), and was consequently log-transformed in order to restore normality for regression analysis. To ensure homogeneity of variance and normality of error, we used the “ncvTest” function in the *cars* package (Fox & Weisberg 2011) and graphically assessed histograms of residuals.

We began by constructing linear mixed effects models with grass leaf Si as the response variable, experimental treatments (NPK and enclosures), climate variables (MAP, MAT, potential evapotranspiration [PET]) and soil conditions (particle size distribution, pH, organic matter, total carbon and total nitrogen) as fixed effects and sites as a random effect. Models were fit using the R package *lme4* (Bates et al. 2014), and the conditional and marginal coefficients of determination were calculated using the R package MuMIn (Barton 2016, Nakagawa & Schielzeth 2013). A multi-model inference approach based on Akaike information criterion (AIC) was used to identify the top models which best predicted grass leaf Si at the 17 study sites. Included in our model selection were all additive models and, where logical, models including 2-way interactions. Models with a $\Delta AIC < 2$ were considered equivalent (Burnham & Anderson 2002), while ΔAIC values above 7 indicate poor fit relative to the best model. Finally, we used ordinary least

squares (OLS) regression to explore the linear relationship between leaf Si and leaf C across all grass samples.

Where data showed threshold effects between leaf Si and environmental predictors, we used the R package 'rpart' to perform regression tree analysis (Therneau et al. 2015). Regression trees were pruned by indicating the complexity parameter associated with the minimum error.

RESULTS

Effect of exclosures, NPK addition, soils, and climate on leaf Si

Model selection identified two equivalent models which predicted grass leaf Si: one with NPK addition as the sole explanatory variable and one with NPK + soil carbon as explanatory variables. In the mixed model, allowing for random intercepts of sites, leaf Si concentration was significantly lower in plots where NPK was added (mean = 1.71 ± 0.19 % dw Si, difference = -0.43 ± 0.09 SE, $t = -4.74$, $p < 0.01$), while plots inside and outside of exclosures showed no significant difference in leaf Si concentration ($p = 0.79$, $t = 0.26$; see Fig 2). NPK treatment explained 71% of variation in leaf Si after accounting for the random effect of site (Table 1, R^2_c). The equivalent model, NPK treatment + soil C ($\Delta AIC = 0.9$), resulted in an identical conditional coefficient of determination, but with an improved explanation of marginal variance (Table 1, $R^2_m = 0.24$). In total, four models were identified within ΔAIC of 7, all of which identified NPK treatment as an important predictor of grass leaf Si and none of which identified grazers as a predictor of grass leaf Si (Table 1; see supplement for complete AIC table of all models tested).

The slope of relationship between leaf Si and soil C was negative, although inspection of the residuals showed non-constant variance, with large variability in leaf Si

below soil C values of ~ 6% (Fig 3). To further understand this pattern, we used a regression tree analysis to explore potential threshold relationships between leaf % Si and soil % C with and without NPK fertilization. The branching pattern of our pruned tree indicated that NPK addition was an important driver of leaf Si above a threshold in soil C (Fig 3 inset).

Linear regression demonstrated that leaf Si generally declined along a gradient of increasing precipitation and showed a positive relationship with mean annual temperature (Supplementary Fig 2). Precipitation also played an important role in determining the strength of tradeoff observed between C and Si.

Leaf chemistry

Grass leaf carbon varied from 38 to 48% across sites and was, in general, negatively related to grass leaf Si concentration across sites (Fig 4 lower). However, the sign and strength of the individual slopes of the C ~ Si relationship within sites changed with rainfall. At arid sites the linear correlation between Si and C was strongly negative, whereas the slope of the correlation shifted to near-zero, or even slightly positive, along a gradient of increasing precipitation (Fig 4 inset).

DISCUSSION

By experimentally manipulating herbivory and NPK availability at grassland plots which occur in dramatically different climate space, we have provided novel insight into those processes which drive global patterns of silicification in grasses, one of earth's dominant plant functional types (Edwards et al. 2010). Our results show that Si concentration of grasses did not respond to herbivore removal, but was clearly influenced by soil fertility.

Plots which received NPK fertilizer generally had lower Si concentrations than untreated plots, specifically for sites which had relatively low soil carbon.

The observed decline in grass leaf Si at plots amended with NPK fertilizer indicates that soil nutrients, beyond the availability of soil Si itself, influence plant silicon accumulation. Increased leaf silification has been previously reported for turfgrasses and important food crops following N fertilization (Jones & Handreck 1967, Street 1974, Wallace et al. 1976, Gali-Muhtasib et al. 1992, but see also Carey & Fulweiler 2013), but the little consideration has been given to the underlying process driving this pattern. We provide three possible mechanistic explanations. First, the observed shift toward lower leaf Si following NPK addition may be a result of species turnover, rather than plasticity of individual plants (i.e. an induced Si response). We explored this option at one site where species-specific data were available (site Chichaqua Bottoms from Supplementary Table 1). Including species in a linear model indicated that there was no significant effect of species on leaf Si accumulation after accounting for the main effect of NPK; however, including species as a random effect did improve model fit. This suggests that species have different baseline Si values, but respond similarly to NPK addition. Interestingly, we also noted that the one C₄ grass species analyzed at this site, *Andropogon gerardii*, had significantly lower foliar Si concentration than all five C₃ species analyzed (Supplementary Fig 3). Although anecdotal, this observation may suggest that after controlling for environmental factors (soil, climate, etc.) C₄ species have a decreased demand for Si investment due to a greater C assimilation efficiency associated with Kranz anatomy; we suggest future analyses investigate this possibility.

Next, stoichiometric tradeoffs may be playing some role in the observed decline in leaf Si concentration. Consistent with this idea, leaf N, P, and K all showed a significant increase following NPK addition (data not shown). Mass balance for most

essential elements is tightly regulated above some minimum requirement, whereas for non-essential elements such as Si, accumulation is much more plastic and active uptake mechanisms may be down-regulated. Finally, it is likely that under abundant supply of NPK, grasses shift resource allocation strategy to optimize photosynthesis rather than investing in stress-tolerance and/or defensive compounds in accordance with the resource availability hypothesis framework of Coley et al. (1983). Resource availability is a major determinant of allocation patterns (Coley et al. 1985); thus, if sites are stressful due to resource-poor soils (i.e. low NPK availability) a low metabolic demand conferred by slow growth/turnover is expected. Our results support this hypothesis since plants growing in soils which were not amended with NPK fertilizer had higher leaf Si concentrations and were generally associated with lower leaf carbon. Because we did not find evidence for herbivore-driven patterns of leaf silicification, however, it remains unclear exactly how leaf silification fits into the resource availability hypothesis (RAH). It has been suggested that the rigid and immobile nature of phytoliths incurs a cost, but further investigation of trait-based patterns (i.e. Cooke & Leishman 2012) are needed to understand the costs and benefits associated with Si rather than C investment. That NPK addition appears to specifically drive patterns of leaf silicification in soils with low soil C suggests that soil C is an additional parameter which reflects overall soil fertility. Soil organic C in particular is associated with accelerated dissolution of important soil minerals, such as phosphates and calcium and magnesium carbonates.

Leaf silicon was negatively associated with leaf carbon, an overall pattern which appeared to shift across sites due to differences in precipitation. Similar studies have also reported this trend: in two distinct vegetation communities in Australia, Cooke and Leishman (2012) observed a negative correlation between leaf silica and carbon, although neither lignin nor cellulose were negatively correlated with silica; these

relationships were interpreted to indicate that silicon is not being used as a structural substitute. They also observed lower concentrations of total phenols and tannins in plants with high silicon concentration, suggesting a tradeoff in the type of defense used, rather than a tradeoff between growth and defense (Cooke and Leishman 2012). These patterns were, however, reported across a diverse vegetation community of 47 species, which tend not to exhibit the extreme within-taxon variation in leaf silicification that is observed among graminoids. The lack of a leaf silicification response to herbivore removal in our study sheds doubt on its defensive role and points toward a role in structural support or stress tolerance.

The precipitation component of the Si ~ C pattern implies that the benefit/cost ratio of silicon-accumulation is highest in dry climates, and this global pattern is in accordance with numerous studies of individual plant physiology. Maize plants treated with silicon decrease stomatal conductance and transpiration rate (Gao et al. 2004), and, under drought conditions, silicon treated *Sorghum* exhibits facilitated root growth which allows plants to extract greater amounts of water from dry soils (Hattori et al. 2005). It has also been shown that silicon prevents cell membrane deterioration of rice plants exposed to drought stress (Agarie et al. 1998), and wheat plants have been shown to increase antioxidant enzyme activity with Si application, which ameliorates damaging reactive oxygen species (ROS) produced under drought stress (Gong et al. 2005). Collectively, Si-accumulation appears to improve water use efficiency under drought conditions and outweigh potential costs of permanently incorporating rigid phytoliths into the leaf surface, although this beneficial gain dissipates under high rainfall availability. Given this observation, it appears that the benefits of improved cell wall rigidity become less important in mesic environments where plants are able to maintain turgor pressure, and water loss is trivial. Finally, it has been reported that plants utilize both passive (i.e.

transpirational flow) and active mechanisms for Si uptake, and active mechanisms have been identified as the major mechanism for Si uptake by grass species (Liang et al. 2006). That plants at dry sites, where transpiration and passive flow should be lowest, have high Si concentrations further suggests that active accumulation mechanisms dominate among grasses and merits further studies comparing the regulation of silicon transporters (i.e. *LSi1*, Ma et al. 2006) expressed in roots.

While Si-accumulation is repeatedly cited as a defense against grazing herbivores, we did not observe any signal of Si response to herbivore exclusion at the global level (Fig 1a), and recent lines of evidence highlight that grazing-induced Si-accumulation may not be the norm. In a field experiment excluding small (rodent) and large mammalian (reindeer) herbivores, 4 of 5 grass species tested showed no significant difference in Si concentration inside and outside of exclosures (Soininen et al. 2012). These researchers also found significantly different baseline Si levels among localities at multiple spatial scales but no relationship to variation in herbivore density among sites, further emphasizing the role of abiotic conditions like climate and soil. In contrast, leaf Si concentrations of Serengeti grasses were reported to be higher in regions which had evolved under heavy grazing pressure relative to lightly grazed areas, a notion which was further supported by greenhouse studies (McNaughton & Tarrants 1983, McNaughton et al. 1985). These patterns were initially attributed to natural selection for an inducible herbivore defense, but recent work in the Serengeti ecosystem suggests that environmental gradients explain much of regional variation in grass leaf Si (Quigley et al. 2016). High plant silicon concentrations have also been observed for heavily, relative to lightly, grazed grasslands of the American midwest, although microclimate conditions at sites were not considered (Brizuela et al. 1986). Despite this observation, authors questioned the efficacy of Si-induction as a short-term defense

against herbivores citing the preferential grazing succession of bison and gazelles following defoliation by prairie dogs in the US, and wildebeest in Serengeti, respectively (Coppock et al. 1983a, McNaughton 1976). Successional grazing occurs because grazing tends to select for fast-growing and easily digestible N-rich grasses (Coppock et al. 1983b, McNaughton 1984). Thus, the lack of Si-response to grazing may be alternatively be explained by a dilution effect resulting from greater productivity of defoliated plants (e.g. Cid et al. 1990).

Although we did not observe a relationship between exclosures or grazing intensity and leaf Si concentration, our exclosures were designed to exclude mammalian herbivores, and, similarly, grazing intensity scores were calculated based on mammalian herbivores. It is worth noting, however, that there is strong evidence for leaf silicification as an effective deterrent against small herbivores, especially insects. Leaf silica is known to act as a physical defense against insects, causing mandibular wear (Massey & Hartley 2009), improved protection of chlorophyll within chlorenchyma cells (Hunt et al. 2008), and as a nutritional hindrance which may reduce digestive efficiency (Massey et al. 2006) and slow the development of destructive crop pests (Han et al. 2015). Because no attempts were made to exclude grazing by insects, we conclude that further studies are needed to distinguish between the impacts of insect and large herbivores on grass leaf silicification.

CONCLUSIONS

Because of their recent origins, global distribution, and uniquely high silicification among angiosperms, grasses are a perfect study system for investigating the evolutionary drivers of phytogenic Si. Grasses have played an important role in the recent geological history of earth, and the Miocene expansion of Si-rich grassland ecosystems had a measurable impact on terrestrial Si flux, For instance, Si flux to oceans may have played

a role in diatom evolution during the Cenozoic (Cermeño et al. 2015). Investigating global scale environmental correlates of plant silicification offers the potential to improve biogeochemical models of the terrestrial silicon cycle, with implications for modeling the marine Si cycle and the global carbon cycle which is linked to the Si cycle via silicate weathering (Conley 2002). In presenting the first global scale study linking leaf Si concentration in grasslands to climate, soil nutrients, and grazing, we found that soil fertility best explained variation in foliar Si concentration, but mammalian herbivore exclusion did not elicit a consistent change in silicification. While the literature shows that Si may confer anti-herbivore benefits in a contemporary setting, we failed to observe such a pattern at the global scale. Instead, our results demonstrate that water and soil nutrient availability represent selective pressures that drive contemporary patterns of grass leaf silicification. Silicon concentration, both among and within plant species, varies more considerably than that of any other element (Epstein 2009), and despite the many physiological benefits conferred by silicification, this trait's adaptive origins remain unclear. Merging contemporary patterns of plant silicification and prehistoric changes in the earth's lithosphere, atmosphere, and biosphere is the next necessary step in understanding the adaptive origins of this important, yet perplexing plant trait.

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REFERENCES

- Agarie, S., Hanaoka, N., Ueno, O., Miyazaki, A., Kubota, F., Agata, W., & Kaufman, P.B. (1998). Effects of Silicon on Tolerance to Water Deficit and Heat Stress in Rice Plants, Monitored by Electrolyte Leakage. *Plant Prod. Sci.* 1 (2): 96–103.
- Anderson et al. in review
- Barton, K. (2016). MuMIN: multi-model inference. <https://CRAN.R-project.org/package=MuMIn>.
- Bates, D., Machler M., & Bolker, B. (2015). Fitting linear mixed-effects models using lme4. *J. Statistical Software*, 67, 1-48.
- Borer, E.T., Harpole, W.S., Adler, P.B., Lind, E.M., Orrock, J.L., Seabloom, E.W., & Smith, M.D. (2014). Finding Generality in Ecology: A Model for Globally Distributed Experiments. *Methods Ecol. Evol.*, 5, 65–73.
- Burnham, K.P. & Anderson, D.R.. (2002). *Model Selection and Multimodel Inference. A Practical Information-Theoretical Approach*. Springer-Verlag: New York.
- 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.
- Carey, J.C. & Fulweiler, R.W. (2013). Nitrogen Enrichment Increases Net Silica Accumulation in a Temperate Salt Marsh. *Limnol. Oceanography*, 58, 99–111.
- Cermeño, P., Falkowski, P.G., Romero, O.E., Schaller, M.F., & Vallina, S.M. (2015). Continental Erosion and the Cenozoic Rise of Marine Diatoms. *Proc. Natl. Acad. Sci. USA*, 112, 201412883.
- Che, J., Yamaji, N., Shao, J.F., Ma, J.F., & Shen, R.F. (2016). Silicon Decreases Both Uptake and Root-to-Shoot Translocation of Manganese in Rice. *J. Exp. Bot.*, 67, 1535-1544.
- 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. *J. Range Man.*, 43, 344–46.
- Coley, P.D. (1983). Herbivory and Defensive Characteristics of Tree Species in a Lowland Tropical Forest. *Ecol. Monogr.*, 53, 209–34.
- Coley, P.D., Bryant, J.P., Chapin, F.S. (1985). Resource Availability and Plant Antiherbivore Defense. *Science*, 230, 895–99.

- Conley, D.J. (2002). Terrestrial Ecosystems and the Global Biogeochemical Silica Cycle. *Global Biogeochem. Cycles*, 16, 1–8.
- Cooke, J. & M.R. Leishman. (2012). Tradeoffs between Foliar Silicon and Carbon-Based Defences: Evidence from Vegetation Communities of Contrasting Soil Types. *Oikos*, 121, 2052–2060.
- Cooke, J. & M.R. Leishman. (2011). Silicon Concentration and Leaf Longevity: Is Silicon a Player in the Leaf Dry Mass Spectrum? *Func. Ecol.*, 25, 1181–88.
- Coppock, D.L., Ellis, J.E., Detling, J.K., & Dyer, M.I. (1983a). Plant-Herbivore Interactions in a North American Mixed-Grass Prairie . II . Responses of Bison to Modification of Vegetation by Prairie Dogs. *Oecologia*, 56, 10–15.
- Coppock, D.L., Ellis, J.E., Detling, J.K., & Dyer, M.I. (1983b). Plant-Herbivore Interactions in a North American Mixed-Grass Prairie I. Effects of Black-Tailed Prairie Dogs on Intraseasonal Aboveground Plant Biomass and Nutrient Dynamics and Plant Species Diversity. *Oecologia*, 56, 10–15.
- Corrales, I., Poschenrieder, C., & Barceló, J. (1997). Influence of Silicon Pretreatment on Aluminium Toxicity in Maize Roots. *Plant Soil*, 190, 203–9.
- Cunha, K., Vieira, P., & Nascimento, C.W.A. (2008). Silicon Effects on Metal Tolerance and Structural Changes in Maize (*Zea Mays* L.) Grown on a Cadmium and Zinc Enriched Soil. *Water, Air, Soil Poll.*, 197, 323–30.
- Edwards, E.J., Osborne C.P., Strömberg C.A.E., Smith, S.A., Bond, W.J., Christin, P., Cousins, A.B., et al. (2010). The Origins of C4 Grasslands: Integrating Evolutionary and Ecosystem Science. *Science*, 328, 587–91.
- Epstein, E. (2009). Silicon: Its Manifold Roles in Plants. (2009). *Ann. App. Biol.*, 155, 155–60.
- Fang, C., Xun, Q., Wang, S. Yu, Y., Li, Q.M., Zhang, H.L., Wu, X.C., Chen, T., & Lin, W.X. (2011). Suppression and Overexpression of Lsi1 Induce Differential Gene Expression in Rice under Ultraviolet Radiation. *Plant Growth Reg.*, 65, 1–10.
- Fox, J. & Weisburg, S. (2011). *An R Companion to Applied Regression, Second Edition*. Thousand Oaks, CA: Sage.
- Gali, H.U. & Smith, C.C. (1992). Effect of Silicon Supply on Growth, Fertility, and Mineral Composition of an Annual Brome, *Bromus Secalinus* L. (Gramineae). *Am. J. Bot.*, 79, 1259–63.
- 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, 1724–29.

- Garbuzov, M., Reidinger, S., & Hartley, S.E. (2011). Interactive Effects of Plant-Available Soil Silicon and Herbivory on Competition between Two Grass Species. *Ann. Bot.*, 108, 1355–63.
- Gao, X., Zou, C., Wang, L., & Zhang, F. (2004) Silicon improves water use efficiency in maize plants. *J. Plant Nut.*, 27, 1457 – 1470.
- Gong, H., Zhu, X., Chen, K., Wang, S., & Zhang, C. (2005). Silicon Alleviates Oxidative Damage of Wheat Plants in Pots under Drought. *Plant Sci.*, 169, 313–21.
- Grégoire, C., Rémus-Borel, W., Vivancos, J., Labbé, C., Belzile, F., & Bélanger R.R. (2012). Discovery of a Multigene Family of Aquaporin Silicon Transporters in the Primitive Plant *Equisetum Arvense*. *Plant J.*, 72, 320–30.
- Griffith, D & Anderson T. In review.
- Han, Y., Lei, W., Wen, L., & Hou, M. (2015). Silicon-Mediated Resistance in a Susceptible Rice Variety to the Rice Leaf Folder, *Cnaphalocrocis Medinalis* Guenee (Lepidoptera: Pyralidae). *PLoS ONE*, 10, 1–14.
- Hartley, S.E., Fitt, R.N., McLarnon, 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. *Front. Plant Sci.*, 6, 1–8.
- Hattori, T., Inanaga, S., Araki, H., An, P., Morita, S., Luxova, M., & Lux, A. (2005). Application of Silicon Enhanced Drought Tolerance in Sorghum Bicolor. *Physiologia Plantarum*, 123, 459–66.
- Hodson, M.J., White, P.J., Mead, A., Broadley, M.R. (2005). Phylogenetic Variation in the Silicon Composition of Plants. *Ann. Bot.*, 96, 1027–46.
- Hunt, J.W., Dean, A.P., Webster, R.E., Johnson, G.N., & A.R. Ennos. (2008). A Novel Mechanism by Which Silica Defends Grasses Against Herbivory. *Ann. Bot.*, 102, 653–56.
- Jones, L.H.P. & Handreck. K.P (1969). Uptake of Silica by *Trifolium Incarnatum* in Relation to the Concentration in the External Solution and to Transpiration. *Plant Soil*, 1, 71–80.
- Jones, L.H.P & Handreck, K.A. (1967). Silica in Soils, Plants, and Animals. *Adv. Agron.*, 19, 107–49.
- Karban, R. & Myers, J.H. (1989). Induced Plant Responses to Herbivory. *Ann. Rev. Ecol. Syst.*, 20, 331–48.
- Kaufman, P.B., Cseke, L.J., Warber, S., Duke, J.A., Briemann, H.L. (1999). Natural products from plants. Boca Raton, FL, USA: CRC Press LLC.

- Kennard, R.W. & Stone, L.A. 1969. Computer Aided Design of Experiments. *Technometrics*, 11, 137–48.
- Kindomihou, V., Sinsin, B., & Meerts, P.. (2006). Effect of Defoliation on Silica Accumulation in Five Tropical Fodder Grass Species in Benin. *Belg. J. Bot.*, 139, 87–102.
- Li, P., Song, A., Li, Z., Fan, F., & Liang, Y. (2012). Silicon Ameliorates Manganese Toxicity by Regulating Manganese Transport and Antioxidant Reactions in Rice (*Oryza Sativa* L.). *Plant Soil*, 354, 407–19.
- Liang, Y. (1998). Effect of Silicon on Leaf Ultrastructure, Chlorophyll Content and Photosynthetic Activity of Barley under Salt Stress. *Pedosphere*, 8, 289–96.
- Liang, Y. (1999). Effects of Silicon on Enzyme Activity and Sodium, Potassium and Calcium Concentration in Barley under Salt Stress. *Plant Soil*, 209, 217–24.
- Liang, Y., Hua, H., Zhu, Y.G., Zhang, J., Cheng, C., & Römheld, V.. (2006). Importance of Plant Species and External Silicon Concentration to Active Silicon Uptake and Transport. *New Phyt.*, 172, 63–72.
- Liu, Q. & Zhu, Z. (2010). Functional Divergence of the NIP III Subgroup Proteins Involved Altered Selective Constraints and Positive Selection. *BMC Plant Biol.*, 10, 256.
- Loneragan, J.F., Snowball, K., & Robson, A.D. (1976). Remobilization of Nutrients and its significance in plant nutrition. In *Transport and Transfer Process in Plants*, 463-469. Academic Press: New York.
- Ma, J.F & Takahashi, E. (2002). Appendix 3-A. In *Soil, Fertilizer, and Plant Silicon Research in Japan*, 205–33. Amsterdam: Elsevier.
- Ma, J.F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., & Yano, Y. (2006). A Silicon Transporter in Rice. *Nature*, 440, 688–91.
- 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 R. Soc. B Biol. Sci.*, 273, 2299–2304.
- Massey, F.P. & Hartley, S.E. (2009). Physical Defences Wear You down : Progressive and Irreversible Impacts of Silica on Insect Herbivores. *J. An. Ecol.*, 78, 281–91.
- 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 App. Ecol.*, 10, 622–30.
- 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. *J. An. Ecol.*, 75, 595–603.

- Massey, F.P., Ennos, A.R., & Hartley, S.E. (2007). Grasses and the Resource Availability Hypothesis: The Importance of Silica-Based Defences. *J. Ecol.*, 95, 414-424.
- McNaughton, S. J. (1976). Serengeti Migratory Wildebeest : Facilitation of Energy Flow by Grazing. *Science*, 191, 92–94.
- McNaughton, S J. (1984). Grazing Lawns: Animals in Herds, Plant Form, and Coevolution. *The Am. Nat.*, 124, 863–86.
- McNaughton, S. J. & Tarrant, J.L. (1983). Grass Leaf Silicification: Natural Selection for an Inducible Defense against Herbivores. *Proc. Nat. Acad. Sci. USA*, 80, 790–91.
- McNaughton, S.J., Tarrant, J.L, McNaughton, M.M., & Davis, R.H. (1985). Silica as a Defense against Herbivory and a Growth Promotor in African Grasses. *Ecology* 66 (2): 528–35.
- Mitani, N. & Ma, J.F. (2005). Uptake System of Silicon in Different Plant Species. *Journal of Exp. Bot.*, 56, 1255–61.
- Nakagawa, S. & Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4, 133-142.
- O'Halloran, L.R., Borer, E.T., Seabloom, E.W., MacDougall, A.S., Cleland, E.E., McCulley, R.L., Hobbie, S., Harpole, W.S., DeCrappeo, N.M., Chu, C. and Bakker, J.D., 2013. Regional contingencies in the relationship between aboveground biomass and litter in the world's grasslands. *PLoS One*, 8(2), p.e54988.
- Parry, D.W. & Winslow A. (1977). Electron-Probe Microanalysis of Silicon Accumulation in the Leaves and Tendrils of *Pisum Sativum* Following Root Severance. *Ann. Bot.*, 41, 275–78.
- Piperno, D.R. (2006). *Phytoliths: A comprehensive guide for archaeologists and paleoecologists*. AltaMira Press: Lanham, MD.
- 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. *Front. Pl. Sci.*, 5, 1–10.
- Quigley, K.M., Althoff, A.G., & Donati, G.L. (2016a). Inductively Coupled Plasma Optical Emission Spectrometry as a Reference Method for Silicon Estimation by near Infrared Spectroscopy and Potential Application to Global-Scale Studies of Plant Chemistry. *Microchem. J.*, 129, 231–35.
- Quigley, K.M., Donati, G.L., & Anderson, T.M. (2016b). Variation in the Soil 'silicon Landscape' Explains Plant Silica Accumulation across Environmental Gradients in Serengeti. *Plant Soil*.

- R Development Core Team. (2012). *R: A Language and Environment for Statistical Computing*, R Development Core Team.
- Raven, J.A. (1983). The Transport and Function of Silicon in Plants. *Biol. Rev. Camb. Phil. Soc.*, 58, 179–207.
- Rémus-Borel, W., Menzies, J.G., & Bélanger, R.R. (2005). Silicon Induces Antifungal Compounds in Powdery Mildew-Infected Wheat. *Phys. Mol. Pl. Path.*, 66, 108–15.
- Reynolds, J.H., Lambin, X., Massey, F.P, Reidinger, S., Sherratt, J.A., Smith, M.J., White, A., & Hartley, S.E. (2012). Delayed Induced Silica Defences in Grasses and Their Potential for Destabilising Herbivore Population Dynamics. *Oecologia*, 170, 445–56.
- Saporo, A., Tadé, M.O., & Vuthaluru, H. (2012). A Modified Kennard-Stone Algorithm for Optimal Division of Data for Developing Artificial Neural Network Models. *Chem. Prod. Proc. Mod.*, 7, 1–14.
- Schoelynck, J., Bal, K., Backx, H., Okruszko, T., Meire, P., & Struyf, E. (2010). Silica Uptake in Aquatic and Wetland Macrophytes: A Strategic Choice between Silica, Lignin and Cellulose? *New Phyt.*, 186, 385–91.
- Snee, R.D. (1977). Validation of Regression Models: Methods and Examples. *Technometrics*, 19, 415–28.
- Soininen, E.M., Bråthen, K.A., Jusdado, J.G.H., Reidinger, S., & Hartley, S.E. (2012). More than Herbivory: Levels of Silica-Based Defences in Grasses Vary with Plant Species, Genotype and Location. *Oikos*, 122, 30–41.
- Stebbins, G.L. (1981). Coevolution of Grasses and Herbivores. *Ann. Miss. Bot. G.*, 68, 75–86.
- Street, J.R. 1974. The Influence of Silica Concentration on the Chemical Composition and Decomposition Rates of Turfgrass Tissue and Water Absorption Rates among Three Turfgrass Species. Ohio State University.
- Stromberg, C.A.E., Di Stilio, V.S., & Song, V. (2016). Functions of phytoliths in vascular plants: an evolutionary perspective. *Func.Ecol.*, In press.
- Therneau T., Atkinson, B., & Ripley, B. Rpart: Recursive partitioning and regression trees. <https://CRAN.R-project.org/package=rpart>.
- Trembath-Reichert, E., Wilson, J.P., McGlynn, S.E., & Fischer, W.W. (2015). Four Hundred Million Years of Silica Biomineralization in Land Plants. *Proc. Nat. Acad. Sci. USA*, 112, 5449-5454.
- Vaculík, M., Landberg, T., Greger, M., Luxová, M., Stoláriková, M., & Lux, A. (2012). Silicon Modifies Root Anatomy, and Uptake and Subcellular Distribution of Cadmium in Young Maize Plants. *Ann.Bot.*, 110, 433–43.

- VanDerVorm, P.D.J. (1980). Uptake of Si by Five Plant Species, as Influenced by Variations in Si-Supply. *Plant Soil*, 56, 153–56.
- Wallace, A., Romney, E.M., & Mueller, R.T. (1976). Nitrogen-Silicon Interaction in Plants Grown in Desert Soil with Nitrogen Deficiency. *Agron. J.*, 68, 529–30.
- Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., et al. (2004). The Worldwide Leaf Economics Spectrum. *Nature*, 428, 821–27.
- Yao, X., Chu, J., Cai, K., Liu, L., Shi, J., & Geng, W. (2011). Silicon Improves the Tolerance of Wheat Seedlings to Ultraviolet-B Stress. *Biol. Trace El. Res.*, 143, 507–17.
- Yin, L., Wang, S., Tanaka, K., Fujihara, S., Itai, A., Den, X., & Zhang, S. (2015). Silicon-Mediated Changes in Polyamines Participate in Silicon-Induced Salt Tolerance In *Sorghum Bicolor* L. *Plant, Cell Env.*, 39, 245-258.

Table 1. Summary of models within 7 Δ AIC of the best fit model for predicting grass leaf Si. The response variable (Si) was log transformed in all models to correct for skew. Models are ranked according to AIC score. R^2_c represents the coefficient of determination for fixed effects only (conditional), and R^2_m represents the coefficient of determination which accounts for a random effect of site (marginal) as described by Nakagawa & Schielzeth (2013). The Akaike weight (w_i) indicates the probability that a model from the respective set is the best one.

Model components	df	AIC	Δ AIC	w_i	R^2_M	R^2_C
NPK	4	168.81	0.0	0.57	0.05	0.71
NPK + soil C	5	169.71	0.9	0.36	0.24	0.67
NPK + soil C + soil N + pH	7	174.31	5.5	0.04	0.23	0.69
fence + NPK	5	174.41	5.6	0.03	0.05	0.71

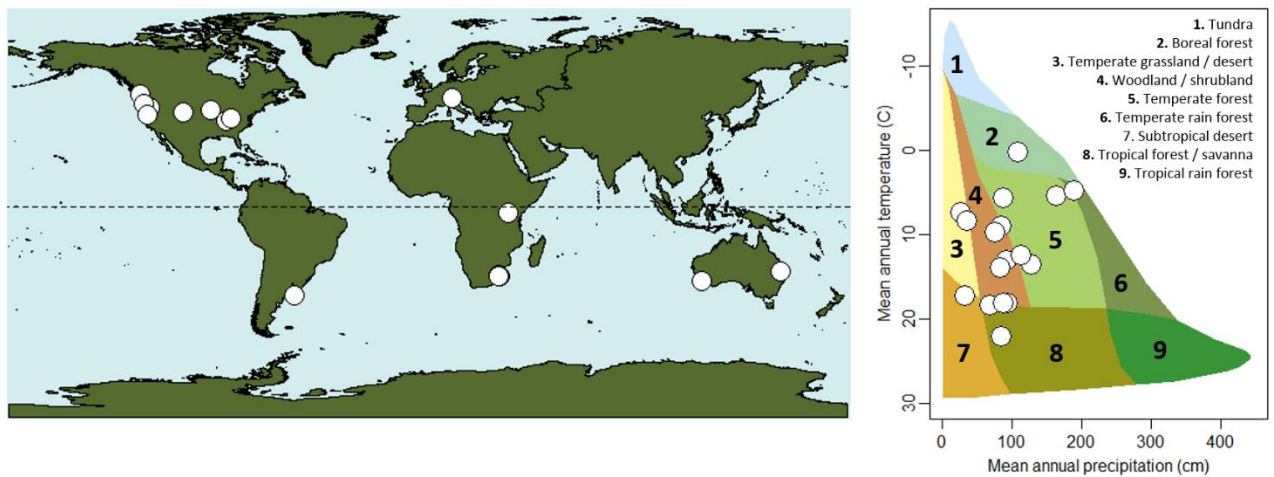


Figure 1. Distribution of NutNet sites analyzed in this study considered from (A) a global perspective, and (B) in biome space. While all sites are considered grasslands, they span far outside of the traditional (Whittaker) biome space (#4).

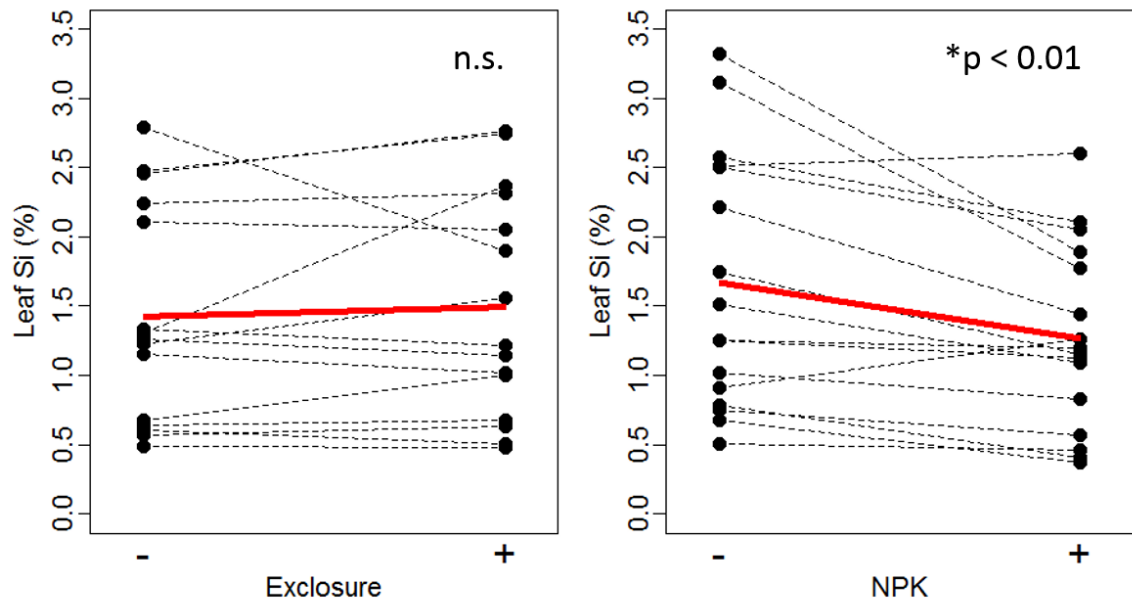


Figure 2. Leaf Si did not differ significantly inside and outside of grazing exclosures, but a significant decline in leaf Si was observed following the addition of NPK fertilizer. Dashed lines represent mean leaf Si (%) at individual sites, and solid lines represent treatment means. Panel A excludes two sites (Summerveld and Ukulinga; n=15) which did not have exclosures constructed, and panel B excludes one site (Hall; n=16) which did not have grass samples representing both full NPK treatments. Points represent mean site values across all blocks.

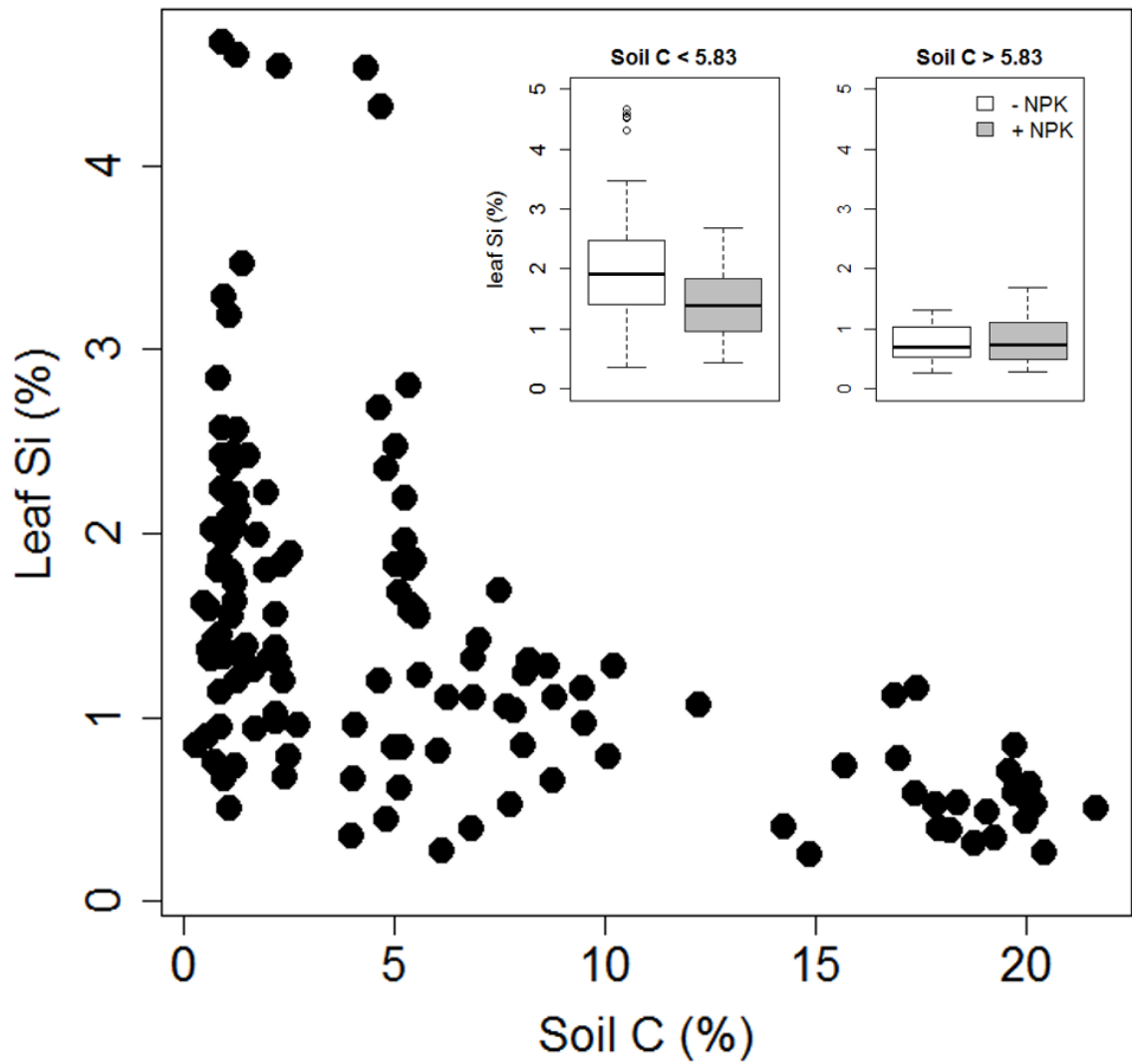


Figure 3. Grass leaf silicon plotted against total soil carbon. A mixed model which included soil C in addition to NPK treatment provided an equivalent predictive strength to the NPK model (Table 1).

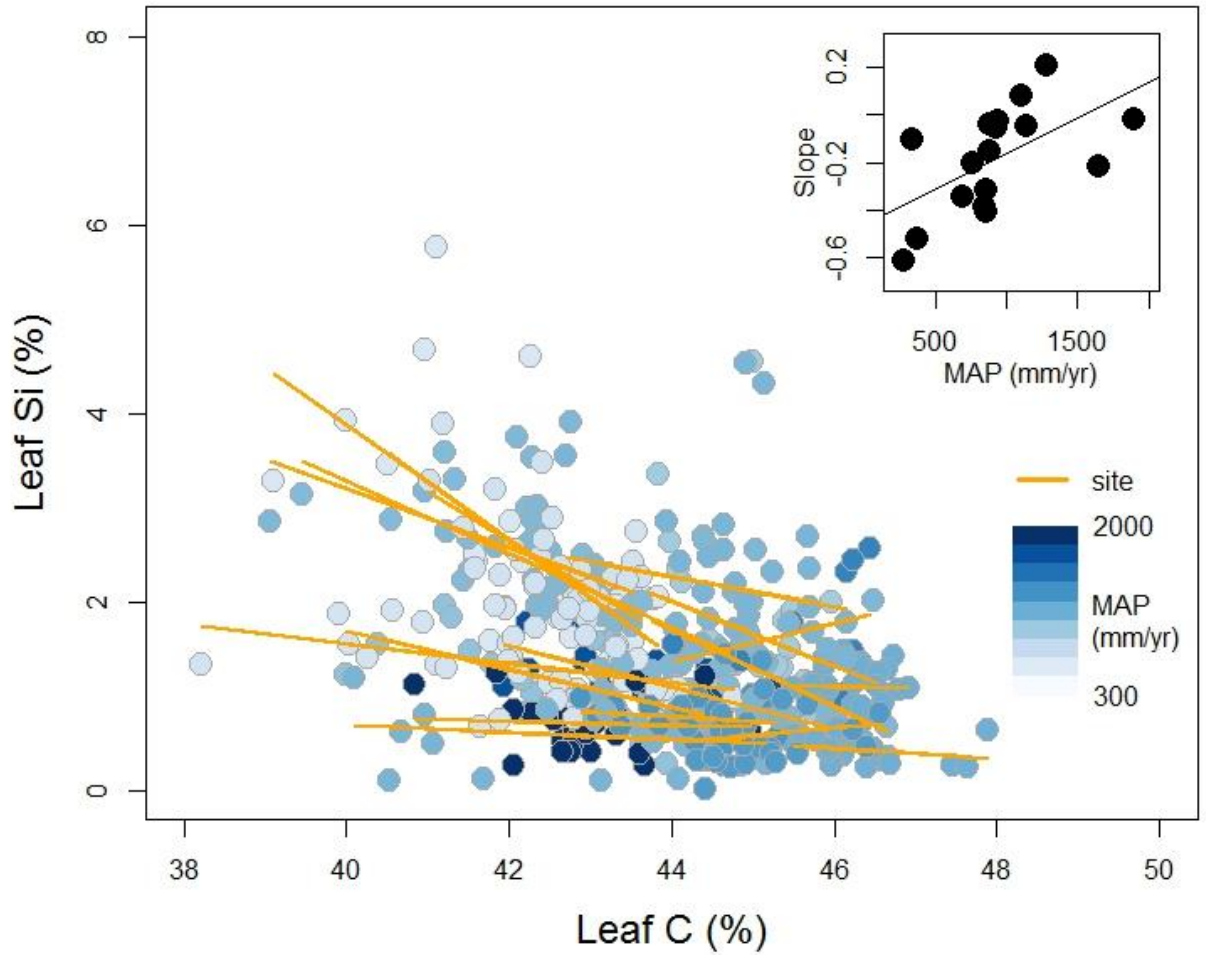


Figure 4. Leaf silicon exhibited a strong negative correlation with leaf carbon, especially at arid sites. The strength of this relationship generally diminished at mesic sites, even shifting to a positive relationship at two of the sites (see inset at top right).

CHAPTER 5

WHY DO GRASSES ACCUMULATE SILICA? A META-ANALYSIS AND EOVLUTIONARY CONSIDERATIONS

The following manuscript is formatted for and to be submitted to *New Phytologist*.

Stylistic variations are due to the requirements of the journal.

Abstract

Si-accumulation is prevalent among non-vascular plants, while remaining plant groups tend to passively absorb aqueous silicon to 1-2% of dry weight. Because of their high productivity and widespread distribution, Poales (grasses and close relatives) are arguably the most ecologically important angiosperms which accumulate silicon. Previous studies have attempted to reveal the abiotic and biotic drivers of Si accumulation by grasses, but are often limited to very few species in the laboratory or to a limited spatial scale in natural systems. Each of these shortcomings can result in a lack of clarity about what drives foliar silicification. I performed an exhaustive literature search to identify studies in which silicon content of leaf tissue was quantified and compiled these data to perform a meta-analysis exploring broad patterns and trends within the Poales in response to stress and in relation to key plant traits. Meta-analysis revealed that Leaf Si was generally correlated with maximum plant height, reinforcing previous conclusions that Si promotes plant growth. I also found that leaf Si increased under high soil Si availability and decreased under high nitrogen availability, suggesting that 1) the evolution and expansion of grasslands likely resulted in a positive feedback loop in the terrestrial biogeochemical Si cycle and 2) grasses may substitute Si in place of other major leaf constituents (C, N) under nutrient-poor soil conditions.

Introduction

Silicon is abundant on earth, second only to oxygen (Wedepohl 1995), and plants act as major conduits of silicon transport. Plant roots absorb silicon primarily in the form of aqueous monosilicic acid (H_4SiO_4) present in the soil water (Ma & Yamaji 2006). Silicic acid is a relatively bulky solute, which requires specialized transporters for

absorption. Si-transport proteins are modified from aquaporins, which are ubiquitous among plants because of their vital role in water transport. The specific aquaporins of the nodulin 26-like intrinsic protein (NIP) subfamily which selectively filter silicic acid have only recently been discovered (Ma et al. 2006), and further work has demonstrated the coupled action of influx and efflux transporters (Ma & Yamaji 2015). Advances in high-throughput sequencing and genome availability have allowed researchers to characterize the presence or absence of these specialized aquaporins among plant groups and accurately classify species as “Si competent” or not, based on genome scans (Deshmukh & Bélanger 2015). Thus, the genomic basis for variation in plant Si accumulation is being unraveled, but the evolutionary pressures which led to gains or losses of this function are unknown.

As has been previously pointed out, the benefits of silicon were first recognized and investigated by agricultural researchers interested in improving crop growth (Cooke et al. 2016). However, recent studies have begun asking questions to understand the ecology and evolution of plant Si-accumulation. The role of silicon in abiotic stress alleviation in particular has been emphasized, and plants appear to consistently show improved stress tolerance when supplied with silicon (see meta-analysis by Cooke & Leishman 2016). The other line of evidence that has been largely cited as an explanation for Si accumulation by grasses is the role of Si in the so-called evolutionary “arm’s race” between grasses and grazing herbivores. Early ecological studies suggested that silicon acted as an inducible defense against Serengeti’s iconic herds of grazing mammals (McNaughton & Tarrants 1983), and further work has demonstrated that plant phytoliths abrade insect mouthparts (Massey & Hartley 2009) and absorptive gut surfaces (Wieczorek et al. 2015), and a high silica diet results in negative fitness consequences via reduced growth rates (Massey & Hartley 2006, Massey & Hartley

2009). While studies such as these implicate silicon as an important extant deterrent of herbivory, a recent evolutionary perspective of phytolith function has shed doubt on grass-grazer coevolution as a functional demand for plant silica-accumulation (Stromberg et al. 2016).

The literature on plant silicification is rife with among-study discrepancies, and several questions remain unclear: Which environmental stimuli consistently elicit a response in Si-uptake? Are plant traits broadly correlated with silicification? What are the adaptive forces which most likely caused grasses to become Si-accumulators? In order to address these questions, I conducted an extensive literature search identifying studies which quantified foliar silicon concentration for members of the plant order Poales and investigated Si-uptake patterns in response to experimental manipulation and in relationship to plant traits such as photosynthetic strategy and plant height. By searching for consistent responses and relationships, I sought to provide an improved understanding of the adaptive pressures which may have resulted in Si being the most highly variable plant nutrient and grasses becoming the most heavily silicified extant angiosperms.

Methods

Database compilation

I began by conducting a bibliographic search using Google Scholar to identify studies which quantified shoot Si concentration of plants in the order Poales. I used a keyword search which required the presence of one silicon identifier term (Si OR silicon OR silica OR silicification OR phytolith) and one plant identifier term (Poales OR Poaceae OR Cyperaceae OR grass OR graminoid OR bamboo OR sedge OR reed OR

leaf OR leaves OR shoot OR plant). When necessary, silica or phytolith concentrations were converted to Si concentration by molar ratio conversion ($\text{Si} = \text{SiO}_2 * 0.4674$) or phytolith-transfer functions (see Song et al. 2013, Yang et al. 2015). For papers which only presented Si concentrations in figures, I used the program Digitizelt (Bormann 2001) to extract values. For each study, I recorded treatment * species means and sample sizes (as n experimental units). Studies which had a sample size of 1 or which did not report sample size were excluded from analysis.

Each study was assigned a unique 3 digit identifier and classified by a range of conditions in order to appropriately subset data for specific analyses. To investigate the interest driving plant Si studies, we first classified each study into one of two types: “agronomy” or “ecology”. Studies which focused on improving crop yield, investigating forage quality, or otherwise focusing on cereal crops were broadly defined as agronomy studies. Studies which focused on organism-environment interactions or quantifying plant Si in natural systems were broadly defined as ecology studies. I also classified the study setting as either “field” or “lab” (including greenhouse studies). Next, plants were phylogenetically classified within the order Poales to identify family, subfamily, and, for members of Poaceae, tribe of each species.

I identified five main treatments for which Si was quantified under control and experimental conditions: defoliation, fire, drought, Si addition, and N addition. Defoliation studies were further identified as “clip” or “graze”, as these two types of defoliation are thought to elicit different responses (Quigley & Anderson 2014). In addition, relative levels of each treatment were recorded. For instance, for factors with multiple levels tested (i.e. N and Si availability) I assigned each level to a relative, qualitative group as “low”, “moderate”, or “high”. For studies which recorded Si concentration seasonally or at multiple time points, temporal values were averaged. Beyond the five main treatments,

all other treatments were specified in a column labeled “other treatments”. Trait data for 1) maximum plant height, 2) photosynthetic strategy (C3 vs. C4), and 3) growth habit (annual vs. perennial), were acquired for each species from reputable plant databases (KEW PlantList, GrassPortal, FAO GrassIndex, USDA, and eFloras) and linked by species to the Si database. Wetland species, such as *Spartina* species, were excluded from this analysis as they tend to have very different ecology than typical terrestrial species.

Data analysis

Once the database was constructed, I explored general trends in Si uptake in response to the five main treatments (N availability, Si availability, defoliation, drought, and fire) and in relation to photosynthetic type (C3 vs. C4), growth habit (annual vs. perennial), and maximum plant height. To analyze whether treatments elicited consistent Si responses, I calculated mean study-by-species response ratios as the natural logarithm of (Si conc. of treated plants / Si conc. of untreated plants). Following this calculation, plants which experienced an increase in Si following treatment have a positive value, while those which have decreased Si following treatment have a negative value. In order to understand whether values were consistent among studies and species, I calculated a mean response ratio and standard deviation for each treatment. Standard errors which do not overlap zero indicate a consistent and significant treatment response. Thus, mean response ratios and standard deviations for each treatment were plotted alongside individual study-by-species values in order to clearly visualize how each treatment affected leaf Si.

When considering relationships between plants traits (maximum height, photosynthesis, and growth habit) and Si concentration, I focused on Si data for plants within the family Poaceae which did not receive any treatment/manipulation. I then used

linear modeling to investigate whether Si was related to any of these traits. For plant height, Si was modeled using linear mixed models, which allowed for continuous x and y variables (height and Si, respectively). Study was included as a random effect, in order to account for general among-study differences in growth conditions, such as watering regimes and soils. The categorical predictors of Si, photosynthesis and growth habit, were modeled similarly to leaf height, but using an analysis of variance (ANOVA). Mixed models were evaluated using the *lme4* package in the R statistical programming environment, and figures were created using the *ggplot2* package (Wickham 2009, R Core Development Team 2014, Bates et al. 2015).

Results

The database

In sum, I identified 128 studies published between 1953 – present comprising 1839 unique measurements of plant Si, consisting of both experimental and observational studies (see supplement for citation list). 440 species were represented, although the dataset is biased toward true grasses (Poaceae, n = 390 species) since limited data were available for Typhaceae (n = 2), Juncaceae (n = 5), and Cyperaceae (n = 43). Consideration of the types of studies conducted on Si research showed a clear dominance of agronomy research in the literature, although there has been a steady rise in ecological studies (see Fig 1). For instance, the genus *Oryza* (rice) comprised approximately 10% of the datapoints (n = 189), and the three remaining most important cereal crops (corn, wheat, sugar) collectively comprised 5% of the data.

Treatment effects

Of the five treatments investigated, two elicited consistent and uni-directional responses in terms of leaf silicification: Si addition and N addition (Fig 2). Adding silicon to soils consistently resulted in plants with greater leaf Si, while adding nitrogen to soils consistently caused a reduction in leaf Si. Fire tended to induce Si uptake, but Si data for this stress were very limited; I only identified three studies concerning plant silicon in burned and unburned areas, one of which did not provide species-specific values. Drought or water deficit generally resulted in plants with lower silicon, although this response was not significant. Similarly, defoliation tended to result in plants with increased Si concentration relative to controls, but this treatment overlapped the most with 0, indicating that the directionality of response to defoliation was highly variable.

Si ~ trait relationships

Si concentration was positively correlated with maximum plant height, but several outliers occurred, predominantly from the genus *Oryza* (Supplement S1). Thus, I excluded this taxonomic group from the final linear regression model. After accounting for the random effect of site, the relationship between maximum plant height and leaf Si was positive with a moderate fit (Fig 3; conditional $R^2 = 0.23$, $p < 0.01$). Remaining outliers were from the Bambusoideae subfamily, which, as the tallest members of Poaceae, also appeared to drive the overall positive relationship between maximum plant height and leaf Si. ANOVA revealed that neither growth habit ($F_{1,368} = 1.02$, $p = 0.31$) nor photosynthetic type ($F_{1,369} = 0.47$, $p = 0.49$) was statistically related to foliar Si.

Discussion

The results presented here provide evidence that soil nutrients, namely Si and N availability, influence leaf Si of grasses and their close relatives in a predictable manner.

Soil silicon addition results in plants with greater Si concentration, while nitrogen addition causes plants to take up less silicon. Fire generally resulted in plants with increased Si, while water deficit resulted in plants with decreased Si; response ratios for both of these treatments were not significantly different from zero, however, likely because of the small sample sizes within each treatment. Finally, defoliation tended to increase leaf Si, but this treatment showed the greatest range of variation in response ratios, with several values extending into the negative range.

Studies which have measured plant height and corresponding leaf Si have observed a strong positive relationship between these two traits (Struyf et al. 2005, Querne et al. 2012, Quigley unpublished data), suggesting that the overall weakness of the pattern observed via meta-analysis may be an artefact of obtaining mean plant height values from databases. Including distantly related species in this analysis, namely members of Bambusoideae which tended to have very high leaf Si regardless of plant height, may have also resulted in a poor overall relationship between height and Si (Fig 3). Among the species included in these analyses, there was a clear phylogenetic signal at the taxonomic level of Poaceae subfamily (Supplement S2), which is in line with previous studies performed at a broader taxonomic level (Hodson et al. 2005, Trembath-Reichert et al. 2015). This signal indicates that including taxonomic information as an additional fixed factor in models of foliar Si concentration may significantly improve predictive strength for predicting Si variation. However, the currently available data are insufficient at finer level taxonomic groups such as species or genera.

In an early study of Si-accumulation by grasses and *Equisetum*, Lovering (1967) estimated that Si-accumulating plants such as these mobilize approximately 340 kg/acre of SiO₂ from soil annually. This fact, coupled with the finding that across all previous studies Si supply results in plants with significantly increased leaf Si suggests that plants

likely create positive feedback loops in soil Si availability. Because some of the earliest land plants accumulate large amounts of silica in their aboveground biomass (i.e. bryophytes, lycophytes; Ma & Takahashi 2002), plant invasion of terrestrial environments should have resulted in rapid and profound changes in the terrestrial biogeochemical Si cycle as early as the Silurian (> 400 mya; Edwards et al. 1995). This hypothesized change is because after plants translocate silicic acid from weathered soil minerals, their phytolith-rich leaf tissue is eventually returned to the soil via either leaf litter or dung. Soil phytoliths are much more labile than are parent rock sources (Lindsay 1979), especially after animal digestion (Vandevenne et al. 2013). The evolution and subsequent radiation of grasses in the Miocene (Spriggs et al. 2014) likely caused a second major perturbation in the terrestrial Si cycle, due to their ability to accumulate extremely high amounts of Si, their global pervasiveness, and the coupled radiation of grazing mammalian herbivores which create Si “dissolution hotspots” in dung (Herrera 1985, Vandevenne et al. 2013). Finally, phytolith formation and silicate weathering are linked to CO₂ transfer between the lithosphere and atmosphere, which suggests that vegetation-induced changes to the Si-cycle could have affected pre-historic global CO₂ concentrations and climate (Conley 2002).

The observed trend of high Si plants on soils with relatively low nitrogen availability supports the previously suggested hypothesis that Si may substitute for otherwise costly structural components under nutrient-poor conditions (Cooke & Leishman 2012). Silicon is metabolically cheaper to obtain and incorporate into leaf tissue than carbon (Raven 1983), and may represent an alternative structural and/or defensive strategy that results in favorable carbon balance, specifically in short-lived plants where leaf construction is especially costly (Cooke & Leishman 2011). I was, however, unable to observe a difference in Si concentration between perennial and

annual plants within the Poales in the presented meta-analysis. Because Si is less costly than is C fixation and confers a suite of stress alleviation benefits (Ma 2004, Liang et al. 2007) the cost of Si accumulation is intriguing. At first glance, inducible stress alleviation is cost-saving, since plants that are not experiencing stress avoid allocating resources to off-setting stress. Responses which are highly inducible, however, assumedly incur a maintenance cost for the sensory and regulatory machinery necessary for plasticity (DeWitt 1998, Dorn 2000). Thus, fitness costs are likely associated with the plastic nature of Si uptake.

General trends were observed for Si response to fire, water deficit, and defoliation, but none were statistically significant. Fire appears to increase Si uptake, although due to very limited sample size in this treatment group, two slightly negative response ratio values made the overall treatment response non-significant. Fire affects vegetation most generally by removing aboveground biomass and stimulating new growth (Baxter et al. 1994, Langevelde et al. 2003). The observed trend of fire to increase leaf Si suggests that silicon allows recently burned plants to re-grow rapidly and out-compete slow-growing species via utilizing Si as a cheap structural substitute (previous section). Of the five treatments investigated, defoliation exhibited the most inconsistent response in terms of Si induction. An inconsistent defoliation response has been discussed in detail previously (Quigley & Anderson 2014) so we provide a brief explanation here. Inconsistencies among studies appear to be due to species-specific responses to defoliation (i.e. Soininen et al. 2012), while intensity and frequency of defoliation also influence the extent of Si induction (Reynolds et al. 2012). In summary, the vastly variable response of leaf Si to defoliation, coupled with the lack of evidence for Cenozoic grass-grazer coevolution (Stromberg et al. 2016) sheds doubt the on the

existing paradigm that heavy grass leaf silicification evolved as an adaptive response to increased herbivory pressure.

Wetland species were initially included in our database and analyses, but I quickly observed that these species (i.e. *Spartina spp*) had a tendency to act oppositely of dryland species in terms of Si response (Euliss 2005, Carey 2013). Because there is not adequate data available to test for differences between dryland and wetland species treatment responses, wetland species were excluded from these analyses. Wetland plants are ecologically very dissimilar from terrestrial plants, especially in terms of environmental stressors. For instance, *Spartina* plants accumulate extremely high levels of salt (Mateos-Naranjo & Andrades-Moreno 2013), but do not typically experience fire disturbance or water limitation like many terrestrial grass species. This observation suggests a role of plant water relations in determining foliar Si concentration, and differences in the relative costs and benefits of silicification between wetland and terrestrial plants necessitates further investigation.

Finally, a bias was introduced in some of the presented analyses as a result of including agricultural cultivars in the dataset, as observed by outliers within the Andropogoneae (corn, sorghum), Oryzeae (rice), Poaceae (oats), and Tritaceae (wheat) grass subfamilies. That crop species appear to be outliers indicates that, while ecological studies of leaf Si are on the rise, the currently available data may better represent agricultural cultivars, which are subject to intense artificial selection, than species found in natural grassland ecosystems. Furthermore, natural systems are subject to many or all of the stimuli presented here simultaneously. A previous study in arctic grasslands revealed that plant Si response to abiotic and biotic conditions interacts with species and genotype in a complex manner (Soininen et al. 2012), further stressing the need for ecological studies to investigate how multiple stresses interact in grassland

ecosystems. Similarly, a system-level model of grass silicification along interacting natural gradients in Serengeti highlighted the importance of soil water and nutrients (Quigley & Anderson 2016), but further consideration of the disturbances vital to grassland maintenance (e.g. herbivores and fire) is needed.

Conclusion

Anthropocene-associated climate change, such as fire suppression, drought, and eutrophication have the potential to influence the C cycle via their effects on plant Si storage. Because stress is known to influence mineral nutrition of plants, and silicon is coupled to the carbon cycle, understanding how grass Si accumulation responds to various forms of stress is important for predicting the role of plant Si uptake in an uncertain climate. Major perturbations in the global C and Si cycles are expected have occurred with 1) the appearance of Si-accumulating plants on land (bryophytes etc.), 2) major radiations of grazing herbivores, and 3) the rise of grasslands in the late Cenozoic (Stromberg & Feranec 2004, Stromberg 2005). The frequent citing of Si as an anti-herbivore defense in modern studies does not appear to be consistently supported by induced Si uptake following defoliation, and observing an induced response to herbivory in some studies does not necessarily provide an explanation for the evolution of the induced response (Karban and Myers 1989). Contemporary selective pressures undoubtedly differ from historical pressures, and plants face several other stresses (e.g. disease, nutrient stress) which are also ameliorated by silicon accumulation (Ma 2004). The meta-analysis presented here emphasizes a strong role for changes in Si and N availability in driving patterns of grass leaf silicification, and further consideration of these patterns in relation to pre-historic appearances of Si-accumulating plants is needed in order to understand the adaptive forces which caused grass to become glass.

References

- Bates D, Maechler M, Bolker B, and Walker S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67(1): 1-48.
- Baxter, B. J. M., VanStaden, J., Granger, J. E., & Brown, N. A. C. (1994). Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra*. *Environmental and Experimental Botany*, 34(2), 217–223.
- Carey, J. C., & Fulweiler, R. W. (2013). Nitrogen enrichment increases net silica accumulation in a temperate salt marsh. *Limnology and Oceanography*, 58(1), 99–111.
- Conley, D. J. (2002). Terrestrial ecosystems and the global biogeochemical silica cycle. *Global Biogeochemical Cycles*, 16(4), 1–8.
- Cooke, J., DeGabriel, J. L., & Hartley, S. E. (2016). The functional ecology of plant silicon: geoscience to genes. *Functional Ecology*, 30(8), 1270–1276.
- Cooke, J., & Leishman, M. R. (2016). Consistent alleviation of abiotic stress with silicon addition: a meta-analysis. *Functional Ecology*, 1340–1357.
- Cooke, J., & Leishman, M. R. (2011). Silicon concentration and leaf longevity: is silicon a player in the leaf dry mass spectrum? *Functional Ecology*, 25(6), 1181–1188.
- Cooke, J., & Leishman, M. R. (2012). Tradeoffs between foliar silicon and carbon-based defences: evidence from vegetation communities of contrasting soil types. *Oikos*, 121(12), 2052–2060.
- Deshmukh, R., & Bélanger, R. R. (2015). Molecular evolution of aquaporins and silicon influx in plants. *Functional Ecology*, 30: 1277-1285.
- Dewitt, T. J., Sih, a, & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, 13(2), 77–81.
- Dorn, L. a, Pyle, E. H., & Schmitt, J. (2000). Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution; International Journal of Organic Evolution*, 54(6), 1982–94.
- Edwards, D., Duckett, J., & Richardson, J. (1995). Hepatic characters in the earliest land plants. *Nature*, 374(13).
- Euliss, K. W., Dorsey, B. L., Benke, K. C., Banks, M. K., & Schwab, a. P. (2005). The use of plant tissue silica content for estimating transpiration. *Ecological Engineering*, 25(4), 343–348.
- Herrera, C. M. (1985). Grass/grazer radiations : an interpretation of silica-body diversity. *Oikos*, 45(3), 446–447.

- Hodson, M. J., White, P. J., Mead, a, & Broadley, M. R. (2005). Phylogenetic variation in the silicon composition of plants. *Annals of Botany*, 96(6), 1027–1046.
- Karban, R., & Myers, J. H. (1989). Induced plant responses to herbivory. *Annual Review of Ecology and Systematics*, 20, 331–348.
- Langevelde, F. Van, VanDeVijver, C. A. D. M., Kumar, L., VanDeKoppel, J., DeRidder, N., VanAndel, J., ... Rietkerk, M. (2003). Effects of fire and herbivory on the stability of savanna ecosystems. *Ecology*, 84(2), 337–350.
- Liang, Y., Sun, W., Zhu, Y.-G., & Christie, P. (2007). Mechanisms of silicon-mediated alleviation of abiotic stresses in higher plants: a review. *Environmental Pollution*, 147(2), 422–428.
- Lindsay, W. L. (1979). Silica. In *Chemical equilibria in soils* (pp. 51–55). John Wiley & Sons.
- Lovering, T., & Engel, C. (1967). Translocation of Silica and Other Elements from Rock into Equisetum and Three Grasses. *Geological Survey Professional Paper*, 594–B, B1–B16.
- Ma, J. F. (2004). Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Science and Plant Nutrition*, 50(1), 11–18.
- Ma, J. F., & Takahashi, E. (2002). Appendix 3-A. In *Soil, Fertilizer, and Plant Silicon Research in Japan* (pp. 205–233). Amsterdam: Elsevier.
- Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., ... Yano, M. (2006). A silicon transporter in rice. *Nature*, 440(7084), 688–91.
- Ma, J. F., & Yamaji, N. (2006). Silicon uptake and accumulation in higher plants. *Trends in Plant Science*, 11(8), 392–397.
- Ma, J. F., & Yamaji, N. (2015). A cooperative system of silicon transport in plants. *Trends in Plant Science*, 20(7), 435–442.
- 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(1), 281–291.
- 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.
- Mateos-Naranjo, E., Andrades-Moreno, L., & Davy, A. J. (2013). Silicon alleviates deleterious effects of high salinity on the halophytic grass *Spartina densiflora*. *Plant Physiology and Biochemistry*, 63, 115–21.

- 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*, 80(3), 790–791.
- Querné, J., Ragueneau, O., & Poupart, N. (2012). In situ biogenic silica variations in the invasive salt marsh plant, *Spartina alterniflora*: A possible link with environmental stress. *Plant and Soil*, 352(1–2), 157–171.
- 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(October), 1–10.
- Quigley, K. M., Donati, G. L., & Anderson, T. M. (2016). Variation in the soil “silicon landscape” explains plant silica accumulation across environmental gradients in Serengeti. *Plant and Soil*.
- R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Raven, J. A. (1983). The transport and function of silicon in plants. *Biological Reviews of the Cambridge Philisophical Society*, 58(2), 179–207.
- Reynolds, J. J. H., Lambin, X., Massey, F. P., Reidinger, S., Sherratt, J. a, Smith, M. J., ... Hartley, S. E. (2012). Delayed induced silica defences in grasses and their potential for destabilising herbivore population dynamics. *Oecologia*, 170(2), 445–56.
- Soininen, E. M., Bråthen, K. A., Jusdado, J. G. H., Reidinger, S., & Hartley, S. E. (2012). More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. *Oikos*, 122(1), 30–41.
- Song, Z., Liu, H., Si, Y., & Yin, Y. (2012). The production of phytoliths in China's grasslands: implications to the biogeochemical sequestration of atmospheric CO₂. *Global Change Biology*, 18(12), 3647–3653.
- Spriggs, E. L., Christinb, P. A., & Edwards, E. J. (2014). C4 photosynthesis promoted species diversification during the miocene grassland expansion. *PLoS ONE*, 9(5).
- Strömberg, C. a E. (2005). Decoupled taxonomic radiation and ecological expansion of open-habitat grasses in the Cenozoic of North America. *Proceedings of the National Academy of Sciences of the United States of America*, 102(34), 11980–11984.
- Stromberg, C. A. E., & Feranec, R. S. (2004). The evolution of grass-dominated ecosystems during the late Cenozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 207, 199–201.
- Stromberg, C. A., DiStilio, V. S., & Song, Z. (2016). Functions of phytoliths in vascular plants : an evolutionary perspective. *Functional Ecology*, 30, 1286–1297.

- Struyf, E., Van Damme, S., Gribsholt, B., Middelburg, J., & Meire, P. (2005). Biogenic silica in tidal freshwater marsh sediments and vegetation (Schelde estuary, Belgium). *Marine Ecology Progress Series*, 303, 51–60.
- Trembath-Reichert, E., Wilson, J. P., McGlynn, S. E., & Fischer, W. W. (2015). Four hundred million years of silica biomineralization in land plants. *Proceedings of the National Academy of Sciences*, 112(17), 5449–5454.
- Vandevenne, F. I., Barão, A. L., Schoelynck, J., Smis, A., Ryken, N., Van, S., ... Damme, S. Van. (2013). Grazers : biocatalysts of terrestrial silica cycling. *Proceedings of the Royal Society B*, 280, 1–9.
- Wedepohl, K. H. (1995). Composition of the Continental Crust. *Geochimica et Cosmochimica Acta*, 59(7), 1217–1232.
- Wickham H. 2009. ggplot2: Elegant graphics for data analysis. Springer-Verlag: New York.
- Wieczorek, M., Szafranska, P. a., Labecka, a. M., Lazaro, J., & Konarzewski, M. (2015). Effect of the abrasive properties of sedges on the intestinal absorptive surface and resting metabolic rate of root voles. *Journal of Experimental Biology*, 218(2), 309–315.
- Yang, X., Song, Z., Liu, H., Bolan, N. S., Wang, H., & Li, Z. (2015). Plant silicon content in forests of north China and its implications for phytolith carbon sequestration. *Ecological Research*, 30(2), 347–355.

Tables and Figures

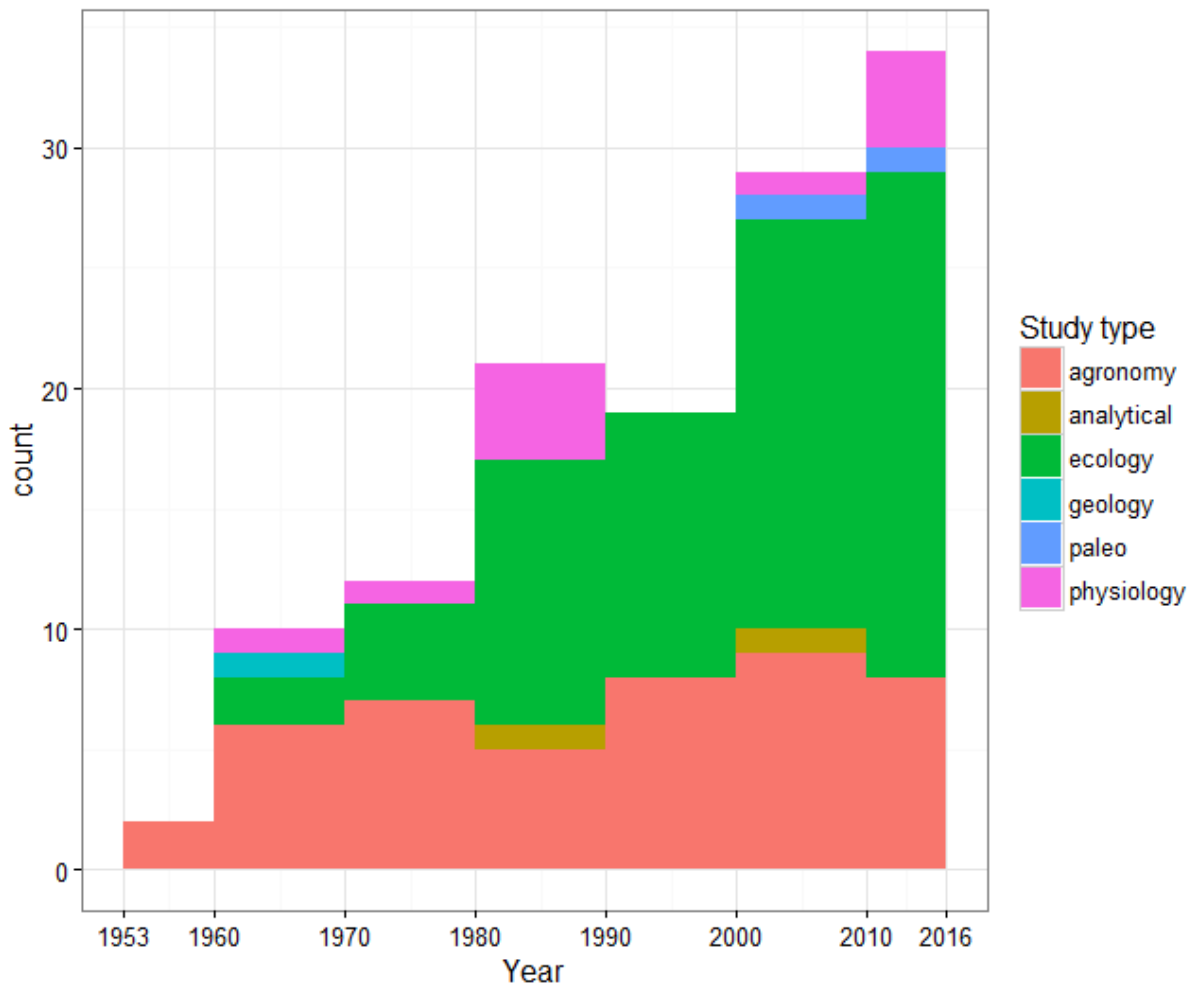


Figure 1. A historical representation on the types of studies recording grass Si concentration. Only recently have ecological studies (green) begun dominating the literature.

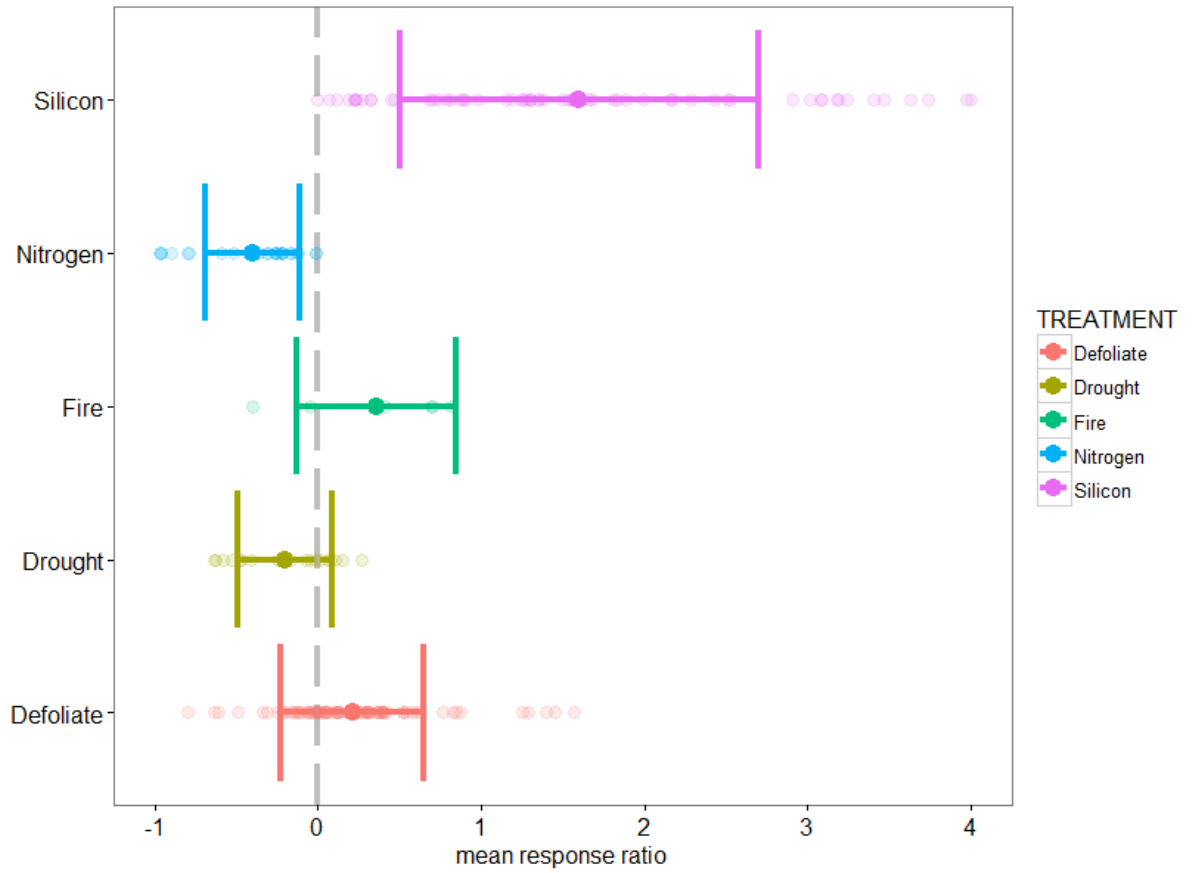


Figure 2. Response ratios for each of the five main treatments investigated. Among all studies, only soil Si addition (purple) and soil N availability (blue) had a strong, uni-directional effect on leaf Si concentration.

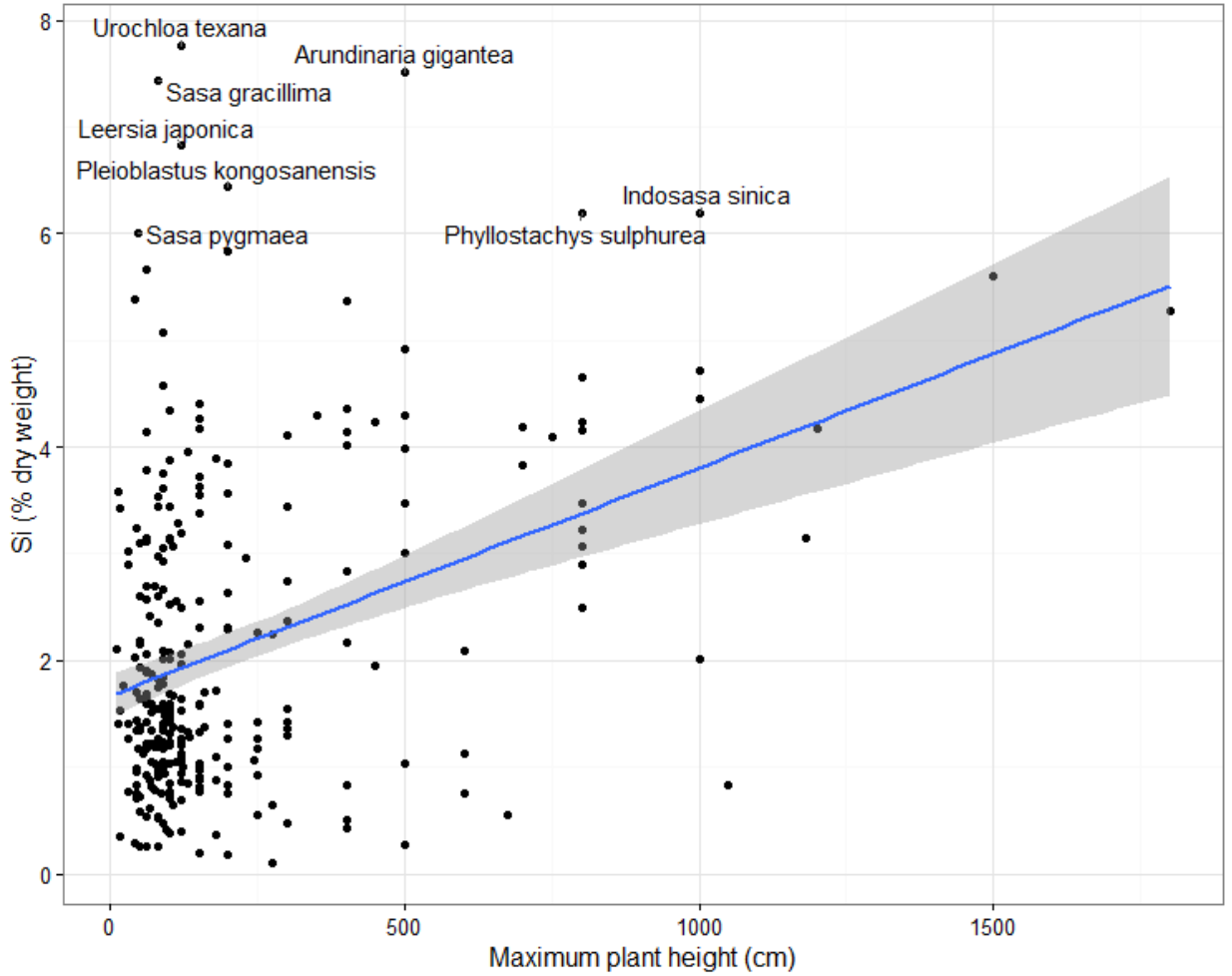
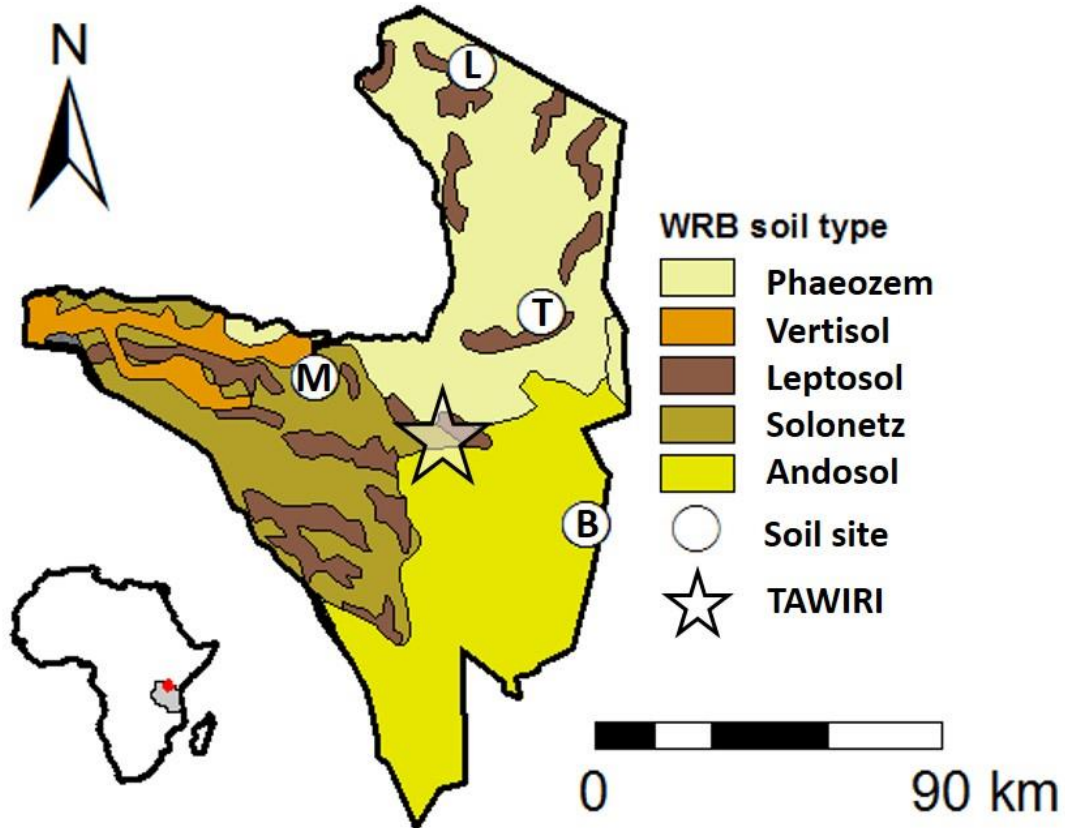
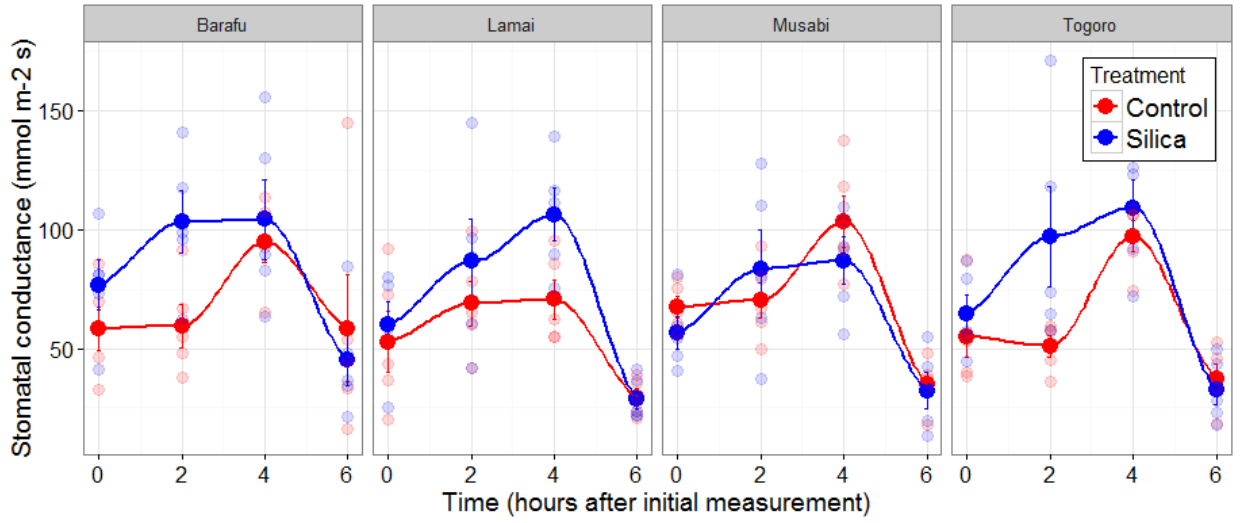


Figure 3. Linear regression for the relationship between maximum plant height and foliar Si. Individual points represent species means ($n = 363$ species within Poaceae). Blue line shows linear model fit, and gray shaded area represents standard error around the linear model. Data points with Si concentration $> 6\%$ dry weight are labeled to indicate species names; the majority of these species are bamboos.

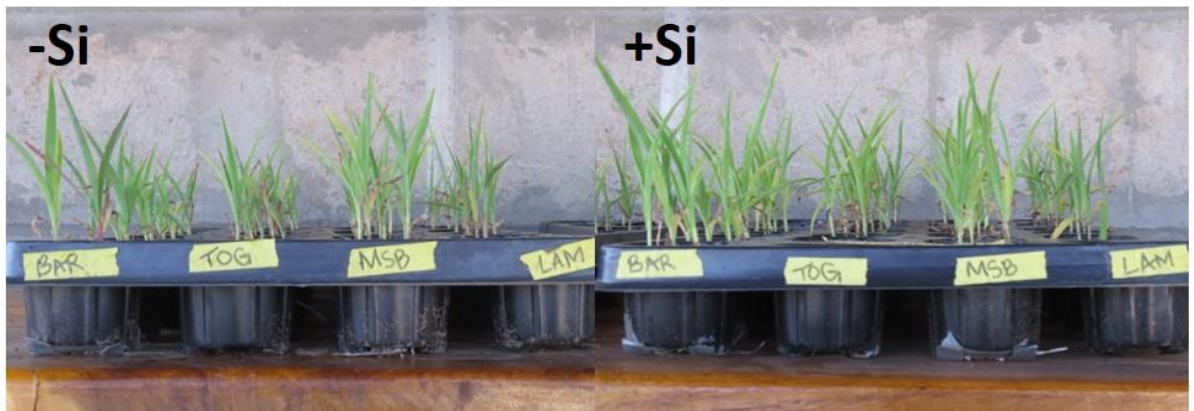
APPENDIX A
SUPPORTING INFORMATION FOR CHAPTER 1



S1. Map showing the collection sites of the four soils used in the pot experiment. Inset at lower left shows the location of Serengeti National Park within Tanzania, east Africa. Labels indicate first letters of site names; B = Barafu, L = Lamai, M = Musabi, and T = Togoro. The experiment took place at the Tanzania wildlife research center (TAWIRI) near the center of the ecosystem.



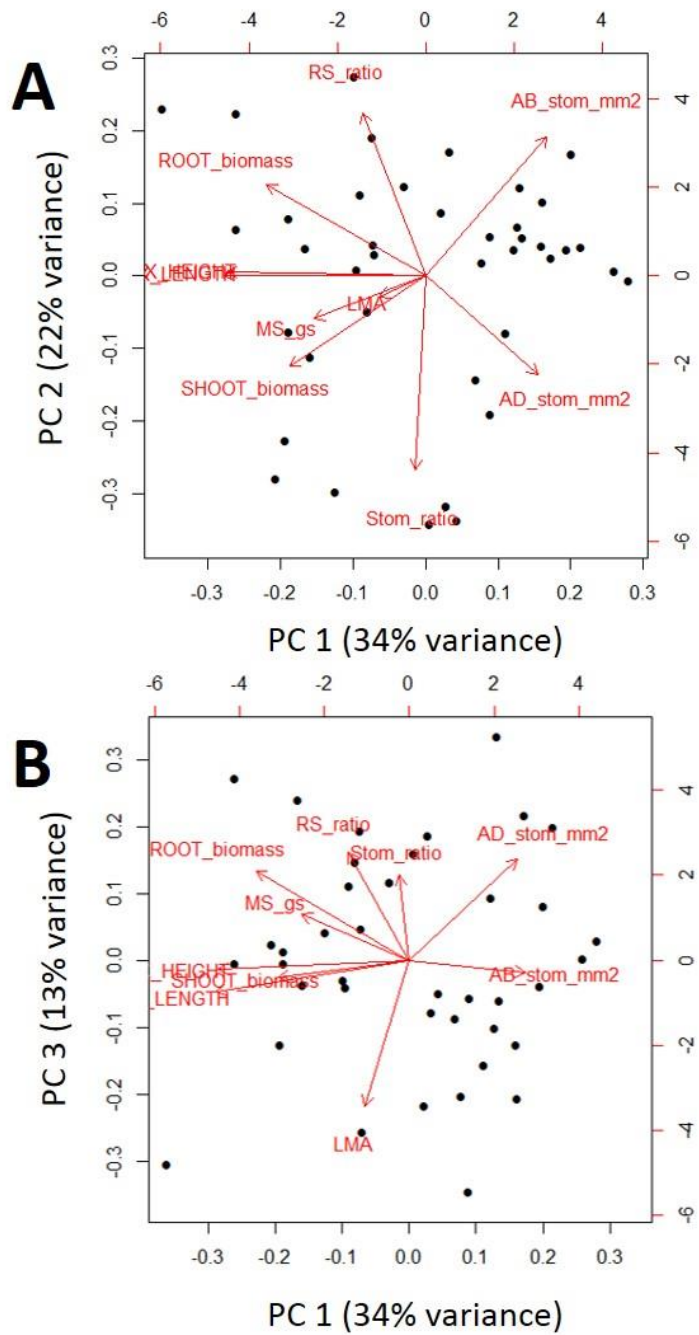
S2. Time series data for stomatal conductance measurements for each of the four soil types.



S3. Chlorosis accompanied by red spotting was commonly observed in plants grown in untreated soils (top), while sorghum seedlings provided with Si fertilizer exhibited improvement in several growth traits.

Table S4. Variance explanation of principal components vectors. PCs 1-5 provided 88% of cumulative variance explanation, and the remaining 5 PCs explained the remaining 12% of variance.

Component	SD	Proportion Var.	Cum. Proportion
1	1.85	0.34	0.34
2	1.50	0.22	0.56
3	1.16	0.13	0.70
4	0.99	0.10	0.80
5	0.94	0.10	0.88
...10	0.08	<0.01	1.00



S5. Ordination diagrams for PC1 vs. PC2 and PC3. Points show locations of individual plants along these axes.

APPENDIX B
SUPPORTING INFORMATION FOR CHAPTER 2

Table 1.

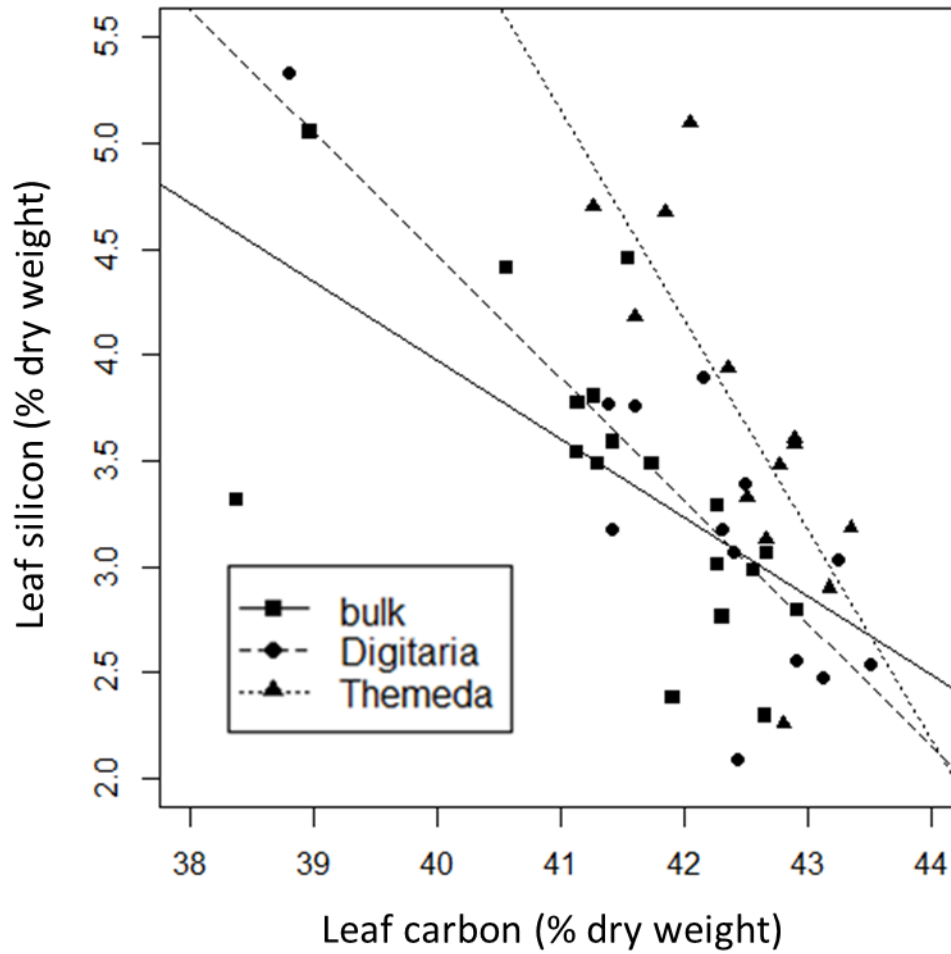
	Estimate	SE	t value	Pr (> t)
Intercept	2.644	0.344	7.686	0.002
Species	1.766	0.320	5.515	<0.001
Water	0.060	0.232	0.259	0.797
Species x Water	-1.345	0.390	-3.448	0.001

Table 2.

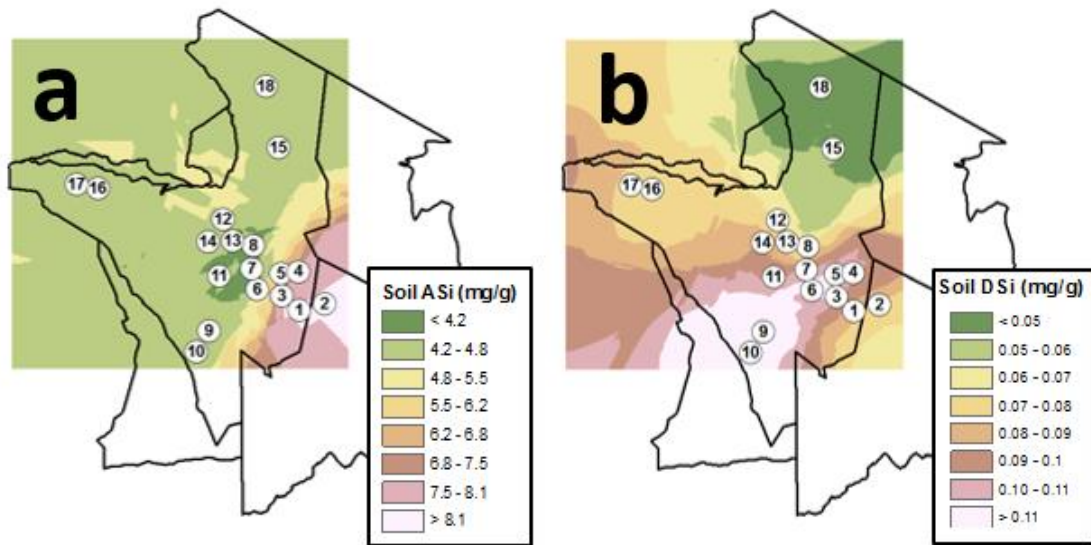
UID	Study	Species	Method	Grazer type	Defoliation Response
1	Brizuela 1986	<i>Schyzachyrium scoparium</i>	clip		-
2	Cid 1990	<i>Pascopyrum smithii</i>	clip		-
3	Quigley 2014	<i>Digitaria macroblephera</i>	clip		0
4	Quigley 2014	<i>Themeda triandra</i>	clip		0
5	Soininen 2012	<i>Deschampsia flexuosa</i>	clip		0
6	Soininen 2012	<i>Festuca ovina</i>	clip		0
7	Massey 2007	<i>Festuca ovina</i>	clip		+
8	Massey 2007	<i>Lolium perenne</i>	clip		+
9	McNaughton 1985	<i>Eustachys paspaloides</i>	clip		+
10	Soininen 2012	<i>Anthoxanthum odoratum</i>	clip		+
11	Soininen 2012	<i>Deschampsia caespitosa</i>	clip		+
12	Garbuzov 2011	<i>Poa annua</i>	graze	insect small	0
13	Reynolds 2012	<i>Deschampsia caespitosa</i>	graze	mammal small	0
14	Soininen 2012	<i>Deschampsia caespitosa</i>	graze	mammal small	0
15	Soininen 2012	<i>Deschampsia flexuosa</i>	graze	mammal small	0
16	Soininen 2012	<i>Anthoxanthum odoratum</i>	graze	mammal large	0
17	McNaughton 1985	<i>Eustachys paspaloides</i>	graze	mammal	0

18	Morton 1974	<i>Festuca arundinacea</i>	graze	large mammal	0
19	Soininen 2012	<i>Calamagrostis phragmitoides</i>	graze	large mammal	0
20	Soininen 2012	<i>Deschampsia caespitosa</i>	graze	large mammal	0
21	Soininen 2012	<i>Deschampsia flexuosa</i>	graze	large mammal	0
22	Soininen 2012	<i>Phleum alpinum</i>	graze	large mammal	0
23	Soininen 2012	<i>Anthoxanthum odoratum</i>	graze	large mammal	0
24	Banuelos 2000	<i>Agrostis capillaris</i>	graze	insect	+
25	Garbuzov 2011	<i>Lolium perenne</i>	graze	insect	+
26	Massey 2007	<i>Festuca ovina</i>	graze	insect	+
27	Massey 2007	<i>Lolium perenne</i>	graze	insect small	+
28	Brizuela 1986	<i>Pascopyrum smithii</i>	graze	mammal small	+
29	Brizuela 1986	<i>Schizachyrium scoparium</i>	graze	mammal small	+
30	Massey 2007	<i>Festuca ovina</i>	graze	mammal small	+
31	Massey 2007	<i>Lolium perenne</i>	graze	mammal small	+
32	Reynolds 2012	<i>Deschampsia caespitosa</i>	graze	mammal small	+
33	Soininen 2012	<i>Calamagrostis phragmitoides</i>	graze	mammal large	+
34	McNaughton 1983	<i>Eustachys paspaloides</i>	graze	mammal	+

APPENDIX C
SUPPORTING INFORMATION FOR CHAPTER 3



Supplementary Figure 1. Scatterplot showing the relationships between leaf carbon and leaf silica (% dry weight) for each species pool. Statistics are provided in text.



Supplementary Figure 2. Interpolated (Krig) surfaces of soil ASi (a) and soil DSi (b) across Serengeti and surrounding protected areas. Site numbers correspond with those from Table 1. Maps were created using the Spatial Analyst tool in ArcGIS 10, and data breaks represent eight equal intervals.

Supplementary Table 1. Species composition of the bulk plant sample collected from each site. Sites are arranged in order of increasing precipitation.

SITE	Species 1	Species 2	Species 3	Species 4
1	Cynodon dactylon	Sporobolus fimbriatus	Sporobolus ioclados	Eustachys paspaloides
2	Cynodon dactylon	Sporobolus ioclados	Eustachys paspaloides	
3	ioclados	Cynodon dactylon		
4	Pennisetum mezianum	Panicum coloratum	Sporobolus pellucidus	Microchloa kunthii
5	Bothriochloa insculpta	Pennisetum mezianum	Sporobolus stapfianus	
6	Pennisetum mezianum	Cynodon dactylon		
7	Sporobolus fimbriatus	Sporobolus pellucidus	Cynodon dactylon	
8	Sporobolus ioclados	Pennisetum mezianum		
9	Digitaria scalarum	Setaria pumila	Pennisetum stramineum	Bothriochloa insculpta
10	Cynodon dactylon	Dactylon aegypticum	Eriochloa nubica	
11	Cymbopogon excavatus	Aristida adoensis	Digitaria scalarum	
12	Eragrostis tenuifolia	Eragrostis racemosa	Aristida adoensis	
13	Chloris roxburghiana	Chloris roxburghiana		
14	Cynodon dactylon	Eragrostis tenuifolia	Bothriochloa insculpta	Chrysochloa orientalis
15	Panicum coloratum	Heterpogon contortus		
16	Cenchrus ciliaris	Sporobolus pyramidalis	Panicum coloratum	
17	Chrysochloa orientalis	Sporobolus fimbriatus		
18	Cynodon dactylon	Bothriochloa insculpta	Panicum coloratum	Microchloa kunthii
18	Eriochloa nubica			

APPENDIX D
SUPPORTING INFORMATION FOR CHAPTER 4

Full description of methods

Analytical methods

Following sample grinding, all plant samples were oven dried and stored in a dessicator until near infrared spectroscopy (NIRS) analysis. After NIRS analysis, an autoclave-induced base digestion procedure was used to extract silicon from plant samples.

Briefly, plant material was placed in a matrix of H₂O₂ and NaOH, and digested at high temperature and pressure (126 °C, 138 kPa) for 1 hour. Under these conditions, the organic plant tissue matrix is dissolved by NaOH and undergoes oxidation by the peroxide; organic material remains bound to the oxidant, and Si is then released from the matrix and dissolved to completion by NaOH (Elliott & Snyder 1991). Immediately following digestion (< 24hr), 1 ml of 5 mmol NH₄F (Kraska & Breitenbeck 2010) was added to each sample and they were diluted to 50 ml in distilled-deionized water. A final dilution of 100 ul diluted extractant in 10 ml of 1% HCl was performed prior to Si quantification by inductively couple plasma optical emission spectroscopy (ICP OES). Recovery following digestion was validated by use of a certified reference material (Community Bureau of Reference, BCR, Reference material No. 129, hay powder).

A Prodigy ICP OES system (Teledyne Leeman Labs, Hudson, NH, USA) composed of an automatic sampler, a double-pass spray chamber and a concentric nebulizer was used to quantify Si content of selected calibration samples. The operating conditions were: axial view mode, radio-frequency applied power 1.3 kW, plasma gas flow rate 18 L/min, nebulizer pressure 30 psi, sample flow rate 0.6 mL/min, and atomic emission integration time 15 s, with three replicates per sample. Analytical calibration curves were

prepared by diluting a silicon stock solution (1000 mg/L, High Purity Standards, Charleston, SC, USA) in 1% HCl (Certified ACS Plus, Fisher, Pittsburgh, PA, USA) and distilled-deionized water (18.2 MΩ cm, Milli-Q, Millipore, Bedford, MA, USA), and mean peak intensity of the 3 replicates was analyzed at 251 nm. The limit of detection (LOD) for the ICP OES, calculated according to IUPAC's recommendations as 3 times the standard deviation of the blank solution (n = 10) divided by the calibration curve slope, was 0.05% silicon in dry mass. Samples which fell below the LOD were eliminated from our calibration dataset.

NIRS Model

We used the R package *LeafSpec* (Griffith & Anderson *in prep*) to conduct pre-processing of scans (outlier identification, spectral averaging) and conduct sample selection for our calibration dataset. Kennard Stone run on averaged scans for all samples. To increase robustness of predictive power for grass leaf Si, the functional type of greatest interest, an additional Kennard Stone selection was run on the subset of grass samples to ensure that wet chemistry (ICP OES) was performed for 20% of grass samples. In total, 345 samples were used for calibration. The distribution of these samples across functional types is provided in Figure S1.

We developed our Si database with Near Infrared Spectroscopy (NIRS). Each sample was then scanned 3 times using a Bruker Multipurpose Analyzer (Bruker Optics Inc., Billerica, MA, USA), recorded as the logarithmic inverse reflectance from 1300 to 2650 nm and utilizing a macrosample rotator (ca. 15 mm) when sufficient plant biomass was available. A previous study of plant Si using NIRS suggested that creating densely packed "tablets" of pulverized plant material may improve NIRS prediction, but we

compared loose and pelleted plant material and saw no difference in predictive quality (Smis et al. 2014, Quigley et al. 2016). A representative calibration dataset, consisting of > 20% of all NIR scans (n = 345), was selected with the Kennard-Stone algorithm (Kennard & Stone, 1969). These samples were prepped and analyzed for Si in the lab using the wet lab technique described above. The model with the lowest Root Mean Squared Error of Prediction (RMSEP = 0.52; 25 latent vectors) was one in which spectra were first preprocessed with Vector Normalization and then restricted to between 7500 to 6100 cm^{-1} and 5450 to 4600 cm^{-1} which corroborate the wavenumbers used by Smis *et al.*, (2014). In addition, the model was greatly improved by the removal of bryophyte and Mt. Caroline (Australia) samples, which each had unique spectral properties. Therefore, when analyzing the final dataset, neither bryophytes nor predicted data from Mt. Caroline were included. The final calibration model was validated on the test set and performed well (validation $R^2 = 0.83$; calibration $R^2 = 0.86$) (Supplementary Figure S4).

Supplementary Tables

Table S1. Sites included in data analysis. Site locations are displayed in Figure 4 (main text), and MAP, MAT, and grazing index calculations are described in supplemental methods.

Site name	Country	Longitude	Latitude	1st year	Habitat type	MAP (mm/yr)	MAT (°C)	Grazing index
Bunchgrass	US	-121.97	44.28	2007	montane grassland semiarid	1647	5.5	8
Burrawan	AU	151.14	-27.73	2008	grassland tallgrass	683	18.4	13
Chichaqua	US	-93.21	41.79	2009	prairie	855	9	10
Bottoms	CA	-123.38	48.46	2007	old field	764	9.8	3
Cowichan	CA	-123.38	48.46	2007	old field	764	9.8	3
Mt. Gilboa	ZA	30.29	-29.28	2010	montane grassland tallgrass	926	13.1	5
Hall's Prairie	US	-86.70	36.87	2007	prairie shrub	1282	13.6	5
Hart	US	-119.50	42.72	2007	steppe	272	7.4	11
Mountain	US	-119.50	42.72	2007	steppe	272	7.4	11
Lookout	US	-122.41	44.21	2007	montane grassland	1898	4.8	8
Mar	US	-122.41	44.21	2007	montane grassland	1898	4.8	8
Chiquita	AR	-57.42	-37.72	2012	grassland	838	13.9	8
Mt. Caroline	AU	117.61	-31.78	2008	savanna	330	17.3	9
Sagehen	US	-120.24	39.43	2007	montane grassland	882	5.7	9
Creek	US	-120.24	39.43	2007	grassland	882	5.7	9
Serengeti	TZ	34.51	-2.25	2008	savanna	854	22.1	29
Shortgrass	US	-104.77	40.82	2007	shortgrass	365	8.4	14
steppe	US	-104.77	40.82	2007	prairie	365	8.4	14
Spindletop	US	-84.50	38.14	2007	pasture	1140	12.5	4
Summerveld	ZA	30.72	-29.81	2009	mesic grassland	939	18.2	
Ukulinga	ZA	30.40	-29.67	2009	mesic grassland	880	18.1	0
Val Mustair	CH	10.37	46.63	2008	alpine grassland	1098	0.3	9

Table S2.

Model components	df	AIC	Δ AIC	w_i	R^2_M	R^2_C
NPK	4	168.81	0.0	0.5647	0.05	0.71
NPK + soil C	5	169.71	0.9	0.3520	0.24	0.67
NPK + soil C + soil N + pH	7	174.31	5.5	0.0370	0.23	0.69
fence + NPK	5	174.41	5.6	0.0340	0.05	0.71
fence * NPK	6	178.11	9.3	0.0055	0.06	0.71
NPK * soil C	6	178.71	9.9	0.0040	0.24	0.67
NPK + MAP	5	180.91	12.1	0.0013	0.24	0.72
soil N	4	183.01	14.2	0.0005	0.21	0.61
soil pH	4	183.01	14.2	0.0005	0.11	0.64
NPK * soil % sand	6	184.41	15.6	0.0002	0.08	0.73
All soil variables	8	184.91	16.1	0.0002	0.22	0.69
fence + NPK + MAP	6	186.61	17.8	0.0001	0.24	0.72
soil C	4	186.91	18.1	0.0001	0.22	0.62
MAT	4	192.51	23.7	0.0000	0.12	0.66
fence	4	193.31	24.5	0.0000	0.00	0.06
NPK * MAP	6	196.21	27.4	0.0000	0.24	0.73
soil % sand	4	198.01	29.2	0.0000	0.02	0.66
MAP	4	200.31	31.5	0.0000	0.18	0.67
fence + MAP	5	205.81	37.0	0.0000	0.18	0.66
MAP + MAT	5	206.51	37.7	0.0000	0.23	0.68
soil C * soil % sand	6	208.31	39.5	0.0000	0.18	0.64
fence * soil % sand	6	214.61	45.8	0.0000	0.02	0.66
fence * MAP	6	223.81	55.0	0.0000	0.18	0.66
MAP * MAT	6	224.81	56.0	0.0000	0.24	0.68
MAP * soil % sand	6	232.21	63.4	0.0000	0.21	0.68

Supplementary Figures

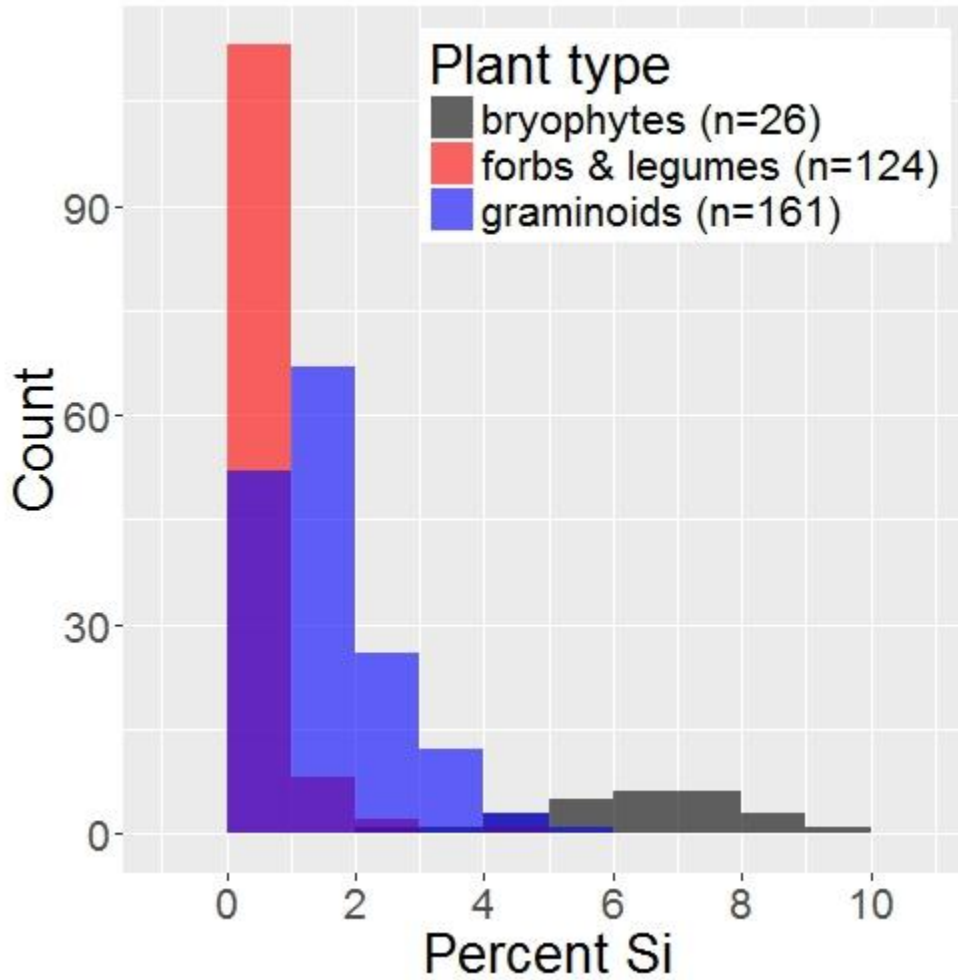


Figure S1. Distribution of leaf Si accumulation for the major plant functional type groups included in the NIRS calibration model ($n = 311$). 34 additional samples (not displayed), were not assigned to any of these four functional types resulting in a total calibration dataset $n = 345$. Forbs and legumes overlapped significantly in their range of Si concentrations so were combined here.

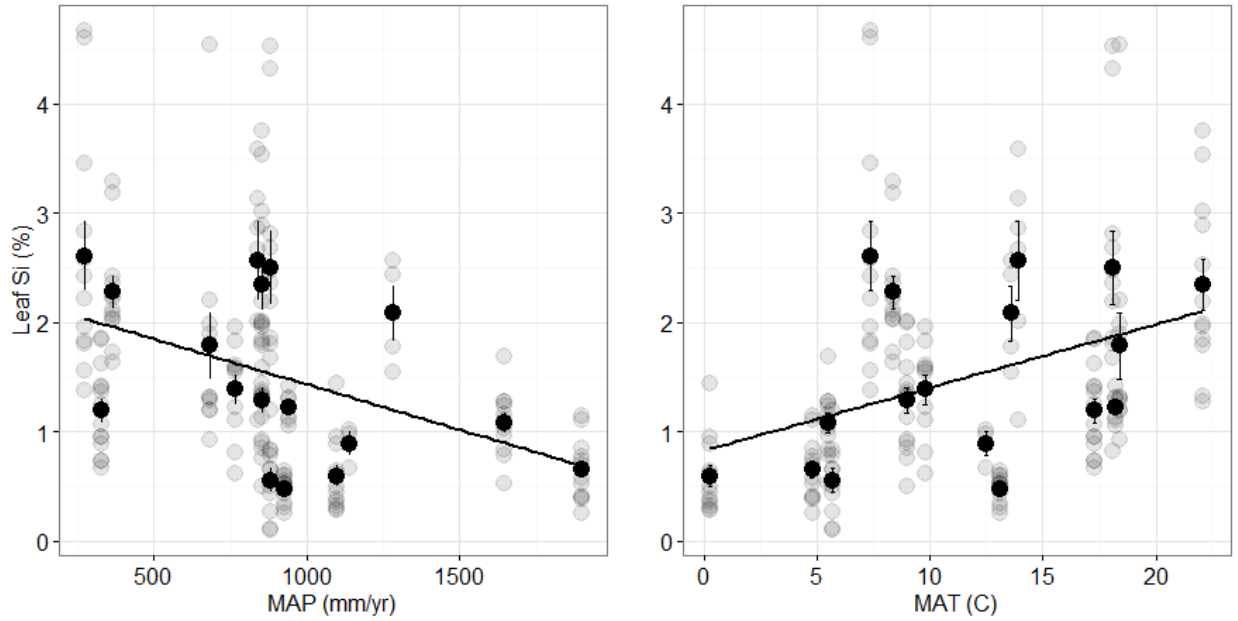


Figure S2. Linear regressions for leaf Si (%) on the two main climate variables of interest. Solid black dots show site-specific averages for Si, and transparent gray points represent individual plant samples. In general, leaf Si concentration declined with increasing precipitation ($r^2 = 0.15$, $p < 0.05$) and was greatest at sites with high mean annual temperature ($r^2 = 0.13$, $p < 0.05$). A linear model combining MAP and MAT explained 20% of variation in grass leaf Si ($p < 0.05$).

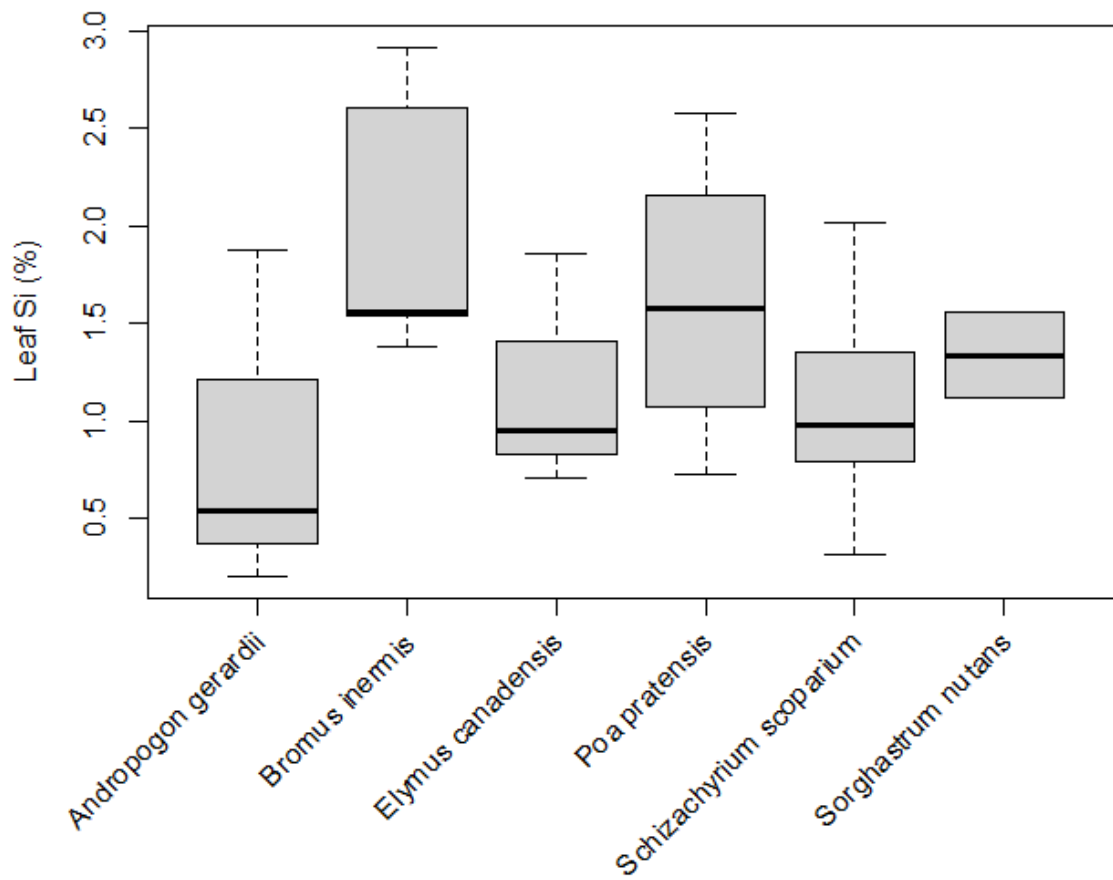


Figure S3. Species-specific Si-accumulation of grass species from the site Chichaqua Bottoms. *Andropogon gerardii* is a C4 grass, and the remaining 5 species are all C3.

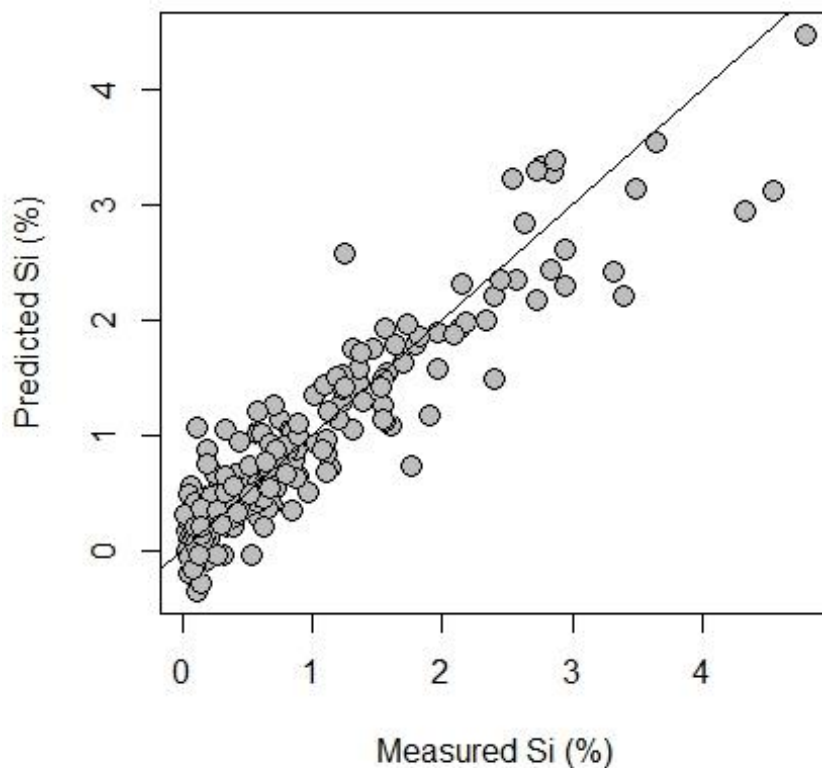


Figure S4. Diagnostic plot for NIR Si calibration showing model predictions plotted against measured wet-lab chemistry. This model excluded bryophytes, litter, and samples from a single site (Mt. Caroline).

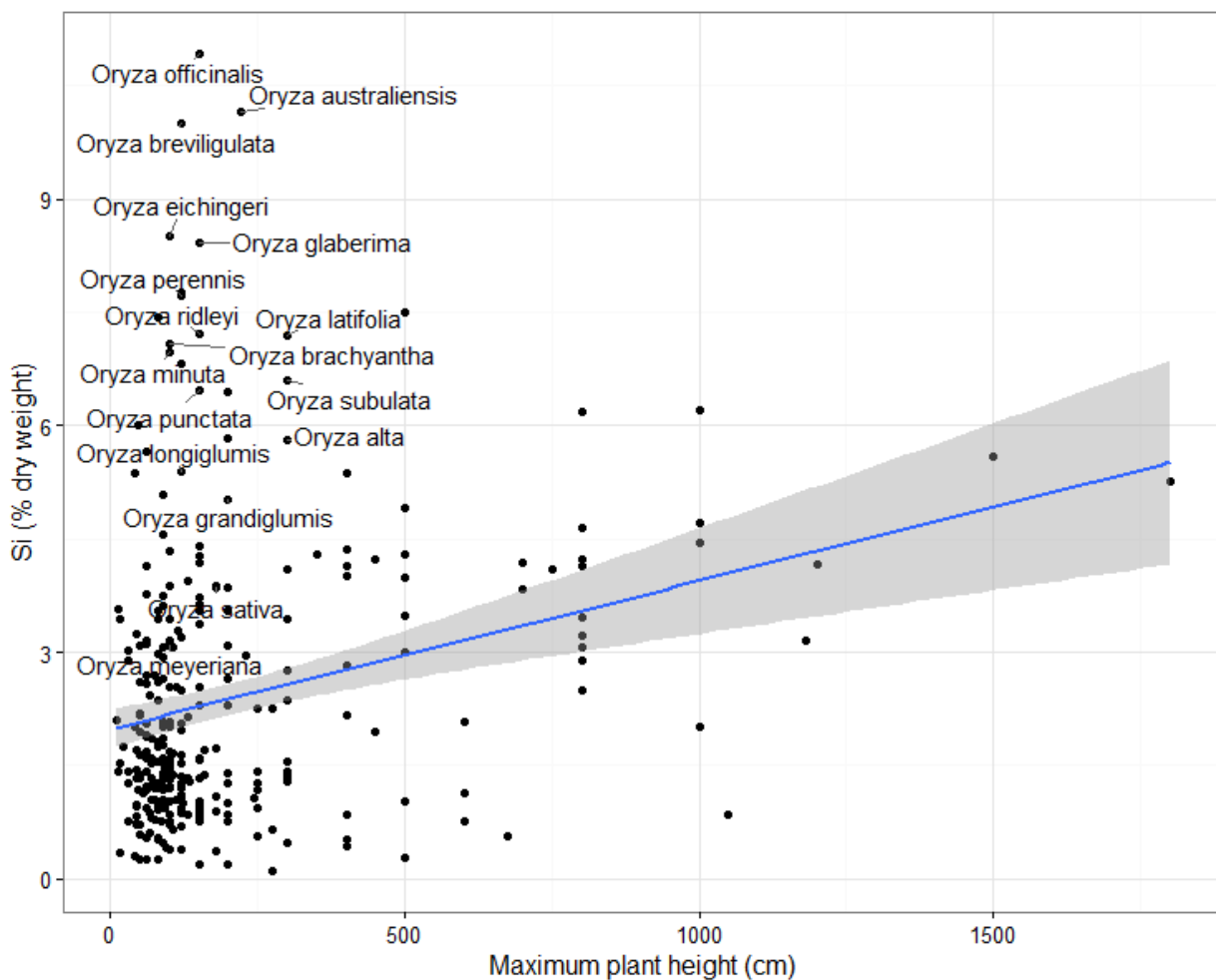
Citations:

Quigley, Kathleen M., Amanda G. Althoff, and George L. Donati. 2016. "Inductively Coupled Plasma Optical Emission Spectrometry as a Reference Method for Silicon Estimation by near Infrared Spectroscopy and Potential Application to Global-Scale Studies of Plant Chemistry." *Microchemical Journal* 129: 231–35.

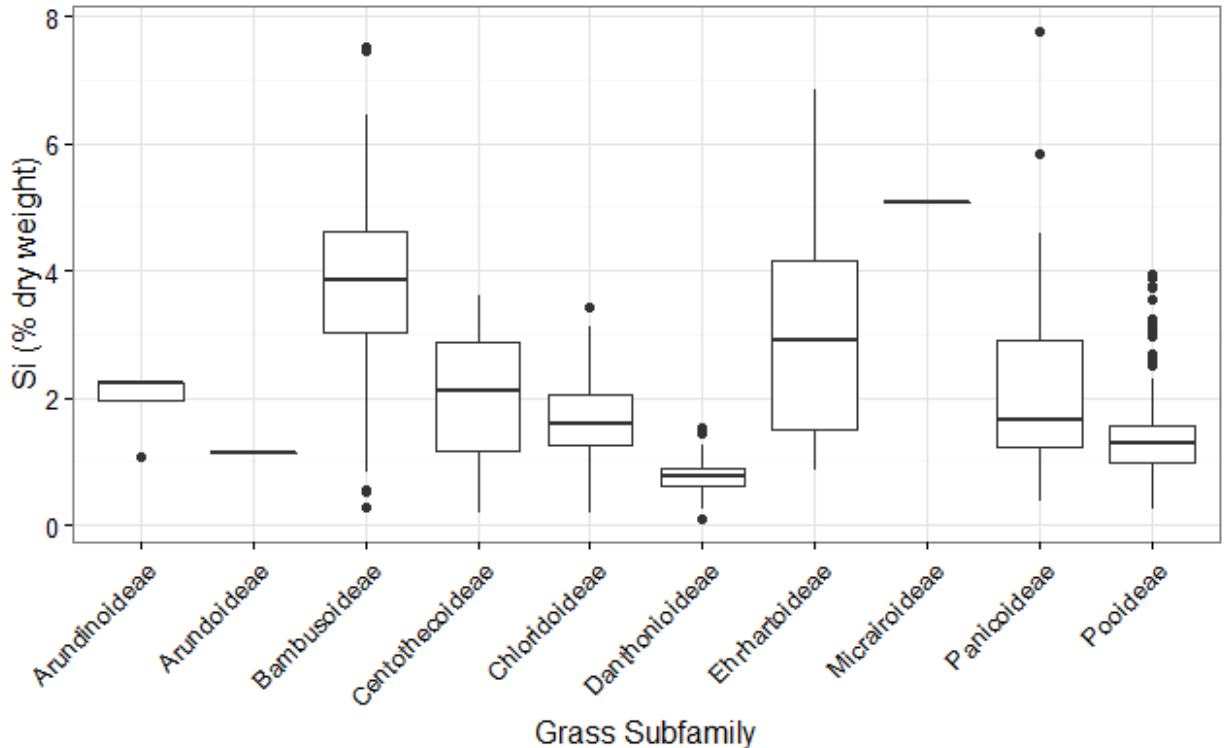
Smis, Adriaan, Francisco Javier Ancin Murguzur, Eric Struyf, Eeva M Soinen, Juan G Herranz Jusdado, Patrick Meire, and Kari Anne Bråthen. 2014. "Determination of Plant Silicon Content with near Infrared Reflectance Spectroscopy." *Frontiers in Plant Science* 5: 496.

APPENDIX E

SUPPORTING INFORMATION FOR CHAPTER 5



Supplement S1. Linear regression between maximum plant height and Si concentration, showing several *Oryza* species as short-statured, but high Si outliers. *Oryza* species were removed from our final analysis of this relationship.



Supplement S2. Boxplot showing the variation in leaf Si among grass (Poaceae) subfamilies.

Supplement S3. Citation information for all studies included in the database.

Agarie, S., Agata, W., Uchida, H., Kubota, F., & Kaufman, P. B. (1996). Function of silica bodies in the epidermal system of rice (*Oryza sativa* L.): testing the window hypothesis. *Journal of Experimental Botany*, 47(298), 655–660. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/11539362>

Alexandre, A., Bouvet, M., & Abbadie, L. (2011). The role of savannas in the terrestrial Si cycle: A case-study from Lamto, Ivory Coast. *Global and Planetary Change*, 78(3–4), 162–169. <http://doi.org/10.1016/j.gloplacha.2011.06.007>

Alexandre, A., Meunier, J.-D., Colin, F., & Koud, J.-M. (1997). Plant impact on the biogeochemical cycle of silicon and related weathering processes. *Geochimica et Cosmochimica Acta*, 61(3), 677–682. [http://doi.org/10.1016/S0016-7037\(97\)00001-X](http://doi.org/10.1016/S0016-7037(97)00001-X)

Ando, H., Kakuda, K., Fujii, H., Suzuki, K., & Ajiki, T. (2002). Growth and canopy structure of rice plants grown under field conditions as affected by Si application. *Soil Science and Plant Nutrition*, 48(3), 429–432.

- 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(1), 45–50.
- Barbehenn, R. V. (1993). Silicon: an indigestible marker for measuring food consumption and utilization by insects. *Entomologia Experimentalis et Applicata*, 67(3), 247–251.
- Barker, G. M. (1989). Grass host preferences of *Listronotus bonariensis*. *Journal of Economic Entomology*, 82(6), 1807–1816.
- Bezeau, L. M., Johnston, A., & Smoliak, S. (1966). Silica and protein content of mixed prairie and fescue grassland vegetation and its relationship to the incidence of silica urolithiasis. *Canadian Journal of Plant Science*, 46, 625–631.
- Bilbro, J. D., Undersander, D. J., Fryrear, D. W., & Lester, C. M. (1991). A survey of lignin, cellulose, and acid detergent fiber ash contents of several plants and implications for wind erosion control. *Journal of Soil and Water Conservation*, 46(4), 314–316.
- Blank, R. R., Allen, F., & Young, J. a. (1994). Growth and elemental content of several sagebrush-steppe species in unburned and post-wildfire soil and plant effects on soil attributes. *Plant and Soil*, 164(1), 35–41. <http://doi.org/10.1007/BF00010108>
- Blecker, S. W., McCulley, R. L., Chadwick, O. a, & Kelly, E. F. (2006). Biologic cycling of silica across a grassland bioclimosequence. *Global Biogeochemical Cycles*, 20(3), 1–11. <http://doi.org/10.1029/2006GB002690>
- Brizuela, M. A., Detling, J. K., & Cid, M. S. (1986). Silicon Concentration of Grasses Growing in Sites With Different Grazing Histories. *Ecology*, 67(4), 1098–1101.
- Butler, J. D., & Hodges, T. K. (1967). Mineral composition of Turfgrasses. *Horticultural Science*, 2(2), 62–63.
- Carey, J. C., & Fulweiler, R. W. (2013). Nitrogen enrichment increases net silica accumulation in a temperate salt marsh. *Limnology and Oceanography*, 58(1), 99–111. <http://doi.org/10.4319/lo.2013.58.1.0099>
- Carnelli, A.L., Madella, M., Theurillat, J. . (2001). Biogenic Silica Production in Selected Alpine Plant Species and Plant Communities. *Annals of Botany*, 87(4), 425–434. <http://doi.org/10.1006/anbo.2000.1355>
- Chen, W., Yao, X., Cai, K., & Chen, J. (2011). Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrient absorption. *Biological Trace Element Research*, 142(1), 67–76. <http://doi.org/10.1007/s12011-010-8742-x>
- 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(4), 344–346. Retrieved from <http://www.jstor.org/stable/3898929>

- 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(2), 268–271.
- Conley, D. J., Likens, G. E., Buso, D. C., Saccone, L., Bailey, S. W., & Johnson, C. E. (2008). Deforestation causes increased dissolved silicate losses in the Hubbard Brook Experimental Forest. *Global Change Biology*, 14(11), 2548–2554. <http://doi.org/10.1111/j.1365-2486.2008.01667.x>
- Cornelissen, J. H. C., & Thompson, K. (1997). Functional leaf attributes predict litter decomposition rate in herbaceous plants. *New Phytologist*, 135, 109–114.
- 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(3), 247–253. <http://doi.org/10.1002/ps>
- DeBakker, N. V. J., Hemminga, M. A., & Soelen, J. Van. (1999). The relationship between silicon availability , and growth and silicon concentration of the salt marsh halophyte *Spartina anglica*. *Plant and Soil*, 215, 19–27.
- Ellis, J. R., Watson, D. M. H., Varvel, G. E., & Jawson, M. D. (1995). Methyl Bromide Soil Fumigation Alters Plant Element Concentrations. *Soil Science Society of America*, 59, 848–852.
- Eneji, A. E., Inanaga, S., Muranaka, S., Li, J., Hattori, T., An, P., & Tsuji, W. (2008). Growth and Nutrient Use in Four Grasses Under Drought Stress as Mediated by Silicon Fertilizers. *Journal of Plant Nutrition*, 31(2), 355–365. <http://doi.org/10.1080/01904160801894913>
- Euliss, K. W., Dorsey, B. L., Benke, K. C., Banks, M. K., & Schwab, a. P. (2005). The use of plant tissue silica content for estimating transpiration. *Ecological Engineering*, 25(4), 343–348. <http://doi.org/10.1016/j.ecoleng.2005.06.003>
- 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(1), 35–40.
- Fox, R., Silva, J., Younge, O., Plucknett, D., & She, G. (1967). Soil and plant silicon and silicate response by sugar cane. *Proceedings of the Soil Science Society of America*, 31(6), 775–779.
- Fu, F., Akagi, T., Yabuki, S., & Iwaki, M. (2001). The variation of REE (rare earth elements) patterns in soil-grown plants : a new proxy for the source of rare earth elements and silicon in plants. *Plant and Soil*, 235, 53–64.

- Gadallah, F. L., & Jefferies, R. L. (1995). Forage quality in brood rearing areas of the lesser snow goose and the growth of captive goslings. *Journal of Applied Ecology*, 32(2), 276–287.
- Gali, H. U., & Smith, C. C. (1992). Effect of silicon supply on growth, fertility, and mineral composition of an annual brome, *Bromus secalinus* L. (Gramineae). *American Journal of Botany*, 79(11), 1259–1263.
- 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(5), 1724–1729.
- Gao, X., Zou, C., Wang, L., & Zhang, F. (2006). Silicon Decreases Transpiration Rate and Conductance from Stomata of Maize Plants. *Journal of Plant Nutrition*, 29(9), 1637–1647. <http://doi.org/10.1080/01904160600851494>
- 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(7), 1355–1363. <http://doi.org/10.1093/aob/mcr230>
- Geis, J. W. (1978). Biogenic opal in three species of Gramineae. *Annals of Botany*, 42, 1119–1129.
- Georgiadis, N. J., & McNaughton, S. J. (1988). Interactions between grazers and a cyanogenic grass, *Cynodon plectostachyus*. *Oikos*, 51, 343–350.
- Georgiadis, N. J., & McNaughton, S. J. (1990). Elemental and fibre contents of savanna grasses: variation with grazing, soil type, season, and species. *Journal of Applied Ecology*, 27(2), 623–634.
- Griffin, M., Hogan, B., & Schmidt, O. (2015). Silicon reduces slug feeding on wheat seedlings. *Journal of Pest Science*, 88(1), 17–24. <http://doi.org/10.1007/s10340-014-0579-1>
- Grobe-Brauckmann, E. (1953). Über die Kieselsaureaufnahme von Pflanzen nach Düngung mit kohlen-saurem Kalk Imprint: Weinheim ; Verlag Chemie. *Journal of Plant Nutrition*, 62(1), 19–23.
- Han, W. X., Fang, J. Y., Reich, P. B., Ian Woodward, F., & Wang, Z. H. (2011). Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. *Ecology Letters*, 14(8), 788–796. <http://doi.org/10.1111/j.1461-0248.2011.01641.x>
- Han, Y., Lei, W., Wen, L., & Hou, M. (2015). Silicon-mediated resistance in a susceptible rice variety to the rice leaf folder, *Cnaphalocrocis medinalis* Guenee (Lepidoptera: Pyralidae). *PLoS ONE*, 10(4), 1–14. <http://doi.org/10.1371/journal.pone.0120557>
- Hartley, S. E., Fitt, R. N., McLarnon, 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(February), 1–8. <http://doi.org/10.3389/fpls.2015.00035>
- Hattori, T., Inanaga, S., Araki, H., An, P., Morita, S., Luxova, M., & Lux, A. (2005). Application of silicon enhanced drought tolerance in *Sorghum bicolor*. *Physiologia Plantarum*, 123(4), 459–466. <http://doi.org/10.1111/j.1399-3054.2005.00481.x>
- Helander, M., Jia, R., Huitu, O., Sieber, T. N., Jia, J., Niemelä, P., & Saikkonen, K. (2013). Endophytic fungi and silica content of different bamboo species in giant panda diet. *Symbiosis*, 61(1), 13–22. <http://doi.org/10.1007/s13199-013-0253-z>
- Hohne, H. (1963). Der mineralstoff- und stickstoffgehalt von waldbodenpflanzen in abhängigkeit vom standort. *Archiv Fur Forstwesen*, 12, 791–805.
- Hossain, K. A., Horiuchi, T., & Miyagawa, S. (1998). Effects of powdered rice chaff application on lodging resistance, Si and N contents and yield components of rice (*Oryza sativa* L.) under shaded conditions. *Acta Agronomica Hungarica*, 46(3), 273–281.
- Hou, M., & Han, Y. (2010). Silicon-mediated rice plant resistance to the Asiatic rice borer (Lepidoptera: Crambidae): effects of silicon amendment and rice varietal resistance. *Journal of Economic Entomology*, 103(4), 1412–1419. <http://doi.org/10.1603/EC09341>
- Huitu, O., Forbes, K. M., Helander, M., Julkunen-Tiitto, R., Lambin, X., Saikkonen, K., ... Hartley, S. (2014). Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores. *Frontiers in Plant Science*, 5(September), 1–10. <http://doi.org/10.3389/fpls.2014.00478>
- 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(4), 653–656. <http://doi.org/10.1093/aob/mcn130>
- Issaharou-Matchi, I., Barboni, D., Meunier, J.-D., Saadou, M., Dussouillez, P., Contoux, C., & Zirihi-Guede, N. (2016). Intraspecific biogenic silica variations in the grass species *Pennisetum pedicellatum* along an evapotranspiration gradient in South Niger. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 220, 84–93. <http://doi.org/10.1016/j.flora.2016.02.008>
- Johnston, A., Bezeau, M., & Smoliak, S. (1967). Variation in silica content of range grasses. *Canadian Journal of Plant Science*, 47, 65–71.
- Jones, L. H. P., Milne, A. A., & Wadham, S. M. (1963). Studies of silica in the oat plant. *Plant and Soil*, (3), 358–371.
- Katz, O. (2014). Beyond grasses: the potential benefits of studying silicon accumulation in non-grass species. *Frontiers in Plant Science*, 5(376), 1–3. <http://doi.org/10.1093/aob/mci255>

- Keeping, M. (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(June), 1–12. <http://doi.org/10.3389/fpls.2014.00289>
- Kindomihou, V., Sinsin, B., Meerts, P., Belgian, S., 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(1), 87–102.
- Klotzbücher, T., Marxen, A., Jahn, R., & Vetterlein, D. (2016). Silicon cycle in rice paddy fields: insights provided by relations between silicon forms in topsoils and plant silicon uptake. *Nutrient Cycling in Agroecosystems*. <http://doi.org/10.1007/s10705-016-9782-1>
- Klotzbücher, T., Leuther, F., Marxen, a., Vetterlein, D., Horgan, F. G., & Jahn, R. (2015). Forms and fluxes of potential plant-available silicon in irrigated lowland rice production (Laguna, the Philippines). *Plant and Soil*, 393(1–2), 177–191. <http://doi.org/10.1007/s11104-015-2480-y>
- Lanning, F. C. (1966). Silica and calcium deposition in the tissues of certain plants. *Advancing Frontiers in Plant Science*, 13, 55–66.
- Lanning, F. C., & Eleuterius, L. N. (1989). Silica deposition in some C3 and C4 species of grasses, sedges, and composites in the USA. *Annals of Botany*, 64(4), 395–410.
- Lanning, F. C., & Eleuterius, L. N. (1981). Silica and ash in several marsh plants. *Gulf Research Reports*, 7, 47–52.
- Lanning, F. C., & Eleuterius, L. N. (1987). Silica and ash in native plants of the central and south eastern regions of the United States. *Annals of Botany*, 60(4), 361–375.
- Lanning, F. C., & Eleuterius, L. N. (1985). Silica and ash in tissues of some plants growing in the coastal area of Mississippi, USA. *Annals of Botany*, 56(2), 157–172.
- Lanning, F. C., & Eleuterius, L. N. (1983). Silica and ash in tissues of some coastal plants. *Annals of Botany*, 51(6), 835–850.
- Li, B., Song, Z., Li, Z., Wang, H., Gui, R., & Song, R. (2014). Phylogenetic variation of phytolith carbon sequestration in bamboos. *Scientific Reports*, 4, 4710. <http://doi.org/10.1038/srep04710>
- Liang, Y., Hua, H., Zhu, Y. G., Zhang, J., Cheng, C., & Römheld, V. (2006). Importance of plant species and external silicon concentration to active silicon uptake and transport. *New Phytologist*, 172(1), 63–72. <http://doi.org/10.1111/j.1469-8137.2006.01797.x>
- Lovering, T., & Engel, C. (1967). Translocation of Silica and Other Elements from Rock into Equisetum and Three Grasses. *Geological Survey Professional Paper*, 594–B, B1–B16.

- Ma, J. F., & Takahashi, E. (1993). Interaction between calcium and silicon in water-cultured rice plants. *Plant and Soil*, *148*(1), 107–113. <http://doi.org/10.1007/BF02185390>
- Ma, J. F., & Takahashi, E. (1990). Effect of Silicon on the Growth and Phosphorus Uptake of Rice. *Plant and Soil*, *126*, 115–119. <http://doi.org/10.1007/BF00041376>
- Marxen, a., Klotzbücher, T., Jahn, R., Kaiser, K., Nguyen, V. S., Schmidt, a., ... Vetterlein, D. (2015). Interaction between silicon cycling and straw decomposition in a silicon deficient rice production system. *Plant and Soil*. <http://doi.org/10.1007/s11104-015-2645-8>
- Massey, F. P., Smith, M. J., Lambin, X., & Hartley, S. E. (2008). Are silica defences in grasses driving vole population cycles? *Biology Letters*, *4*(4), 419–422. <http://doi.org/10.1098/rsbl.2008.0106>
- 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*(1), 281–291. <http://doi.org/10.1111/j.1365-2656.2007.0>
- Massey, F. P., Massey, K., Roland Ennos, a., & Hartley, S. E. (2009). Impacts of silica-based defences in grasses on the feeding preferences of sheep. *Basic and Applied Ecology*, *10*(7), 622–630. <http://doi.org/10.1016/j.baae.2009.04.004>
- 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*(2), 595–603. <http://doi.org/10.1111/j.1365-2656.2006.01082.x>
- Massey, F. P., Ennos, a R., & Hartley, S. E. (2007). Herbivore specific induction of silica-based plant defences. *Oecologia*, *152*(4), 677–683. <http://doi.org/10.1007/s00442-007-0703-5>
- Massey, F. P., Ennos, R. a, & Hartley, S. E. (2007). Grasses and the resource availability hypothesis: the importance of silica-based defences. *Journal of Ecology*, *95*(3), 414–424. <http://doi.org/10.1111/j.1365-2745.2007.01223.x>
- 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. <http://doi.org/10.1098/rspb.2006.3586>
- Mateos-Naranjo, E., Andrades-Moreno, L., & Davy, A. J. (2013). Silicon alleviates deleterious effects of high salinity on the halophytic grass *Spartina densiflora*. *Plant Physiology and Biochemistry*, *63*, 115–21. <http://doi.org/10.1016/j.plaphy.2012.11.015>
- Mayland, H. F., Johnson, D. A., Asay, K. H., & Read, J. J. (1993). Ash , Carbon Isotope Discrimination , and Silicon as Estimators of Transpiration Efficiency in Crested Wheatgrass. *Australian Journal of Plant Physiology*, *20*, 361–369.

- McManus, W. R. (1977). The physical distribution of mineral material on forage plant cell walls. *Australian Journal of Agricultural Research*, 28, 651–662.
- 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*, 80(3), 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(2), 528–535.
- Melo, S. P., Monteiro, F. a., & Bona, F. D. (2010). Silicon distribution and accumulation in shoot tissue of the tropical forage grass *Brachiaria brizantha*. *Plant and Soil*, 336(1–2), 241–249. <http://doi.org/10.1007/s11104-010-0472-5>
- Melzer, S. E., Knapp, A. K., Kirkman, K. P., Smith, M. D., Blair, J. M., & Kelly, E. F. (2009). Fire and grazing impacts on silica production and storage in grass dominated ecosystems. *Biogeochemistry*, 97(2–3), 263–278. <http://doi.org/10.1007/s10533-009-9371-3>
- Melo, S. P. De, Korndörfer, G. H., Korndörfer, C. M., Lana, R. M. Q., & Santana, D. G. De. (2003). Silicon accumulation and water deficit tolerance in *Brachiaria* grasses. *Scientia Agricola*, 60(4), 755–759. <http://doi.org/10.1590/S0103-90162003000400022>
- Minson, D. J. (1971). The digestibility and voluntary intake of six varieties of *Panicum*. *Australian Journal of Experimental Agriculture and Animal Husbandry*, 11(48), 18–25.
- Morton, B. B., & Jutras, M. W. (1974). Silica concentrations in grazed and ungrazed forage species. *Agronomy Journal*, 66, 10–12.
- Norris, A. R., & Hackney, C. T. (1999). Silica Content of a Mesohaline Tidal Marsh in North Carolina. *Estuarine, Coastal and Shelf Science*, 49, 597–605.
- Pahkala, K., & Pihala, M. (2000). Different plant parts as raw material for fuel and pulp production. *Industrial Crops and Products*, 11(2–3), 119–128. [http://doi.org/10.1016/S0926-6690\(99\)00050-3](http://doi.org/10.1016/S0926-6690(99)00050-3)
- Querné, J., Ragueneau, O., & Poupart, N. (2012). In situ biogenic silica variations in the invasive salt marsh plant, *Spartina alterniflora*: A possible link with environmental stress. *Plant and Soil*, 352(1–2), 157–171. <http://doi.org/10.1007/s11104-011-0986-5>
- 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(October), 1–10. <http://doi.org/10.3389/fpls.2014.00568>

- Quigley, K. M., Donati, G. L., & Anderson, T. M. (2016). Variation in the soil “silicon landscape” explains plant silica accumulation across environmental gradients in Serengeti. *Plant and Soil*. <http://doi.org/10.1007/s11104-016-3000-4>
- Redmond, C. T., & Potter, D. A. (2006). Silicon Fertilization Does Not Enhance Creeping Bentgrass Resistance to Cutworms and White Grubs. *Applied Turfgrass Science*, online. <http://doi.org/10.1094/ATS-2006-1110-01-RS.Abstract>
- Reynolds, J. J. H., Lambin, X., Massey, F. P., Reidinger, S., Sherratt, J. a, Smith, M. J., ... Hartley, S. E. (2012). Delayed induced silica defences in grasses and their potential for destabilising herbivore population dynamics. *Oecologia*, *170*(2), 445–56. <http://doi.org/10.1007/s00442-012-2326-8>
- Robbins, A. C. T., Mole, S., Hagerman, A. E., & Hanley, T. A. (1987). Role of Tannins in Defending Plants Against Ruminants : Reduction in Dry Matter Digestion ? *Ecology*, *68*(6), 1606–1615.
- Rufo, L., Franco, A., & de la Fuente, V. (2013). Silicon in *Imperata cylindrica* (L.) P. Beauv: content, distribution, and ultrastructure. *Protoplasma*. <http://doi.org/10.1007/s00709-013-0594-8>
- Saijonkari-pahkala, K. (2001). *Non-wood plants as raw material for pulp and paper*. University of Helsinki.
- Schaller, J., Brackhage, C., Gessner, M. O., Bäucker, E., & Gert Dudel, E. (2012). Silicon supply modifies C:N:P stoichiometry and growth of *Phragmites australis*. *Plant Biology*, *14*(2), 392–6. <http://doi.org/10.1111/j.1438-8677.2011.00537.x>
- Schaller, J., Brackhage, C., Paasch, S., Brunner, E., Bäucker, E., & Dudel, E. G. (2013). Silica uptake from nanoparticles and silica condensation state in different tissues of *Phragmites australis*. *Science of the Total Environment*, *442*, 6–9. <http://doi.org/10.1016/j.scitotenv.2012.10.016>
- Schnug, E., & von Franck, E. (1985). Untersuchungen zur Silizium-Versorgung von Kulturpflanzen in Schleswig-Holstein. *Journal of Plant Nutrition and Soil Science*, *148*, 1–9.
- Schoelynck, J., Bal, K., Backx, H., Okruszko, T., Meire, P., & Struyf, E. (2010). Silica uptake in aquatic and wetland macrophytes: a strategic choice between silica, lignin and cellulose? *New Phytologist*, *186*(2), 385–391. <http://doi.org/10.1111/j.1469-8137.2009.03176.x>
- Seastedt, T. R., Ramundo, R. a, & Hayes, D. C. (1989). Silica, Nitrogen, and Phosphorus Dynamics of Tallgrass Prairie. In *North American Prairie Conference* (pp. 205–209).
- 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*(2), 122. <http://doi.org/10.2307/3899308>

- Smith, G. S., Nelson, A. B., & Boggino, E. J. A. (1971). Digestibility of forages in vitro as affected by content of silica. *Journal of Animal Science*, 33, 466–471.
- Soininen, E. M., Bråthen, K. A., Jusdado, J. G. H., Reidinger, S., & Hartley, S. E. (2012). More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. *Oikos*, 122(1), 30–41. <http://doi.org/10.1111/j.1600-0706.2012.20689.x>
- Song, Z., Liu, H., Si, Y., & Yin, Y. (2012). The production of phytoliths in China's grasslands: implications to the biogeochemical sequestration of atmospheric CO₂. *Global Change Biology*, 18(12), 3647–3653. <http://doi.org/10.1111/gcb.12017>
- Street, J. R. (1974). *The influence of silica concentration on the chemical composition and decomposition rates of turfgrass tissue and water absorption rates among three turfgrass species*. Ohio State University.
- Struyf, E., Van Damme, S., Gribsholt, B., Middelburg, J., & Meire, P. (2005). Biogenic silica in tidal freshwater marsh sediments and vegetation (Schelde estuary, Belgium). *Marine Ecology Progress Series*, 303, 51–60. <http://doi.org/10.3354/meps303051>
- Taber, H. G. (2002). Extraction of silicon from plant tissue with dilute HCl and HF and measurement by modified inductive coupled argon plasma procedures. *Communications in Soil Science and Plant Analysis*, 33, 1661–1670.
- Tirtapradja, H. (1971). Quantitative Untersuchungen an Kieselzellen in Blättern und Halmen von *Festuca pratensis* Huds. und *F. arundinaceae* Schreb. *Angewandte Botanik*, 231–247.
- Tyler, G. (1971). Distribution and turnover of organic matter and minerals in a shore meadow ecosystem. *Oikos*, 22, 265–291.
- VanDerVorm, P. D. J. (1980). Uptake of Si by five plant species, as influenced by variations in Si-supply. *Plant and Soil*, 56, 153–156.
- Vandevenne, F. I., Barão, A. L., Schoelynck, J., Smis, A., Ryken, N., Van, S., ... Damme, S. Van. (2013). Grazers : biocatalysts of terrestrial silica cycling. *Proceedings of the Royal Society B*, 280, 1–9.
- Vandevenne, F., Struyf, E., Clymans, W., & Meire, P. (2012). Agricultural silica harvest: have humans created a new loop in the global silica cycle? *Frontiers in Ecology and the Environment*, 10(5), 243–248. <http://doi.org/10.1890/110046>
- Wallace, A., Romney, E. M., & Mueller, R. T. (1976). Nitrogen-silicon interaction in plants grown in desert soil with nitrogen deficiency. *Agronomy Journal*, 68, 529–530.
- Wallis, L. (2003). An overview of leaf phytolith production patterns in selected northwest Australian flora. *Review of Palaeobotany and Palynology*, 125(3–4), 201–248. [http://doi.org/10.1016/S0034-6667\(03\)00003-4](http://doi.org/10.1016/S0034-6667(03)00003-4)

Yang, X., Song, Z., Liu, H., Bolan, N. S., Wang, H., & Li, Z. (2015). Plant silicon content in forests of north China and its implications for phytolith carbon sequestration. *Ecological Research*, 30(2), 347–355. <http://doi.org/10.1007/s11284-014-1228-0>

Yoshida, S., Navasero, S., & Ramirez, E. (1969). Effects of silica and nitrogen supply on some leaf characters of the rice plant. *Plant and Soil*, (1), 48–56.

APPENDIX F

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTROSCOPY AS A
REFERENCE METHOD FOR SILICON ESTIMATION BY NEAR INFRARED
SPECTROSCOPY AND POTENTIAL APPLICATION TO GLOBAL-SCALE STUDIES OF
PLANT CHEMISTRY

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ABSTRACT

Inductively coupled plasma optical emission spectrometry (ICP OES) is used to produce reference calibration samples for Si determination in plants by near infrared spectroscopy (NIRS). A certified reference material (CRM) of hay was used to validate the ICP OES reference procedure, which is based on strong base digestion in an autoclave. No statistically significant difference was observed between certified and ICP OES-determined values at a 95 % confidence level (*t*-test). The limit of detection (LOD) for this procedure was calculated as 10 µg/L (0.05 % m/m). A calibration dataset with 346 plant samples (including grass, forbs, legumes, woody plants, and bryophytes) was analyzed by the reference procedure and the results were used to create a partial least squares (PLS) regression model to determine Si in dried, ground plant samples by NIRS. We explored options for optimizing NIRS predictive strength, and excluding bryophytes from the calibration resulted in a PLS model with a validation R^2 of 0.83. The optimal NIRS model was then used to determine Si concentrations in more than 875 plant samples. Plant functional types exhibited significant variation in their Si concentration, with most legumes and woody plants presenting values below the method's LOD. Silicon concentrations in the 0.05-4.46, 0.07-1.00, and 0.11-4.67 % range were found for forbs ($n = 179$), legumes ($n = 23$), and graminoids ($n = 398$), respectively. NIRS is non-destructive and requires virtually no sample preparation and almost no training prior to instrument operation. The rapid determination of Si by NIRS is a powerful strategy for global scale analyses of plants. It is a simple, effective and relatively low-cost approach, with important implications on food crop, livestock forage and ecological theory research.

Keywords: Plant silicon, Strong base digestion, Near infrared spectroscopy, Calibration, Modeling

1. Introduction

Silicon occurs ubiquitously in the Earth's lithosphere and biosphere. It is second only to oxygen in the Earth's crust and accounts for more than 25 % of its mass, most commonly occurring in the form of silicate minerals [1]. In the biosphere, Si plays a vital role in many living organisms: it precipitates as solid silica (SiO_2), forming the skeletons of sponges and diatoms as well as specialized cells of plant tissue, referred to as phytoliths. Most plants accumulate relatively small amounts of Si-rich phytoliths, but some plants (e.g. rice, horsetail, and certain mosses) hyper-accumulate silicon, with dry weight concentrations higher than 10 % m/m [2]. Although not considered an essential plant nutrient, Si is known to alleviate a broad range of abiotic stress, from drought and salinity to toxic-metal stress [3]. It may also prevent or reduce herbivory by insects and mammals [4,5], thus making it of great interest in a broad range of research.

The benefits associated with plant Si accumulation emphasize its biological significance, yet plant Si remains difficult to quantify for several reasons. Simple ashing and gravimetric determination involve many poorly controlled steps, with high potential for contamination and analyte loss [6]. On the detection end, traditional colorimetric methods, such as the one based on molybdenum blue, require the preparation of specifically-optimized reagents and are highly time-sensitive [7]. Silicon commonly occurs as solid silica deposits in a complex organic matrix within the plant tissues. A liquid solution containing the analyte is required for most instrumental analytical techniques, but releasing Si into solution from the sample matrix is no trivial task. Open-vessel digestion may be the most common approach to sample preparation for Si determination using spectrochemical methods [8,9]. However, considering the large amounts of reagents required and potential sources of contamination involved, other alternatives are usually considered. Closed-vessel microwave-assisted

digestion is an efficient example of such sample preparation alternatives. It allows for a more controlled digestion environment, which minimizes contamination and analyte loss, while providing improved precision and accuracy [10,11]. Despite their efficiency, procedures based on closed-vessel microwave-assisted acid digestion may become cumbersome for the specific case of Si determination. Hydrofluoric acid is usually required to dissolve Si compounds. Excess fluoride in the digested sample solutions can then react with instrument components (e.g. quartz torches) releasing Si, which ultimately compromises accuracy and reduces the instrument lifetime. Therefore, an additional step is usually required to remove F⁻ from solution, e.g. H₃BO₃ is added after digestion to form HBF₄ and prevent reactions with glass and quartz surfaces [12,13].

Another approach to solubilizing Si compounds is based on digestion with highly alkaline solutions. Elliot and Snyder used a combination of NaOH and H₂O₂ to digest plant samples using an autoclave [9]. Kraska and Breitenbeck modified this procedure by adding dilute NH₄F to the digested samples to improve accuracy [14]. Barros *et al.* used a two-step closed-vessel microwave-assisted digestion procedure based on diluted HNO₃ and H₂O₂, and then diluted NaOH, to simultaneously determine Si and eleven other elements by inductively coupled plasma optical emission spectrometry (ICP OES). Recoveries for certified reference materials and for plant roots and leaves were in the 91-109 % range [15].

Notably, all of the aforementioned methods are destructive in nature. In spite of the labor-intensive procedures and sometimes hazardous reagents required, Si solubilization and subsequent determination remain difficult, especially considering precision, accuracy and

sample throughput [16,17]. Although there is a great deal of interest in plant Si research, many questions pertaining to the role of this element in natural ecosystems remain unanswered. The constraints associated with sample preparation and analysis and the consequent lack of certified reference materials for Si are largely to blame [18]. Such limitations hinder progress toward answering questions beyond the laboratory or local site scale. Addressing landscape, ecosystem, and even global-scale questions about plant Si accumulation necessitates the use of cost-effective and high-throughput methods. Recently, near infrared spectroscopy (NIRS) has been proposed as a time-efficient and accurate way to estimate plant Si [19,20]. Smis and colleagues, for example, were able to create a strong statistical calibration method based on NIRS spectra and extrapolate Si concentration for a large collection of samples with up to 95 % validation (accuracy) depending upon the breadth of samples included in the calibration model [20]. NIRS is a non-destructive technique that requires minimal user training and no complex or time-consuming sample preparation, while allowing for the analysis of many samples in a reduced amount of time. It has been applied to a broad range of analytical challenges, especially for plant and soil research. For instance, functional biodiversity research has recently benefited from the use of NIRS to predict concentrations of important plant molecules including sugar, starch, and non-structural carbohydrates [21].

In the present work, we used ICP OES and a certified reference material (CRM) of hay to produce reference samples that can be used to construct NIRS calibration models for accurately predicting plant Si. The CRM was used to validate the reference method, which is based on digestion in an autoclave using NaOH, H₂O₂ and NH₄F [14], and Si determination by ICP OES. The ICP OES-based method was then used to analyze a subset of 346 samples and build a calibration dataset for NIRS. The NIRS method was

used as a fast, accurate, and non-destructive strategy to determine Si in 800 plant samples from collection sites around the world.

2. Experimental

2.1. Instrumentation

A Cyclone belt drive sample mill (UDY Corp., Fort Collins, CO, USA) and a Precision drying oven (OV702F, Thermo Scientific, Dubuque, IA, USA) were used for grinding and then drying the plant samples. Sample digestion was carried out in an Amsco Renaissance 3011 autoclave (STERIS Corp., Mentor, OH, USA). A Prodigy ICP OES system (Teledyne Leeman Labs, Hudson, NH, USA) composed of an automatic sampler, a double-pass spray chamber, and a concentric nebulizer was used in all Si determinations. The ICP OES operating conditions are summarized in Table 1.

NIR spectra were measured by diffuse reflection in the 7692-3774 cm^{-1} region using a MPA FT-NIR analyzer (Bruker Optics Inc., Billerica, MA, USA). When possible, a macrosample rotator (ca. 15 mm) was utilized to control for within-sample heterogeneity, and a stationary microsample chamber (ca. 4 mm) was used when plant biomass was below the threshold required for the rotating chamber. Three individual subsamples of each dried, ground plant sample were scanned, and an average spectral signature was calculated in the R statistical programming environment [22].

2.2. Certified reference sample, standard reference solutions and reagents

A standard reference material (CRM) of hay (BCR 129, hay powder, Institute for Reference Materials and Measurements, Geel, Belgium) was used to validate the ICP OES reference method (*i.e.* alkaline digestion and Si determination by ICP OES). Concentrated HCl (Certified ACS Plus, Fisher, Pittsburgh, PA, USA) and distilled-deionized water (18.2 M Ω cm, Milli-Q, Millipore, Bedford, MA, USA) were used to prepare solutions used in the ICP OES determinations. Standard reference solutions used for calibration were prepared by dilution of a Si stock solution (1000 mg/L, High Purity Standards, Charleston, SC, USA) in 1 % v/v HCl. Sodium hydroxide (NaOH, NF/FCC pellets, Fisher Scientific, Waltham, MA, USA), H₂O₂ 50 % v/v (Fisher), 1-octanol (Carolina Biological Supply, Burlington, NC, USA), and NH₄F (Acros Organics, Fair Lawn, NJ, USA) were used for sample digestion.

2.3. Sample collection and preparation

Plant samples were collected from a series of grassland plots around the world as part of an ongoing experimental network, the *Nutrient Network*. The standardized protocol of this collaborative project includes experimental plots which manipulate both grazing and nutrient availability in a fully factorial manner [23]. During collection, each sample was classified to plant functional type by local researchers as either graminoid, forb, legume, woody plant, or bryophyte. Plant samples were shipped to Wake Forest University where they were finely ground into a homogenous powder using a belt drive sample mill, and oven-dried at 60 °C for a minimum of 24 h prior to analysis.

After scanning all samples using NIRS, the top-ranked samples from a Kennard-Stone selection ($n = 346$) were subjected to autoclave-induced base digestion [14]. A sample

aliquot of approximately 100.0 mg was placed in a sterile 50.0 mL polypropylene tube and wetted with 1-octanol to reduce foaming and prevent incomplete digestion. Next, 2.00 mL of H₂O₂ 50 % v/v and 3.50 mL of a 50 % m/v NaOH aqueous solution were added to each tube. Samples were briefly vortexed and immediately digested in an autoclave at 250 °C and 238 kPa for 1 h. Following digestion, samples were allowed to cool and 1.00 mL of a 5.0 mM NH₄F solution was added to each digestion tube, which were then filled to 50.0 mL with distilled-deionized water. Finally, 0.100 mL aliquots of the digested solutions were diluted to 10.0 mL with 1 % v/v HCl.

3. Results and discussion

3.1. Reference method

Alkaline digestion with NaOH and H₂O₂ in an autoclave, and addition of NH₄F to the digested solutions was the approach adopted to solubilize the samples [14]. Because of ICP OES' sensitivity in Si determinations and the relatively high concentrations of this element in plants, we were able to dilute the digested solutions several times. Fluoride ions, for example, were diluted at least 5000-fold, which significantly minimized any potential negative effects on accuracy and equipment integrity. Sample dilution has also reduced the concentrations of Na and other matrix components, further contributing to improved precision and accuracy. Finally, because the already diluted matrix represents only 1 % of the analytical samples (0.100 mL in 10.0 mL of solution), HCl 1 % v/v was used to prepare the reference calibration solutions, with no need for convoluted matrix-matching procedures.

A CRM of hay was used to validate the digestion procedure and the ICP OES determination method. The Si content found (2372 ± 315 mg/kg, $n = 3$) was statistically not different from the reference value (2221 mg/kg Si), as indicated by applying a *t*-test at the 95 % confidence level. The limit of detection (LOD), calculated according to IUPAC's recommendations as 3 times the standard deviation of a blank solution (S_B , $n = 10$) divided by the calibration curve slope (m), is 10 $\mu\text{g/L}$ (0.05 % Si in dry mass). Considering its accuracy, precision and sensitivity, the combination of alkaline digestion and ICP OES determination was used as reference to calibrate the NIRS method for non-destructive, direct determination of Si in plants.

3.2. Calibration samples

All available samples (approximately 1700) were scanned by NIRS to define a subset that would most effectively be used with the ICP OES reference method to build a calibration model for NIRS determinations of Si. Three individual subsamples of each dried, ground plant sample were scanned, and an average spectral signature was calculated in the R statistical programming environment [22]. Although previous researchers have proposed the compression of plant material into "pellets" to reduce random light scatter [20], spectrum comparisons between loose and pelleted plant material for approximately 20 samples have shown no significant differences (Fig. 1). Therefore, uncompressed samples (loose) were used in all subsequent analyses. After all samples were scanned and subsamples averaged, a Kennard-Stone algorithm was used to identify the most adequate samples to be used as calibration references. Kennard-Stone divides an existing dataset into variance-covariance matrices for optimal model building. It allowed us to represent the maximum amount of variation across the entire spectral range of all

samples, while analyzing only approximately 20 % of the total number of samples by ICP OES [24].

3.3. NIRS models

The ICP OES-analyzed calibration samples ($n = 346$) were further subdivided into calibration (model development; 80%) and validation (test set; 20%) subsets using a modified Kennard-Stone algorithm that incorporates variation in both spectra and the Si values [25, 26]. Using the “leaf.spec” R package [22,27], we developed an optimal Partial Least Squares (PLS) regression model with the calibration dataset. We began with a calibration model which included all samples, and then considered multiple calibration model subsets to evaluate different geographic regions and plant functional types using a variety of spectral preprocessing steps and specific spectral regions (Table 2).

The model with the lowest root mean squared error of prediction (RMSEP = 0.47; 19 latent vectors) was one in which spectra were first pre-processed using the second derivative and then restricted to between 4250-5450 cm^{-1} and 6100-7500 cm^{-1} (model 3 in Table 2), which corroborate the wavenumber regions used by Smis *et al.* [20]. In addition, the model was greatly improved by the removal of both bryophytes and samples from a single site (Mount Caroline, Australia), which each had unique spectral properties (see discussion in the next section). Therefore, when analyzing the final dataset, neither bryophytes nor predicted data from Mount Caroline were included. The final calibration model was validated on the test set and performed well, with validation $R^2 = 0.83$, and calibration $R^2 = 0.82$ (Fig. 2). The top three latent vectors alone provided 75 % of the explained variance, with consistently high loading values observed near 5252 cm^{-1} . Figure 3 provides a visual

summary, known as a loading plot, showing these top 3 latent vectors from the optimal calibration model.

3.4. NIRS model limitations

The NIRS spectrum of a plant depends on its complete phytochemical profile, and is likely to show different features depending upon how SiOH groups associate with organic and inorganic molecules present in the tissue matrix by dihydrogen bonds [28-31]. The unaccounted for variation in plant Si associated with our NIRS model likely results from the complex nature of our sampling regime. We not only analyzed a broad array of plant functional types, but additionally, plants were collected from a global range of climatic conditions (mean annual precipitation from 262 to 1898 mm/yr; mean annual temperature from -4.1 °C to 22.1 °C) and were subjected to both nutrient addition and herbivore exclusion. Combined, all of these factors likely resulted in extremely high phytochemical variation and functional type-specific plasticity beyond that which would be observed under natural conditions.

A previous study found that using unique, restrictive models provided optimal prediction of leaf Si, *i.e.*: single species > single functional type > many species [20]. However, even within a single closely-related group of plants, such as the grasses (family: *Poaceae*), silica particles embedded within plant tissue, termed “phytoliths”, occur in various states of hydration [32]. Furthermore, phytoliths are highly variable in their morphology depending upon deposition site [33], which may account for additional unexplained variance during NIRS modeling. Although models including all plant samples had the highest calibration and validation R² values (Table 2), it was clear after plotting known vs.

predicted values that large differences between functional types were inflating these values. For our dataset, removing bryophytes from the initial model significantly improved its predictive strength. A separate model may be needed for bryophytes, perhaps due to high concentrations of aromatic secondary compounds or other molecular traits unique to this group, which includes the earliest diverging land plants [34]. On the other hand, it can be observed in Fig. 2 that samples with very low Si concentrations tend to have the greatest unexplained variance with our NIRS model. Such effect may be explained by the measured values being near the ICP OES LOD for Si (0.05 % m/m), which would corroborate previous classification of legumes as silicon excluders [35]. Again, this functional type may be better predicted by a separate NIRS model, perhaps one that uses a more sensitive reference method such as ICP-MS. Similarly, one site had to be removed from the calibration dataset in order to improve model strength. Upon further investigation, we noticed that many samples from this site (Mount Caroline, Australia) had very pubescent leaves, which presumably resulted in large air pockets and high light scatter during NIRS scanning.

3.5. Silicon determination in forbs, legumes and graminoids

The NIRS method, using the most statistically robust model (model 3 in Table 2, Procedure S1), was applied to 875 plant samples: 360 forbs, 102 legumes, and 413 graminoids. Silicon levels in 275 of those samples were below the LOD. The other 600 samples presented Si concentrations in the 0.05-4.46, 0.07-1.00, and 0.11-4.67 % range for forbs (n = 179), legumes (n = 23), and graminoids (n = 398), respectively. Figure 4 illustrates the major difference in Si-accumulation between forbs and legumes vs. graminoids, and the light grey region represents overlapping Si concentrations between the different groups.

4. Conclusions

Alkaline digestion combined with ICP OES determination of Si may be a powerful strategy to calibrate NIRS models, with great potential to improve sample throughput and accuracy in plant Si estimation. Because the digestion method is relatively simple and requires the use of equipment available in most laboratories, it may be easily applied at institutions with limited research facilities. Because of reasons previously discussed, this approach is more accurate and precise than both colorimetric and gravimetric determinations.

As NIRS becomes more frequently used, global calibration models may be built, compiled, and readily modified for application to a particular dataset. NIRS is non-destructive and requires no reagents for sample preparation, and almost no training prior to instrument operation. Using NIRS to analyze a single batch of 48 samples, for example, can cut the analysis time in half when compared with alkaline digestion and ICP OES determination. The rapid determination of Si by NIRS is a useful alternative for global scale analyses of plants, and leaf chemistry in general. It is a simple, effective and relatively low-cost approach to large-sample studies with important implications for studies of food crops, livestock forage, ecological theory, and more.

Supplementary data

Data file developed in the R statistical programming environment to run the optimized model used in this work (NoBryos.RData), and instructions on how to use it (Procedure

S1). This material is available alongside the electronic version of this article at <http://www.sciencedirect.com>.

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References

- [1] K.H. Wedepohl, Composition of the continental crust, *Geochim. Cosmochim. Acta* 59 (1995) 1217-1232.
- [2] M.J. Hodson, P.J. White, A. Mead, M.R. Broadley, Phylogenetic variation in the silicon composition of plants, *Ann. Bot.* 96 (2005) 1027-1046.
- [3] J. Cooke, M.R. Leishman, Is plant ecology more siliceous than we realise?, *Trends Plant Sci.* 16 (2011) 61-68.
- [4] F.P. Massey, S.E. Hartley, Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates, *Proc. R. Soc. B Biol. Sci.* 273 (2006) 2299-2304.
- [5] O.L. Reynolds, M.G. Keeping, J.H. Meyer, Silicon-augmented resistance of plants to herbivorous insects: a review, *Ann. Appl. Biol.* 155 (2009) 171-18.
- [6] S. Hauptkorn, J. Pavel, H. Seltner, Determination of silicon in biological samples by ICP-OES after non-oxidative decomposition under alkaline conditions, *Fresenius. J. Anal. Chem.* 370 (2001) 246-250.
- [7] H.A. Currie, C.C. Perry, Silica in plants: biological, biochemical and chemical studies, *Ann. Bot.* 100 (2007) 1383-1389.
- [8] R.L. Fox, J.A. Silva, D.L. Plucknett, D.Y. Teranish, Soluble and total silicon in sugarcane, *Plant Soil* 30 (1969) 81-91.
- [9] C. L. Elliot, G. H. Snyder, Autoclave-induced digestion for the colorimetric determination of silicon in rice straw, *J. Agr. Food Chem.* 39 (1991) 1118-1119.

- [10] J.A. Nóbrega, G. L. Donati, Microwave-assisted sample preparation for spectrochemistry, in: R.A. Meyers (Ed.), Encyclopedia of Analytical Chemistry, Wiley, Chichester, 2011.
- [11] D.L. Rocha, A.D. Batista, F.R.P. Rocha, G.L. Donati, J.A. Nóbrega, Greening sample preparation in inorganic analysis, *Trend Anal. Chem.* 45 (2013) 79-92.
- [12] J. Wang, N. Topham, C.Y. Wu, Determination of silica coating efficiency on metal particles using multiple digestion methods, *Talanta* 85 (2011) 2655-2661.
- [13] X. Feng, S. Wu, A. Wharmby, A. Wittmeier, Microwave digestion of plant and grain standard reference materials in nitric and hydrofluoric acids for multi-elemental determination by inductively coupled plasma mass spectrometry, *J. Anal. At. Spectrom.* 14 (1999) 939-946.
- [14] J.E. Kraska and G.A. Breitenbeck, Simple, robust method for quantifying silicon in plant tissue, *Commun. Soil Sci. Plant Anal.* 41 (2010) 2075-2085.
- [15] J.A. Barros, P.F. de Souza, D. Schiavo, J.A. Nóbrega, Microwave-assisted digestion using diluted acid and base solutions for plant analysis by ICP OES, *J. Anal. At. Spectrom.* 31 (2016) 337-343.
- [16] A.P. Krushevska, A. Lasztity, M. Kotrebai, R.M. Barnes, Addition of tertiary amines in the semiquantitative, multi-element inductively coupled plasma mass spectrometric analysis of biological materials, *J. Anal. At. Spectrom.* 11 (1996) 343-352.
- [17] K. Van Dyck *et al.* Spectrometric determination of silicon in food and biological samples: an interlaboratory trial, *J. Anal. At. Spectrom.* 15 (2000) 735-741.
- [18] P.F. Souza, D. Santos Jr., G.G.A. Carvalho, L.C. Nunes, M.S. Gomes, M.B.B. Guerra, F.J. Krug, Determination of silicon in plant materials by laser-induced breakdown spectroscopy, *Spectrochim. Acta Part B* 83-84 (2013) 61-65.
- [19] Z.A. Ostatek-Boczynski, D.E. Purcell, E.C. Keeffe, W.N. Martens, M.G. O'Shea, Rapid determination of carbon, nitrogen, silicon, phosphorus, and potassium in sugar mill by-products, mill mud, and ash using near infrared spectroscopy, *Commun. Soil Sci. Plant Anal.* 44 (2013) 1156-1166.
- [20] A. Smis, F.J.A. Murguzur, E. Struyf, E.M. Soininen, J.G.H. Jurdado, P. Meire, K.A. Brathen, Determination of plant silicon content with near infrared reflectance spectroscopy, *Front. Plant Sci.* 5(496) (2014) 1-9.
- [21] J.A. Ramirez, J.M. Posada, I.T. Handa, G. Hoch, M. Vohland, C. Messier, B. Reu, Near-infrared spectroscopy (NIRS) predicts non-structural carbohydrate concentrations in different tissue types of a broad range of tree species, *Methods Ecol. Evol.* 6 (2015) 1018-1025.
- [22] R Development Core Team (2011), R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: <http://www.R-project.org>.

- [23] Nutrient Network: A Global Research Cooperative (2007), URL: <http://www.nutnet.umn.edu>.
- [24] R.W. Kennard, L.A. Stone, Computer aided design of experiments, *Technometrics* 11 (1969) 137-148.
- [25] R.D. Snee, Validation of regression models: methods and examples, *Technometrics* 19 (1977) 415-428.
- [26] A. Saptoro, M.O. Tade, H. Vuthaluru, A modified Kennard-Stone algorithm for optimal division of data for developing artificial neural network models, *Chem. Prod. Process Model.* 7 (2010) 1-14.
- [27] D.M. Griffith, T.M. Anderson, The 'leaf.spec' R package: a tool for spectral analysis of plant stoichiometry, In prep, *Methods in Ecology and Evolution*.
- [28] A.P. Dayanandan, P.B. Kaufman, C.I. Franklin, Detection of silica in plants, *Am. J. Bot.* 70 (1983) 1079-1084.
- [29] A. Inanaga, A. Okasaka, Calcium and silicon binding compounds in cell walls of rice shoots, *Soil Sci. Plant Nutr.* 41 (1995) 103-110.
- [30] C.C. Harrison, Evidence for intramineral macromolecules containing protein from plant silicas, *Phytochemistry* 41 (1996) 37-42.
- [31] C.C. Perry, T. Keeling-Tucker, Biosilicification: the role of the organic matrix in structure control, *J. Biol. Inorg. Chem.* 5 (2000) 537-550.
- [32] F. Bartoli, L. Wilding, Dissolution of biogenic opal as a function of its physical and chemical properties, *Soil Sci. Soc. Am.* 4 (1980) 873-878.
- [33] D.R. Piperno, D.M. Pearsall, The silica bodies of tropical american grasses: morphology, taxonomy, and implications for grass systematics and fossil phytolith identification, *Smithsonian Contrib. to Bot.* 85 (1998) 1-40.
- [34] C.F. Xie, H.X. Lou, Secondary metabolites in bryophytes: an ecological aspect, *Chem. Biodivers.* 6 (2009) 303-312.
- [35] L.H.P. Jones, K.A. Handreck, Uptake of silica by *Trifolium incarnatum* in relation to the concentration in the external solution and to transpiration, *Plant Soil* 1 (1969) 71-80.

Table 1. ICP OES operating conditions.

Instrumental parameter	Operating condition
Radio frequency (RF) applied power (kW)	1.30
Silicon analytical wavelength (nm)	251.611
Sample flow rate (mL/min)	0.60
Plasma gas flow rate (L/min)	18.0
Nebulizer pressure (psi)	30
Torch view	Axial
Integration time (s)	30
Replicates per sample	3

Table 2. Comparison of various NIRS models.

Model components ^a	n	Calibration R ²	Validation R ²	RMSEP ^b	Pre-processing regions (cm ⁻¹)	Pre-processing method ^c
1	345	0.88	0.92	0.63	4600-6100	D1f, SNV
2	308	0.88	0.92	0.65	4250-4600, 5450-9400	MMN
3	282	0.82	0.83	0.47	4250-5450, 6100-7500	D2f
4	145	0.90	0.77	0.56	4600-5450, 6100-7500	D1f

^a(1) All plant functional types, all collection sites; (2) All plant functional types, Mount Carolina samples excluded; (3) No bryophytes, Mount Carolina samples excluded; (4) Grasses only, Mount Carolina samples excluded.

^bRoot mean squared error of prediction.

^cD1f = first derivative; SNV = vector normalization; MMN = min / max normalization; D2f = second derivative.

Figure Captions

Figure 1. Predicted values for loose vs. “pelleted” samples obtained using the optimal NIRS model ($R^2 = 0.97$). Pelleted samples were compacted in 33 mm diameter aluminum caps to 5 mm depth with a hydraulic press, using 11 tons of pressure for 2 s. Dashed line represents a 1:1 slope.

Figure 2. Measured (ICP OES) vs. predicted (NIRS) Si concentration of plant samples used to build the calibration model ($n = 346$; validation $R^2 = 0.83$, calibration $R^2 = 0.82$).

Figure 3. Loading value plot of the top 3 latent vectors used in the NIRS calibration model. Flat line at 0 represent regions which were removed during pre-processing. Latent vectors had consistently high loading values near 5252 cm^{-1} .

Figure 4. Silicon concentration (% m/m) in plants determined by NIRS using model 3 in Table 2. The histogram illustrates major differences in Si accumulation for distinct plant functional types. Light grey regions represents overlapping concentrations between the different groups.

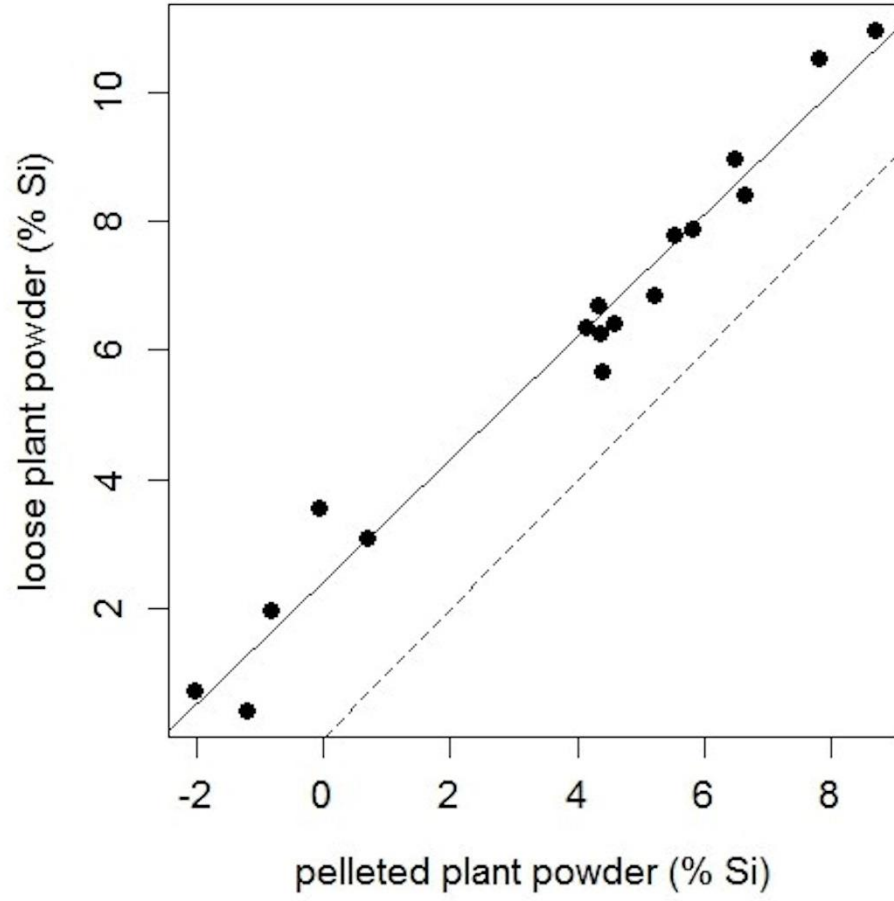


Fig. 1

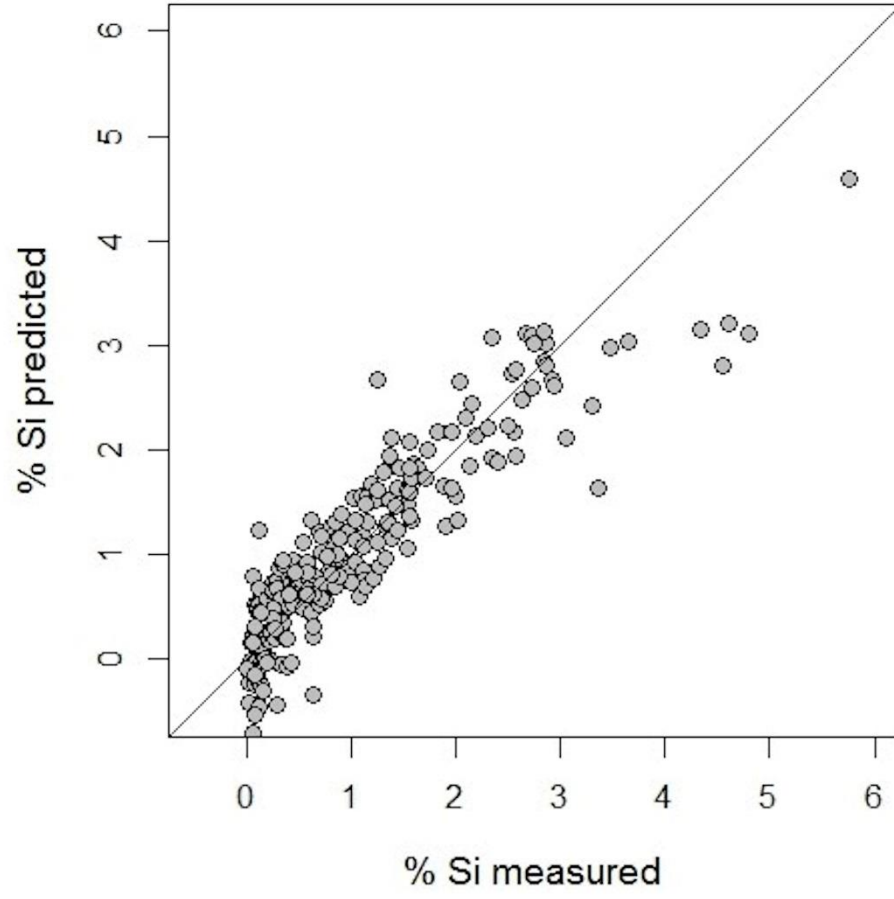


Fig. 2

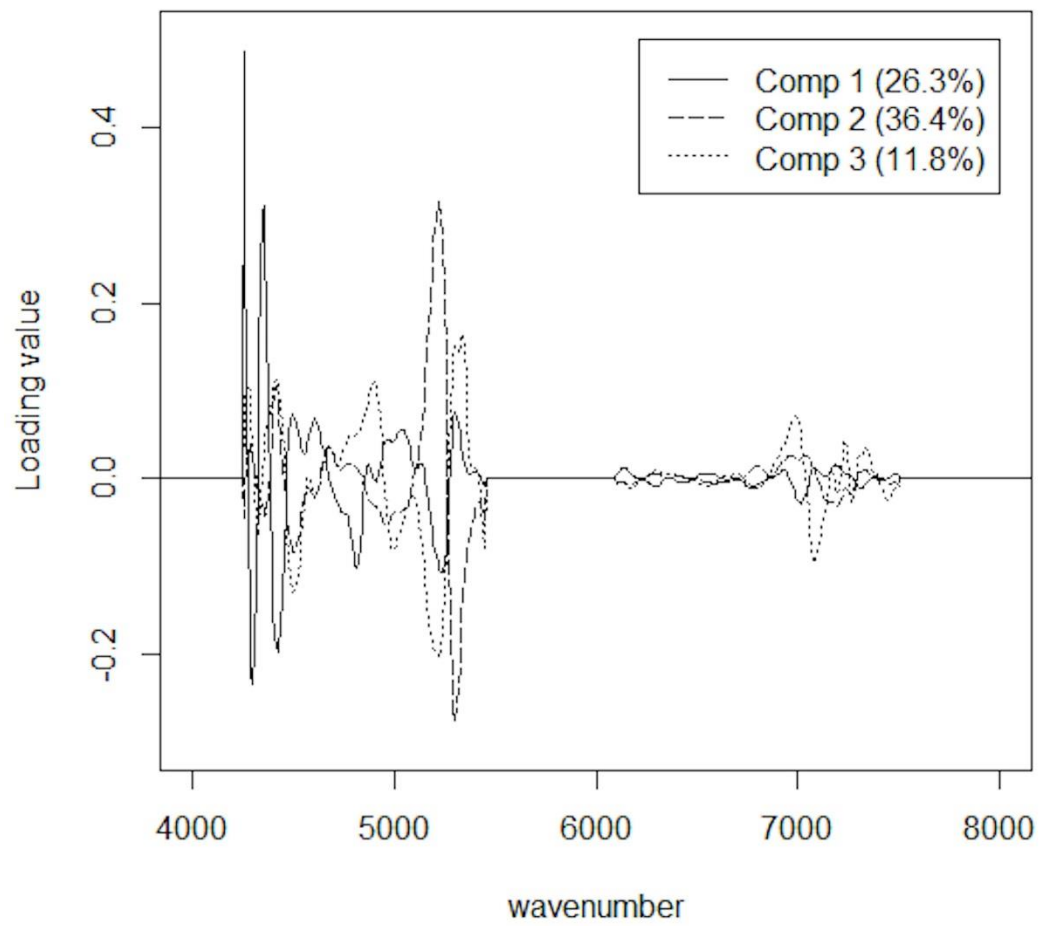


Fig. 3

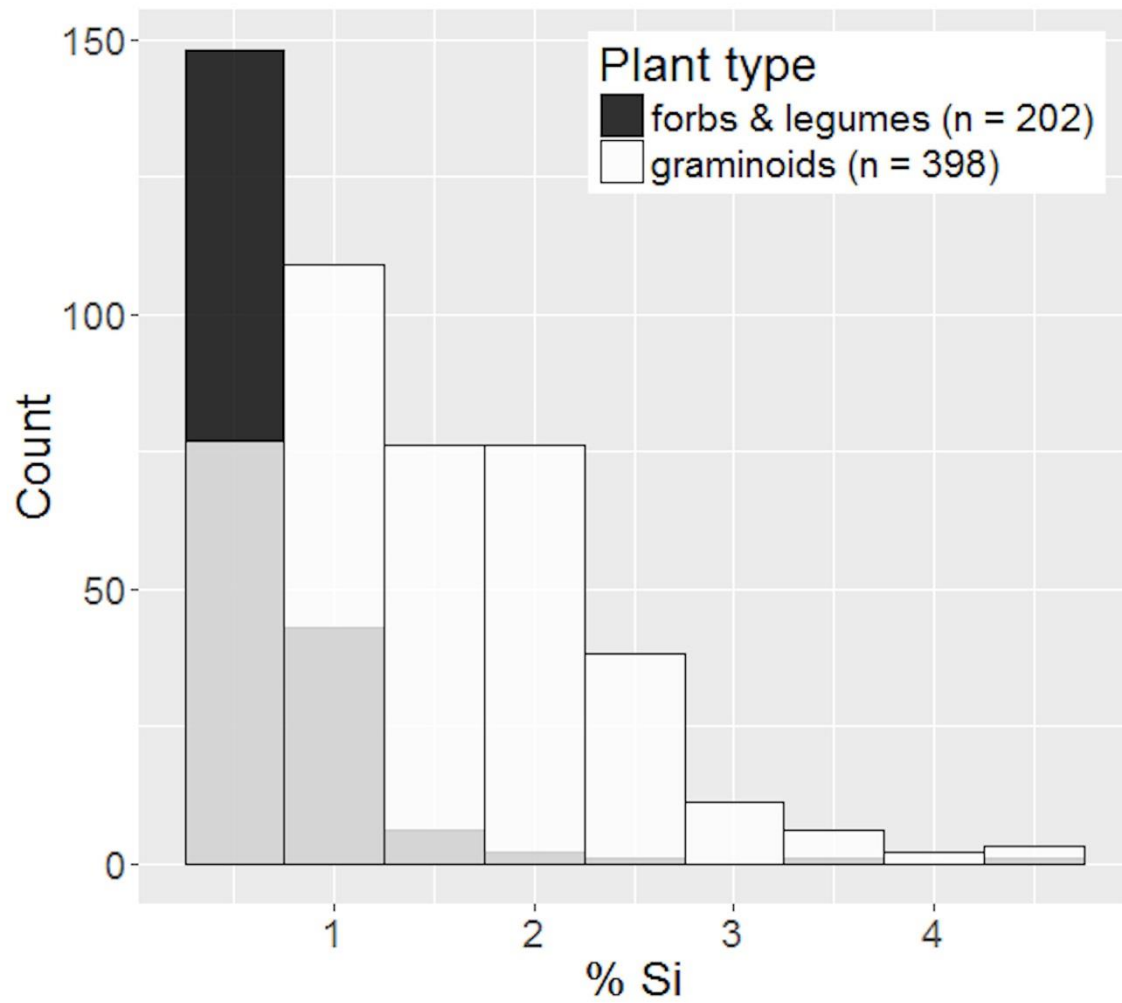


Fig. 4

CURRICULUM VITAE

EDUCATION

Wake Forest University PhD candidate, Biology	Winston-Salem, NC Jan 2011- 2016 (expected)
Monmouth College Bachelor of Arts, Biology (chemistry minor)	Monmouth, IL 2005-2009
University of Dar es Salaam Ecology and Human Origins	Dar es Salaam, Tanzania 2007

RESEARCH APPOINTMENTS & WORK EXPERIENCE

Wake Forest University <i>Graduate Researcher, Dr. T. Michael Anderson</i> <ul style="list-style-type: none">PhD dissertation on the coevolution of plant-herbivore interactions in Serengeti, with an emphasis on soil-plant-herbivore cycling of silicon	Winston-Salem, NC 2011-2016
North Carolina State University <i>Laboratory Manager, Dr. R. Brian Langerhans</i> <ul style="list-style-type: none">Created database of fish specimens, extracted & amplified DNA for analysis	Raleigh, NC 2010
University of Oklahoma Biological Station <i>Laboratory Technician and field assistant, Dr. R. Brian Langerhans</i> <ul style="list-style-type: none">Same duties as above plus field work collecting fish in Bahamian tidal creeks	Norman, OK 2009-2010
Monmouth College <i>Undergraduate Researcher, Dr. Laura Moore</i> <ul style="list-style-type: none">Transformed extremophile genes into <i>E. coli</i> and monitored cultures w/ UV spec.	Monmouth, IL 2008-2009
Northwestern University <i>Laboratory Assistant, Dr. Francesca McInerney</i> <ul style="list-style-type: none">Prepared fossil soils for $\delta^{13}\text{C}$ isotope analysis	Evanston, IL 2008
Chicago Botanic Garden <i>National Science Foundation REU Intern, Douglas Lynch and Dr. Francesca McInerney</i> <ul style="list-style-type: none">Investigated correlation between light intensity and leaf anatomyCollected plant fossils in Wyoming badlands from the Paleocene-Eocene boundary	Glencoe, IL 2008
University of Dar es Salaam <i>Undergraduate Researcher, ACM Program</i> <ul style="list-style-type: none">Studied spatial patterns of elephant damage to baobab trees, in Tarangire N.P.Assisted with research projects on animal behavior, ecology, and anthropology	Dar es Salaam, Tanzania 2007

PUBLICATIONS

4. **Quigley KM**, GL Donati, and TM Anderson. Variation in the soil 'silicon landscape' explains plant silica accumulation across an environmental gradient in Serengeti. *Plant and Soil*, In press.
3. **Quigley KM**, AG Althoff, and GL Donati. 2016. Inductively coupled plasma optical emission spectrometry as a reference method for silicon estimation by near infrared spectroscopy and potential application to global-scale studies of plant chemistry. *Microchemical Journal* 129:231-235.

2. Griffith DM, **KM Quigley**, and TM Anderson. 2016. Leaf thickness controls variation in leaf mass per area (LMA) among grazing-adapted grasses in Serengeti. *Oecologia* 181: 1035-1040.

1. **Quigley KM** and TM Anderson. 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: 1–10.

ACADEMIC AWARDS

WFU Vecellio Award (\$1224)	2015
CSU Summer Soil Institute tuition scholarship (\$500)	2015
Decagon G.A. Harris Instrumentation Fellowship (\$5000)	2014
WFU Vecellio Award (\$1275)	2014
WFU Richter fellowship (\$5000)	2013
Elton Cocke travel award (\$200)	2013
WFU Tuttle-Newhall Award (\$500)	2012
Monmouth College Biology Department Award	2009
Tri-Beta Outstanding Scholarship Award	2009
Frank G. Brooks Award for Excellence in Oral Presentation of Research	2009
President, Tri-Beta National Biology Honor Society, Monmouth College	2009

TEACHING & OUTREACH

Wake Forest University (Winston-Salem, NC)	
Graduate Teaching Assistant, Ecology and Evolution	2013-2014
Graduate Teaching Assistant, Biology and the Human Condition	2011-2012, 2015-2016
Monmouth College (Monmouth, IL)	
Teaching Assistant, Introduction to Ecology, Evolution, and Behavior Lab	2009
Teaching Assistant, Investigating Biological Concepts Lab and Cultural Chemistry Lab	2008
Community outreach	
Ken Carlson Boys and Girls Club (Winston-Salem, NC) Garden Club volunteer	2013-2015
WFU genetics outreach at Winston-Salem public middle and high schools	2012-2015
Hanes Magnet School (Winston-Salem, NC) volunteer science fair judge	2013

PROFESSIONAL DEVELOPMENT

Workshops & Training

Colorado State University Summer Soil Institute, Fort Collins CO	2015
The Georgia R School: Introduction to R for Statistical Analysis I & II	2011

Presentations

Southeastern Ecology and Evolution (SEEC) conference	Orlando, FL
<i>Stress-induced silicon uptake in Serengeti grasses and associated physiological costs</i>	2013
ACM Off-Campus Study Symposium	Chicago, IL
<i>Tembo na Mibuyu: An Investigation of Spatial Utilization Patterns Using GIS</i>	2009
Tri-Beta District NC-1 Convention	Romeoville, IL
<i>Spatial Analysis of Adansonia digitata Utilization by Loxodonta Africana in Tarangire, Tanzania</i>	2009
Chicago Botanic Garden NSF-REU Poster Symposium	Glencoe, IL
<i>Bundle Sheath Extension Density along a Light Gradient</i>	2008

Journal Reviewer

Acta Physiologica Plantarum, Functional Ecology, The Science of Nature, Plant and Soil