

Beneficial effects and intraspecific variation of silicon in wild dicots

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Table of Contents

1 Abstract.....	1
2 Zusammenfassung.....	2
I Synopsis.....	5
1 Introduction.....	5
1.1 Motivation.....	5
1.2 State of the art and objectives.....	7
2 Materials and methods.....	16
2.1 Experimental designs and statistical analyses.....	16
2.2 Species selection and plant material.....	18
2.3 Experimental substrates.....	19
2.4 Chemical analyses.....	19
3 Results and discussion.....	20
3.1 Plant growth and nutrient status.....	21
3.2 Plant defence.....	26
3.3 Drivers of intraspecific variation in tissue Si concentrations.....	30
3.4 Generalizing effects of Si across species and environments.....	33
3.5 Conclusions.....	36
4 References.....	38
II Manuscript 1.....	50
Abstract.....	51
1 Introduction.....	51
2 Materials and methods.....	54
2.1 Study site and overview.....	54
2.2 Study species and plant material.....	54
2.3 Experiment.....	55
2.4 Chemical analysis of soils and leaves.....	56
2.5 Statistical analysis.....	57
3 Results.....	58
4 Discussion.....	61
4.1 Species-specific foliar Si responses to plant-available Si.....	61
4.2 Effects of Si on seedling growth of tropical tree species.....	62
4.3 Inconsistent and contrasting effects of Si on foliar P concentrations and stoichiometry.....	64
4.4 No indication of interactions between Si and C in most species.....	65
4.5 Ecological implications.....	65
5 Acknowledgements.....	65
6 References.....	66
7 Supporting information.....	71
III Manuscript 2.....	75
Abstract.....	76
1 Introduction.....	76

2 Materials and methods.....	78
2.1 Study species and plant material.....	78
2.2 Si treatment.....	79
2.3 Simulation of herbivory.....	79
2.4 Generalist herbivore.....	80
2.5 Feeding trials.....	80
2.6 Si analysis of leaves and soil.....	81
2.7 Statistical analyses.....	81
3 Results.....	83
3.1 Effects of soil Si and simulated herbivory on leaf damage.....	83
3.2 Effects of soil Si and simulated herbivory on leaf Si concentrations.....	84
3.3 Effect of leaf Si concentration on leaf damage.....	85
4 Discussion.....	86
4.1 Si-based defenses in tropical seedlings.....	86
4.2 Si-based defenses were not inducible.....	88
5 Ecological implications.....	90
6 Acknowledgements.....	90
7 References.....	91
8 Supporting information.....	95
IV Manuscript 3	97
Abstract.....	98
1 Introduction.....	98
2 Materials and methods.....	102
2.1 Experimental set-up.....	102
2.2 Study species.....	103
2.3 Chemical analysis.....	104
2.4 Statistics.....	104
3 Results.....	106
3.1 Differences of Si and N concentrations between life-forms and across species.....	106
3.2 Effect of water regimes on Si concentrations.....	107
3.3 Effect of water regimes on N concentrations.....	109
4 Discussion.....	110
5 Conclusions.....	114
6 Acknowledgements.....	114
7 References.....	115
8 Supporting information.....	119
V Author contributions	122
VI Full publication list	124
VII Acknowledgements	125
VIII Eidesstattliche Versicherung und Erklärung	126

1 Abstract

Silicon (Si) is a major constituent of the earth's crust and can be found in almost all plant species. Plants take up Si from the soil solution as mono-silicic acid, which accumulates forming amorphous Si deposits in plant organs, especially in leaves. Si is a non-essential, but beneficial element for plants and Si accumulation can improve growth, herbivory defence and leaf nutrient status in crops and wild grasses. Furthermore, plant-available soil Si concentrations, herbivory damage and soil moisture influence tissue Si concentrations, potentially modulating the beneficial effects of Si on plants.

Tropical forests and temperate grasslands show large spatial and/or temporal variation in plant-available soil Si concentrations, soil moisture and herbivory pressure, suggesting Si might play an important, but so far underestimated, ecological role in these ecosystems. Yet, beneficial effects of Si on growth, defence and nutrient status, the effects of environmental factors on intraspecific variation in tissue Si and how these effects vary across species have not yet been studied in wild dicots, such as tropical trees and temperate forbs. Dicots make up a marked proportion of the plant species composition of tropical forests and temperate grasslands, which together make up a large proportion of the earth's terrestrial area, harbour great biodiversity and offer essential ecosystem services. Thus, beneficial effects and intraspecific variation of Si in wild dicots might have consequences for ecological processes and ecosystem function and services.

To test the effect of Si on plant growth, leaf macronutrient concentrations and stoichiometry as well as leaf Si concentrations a Si fertilization experiment, including 14 different levels of soil Si availability and seedlings of 12 tropical tree species, was undertaken. Furthermore, we tested whether increasing plant-available soil Si increased soil phosphorus (P) availability. The effect of Si on herbivory defence of tropical tree seedlings and the effect of herbivory damage on leaf Si concentrations was assessed in a full-factorial experiment, including a Si fertilization and herbivory simulation treatment, followed by multiple-choice feeding trials with a generalist herbivore. The effect of soil moisture on tissue Si concentrations of 10 temperate herbaceous species, including five forb and grass species, was assessed in a mesocosm experiment in which plants were exposed to four different soil moisture regimes.

Increasing plant-available Si improved growth, herbivory defence or changed leaf nutrient status in about 60% of the tropical tree species we studied. The data presented here is the first showing beneficial effects of Si on plant growth and defence in wild tropical tree species and wild dicots in general. Specifically, three out of the 12 species showed increases in aboveground biomass with increasing plant-available soil Si (by up to 220%). As for herbivory defence, three out of the eight species showed lower leaf damage in seedlings grown on Si-rich compared to Si-poor soil (by up to 72%). In one species increased leaf and soil Si led to higher leaf damage, indicating Si increased the leaves' attractiveness to the herbivore. In terms of leaf

nutrient status, leaf P concentrations increased and decreased in two species. Leaf carbon (C) and nitrogen (N) concentrations decreased with increasing plant-available Si in up to four species. Leaf C:N ratios increased in two and leaf C:P and N:P ratios changed in two species, yet with opposite direction. Taken together, only a subset of two to four species responded to increasing soil Si availability in each leaf nutrient status parameter we measured. The strength of the effects of Si on plant growth, herbivory defence and leaf nutrient status varied across species.

Plant-available Si concentrations and soil moisture influenced tissue Si concentrations in most tropical and temperate species, respectively. Simulated herbivory had no effect on leaf Si concentrations in tropical seedlings. The direction of the effect of plant-available Si on leaf Si was linear in all species and the strength of the effect varied across species, with stronger responses in species with a higher Si uptake capacity. For soil moisture, highly species-specific effects on plant Si concentrations emerged for forbs, while the effects in grasses were uniform.

The findings suggest that variation in plant-available Si across habitats may differently influence species' performance, either directly through effects of soil Si on plant growth or indirectly through effects of soil and/or leaf Si on plant defence. Temporal and spatial variation in soil moisture may additionally influence tissue Si concentrations, and thus herbivory defence. Furthermore, Si-induced changes in leaf nutrient ratios, important drivers of litter decomposition and nutrient release, might influence nutrient availability and also plant performance. Overall, species-specific effects of variation in plant-available Si on plant growth, defence and leaf nutrient status could lead to differences in species performance ranks across habitats varying in plant-available Si and influence species distribution and community composition. Thus, the role of Si in ecological processes and patterns as well as ecosystem function deserves more attention in future studies.

2 Zusammenfassung

Silizium (Si) ist einer der Hauptbestandteile der Erdkruste und kommt in fast allen Pflanzen vor. Pflanzen nehmen Si als Kieselsäure aus der Bodenlösung auf. Die Kieselsäure akkumuliert und bildet amorphe Ablagerungen in den Blättern. Si ist nicht-essentiell, hat aber positive Effekte auf Pflanzen. In Nutzpflanzen und wilden Gräsern kann Si das Wachstum, die Herbivorenabwehr und den Nährstoffhaushalt verbessern. Darüber hinaus, werden Si-Konzentrationen in den Pflanzen von Si-Konzentrationen im Boden, Beschädigungen durch Herbivore und den Bodenwassergehalt beeinflusst, was wiederum Einfluss auf die positiven Effekte von Si haben könnte.

Tropische Wälder und temperate Grasländer zeigen große räumliche und/oder zeitliche Variationen in der Pflanzenverfügbarkeit von Si, Bodenwassergehalte und Herbivorie, was

darauf hindeutet, dass Si eine wichtige ökologische Rolle in diesen Ökosystemen spielen könnte. Allerdings wurden die nützlichen Effekte von Si auf Wachstum, Herbivorenabwehr und Nährstoffhaushalt, sowie die innerartliche Variation von Si-Konzentrationen und die diesbezüglichen Unterschiede zwischen den Arten noch nicht in wilden zweikeimblättrigen Pflanzen, d.h. Dikotyle, untersucht. Dikotyle tragen zu einem großen Teil der Artenzusammensetzung von tropischen Wäldern und temperaten Grasländern bei. Diese beiden Ökosysteme, wiederum, decken einen großen Teil der terrestrischen Fläche der Erde ab, haben große Biodiversität und liefern wichtige Ökosystemdienstleistungen. Von daher sollten potentielle nützliche Effekte von Si für wilde Dikotyle und die innerartliche Variation von Si-Konzentrationen wichtige Konsequenzen für ökologische Prozesse und Ökosystemfunktionen haben.

Um den Effekt von pflanzen-verfügbarem Si auf das Wachstum und den Nährstoffhaushalt sowie die Blatt-Si-Konzentrationen zu testen, wurde ein Düngungsexperiment mit 14 Boden-Si-Level und 12 tropischen Baumarten durchgeführt. Außerdem wurde untersucht ob eine erhöhte Si-Verfügbarkeit die Verfügbarkeit von Phosphor (P) erhöht. Der Effekt von Si auf die Herbivorenabwehr in tropischen Baumarten und der Effekt von Herbivorieschäden auf die Si-Konzentrationen wurde in einem voll-faktoriellen Experiment, das Behandlungen mit Si-Dünger und simulierter Herbivorie beinhaltete, und einem darauffolgendem „multiple-choice“ Fütterungsversuch mit einer Herbivorenart getestet. Der Effekt vom Bodenwassergehalt auf Si-Konzentrationen in 10 temperaten krautigen Arten, darunter fünf Kräuter und fünf Gräser, wurde in einem Mesokosmen-Experiment mit vier Bodenwasserbehandlungen getestet.

Erhöhte Si-Verfügbarkeit im Boden verbesserte das Wachstum, die Herbivorenabwehr oder veränderte den Nährstoffstatus der Blätter in ungefähr 60% der untersuchten tropischen Baumarten. Die hier präsentierten Daten sind die ersten, die positive Auswirkungen von Si auf das Wachstum und die Herbivorenabwehr wilder tropischer Baumarten und wilder Dikotyle im allgemeinen zeigen. Konkret produzierten drei der zwölf Arten mehr oberirdische Biomasse mit zunehmender Si Verfügbarkeit (Anstieg um bis zu 220 %). Was die Herbivorenabwehr anbelangt, so zeigten drei der acht Arten bei Sämlingen, die auf Si-reichen Böden wuchsen, geringere Blattschäden als auf Si-armen Böden (Rückgang um bis zu 72 %). Bei einer Art führte eine erhöhte Si-Konzentration in Blättern und Böden zu mehr Blattschäden, was darauf hindeutet, dass Si die Attraktivität der Blätter für den Herbivor steigerte. Im Hinblick auf den Blattnährstoffstatus nahmen die Blatt-P-Konzentrationen bei zwei Arten zu beziehungsweise ab. Die Konzentrationen von Kohlenstoff (C) und Stickstoff (N) in den Blättern nahmen mit zunehmender Si-Verfügbarkeit bei jeweils bis zu vier Arten ab. Bei zwei Arten stiegen die Blatt-C:N-Verhältnisse und bei zwei Arten veränderten sich die Blatt-C:P- und N:P-Verhältnisse in entgegengesetzter Richtung. Insgesamt reagierten jeweils nur zwei bis vier Arten auf die zunehmende Si-Verfügbarkeit im Boden in jedem von uns gemessenen Blattnährstoffstatusparameter. Die Stärke der Auswirkungen von Si auf Wachstum, Herbivorenabwehr und den Nährstoffstatus variierte je nach Art.

Erhöhte Si-Verfügbarkeit im Boden und Veränderungen in der Bodenfeuchtigkeit beeinflussten die Si-Konzentrationen in den meisten tropischen Baumarten beziehungsweise gemäßigten krautigen Arten. Simulierte Herbivorie hatten keinen Einfluss auf die Blatt-Si-Konzentrationen tropischer Baumsämlinge. Der Zusammenhang zwischen Si-Verfügbarkeit im Boden und Blatt-Si-Konzentrationen war bei allen Arten linear. Die Stärke des Effekts variierte zwischen den Arten, wobei bei Arten mit einer höheren Si-Aufnahmekapazität stärkere Reaktionen auftraten. Für die Bodenfeuchtigkeit zeigten sich bei Kräutern sehr artspezifische Auswirkungen auf die pflanzlichen Si-Konzentrationen, während die Auswirkungen bei Gräsern einheitlich waren.

Die Ergebnisse deuten darauf hin, dass die Variation der Si-Verfügbarkeit im Boden in verschiedenen Lebensräumen die Leistung der Arten unterschiedlich beeinflussen kann, entweder direkt durch Auswirkungen von Boden-Si auf das Wachstum oder indirekt durch Auswirkungen von Boden- und/oder Blatt-Si auf die Herbivorenabwehr. Zeitliche und räumliche Schwankungen der Bodenfeuchtigkeit könnten die Si-Konzentration im Gewebe und damit bei einigen Arten die Herbivorenabwehr zusätzlich beeinflussen. Darüber hinaus könnten durch Si hervorgerufene Veränderungen im Blattnährstoffverhältnis, ein wichtiger Faktor in der Streuzersetzung und Nährstofffreisetzung, die Nährstoffverfügbarkeit und damit auch die Performanz von Pflanzen beeinflussen. Insgesamt könnten artspezifische Auswirkungen der Variation der Si-Verfügbarkeit im Boden auf Pflanzenwachstum, Herbivorenabwehr und Blattnährstoffstatus zu Unterschieden der Artenleistungsränge in Lebensräumen mit unterschiedlicher Si-Verfügbarkeit führen und die Artenverteilung und Artzusammensetzung ganzer Pflanzengemeinschaften beeinflussen. Daher verdient der Einfluss von Si auf ökologische Prozesse und Muster sowie auf Ökosystemfunktionen mehr Aufmerksamkeit in zukünftigen Studien.

I Synopsis

1 Introduction

1.1 Motivation

Traits are measurable morphological, biochemical, physiological and phenological characteristics of plants (Violle et al. 2007). Trait values vary across species and can show pronounced variation within species (Mommer et al. 2006, Albert et al. 2010, Kazakou et al. 2014, Mayor et al. 2014). This intraspecific trait variation is caused, among others, by genetic and environmental factors (Callaway et al. 2003, Albert et al. 2010, Elser et al. 2010, Mayor et al. 2014) and can influence plant performance (e.g. Joshi et al. 2001). Furthermore, intraspecific trait variation can vary across species and this might impact ecological patterns and processes, including plant species distribution, community composition and other trophic levels, such as decomposers and herbivores (Koricheva et al. 1998, Callaway et al. 2003, Cornwell et al. 2008, Cornwell and Ackerly 2009). Out of all traits, leaf chemical traits probably vary the most within species (Kazakou et al. 2014, Siefert et al. 2015). Especially plant silicon (Si) concentrations can show wide intraspecific variation covering several orders of magnitude and being similar to or even exceeding the variation in macronutrient concentrations (Epstein 1999, Han et al. 2011, Guntzer et al. 2012). Yet, our understanding of the environmental drivers of intraspecific variation in tissue Si concentrations and its consequences for plant growth, herbivory defence and nutrient status is largely limited for wild plant species.

Si makes up a substantial proportion of the earth's crust, mostly in the form of silicate minerals (Wedepohl 1995). These minerals are being constantly weathered enriching soils with different forms of Si, such as amorphous Si particles and plant-available, dissolved mono-silicic acid (Conley 2002, Street-Perrott and Barker 2008, Schaller et al. 2021a). Substantial amounts of dissolved mono-silicic acid either run off into rivers feeding the oceanic Si cycle or remain in the terrestrial Si cycle (Conley 2002). Vegetation is an important component of the terrestrial Si cycle as Si is fixed by plants as amorphous Si and recycled after litter-fall through dissolution and subsequent plant (re-)uptake of dissolved mono-silicic acid (Conley 2002, Street-Perrott and Barker 2008, Schaller et al. 2021a). In fact, most of the plant-available soil Si stems from recycled biogenic Si rather than silicate minerals (Street-Perrott and Barker 2008, Schaller et al. 2021a). All in all, Si is very much ubiquitous in the ecosphere and found in almost all marine and terrestrial organisms to some degree (Perry 2003). Diatoms, sponges, and early land plants like liverworts, ferns and horsetails and later on angiosperms, including dicots and monocots, evolved the ability to take up Si (Street-Perrott and Barker 2008, Trembath-Reichert et al. 2015). Monocots, such as grasses, typically accumulate higher amounts of Si than dicots, such as most forbs and trees (Hodson et al. 2005). Tissue Si concentrations range from ca. 0.1 mg g⁻¹ to 100 mg g⁻¹ on a dry weight basis across species (Epstein 1999, Hodson et al. 2005).

Typically, Si accumulated in plants is bound in amorphous deposits (Raven 1983). While Si is essential to diatoms, it is thought to be a non-essential but beneficial element for plants (Epstein 1999).

In plants, Si can alleviate many stresses including herbivory, nutrient limitation, pathogens, drought, salinity, metal toxicity, UV and mechanical stress induced by water currents or wind (reviewed in Guntzer et al. 2012, Cooke and Leishman 2016, Schoelynck and Struyf 2016, Frew et al. 2018, Greger et al. 2018, Singh et al. 2020 and Thorne et al. 2020). Most research into the beneficial effects of Si has been done on cultivated grass species (including crops and forage species) and some cultivated dicot and wild aquatic species (Schoelynck et al. 2010, Cooke and Leishman 2011a, 2016, Katz 2014). The bias towards grasses is not surprising considering their major contribution to global food production and their high ability to take up Si, with some species reaching tissue concentrations of up to 10% on a dry weight basis (e.g. rice, sugar cane and wheat; Hodson et al. 2005, Guntzer et al. 2012). As a consequence many crops in Asia and America are being fertilized with Si-rich compounds (Guntzer et al. 2012). Besides agricultural systems, the role of Si in natural systems has also been gaining more attention, especially during the last decade (reviewed in Cooke et al. 2016), and beneficial effects of Si on plant growth, defence and nutrient status and the environmental factors driving intraspecific variation in tissue Si concentrations have been studied in a number of wild grass species (e.g. McNaughton et al. 1985, Massey et al. 2006, Xu et al. 2020, Hao et al. 2022).

For wild dicot species, however, almost no experimental data on beneficial effects of Si and the factors driving intraspecific variation in tissue Si concentrations are available. Especially species of tropical forests and temperate grasslands (i.e. azonal/extrazonal and semi-natural temperate grasslands, Dengler et al. 2014) have received little to no attention (but see Gómez-Vera et al. 2021 and Parecido et al. 2022 for work on cocoa and coffee cultivars). This is surprising, because Si accumulation in these species can be substantial and similar to grasses (Schaller et al. 2018, Ishizawa et al. 2019, Nakamura et al. 2019, Song et al. 2020, Klotz et al. 2021) and stresses alleviated by Si, such as nutrient limitation, herbivory and drought, have pervasive negative effects on plant performance in tropical forests and temperate grasslands (Engelbrecht and Kursar 2003, Eichhorn et al. 2010, Vitousek et al. 2010, Wright 2019). Furthermore, mean tissue Si concentrations vary up to 5000- and 70-fold across tropical and temperate dicot species, respectively (Schaller et al. 2018, Klotz et al. 2021), suggesting species should differ in how much their growth, defence and nutrient status should benefit from Si.

Tropical forests and temperate grassland exhibit pronounced spatial variation in soil Si availability at local- and regional-scale (Schaller et al. 2018, Ishizawa et al. 2019, Nakamura et al. 2019, Caubet et al. 2020, Rausch 2021, Ried 2022), potentially influencing intraspecific variation in tissue Si (Klotz et al. 2021). For instance, a 27-fold variation in plant-available soil

Si was found across tropical lowland forests of central Panama (Schaller et al. 2018) and a 3-fold variation within a 2 ha plot in Malaysia (Ishizawa et al. 2019). Similar values have been reported for temperate grasslands of central Europe (Caubet et al. 2020, Rausch 2021, Ried 2022). Besides plant-available Si, soil moisture and herbivory damage can drive intraspecific variation in tissue Si (Soininen et al. 2013, Quigley and Anderson 2014, Brightly et al. 2020). As tropical forests and temperate grasslands also exhibit strong temporal and/or spatial variation in soil moisture and herbivory (Comita and Engelbrecht 2014, Silvertown et al. 2015, Alberti et al. 2017, Weissflog et al. 2018), both factors might additionally influence intraspecific variation in tissue Si concentration in wild dicots, potentially affecting their growth, defence and nutrient status. This could have repercussions for other ecological processes, including performance ranks and distributions of plant species as well as their interactions with herbivores and decomposer. Overall, the ecological importance of Si for wild dicots might have been largely underestimated and, therefore, a deeper understanding of the environmental drivers of intraspecific tissue Si variation and beneficial effects of Si is needed for this group of species.

Tropical forests and temperate grasslands together make up about 20% of the earths' terrestrial area (Dinerstein et al. 2017), harbour great biodiversity (Dengler et al. 2014, Brancalion et al. 2019) and offer essential ecosystem services (Gibson 2009, Lewis et al. 2015). Furthermore, both biomes play a central role in mitigating the projected impacts of climate change (IPCC 2021), e.g. by carbon sequestration and atmospheric cooling (Lewis et al. 2015, Petermann and Buzhdygan 2021). Since dicots make up a marked proportion of the plant species composition of tropical forests and temperate grasslands (Knight 1975, Gibson 2009), intraspecific variation in tissue Si concentrations and beneficial effects of Si in this group of species might have consequences for ecosystem function and services, including the mitigation of climate change impacts.

To better understand the ecological importance of Si for wild dicots and potentially plant communities and whole ecosystems my thesis addresses the following research questions: **(i)** Does Si improve plant growth, herbivory defence and leaf (macro)nutrient status in tropical tree species? **(ii)** How does plant-available soil Si, soil moisture and herbivory influence tissue Si concentrations in species of temperate grasslands and/or tropical forests? **(iii)** How do the effects of Si on plant growth, herbivory defence and leaf (macro)nutrient status as well as the effects of plant-available soil Si, soil moisture and herbivory damage on tissue Si concentrations vary across species?

1.2 State of the art and objectives

In this section I present the existing knowledge about the effect of Si on herbivory defence, plant growth and leaf (macro)nutrient status in wild temperate and tropical grasses (including

economically important pasture species). Furthermore, I introduce the hypotheses (Figure 1a) tackled in the three manuscripts of my thesis.

1.2.1 Effect of Si on herbivory defence

Grasses have been the major focus of plant physiological and agricultural Si research (Cooke and Leishman 2011a, Katz 2014). Not surprisingly, most of our ecological knowledge about Si in wild plants is coming from studies on temperate and tropical grasses. In this group of species the effects of Si on herbivory defence have been studied the most, while the effects of Si on plant growth and nutrient status received relatively little attention.

Seminal work by McNaughton et al. (1985) on savannah grasses demonstrated that Si uptake can be induced by herbivory damage and that Si has the potential to deter mammalian herbivores. A little later Gali-Muhtasib et al. (1992) provided the first experimental evidence that constitutive Si-based defences can effectively fend off herbivores in savannah grasses. Two decades later constitutive and induced Si-based defences against small mammals and insects were also reported for a number of temperate grasses, with the defensive effect of Si

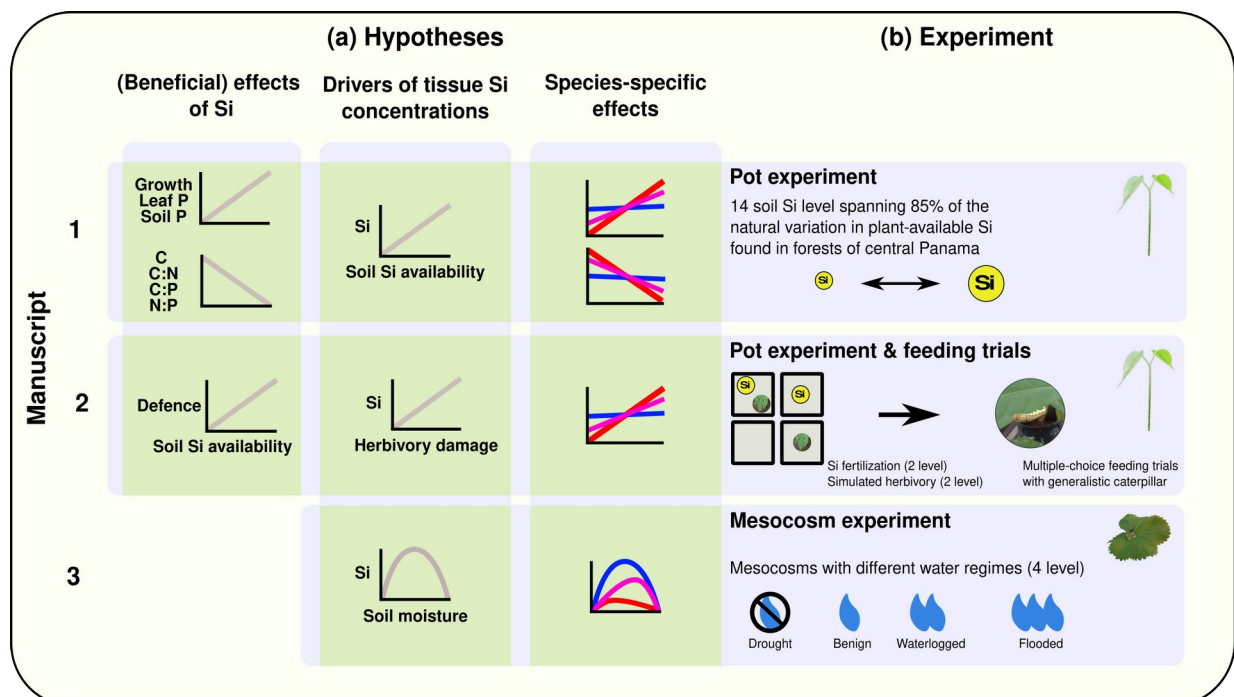


Figure 1: Overview of the (a) hypotheses and (b) experimental designs of the three studies presented in my thesis. Manuscripts 1 and 2 address the effects of plant-available soil Si on plant growth and leaf nutrient status or herbivory defence in tropical tree species. Manuscripts 1, 2 and 3 contain results on the effect of plant-available soil Si, herbivory or soil moisture on plant (i.e. total aboveground biomass) and/or leaf Si concentrations of tropical trees (manuscript 1 and 2) or temperate herbaceous species (manuscript 3). The degree of species-specific effects was assessed for each parameter.

varying across species (Massey et al. 2006, 2007a, Massey and Hartley 2006). The same studies demonstrated that the defensive function of Si is likely to be a result of increases in the abrasiveness of leaves due to deposition of amorphous Si, which deters herbivores and can lead to wear-down of their mouth parts and digestive tract after ingestion (Massey and Hartley 2006, 2009, Massey et al. 2006) - probably the best supported mechanism underlying Si-based defences (for the mechanism underlying induced Si uptake and defence see Table 1 and page 15). In a next step, Massey et al. (2007a) showed that the predictions of the resource-availability hypothesis, i.e. slow-growing species are better defended than fast-growing species (Endara and Coley 2011), which had before then only been studied in woody species, also hold in grasses and this was attributed to the major contribution of Si-based defences. In fact, this was the first study integrating Si into widely accepted ecological concepts. Since then Si-based herbivory defences have been studied extensively in grasses, from the physiological to the population and community level (Table 1).

Table 1: Overview of the main findings of a selection of studies on Si-based defences in wild grasses (including some economically important pasture grasses). For studies on cultivated crop species, such as rice, wheat and sugar cane please see references in Singh et al. (2020).

Study	Study species/system	Main finding
Garbuzov et al. 2011	<i>Poa annua</i> , <i>Lolium perenne</i>	Competitive differences between two grass species were jointly modulated by soil Si availability and herbivory
Reynolds et al. 2012	<i>Deschampsia caespitosa</i>	Delayed induction of Si-based defences influenced herbivore population dynamics in a grass–herbivore population model
Huitu et al. 2014	<i>Schedonorus pratensis</i>	Si, endophytes and secondary metabolites together defended grasses against mammalian herbivores
Hartley et al. 2015	<i>Festuca arundinacea</i> , <i>F. ovina</i> , <i>F. rubra</i> , <i>Deschampsia caespitosa</i>	Changes in the location and morphology of amorphous Si deposits at the leaf surface changed with Si fertilization
McLarnon et al. 2017	<i>Festuca arundinacea</i>	Herbivory-induced Si uptake probably was a result of active processes, e.g. gene expressions
Ryalls et al. 2017	<i>Phalaris aquatica</i>	Si-based defences reduced growth of herbivores and their predators under ambient and increased atmospheric CO ₂ concentrations.

Table 1 continued

Johnson et al. 2019	<i>Phalaris aquatica</i>	Si-based defences were less effective under warmer temperatures
Hall et al. 2020a	<i>Brachypodium distachyon</i>	Jasmonic acid induced Si uptake and increased atmospheric CO ₂ reduced Si-based defences, mediated by changes in jasmonic acid signalling
Vandegeer et al. 2021b	<i>Festuca arundinacea</i>	Si-based defences acted in droughted plants
Cibils-Stewart et al. 2022	<i>Festuca arundinacea, Lolium perenne</i>	Si-based defences interacted with alkaloid-based defences produced by endophytes and this interaction in turn varied across grass species
Hall et al. 2021	<i>Phalaris aquatica</i>	Si-based defences influenced secondary metabolite concentrations and reduced performance of aphids less compared to their parasitoids
Islam et al. 2022	<i>Phaseolus vulgaris</i>	Si fertilization led to changes in plant volatile emission and fostered the attraction of a herbivores' predator
Petit Bon et al. 2022	Tundra grass communities	Leaf damage by different herbivores differently influenced tissue Si and N concentrations across grass species

Given the strong evidence for Si-based defences in grasses, Si should have a similar function in wild dicot species of tropical forests and temperate grasslands, but empirical evidence is largely missing. Only recently, Pickelmann (2023) demonstrated in interspecific dual-choice feeding trials that Si-based defences might protect some wild temperate dicot species against leaf-eating insects. In this study, pairs of closely related temperate forb species were offered to a chewing herbivore and the species with lower Si concentrations was preferably eaten in three out of the seven species pairs (Pickelmann 2023). Yet, interspecific feeding trials can only capture the joint efficacy of all plant defences acting in a species, which are known to be numerous in dicots and can vary markedly across species (Koricheva et al. 2004, Kursar et al. 2009, Endara and Coley 2011). To separate the effect of Si-based defences on herbivore preferences from other plant defences, intraspecific feeding trials with con-specific individuals varying in tissue Si are needed (see Massey et al. 2006). Such experimental assessments of Si-

based defences have not yet been carried out for tropical tree species, although herbivory pressure is high in tropical forests and has substantial negative effects on seedling survival (Coley and Barone 1996, Eichhorn et al. 2010). Hence, whether herbivory defence of wild dicots, specifically seedlings of tropical tree species, is improved by Si and whether the effects of Si are species-specific is addressed in manuscript 2 (Figure 1a). In our study leaves of seedlings of eight tropical tree species, which have been treated with Si fertilization and simulated herbivory, were offered to a generalist herbivore in intraspecific (i.e. leaves of the same tree species) multiple-choice feeding trials. We hypothesized that, if Si improves herbivory defence, leaves of seedlings growing in Si-rich soil and/or showing high leaf Si concentrations should show less leaf damage than leaves of seedlings growing in Si-poor soil and/or showing low leaf Si concentrations. Additionally, if Si uptake and defence is inducible, leaf Si concentrations should be higher and leaf damage should be lower in seedlings exposed to simulated herbivory.

1.2.2 Effect of Si on plant growth and leaf nutrient status

Effects of Si on plant growth have also been shown in a few studies on wild species from savannah and alpine grasslands (McNaughton et al. 1985, Xu et al. 2015, 2020) and semi-natural temperate grasslands (Garbuzov et al. 2011). Again, experimental work on the effects of Si on plant growth in wild dicot species of temperate grasslands and tropical forests is scarce. To my knowledge performance-enhancing effects of Si in temperate and tropical dicots have only been studied in Alfalfa (non-native forb species occasionally found in temperate grasslands; Johnson et al. 2017, 2018, Putra et al. 2021) and modern cultivars of cocoa and coffee (Gómez-Vera et al. 2021, Parecido et al. 2022), all showing positive effects of Si on plant growth. Apart from that, indirect evidence for an influence of Si on plant growth of wild dicots can be gleaned from a few cross-species comparisons. For instance, Cooke and Leishman (2011b) demonstrated that plant Si concentrations correlate negatively with leaf life span, a functional plant trait reflecting the fast-slow continuum of leaf nutrient investments and plant growth strategies (Westoby et al. 2002, Reich 2014). The results are intriguing, but the implications for plant growth remain vague and tropical species were hardly considered.

Beneficial effects of Si on plant growth can be due to changes in physiological and/or soil processes. So far, most of our mechanistic understanding of the effect of Si on plant growth is based on studies comparing physiological parameters between Si-fed stressed and unstressed crop species. Si fertilization has been demonstrated to enhance several physiological parameters, including photosynthesis and antioxidant activity (Cooke and Leishman 2016, Frew et al. 2018). However, the interactions between these physiological parameters and Si and how they influence growth are contentious, mainly because Si is biochemically unreactive and any changes and covariation in physiological parameters observed after Si fertilization only bear correlational rather than causal evidence (Coskun et al. 2019). Furthermore, it was argued that beneficial effects of Si on growth should only come up in stressed plants, although there is

evidence for Si-mediated growth improvements in unstressed plants (e.g. Detmann et al. 2012, Johnson et al. 2017, 2018, Neu et al. 2017, Putra et al. 2021). Besides biochemical mechanisms, it was suggested that amorphous Si deposits may support the structural integrity and erectness of leaves and thereby improve light interception and photosynthesis (McNaughton et al. 1985, Quigley and Anderson 2014), yet mechanistic evidence is again lacking. Taken together, the physiological underpinnings of the effect of Si on plant growth are not straightforward and potentially vary widely across species. As an alternative explanation some authors argued that the changes in plant physiology of Si-fed plants could be caused by changes in soil chemistry (Neu et al. 2017), which in turn can influence leaf nutrient status. Nutrient concentrations in leaves play an important role in maintaining protein production and photosynthesis (Lambers and Oliveira 2019) and factors influencing leaf chemistry might therefore have far-reaching consequences for plant growth (Lambers and Oliveira 2019).

Many experiments showed that Si fertilization can influence leaf nutrient status, i.e. carbon (C), nitrogen (N) and phosphorus (P) concentration and their stoichiometry, in crop species, including grasses and some dicots (e.g. Eneji et al. 2008, Neu et al. 2017, Haddad et al. 2018, Greger et al. 2018, Islam et al. 2020, Schaller et al. 2021b). Again, most work on within-species effects of Si on leaf nutrient status in wild species has been done on monocots, including grasses, wetland or aquatic plants (Schaller et al. 2012a, b, Schoelynck and Struyf 2016, Quigley et al. 2017, Johnson and Hartley 2018, Minden et al. 2021, Hao et al. 2022), while dicots are systematically understudied (but see Hao et al. 2022). In many studies, higher plant-available soil Si was associated with higher leaf P and N concentrations and/or reduced C concentrations (Neu et al. 2017, Haddad et al. 2018, but see Greger et al. 2018, Islam et al. 2020, Schaller et al. 2021b), leading to changes in C:P and C:N ratios (e.g. Neu et al. 2017). In some cases the increase in leaf P was associated with improved plant growth (Schaller et al. 2012a, 2021b, Neu et al. 2017).

So far, the most parsimonious explanation for how Si can influence the leaf P status has been put forward by Schaller et al. (2019, 2020b, 2021b). They showed that increasing concentrations of plant-available Si in soils improves P availability to plants, probably because dissolved Si and P compete over binding sites on soil minerals (Schaller et al. 2019, 2020a, 2021b). Most of the work has been done in arctic and temperate soils but later on Gunnarsen et al. (2022) provided evidence for this processes in a P-rich tropical soil. Some physiological explanations for Si-induced improvements in P nutrition exist as well. For instance, Si accumulation in plant tissues may release P previously bound to Mn and Fe through the formation of Si-Mn and/or Si-Fe complexes (Hu et al. 2020) or promote the exudation of P-mobilizing organic acids into the rhizosphere (Kostic et al. 2017). Yet, both physiological processes have not received much attention and are not well-supported. From an ecological perspective, Si-induced improvements in P nutrition might have pervasive implications for the growth of tropical forest species on P-poor soils, which are very common in the tropics (Vitousek et al. 2010). Furthermore, growth and leaf P responses to Si-induced changes in P

availability should vary across tropical tree species. This is because some species can only take up dissolved, plant-available P (Reichert et al. 2022) and should therefore be sensitive to changes in soil P availability. Other species, by contrast, evolved foraging and mining strategies to access organic P and/or P bound to soil minerals and hydroxides (Reichert et al. 2022). These species might not respond to Si-induced increases in dissolved P. In order to test whether Si-induced increases in soil P availability influence P nutrition and growth of seedlings of tropical tree species, we experimentally grew several species along a soil Si gradient and assessed changes in soil P availability, leaf P concentration and plant growth (manuscript 1, Figure 1b). We hypothesised that increasing plant-available soil Si should increase soil P availability and thus leaf P concentrations and aboveground growth, if Si displaces P from soil binding sites. Furthermore, species associated to P-rich soils, which probably are more sensitive to changes in P availability (Zalamea et al. 2016), should respond stronger to Si-induced changes in soil P availability than species associated to P-poor soils. We also expected that an increase in leaf P should lead to a parallel decrease in leaf C:P and N:P ratios.

As with P, the mechanisms underlying the negative correlation between tissue Si and C concentrations remain debated (Hodson and Guppy 2022). The pattern has been interpreted as a result of substitution of C-based compounds, such as phenols or lignin, by Si (Raven 1983, Cooke and Leishman 2011a, 2012, Schaller et al. 2012b, Schoelynck and Struyf 2016, Hodson and Guppy 2022). The rationale is that Si is metabolically cheaper than C-based compounds and functionally equivalent to them, potentially making Si uptake and accumulation more worthwhile than synthesis of C-based compounds (Raven 1983). However, more cell-level studies are needed to provide reliable evidence for this explanation (Hodson and Guppy 2022), which is not within the scope of my work. Nevertheless, a potential substitution of C by Si might have implications for the ecology of many plant species, especially in resource and C-limited ecosystems (Quigley et al. 2020). Species are thought to optimize their leaf C economy to maximize performance in the given environment (Lambers and Oliveira 2019). Seedlings of shade-tolerant tropical forest species experience high herbivore pressure, extremely low light conditions and, in more seasonal forests, severe droughts in the forest understory (Engelbrecht and Kursar 2003, R uger et al. 2009, Eichhorn et al. 2010). All of these factors can impede C assimilation and/or promote C loss, and as a result tropical forest species have evolved strategies optimizing net C gain, such as long-lived and well-defended leaves (Poorter and Bongers 2006, Alvarez-Clare and Kitajima 2007). We explored the role of Si in optimizing net C gain in tropical seedlings and tested whether a negative correlation between plant Si and C concentrations, a potential indicator for Si-induced C substitution, emerges in these species (manuscript 1). We hypothesized that if Si substitutes leaf C, increasing plant-available soil Si should decrease leaf C concentrations. The C responses should be more negative in species showing stronger increases in leaf Si concentrations with increasing plant-available Si. We also expected that a decrease in leaf C should lead to a parallel decrease in leaf C:N and C:P ratios.

1.2.3 Drivers of inter- and intraspecific variation in tissue Si concentrations

1.2.3.1 Root Si uptake and genetic factors

Plants take up Si as mono-silicic acid from the soil solution via their roots (Raven 1983, Ma and Yamaji 2015, Deshmukh and Bélanger 2016). The mono-silicic acid is then transported to aboveground organs through the xylem driven by the transpiration stream (Raven 1983, Ma and Yamaji 2015). When the concentration of mono-silicic acid is high enough, precipitation occurs and amorphous Si deposits are being formed and accumulate in inter- and intracellular spaces, especially in cell walls and around vascular, epidermal and stomatal cells (Raven 1983, Trembath-Reichert et al. 2015, Hodson 2016). Root uptake of Si can be passive or active: passive uptake happens continuously as Si diffuses into the root through membrane channels as a by-product of water uptake and is driven by transpiration (Faisal et al. 2012). Active Si uptake is mediated by ATP-consuming Si transporter and typically leads to higher plant Si concentrations than passive uptake (Ma and Yamaji 2015, Deshmukh and Bélanger 2016). The relative contribution of active and passive mechanisms to Si uptake varies across species (Liang et al. 2006). Furthermore, the active component is largely determined by genetics (Ma and Yamaji 2015, Deshmukh and Bélanger 2016). As a consequence closely related species typically show more similar Si concentrations than distantly related species (Hodson et al. 2005, Deshmukh et al. 2020, Klotz et al. 2021). Overall, passive and active Si uptake mechanism and their genetic underpinnings explain a great proportion of the wide variation in plant Si concentrations found across species.

1.2.3.2 Environmental factors

Intraspecific variation in Si uptake and accumulation is affected by environmental factors, such as variation in plant-available soil Si (Liang et al. 2006, Massey et al. 2007b, Quigley et al. 2017, Klotz et al. 2021), soil moisture (Han et al. 2011, Quigley and Anderson 2014, Quigley et al. 2017, Brightly et al. 2020), herbivory damage (McNaughton et al. 1985, Soininen et al. 2013, McLarnon et al. 2017), light (Kajino and Kitajima 2021), temperature (Han et al. 2011, Johnson and Hartley 2018), soil pH (Han et al. 2011, Quigley et al. 2017, Schaller et al. 2018) and nutrient availability (Quigley et al. 2020, Minden et al. 2021). Most of these factors are thought to modulate the genetically-determined baseline Si accumulation of plant species (e.g. Soininen et al. 2013). This may happen directly through their effects on plant physiology, including passive and active Si uptake mechanisms, or indirectly by modifying soil Si availability.

Out of all environmental factors, changes in soil moisture should influence tissue Si concentrations the most as (passive) Si and water uptake are mechanistically intertwined (see above). Furthermore, unfavourable soil moisture conditions, i.e. too much and too little water, hamper nutrient uptake through reductions in transpiration, ATP availability and protein synthesis (Lambers and Oliveira 2019, Yeung et al. 2019), e.g. for Si transporters. Hence Si uptake should be highest under ample soil moisture. Furthermore, differences in stomatal

regulation (Bartlett et al. 2016), probably influencing passive Si uptake, and in active Si uptake across species (see above, Deshmukh and Bélanger 2016) suggest that their tissue Si concentrations should be differently influenced by soil moisture.

Besides soil Si and moisture, herbivory damage is known to influence tissue Si concentrations (McNaughton et al. 1985, Soininen et al. 2013, McLarnon et al. 2017). Studies on a common pasture grass demonstrated that the induction of Si uptake is mediated by an up-regulation of Si transporter genes after damage (McLarnon et al. 2017). Furthermore, signalling hormones involved in anti-herbivore responses, such as jasmonic acid and its derivative methyl jasmonate, have been shown to up-regulate Si transporters genes and could potentially be responsible for the induction of Si uptake after herbivory damage (Hall et al. 2019, 2020a). However, physical processes might also be important, as damaged leaves usually exhibit higher water loss (e.g. Aldea et al. 2005) elevating the influx of dissolved mono-silicic acid to the leaf and thereby increasing leaf Si concentrations (McLarnon et al. 2017). Furthermore, the strength of induction of Si uptake varies across species (Soininen et al. 2013).

Experimental studies on environmental drivers of intraspecific tissue Si variation have been largely biased towards wild tropical and temperate grasses. For instance, there is strong experimental evidence for herbivory-induced Si uptake in various wild grass species (McNaughton et al. 1985, Massey et al. 2007a, Soininen et al. 2013, Hartley et al. 2015). For dicots, only one field study has been conducted showing rather inconsistent results (Katz et al. 2014). Along the same line, the positive effect of plant-available soil Si on tissue Si concentrations has been shown experimentally many times in various wild grass species (e.g. Gali-Muhtasib et al. 1992, Massey et al. 2006, 2007b, Schaller et al. 2012b), yet only once in wild dicots (temperate forbs; Klotz et al. 2021), with overall positive but highly species-specific effects of plant-available Si. For tropical dicots some inconsistent patterns can be gleaned from field data comparing plant Si concentrations across different habitats (Schaller et al. 2018, Ishizawa et al. 2019, Nakamura et al. 2019). Furthermore, the effect of soil moisture on intraspecific variation in tissue Si concentrations has been mostly assessed in wild temperate and tropical grasses (Quigley and Anderson 2014, Quigley et al. 2017, Ryalls et al. 2018, Brightly et al. 2020), but only once in wild dicots (Katz et al. 2013). Although plant water and Si uptake are mechanistically coupled, the results have been rather inconsistent, with positive, negative and no effects of water availability on tissue Si concentrations. Taken together, most data on how environmental factors influence tissue Si concentrations in wild dicots and on how the effects vary across species has been derived from field studies precluding mechanistic conclusion and generalizations. Even in grasses, conclusive experimental evidence for species-specific tissue Si responses to variation in soil moisture is scarce, because most data available have been derived from single-species studies (but see Brightly et al. 2020), varying in how and to what degree soil moisture was manipulated – a potential explanation for the inconsistent results observed across studies – or studies assessing the effects of drought only (Brightly et al. 2020). This largely hampers thorough cross-species

comparisons of tissue Si responses to environmental factors in both, wild dicots and grasses. This is probably one reason why the generality of effects of environmental factors on tissue Si concentrations, the degree of species-specific tissue Si responses and the cascading effect on plant growth, defence and nutrient status are not well understood. Thus, we experimentally assessed tissue Si responses to variation in soil moisture across multiple temperate grassland species in manuscript 3, and to variation in plant-available Si and herbivory across multiple tropical tree species in manuscripts 1 and 2. We tested the following hypotheses: **(i)** Increasing plant-available soil Si increases leaf Si concentrations. **(ii)** Simulated herbivory should increase leaf Si concentrations, if Si uptake is inducible by herbivory damage. **(iii)** Variation in soil moisture influences plant Si concentrations, with the highest concentrations under benign conditions and the lowest concentrations under drought, waterlogging and flooding. **(iv)** The effects of plant-available soil Si, simulated herbivory and soil moisture on tissue Si concentrations vary across species.

2 Materials and methods

In this section I present the experiments we undertook to address my research questions and hypotheses. At first, I outline for each manuscript the experimental design (Figure 1b) and statistical analysis (all statistical analyses were performed in R version 4.1.0 or 4.2.2, R Core Team 2021, 2022). Then I provide more detailed information on the experimental substrate and plant material.

The experiments reported on in manuscript 1 and 2 were undertaken in Gamboa, Panama (09°07'N, 79°42'W) with a moist tropical climate with a mean annual temperature of 27°C and a mean annual rainfall of 2100 mm (Slot and Winter 2017). The experiment in manuscript 3 was undertaken in the Ecological-Botanical Garden of the University of Bayreuth, Germany (49°55'N, 11°34'E) with a temperate, moderately continental climate with a mean annual temperature of 8.7°C and a mean annual rainfall of 724 mm (Walter et al. 2012).

2.1 Experimental designs and statistical analyses

2.1.1 Manuscript 1 – Pot experiment

To address the effect of plant-available soil Si on leaf nutrient status, i.e. Si, P, C and N concentrations as well as C:N, C:P and N:P ratios, and aboveground growth we grew seedlings of 12 tropical tree species for up to 10 month in pots under 14 different levels of soil Si availability, representing 85% of the soil Si variation found across forests of central Panama (based on Schaller et al. 2018). We used a non-replicated gradient design (Kreyling et al. 2018) in which one individual per species was randomly assigned to each of the soil Si levels. A gradient design enabled us to better capture the strength and shape of responses compared to

replicated designs with only two or three Si levels (Kreyling et al. 2018). To assess the direction and magnitude of each species' response to increasing plant-available Si in each parameter, we ran separate ordinary linear models ($n = 11-13$). To assess whether the effects of plant-available soil Si on aboveground growth and leaf nutrient status varied across species, we ran ANCOVAs and assessed the significance of the species \times Si-level interactions ($n = 151$). Furthermore, we assessed whether increasing plant-available soil Si increased soil P availability using ordinary linear models ($n = 14$).

2.1.2 Manuscript 2 – Pot experiment and multiple-choice feeding trials

To test the effect of plant-available soil Si and leaf Si concentrations on herbivory defence and whether Si uptake is inducible we undertook a full-factorial pot experiment, with a Si fertilization and simulated herbivory treatment, followed by within-species multiple-choice feeding trials. In the pot experiment plants of eight species were exposed to the four treatment combinations for up to 14 months. Herbivory was simulated by clipping 50% of the leaf area of two leaves along the leaf margins combined with application of methyl jasmonate, a hormone inducing defence responses in plants (Mithöfer and Boland 2012). The simulated herbivory treatment enabled us to standardize the damage intensity across and within species (Lehtilä and Boalt 2008). After the pot experiment, we carried out feeding trials, in which leaf disks of each treatment combination of a single species were offered to *Spodoptera frugiperda* (J.E. Smith), a generalist caterpillar. After the trials we measured the remaining leaf area and calculated leaf damage. To analyse the effect of soil Si treatment, simulated herbivory and their interactions as well as leaf Si concentrations on leaf damage we ran zero-inflated generalized linear mixed-effect models (ZIGLMM, $n = 4-7$) separately for each species. Because the data were proportional and had many zeros the residuals were assumed to be beta-distributed and zero-inflated. We used parametric bootstrapping (10000 iterations) to calculate robust 90% and 95% confidence intervals for the model estimates to assess their significance. To test whether simulated herbivory influenced leaf Si concentrations we ran ANOVA.

2.1.3 Manuscript 3 – Mesocosm experiment

To assess the effect of soil moisture on plant (i.e. aboveground biomass) Si concentrations we grew ten temperate grassland species of two life-forms (grasses and forbs) under four different soil moisture regimes, including benign, dry waterlogged and flooded conditions, in mesocosms. Each moisture regime was replicated five times ($n = 5$). Using mesocosms and a standardized soil enabled us to expose plants to four different moisture regimes while, at the same time, reducing variation in nutrient availabilities – conditions which cannot be easily achieved by carrying out moisture regime manipulations in field experiments due to small-scale variation in soil nutrient availability in grasslands (Hodge 2004). All treatments lasted for 19 weeks from mid-May until the end of September 2016. We analysed the effects of species (or life-form) and moisture regime, as well as their interactions by running generalized least

squares models (for species) or generalized linear mixed-effect models (for life-forms). Tukey post-hoc tests were applied to assess the significance of differences in plant Si concentrations across the moisture regimes within each species and life-form. To assess how much of the total variation in plant Si concentrations is explained by species differences, moisture regimes, and by species x moisture regime interactions, we partitioned the variance between these components by fitting random intercept models (see Messier et al. 2010). Furthermore, we tested whether species ranks changed across moisture regimes using Spearman rank correlation.

2.2 Species selection and plant material

Understanding the extent of species-specific effects is an overarching aspect tackled in all experiments. Therefore, the experiments included at least eight species. Species were selected to reflect a wide range of habitat associations to plant-available soil P (manuscript 1, on the basis of species occurrence data along a plant-available P gradient in Panama, Condit et al. 2013) or soil moisture conditions (manuscript 3, based on Ellenberg et al. 2001). Species adapted to different soil moisture conditions or soil P availabilities differ in their ecological strategies in how they cope with drought (Silvertown et al. 2015, Zwicke et al. 2015) and anoxia (Mommer et al. 2006) or in how they acquire P (Reichert et al. 2022). Furthermore, we tried to select species representing the whole spectrum of Si uptake capacities (all manuscripts). The species selection in manuscripts 1 and 2 was informed by data obtained from leaf collections of plants within a 50 ha forest plot in central Panama (K. Kitajima et al. unpublished data). The species selections of manuscripts 1 and 2 overlapped partly and only included shade-tolerant species.

Seeds for the pot experiments in manuscripts 1 and 2 were collected from mature trees in central Panamanian forests in October and November 2019. To maximize genetic diversity we selected seeds from at least three trees, which were at least 100m apart from each other. Seeds were germinated on a substrate consisting of a 1:1 mixture of local forest soil with low plant-available Si and washed river sand in the shade-house. Then seedlings were transferred into separate pots with the experimental substrates. Individuals of similar size (height and number of leaves) were chosen per species to reduce variation in the initial biomass. Seedlings were kept well-watered, under intermediate light conditions (ca. 10% full sunlight) and protected from rainfall. We randomized the position of species and treatments.

Seeds for the mesocosm experiment in manuscript 3 were purchased from a local seed supplier (Rieger-Hofmann GmbH). According to the supplier, seeds of each species contained a mixture of genotypes typically occurring in southern Germany. Seeds were germinated in the greenhouse and after that the seedlings were planted into the mesocosms, arranged in a concentric grid. The position of individuals was randomly selected (for details see *Figure S1* in *Supporting information* of manuscript 3).

2.3 Experimental substrates

The experimental substrate used in both pot experiments was a 1:1 mixture of sieved forest soil (Cerro Pelado, selected on the basis of Schaller et al. 2018 and Condit et al. 2013) and thoroughly washed nutrient-poor river sand. The forest soil had very low plant-available Si and P concentrations. To manipulate plant-available Si we added Aerosil 300 (Evonik Industries AG, Essen, Germany), which is hydrophilic pyrogenic silicon dioxide. Aerosil 300 mirrors the chemical and physical properties of biogenic amorphous Si (Schaller et al. 2020b). Fertilization with Aerosil 300 has been shown to increase plant-available soil Si (Neu et al. 2017, Klotz et al. 2021). Aerosil 300 does not change soil pH (J. Schaller, unpublished data). The lowest Si-level in experiment 1 and the low Si treatment in experiment 2 did not contain Aerosil 300. We fertilized each seedling with 20 ml of a half strength Hoagland solution three times within the first three months of the experiments. In this way, we ensured survival of species adapted to P-rich soil. The experimental substrate used in the mesocosm experiment consisted of local topsoil mixed with crushed limestone.

2.4 Chemical analyses

2.4.1 Plants

Leaves (manuscripts 1 and 2) or aboveground biomass (manuscript 3) were harvested, oven-dried at maximum 70 °C and ground for chemical analyses. Leaf Si was extracted following Schaller et al. (2018) and Rausch (2021). Leaf P was extracted by digesting the leaf material with HNO₃ and H₂O₂ in a closed vessel microwave digestions system (Mars express6, CEM, Germany). The Si and P concentration of the biomass extracts was determined with inductively coupled plasma optical-emission spectrometry (ICP-OES) using an elemental analyser. Leaf C and N concentrations and C isotope ratios ($\delta^{13}\text{C}$) were analysed with EA-IRMS coupling (Elemental Analyzer NA 1108, CE Instruments; Interface ConFlo III, Finnigan MAT; isotope ratio mass spectrometer: delta S, Finnigan MAT) and calculated following Liebel et al. (2010).

2.4.2 Soils

Samples of the experimental substrates in manuscripts 1 and 2 were air-dried, crushed to break up large aggregates, and sieved to remove roots and stones. Plant-available Si was extracted following Schaller et al. (2018). Plant-available P was extracted following the Mehlich-III method (Sims 1989).

3 Results and discussion

Increasing plant-available soil Si improved seedling growth and defence and influenced leaf nutrient status in more than half of the tropical tree species we studied (ca. 60%), with the size of effects varying across species. To my knowledge we were the first to show beneficial effects of Si on plant growth and defence in wild tropical tree species. Furthermore, tissue Si concentrations of tropical tree seedlings and temperate herbaceous species were influenced by variation in plant-available soil Si and soil moisture, respectively. Again, the effects varied strongly across species. No effect of simulated herbivory on leaf Si concentrations was found, i.e. Si uptake and defence was not inducible in tropical tree species.

In the following sections 3.1 – 3.3 I present the results of the three manuscripts in more detail, including discussions on potential mechanistic underpinnings and the ecological implications

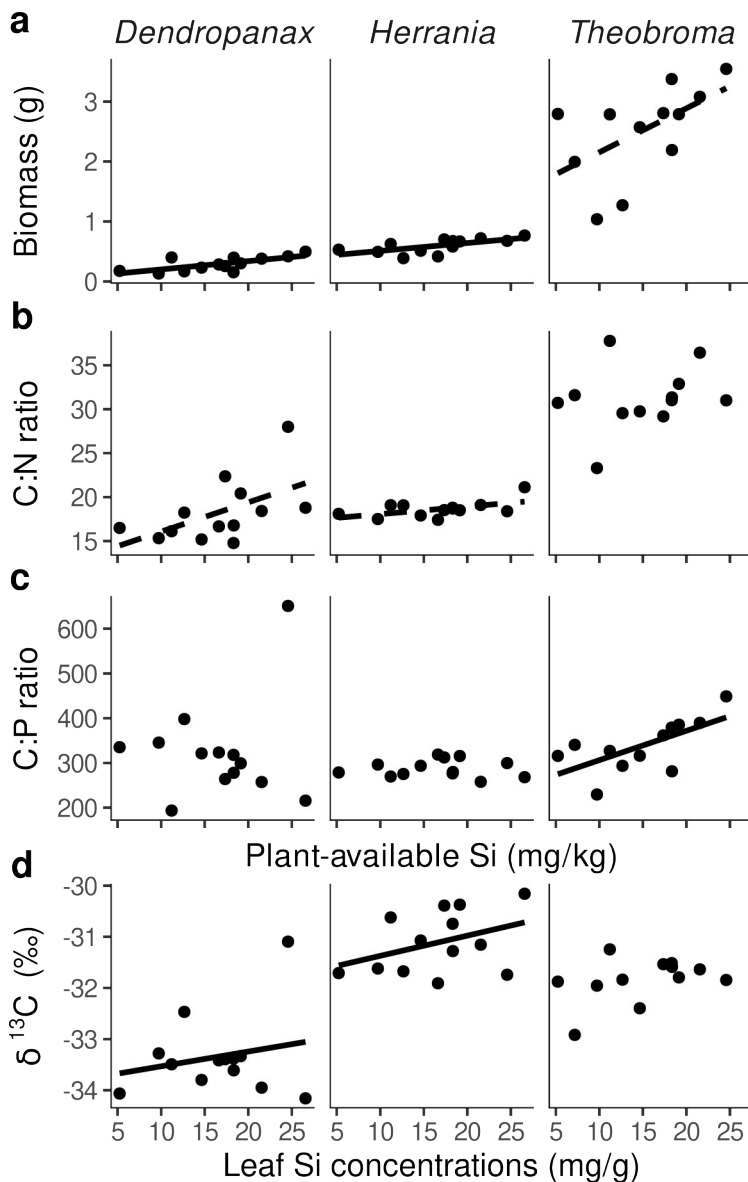


Figure 2: Effect of increasing plant-available soil Si on (a) aboveground biomass, leaf (b) C:N and (c) C:P ratios as well as effect of leaf Si concentrations on (d) water-use efficiency ($\delta^{13}\text{C}$) in the three species, showing positive growth responses to increasing plant-available soil Si. Solid and dashed lines denote significant responses ($p < 0.05$) and trends ($0.05 < p < 0.1$), based on ordinary linear models (for details see *Table S3* in the *supporting information* of manuscript 1; models for $\delta^{13}\text{C}$ are not included in the manuscript: *Dendropanax*: $\beta = 0.77$, $\text{SE} = 0.35$, $\text{adj. } R^2 = 0.25$; *Herrania*: $\beta = 0.71$, $\text{SE} = 0.03$, $\text{adj. } R^2 = 0.65$; *Theobroma*: $\beta = 0.03$, $\text{SE} = 0.30$, $\text{adj. } R^2 = 0.00$).

of our findings, and in some cases, potential future challenges and research directions. I refer to the species by their genus name (see *Table S1* in *Supporting information* of manuscript 1 for full species names).

3.1 Plant growth and nutrient status

3.1.1 Plant growth

Three (*Dendropanax*, *Herrania* and *Theobroma*) out of the 12 species we studied showed increases in aboveground biomass along the soil Si gradient (by up to 220%, Figure 2a). In one of these species (*Theobroma*) a non-significant trend emerged. In the other nine species growth remained unaffected by soil Si. Improved growth was not associated with increasing P availability, which remained unchanged along the soil Si gradient (see *Figure 1* in manuscript 1), not supporting our hypothesis of Si-induced increases in soil P availability and plant growth. These results plus a significant species x soil Si interaction that emerged in the ANCOVA (see *Table S2* in *Supporting information* of manuscript 1) are indicative of species-specific effects of plant-available Si on growth of tropical tree seedlings. However, the responses of the different species were not related to their association with soil P (see *Figure 3* in manuscript 1).

To my knowledge our study was the first multi-species experiment testing whether Si can improve plant growth in tropical tree species. Interestingly, species with both low and high Si uptake capacity showed positive growth responses. Previous studies on cultivated species also found improved growth in Si-fed crop and grass species with low and high Si uptake capacity (reviewed in Cooke and Leishman 2016 and Frew et al. 2018), including cultivars of tropical tree species, such as coffee (Parecido et al. 2022), cocoa (Gómez-Vera et al. 2021) and mango (Helaly et al. 2017). Most of these studies, however, focused on single species or cultivars, which typically are (or being bred to be) fast-growing and nutrient-acquisitive (e.g. Tribouillois et al. 2015). From an ecological perspective, these species represent a small subset of possible plant growth strategies found in natural systems (Westoby et al. 2002, Reich 2014). These strategies are thought to form a spectrum with fast-growing species on one end and slow-growing species, using nutrients more conservatively, on the other end (Westoby et al. 2002, Reich 2014). About 25% of the (slow-growing) species we studied showed positive growth responses. Thus, our results are at odds with a wealth of literature consistently reporting positive effects of Si on plant growth in fast-growing and nutrient-acquisitive species (Cooke and Leishman 2016, Frew et al. 2018). This either suggests that slow-growing species are less likely to benefit from Si (see Cooke and Leishman 2011b) or, as argued previously, beneficial effects of Si are less likely to occur in unstressed plants (Coskun et al. 2019). According to Coskun et al. (2019), the only growth-promoting function of Si should be mitigating the detrimental impact of stress on plant physiology by restoring physiological processes, such as anti-oxidant production or photosynthesis, to the level of unstressed plants - a function that

cannot take effect if physiological processes are already at optimal level, e.g. under unstressed conditions. In our experiment, seedlings were protected from herbivores, grew under intermediate light conditions and were well-watered and -fertilized. By contrast, in the forest understory, seedlings are exposed to various stressors, including drought, herbivory, low nutrient and light levels (Chazdon et al. 1996, Engelbrecht and Kursar 2003, Eichhorn et al. 2010, Wright 2019). Thus, the number of species showing positive effects and the effect sizes we observed might potentially underestimate the prevalence of growth-promoting effects of increased plant-available Si in the field, especially if beneficial effects were more likely to occur under stressed conditions. However, in a field experiment, droughted seedlings of seven species showed no differences in growth and survival between -Si and +Si plants and, in one species, survival was even reduced in Si-fed plants (Knauff 2023). As tropical tree species have evolved numerous strategies to maintain growth under conditions of stress (Alvarez-Clare and Kitajima 2007, Reichert et al. 2022), growth-promoting effects of Si, as commonly found in less stress-resistant crop species, might either be obscured by other strategies, such as those promoting P uptake and drought resistance, and/or did not evolve in most species.

3.1.1.1 No performance trade-off associated with high Si availability and uptake

Given that our experimental soil Si gradient covered about 85% of the variation in soil Si availability found in central Panamanian forests, our results suggest that the growth of species benefiting from Si should consistently be improved in forest sites with Si-rich compared to sites with Si-poor soils. While the direction of growth responses to Si are largely in line with those reported elsewhere, we are the first to show that the increase in growth with increasing plant-available Si is in fact linear. Up to now, the shape of responses could not be evaluated reliably, as most studies had carried out factorial experiments only including two soil Si treatments, a Si-rich and -poor soil (see references in Cooke and Leishman 2016, but see Thorne et al. 2022). Interestingly, a few of these studies found reduced growth in Si-fed plants (Schaller et al. 2012a, Neu et al. 2017), suggesting e.g. toxic effects of Si (Raven 1983). Alternatively, it was hypothesized that, if Si is not beneficial in the given environment, for example because stress-levels are too low (see above), plants might need to trade off the energy costs of active Si uptake against growth under high soil Si availability (de Tombeur et al. 2023). Yet, empirical evidence that the energy costs of Si uptake are in fact high enough to impede growth under high Si availability is scarce and, to my knowledge, is only provided by Garbuzov et al. (2011), showing that a high-accumulating grass species performed worse than a low-accumulating species in Si-rich soil and vice versa in Si-poor soil (Garbuzov et al. 2011). Furthermore, Raven (1983) estimated that Si uptake and accumulation only consume a very small proportion of a plants energy budget (one ATP per Si atom; Raven 1983) demonstrating that the costs of Si uptake are small compared to other physiological processes such as C assimilation (Lambers and Oliveira 2019). In line with that, we did not find indication for reduced growth and performance trade-offs within the range of soil Si availabilities typical for tropical forests of central Panama.

3.1.1.2 Potential mechanism underlying Si-induced growth improvements

The mechanisms underlying positive growth responses to Si might be soil- and/or plant-based (Frew et al. 2018, Schaller et al. 2019, 2020a, Coskun et al. 2019). Si-mediated changes in soil processes that had been shown to improve plant growth elsewhere (Schaller et al. 2019, 2020a, b, Kuhla et al. 2021, Gunnarsen et al. 2022) may not have contributed to plant growth in our study. Firstly, we did not find improvements in soil P availability along the soil Si gradient (see above). Secondly, although biogenic amorphous Si has been shown to improve soil water-holding capacity (Schaller et al. 2020b, a, Kuhla et al. 2021), potential differences in water-holding capacities between the low and high Si levels should not have caused the differences in plant growth, because our experimental substrates were kept well-watered throughout the experiment. Hence, our results suggest that plant-based processes were more important than soil-based processes in our study species.

In the following section I discuss patterns in leaf nutrient stoichiometry and carbon isotope ratios ($\delta^{13}\text{C}$) that emerged in the species showing improved growth with increasing plant-available soil Si. Although anecdotal evidence, these patterns might offer insights into the mechanistic underpinnings of Si-induced growth improvements in the species we studied. Firstly, the increase in C:N and C:P ratios in the species showing positive growth responses (Figure 2b,c) to increasing plant-available Si could indicate a better nutrient-use efficiency, i.e. more C was assimilated while plant nutrient uptake and concentrations remained unchanged (Lambers and Oliveira 2019). Evidence for a better photosynthetic nutrient use efficiency in Si-fed plants was previously found in coffee and cacao cultivars (Gómez-Vera et al. 2021, Parecido et al. 2022). Alternatively, the increasing leaf C:N and C:P ratios could reflect diluted N and P concentrations. Macronutrients can become diluted when a plant maintains high growth and thus C assimilation in a nutrient-poor soil (Elser et al. 2010, Anderson et al. 2018). As we did not measure nutrient-use efficiency directly, other processes, such as nutrient dilution, cannot be ruled out.

Secondly, in *Dendropanax* and *Herrania* not only growth was improved with increasing plant-available Si but $\delta^{13}\text{C}$ values increased with leaf Si concentrations (Figure 2d; only in *Herrania* leaf Si concentrations increased with increasing plant-available Si, see *Table S3* in the *Supporting information* of manuscript 1), potentially indicating a higher water-use efficiency (Lambers and Oliveira 2019) when leaf and/or soil Si concentrations are high. Yet, changes in $\delta^{13}\text{C}$ cannot always be attributed to changes in water-use efficiency, as $\delta^{13}\text{C}$ is also sensitive to changes in leaf morphology, e.g. mesophyll conductance, and the environment, e.g. leaf boundary layer conductance and water vapour deficit (Seibt et al. 2008). In our experiment environmental conditions, such as atmospheric CO_2 concentration, water vapour deficit and temperature, were the same for all individuals. Intraspecific differences in morphology should have also been minimal, because all individuals initially had similar age and height. Changes in $\delta^{13}\text{C}$ within species should thus very likely reflect inter-individual differences in water-use efficiency, i.e. the ratio of C assimilation to stomatal conductance integrated over the

experimental period (Lambers and Oliveira 2019). This implies either changes in gas exchange and/or photosynthesis have occurred with increasing plant-available Si and/or leaf Si concentrations. In fact, Si-fed plants have been shown to have reduced cuticular conductance and more sensitive guard cells, potentially leading to earlier stomata closure (Vandegeer et al. 2021b), as well as higher C assimilation rates and chlorophyll contents (Cooke and Leishman 2016). Whether similar effects of Si emerged in our species was not studied explicitly, but in another experiment with *Herrania* no effects of plant-available and leaf Si on stomatal and cuticular conductance were found (Knauff 2023), suggesting that Si fertilization and accumulation might have promoted photosynthesis in *Herrania* in our experiment. Yet, the exact mechanisms underlying Si-induced effects on plant photosynthesis and performance remain highly debated (Frew et al. 2018, Coskun et al. 2019).

Besides effects of Si on plant metabolism, the growth increments we observed could be explained by the additional mass cost incurred by plants due to Si accumulation (Raven 1983). Amorphous Si deposits have a density between 1.5 - 2.3 g cm⁻² (Hodson 2016) and are up to five-fold denser than leaves of woody evergreens (mean: 0.42 g cm⁻², Poorter et al. 2009). Thus, our measure of plant growth, i.e. weight changes in aboveground biomass during the experimental period, did not only reflect changes in C assimilation, but also weight changes caused by deposition of amorphous Si (see O'Reagain and Mentis 1989). Many previous studies have also used (aboveground) dry mass as growth parameter (Johnson et al. 2017, 2018, Xu et al. 2020). Instead, changes in leaf area could have been a more accurate measure of plant growth, only reflecting actual changes in plant primary metabolism leading to increments in metabolically active biomass and not the changes in metabolically inactive amorphous Si. Especially in light-limited environments like the tropical forest understory (Chazdon et al. 1996), but in parts also temperate grasslands (Weisser et al. 2017), information on potential Si-induced changes in leaf area and thus light interception and competition might be valuable and can greatly improve our ecological understanding of Si. Thus, the contribution of Si-induced weight costs vs. actual effects on plant metabolism deserves more attention in future studies on growth-promoting effects of Si in natural systems.

3.1.1.3 Ecological implications

Irrespective of the underlying mechanisms, our findings of species-specific effects of plant-available soil Si on plant growth suggest that spatial variation in plant-available soil Si in tropical forests may affect performance differently across plant species. This may lead to differences in species performance ranks across habitats varying in plant-available soil Si, with potential consequences for species distribution and community composition (Baraloto et al. 2005, Russo et al. 2005).

3.1.2 Nutrient status

As for leaf nutrient status, leaf P concentrations increased with increasing plant-available soil Si in only one out of the 12 species we studied (see *Figure 4* in manuscript 1), not in line with our hypothesis of Si-induced increases in P availability and leaf P concentrations (see above). Leaf C concentrations decreased significantly with higher plant-available Si in four species, consistent with our hypothesis of Si-induced substitution of C-based compounds. Leaf C:N ratios increased in two (a non-significant trend, *Figure 2b*) and leaf C:P and N:P ratios increased and decreased in two species, respectively, not consistent with our hypotheses (see *Figure 5* in manuscript 1). Leaf N concentrations decreased along the soil Si gradient in four species (a non-significant trend in two species). Taken together, only a subset of two to four species responded to increasing soil Si availability in each leaf nutrient status parameter we measured, suggesting species-specific effects of plant-available soil Si on leaf nutrient status.

Our results showed that effects of Si on leaf chemistry in tropical dicot species are possible. In most cases (macro-)nutrient concentrations decreased with increasing plant-available Si. In only one of the species we studied leaf P concentrations increased and C:P and N:P ratios decreased along the soil Si gradient. As effects of dissolved Si on P availability have been ruled out (see above), physiological processes related to plant P nutrition must have changed with increasing Si availability. Indeed, evidence for Si-induced changes in P-mobilizing exudate production and internal P availability can be found in Kostic et al. (2017) and Hu et al. (2020). In terms of C, negative correlations between leaf C concentrations and leaf and/or soil Si concentrations and have been argued to be a result of substitution of C-based compounds, such as lignin and phenols, by Si (Cooke and Leishman 2011a, Hodson and Guppy 2022). Alternatively, leaf C could have become diluted by the greater accumulation of dense amorphous Si deposits along the soil Si gradient (see above, Hodson and Guppy 2022). This would also explain the decreasing leaf P and N concentrations in most species. Overall, the responses in leaf P, N and C concentrations we observed were consistent with those reported in previous studies on crop species, including some dicots (Schaller et al. 2012a, Neu et al. 2017, Kostic et al. 2017, Greger et al. 2018, Hodson and Guppy 2022 and references therein). However, our findings of only a subset of species showing Si-induced changes in leaf P and C concentrations is at odds with the compelling hypotheses of other authors, putting forward that species of resource-poor environments, such as tropical forests, should benefit from Si-induced substitution of C-based compounds (Quigley et al. 2020) and that Si might play an important role in P nutrition in tropical tree species (Schaller et al. 2018).

3.1.2.1 Ecological implications

Si-induced changes in leaf nutrient status, especially leaf C:N and C:P ratios, in combination with the pronounced spatial variation in plant-available soil Si may have pervasive ecological consequences in tropical forests. This is because leaf C:N and C:P ratios influence litter decomposition, nutrient release and nutrient availability in tropical soils (Laird-Hopkins et al.

2017, Camenzind et al. 2018, Sayer et al. 2020). Nutrient availability, on the other hand, is an important determinant of species performance and distribution as well as community composition (Condit et al. 2013, Wright 2019). Thus, variation in plant-available soil Si may indirectly contribute to species performance and distribution via effects on leaf C:N and C:P ratios.

3.1.3 Future challenges and research directions

Some limitations of our study design should be considered when applying our findings of Si-mediated effects on plant growth and leaf nutrient status to plants in the field. Firstly, manipulating Si availability by adding artificial, biogenic Si might have changed soil factors other than plant-available Si concentrations, such as amorphous Si concentrations and soil texture. Both are known to influence water-holding capacity (Schaller et al. 2020b, a, Kuhla et al. 2021) and/or root growth (Jones 1983, Dexter 2004) which in turn might have impacted growth and nutrient status at the highest soil Si levels. Data on changes in rooting morphology and soil texture was not obtained in our study and a comparison of water-holding capacity of a local forest soil with and without artificial amorphous Si yielded significant differences (Knauff 2023), with the Si-treated soil showing a higher water-holding capacity. However, as the plants were kept well-watered the difference in water-holding capacity should not have influenced plant growth and nutrient responses at any point of the experiment. Secondly, the experimental substrate was extremely P-poor, mitigating competition between dissolved P and Si for soil binding sites and potentially explaining why soil P availability did not change along the soil Si gradient. In fact, a recent study found evidence for Si-induced increases in P availability in a tropical soil with greater P availability (Gunnarsen et al. 2022). Although our results might only apply to P-poor conditions, they should be representative for most tropical lowland forests, because they typically occur on highly weathered, P-impooverished soils (Vitousek et al. 2010, Wright 2019). However, to address these caveats a screening of leaf chemical traits and seedling growth along an soil Si gradient in the field is needed.

3.2 Plant defence

Three (*Calophyllum*, *Ormosia* and *Sorocea*) of the eight species we studied showed significantly lower leaf damage and thus improved herbivory defence when grown in Si-rich compared to Si-poor soil (Figure 3a), consistent with our hypothesis that Si-fed plants should be better defended. Si-based defences showed relatively consistent effect sizes (a 11% to 72% reduction in leaf damage in high compared to low Si soil, i.e. six-fold variation) given that the species' Si uptake capacity varied 30-fold. Additionally, in two species higher leaf Si concentrations were related to lower leaf damage (Figure 3b, a non-significant trend for *Sorocea*), suggesting a direct positive effect of leaf Si on herbivory defence. In one species no association between the changes in leaf damage and leaf Si concentrations emerged, suggesting soil Si improved herbivory defence via changes in other defence types. In four species no effect

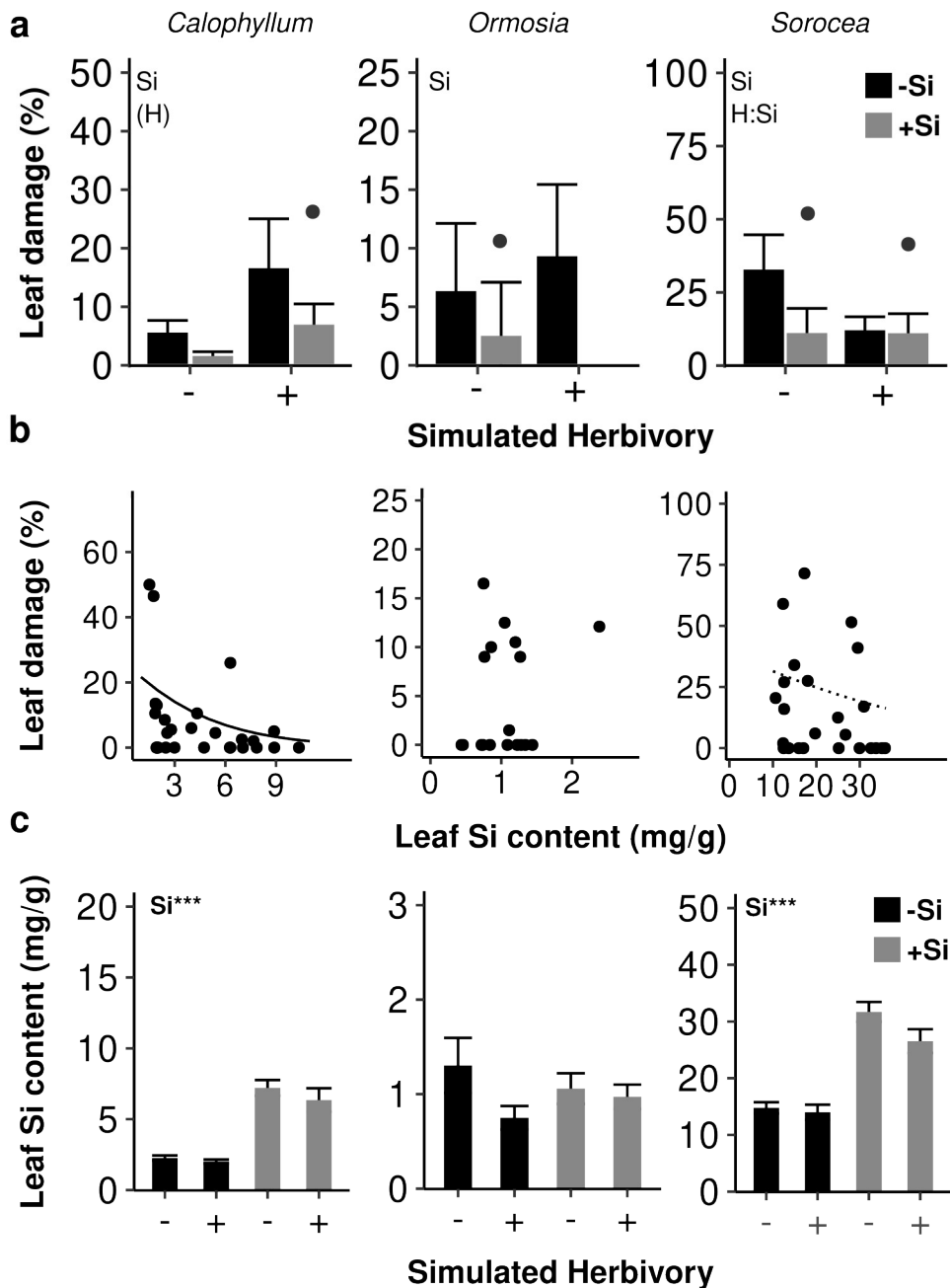


Figure 3: Effect of (a) soil Si (Si), simulated herbivory (H) and their interaction (H:Si) and (b) leaf Si concentrations on leaf damage (% of leaf area removed) in intraspecific multiple-choice feeding trials and (c) effect of soil Si (Si) and simulated herbivory on leaf Si concentrations in the preceding pot experiment. Data are shown for the three species in which we found evidence for Si-based defence. In (a) letters with and without parentheses and in (b) solid and dashed lines indicate significant effects ($p < 0.05$) and trends ($0.05 < p < 0.1$) based on bootstrapped confidence intervals using ZIGLMMs. In (c) results of ANOVA are shown (** $p < 0.001$). For details see manuscript 2. The figures originated from Klotz et al. (2023), which is distributed under the terms of the Creative Commons CC BY 4.0 license. To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>. The panels were rearranged, the colour changed, the letters “a”, “b”, “c” added and the word “concentrations” replaced by “content”.

of Si emerged and in one species increased leaf and soil Si led to increased leaf damage (see *Figure 1* in manuscript 2), indicating Si increased the leaves’ attractiveness to the herbivore. Si-based defences were constitutive throughout as Si uptake was not inducible by simulated herbivory in any of the species we studied (Figure 3c), not consistent with our hypothesis.

To our knowledge we are the first to show that Si-based defences also act in tropical tree species and thus wild dicots. A wealth of studies has demonstrated Si-based defences in grasses, including wild species, and dicot crops (e.g. Massey et al. 2006, 2007b, Ryalls et al. 2017, Islam et al. 2020). Furthermore, the range of effect sizes we observed is comparable to those reported for grasses (Massey et al. 2006). Overall, we found evidence for Si-based defence in 40% of our species, suggesting the effect of Si on herbivory defence may vary across species. This might be because tropical dicots species show strong interspecific variation in herbivory defences, with many defences particularly fending off specialist herbivores (Coley and Barone 1996, Kursar et al. 2009, Becerra 2015). Thus, other defences are more likely to either obscure effects of changing leaf Si concentrations or even reduce the necessity for Si-based defences, which appear to be less targeted than other defences, such as those directed against specialists or mediated by extrafloral nectars (Kursar et al. 2009). Nonetheless, our results show that constitutive Si-based defences act in tropical tree species and should be considered part of the vast array of anti-herbivores defences in tropical tree species.

3.2.1 Potential mechanism underlying Si-based defences

Our results suggests that the mechanisms underlying Si-based defences vary across tropical tree species. For instance, in two species (*Calophyllum* and *Sorocea*) direct effects of amorphous Si deposited in leaves may have mediated the negative effect of increased soil Si on leaf damage. Amorphous Si has been shown to increase the abrasiveness of leaves (Massey et al. 2006, Massey and Hartley 2009, Hartley et al. 2015); a well-established and empirically supported mechanism of Si-based defences. In one species (*Ormosia*), on the other hand, changes in leaf damage were only associated with changes in plant-available Si. Here, the Si fertilization treatment might have caused changes in other plant defence types, such as secondary metabolites and trichomes, and/or changes in C:N ratios, which have all been shown to vary with plant-available Si (McLarnon et al. 2017, Neu et al. 2017, Hall et al. 2019, Singh et al. 2020). According to our data on Si-mediated changes in leaf nutrient status (see above), changes in in leaf C:N ratios did not occur and can be ruled out for this species (see *Table S3* in *Supporting information* of manuscript 1). All in all, variation in plant-available and leaf Si concentrations and variation in environmental factors, leading to intraspecific variation in leaf Si concentrations, might improve seedling defence in tropical forests.

3.2.2 Ecological implications

As herbivore pressure is high in tropical forests and herbivory on mature leaves can cause substantial mortality, especially among seedlings (Eichhorn et al. 2010), effective plant defence is pivotal for the survival of tree seedlings in these habitats. However, in the shady forest understory, where light levels can be as low as 1-2% of full sunlight (Chazdon et al. 1996), C assimilation and growth is largely impeded and leaves need to be well-defended to increase their life-span and make up for the high costs for leaf production (Alvarez-Clare and Kitajima

2007). Yet, leaf defence also largely depends on costly C-based compounds, such as secondary metabolites (Kursar et al. 2009), which can compete with C allocation into growth (Züst and Agrawal 2017). Thus, in this C-limited environment, Si-based defences, especially amorphous Si deposits in leaves, which are metabolically cheaper than C-based defence compounds (Raven 1983) could play an important role in herbivory defence on Si-rich soils. Furthermore, our findings of species-specific effects of soil Si on herbivory defence suggest that spatial variation in soil Si concentrations may influence herbivory defence differently across species. This may lead to species' performance differences across habitats varying in soil Si. Again, such performance differences may eventually contribute to species distribution and community composition (Baraloto et al. 2005, Russo et al. 2005).

3.2.3 Future challenges and research directions

Our findings clearly demonstrated that Si-based defences act in seedlings of tropical tree species. However, to fully appreciate the importance of Si-based defences for tropical tree seedlings in the field, additional processes have to be taken into account. Firstly, Si-based defence may not only improve plant performance but may also have detrimental effects on insect performance and their larvae, because ingested amorphous Si damages their digestive tract impeding nutrient uptake (Massey et al. 2006, Massey and Hartley 2009, Islam et al. 2020). Furthermore, Si-based defence was shown to both increase and decrease predation and parasitism of insect herbivores (Ryalls et al. 2017, Hall et al. 2021, Islam et al. 2022), potentially leading to changes in herbivore pressure on seedlings. Secondly, most herbivory on tropical tree seedlings in the field is restricted to the brief period of leaf development (Coley and Barone 1996). Expanding leaves are not well-defended and they are rich in nitrogen, making them highly attractive for herbivores (Coley and Barone 1996). Furthermore, they might contain only little amounts of amorphous Si as Si accumulation is a function of leaf age (Motomura et al. 2002, but see Schaller et al. 2022). On the other hand, Si-based defences can show an effect within no longer than 72 hours after Si fertilization (Waterman et al. 2021). Thirdly, the effectiveness and/or types of herbivory defence might vary with environmental factors and levels of stress in woody plants (Koricheva et al. 1998). In fact, no difference in herbivory rates between +Si and -Si plants was found across seven species in a field drought experiment (Knauff 2023), potentially because low tissue water content or drought-induced increases in secondary metabolites (Gely et al. 2020) in both treatment groups might have obscured effects of Si-based defences. All in all, to fully appreciate the ecological importance of Si-based defences in tropical forest species, field studies need to be undertaken that specifically look at changes in leaf damage across habitats differing in plant-available Si throughout different seasons.

3.3 Drivers of intraspecific variation in tissue Si concentrations

Increasing plant-available soil Si concentrations increased leaf Si concentrations in more than half of the tropical tree species we studied (ca. 60%), while simulated herbivory had no effect on leaf Si concentration in any species. In temperate forbs and grasses, variation in soil moisture strongly influenced plant Si concentrations. The strength and direction of the effects of plant-available Si and soil moisture on tissue Si concentrations varied across species. The effect of plant-available Si on leaf Si was linear and positive in all species responding to Si addition, but the effect sizes varied across species. By contrast, the effect of soil moisture on plant Si were highly idiosyncratic across forb species and uniform, but non-linear, across grasses, with all grasses showing the lowest Si concentrations under drought, control and flooded conditions.

3.3.1 Soil Si availability

The increase in leaf Si concentrations with increasing plant-available soil Si in most species (see *Figure 2* in manuscript 1) was consistent with our hypothesis and is largely in line with previous work (e.g. Liang et al. 2006, Neu et al. 2017, Greger et al. 2018, Klotz et al. 2021). Most of these studies, however, used a factorial design, mostly consisting of two soil Si levels, with plant-available Si concentrations not always corresponding to or even exceeding the soil concentrations of natural systems. Furthermore, the shape of responses could not be assessed and thus remained unknown. In our study, the strength of leaf Si responses varied across species, with stronger responses in species with a higher Si uptake capacity (Pearson correlation coefficient between the species' leaf Si responses and Si uptake capacity: $r = 0.97$, $p < 0.001$). The different responses probably reflect interspecific variation in the active component of Si uptake and thus different efficiencies of how the species extract mono-silicic acid from the soil solution (Liang et al. 2006, Deshmukh and Bélanger 2016). The species not responding to Si fertilization probably lacked Si membrane channels or functional Si transporter (Deshmukh and Bélanger 2016). Furthermore, the finding that species with high Si uptake capacity are more sensitive to changes in plant-available Si is largely in line with the few previous studies assessing plant Si responses in wild dicot species (Cooke and Leishman 2012, Klotz et al. 2021). In field studies, however, some species with (presumably) low Si uptake capacity even showed negative responses (Cooke and Leishman 2012, Schaller et al. 2018), which was not found in our species. Under natural conditions factors, such as soil moisture (Quigley and Anderson 2014, Quigley et al. 2017), light (Kajino and Kitajima 2021), nutrient availability (Minden et al. 2021) and herbivory (Soininen et al. 2013) additionally influence Si uptake and accumulation, and might modulate the effects of plant-available Si. Overall, leaf Si concentrations of seedlings of tropical tree species, especially those with high Si uptake capacity, should be sensitive to the pronounced spatial variation in plant-available soil Si in tropical forests.

3.3.1.1 Ecological implications

Our findings of effects of plant-available soil Si on leaf Si concentrations suggest that variation in plant-available soil Si may have pervasive consequences for tropical tree seedlings in the field. For instance, variation in leaf Si concentrations in response to varying soil Si might influence herbivory defence (see above). Furthermore, there is evidence showing that some tropical tree species can perform better under UV-stress when their leaf Si concentrations are increased (Rittelmann 2023). Previous work also showed that higher leaf Si concentrations can decrease rates of litter decomposition by microbes and arthropods (Schaller et al. 2014, Nakamura et al. 2022). Over time hampered litter decomposition might reduce nutrient release and availability (Sayer et al. 2020). Decreasing nutrient availability in turn might influence plant performance differently across species (Zalamea et al. 2016). Species adapted to high nutrient availability might experience higher performance losses than those adapted to low nutrient availability (Zalamea et al. 2016) and this might eventually lead to shifts in community composition (Russo et al. 2005).

3.3.2 Soil moisture

Moisture regimes influenced plant Si concentrations in almost all 10 species we studied, specifically four forb and five grass species (see *Figure 2* in manuscript 3). Waterlogging led to the highest concentrations in most species, not supporting our hypothesis, that stressful water regimes, including waterlogging, should decrease plant Si concentrations. In grasses, plant Si responses were stronger and more uniform, with the changes in water regimes explaining most of the variation in plant Si (73%, see *Figure 5* in manuscript 3). In forbs, the species' plant Si responses to moisture regimes were generally weak and highly species-specific. The species x moisture regime interaction, i.e. the species-specific responses in plant Si concentrations to moisture regimes, explained 54% of the variation in plant Si for forbs. Interestingly, in both life-forms species identity explained the least of the total variation in plant Si concentrations (4-7%). The higher importance of intraspecific over interspecific variation, especially in forbs, led to changes in the species ranks of plant Si concentrations in the different moisture regimes, with the ranking of grasses being more stable than those of forbs (see *Table 3* in manuscript 3).

Physiological differences in Si uptake mechanisms can explain the different plant Si responses of grasses and forbs. Grasses typically take up Si actively, mediated by Si transporters, while in forbs, the relative contribution of passive versus active uptake varies more strongly across species (Deshmukh and Bélanger 2016, Deshmukh et al. 2020). Furthermore, Si uptake and its molecular underpinnings are phylogenetically determined, i.e. more closely related species show more similar leaf Si concentrations (Hodson et al. 2005, Deshmukh et al. 2020, Klotz et al. 2021), and potentially more similar responses to variation in moisture regimes. Our results also implied that other factors linked to passive and active Si uptake, like stomatal regulation and energy availability (Liang et al. 2006, Faisal et al. 2012, Deshmukh and Bélanger 2016), may influence plant Si responses to variation in moisture regimes. These factors are strongly

influenced by moisture regimes, with lower energy availability and stomatal conductance under more stressful moisture regimes (Silvertown et al. 2015, Bartlett et al. 2016, Yeung et al. 2019). Furthermore, species vary in how they cope with drought, waterlogging and flooding (Mommer et al. 2006, Zwicke et al. 2015), and thus can show different stomatal responses to changing soil moisture and different physiological responses to changing energy availability – potentially explaining the species-specific responses in forbs. Future ecological studies into the effects of moisture regimes on intraspecific variation in tissue Si concentrations should focus on these factors in order to understand the mechanistic underpinnings of species-specific plant Si responses to moisture regimes.

3.3.2.1 Ecological implications

Species-specific plant Si responses to variation in moisture regimes and changing species rankings in plant Si concentrations can have cascading effects on insect herbivory in temperate grasslands. Given that plant Si can improve herbivory defence in temperate herbaceous species (Massey et al. 2006, Pickelmann 2023), herbivore food preferences might be influenced by soil moisture-induced changes in plant Si concentrations. Furthermore, our results suggest that the species' efficacy of Si-based defences and thus herbivore food preferences may vary across moisture regimes. This should influence species' performance differently across moisture regimes. In fact, a wealth of research has already shown shifts in insect food preferences in response to drought, mediated by concurrent changes in leaf hydration and/or N concentrations (e.g. Huberty and Denno 2004, Gutbrodt et al. 2011). However, whether herbivory rates are more likely to increase or decrease across water regimes, to what degree the change is mediated by leaf hydration and N concentrations and the potential role of flooding, waterlogging and intraspecific variation in tissue Si remains largely unknown (e.g. Huberty and Denno 2004, but see Vandegeer et al. 2021a). Our results suggest that intraspecific variation in tissue Si concentrations may be important and should be accounted for in studies on environmental drivers of herbivory in temperate grasslands and other ecosystems.

3.3.3 Herbivory

We did not find evidence for the induction of Si uptake after simulated herbivory in seedlings of tropical tree species, not consistent with our hypothesis that plants exposed to simulated herbivory should show higher leaf Si concentrations than control plants. By contrast multiple previous studies on wild and cultivated grasses as well as some on dicot crops showed that Si uptake can be induced by artificial and natural herbivory damage (McNaughton et al. 1985, Massey et al. 2007a, Soininen et al. 2013, Hall et al. 2019, Islam et al. 2020), leading to higher leaf Si concentrations and better protection against herbivores. Various methodological and biological reasons might explain our findings. Firstly, the amounts of methyl jasmonate and/or damage might not have been sufficient to elicit plant responses (Hartley and DeGabriel 2016). Secondly, artificial herbivory cannot fully mimic natural herbivory, e.g. due to the absence of saliva, which is known to play a central role in eliciting plant responses and hormonal

signalling after herbivory damage (Hartley and DeGabriel 2016). Thirdly, tropical trees are thought to have evolved mainly constitutive defence types which are more cost-effective than induced defence in habitats with constantly high herbivore pressure (but see Barton 2016, Bixenmann et al. 2016).

3.4 Generalizing effects of Si across species and environments

In this section I discuss our results in the context of trait-based ecology. After that, in section 3.4.1, I present two examples that could advance our ability to generalise effects of Si on the ecology of wild plant species.

The effects of Si on growth, defence and leaf nutrient status we found were highly species-specific and probably mediated by both soil and physiological mechanisms. These species-specific effects preclude generalizations about the effects of Si on wild dicot species. Trait-based ecology is based on the idea that information on functional traits, which can be quantitatively compared across species, communities and ecosystems, rather than species identity, allows for generalized predictions of how, for instance, environment factors influence species distribution and community composition across different scales and ecosystems (Mcgill et al. 2006, Shipley et al. 2016). Functional traits are mechanistically linked to processes maintaining fitness, i.e. growth, survival and reproduction, of an individual and functional trait values should therefore correlate with the different fitness measures within and across species (Violle et al. 2007, Shipley et al. 2016). In terms of Si, a specific trait reflecting the plant Si status that correlates with the beneficial effect of Si on plant performance across and within species, i.e. is a ‘functional’ trait (Violle et al. 2007), should be identified. Although tissue Si concentration, like every other measurable characteristic of the plant Si status (e.g. Si uptake mechanisms), undoubtedly is a plant trait¹, it is unclear whether it is in fact functionally important. Understanding the functional importance of a trait, however, might play a key role for generalizations (Shipley et al. 2016). In the past, any attempts of assigning functional importance to leaf Si concentrations were confounded by the quality and amount of data available. Most data came from single-species studies on a limited selection of mostly fast-growing crop species and grasses (see above). To my knowledge, we were the first to carry out comparative multi-species experiments with wild plant species, which enabled us to evaluate whether plant Si concentrations may be functionally important.

Prior to our work, perhaps the most prominent example attempting to assign functional importance to tissue Si concentrations was done by Cooke and Leishman (2011b). They tested the relationship between leave Si concentrations and the functional traits of the leaf economic

¹ Violle et al. (2007) defined a plant trait as “any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization.”

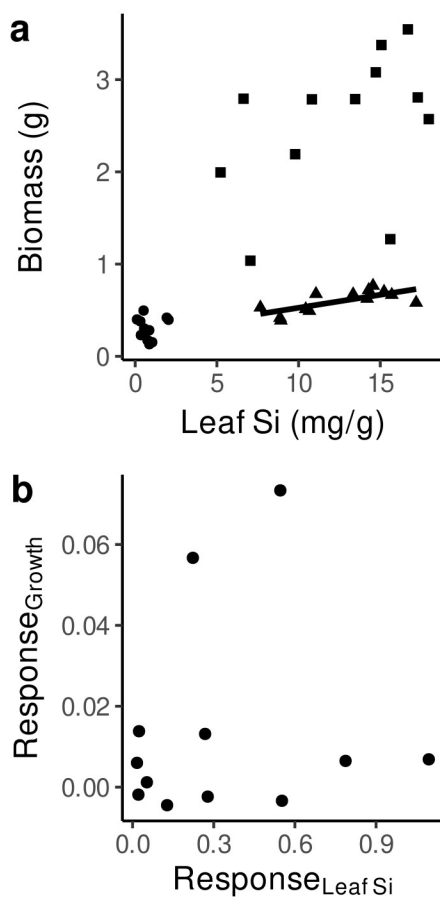


Figure 4: Relationship between (a) leaf Si concentrations and aboveground biomass for the three species with positive growth responses along the soil Si gradient (circle: *Dendropanax*, square: *Theobroma*, triangle: *Herrania*) and (b) between the leaf Si and growth responses across all 12 species of manuscript 1. Solid lines denote a significant Pearson correlation coefficient ($p < 0.05$).

Si concentrations showed a positive correlation with growth in only one out of the 12 species we studied (*Herrania*; $r = 0.72$, $p = 0.006$, Figure 4a) and the strength of responses in leaf Si concentrations and growth did not correlate across species ($r = 0.12$, $p = 0.72$, Figure 4b). Furthermore, the positive growth responses along the soil Si gradient in *Dendropanax* and *Theobroma* were better explained by changes in plant-available Si ($R^2 = 44\%$ & 25% , see Table S3 in manuscript 1) than the parallel increase in leaf Si concentrations ($R^2 = 0\%$ & 11% ; obtained from linear regression of aboveground biomass on leaf Si concentrations for both species). In terms of survival, no positive effect of increased leaf Si concentration was observed in a field drought experiment with seven species (Knauff 2023) - in one species higher leaf Si even decreased survival. Under UV-stress, one out of six tropical tree species showed reduced photo inhibition, yet without positive effect on growth (Rittelmann 2023).

spectrum (Wright et al. 2004) across 155 species. They showed that decreasing leaf-life span correlated positively with leaf Si concentrations across species. They hypothesized that species with short-lived leaves, which typically are fast-growing with an acquisitive resource-use strategy (Westoby et al. 2002, Reich 2014), should take up more Si than species with long-lived leaves, because Si is a metabolically cheap substitute to C-based compounds potentially improving the species' C balance (Cooke and Leishman 2011b). On the other hand and despite the advantages of taking up Si, species with long-lived leaves should take up less Si, because amorphous Si may not be translocated after deposition, is biochemically less versatile than C-based compounds and potentially incurs mass costs (Cooke and Leishman 2011b). Although the correlation between leaf Si concentration and leaf-life span is intriguing and merits focused research (for research directions see Cooke and Leishman 2011b), the non-experimental approach did not allow conclusions about the functional importance of leaf Si concentrations (see Kurze 2023, Seebacher and Franklin 2012).

Despite the amount of experimental work required to thoroughly understand the functional importance of traits (Shiple et al. 2016, Kurze 2023), some authors suggested, more or less explicitly, that plant and leaf Si concentrations are a functional trait (Schoelynck et al. 2014, Katz 2019, de Tombeur et al. 2023), which is largely in conflict with our results, including work of Knauff (2023) and Rittelmann (2023). In our data, leaf

Data on the effect of tissue Si concentrations on seed production of tropical trees is not yet available. All in all, we did not find conclusive support for the idea that leaf Si concentrations are functionally important in tropical dicots, because plant Si concentrations did not consistently correlate with fitness components within and across species.

3.4.1 Future challenges and research directions

3.4.1.1 Morphology and location of amorphous Si deposits

An increasing body of work suggests that the morphology and location of amorphous Si deposits in leaf tissues may play an important role in improving plant performance and defence in Si-rich soils. For example, Si deposition at the epidermis, on stomatal guard cells and in vessel tubes and pits is thought to improve the plant water status under drought (Vandegeer et al. 2021b, Wang et al. 2021). Si-filled trichomes and Si deposition at the leaf surface might contribute to Si-based defence (Hartley et al. 2015, Hall et al. 2020b). Furthermore, Coskun et al. (2019) proposed that Si deposited in cell walls might have a central function in general stress alleviation. Such silicification patterns, on the other hand, can be influenced by soil Si availability (Schaller et al. 2022). Thus, a more thorough understanding of the link between soil Si, the location and morphology of amorphous Si deposits and their specific effects on growth and defence is needed and might enable us to generalize the effects of Si on plant growth and defence across scales, communities and ecosystems without information on species identity. The only information required is data on the number, morphology and location of amorphous Si deposits in leaves. Yet to date, mostly archaeologists and palaeobotanists have been assessing the inter- and intraspecific variation in the morphology of Si deposits in wild plant species (e.g. Carnelli 2001, Fernandez Honaine et al. 2006, Hošková et al. 2022). Future ecological studies, for instance those that aim to generalize the effects of Si across species and environments, should pay more attention to systematic changes in inter- and intraspecific variation in the location and morphology of Si deposits in plant tissues by either collecting the relevant data themselves, by collaborating with archaeologists and palaeobotanists or by screening the available archaeological and palaeobotanical literature.

3.4.1.2 Life stage differences in tropical trees

We assessed the effects of Si on plant growth, herbivory defence and nutrient status as well as the drivers of intraspecific variation in leaf Si concentrations at the seedling stage of tropical tree species. The seedling stage is thought to be the most important life-stage in a species' life cycle (Poorter 2007), because seedling performance determines whether the transition from the seedling to the adult stage and thus the establishment of a species is successful or not in the given environment. Thus, from an evolutionary perspective, species are adapted to environmental conditions in the forest understory rather than in the canopy (Poorter 2007). This not only means that looking at the seedling stage is pivotal to understand the ecological impact of Si, but at the same time, as a species transitions between life stages, ecological

strategies related to growth and defence (Poorter 2007, Wright et al. 2010) and potentially the role of Si might change. Adult trees, on the other hand, play an important role in the overall biomass accumulation of tropical forests (Slik et al. 2013) and might help to mitigate the projected impacts of climate change (Lewis et al. 2015, IPCC 2021). Thus, understanding the effects of environmental factors on adult trees is important as well. Yet, whether the role of Si as potential growth and defence promoter changes between life stages and also plays a role in adult trees is unknown and merits focused research.

3.5 Conclusions

We showed that Si can promote seedling growth and, to a greater extent, herbivory defence in wild tropical tree species. About 40% and 25% of the species we studied showed better herbivory defence and aboveground growth in Si-rich soil, respectively, suggesting that Si-based defences and growth improvements might be common in tropical tree species. Besides defence and growth, we found effects of soil Si on leaf macronutrient concentrations and stoichiometry in about 30% of the species. As our species selection only represented a small proportion of the total species pool of central Panamanian tropical lowland forests, which is well within a several hundreds (e.g. Knight, 1975), a large absolute number of tree species benefiting from Si might exist in central Panamanian tropical lowland forests. We also found

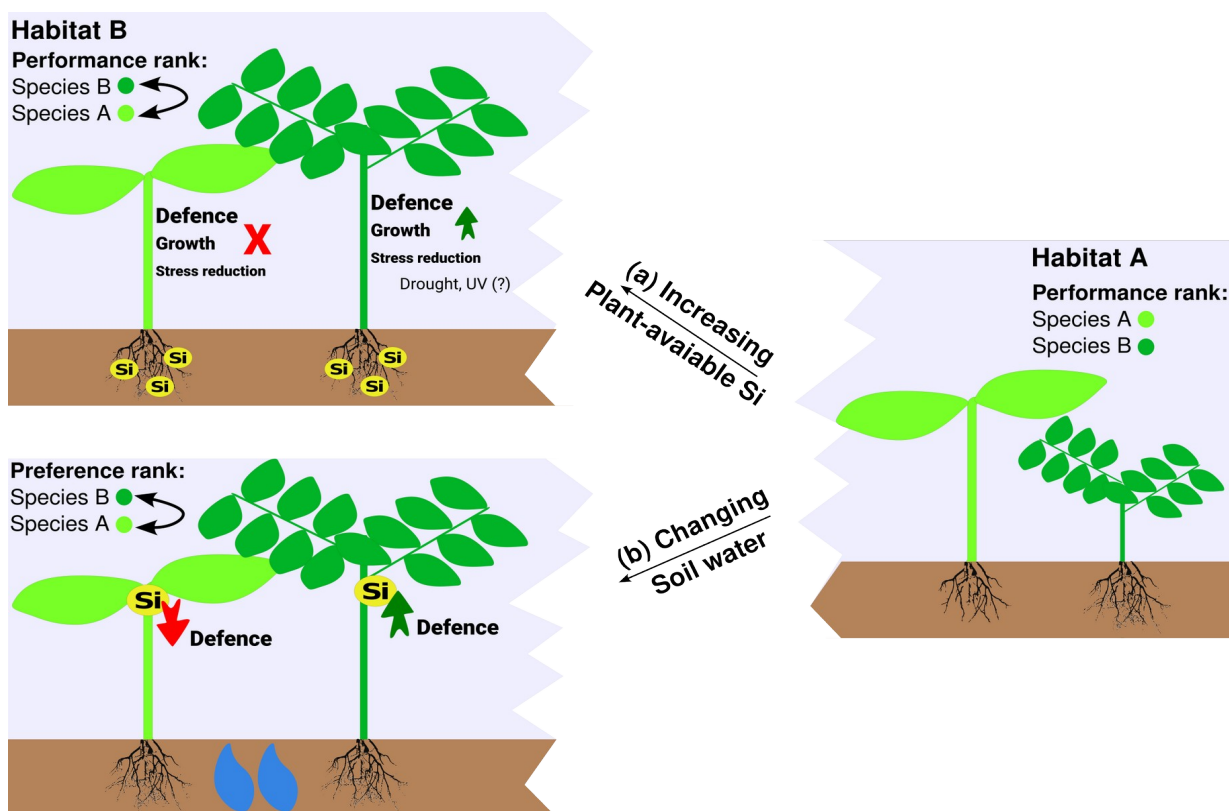


Figure 5: Scheme showing how performance ranks of co-occurring species may change across habitats varying in (a) plant-available soil Si and/or (b) soil moisture. Compared to species A, species B does benefit from Si and may perform better on the Si-rich and/or moister soil in habitat B.

strong effects of plant-available soil Si and soil moisture on tissue Si concentrations in tropical trees or temperate herbaceous species, respectively. Perhaps the most important finding from an ecological perspective is that responses in intraspecific variation in tissue Si to soil Si and moisture as well as the effects of Si on growth, defence and nutrient status were highly species-specific.

Our findings suggest that variation in soil and leaf Si may have important ecological implications in natural systems, such as tropical forests and temperate grasslands. Firstly, variation in plant-available soil Si across and within forest and grassland habitats might differently influence species' performance, either directly through effects of soil Si on plant growth or indirectly through effects of soil or leaf Si on plant defence (Figure 5a). Secondly, temporal and spatial variation in soil moisture, which is very common in tropical forests and temperate grasslands (Comita and Engelbrecht 2014, Silvertown et al. 2015), might cause species-specific tissue Si responses and thus can additionally modulate the efficacy of Si-based defences (Figure 5b), potentially leading to changes plant performance in some species. Apart from that, species-specific Si-induced changes in leaf nutrient stoichiometry, an important driver of litter decomposition and nutrient availability (Cornwell et al. 2008, Sayer et al. 2020), could influence nutrient availability and thus plant performance. As a consequence, variation in soil and plant Si in tropical forests and temperate grasslands could contribute to differences in species performance ranks across habitats varying in plant-available Si, species coexistence and eventually species distribution and community composition.

4 References

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II Manuscript 1

Effects of plant-available soil silicon on seedling growth and foliar nutrient status across tropical tree species

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Abstract

Plant-available silicon (Si) concentrations vary considerably across tropical soils, yet the ecological importance of that variation remains largely unresolved. Increased Si availability can enhance growth and modulate foliar nutrient status in many crop species suggesting similar effects might occur in natural systems. However, how growth, foliar Si and macronutrient concentrations as well as their stoichiometry respond to plant-available Si and how these responses differ across tropical tree species is unknown. We experimentally exposed seedlings of 12 tropical tree species to a gradient of plant-available Si concentrations, representing 85% of the variation found across central Panama, and assessed responses in aboveground growth and foliar nutrient status. Furthermore, we assessed whether higher plant-available Si increases P availability. Increasing plant-available Si led to increased foliar Si concentrations (by up to 140%). It also led to higher aboveground growth (by up to 220%), and it affected foliar C and N concentrations, and nutrient stoichiometry across species. However, at the species level only a small subset of two to four species showed significant growth and foliar nutrient responses. At the soil-level, plant-available P remained unchanged along the experimental soil Si gradient. Our results showed that Si can improve growth and/or modulate foliar nutrient status in a number of tropical tree species. Furthermore, species' growth and foliar nutrient concentrations might vary differently across tropical forest sites varying in plant-available Si. Additionally, Si-induced responses in foliar nutrient stoichiometry have the potential to affect herbivory and litter decomposition. Taken together, natural variation in plant-available Si might influence plant performance unequally across tropical tree species, and change trophic interactions, with potential implications for ecosystem processes.

1 Introduction

Plants require nitrogen (N), phosphorus (P) and carbon (C) to maintain protein production, photosynthesis and growth (Lambers and Oliveira 2019). Their foliar concentrations and stoichiometric ratios govern important ecological processes, including herbivory and litter decomposition (Poorter et al. 2004, Coley et al. 2006, Cornwell et al. 2008), and any changes in these concentrations or ratios can have pronounced consequences at the individual, species and community level. Foliar macronutrient concentrations and their stoichiometry are physiologically, biochemically and genetically constrained (Elser et al. 2010), yet they respond plastically to environmental factors, such as changing soil chemistry (Reich and Oleksyn 2004, Mayor et al. 2014). The availability of Si to plants has also been shown to modulate foliar nutrient status, to promote biomass production and to alleviate multiple abiotic and biotic stressors (Cooke and Leishman 2016, Neu et al. 2017, Frew et al. 2018, 2019, Greger et al. 2018). Such effects of Si have been studied primarily in agricultural species, where Si fertilization is widely used to improve plant performance and resilience (Cooke and Leishman 2011). Yet, potential effects of Si on plant growth and foliar nutrient status remain understudied in natural systems, such as tropical forests.

Tropical forests display a remarkable tree species diversity exhibiting pronounced variation in foliar chemistry (Hättenschwiler et al. 2008, Wright 2019). Recent studies demonstrated that concentrations of plant-available Si vary considerably in tropical soils, and that trees show pronounced inter- and intraspecific variation in foliar Si concentrations (Schaller et al. 2018, Ishizawa et al. 2019). Maximum foliar Si concentrations in these trees were similar to those of high-accumulating grass species (Ma and Yamaji 2006), which have long been the main focus of Si research (Cooke and Leishman 2011). The ecological consequences of variation in plant-available and foliar Si might be pervasive considering that Si can alleviate important stressors that shape species performance and distribution in tropical forests, such as P limitation (Condit et al. 2013, Wright 2019), herbivory (Eichhorn et al. 2010, Bagchi et al. 2014) and drought (Engelbrecht et al. 2007, Condit et al. 2013). To advance our understanding of the ecological role of Si in tropical forests, we need to elucidate the responses of plant growth, foliar Si and macronutrient concentrations as well as stoichiometry to plant-available Si, and how responses differ across species.

Plant roots take up Si as dissolved mono-silicic acid from the soil. Then, Si is carried to aboveground organs with the transpiration stream, where it accumulates and forms amorphous Si deposits (Raven 1983, Ma and Yamaji 2006). In most species and under experimental conditions higher plant-available Si routinely led to increased foliar Si concentrations (Liang et al. 2006, Neu et al. 2017, Greger et al. 2018, Klotz et al. 2021). The contributions of different Si uptake mechanisms, i.e. active Si uptake by transporters versus passive uptake by diffusion, vary across species and determine their capacity to take up Si and to respond to plant-available Si (Liang et al. 2006, Deshmukh and Bélanger 2016). Indeed, in temperate herbaceous species, the ability to respond to plant-available Si was related to the physiological capacity for Si uptake, with stronger responses in species with higher Si uptake capacity (Klotz et al. 2021). As with previous studies, tropical tree species with high Si uptake capacities should show stronger foliar Si responses to increasing plant-available Si.

Performance-enhancing effects of increased plant-available Si were reported across species and families showing a wide range of Si uptake capacities (Cooke and Leishman 2016, Frew et al. 2018). They can result either from altered physiological and/or soil processes. Si fertilization has been shown to improve several physiological parameters, including photosynthesis, nutritional status and antioxidant activity (reviewed by Frew et al. 2018). Yet, the mechanistic interactions between these physiological parameters and Si, and how they influence growth, are far from clear (Coskun et al. 2019). Alternatively, Si-induced changes in soil processes might be the reason for the enhanced growth observed. For instance, dissolved mono-silicic acid has been shown to increase plant-available P by displacing inorganic P from binding sites on soil minerals (Schaller et al. 2019, 2020b). Work on tropical soils in Panama showed a positive correlation between plant-available Si and P, suggesting that a similar mechanism may exist in the tropics (Schaller et al. 2018), yet experimental evidence is missing.

Tropical trees should be particularly responsive to Si-induced increases in plant-available P, which is generally low in tropical soils, making P limitation of tree growth pervasive (Vitousek et al. 2010, Mayor et al. 2014, Wright 2019). However, the strength of foliar P and growth responses varies considerably across species (Mayor et al. 2014, Zalamea et al. 2016), which is attributed to their diverse array of P uptake strategies (Zalamea et al. 2016). For instance, species associated to P-poor soils invest substantial energy into foraging and mining strategies to access chemically unavailable forms of soil P (Reichert et al. 2022). As a result, in these species, foliar P and growth responses to Si-induced increases in plant-available P should be weak (Zalamea et al. 2016). By contrast, species associated to P-rich soils rely entirely on plant-available P and should exhibit a stronger Si-induced increase in foliar P concentrations and growth as well as decrease in foliar N:P ratios (Mayor et al. 2014, Zalamea et al. 2016).

Besides potential interactions between Si and P, Si can also influence foliar C concentrations. Negative correlations between plant C and Si concentrations within species have been shown in many field and experimental studies (reviewed by Hodson and Guppy 2022). Substitution of C-based compounds (e.g. lignin and phenols) by metabolically cheap and functionally equivalent Si deposits is a widely held, yet still controversial, explanation for that pattern (Cooke and Leishman 2011, reviewed by Hodson and Guppy 2022). In this way plants may allocate more C into growth and reproduction (Raven 1983), which should particularly benefit species in low resource environments (Quigley et al. 2020). Seedlings of tropical tree species are strongly limited by light, nutrients and occasional droughts in the forest understory (Engelbrecht et al. 2007, Rüger et al. 2009, Vitousek et al. 2010), and thus should benefit from Si substitution of C-based compounds. If Si substitutes C in tropical seedlings, increasing plant-available Si should lead to reduced foliar C concentrations with parallel changes in foliar C:N and C:P ratios. The responses in foliar Si and C should be coupled, so that species with stronger increases in foliar Si concentrations should show more pronounced decreases in foliar C concentrations.

Up to now, most experimental studies on how plant-available Si influences growth and foliar nutrient status focussed on single species, mostly grasses and/or agricultural species (e.g. for tropical species: *Theobroma*, Gómez-Vera et al. 2021, *Coffea*, Parecido et al. 2022, *Oryza*, de Tombeur et al. 2021). Yet, to understand Si's ecological role in species-rich tropical forests, the growth and foliar nutrient responses must be compared across species. Additionally, most studies compared only two soil categories, i.e. Si-enriched versus non-manipulated soil, and thus cannot reflect responses to the wide range of plant-available Si found across tropical landscapes (Schaller et al. 2018, Ishizawa et al. 2019). To capture the strength and shape of responses (Kreyling et al. 2018) and to assess ecological consequences of variation of soil Si in tropical forests, studies of nutrient and growth responses along a gradient of plant-available Si are necessary.

Here, we comparatively assessed growth and foliar chemistry in seedlings of 12 tropical tree species along an experimental soil Si gradient, mirroring the wide range of plant-available Si of lowland tropical forests in central Panama. We tested the following hypotheses: 1) responses in foliar Si concentrations to plant-available soil Si vary across species, with stronger responses in species with higher Si uptake capacity, 2) increased plant-available soil Si should increase plant-available soil P, foliar P concentrations and growth, if plant-available soil Si displaces P from soil binding sites and thereby improves P provisioning. Species associated to P-rich soils should show stronger responses than those associated to P-poor soils, 3) if foliar Si substitutes foliar C, increased plant-available soil Si should decrease foliar C concentrations, with more negative responses in species showing strong foliar Si responses, and 4) furthermore, increased plant-available soil Si should lead to changes in foliar nutrient stoichiometry, reducing foliar N:P, C:N and C:P ratios in species experiencing Si-induced improvements in P nutrition and/or substitution of foliar C.

2 Materials and methods

2.1 Study site and overview

We conducted a Si fertilization experiment with potted seedlings of 12 tree species in Gamboa, Panama (09°07'N, 79°42'W), which has a moist tropical climate with a mean annual temperature of 27°C and a mean annual rainfall of 2100 mm (Slot and Winter 2017). To assess the strength and shape of plant responses to plant-available soil Si and to compare them across species, we used a non-replicated gradient design (Kreyling et al. 2018), with one individual per species randomly assigned to each of 14 soil Si levels, i.e. 14 plants per species and 168 plants in total.

2.2 Study species and plant material

We studied species from moist tropical forests in central Panama (Table S1). Species were selected based on the following criteria: 1) a wide range of foliar Si concentrations (based on leaf collections of plants within a 50 ha forest plot in the area, K. Kitajima et al. unpubl.) suggesting different physiological Si uptake capacities, 2) a wide range of habitat associations to plant-available P (based on species occurrence records along a regional gradient of plant-available soil P; Condit et al. 2013), 3) shade-tolerant species (Rüger et al. 2009), which contribute the largest proportion of species in the area, and 4) availability of seeds before the onset of the experiment. The focal species were in 10 different plant families. In the following we refer to the species by their genus name.

Seeds were collected in forests of the Panama Canal area in October and November 2019 from at least three individual trees per species with a minimum distance of 100 m between them.

Seeds were germinated and raised until cotyledon stage or development of first foliage leaves in trays on a substrate consisting of 50% local forest soil and 50% washed river sand.

Seedlings were then transplanted into individual pots (diameter: 6.5 cm, depth: 36 cm) with the experimental substrates. For each species, we selected individuals of similar size (height and number of leaves) to reduce variation in the initial biomass across individuals. Species were transplanted in two batches which grew for ca 7.5 and 10 months in the experimental substrates, respectively. Species batches explained only negligible variation when included as random factor in the statistical models and were therefore not distinguished in further analyses.

2.3 Experiment

To manipulate plant-available Si of the base substrate we added hydrophilic pyrogenic silicon dioxide (Aerosil 300, Evonik Industries AG). Aerosil 300 closely resembles the chemical and physical properties of biogenic amorphous Si (Schaller et al. 2020a) and supplementing soils with Aerosil has been shown to increase plant-available Si (Klotz et al. 2021) without changing soil pH (J. Schaller unpubl.). The base substrate was a 1:1 mixture of sieved local forest soil with very low plant-available Si and P (Cerro Pelado, selected based on Schaller et al. 2018 and Condit et al. 2013) and thoroughly washed river sand. To create the 14 experimental substrates we added Aerosil 300 in amounts ranging regularly between 0 and 45 g l⁻¹ to the base substrate. The local soil, washed river sand and amorphous Si were thoroughly homogenized using a cement mixer. Addition of Aerosil 300 led to a linear increase of plant-available Si concentrations, ranging from 5.23 to 26.54 mg kg⁻¹ in the 14 experimental substrates and covered about 85% of the natural variation of plant-available Si found in forest soils in the region (2.25–39.88 mg kg⁻¹; Schaller et al. 2018). Adding the sand to the forest soil reduced plant-available P concentrations to 0.47 mg kg⁻¹. To ensure survival of species associated to P-rich soils, we fertilized each plant using 20 ml of a half strength Hoagland solution three times within the first three months of the experiment. Throughout the experiment, plants were regularly watered and kept under intermediate light conditions (ca 10% full sunlight) and protected from rainfall. The position of species and treatments was randomized.

We sampled each of the 14 thoroughly mixed experimental substrates (one sample per Si level) before the experiment to analyse the maximum plant-available Si concentration the plants were exposed to. To ensure the detection of interactions between plant-available Si and P in the soil, we additionally analysed plant-available P in the experimental substrates (without fertilization or plants) at the end of the experiment.

At the end of the experiment, leaves and stems of each individual were harvested, cleaned to remove any potential residual soil material, oven-dried for 48 h at 65°C, and weighed to

quantify aboveground growth. Foliar Si, C, N and P concentrations were analysed, and foliar stoichiometric ratios calculated. Total foliar nutrient contents were calculated multiplying the respective foliar concentration by foliar biomass.

2.4 Chemical analysis of soils and leaves

2.4.1 Silicon and phosphorus

Samples of the experimental substrates were air-dried, crushed to break up large aggregates, and sieved to remove roots and stones. Plant-available Si was extracted in CaCl₂ following Schaller et al. (2018). Three g of sifted soil were shaken with 30 ml of 0.01 M CaCl₂ for 1 h at ambient laboratory temperature. The suspension was centrifuged (8000×g, for 10 min) and the supernatant decanted.

Plant-available P was extracted following the Mehlich-III method (Sims 1989). Five g of sifted soil was extracted using 10 ml g⁻¹ Mehlich III solution (0.015 M NH₄F, 0.001 M EDTA, 0.25 M NH₄NO₃, 0.00325 M HNO₃, 0.2 M HAc). Samples were shaken for 5 min at 200 min⁻¹ and centrifuged for 5 min at 10000×g. Afterwards, the supernatant was filtered using a 0.2 µm cellulose acetate filter.

Leaves were ground for chemical analyses. Foliar Si was extracted for five h by an alkaline method using 30 mg of leaf material and 30 ml of 0.1 M sodium carbonate solution (Na₂CO₃) in a regularly shaken water bath at 85°C (Katz et al. 2021). The solution was centrifuged (3000×g, for 5 min) and passed through a 0.45 µm cellulose acetate filter (Rausch 2021). Foliar P was extracted by digesting the leaf material with HNO₃ and H₂O₂ in a closed vessel microwave digestions system (Mars express6, CEM, Germany).

The Si and P concentration of the leaf and soil extracts was determined with inductively coupled plasma optical-emission spectrometry (ICP-OES) using an elemental analyser.

2.4.2 Carbon and nitrogen

Foliar C and N concentrations were analysed with EA-IRMS coupling (Elemental Analyzer NA 1108, CE Instruments; Interface ConFlo III, Finnigan MAT; isotope ratio mass spectrometer: delta S, Finnigan MAT) and calculated from samples weights and peak areas as detailed by Liebel et al. (2010).

2.5 Statistical analysis

Data of individuals that did not survive the experiment or produced extremely low aboveground biomass (individuals below the 5% percentile for aboveground biomass) were removed before analyses. We calculated pairwise Spearman rank correlations among all parameters (foliar nutrient concentrations, total nutrient contents and stoichiometry, and foliar and total aboveground growth) across all species to check for collinearity (Figure S5). We excluded foliar growth and total nutrient contents from further analyses due to their high positive correlation with total aboveground growth ($r > 0.8$), and excluded Si:C ratios based on their strong correlation with foliar Si concentrations ($r = 0.99$).

To assess effects of increasing plant-available Si (treatment) on foliar Si and aboveground growth as well as on foliar P, C, and N concentrations and their stoichiometry (N:P, C:N and C:P), we used linear mixed-effects models including species as random effect ($n = 151$). To evaluate whether the effects of plant-available Si on growth and foliar nutrient status varied across species, we ran ANCOVAs and tested for species \times treatment interactions ($n = 151$). Furthermore, to assess the direction and strength of each species' response in each parameter, we ran separate ordinary linear models to get the respective slope estimate (β , $n = 11-13$). We determined the species' capacities to take up Si from the linear models for foliar Si, using their fitted foliar Si concentration at maximum plant-available Si. We used a threshold value of 5 mg g⁻¹ to separate species with low and high Si uptake capacities (for similar approach compare Ishizawa et al. 2019). To test if experimentally increased plant-available Si leads to higher plant-available P, we ran an ordinary linear model ($n = 14$). We applied graphical model diagnostics to ensure normality and homogeneity in residuals. If necessary, we log₁₀-transformed the respective response parameter. The % increase (or decrease) in the parameters under the highest compared to the lowest Si availability (based on fitted values) is presented in the text where appropriate.

We tested if the responses in foliar Si and C concentrations are related to the species' Si uptake capacities by correlating the slopes of the single-species models (i.e. responses in foliar Si and C) to the fitted foliar Si concentrations at maximum plant-available Si (Pearson correlation, $n = 12$). We also tested if the responses in aboveground growth and foliar P concentrations are related to the species' habitat association to plant-available P (i.e. P effect sizes from Condit et al. 2013) by correlating them with the slopes of the single-species models (i.e. responses in aboveground growth and foliar P; Pearson correlation, $n = 11$).

All statistical analyses were performed in R version 4.1.0 (R Core Team 2021). Linear mixed-effect models were fit using the 'lmerTest' package (Kuznetsova et al. 2017).

3 Results

Table 1: Effects of plant-available Si on foliar Si concentration, aboveground growth (BM), foliar P, C and N concentrations and foliar N:P, C:N and C:P ratios across the 12 study species. Given are the results of linear mixed-effect models. Foliar Si, C, C:N and C:P as well as aboveground growth were log₁₀-transformed. Significant p-values ($p \leq 0.05$) and p-values indicating weak evidence for an effect ($0.05 < p \leq 0.1$) are marked in bold and italics.

	β	SE	df	t	p
Si	0.013	0.002	139.00	6.61	<0.001
BM	0.005	0.002	139.07	2.44	0.016
P	-0.000	0.003	139.20	-0.09	0.931
C	-0.000	0.000	139.10	-1.97	<i>0.052</i>
N	-0.008	0.003	139.08	-2.43	0.017
N:P	-0.088	0.042	139.16	-2.09	0.039
C:N	0.001	0.001	139.10	1.88	<i>0.063</i>
C:P	-0.000	0.001	139.10	-0.43	0.670

Of the 12 focal species, eight reached foliar Si concentrations considered to reflect a high physiological Si uptake capacity ($> 5 \text{ mg g}^{-1}$), while the remaining species exhibited low Si uptake capacities. Overall, increasing plant-available Si led to increased foliar Si concentrations and higher aboveground growth, and it affected foliar C and N concentrations, and nutrient stoichiometry (Table 1). All responses were linear and we did not find any indication for non-linear responses, e.g. patterns in the data or model residuals indicating saturation or hump-shaped relationships. However, effects were concentrated on four of the 12 species and were strongly species-specific.

Below, we will present for each of the analysed parameters the overall effect of plant-available Si (treatment effect in linear mixed effect model with species as random effect), whether effects differed across species (species \times treatment in ANCOVA), how many individual species responded to soil Si (linear models for single species) and how the species' responses correlate with their Si uptake capacities or habitat associations to plant-available P.

Foliar Si concentrations increased significantly with plant-available Si across species (Table 1) and the direction of the species' responses was consistent (no species \times treatment interaction, Table S2). The increase was significant in seven species (Figure 2a, Table S3), which displayed a

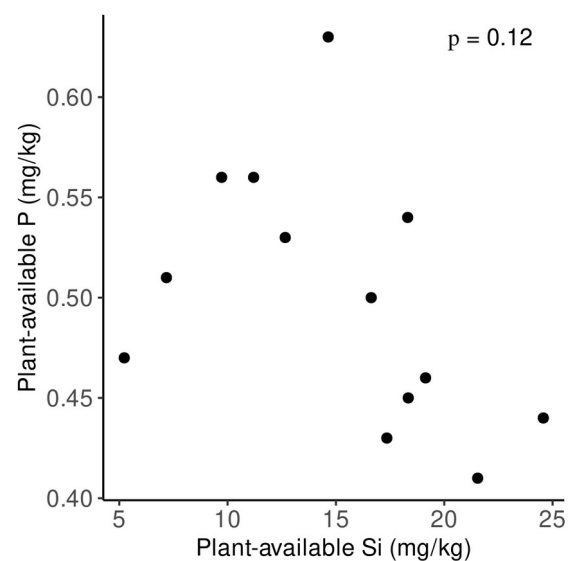


Figure 1: Relationship between plant-available P and Si concentrations along the experimental soil Si gradient ($n = 14$).

64–140% increase in foliar Si under the highest compared to the lowest Si availability. For three other species there was weak evidence (i.e. $0.05 \leq p \leq 0.10$, compare Muff et al. 2022) for an increase in foliar Si concentrations. Only two species showed no response. In line with our first hypothesis, the relationship between the species' foliar Si responses to soil Si (β in Table S3) and their fitted foliar Si concentration at maximum plant-available Si, reflecting their physiological uptake capacity, was positive and statistically significant (Figure 2b).

Aboveground biomass, reflecting growth over the experimental period, increased significantly with plant-available Si across species (Table 1), and the species' responses were again consistent (no species \times treatment interaction, Table S2). In *Herrania* and *Dendropanax* the growth increase with plant-available Si was significant (increase by 63 and 220%, respectively; Figure 3a, Table S3). In one species (*Theobroma*) there was weak evidence for an increase in growth along the soil Si gradient (increase by 79%). Yet, at the soil-level, increasing plant-available Si did not enhance P availability to plants ($\beta = -0.005$, $t = -1.70$, $p = 0.117$; Figure 1), as we had hypothesized. Furthermore, no relationship emerged between the species' growth responses and their habitat association to plant-available P (Figure 3b).

Increasing plant-available Si did not influence foliar P concentrations across species, but significantly decreased foliar N. We found weak evidence that plant-available Si decreases foliar C concentrations (Table 1). Significant, but opposite, responses in foliar P concentrations occurred in two species, with *Sorocea* showing an increase of 52% and *Theobroma* showing a 50% reduction (Figure 4a, Table S3).

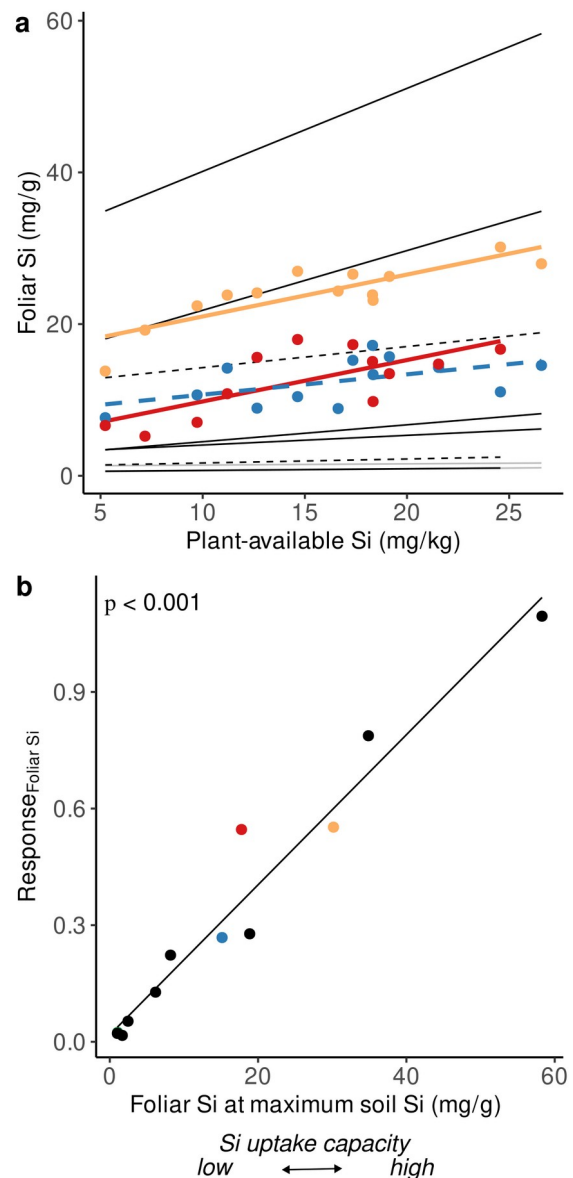


Figure 2: (a) Relationship between plant-available Si and foliar Si concentrations for 12 tropical tree species and (b) correlation ($r = 0.97$) between the species' foliar Si responses to increasing plant-available Si (Response_{Foliar Si}, β of species model) and their fitted foliar Si concentrations at maximum soil Si. Black and coloured lines indicate significant relationships (solid line; $p \leq 0.05$ in (a) and $p \leq 0.001$ in (b)) and relationships with weak evidence in the data (dashed line, $0.05 < p \leq 0.10$). Grey lines indicate relationships without evidence. In (a) p-values are based on species-level ordinary linear models and in (b) on Pearson correlation. Colours indicate species highlighted in the text: *Sorocea* (yellow), *Theobroma* (red), *Dendropanax* (green) and *Herrania* (blue). In (b) the solid line represents a fitted linear regression for visualization.

Nevertheless, no species \times treatment interaction emerged for foliar P (Table S2). Responses in foliar C concentrations were species-specific (species \times treatment interactions, Table S2) with four species showing a significant decrease between 4 and 13% (Figure 4b, Table S3), consistent with our hypothesis. Foliar N responses did not vary across species (no species \times treatment interaction, Table S2), yet we found a significant reduction by 14 and 21% in foliar N along the soil Si gradient in two species. In two other species, there was weak evidence for a decrease in foliar N concentrations (Figure 4c, Table S3). No relationship emerged between foliar P responses and the species' habitat association to plant-available P ($r = -0.16$, $p = 0.64$). The relationship between foliar C and Si responses was negative and significant ($r = -0.60$, $p = 0.04$).

As for foliar nutrient stoichiometry, foliar N:P decreased significantly and there was weak evidence for an increase in C:N ratios with plant-available Si across species (Table 1), while C:P ratios remained unaffected. The responses did not vary across species (no species \times treatment interaction, Table S2). In *Sorocea* and *Theobroma* significant responses in foliar N:P and C:P ratios emerged, but with opposite direction. *Sorocea* displayed a 47% decrease in foliar C:P and a 39% decrease in N:P ratios (from 22 to 16) along the soil Si gradient, while in *Theobroma* foliar C:P increased by 46% and N:P by 33% (from 9 to 12; Figure 5a, c, Table S3). In two other species (*Herrania* and *Dendropanax*) we found weak evidence for increases in foliar C:N ratios (Figure 5b, Table S3).

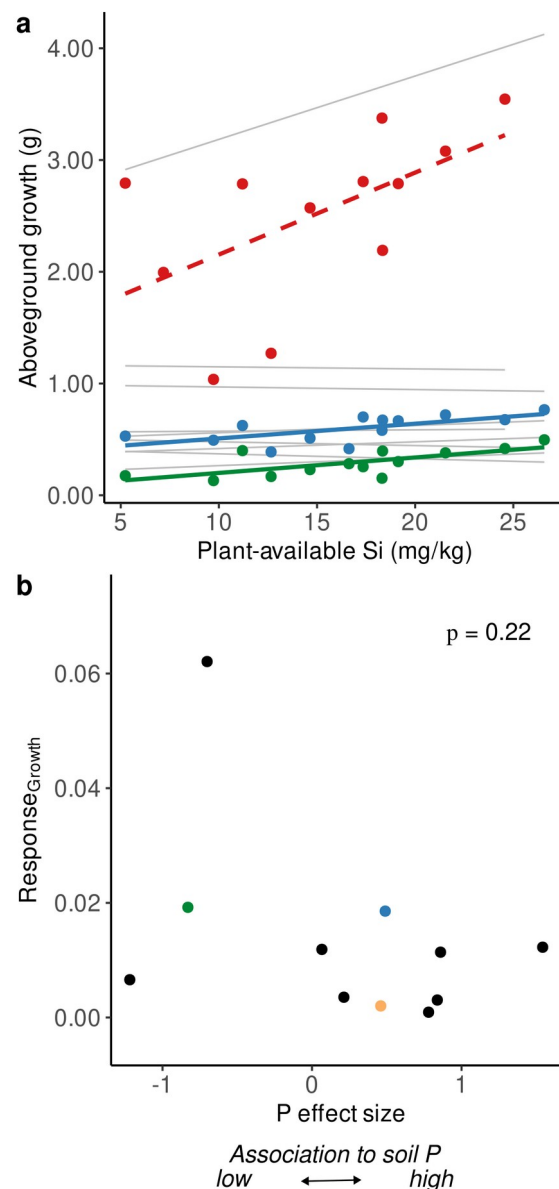


Figure 3: (a) Relationship between plant-available Si and aboveground growth for 12 tropical tree species, and (b) correlation ($r = -0.40$, $p = 0.22$) between the species' aboveground growth responses to increasing plant-available Si ($\text{Response}_{\text{Growth}}$, β of species model) and their habitat associations to plant-available P (P effect sizes in Condit et al. 2013). In (a) coloured lines indicate significant relationships (solid line, $p \leq 0.05$) and relationships with weak evidence in the data (dashed line, $0.05 < p \leq 0.1$). Grey lines indicate relationships without evidence. In (a) p-values are based on species-level ordinary linear models and in (b) on Pearson correlation. Colours indicate species highlighted in the text: *Sorocea* (yellow), *Theobroma* (red), *Dendropanax* (green) and *Herrania* (blue).

4 Discussion

Increasing plant-available Si led to increased foliar Si concentrations and higher aboveground growth, and it affected foliar C and N concentrations, and nutrient stoichiometry across species. More than half of the species showed positive growth responses or changes in foliar macronutrient concentrations and/or stoichiometry along the experimental soil Si gradient. Yet, within the single parameters only a small subset of two to four species showed significant responses.

4.1 Species-specific foliar Si responses to plant-available Si

Responses in foliar Si concentrations to increasing plant-available Si were highly species-specific and stronger in species with high Si uptake capacities. Both active, transporter-driven and passive, transpiration-driven, Si uptake determine a species' capacity to take up Si (Liang et al. 2006), and their relative contributions vary substantially across species (Liang et al. 2006). Since we kept environmental factors known to influence passive Si uptake (e.g. light

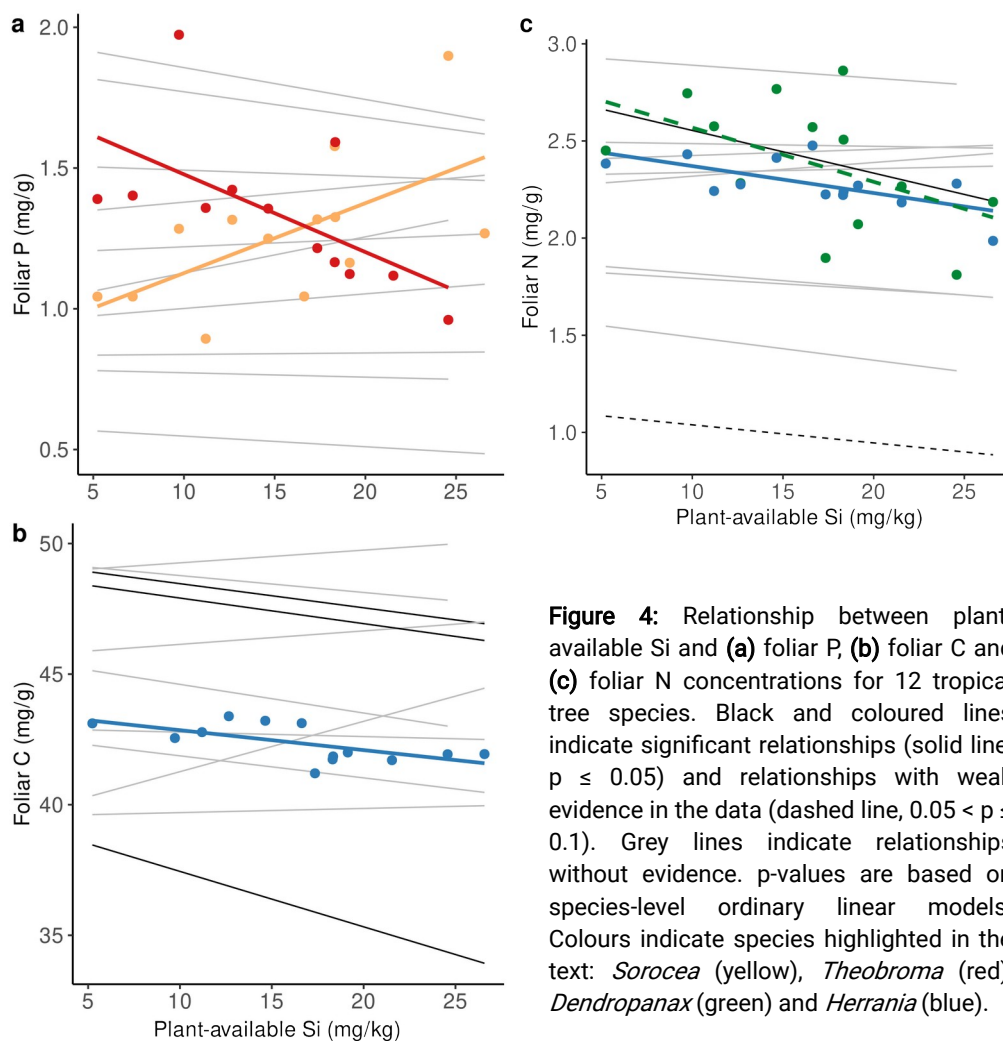


Figure 4: Relationship between plant-available Si and (a) foliar P, (b) foliar C and (c) foliar N concentrations for 12 tropical tree species. Black and coloured lines indicate significant relationships (solid line; $p \leq 0.05$) and relationships with weak evidence in the data (dashed line, $0.05 < p \leq 0.1$). Grey lines indicate relationships without evidence. p-values are based on species-level ordinary linear models. Colours indicate species highlighted in the text: *Sorocea* (yellow), *Theobroma* (red), *Dendropanax* (green) and *Herrania* (blue).

and water availability; Kajino and Kitajima 2021, Quigley and Anderson 2014), constant across species and along the soil Si gradient, the species-specific foliar Si responses we observed were probably driven by physiological differences in active instead of passive Si uptake. Only two species did not respond to plant-available Si and their Si uptake capacities were the lowest of all species studied. These species might lack membrane channels (aquaporins) permeable to Si and facilitating the diffusion of Si, and/or functional active Si transporters (Deshmukh and Bélanger 2016). Furthermore, in line with responses found in temperate grassland species (Klotz et al. 2021), but contrary to findings in a crop species (Faisal et al. 2012), we found no indication that species with high Si uptake capacity upregulated Si uptake under low soil Si availability, e.g. to maintain possible performance-enhancing effects of high foliar Si concentrations. Interestingly, the Si uptake capacities determined in our study correlated with mean foliar Si concentrations measured in conspecifics on Barro Colorado Island, Panama ($r = 0.71$, $p = 0.02$), within a 50 ha forest plot (K. Kitajima et al. unpubl.). In line with our findings, another field study in the area showed that species with low Si uptake capacities had relatively weak and inconsistent foliar Si responses across sites differing in plant-available Si (Schaller et al. 2018). Hence, field data may provide a useful proxy for Si uptake capacities of species and their foliar Si responses to changing plant-available Si. Overall, our data suggests pronounced foliar Si responses across sites varying in plant-available Si are more likely in species with high Si uptake capacities, while species with low Si uptake capacities might not respond and/or their foliar Si responses might be overruled by other environmental factors.

4.2 Effects of Si on seedling growth of tropical tree species

Increasing plant-available Si led to overall higher aboveground growth across species. Nevertheless, at the species level, evidence of a positive (and with about 60–220% rather strong) growth response emerged only in three of the 12 species studied. Performance-enhancing effects of Si have been studied mainly in agricultural species (Cooke and Leishman 2011, 2016), which are typically fast-growing and exposed to high resource availability. In contrast, shade-tolerant tropical forest species are slow-growing and their performance is strongly limited by multiple environmental factors, including light and nutrients (Rüger et al. 2009, Vitousek et al. 2010, Wright 2019). Studies have demonstrated positive effects of Si fertilization on the growth of cultivated tropical tree species, specifically cacao *Theobroma* (Gómez-Vera et al. 2021) and coffee *Coffea* (Parecido et al. 2022). Yet, to our knowledge, we are the first to show positive growth responses to plant-available Si in tropical tree species not used for agriculture (*Herrania* and *Dendropanax*). Furthermore, these responses emerged in species with both low and high Si uptake capacities, consistent with previous work showing that a high physiological Si uptake capacity is not necessary for plants to gain beneficial effects from Si (Cooke and Leishman 2016). The effect of Si in plants might depend on whether Si is primarily bound as amorphous Si deposits which consume high amounts of mono-silicic acid or remains dissolved and thus potentially more reactive (but see Coskun et al. 2019), in which case low amounts of Si might be sufficient to cause beneficial effects. All in all, considering the vast local- and regional-scale variation in plant-available Si in tropical forests, the effects of

Si on the growth of seedlings of tropical tree species might have consequences for species performance rankings across sites varying in plant-available Si.

We specifically tested whether seedling growth was improved by Si-induced increases in plant-available P. No such effect was observed, i.e. Si fertilization did not increase plant-available P nor foliar P, and growth responses were independent of the species' habitat association to plant-available P. Our substrate was extremely P-poor and, as a result, the amount of P bound to soil minerals may have been insufficient to allow for P release through competition with dissolved mono-silicic acid – a mechanism experimentally shown in temperate and arctic soils with substantially higher total P concentrations (Schaller et al. 2019, 2020b). Whether a comparable mechanism may occur in tropical soils with higher P concentrations remains to be investigated. Since plant-available P was not increased in our study, the growth enhancements we observed must have been due to modulation of physiological mechanisms along the soil Si gradient. Many studies, including those on cacao and coffee, reported that Si increased photosynthesis (Cooke and Leishman 2016, Gómez-Vera et al. 2021) and nutrient use efficiency (Neu et al.

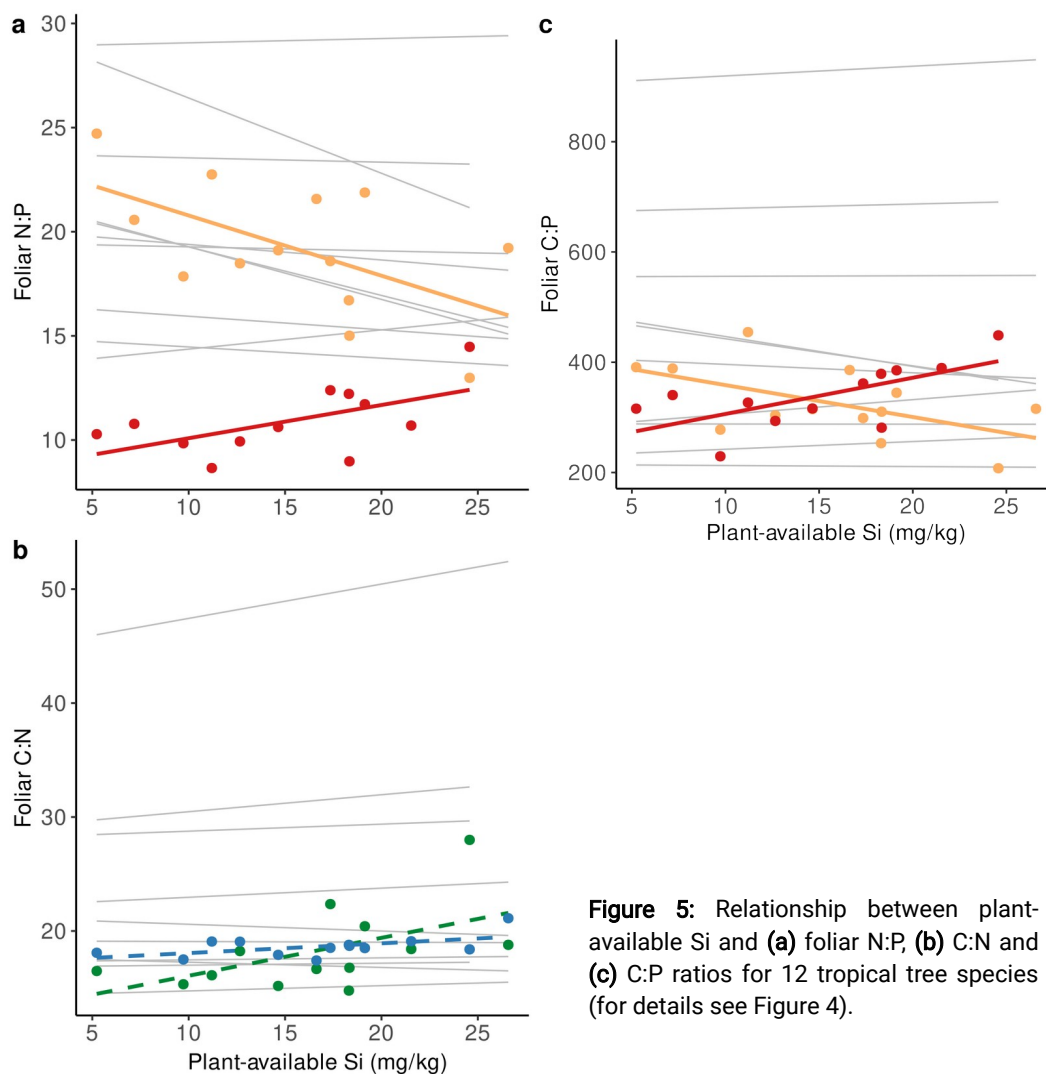


Figure 5: Relationship between plant-available Si and (a) foliar N:P, (b) C:N and (c) C:P ratios for 12 tropical tree species (for details see Figure 4).

2017, Gómez-Vera et al. 2021, Parecido et al. 2022). We also found that higher growth was coupled with increased foliar C:N (weak evidence for *Herrania* and *Dendropanax*) or C:P ratios (*Theobroma*), suggesting improved nutrient-use efficiency with higher plant-available and/or foliar Si. However, the mechanisms of how Si may influence plant physiology and, e.g. improve nutrient-use efficiency, remain debated (Frew et al. 2018, Coskun et al. 2019).

In nine of the 12 species no evidence for performance-enhancing effects of Si emerged, which might have several reasons. Firstly, it was argued that beneficial effects of Si on plant performance can only be expected under biotic or abiotic stress (Coskun et al. 2019). In our experiment, for most species stress levels might have been too low for beneficial effects of Si to occur (we did not aim to stress plants in the first place). Secondly, active Si uptake requires energy and can be relatively costly (de Tombeur et al. 2023), so that only species taking up Si in a cost-efficient way should benefit from Si or if the benefits of Si uptake outweigh its cost (de Tombeur et al. 2023). Thirdly, the precise location and morphology of accumulated amorphous Si deposits might influence whether Si can be beneficial or not (Hartley et al. 2015).

4.3 Inconsistent and contrasting effects of Si on foliar P concentrations and stoichiometry

Most species (10 out of 12) did not show any foliar P responses to increasing plant-available Si. According to our data it seems unlikely that Si plays a significant physiological role in P uptake in most tropical tree species studied. However, foliar P concentrations, N:P and C:P ratios responded to plant-available Si in two species, *Sorocea* and *Theobroma*. In *Sorocea*, foliar P increased with plant-available Si, although plant-available P did not increase in the bulk soil, which suggests Si influenced physiological processes associated with P nutrition. Indeed, Si was shown to mobilise P from soil binding sites in the rhizosphere and in root cells through increased release of root exudates or complexation between dissolved mono-silicic acid and metals (e.g. Mn and Fe) in the roots (Kostic et al. 2017, Hu et al. 2020). The increase in foliar P led to a parallel reduction of foliar N:P ratios below 20, suggesting a change from P-limitation to N- and P co-limitation along the soil Si gradient (Güsewell 2004), yet no growth response emerged. In *Theobroma*, foliar P concentrations decreased with plant-available Si. This could be because the improved growth on a P-poor substrate can dilute foliar P concentrations (Quigley et al. 2020). Alternatively, it could be due to Si deposition in roots that can impede colonization of arbuscular mycorrhizal fungi (Johnson et al. 2022) and hence reduce mycorrhiza-mediated P uptake. Taken together, the different foliar P, N:P and C:P responses to plant-available Si in *Sorocea* and *Theobroma* indicated that Si modulated physiological processes governing P uptake and/or metabolism rather than soil-level processes and that such processes vary strongly across species.

4.4 No indication of interactions between Si and C in most species

Most species in our study (8 out of 12) lacked significant responses in foliar C showing that no or only very weak interaction (i.e. substitution or dilution) between Si accumulation and foliar C occurred. Thus, our data did not provide support that low resource environments, such as tropical forests, generally promote the substitution of C-based compounds by Si (Quigley et al. 2020), a metabolically cheap alternative to C (Raven 1983). Nevertheless, foliar C concentrations decreased with increasing plant-available Si in a third of the species studied, and more negative responses emerged in species with strong foliar Si responses, indicating that increased Si accumulation in leaves decreased foliar C concentrations. For these species, our findings are consistent with the widely suggested notion of Si-induced substitution of C-based compounds (Cooke and Leishman 2011, Quigley et al. 2020, Hodson and Guppy 2022). Alternatively, negative relationships between foliar Si and C concentrations can emerge because the high mass of foliar Si deposits can dilute macronutrient concentrations, including foliar C (Hodson and Guppy 2022). Overall, the physiological underpinnings of a Si-induced substitution of C-based compounds remain debated and their full appreciation requires study of cell-level processes (Hodson and Guppy 2022).

4.5 Ecological implications

Seedlings in the forest understory of tropical lowland forests experience high herbivory pressure and seasonal drought, and tree-fall disturbances expose seedlings to UV-stress (Krause et al. 2003, Engelbrecht et al. 2007, Eichhorn et al. 2010, Bagchi et al. 2014). Furthermore, the high activity of decomposers, including litter arthropods and microbes, is important for nutrient provisioning (Camenzind et al. 2018, Sayer et al. 2020). Considering that Si can deter herbivores (Alhousari and Greger 2018), hamper litter decomposition of litter arthropods and microbes (Schaller et al. 2014, Nakamura et al. 2022) and mitigate the detrimental effects of drought and UV-radiation (Cooke and Leishman 2016, Frew et al. 2018), species-specific responses in foliar Si to the wide variation of plant-available Si in tropical forest soils (Schaller et al. 2018, Ishizawa et al. 2019) may differently modulate species' responses to adverse conditions and their interactions with herbivores and decomposers. Furthermore, indirect effects of plant-available Si on ecological processes may occur through shifts in foliar C:N and C:P ratios, which govern rates of herbivory (Coley et al. 2006, Poorter et al. 2004) and litter decomposition (Laird-Hopkins et al. 2017, Camenzind et al. 2018). This suggests that complex synergistic and/or antagonistic effects of foliar Si, C:N and C:P ratios on plant performance might be possible across species and sites, with implications for species distribution and ecosystem processes.

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7 Supporting information

Table S1: Species included in the greenhouse experiment with their name, abbreviation, family, order, habitat association to soil P following Condit et al. (2013), and the number of individuals in the statistical analysis (n).

Scientific Name	Abbreviation	Family	Order	Association to P	n
<i>Aspidosperma spruceanum</i> Benth. ex Müll.Arg.	ASPICR	Apocynaceae	Gentianales	Low P	13
<i>Calophyllum longifolium</i> Willd.	CALOLO	Clusiaceae	Malpighiales	Low P	13
<i>Dendropanax arboreus</i> (L.) Decne. & Planch.	DENDAR	Araliaceae	Apiales	Low P	13
<i>Eugenia oerstediana</i> O.Berg	EUGEOE	Myrtaceae	Myrtales	Indifferent	11
<i>Herrania purpurea</i> (Pittier) R.E.Schult.	HERRPU	Malvaceae	Malvales	High P	13
<i>Inga nobilis</i> Willd.	INGAQU	Fabaceae	Fabales	High P	13
<i>Ormosia macrocalyx</i> Ducke	ORMOMA	Fabaceae	Fabales	Indifferent	12
<i>Randia armata</i> (Sw.) DC.	RANDAR	Rubiaceae	Gentianales	High P	13
<i>Sorocea affinis</i> Hemsl.	SOROAF	Moraceae	Rosales	Indifferent	13
<i>Theobroma cacao</i> L.	THEOCA	Malvaceae	Malvales	No data	12
<i>Trichilia tuberculata</i> (Triana & Planch.) C.DC.	TRI2TU	Meliaceae	Sapindales	High P	13
<i>Trophis caucana</i> (Pittier) C.C.Berg	TROPCA	Moraceae	Malvales	High P	12

Table S2: Results of ANCOVA testing whether different species responded differently to increasing plant-available Si (treatment), i.e. whether a significant species x treatment interaction emerged. Values for foliar Si concentrations, aboveground biomass, foliar C concentrations, C:N ratios and C:P ratios were \log_{10} -transformed. Significant p-values ($p \leq 0.05$) and p-values indicating weak evidence for an effect ($0.05 < p \leq 0.1$) are marked in bold and italics.

		$df_{\text{nom, den}}$	F	p
a) Foliar Si concentration	treatment	1, 127	75.893	< 0.001
	species	11, 127	230.270	< 0.001
	species x treatment	11, 127	0.685	0.750
b) Aboveground growth	treatment	1, 127	3.517	<i>0.063</i>
	species	11, 127	77.649	< 0.001
	species x treatment	11, 127	1.381	0.189
c) Foliar P concentration	treatment	1, 127	0.233	0.630
	species	11, 127	34.194	< 0.001
	species x treatment	11, 127	1.338	0.211
d) Foliar C concentration	treatment	1, 127	8.969	0.003
	species	11, 127	100.611	< 0.001
	species x treatment	11, 127	2.590	0.005
e) Foliar N concentration	treatment	1, 127	8.611	0.004
	species	11, 127	66.233	< 0.001
	species x treatment	11, 127	0.879	0.563

Table S2 continued

f) N:P				
	treatment	1, 127	4.252	0.041
	species	11, 127	34.981	< 0.001
	species x treatment	11, 127	1.144	0.333
g) C:N				
	treatment	1, 127	4.427	0.037
	species	11, 127	103.486	< 0.001
	species x treatment	11, 127	0.817	0.623
h) C:P				
	treatment	1, 127	0.011	0.916
	species	11, 127	69.480	< 0.001
	species x treatment	11, 127	1.142	0.334

Table S3: Species responses in (a) foliar Si concentrations, (b) aboveground growth, (c) foliar P concentrations, (d) foliar C concentrations, (e) foliar N concentrations, (f) foliar N:P ratios, (g) foliar C:N ratios and (h) foliar C:P ratios to plant-available Si. Results of linear models are given. Significant p-values ($p \leq 0.05$) and p-values indicating weak evidence for an effect ($0.05 < p \leq 0.1$) are marked in bold and italics.

		β	t	p	adj. R ²		β	t	p	adj. R ²
a) Foliar Si	ASPICR	<i>0.053</i>	<i>1.871</i>	<i>0.088</i>	<i>0.17</i>	b) Growth	0.001	0.113	0.912	-0.09
	CALOLO	0.223	2.903	0.014	0.38		0.057	1.003	0.337	0.00
	DENDAR	0.024	0.841	0.418	-0.02		0.014	3.223	0.008	0.44
	EUGEOE	0.787	6.072	<0.001	0.78		0.006	0.949	0.367	-0.01
	HERRPU	<i>0.268</i>	<i>2.060</i>	<i>0.064</i>	<i>0.21</i>		0.013	2.892	0.015	0.38
	INGAQU	<i>0.278</i>	<i>1.799</i>	<i>0.099</i>	<i>0.16</i>		-0.002	-0.261	0.799	-0.08
	ORMOMA	0.022	3.547	0.005	0.51		-0.002	-0.065	0.949	-0.10
	RANDAR	0.016	0.661	0.522	-0.05		0.006	1.026	0.327	0.00
	SOROAF	0.552	5.156	<0.001	0.68		-0.003	-0.410	0.689	-0.07
	THEOCA	0.546	3.295	0.008	0.47		<i>0.073</i>	<i>2.148</i>	<i>0.057</i>	<i>0.25</i>
TRI2TU	0.128	2.849	0.016	0.37	-0.004	-0.731	0.480	-0.04		
TROPICA	1.095	3.734	0.004	0.54	0.007	1.527	0.158	0.11		
c) Foliar P	ASPICR	-0.002	-0.153	0.881	-0.09	d) Foliar C	0.049	0.331	0.747	-0.08
	CALOLO	-0.004	-1.060	0.312	0.01		-0.092	-3.658	0.004	0.51
	DENDAR	0.006	0.322	0.753	-0.08		0.193	1.686	0.120	0.13
	EUGEOE	0.005	0.571	0.582	-0.07		-0.084	-1.600	0.144	0.13
	HERRPU	-0.002	-0.435	0.672	-0.07		-0.076	-2.758	0.019	0.35
	INGAQU	0.001	0.144	0.888	-0.09		0.052	0.860	0.408	-0.02
	ORMOMA	0.013	1.121	0.289	0.02		-0.065	-1.001	0.340	0.00
	RANDAR	-0.011	-0.521	0.613	-0.06		-0.017	-0.451	0.661	-0.07
	SOROAF	0.025	2.509	0.029	0.31		0.016	0.246	0.810	-0.08
	THEOCA	-0.028	-2.489	0.032	0.32		-0.110	-1.538	0.155	0.11
TRI2TU	0.003	0.420	0.683	-0.07	-0.098	-5.078	<0.001	0.67		
TROPICA	-0.009	-1.613	0.138	0.13	-0.212	-4.206	0.002	0.60		

Table S3 continued

e) Foliar N	ASPICR	-0.006	-0.307	0.765	-0.08	f) N:P	-0.021	-0.137	0.893	-0.09
	CALOLO	<i>-0.009</i>	<i>-1.968</i>	<i>0.075</i>	<i>0.19</i>		-0.075	-0.587	0.569	-0.06
	DENDAR	<i>-0.028</i>	<i>-1.917</i>	<i>0.082</i>	<i>0.18</i>		-0.233	-1.087	0.300	0.01
	EUGEOE	-0.007	-1.011	0.338	0.00		-0.253	-1.514	0.164	0.11
	HERRPU	-0.014	-2.816	0.017	0.37		-0.065	-0.820	0.429	-0.03
	INGAQU	0.003	0.593	0.565	-0.06		0.021	0.206	0.840	-0.09
	ORMOMA	-0.007	-0.436	0.672	-0.08		-0.361	-1.785	0.105	0.17
	RANDAR	-0.001	-0.084	0.934	-0.09		0.092	0.396	0.700	-0.08
	SOROAF	0.007	0.743	0.473	-0.04		-0.289	-2.290	0.043	0.26
	THEOCA	-0.012	-1.232	0.246	0.05		0.159	2.269	0.047	0.27
	TRI2TU	0.002	0.244	0.812	-0.09		-0.020	-0.207	0.840	-0.09
	TROPCA	-0.022	-2.490	0.032	0.32		-0.054	-0.898	0.390	-0.02
g) C:N	ASPICR	0.062	0.208	0.839	-0.09	h) C:P	0.798	0.097	0.925	-0.09
	CALOLO	0.301	1.353	0.203	0.06		1.765	0.281	0.784	-0.08
	DENDAR	<i>0.333</i>	<i>2.149</i>	<i>0.055</i>	<i>0.23</i>		2.682	0.475	0.644	-0.07
	EUGEOE	0.080	0.669	0.520	-0.06		-4.914	-1.467	0.176	0.10
	HERRPU	<i>0.086</i>	<i>2.154</i>	<i>0.054</i>	<i>0.23</i>		-0.029	-0.029	0.977	-0.09
	INGAQU	-0.006	-0.117	0.909	-0.09		0.098	0.037	0.971	-0.09
	ORMOMA	0.017	0.221	0.830	-0.09		-5.415	-1.433	0.182	0.09
	RANDAR	0.018	0.138	0.893	-0.09		1.395	0.567	0.582	-0.06
	SOROAF	-0.046	-0.544	0.597	-0.06		-5.787	-2.200	0.050	0.24
	THEOCA	0.149	0.790	0.448	-0.04		6.569	2.813	0.018	0.39
	TRI2TU	-0.059	-0.861	0.408	-0.02		-1.524	-0.756	0.466	-0.04
	TROPCA	0.046	0.954	0.363	-0.01		-0.190	-0.276	0.788	-0.09

	Foliar biomass	Total Biomass	C:N	C:P	N total	P total	C	N:P	Soil DSi	Si	Si:C	P	N	
C total	1.00	0.98	0.84	0.69	0.83	0.77	0.37			-0.18	-0.20	-0.53	-0.68	
Foliar biomass		0.98	0.83	0.66	0.83	0.79	0.33			-0.15	-0.17	-0.51	-0.68	
Total Biomass			0.82	0.60	0.83	0.84	0.32			-0.15	-0.17	-0.47	-0.69	
C:N				0.80	0.49	0.54	0.46			-0.23	-0.25	-0.60	-0.91	
C:P					0.47	0.19	0.65	0.51		-0.30	-0.33	-0.88	-0.61	
N total						0.80	0.38	0.22		-0.19	-0.21	-0.42	-0.31	
P total								-0.34					-0.52	
C									0.49	-0.73	-0.75	-0.56	-0.22	
N:P										-0.22	-0.24	-0.66	0.29	
Soil DSi											0.18	0.17		
Si												0.99	0.19	
Si:C													0.23	
P														0.47

Figure S1: Pairwise correlations among foliar and total aboveground growth (foliar and total biomass) as well as foliar nutrient concentrations and ratios based on Spearman rank correlation coefficients across 12 tropical tree species. Only significant ($p \leq 0.05$) correlation coefficients are shown.

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III Manuscript 2

Silicon-based anti-herbivore defense in tropical tree seedlings

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Abstract

Silicon-based defenses deter insect herbivores in many cultivated and wild grass species. Furthermore, in some of these species, silicon (Si) uptake and defense can be induced by herbivory. Tropical trees also take up Si and leaf Si concentrations vary greatly across and within species. As herbivory is a major driver of seedling mortality and niche differentiation of tropical tree species, understanding anti-herbivore defenses is pivotal. Yet, whether silicon is a constitutive and inducible herbivory defense in tropical forest tree species remains unknown. We grew seedlings of eight tropical tree species in a full factorial experiment, including two levels of plant-available soil Si concentrations (-Si/+Si) and a simulated herbivory treatment (-H/+H). The simulated herbivory treatment was a combination of clipping and application of methyl jasmonate. We then carried out multiple-choice feeding trials, separately for each tree species, in which leaves of each treatment combination were offered to a generalist caterpillar (*Spodoptera frugiperda*). Leaf damage was assessed. Three species showed a significant decrease in leaf damage under high compared to low Si conditions (by up to 72%), consistent with our expectation of Si-based defenses acting in tropical tree species. In one species, leaf damage was increased by increasing soil Si and in four species, no effect of soil Si on leaf damage was observed. Opposite to our expectation of Si uptake and defense being inducible by herbivory damage, simulated herbivory increased leaf damage in two species. Furthermore, simulated herbivory reduced Si concentrations in one species. Our results showed that tropical tree seedlings can be better defended when growing in Si-rich compared to Si-poor soils, and that the effects of Si on plant defense vary strongly across species. Furthermore, Si-based defenses may not be inducible in tropical tree species. Overall, constitutive Si-based defense should be considered part of the vast array of anti-herbivore defenses of tropical tree species. Our finding that Si-based defenses are highly species-specific combined with the fact that herbivory is a major driver of mortality in tropical tree seedling, suggests that variation in soil Si concentrations may have pervasive consequences for regeneration and performance across tropical tree species.

1 Introduction

In tropical forests, most herbivory is caused by generalist leaf-chewing insects (Coley and Barone 1996, Eichhorn et al. 2007, Bixenmann et al. 2016), leading to performance losses in the attacked plants (Zangerl et al. 2002). Specifically in seedlings, which are thought to be the most vulnerable life stage of tropical trees (Poorter 2007), herbivory can reduce survival substantially (Eichhorn et al. 2010). Yet, anti-herbivore defenses can minimize the negative impacts of herbivory (Coley and Barone 1996, Eichhorn et al. 2007). Many types of constitutive and inducible defenses that mechanically or chemically protect tropical plants from leaf damage have been identified (Coley and Barone 1996, Eichhorn et al. 2007, Kursar et al. 2009, Barton 2016). However, whether silicon-based defenses, which can effectively deter insect herbivores in many cultivated and wild grass species (Massey et al. 2006, 2007a, Singh et al. 2020), also act in tropical tree species has not been studied.

Plants take up silicon (Si) as dissolved mono-silicic acid from the soil, which is then carried with the transpiration stream to the leaves, where it accumulates as amorphous Si (Raven 1983). Si accumulation is widespread among tropical trees and leaf Si concentrations vary strongly across species (Schaller et al. 2018). Leaf Si concentrations also vary within species (Schaller et al. 2018, Ishizawa et al. 2019), e.g. due to changes in plant-available soil Si concentrations (Klotz et al. 2023a), which differ considerably across tropical soils (Schaller et al. 2018, Ishizawa et al. 2019). Thus, the consequences of variation in soil and leaf Si concentrations for herbivory might be pervasive.

Si-based defenses act both physically and chemically (reviewed in Singh et al. 2020). Amorphous Si deposits make leaves more abrasive which can deter herbivores and thus reduces leaf damage (Massey et al. 2006, 2007a, Massey and Hartley 2009, Hartley et al. 2015, Singh et al. 2020). Furthermore, ingestion of Si-rich diet can cause lasting damage to the insects' mouth parts and digestive tract (Massey and Hartley 2009), impairing their overall fitness (Massey et al. 2006, Massey and Hartley 2009, Islam et al. 2020, 2022, Johnson et al. 2020). Besides such physical effects, Si can also improve plant defense by modulating molecular and biochemical plant responses to herbivory leading to greater production of defense-related enzymes and secondary metabolites (Fauteux et al. 2006, Ye et al. 2013, Hall et al. 2019, Singh et al. 2020). If constitutive Si-based defenses also act in tropical forest species, seedlings grown under high soil Si availability and/or having leaves with increased Si concentrations should show less damage by leaf-chewing insects.

There is strong evidence from grasses and crops that Si can act not only as a constitutive but also as an induced defense, i.e. herbivory damage can lead to greater Si uptake and accumulation in leaf tissues, which in turn reduces subsequent leaf damage (Massey et al. 2007a, McLarnon et al. 2017, Johnson et al. 2021). Leaf damage and signaling hormones involved in anti-herbivore responses, such as jasmonic acid and its derivative methyl jasmonate, have been shown to promote Si uptake and accumulation and might play a role in the induction of Si-based defenses (Ye et al. 2013, McLarnon et al. 2017, Hall et al. 2019, Johnson et al. 2021), although the exact interplay between Si and plant biochemistry is not yet fully understood (Hall et al. 2019). Thus, if Si-based defense is inducible in seedlings of tropical tree species, leaf damage should lead to increased leaf Si concentration and improved protection against herbivory.

Herbivory plays a central role in the ecology and evolution of tropical forest tree species, shaping their physiology and distribution (Coley and Barone 1996, Fine et al. 2004, Kursar et al. 2009, Muehleisen et al. 2020), forest community composition (Fine et al. 2004, Kursar et al. 2009, Muehleisen et al. 2020) and other ecosystem processes, such as nutrient cycling (Metcalfe et al. 2014). Furthermore, projected shifts in rainfall and temperature with global

climate change may influence plant-insect interactions, including herbivory (Hamann et al. 2021). Thus, understanding factors that influence herbivory rates in tropical forests is pivotal.

To test whether Si-based herbivory defenses act in seedlings of tropical tree species and whether they can be induced by herbivory, we experimentally exposed seedlings of eight common species to contrasting soil Si availability and simulated herbivory, and then carried out multiple-choice feeding trials with a generalist caterpillar. We hypothesized that within species (1) leaf damage should be lower in plants grown under high than low soil Si availability (and/or plants with high than low leaf Si concentrations), if Si acts as a herbivore defense. Additionally, (2) if Si accumulation is an inducible herbivore defense (a) leaf Si concentrations should increase, and (b) leaf damage should decrease after exposure to simulated herbivory.

2 Materials and methods

We conducted a full-factorial experiment, including two levels of plant-available soil Si (-Si/+Si) and a simulated herbivory treatment (-H/+H), with potted seedlings of eight tropical tree species in Gamboa, Panama (9°07'0"N, 79°42'0"W). We then compared herbivore preferences for plants grown under the different treatment combinations in intraspecific multiple-choice feeding trials.

2.1 Study species and plant material

Table 1: Species included in the experiment with their scientific name, abbreviation, family, order, and the number of multiple-choice feeding trials conducted per species (n).

Scientific Name	Abbreviation	Family	Order	n
<i>Calophyllum longifolium</i> Willd.	CALOLO	Clusiaceae	Malpighiales	7
<i>Dendropanax arboreus</i> (L.) Decne. & Planch.	DENDAR	Araliaceae	Apiales	4
<i>Eugenia oerstediana</i> O.Berg	EUGEOE	Myrtaceae	Myrtales	5
<i>Herrania purpurea</i> (Pittier) R.E.Schult.	HERRPU	Malvaceae	Malvales	5
<i>Inga nobilis</i> Willd.	INGAQU	Fabaceae	Fabales	7
<i>Ormosia macrocalyx</i> Ducke	ORMOMA	Fabaceae	Fabales	5
<i>Randia armata</i> (Sw.) DC.	RANDAR	Rubiaceae	Gentianales	7
<i>Sorocea affinis</i> Hemsl.	SOROAF	Moraceae	Rosales	6

We studied eight tree species commonly found in tropical forests of central Panama and belonging to eight different families (Table 1). Species were selected based on the following criteria: (1) a wide range of leaf Si concentrations (K. Kitajima, J. Westbrook and S. J. Wright, unpublished data) suggesting different physiological Si uptake capacities, (2) shade-tolerant species (Rüger et al. 2009), which make up the largest proportion of species in the area, and (3) availability of seeds before the onset of the experiment. In the following we refer to the species by their genus name or abbreviation (Table 1).

Seeds were collected in forests of the Panama Canal area in October and November 2019 from at least three individual trees per species with a minimum distance of 100 m between them. Seeds were germinated and raised until cotyledon stage or development of first foliage leaves in trays on a nutrient- and Si-poor substrate consisting of 50% local forest soil and 50% washed river sand (equivalent to substrate for Si treatment, see below).

Seedlings were then transplanted into individual pots (Deepot Cells, Stuewe & Sons, Oregon, USA; diameter: 6.5 cm, depth: 36 cm) with the two experimental substrates (+Si or -Si, see below) in December 2019 or January 2020. The mean experimental growing period varied between ca. 11 - 12.5 months, depending on the species. The species' mean durations of the growing period did not correlate (Pearson correlation) with their leaf Si concentrations of the +Si/-H treatment. Throughout the experiment, all plants were kept well-watered, under intermediate light conditions (ca. 10% of full sunlight) and protected from rainfall. Additionally, -H plants were protected from herbivores by fly screens. The position of species and treatments was randomized. We fertilized each plant using 20 ml of a half strength Hoagland solution three times within the first three months of the experiment to ensure survival of species associated to nutrient-rich soils.

2.2 Si treatment

We manipulated plant-available soil Si concentrations (also termed soil Si in the following). Plants of the -Si treatment grew in a Si- and nutrient-poor substrate consisting of 50% local forest soil (Cerro Pelado, selected based on Schaller et al. 2018 and Condit et al. 2013) and 50% washed river sand. Plants of the +Si treatment grew in the same substrate supplemented with amorphous Si (Aerosil 300, Evonik Industries AG, Essen, Germany; 18 g L⁻¹ substrate), a hydrophilic pyrogenic silicon dioxide. Aerosil 300 has similar chemical and physical properties as biogenic amorphous Si (Schaller et al. 2020) and supplementing soils with Aerosil 300 increases plant-available Si without changing soil pH (J. Schaller, unpublished data). The amorphous Si and substrate were mixed thoroughly. Resulting plant-available soil Si concentrations were 5.23 and 18.27 mg kg⁻¹ for the -Si and +Si treatment, respectively (for analyses see below), corresponding to the minimum and mean values found in the region (Schaller et al. 2018).

2.3 Simulation of herbivory

To elicit potential inducible herbivory defenses we treated half of the plants of both Si treatments with simulated herbivory (+H), a combination of clipping and application of methyl jasmonate (MeJa), a hormone inducing systemic defense responses (Mithöfer and Boland 2012). First, two leaves of each +H plant were clipped along the lamina edges with scissors removing about 50% of the leaf area. Then MeJa mixed with lanolin (4.5 µmol in 100 mg lanolin paste) was applied to the surface of the leaves (10 mg on an area of ca. 0.5 x 0.5 cm per

leaf). The -H plants were not clipped and received the same amount of lanolin paste without MeJa. The treatment was repeated six times throughout the whole experimental period, with the last application not more than three weeks before the onset of the feeding trials. Leaf clipping combined with MeJa application enabled us to standardize the intensity and amount of (simulated) herbivory across and within species, although some additional chemical and physical stimuli of natural herbivores triggering plant responses may be missing (Waterman et al. 2019).

2.4 Generalist herbivore

Multiple-choice feeding trials were conducted with 3rd or 4th instar caterpillars of the moth *Spodoptera frugiperda* (J.E. Smith), a generalist herbivore that does not occur in forest habitats in Panama. Using a herbivore that does not co-occur with the focal plant species enabled us to avoid potential confounding effects of co-evolution, and using a generalist allowed us to use the same herbivore across tree species. The caterpillars were picked from corncoobs purchased at a local market the day before the respective feeding trials, were starved for 10 h and kept in the laboratory under standardized conditions. Each individual caterpillar was only used for one feeding trial (see below) to prevent learning effects.

2.5 Feeding trials

We carried out multiple-choice feeding trials separately for each tree species. In each trial, we offered one leaf disk per treatment combination to one caterpillar in a Petri dish (90 mm diameter) and assessed leaf damage. Additionally, a leaf disk from a standard plant species (*Ixora coccinea*) was included in each trial, but was not considered further in the analyses. Four to seven trials were conducted per species (n = 4–7, Table 1). One seedling per treatment combination was randomly selected for each trial. Fully developed leaves or leaves of the same developmental stage were selected, cleaned, stored in plastic bags and kept in a fridge. Directly before the trials leaf disks of 2 cm² size were punched out with a cork borer (avoiding the main leaf rib). They were pinned to moist sponges to retain tissue moisture and then placed into a Petri dish. The leaf disks were arranged in a circle with the caterpillar placed in the center. The positions of the four treatment groups was randomized and recorded to identify them after the trial. Each trial took 6h. If the caterpillar did not feed on any of the leaf disks the trial was repeated with a new caterpillar and leaf disks from new leaves. In cases where the caterpillar did not feed again, a second (and sometimes third) repetition was carried out, exchanging only the caterpillar (not the leaves, to ensure to keep enough leaf material for Si analysis, see below) and extending the feeding period to 8 h.

After the feeding trials, the remaining area of the leaf disks (LA_{remain}) was photographed (NIKON Coolpix 500) and measured using image software ‘ImageJ’ (Schneider et al. 2012).

For the statistical analysis, we calculated the proportion of leaf area consumed as $1 - (LA_{\text{remain}}/LA_{\text{offered}})$. In the figures we present the percentage of leaf area consumed.

Some individuals of one species (*Dendropanax*) showed signs of herbivory damage before the feeding trials in the -H treatment. We kept data of these individuals in the analyses, so that the effect of simulated herbivory on subsequent leaf damage should be interpreted with care for this species.

2.6 Si analysis of leaves and soil

The leaves that remained on the plants (i.e. leaves not used in the feeding trials plus the leaf area remaining after punching out the leaf disks) were harvested and cleaned to remove any potential residual soil material. They were oven-dried for 48h at 65°C and ground to a fine powder. Leaf Si was extracted for 5 h by an alkaline method using 30 mg of leaf material and 30 ml of 0.1 M sodium carbonate solution (Na_2CO_3) in a regularly shaken water bath at 85 C° (Schaller et al. 2018). The solution was centrifuged (3000×g, for 5 min) and passed through a 0.45 µm cellulose acetate filter (Rausch 2021).

Both experimental soil substrates were sampled before the start of the experiment to analyze the maximum plant-available soil Si concentration the plants were exposed to (one sample per Si level). The samples were air-dried, crushed to break up large aggregates, and sieved to remove roots and stones. Plant-available Si was extracted in CaCl_2 following Schaller et al. (2018). Three g of sifted soil were shaken with 30 ml of 0.01 M CaCl_2 for 1 h at ambient laboratory temperature. The suspension was centrifuged (8000×g, for 10 min) and the supernatant decanted and subsequently passed through a 0.2 mm syringe filter.

The Si concentration of the leaf and soil extracts was determined with inductively coupled plasma optical-emission spectrometry (ICP-OES) using a Varian Vista-Pro Radial element analyzer (Varian Inc., Palo Alto, USA).

2.7 Statistical analyses

2.7.1 Effect of soil Si and simulated herbivory on leaf damage

To assess the effect of the soil Si and simulated herbivory treatments as well as their interaction on leaf damage we run zero-inflated generalized linear mixed-effect models (ZIGLMM) for each species, using the function *glmmTMB* from the R-package ‘glmmTMB’ (Brooks et al. 2017). As our data was proportional and contained many zeros we assumed the residuals to be beta-distributed and zero-inflated (Geissinger et al. 2022). If the whole leaf disk was consumed,

i.e. if leaf damage = 1, we subtracted a trace value of 0.0001, as responses = 1 cannot be modeled with the R-function we used. We included “Feeding trial” as random effect. For two species the full model did not converge so we re-run the model without the interaction term (*Ormosia*) or without the random effect (*Dendropanax*). We assessed significance of the factors using 90% and 95% confidence intervals from parametric bootstrapping (based on 10000 iterations in which the model successfully converged). We predicted marginal means for all four factor levels based on the ZIGLMMs using the R-package ‘ggeffects’ (Lüdtke 2018), yet without consideration of the zero-inflation component. We assessed the effect size of soil Si and simulated herbivory on leaf damage as the % increase or decrease of the marginal means of the +Si and +H compared to control (i.e. the -Si and -H treatments), respectively. We performed model diagnostics using the R-package ‘DHARMA’ (Hartig 2022).

2.7.2 Effect of soil Si and simulated herbivory on leaf Si concentration

To test whether the soil Si and the (simulated) herbivory treatments and their interaction had an effect on leaf Si concentrations we run ANOVA for each species. We applied graphical model diagnostics to ensure normality and homogeneity of residuals. To interpret the effects of soil Si and simulated herbivory, we predicted marginal means for all factor levels based on the ANOVAs.

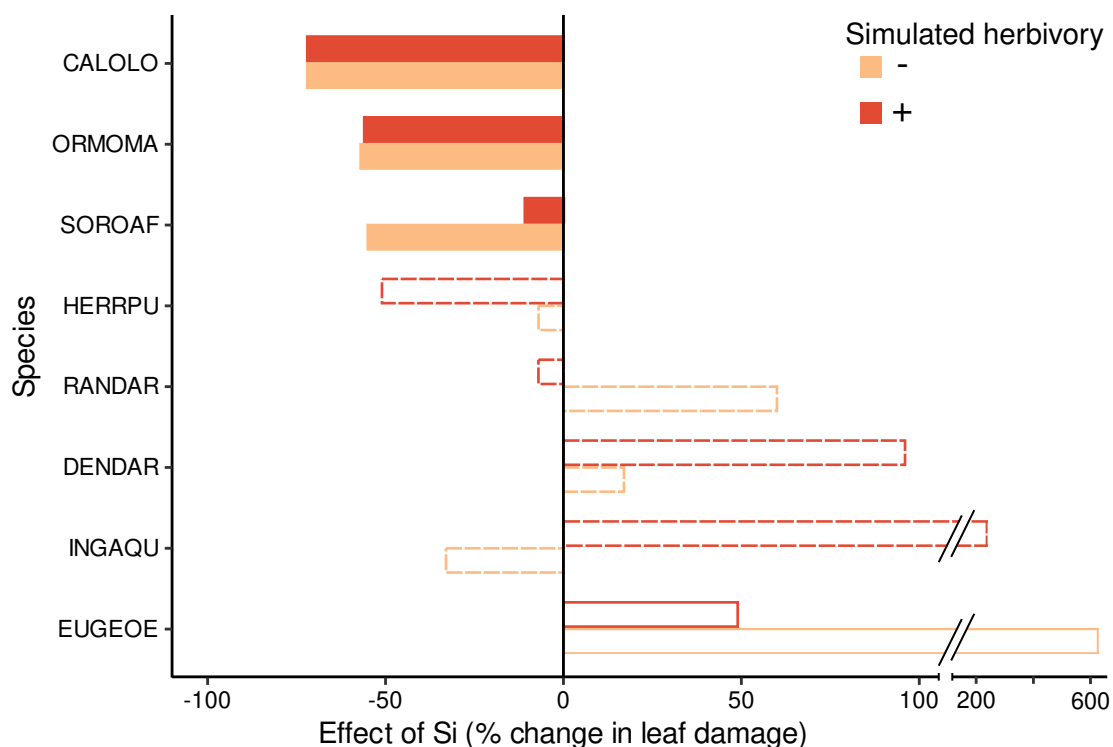


Figure 1: Effect size of the Si treatment on leaf damage for eight tropical tree species. Effects are shown for plants without herbivory (orange bars) and for plants subjected to simulated herbivory (red bars). The effect size indicates the % change of leaf damage in the predicted marginal means in the +Si relative to the -Si treatment. Significant effects and effects with weak or no evidence (for details see Table 2) are indicated by filled, empty and dotted bars, respectively. Species codes are given in Table 1.

2.7.3 Effect of leaf Si concentrations on leaf damage

To assess the direct effect of leaf Si concentrations on leaf damage for each species we also run separate ZIGLMMs (see above) for each species, including “Feeding trial” as random effect (except for *Dendropanax*). We assessed the significance of the slope estimate using 90% and 95% confidence intervals from parametric bootstrapping (based on 10000 iterations in which the model successfully converged). We assessed the effects of leaf Si concentrations on leaf damage for each species as the difference between the predicted marginal means of leaf damage at the lowest and highest leaf Si concentration measured in each species, yet without consideration of the zero-inflation component.

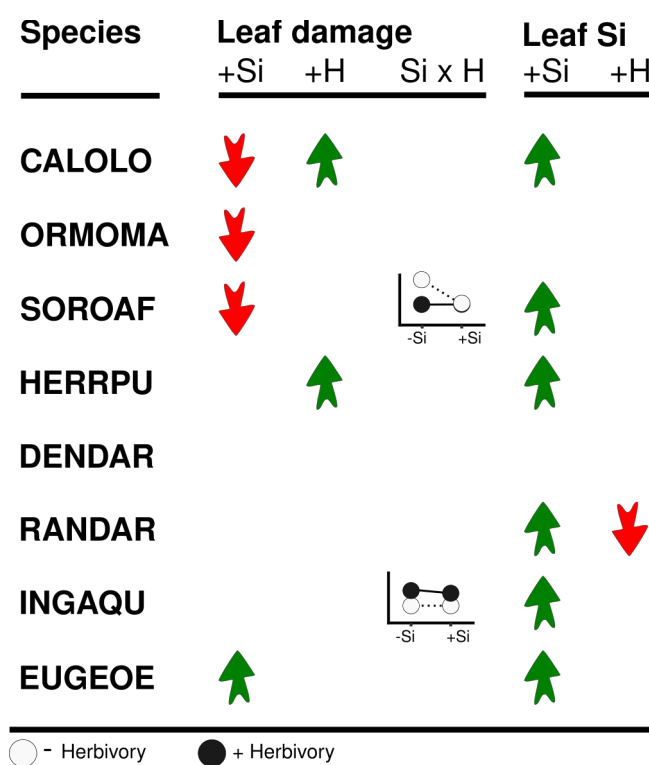


Figure 2: Overview of the effects of high soil Si (+Si) and simulated herbivory (+H) on leaf damage and Si concentrations. Red and green arrows denote significant reductions and increases, respectively. Graphs show significant interactions between soil Si and simulated herbivory (Si x H).

herbivory defenses. In contrast, in one species (*Eugenia*), we found weak evidence for an increase in leaf damage under high compared to low Si conditions. In the four remaining species no evidence for an effect of soil Si on leaf damage emerged (*Herrania*, *Dendropanax*, *Randia*, *Inga*).

We considered p-values of $p < 0.05$ as evidence for a significant effect, while $0.05 < p < 0.1$ indicated weak evidence, and $p > 0.1$ no evidence for an effect (compare Muff et al. 2022). All statistical analyses were performed in R version 4.2.1 (R Core Team 2022).

3 Results

3.1 Effects of soil Si and simulated herbivory on leaf damage

Several species showed significant effects of Si and/or simulated herbivory on leaf damage, but the size and direction of responses was not consistent across species. Three species (*Calophyllum*, *Ormosia* and *Sorocea*) showed a significant decrease in leaf damage under high compared to low Si conditions (by 11% to 72%, Figures 1, 2, S2, Table 2), consistent with the hypothesis of Si-based

Simulated herbivory significantly increased leaf damage (by up to 700%, Figures 2, 3, Table 2) in one species (*Herrania*), and we found weak evidence for an positive effect in another species (*Calophyllum*). The remaining six species showed no evidence for an effect of simulated herbivory on leaf damage.

Significant interactive effects of soil Si and simulated herbivory on leaf damage emerged in two species (Table 2), yet they were not in line with our hypothesis of induced Si-based defense (i.e. lowest herbivory in +Si plants receiving simulated herbivory). Instead, the decrease of leaf damage with soil Si was stronger in plants without simulated herbivory in one species (*Sorocea*). In another species (*Inga*) Si fertilization increased leaf damage in plants treated with simulated herbivory and decreased it in plants without simulated herbivory.

3.2 Effects of soil Si and simulated herbivory on leaf Si concentrations

In six of the eight species leaf Si concentrations were significantly higher in plants growing under high Si compared to low Si conditions (up to 219%, Figures 2, 4; Table 3). In two species (*Dendropanax*, *Ormosia*), which showed the lowest Si uptake capacity out of all species we studied (Figure S1), leaf Si concentrations did not increase with higher soil Si.

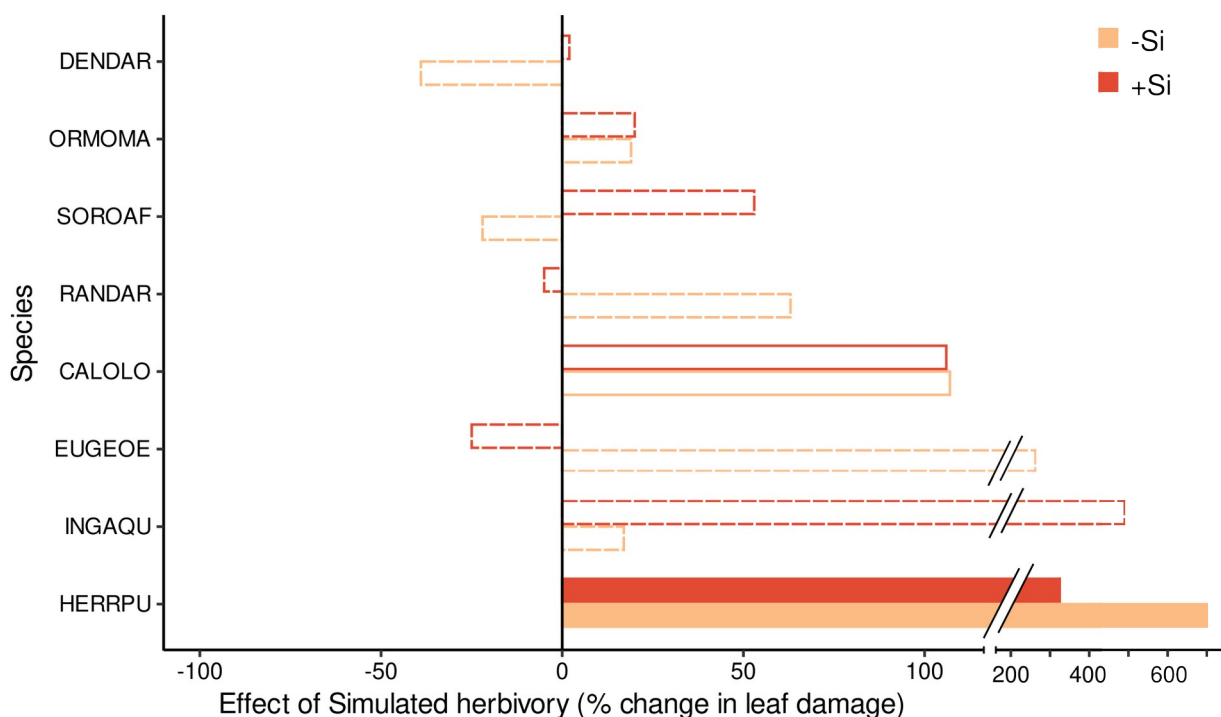


Figure 3: Effect size of the simulated herbivory treatment on leaf damage for eight tropical tree species. Effects are shown for plants of the +Si (red bars) and -Si (orange bars) treatment. The effect size indicates the % change of leaf damage in the predicted marginal means in the +H relative to the -H treatment. Significant effects and effects with weak or no evidence (for details see Table 2) are indicated by filled, empty and dotted bars, respectively. Species codes are given in Table 1.

Overall, the species showed a 30-fold variation in Si uptake capacity (i.e. the leaf Si concentrations of +Si plants not treated with simulated herbivory).

Simulated herbivory did not lead to an increase of leaf Si concentrations in any of the species, again contrary to our expectation of Si being an induced defense. In contrast, simulated herbivory reduced leaf Si concentrations in one species (*Randia*), by about 32% and 12% under low and high Si availability, respectively.

3.3 Effect of leaf Si concentration on leaf damage

Leaf Si concentrations affected leaf damage, both negatively and positively, in three of the eight species studied (Figure 5, Table 4), while leaf Si concentrations did not influence leaf damage in the remaining five species. Increasing leaf Si concentrations significantly decreased leaf damage in one species (by 91%, *Calophyllum*), in line with our hypothesis, and, in another species (*Sorocea*), we found weak evidence for such an effect. By contrast, in one species (*Eugenia*) there was weak evidence for the opposite effect, i.e. leaf damage increased with increasing leaf Si concentration (by 685%).

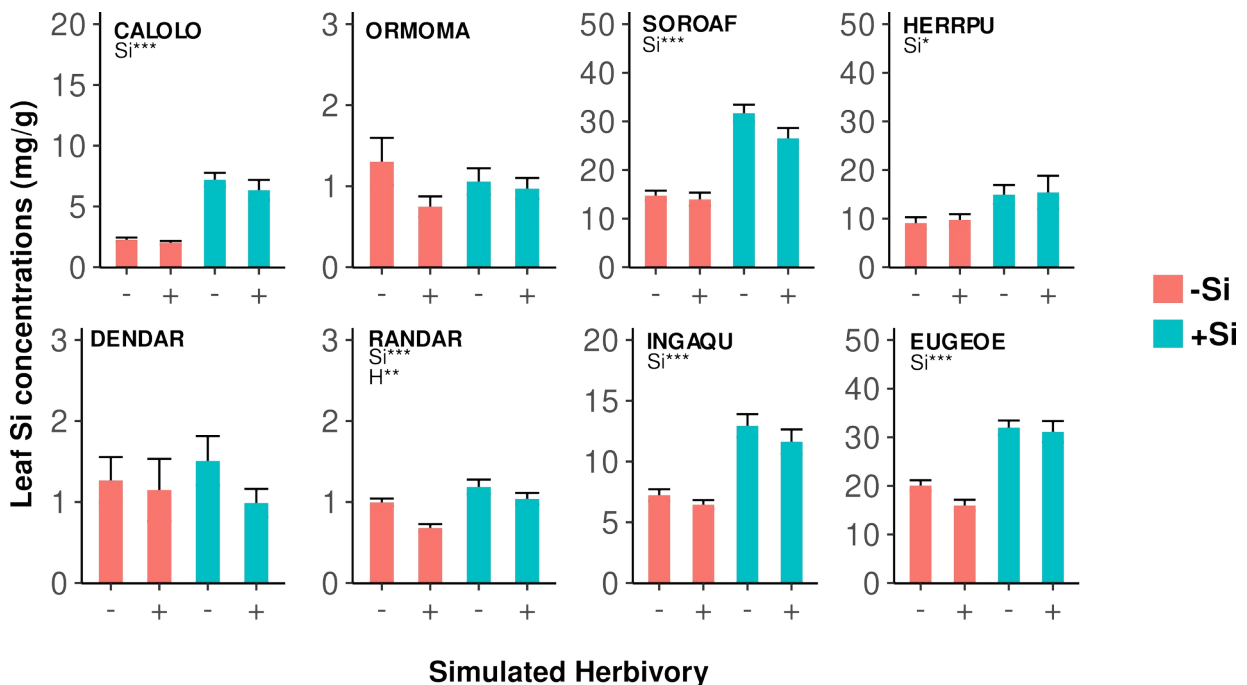


Figure 4: Leaf Si concentrations in seedlings of eight tropical tree species under different plant-available soil Si (-Si/+Si) and simulated herbivory (-H/+H). Results of ANOVA are shown (significant effects of Si (Si), simulated herbivory (H), and the interaction (Si:H); *p < 0.05, ** p < 0.01, *** p < 0.001). Values are means \pm SD per treatment combination and species. Species codes are given in Table 1.

4 Discussion

Effects of soil or leaf Si concentrations and simulated herbivory on leaf damage emerged in seedlings of several tropical tree species and exhibited strong interspecific variation. Higher soil and/or leaf Si concentrations decreased leaf damage by a generalist herbivore in three of the eight species we studied, indicating that Si improved their herbivory defense. In the other species, however, leaf damage either showed no or even a positive relationship to soil Si and leaf Si concentrations. We did not find evidence for inducible Si-based defenses.

Table 2: Effect of plant-available soil Si (Si), simulated herbivory (H) and their interaction (Si:H) on leaf damage (% of leaf area removed) in intraspecific multiple-choice feeding trials with seedlings of eight tropical tree species. Slope estimates (β) of zero-inflated generalized linear mixed-effect models and 95% (CI95%) and 90% (CI90%) bootstrapped confidence intervals are shown. Significant effects (i.e. the 95% CI does not include zero) and effects with weak evidence (i.e. only the 90% CI does not include zero) are marked bold and italic, respectively. Species codes are given in Table 1.

	+Si			+H			Si:H		
	β	CI95%	CI90%	β	CI95%	CI90%	β	CI95%	CI90%
CALOLO	-1.36	-2.64 -0.32	-2.38 -0.52	<i>0.88</i>	-0.02 -1.80	0.18 -1.60	-0.12	-1.56 -1.43	-1.29 -1.12
ORMOMA	-0.90	-1.72 -0.22	-1.58 -0.34	0.08	-0.64 -0.81	-0.50 -0.67		†	
SOROAF	-1.02	-1.6 -0.45	-1.48 -0.57	-0.35	-0.90 -0.19	-0.77 -0.08	0.87	0.02 -1.68	0.22 -1.51
HERRPU	-0.08	-2.48 -1.04	-1.24 -0.73	7.27	3.84 -10.01	5.26 -9.41	-5.29	-8.04 -0.00	-7.46 -0.00
DENDAR	0.18	-0.96 -1.35	-0.73 -1.14	-0.54	-1.90 -0.70	-1.67 -0.48	0.57	-1.06 -2.28	-0.76 -2.00
RANDAR	0.54	-0.63 -1.77	-0.41 -1.57	0.56	-0.56 -1.77	-0.35 -1.57	-0.62	-2.29 -0.93	-2.00 -0.65
INGAQU	-0.44	-1.32 -0.51	-1.16 -0.28	0.19	-0.56 -1.10	-0.43 -0.91	2.29	0.74 -3.42	1.14 -3.22
EUGEOE	<i>2.23</i>	0.00 -2.75	0.13 -2.67	1.39	-0.34 -1.94	-0.20 -1.85	-1.76	-2.36 -0.38	-2.24 -0.27

† Convergence error in model with interaction term.

4.1 Si-based defenses in tropical seedlings

In about 40% of the species we studied Si-based defenses reduced leaf damage, consistent with our hypothesis. Furthermore, the species in which Si improved defense varied greatly in their Si uptake capacities (up to 30-fold). This is in line with work on several grass and crop species, including low-accumulating dicots, that showed reduced leaf damage by insect herbivores in plants fertilized with Si (e.g. Massey et al. 2006, Ryalls et al. 2017, Islam et al. 2020). The range of reduction of leaf damage with Si fertilization in our study, i.e. a 11% to 72% reduction, is similar to Massey et al. (2006), who reported a ca. 43% to 75% reduction across five grass species. In contrast to our finding of Si-based defenses occurring only in a subset of tropical tree species, effects of Si consistently emerged in all grass species studied (Massey et al. 2006), probably because Si-based defenses are one of the main defense types in grasses (Massey et al. 2007a, Huitu et al. 2014). Tropical trees, however, have evolved a wide diversity of chemical as well as physical herbivore defenses, which vary substantially across species

(Coley and Barone 1996, Kursar et al. 2009, Eichhorn et al. 2010, Barton 2016). Hence, finding evidence for Si-based defenses in about 40% of the tree species indicates that Si can play an important but so far widely ignored role in anti-herbivore defense in tropical forests. Conversely and opposite to our expectations, high leaf Si concentrations led to increased leaf damage in one species (*Eugenia*), indicating that, in some species, Si-rich leaves can also be more susceptible to herbivory. This might be due to increases in nutritional quality, e.g. tissue N and/or P concentrations, with higher leaf and/or soil Si concentrations as previously shown for tropical tree and crop species (e.g. Neu et al. 2017, Klotz et al. 2023a). To our knowledge we are the first to show that Si-based defenses act in seedlings of tropical tree species. Our results suggest that Si should be considered part of the vast array of anti-herbivores defenses in tropical trees (Coley and Barone 1996, Kursar et al. 2009, Barton 2016).

Table 3: Effect of plant-available soil Si (Si), simulated herbivory (H) and their interactions (Si:H) on leaf Si concentrations in seedlings of eight tropical tree species. Results of ANOVAs are shown. Significant responses ($p < 0.05$) are shown in bold. Species codes are given in Table 1.

	H			Si			Si:H		
	df _{nom, den}	F	p	df _{nom, den}	F	p	df _{nom, den}	F	p
ORMOMA	1, 15	2.47	0.137	1, 15	0.02	0.899	1, 15	1.40	0.254
CALOLO	1, 24	1.17	0.290	1, 24	82.51	<0.001	1, 24	0.36	0.557
DENDAR	1, 12	1.16	0.304	1, 12	0.02	0.895	1, 12	0.46	0.512
EUGEOE	1, 16	2.63	0.124	1, 16	78.89	<0.001	1, 16	1.13	0.304
SOROAF	1, 20	3.42	0.079	1, 20	84.65	<0.001	1, 20	1.88	0.186
INGAQU	1, 24	2.92	0.100	1, 24	31.92	<0.001	1, 24	1.02	0.322
RANDAR	1, 24	12.18	0.002	1, 24	17.25	<0.001	1, 24	1.60	0.219
HERRPU	1, 16	0.07	0.794	1, 16	7.32	0.016	1, 16	0.00	0.965

The mechanisms underlying the Si-based defenses we observed likely varied across our study species. Si-based defenses were independent of the species' Si uptake capacity, i.e. they can occur in both high- and low-accumulating species. In two species (*Calophyllum* and *Sorocea*) higher leaf Si concentrations were directly related to reduced leaf damage. In these species, amorphous Si deposits may have directly lead to more abrasive leaves, as found elsewhere (Massey et al. 2006, Massey and Hartley 2009). Indeed, the best known direct constitutive anti-herbivore effect of Si is a higher abrasiveness of Si-rich leaves, which can wear down the herbivores' mouthparts and reduce their digestive efficacy (Massey et al. 2006, Massey and Hartley 2009, Hartley et al. 2015, Singh et al. 2020). In another of our study species (*Ormosia*), however, the reduction in leaf damage was not related to higher leaf Si concentrations but only soil Si had an effect on leaf damage, suggesting that the Si-enriched soil conditions must have indirectly influenced defensive leaf properties. Such indirect effects may include Si-mediated changes in soil nutrient availability and plant nutrient status (see above), which may also influence the production and composition of defensive secondary metabolites (Moore et al. 2014), and lead to changes in nutritional quality. Alternatively, high soil Si may have modulated the morphology and/or location of Si deposits in leaves in a way that improved their

defensive properties (Hartley et al. 2015, Schaller et al. 2022). Indeed, previous work on grass species suggested that the morphology and location of leaf Si deposits might be more important factors for Si's protective effects than leaf Si concentrations per se (Hartley et al. 2015). Changes in the morphology and location of Si deposits without concurrent changes in leaf Si concentrations might be related to differences in the amount and/or structure of silicification templates, such as cell walls (Kumar et al. 2017). Yet, whether and how leaf Si deposits of *Ormosia* have changed was not assessed in our study. Overall, the mechanisms underlying Si-based defenses, e.g. whether or not high soil Si alone is sufficient to improve herbivory defense, vary across species and disentangling this variation will contribute to our ecological understanding of Si in tropical forests.

Despite the large effect sizes and robust calculation of confidence intervals (see above), our sample size of $n = 4-7$ is relatively small and the minimum for feeding trials, which remains a caveat of our study.

Table 4: Effect of leaf Si concentrations on leaf damage (% leaf area removed) in intraspecific multiple-choice feeding trials. Slope estimates (β) of zero-inflated generalized linear mixed-effect models and 95% (CI95%) and 90% (CI90%) bootstrapped confidence intervals are shown. Significant effects (i.e. the 95% CI does not include zero) and effects with weak evidence (i.e. only the 90% CI does not include zero) are marked bold and italic, respectively. Species codes are given in Table 1.

	Foliar Si concentration		
	β	CI95%	CI90%
CALOLO	-0.26	-0.46 - -0.10	-0.42 - -0.13
ORMOMA	0.08	-1.49 - 1.52	-1.12 - 1.20
SOROAF	<i>-0.03</i>	-0.07 - 0.00	-0.06 - -0.01
HERRPU	0.01	-0.42 - 0.12	-0.34 - 0.06
DENDAR	-0.31	-0.97 - 0.39	-0.71 - 0.14
RANDAR	-0.86	-2.64 - 0.8	-2.32 - 0.52
INGAQU	-0.02	-0.18 - 0.17	-0.14 - 0.13
EUGEOE	<i>0.08</i>	-0.02 - 0.19	0.01 - 0.17

4.2 Si-based defenses were not inducible

In none of our species simulated herbivory led to higher leaf Si concentrations or to lower leaf damage under high soil Si, suggesting that Si uptake and the associated improvements in herbivory defense may not be inducible in tropical tree seedlings. Previous work had demonstrated that Si uptake can be induced by natural and simulated herbivory in grasses and some dicot species (Massey et al. 2007b, Quigley and Anderson 2014, McLarnon et al. 2017, Islam et al. 2020, Johnson et al. 2020). Application of MeJa, which was also a part of the simulated herbivory treatment in our study, has successfully induced Si uptake in several previous studies (e.g. Hall et al. 2020, Johnson et al. 2021). An ecological explanation for not finding induced Si-based defenses in the tropical tree species may be that under conditions of

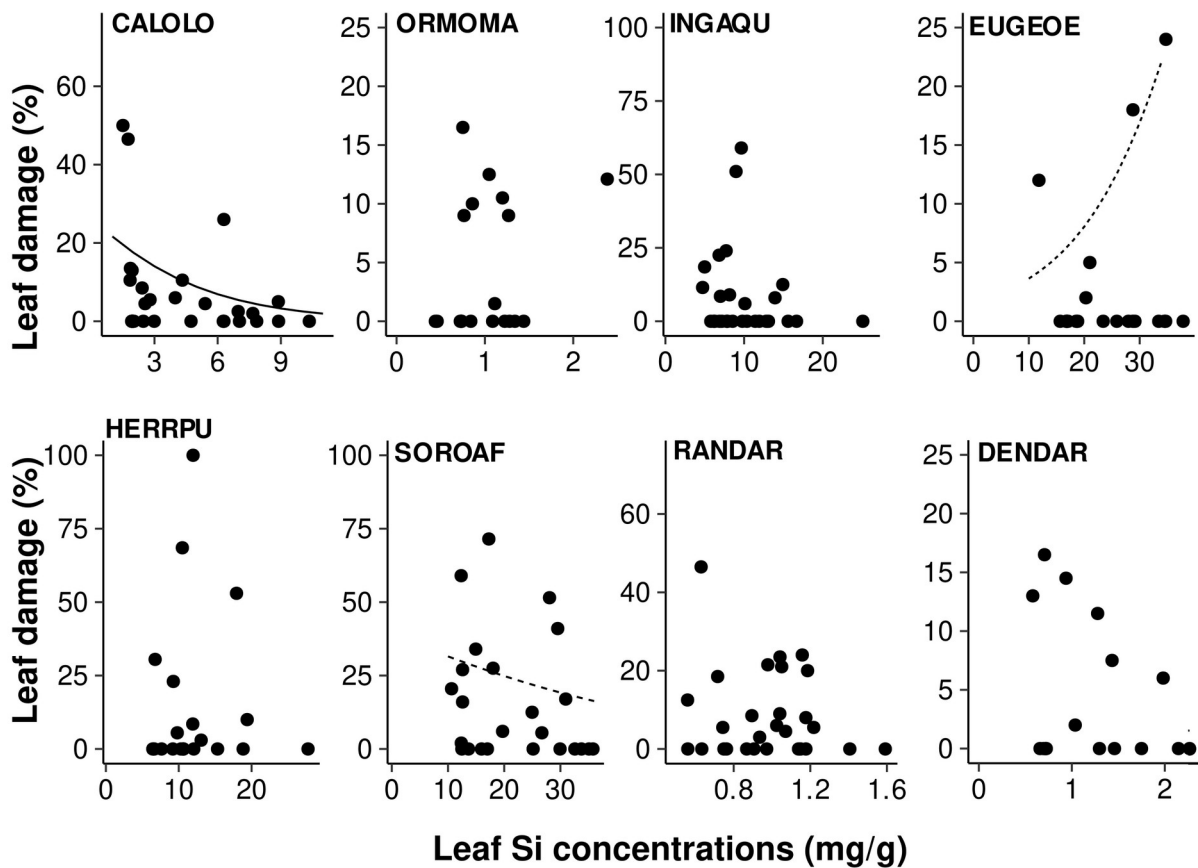


Figure 5: Effect of leaf Si concentrations on leaf damage (% leaf area removed). Results of zero-inflated generalized linear mixed-effect models are shown (bold line: $p < 0.05$, dashed line: $p < 0.1$; significance was assessed based on bootstrapped confidence intervals). Species codes are given in Table 1.

constantly high herbivore pressure, such as in tropical forests, induced herbivory defense is overall less cost-effective and thus less common than constitutive defense (but see Barton 2016, Bixenmann et al. 2016). In fact, the costs of Si uptake and thus Si-based defenses can be high (Garbuzov et al. 2011, de Tombeur et al. 2023), so that additional herbivory-induced Si uptake might not be worthwhile, e.g. because it may not add to the protection already present in the form of constitutive Si-based defense. We note though that in some studies repeated leaf damage was necessary to induce Si uptake (Massey et al. 2007b, Hartley and DeGabriel 2016), and that simulated herbivory through leaf clipping and MeJa application may be less effective in inducing Si uptake than natural herbivory, potentially due to the absence of chemical and physical stimuli associated with herbivore damage, e.g. saliva, that trigger additional (hormonal) plant responses (Hartley and DeGabriel 2016, Waterman et al. 2019). Thus, the amount of MeJa applied and/or the amount or mode of mechanical damage applied in our study might not have been adequate to induce Si uptake. Si accumulation may also have occurred only locally in damaged leaves (but see Islam et al. 2020, see Thorne et al. 2023) and thus remained undetected in our study since we pooled the total leaf biomass per individual to measure Si concentrations. At this point we can therefore not conclusively rule out that induced Si-based defenses occur in some tropical tree species.

5 Ecological implications

Considering the vast spatial variation of plant-available soil Si in tropical forests (Schaller et al. 2018, Ishizawa et al. 2019) Si-based defenses and their strong intra- and interspecific variation might have pervasive implications for the ecology of tropical tree seedlings. Firstly, defensive effects of both soil and leaf Si have been suggested to provide an alternative to C-based defense compounds (Raven 1983, Schaller et al. 2012). This may be especially important in seedlings in the shaded forest understory of tropical forests, which are strongly light- and thus C-limited (Chazdon 1988). Secondly, for species in which Si has protective effects, seedlings growing in sites with Si-rich soil should be better protected against herbivores than conspecifics on Si-poor soil, contributing to intraspecific performance variation. Thirdly, the pronounced interspecific differences of the efficacy of Si-based defenses may lead to changes in performance rankings and competitive balance across sites varying in plant-available soil Si (Garbuzov et al. 2011), with repercussions for tree community composition. Furthermore, the ecology of herbivores may also be affected by variation of soil Si, because a Si-rich diet not only reduces the herbivores' fitness, but it can also both increase and decrease their susceptibility to predation and parasitism (Ryalls et al. 2017, Hall et al. 2021, Islam et al. 2022). Overall, our results suggest Si-based defenses in tropical tree seedlings may have pervasive consequences for seedling performance and ecological processes, such as plant-herbivore interactions, and thus should be considered more in further studies.

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Author contributions: BE and JS conceived the idea. BE, JS and MK designed the study. The implementation of the experiment was coordinated by MK. Chemical analyses were coordinated by BE, JS and MK. The data were analyzed, and figures and tables created by MK with input from BE and JS. MK wrote the manuscript. All co-authors revised and commented subsequent drafts and gave final approval for publication.

Data availability statement: The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Dryad Digital Repository at <https://doi.org/10.5061/dryad.j0zpc86mf> (Klotz et al., 2023b).

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8 Supporting information

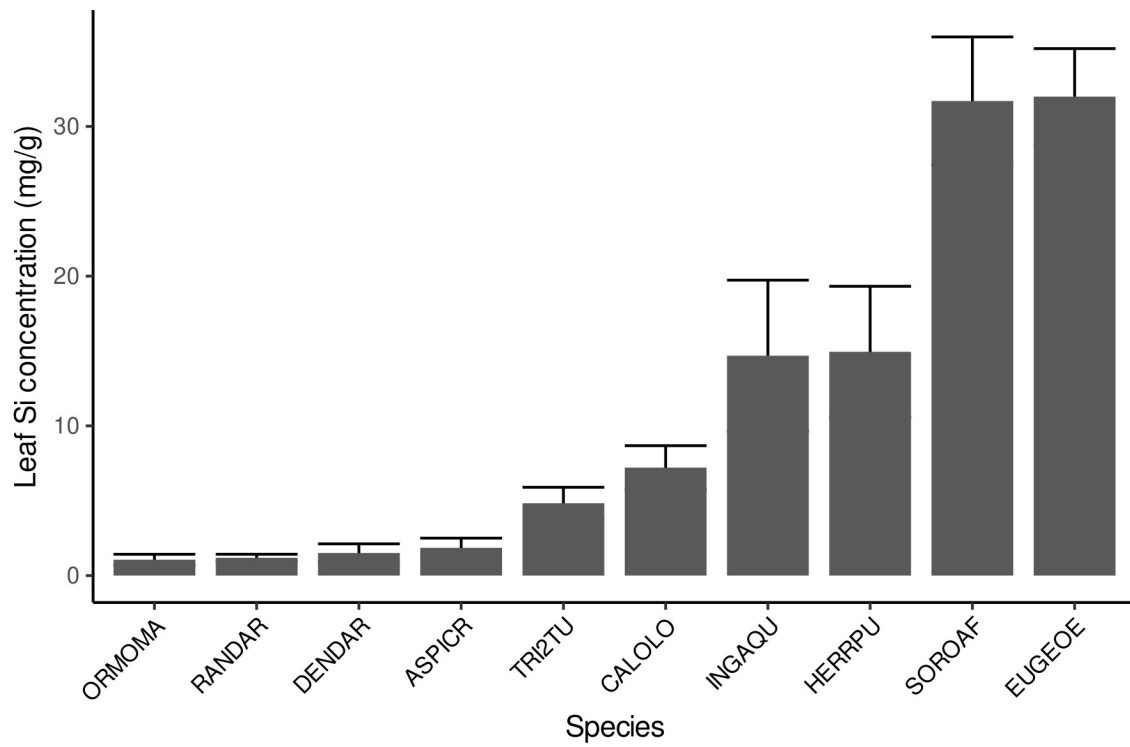


Figure S1: The species' Si concentrations under +Si/-H treatment, reflecting their Si uptake capacities.

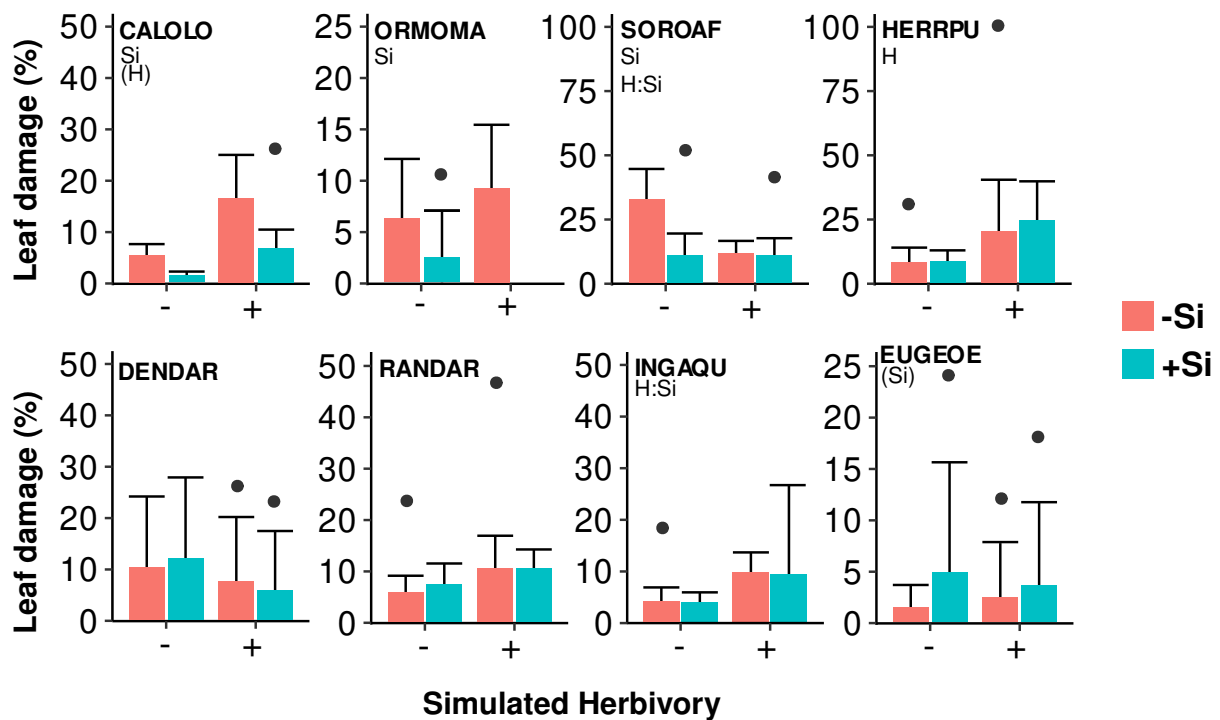


Figure S2: Leaf damage (% of leaf area removed) under different soil Si (+Si, -Si) and simulated herbivory (+H, -H) in intraspecific multiple-choice feeding trials with seedlings of eight tropical tree species. Values are means \pm SD and outliers per treatment combination and species. Results of zero-inflated generalized linear mixed-effect models are shown (significant effect of Si (Si), simulated herbivory (H) and interaction (H:Si). Significant effects (i.e. the 95% CI does not include zero) and effects with weak evidence (i.e. only the 90% CI does not include zero) are shown with and without parentheses, respectively. Species codes are given in Table 1.

IV Manuscript 3

Plasticity of plant silicon and nitrogen concentrations in response to water regimes varies across temperate grassland species

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Abstract

1. Temperate grasslands exhibit strong spatial and temporal variation in water regimes. Thus, grassland plants experience potentially stressful water regimes, which may influence their tissue silicon (Si) and nitrogen (N) concentrations. Plant Si and N concentrations play important ecological roles in temperate grasslands, for example, by influencing plant performance and herbivory, yet comparisons of species' responses to a broad range of water regimes, including drought, waterlogging and flooding, are lacking.

2. We conducted a mesocosm experiment with 10 temperate grassland species of two life-forms (grasses and forbs) exposed to four different soil water regimes (drought, a benign control, waterlogged and flooded conditions), and analysed their Si and N concentrations.

3. Grasses showed lower Si concentrations under drought and flooding compared to the benign control and the highest concentrations emerged under waterlogging. Overall, plant Si responses of grasses were more uniform, while in forbs, responses varied both in direction and magnitude across species. For N concentrations, all species and life-forms showed the highest concentrations under drought compared to the benign control, while half of the species exhibited decreasing concentrations under waterlogging and/or flooding. The water regimes, especially waterlogging and flooding, induced changes in species rankings of plant Si and N concentrations, with stronger shifts in forbs than in grasses.

4. Our results indicate that spatial and temporal variation of water regimes may influence plant Si and N concentrations in temperate grassland species. Plant Si responses to water regimes might be highly species-specific in forbs but more similar in grasses, whereas plant N responses are likely to be relatively uniform across species and life-forms.

5. The strong plasticity in plant Si and N concentrations we observed might have pervasive consequences for ecological processes, such as herbivory.

1 Introduction

Temperate grasslands are species rich and offer essential ecosystem services (Gibson 2009, Dengler et al. 2014). They exhibit strong spatial and temporal variation in water availability, ranging from dry to flooded conditions (Silvertown et al. 2015). Both ends of the water gradient impose stress on plants: under drought, plant performance is limited by a lack of water while under waterlogging and flooding, plants can be limited by anoxic conditions (Silvertown et al. 2015). Thus, water availability influences plant physiology, including silicon (Si) and

nitrogen (N) concentrations (He and Dijkstra 2014, Lambers and Oliveira 2019, Brightly et al. 2020), which, in turn, influence essential ecological processes such as herbivory (Loranger et al. 2012, Descombes et al. 2020, Singh et al. 2020).

In plants, Si accumulation can alleviate multiple stressors, including drought and herbivory (Cooke and Leishman, 2016, Singh et al. 2020, Thorne et al. 2020). Therefore, understanding how plant Si concentrations respond to environmental drivers and the generality of these responses is pivotal. Water availability can influence Si concentrations in temperate and tropical grasses as well as crops (Quigley and Anderson 2014, Quigley et al. 2017, 2020, Ryalls et al. 2018, Brightly et al. 2020, Thorne et al. 2020 and references therein). However, results have been inconsistent, with positive (e.g. Quigley and Anderson, 2014, Ryalls et al. 2018), negative (e.g. Brightly et al. 2020) or no (e.g. Quigley and Anderson 2014) effects of water availability on Si concentrations.

Grassland species vary tremendously in Si concentrations, with monocots (e.g. grasses) commonly showing higher concentrations than dicots (e.g. most forbs; Hodson et al. 2005, Strömberg et al. 2016, Klotz et al. 2021). Physiological mechanisms in roots govern plant Si uptake and contribute to Si plasticity. Plants with low Si concentrations, for example, many forbs, take up Si largely via passive diffusion, driven by the transpiration stream (Mitani and Ma 2005, Faisal et al. 2012). Additionally, many species, including most grasses and some forbs, take up Si actively mediated by energy-dependent transporters (Ma and Yamaji 2015, Deshmukh and Bélanger 2016), leading to high plant Si concentrations. As transpiration, energy (ATP) production and protein synthesis (e.g. for Si transporters) function optimally under ample water and oxygen supply (Lambers and Oliveira 2019), plant Si concentrations should be highest under benign, non-stressing water regimes, and reduced under drought, waterlogging and flooding. Potential indirect effects of water regimes on plant Si concentrations via changes in soil Si availability may occur (Schaller et al. 2021), but should be minimal, because plant Si uptake and responses to plant-available Si are thought to be strongly constrained by physiological factors (Faisal et al. 2012, Deshmukh and Bélanger 2016, Klotz et al. 2021). Furthermore, substantial differences in stomatal regulation (Bartlett et al. 2016) as well as in the physiology of active Si uptake across species and life-forms (Deshmukh and Bélanger 2016) suggest that species vary in how their Si concentrations respond to water regimes.

The physiological and ecological role of plant N concentrations has received a lot of attention with respect to leaf economics, plant performance and herbivory in temperate grasslands (e.g. Aerts and Chapin 1999, Loranger et al. 2012, Belluau and Shipley 2018). Plant N concentrations can be strongly affected by soil water regimes (Araya et al. 2013; meta-analyses of drought effects on plant N concentrations in He and Dijkstra 2014 and Sardans et al. 2017). However, the responses observed in previous studies vary widely, for example, effects of

drought on plant N concentrations ranged from positive to negative (He and Dijkstra 2014). Processes of plant N provisioning, including decomposition and mineralization of organic matter, as well as N transport to and uptake by the root, require ample water as well as oxygen supply and are partly governed by plant transpiration (Oyewole et al. 2014, Lambers and Oliveira 2019). These processes are impaired by drought, waterlogging and flooding (Sanaullah et al. 2012, Araya et al. 2013, Yeung et al. 2019), suggesting plant N concentrations should be - as with Si - highest under benign, non-stressing water regimes. However, plants can acclimate to adverse water regimes by regulating N uptake and leakage (Aerts and Chapin 1999, He and Dijkstra 2014) and these responses may vary across plant species due to variation in the preferred sources of N, root anatomy as well as type and density of N transporters located in root membranes, but also due to variation in stomatal regulation (Aerts and Chapin 1999, Bartlett et al. 2016, Lambers and Oliveira 2019).

Plant Si and N are the plant nutrients that are probably most important for influencing insect herbivory in grasslands (Awmack and Leather 2002, Massey et al. 2006, Loranger et al. 2012, Descombes et al. 2020). Generalist insect herbivores preferably feed on plants with high N concentrations (Pérez-Harguindeguy et al. 2003, Loranger et al. 2012) as insect performance is largely N limited (Elser et al. 2000, Awmack and Leather 2002). In contrast, high plant Si concentrations deter insect herbivores by decreasing the nutritional quality of plant tissues and the forming of physical barriers that protect plant cells against penetration and increase the abrasion of mouthparts (Massey et al. 2006, reviewed by Singh et al. 2020). Furthermore, plant Si and N concentrations have been shown to jointly influence the nutritional quality of grassland species (Massey et al. 2007, Descombes et al. 2020) as well as rates of insect herbivory in a grassland community (Descombes et al. 2020). Thus, plasticity in tissue concentrations of both nutrients in response to water regimes might have major implications for insect herbivory and associated decreases in plant performance. Assessing Si and N plasticity in response to water regimes across plant species may contribute to better understand spatial and temporal patterns in insect feeding preferences and herbivory rates along gradients of water availability in temperate grasslands.

Multi-species experiments that directly assess the effect of different water regimes on plant Si and N concentrations and compare it across species are lacking for temperate grasslands. Studies on temperate species focussed on single crop or horticultural species, precluding cross-species comparisons, and/or considered the effects of drought only (Ryalls et al. 2018, Thorne et al. 2020 and references therein). To our knowledge, the only study that comparatively assessed the effects of water availability on plant Si across multiple species, focused on tropical grasses (see Quigley and Anderson 2014 and Quigley et al. 2017 for comparisons of two grass species, Brightly et al. 2020). For plant N concentrations in temperate grassland species, experimental studies that tested the effects of water regimes usually covered a rather limited gradient of water availability, focussing on drought but often disregarding waterlogging and flooding (see references in He and Dijkstra 2014, Sardans et al. 2017). Studies including

several water regimes did not compare species' plasticity across multiple species or life-forms but assessed community-level responses (e.g. Luo et al. 2018). In field experiments that assessed species plasticity (e.g. Ren et al. 2011) small-scale spatial variation in soil N availability may have confounded direct effects of water availability on N plasticity (Hodge 2004).

Here, we experimentally assessed the effects of different water regimes on Si and N concentrations of plants in temperate grasslands. We grew 10 grassland species belonging to two life-forms (grasses and forbs) under four water regimes, ranging from dry to flooded conditions, in mesocosms with uniform substrate. We hypothesized that plant Si and N concentrations vary across water regimes, with the concentrations of both elements being highest under non-stressing, benign conditions and reduced under dry, waterlogged and flooded conditions. We further expected that plant Si and N responses to different water regimes vary across species and life-forms, reflecting species-specific nutrient uptake mechanisms, and that this results in changes of species rankings of Si and N concentrations across different water regimes.

2 Materials and methods

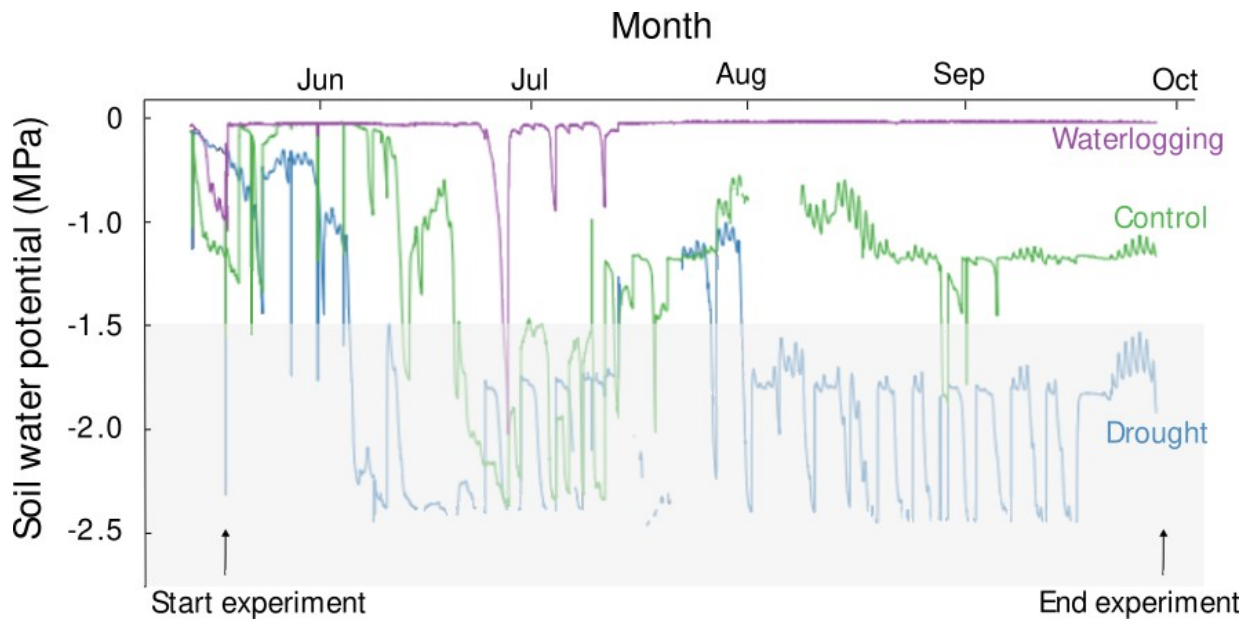


Figure 1: Soil water potentials during the mesocosm experiment in the drought, control and waterlogged treatment. Black arrows indicate start and end of the experiment and coloured lines represent different water regimes. No measurements were taken in the flooded treatments as water potentials in the fully submerged soil were assumed to be 0 MPa. The gypsum blocks used to monitor water potentials have a measurement range down to -1.5 MPa. Lower readings (grey area) therefore indicate water potentials below -1.5 MPa (considered the permanent wilting point) but do not represent the actual water potentials. For visualization values were aggregated into hourly values. See methods for details.

2.1 Experimental set-up

The experiment was conducted in the Ecological-Botanical Garden of the University of Bayreuth, Germany ($49^{\circ}55'19''\text{N}$, $11^{\circ}34'55''\text{E}$). The climate is temperate with a mean annual temperature of 8.7°C and 745 mm mean annual precipitation (1998–2007).

Mesocosms (round plastic containers, 67 cm diameter, 35 cm height, 90 L volume) with a homogenized soil mixture (see below) were exposed to four different watering treatments with five replicates each (i.e. 20 experimental units in total): (1) a ‘control’ treatment of benign, non-stressing water conditions, which consisted of a three-time weekly watering regime, initially applying the amount of water equivalent to average rainfall in the growing season (ca. 70 mm per month, based on monthly mean rainfall from May to September for the years 1998–2007), and increasing the amount as required to maintain the soil moist during hot and dry periods (assessed by finger probing); (2) a ‘drought’ treatment that, in total, received half the amount of water of the ‘control’ treatment by doubling the irrigation interval, but keeping the water supply constant at each watering; (3) a ‘waterlogged’ treatment that was waterlogged up to 15 cm below the soil surface; and (4) a ‘flooded’ treatment that was completely inundated to the rim of the mesocosm ca. 2 – 3 cm above soil surface (but plants emerged above the water

level). For the drought and control treatments, soil water drainage was ensured through holes at the bottom of the containers, whereas in the waterlogged and flooded treatments holes were drilled in the sides of the containers, and water level was maintained through irrigation three times a week until overflow occurred from the holes. Treatments were maintained for 19 weeks from mid-May until the end of September 2016. Soil water potential in three randomly selected mesocosms of the drought, control and waterlogged treatment was monitored every 10 min using gypsum blocks (GB-1; Delmhorst, NJ) connected to a digital soil moisture tester (KS-D1; Delmhorst, NJ) at 10 cm depth. Soil water potential, aggregated into hourly values, differed strongly across water regimes (ANOVA: $p = 0.003$, Figure 1). In the drought treatment, soil water potentials remained below the permanent wilting point for most of the experimental period. In the control, soil water potentials stayed above the permanent wilting point, except for a short period end of June and beginning of July. Waterlogging consistently led to soil water potentials (near) zero, except for some brief reductions in June and July. All treatments were spatially randomized and implemented under transparent rain-out shelters, to assure similar light and temperature conditions. The substrate consisted of local topsoil (brown earth sensu IUSS with a sandy loam texture) mixed with 20% (v/v) crushed limestone to improve soil drainage, and had the following properties: pH = 7.0, 0.2% N, 2.9% C and 45% skeleton content.

2.2 Study species

This study focussed on five grass species and five forb species (Table 1) typical for semi-natural grasslands of central Europe with differing habitat associations to moisture (Ellenberg et al. 2001). In temperate grassland communities, plant species with a range of soil moisture associations often coexist due to small-scale variation of hydrological parameters (Silvertown et al. 2015).

Seeds were obtained from a commercial supplier (Rieger-Hofmann GmbH), and comprised a mixture of genotypes representing the genetic diversity of the species in southern Germany. Seeds were stratified and germinated in the greenhouse. Seedlings were then transplanted to the mesocosms in a concentric grid with ca. 6–8 cm distance between individuals (Figure S1), and with two individuals of each forb species, and four individuals of each grass species randomly assigned to each position. All plants were regularly watered until treatments were started, approximately 4 weeks after the plants were transplanted to the mesocosms.

Table 1: Plant species included in the experiment with their scientific name, abbreviation, family, life-form and Ellenbergs' indicator value for moisture (M-value; Ellenberg et al. 2001)

Scientific Name	Abbreviation	Family	Life-form	M-value
<i>Artemisia campestris</i> L.	AC	Asteraceae	Forb	2
<i>Sanguisorba minor</i> Scop., s. l.	SM	Rosaceae	Forb	3
<i>Silene dioica</i> (L.) Clairv.	SD	Caryophyllaceae	Forb	6

Table 1 continued

<i>Silene flos-cuculi</i> L.	SF	Caryophyllaceae	Forb	7
<i>Silene vulgaris</i> (Moench) Garcke	SV	Caryophyllaceae	Forb	4
<i>Festuca pratensis</i> Huds., s. l.	FP	Poaceae	Grass	6
<i>Festuca valesiaca</i> Schleich. ex Gaudin. s. l.	FV	Poaceae	Grass	2
<i>Koeleria pyramidata</i> (Lam.) P. Beauv.	KP	Poaceae	Grass	4
<i>Poa angustifolia</i> L.	PA	Poaceae	Grass	indifferent
<i>Poa palustris</i> L.	PP	Poaceae	Grass	9

2.3 Chemical analysis

Plants (aboveground biomass) were individually harvested twice, once in summer (mid-July) and once in autumn (end of September), simulating two cuts in managed grasslands. The aboveground biomass from the second harvest was dried at 70°C, ground to a fine powder and used for the analyses. One individual per species and mesocosm was used (n = 5).

Silicon was extracted from the leaves for 5 h by an alkaline method using 30 mg of plant material and 30 ml of 0.1 M sodium carbonate solution (Na₂CO₃) in a regularly shaken water bath following Katz et al. (2021). The solution was subsequently passed through a 0.2 µm syringe filter (ChromafilXtra CA-20/25). The Si concentration of the leaf extract was determined with inductively coupled plasma optical-emission spectrometry (ICP-OES) using a Varian Vista-Pro Radial element analyser (Varian Inc.).

Plant N concentrations were obtained with EA-IRMS coupling (Elemental Analyser NA 1108, CE Instruments; Interface ConFlo III, Finnigan MAT; Isotope ratio mass spectrometer: delta S, Finnigan MAT) and calculated from samples weights and peak areas as detailed by Liebel et al. (2010).

2.4 Statistics

We analysed the effects of species (or life-form) and watering treatment, as well as their interactions separately for Si or N concentration using generalized least squares (GLS, for species) models or generalized linear mixed-effect models (GLMMs, for life-forms). Tukey post-hoc tests were applied to assess the significance of differences of Si or N concentrations across the watering regimes within each species and life-form. We ran models across all species, as well as for grasses and forbs separately. For graphic representation of the responses to the different water regimes, we calculated response ratios (RRs) of plant Si and N concentrations to the respective water regimes compared to control conditions as $RR = \log(\text{treatment/control})$, compare Hedges et al. (1999).

In the models, we used variance structures allowing for heterogeneous residuals (Zuur et al. 2009), so that Si and N concentrations could be kept untransformed to retain valuable biological information on variability and to ease interpretability of the results. To find the appropriate variance structure for each model we built four competing models: one linear model and three GLSs for species as well as one linear mixed-effect model (LMM) and three GLMMs for life-forms, respectively, with each GLS and GLMM having different variance structures either allowing for heterogeneous residuals in species, watering treatment or both plus their interactions. We selected the model with the best fit based on AIC values. We ran graphical model diagnostics to ensure normality of ordinary residuals and homogeneity as well as independence in normalized residuals.

To fit the models we used the R-function *gls* for the GLSs and *lme* for the LMMs and GLMMs and included the variance structures by using the function *varIdent* from the R-package ‘nlme’ (Pinheiro et al. 2020). To conduct post-hoc tests we used the functions *emmeans* and *pairs* from the package ‘emmeans’ (Lenth et al. 2020).

In the flooded treatment the sample size of six species was reduced to $n = 2-4$ for both plant N and Si concentrations (*Artemisia campestris*, *Festuca valesiaca*, *Poa palustris*, *Sanguisorba minor*, *Silene dioica*, *Silene vulgaris*) and for plant Si concentrations one forb species (*Silene vulgaris*) was missing entirely because plant material for analyses was not sufficient due to low growth and/or high mortality. We therefore conducted the analyses for the complete dataset, and for a subset only containing data for the drought, control and waterlogging treatment. Results differed only marginally, so we present the results for the complete dataset in the main text. The missing data, however, led to rank deficient design matrices in all models fitted to plant Si concentrations that included forb species. To circumvent this issue, we tested the effect of the interaction term as a single factor in a separate model, using the *gls* function, in which all available species-treatment combinations were included as factor levels.

To assess how much of the total variation in plant Si and N concentrations is explained by species differences, water regimes and by species \times water regime interactions, we partitioned the variance between these components. We fitted random intercept models (see Messier et al. 2010) separately for grasses and forbs, with each model including species, treatment and the species \times treatment interaction as random effects and no fixed effects. The percentage of variation explained by each random effect and the residuals was calculated based on their variance estimates. We ran graphical model diagnostics to ensure normality in residuals. Since we were interested in variance estimates instead of precisely calculated p-values, it was not necessary to fulfil the assumption of homogeneous variances of residuals and estimates within grouping variables. To fit the models we used the R-function *lmer* from the R-package ‘lme4’ (Bates et al. 2015).

2.4.1 Species rankings across water regimes

To test for changes in species rankings of Si or N concentrations across water regimes, we calculated pairwise Spearman rank correlation coefficients between the control and the different watering treatments (control vs. drought, control vs. waterlogging, control vs. flooding). We conducted these analyses across all species and separately for grasses and forbs.

All statistical analyses were performed in R version 4.1.0 (R Core Team 2021).

3 Results

Table 2: Effects of water regime (treatment), life-form (or species) and their interaction on plant Si and N concentrations. Given are the results of generalized linear (mixed-effects) models including either life-form ((a); two levels: Grasses and forbs; species included as random factor) or species identity ((b), 10 species), and of separate models for forbs and grasses ((c), (d); five species each). For details see methods.

	Si			N		
	df _{nom, den}	F	p	df _{nom, den}	F	p
a)						
life-form	1, 8	114.30	<0.001	1, 8	2.40	0.160
treatment	3, 167	13.53	<0.001	3, 175	111.87	<0.001
life-form x treatment	3, 167	97.66	<0.001	3, 175	2.27	0.082
b)						
species	9, 170	190.99	<0.001	9, 151	252.83	<0.001
treatment	3, 170	11.75	<0.001	3, 151	271.92	<0.001
species x treatment	38, 144	107.87	<0.001	27, 151	4.02	<0.001
c) within forbs						
species	4, 79	46.78	<0.001	4, 72	315.23	<0.001
treatment	3, 79	13.74	<0.001	3, 72	141.44	<0.001
species x treatment	18, 68	36.44	<0.001	12, 72	2.25	0.018
d) within grasses						
species	4, 76	17.52	<0.001	4, 79	24.47	<0.001
treatment	3, 76	159.95	<0.001	3, 79	137.83	<0.001
species x treatment	12, 76	10.39	<0.001	12, 79	4.95	<0.001

3.1 Differences of Si and N concentrations between life-forms and across species

Grasses showed significantly higher Si concentrations than forbs (8.95 mg g⁻¹ vs. 0.67 mg g⁻¹), while N concentrations did not vary between life-forms (Table 2a). Si and N concentrations varied significantly across all species, as well as within forbs and grasses separately (Table 2b–d). Si concentrations varied ca. 42-fold across species, with species means ranging between 0.27 and 11.37 mg g⁻¹, while N concentrations varied ca. two-fold between 12.32 and 22.80 mg g⁻¹.

3.2 Effect of water regimes on Si concentrations

Water regimes significantly influenced Si concentrations across life-forms (Table 2a) and across all species, as well as across grasses and forbs separately (Table 2b–d). Significant effects of water regimes emerged within all species, except *Silene dioica* (Figure 2, Table S1), with up to a four-fold intraspecific variation of Si concentrations across treatments in forbs (*Artemisia campestris*) and up to six-fold in grasses (*Koeleria pyramidata*). The effect of water regimes on Si concentrations differed between grasses and forbs (life-form \times treatment interaction, Table 2a), and across species, also within grasses and forbs separately (species \times treatment interaction, Table 2b–d).

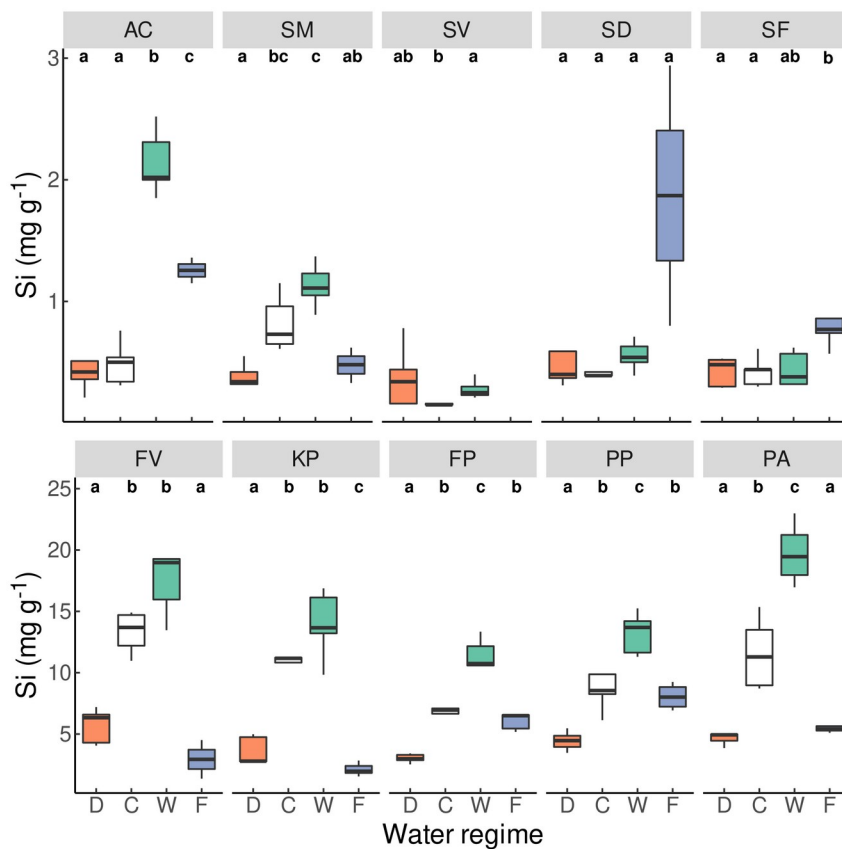


Figure 2: Plant Si concentrations of forbs (upper panel) and grasses (lower panel; five species each) under four water regimes (D = drought; C = control, i.e. benign conditions; W = waterlogging; F = flooding). Box plots show medians, 25th and 75th percentiles (interquartile range), and 1.5 \times interquartile ranges without outliers. Results of Tukey post-hoc tests conducted within each species are given as letters with each letter indicating groups which did not differ significantly. See Table 2 and Table S1 for details and Table 1 for species abbreviations.

In all grasses, Si concentrations consistently decreased under drought and under flooded conditions relative to the control, consistent with our hypothesis, but they increased under waterlogging. The responses to the water regimes were strong and uniform in direction, but species responses varied in magnitude (species \times treatment interaction, Table 2d), with most responses being significant (Figure 4a).

In contrast, in forbs, Si concentrations did not respond to water regimes as we had hypothesized. The responses differed across species in direction as well as in magnitude, with five responses being significant (Figure 4a): Relative to benign conditions, *Artemisia*

campestris exhibited a strong increase in Si concentrations in response to both waterlogging and flooding (but no response to drought), and *Silene flos-cuculi* and *Silene vulgaris* exhibited a significant increase in response to flooding and waterlogging, respectively (but no response to the remaining water regimes). In *Sanguisorba minor* Si concentrations decreased with drought, which was the only forb response supporting our hypothesis.

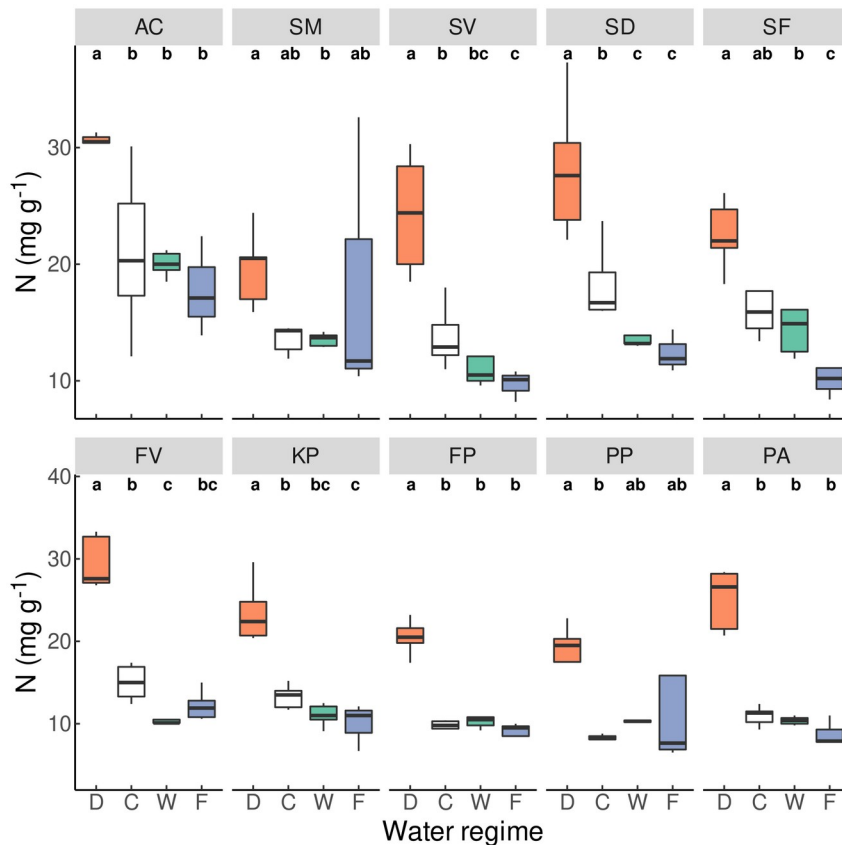


Figure 3: Plant N concentrations of forbs (upper panel) and grasses (lower panel; five species each) under four water regimes (D = drought; C = control, i.e. benign conditions; W = waterlogging; F = flooding). Box plots show medians, 25th and 75th percentiles (interquartile range), and 1.5 × interquartile ranges without outliers. Results of Tukey post-hoc tests conducted within each species are given as letters with each letter indicating groups which did not differ significantly. See Table 2 and Table S1 for details and Table 1 for species abbreviations.

In grasses, the vast majority of the observed variation of Si concentrations was explained by plasticity due to different water regimes (73%), whereas species-specific plasticity and species differences accounted for 13% and 4%, respectively. In contrast, in forbs, most of the observed variation in Si concentration was explained by species-specific plasticity in response to water regimes (i.e. species × treatment interaction, 54%, Figure 5), whereas the effect of water regimes and species differences explained only 13% and 7%, respectively.

Species ranking of Si concentrations under flooded conditions was unrelated to that under control conditions, but it was maintained under drought and waterlogging (Table 3a). In grass species alone, the same pattern was observed (with correlation coefficients being marginally significant). In contrast, in forbs species ranking changed in all water regimes compared to the control conditions.

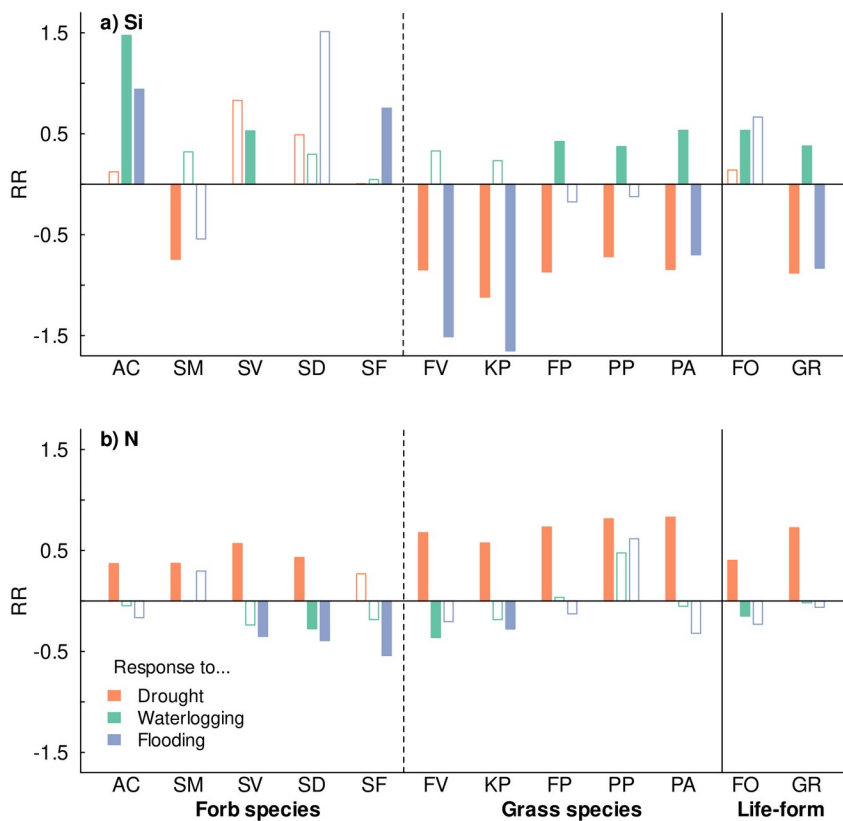


Figure 4: Responses of plant (a) Si and (b) N concentrations to different water regimes compared to benign control conditions in 10 grassland species and summarized for forbs (FO) and grasses (GR). Response ratios (RRs, compare Hedges et al. 1999) are given. Filled bars indicate significant ($p < 0.05$) responses based on Tukey post-hoc tests on the models for life-forms and species. See Table 2 and Table S1 for details and Table 1 for species abbreviations.

3.3 Effect of water regimes on N concentrations

Water regimes significantly influenced plant N concentrations across life-forms (Table 2a) and species, also within grasses and forbs separately (Table 2b–d). Within each individual species, the effect of water regime was significant (Figure 3, Table S1), with up to three-fold variation in N concentrations across all treatments (*Poa angustifolia*). The effect of water regime on N concentrations did not differ between grasses and forbs (no life-form \times treatment interaction, Table 2a), but across species, also within grasses and forbs separately (species \times treatment interactions, Table 2b–d).

Contrary to our hypothesis, under drought, all species consistently exhibited an increase in N concentration compared to control conditions, which was significant in nine of the 10 species

(Figure 4b). Responses to waterlogging and flooding were less pronounced, but in line with our hypothesis: Most species showed a trend towards decreasing N concentrations in response to both waterlogging and flooding. The effect was significant in two species for waterlogging and four species for flooding.

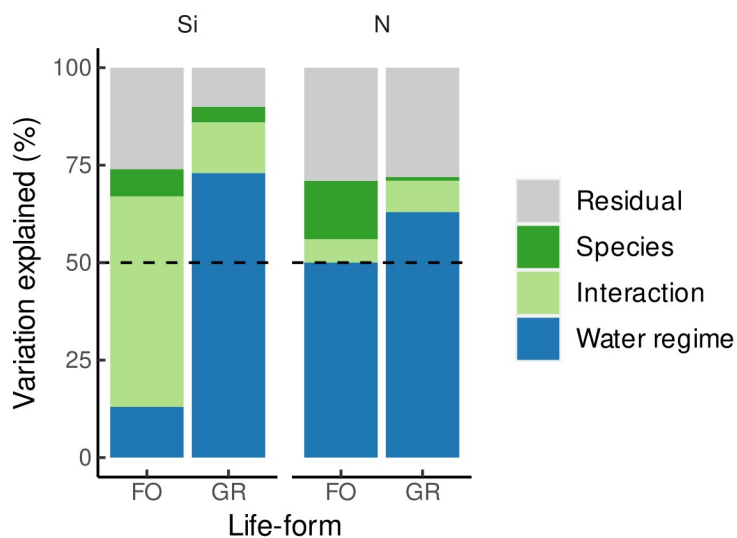


Figure 5: Partitioning of the observed total variation of plant Si and N concentrations in five grass and five forb species under four water regimes ranging from dry to flooded conditions. Colours indicate the amount of variation explained by plasticity in response to water regimes (dark blue) and species-specific responses (interaction, light green), as well as species differences (dark green) and residual variation not explained by any of these factors (grey). Values were derived from variance estimates of random factors of random intercept models. For details see Section 2.

Most of the observed overall variation in plant N concentration was explained by plasticity due to the effects of water regimes both in forbs (50%) and grasses (63%). Species differences explained only 15% and 1% and species-specific responses explained 6% and 8% of the variation in forbs and grasses, respectively (Figure 5).

Similar to Si concentrations, species rankings of N concentrations changed under flooding but were maintained under drought and waterlogging compared to control conditions (Table 3b). When analysed separately, species rankings of

both grasses and forbs changed under waterlogging and flooding and were only maintained under drought (marginally significant correlation).

4 Discussion

Water regimes strongly influenced plant Si and N concentrations of temperate grassland species. Our hypothesis, that plant Si and N concentrations are highest under benign conditions and respond negatively to drought, waterlogging and flooding was only partially supported. Overall, plant Si responses differed strongly across species and life-forms, while plant N responses were more uniform, but still varied across species. Species plasticity, especially species-specific responses, rather than differences across species accounted for most of the variation found in plant Si and N concentrations across water regimes. By experimentally growing multiple plant species under different water regimes with standardized substrate, soil processes in the different treatments influenced all species similarly. Thus, the species-specific

responses (and/or life-form specific responses) we observed reflect differences in the species' physiological capacity to respond plastically to different water regimes rather than differences in soil Si and N provisioning.

Table 3: Results of rank correlations between plant **(a)** Si concentrations and **(b)** N concentrations in the control versus the different water regimes (drought, waterlogging, flooding). Spearman rank correlation coefficients are given with the respective p-values in parentheses (significant values at $p = 0.05$ and $p = 0.10$ are given in regular and italic font respectively). No correlation (n.s.) indicates a change in species rankings in the respective water regimes relative to the control, while a significant positive correlation indicates that species ranking was maintained.

		All species	Grasses	Forbs
a) Si	Control			
	Drought	0.88 (0.002)	<i>0.90 (0.083)</i>	n.s.
	Waterlogging	0.96 (<0.001)	<i>0.90 (0.083)</i>	n.s.
	Flooding	n.s.	n.s.	n.s.
b) N	Drought	0.71 (0.028)	<i>0.90 (0.083)</i>	<i>0.90 (0.083)</i>
	Waterlogging	0.67 (0.039)	n.s.	n.s.
	Flooding	n.s.	n.s.	n.s.

In grasses, drought and flooding consistently led to strong decreases in Si, as we had hypothesized, while waterlogging led to increases compared to benign control conditions. Drought and flooding can elicit similar plant stress responses, such as lowered transpiration rates and aquaporin expression, which ultimately reduce water uptake, photosynthesis and energy (ATP) supply (Bartlett et al. 2016, Lambers and Oliveira 2019, Yeung et al. 2019, Thorne et al. 2020). This might have led to the downregulation of non-essential and energy-demanding physiological processes (Lambers and Oliveira 2019), such as active Si uptake (see Coskun et al. 2019) and the production of Si-dissolving exudates (see de Tombeur et al. 2021). Additionally, reduced transpiration and aquaporin expression may have limited passive Si uptake via diffusion as well as Si transport from the roots to aboveground organs (Mitani and Ma 2005, Deshmukh and Bélanger 2016). In contrast, positive responses to waterlogging suggest that our treatment (waterlogged to 15 cm below the soil surface) did not lead to relevant oxygen limitation in the rooting zone, providing ideal conditions for grasses to maintain active Si uptake and high transpiration rates that resulted in high plant Si concentrations. Opposite responses of grasses to drought than we consistently observed in our study were shown in a recent study on tropical grasses, where most species (39 of 53) exhibited higher Si concentrations under drier than under well-watered conditions (Brightly et al. 2020). Different experimental conditions might have caused the conflicting pattern: very frequent irrigation may have led to Si leaching from the growth substrate in pots under well-watered conditions, while the drier conditions likely were less intense (Brightly et al. 2020).

Furthermore, previous studies suggest increased Si accumulation due to drought might reflect an adaptive response in grasses to mitigate detrimental effects of drought stress (Quigley and Anderson 2014, Strömberg et al. 2016, Brightly et al. 2020), yet this was not supported by our data for temperate grasses. Instead, our results suggest energy availability and stomatal responses mediate the consistent decrease in plant Si concentrations under drought and flooding across temperate grass species.

In forbs, Si concentrations were not higher under benign than under stressful water regimes, as we had hypothesized. Compared to grasses, the responses of forbs were generally weaker (but still pronounced) and more species-specific. Physiological differences in Si uptake might underlie these findings, as grasses and forbs exhibit varying degrees of passive and active uptake. Most grasses are considered to have functional, energy-dependent Si transporters that mediate active Si uptake (Ma and Yamaji 2015, Deshmukh and Bélanger 2016). In contrast, the relative contribution of active versus passive uptake varies strongly across forbs as Si transporters are often absent or less functional than in grasses; some forbs even reject Si (Liang et al. 2006, Deshmukh and Bélanger 2016). Phylogenetic factors influence species differences in the genetic underpinnings of Si uptake (Hodson et al. 2005, Strömberg et al. 2016, Klotz et al. 2021), potentially explaining the uniform responses we observed across closely related grasses and the strong variation across forbs. Overall, the strong differences across forb species in their responses to water regimes suggest that differences in their active component of Si uptake (Deshmukh and Bélanger 2016), in stomatal behaviour (Bartlett et al. 2016, Yeung et al. 2019) and/or in their physiological strategies to cope with flooding and drought (Mommer et al. 2006, Zwicke et al. 2015) might be important drivers of Si plasticity in forbs.

The direction of plant N responses exhibited a consistent pattern across species and life-forms. In all species, N concentrations were higher under drought compared to the unstressed control, not supporting our hypothesis. The responses to waterlogging and flooding were negative, consistent with our hypotheses, yet they were less pronounced than those to drought and differed stronger across species. The positive plant N response to drought we observed is in line with some previous studies (Sardans et al. 2017 and references therein), yet contradicts others, which reported negative or no effects of drought on plant N concentrations for grassland species (Naudts et al. 2011, He and Dijkstra 2014 and references therein). Variation in the duration and intensity of drought and irrigation events can modulate plant N responses to drought (He and Dijkstra 2014), potentially explaining the conflicting results. Soil processes affecting N availability cannot explain the increased plant N concentrations under drought, as soil N availability should be reduced under drought compared to benign conditions (Sanaullah et al. 2012). Instead, plant physiological processes are likely to be important: under drought, growth was substantially reduced in all species (unpublished data), a combined effect of stomatal closure limiting carbon assimilation and reduced cell turgor. Stronger limitation of growth than N availability and uptake can eventually increase N concentrations in plant tissues (Aerts and Chapin 1999, He and Dijkstra 2014). Additionally, it is known that drought-resistant

species can increase N allocation to leaves, enhancing their photosynthetic capacity and water-use efficiency (Wright et al. 2003, Lambers and Oliveira 2019). The more species-specific plant N responses to waterlogging and flooding than to drought might be due to variation in root anatomy and N transporters located in root membranes and/or to variation in stomatal regulation (Aerts and Chapin 1999, Bartlett et al. 2016, Lambers and Oliveira 2019), but also due to strong differences in the physiological capacity to acclimate to excess water, with potential consequences for N metabolism (Mommer et al. 2006). Overall, we showed that N concentrations in grasses and forbs of temperate grasslands are influenced by water regimes and are particularly responsive to drought, leading to strong increases in plant N concentrations across species.

The strong plant Si and N responses to different water regimes across species may lead to shifts in species rankings of Si and N concentrations. We showed that species rankings based on Si concentrations changed across all water regimes in forbs, while in grasses shifts occurred only under flooding. For N concentrations species rankings changed under waterlogged and flooded conditions in both, forbs and grasses. As plants exhibiting higher N concentrations are generally preferred by insect herbivores (Pérez-Harguindeguy et al. 2003, Loranger et al. 2012), whereas plants accumulating more Si are more likely to be rejected (Massey et al. 2006, Singh et al. 2020), such shifts may lead to changes in feeding preferences of insect herbivores across water regimes, with implications for herbivore pressure and plant performance. In fact, effects of drought on plant nutritional quality and associated changes in insect feeding preferences are well-studied for temperate herbaceous species. Changes in plant N concentrations, tissue hydration and secondary metabolites have been suggested to be important drivers (e.g. Huberty and Denno 2004, Gutbrodt et al. 2011), yet results are conflicting, as increased and decreased feeding preferences were observed under drought (Huberty and Denno 2004, Gutbrodt et al. 2011). Furthermore, the role of Si as well as waterlogging and flooding has been hardly considered in this context (but see Vandegeer et al. 2021). Our data suggest that, for forbs, highly species-specific effects of Si on herbivory damage across water regimes, including waterlogging and flooding, are likely. In most grass species, high Si concentrations under benign and waterlogged conditions might diminish their nutritional quality, potentially reducing herbivory damage. In contrast, low Si concentrations under drought and flooding might lead to higher herbivory damage. Furthermore, Si accumulation itself can increase plant N concentrations (Putra et al. 2022), reduce secondary metabolites in plants (Schaller et al. 2012) and improve tissue hydration under drought (Thorne et al. 2020). The species-specific plant Si responses to water regimes we observed, combined with the known defensive effect of Si, and its interaction with other plant traits which also affect herbivory imply that Si plasticity should influence insect herbivory across water regimes in temperate grasslands.

5 Conclusions

We showed strong plasticity in plant Si and N concentration of temperate grassland plants in response to drought, waterlogging and flooding. The responses of plant Si concentrations were highly species specific in forbs, while plant N responses were more uniform across grassland species and life-forms. Our results suggest that spatial and temporal variation of soil water regimes, for example, with microtopography (Silvertown et al. 2015) or following severe drought and precipitation events as projected with climate change (IPCC 2021), might influence plant Si and N concentrations differently across species. This might have pervasive consequences for plant performance and ecological processes, such as herbivory. Our results call for further multi-species experiments to elucidate the ecological consequences of changing plant Si and N concentrations in response to water regimes, including both excess and lack of water.

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Author contributions: Bettina M. J. Engelbrecht, Jürgen Dengler and Gregor Aas conceived the idea and designed the study with input from Jörg Schaller, Gerhard Gebauer, Heike Feldhaar and Anita Weissflog. The experiment was coordinated by Jürgen Dengler and Anita Weissflog, and sample analyses coordinated by Jörg Schaller, Gerhard Gebauer and Bettina M. J. Engelbrecht. The data were analysed, and figures and tables created by Marius Klotz with input from Bettina M. J. Engelbrecht and Jörg Schaller. Marius Klotz wrote the manuscript. All coauthors revised and commented subsequent drafts and gave final approval for publication.

Data availability statement: Data are available in the DRYAD Digital Repository <https://doi.org/10.5061/dryad.7d7wm37zf> (Klotz et al. 2022).

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8 Supporting information

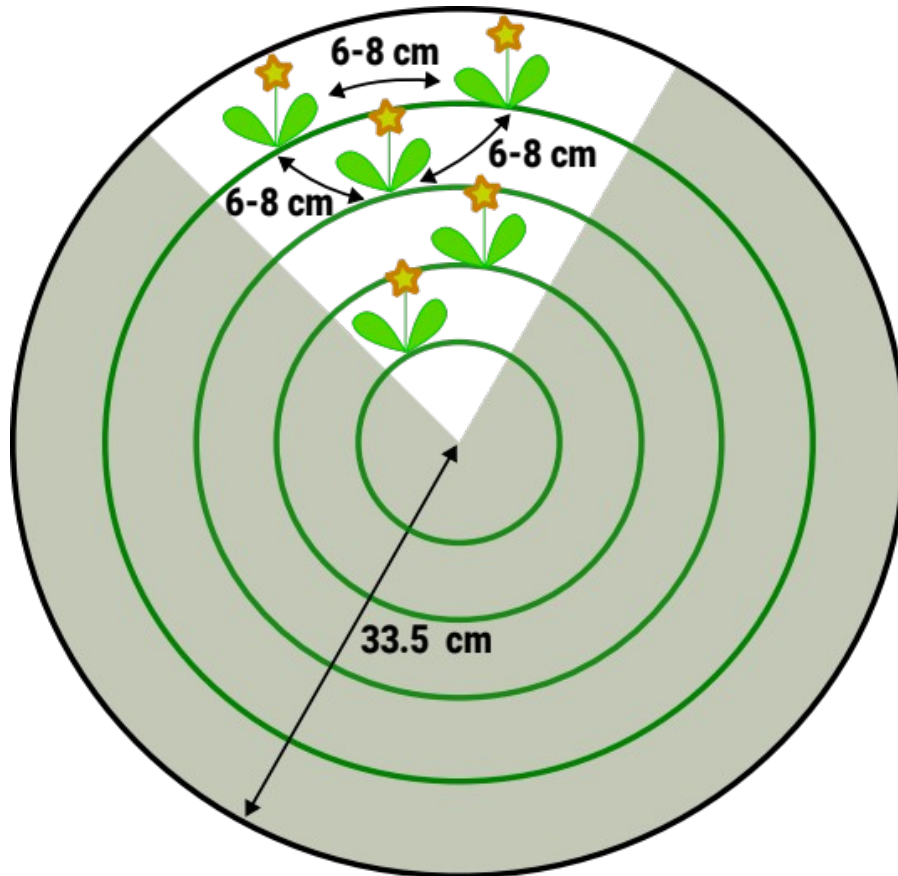


Figure S1: Arrangement and distance of plants in each mesocosm (plastic container with 33.5 cm radius). Plants were arranged in four concentric rings with ca. 6-8 cm distance between individuals. Species were assigned so that both the three inner circles and the outer circle contained every species, distributing potential edge effects evenly across species.

Table S1: Differences in plant Si and N concentrations across different water regimes. Shown are p-values for comparisons **(a)** within species and **(b)** within life-form based on Tukey post-hoc tests on the models including all species or life-form, treatments and their interaction as categorical variables. Significant values ($p < 0.05$) are denoted in bold font. See Table 1 for species abbreviations. Note that the column denoted "control" provides the significance of the responses relative to control conditions, which are presented in Figure 4.

		Si			N		
		Control	Dry	Waterlogged	Control	Dry	Waterlogged
a) Forbs	AC						
		Dry	0.989		0.017		
		Waterlogged	<0.001	<0.001	0.990	<0.001	
		Flooded	<0.001	0.007	<0.001	0.852	<0.001
							0.816
		SM					
			Dry	0.001		0.001	
			Waterlogged	0.087	<0.001	1.000	<0.001
			Flooded	0.050	0.796	<0.001	0.916
						0.997	0.915
		SD					
			Dry	0.753		0.008	
			Waterlogged	0.155	0.970	0.032	<0.001
			Flooded	0.525	0.697	0.610	0.006
						<0.001	0.587
	SF						
		Dry	1.000		0.122		
		Waterlogged	0.995	0.996	0.535	<0.001	
		Flooded	0.040	0.040	0.060	0.003	
					<0.001	<0.001	
	SV						
		Dry	0.159		<0.001		
		Waterlogged	0.005	0.691	0.129	<0.001	
		Flooded			0.028	<0.001	
						0.605	
Grasses	FP						
		Dry	<0.001		<0.001		
		Waterlogged	0.004	<0.001	0.779	<0.001	
		Flooded	0.393	<0.001	<0.001	0.472	<0.001
							0.269
		FV					
			Dry	<0.001		<0.001	
			Waterlogged	0.057	<0.001	<0.001	<0.001
			Flooded	<0.001	0.368	<0.001	0.127
						<0.001	0.165
		KP					
			Dry	<0.001		<0.001	
			Waterlogged	0.136	<0.001	0.060	<0.001
			Flooded	<0.001	<0.001	<0.001	0.038
						<0.001	0.836
	PA						
		Dry	<0.001		<0.001		
		Waterlogged	<0.001	<0.001	0.771	<0.001	
		Flooded	<0.001	0.583	<0.001	0.111	
					<0.001	0.198	
	PP						
		Dry	<0.001		<0.001		
		Waterlogged	0.012	<0.001	0.373	0.459	
		Flooded	0.822	<0.001	<0.001	0.812	
						0.977	
						0.995	

Table S1 continued

b) Forbs							
	Dry	0.994			<0.001		
	Waterlogged	0.003	0.019		0.047	<0.001	
	Flooded	0.124	0.184	0.997	0.132	<0.001	0.806
Grasses							
	Dry	<0.001			<0.001		
	Waterlogged	<0.001	<0.001		0.966	<0.001	
	Flooded	<0.001	0.352	<0.001	0.912	<0.001	0.988

V Author contributions

Manuscript 1

Title: Effects of plant-available soil silicon on seedling growth and foliar nutrient status across tropical tree species

Authors: Marius Klotz, Jörg Schaller and Bettina M. J. Engelbrecht

Status: Published in *Oikos*

Klotz, M., Schaller, J. and Engelbrecht, B. M. J. 2023. Effects of plant-available soil silicon on seedling growth and foliar nutrient status across tropical tree species. - *Oikos*: e10030. doi: [10.1111/oik.10030](https://doi.org/10.1111/oik.10030)

Contribution MK (in %): Study design 50%, implementation of experiments and data collection (including management of assistants, see acknowledgements) 95%, coordination of sample processing and analysis 80%, data analysis 100%, creation of figures 100%, writing first draft of the manuscript 100%, revision of manuscript 80%, corresponding author.

Author contributions: BMJE and JS conceived the idea. BMJE, JS and MK specified the study design. The experiment was coordinated and carried out by MK with the support of two assistants and additional helpers (see acknowledgements). Chemical analyses were coordinated by BMJE, JS and MK and carried out by the Biogeochemistry Laboratory at the Smithsonian Tropical Research Institute, the BayCEER Laboratories of Analytical Chemistry and of Isotope Biogeochemistry at the University of Bayreuth, and the Central Laboratory at Leibniz Centre for Agricultural Landscape Research. The data were analysed, and figures and tables created by MK with input from BMJE and JS. MK wrote the first draft of the manuscript, and revised it with input from BMJE and JS. MK is corresponding author.

Manuscript 2

Title: Silicon-based herbivory defence in tropical tree seedlings

Authors: Marius Klotz, Jörg Schaller and Bettina M. J. Engelbrecht

Status: Published in *Frontiers in Plant Science*

Klotz, M., Schaller, J. and Engelbrecht, B. M. J. 2023. Silicon-based anti-herbivore defense in tropical tree seedlings. - *Frontiers in Plant Science* 14: 1250868. doi: [10.3389/fpls.2023.1250868](https://doi.org/10.3389/fpls.2023.1250868)

Contribution MK (in %): Study design 60%, implementation of experiments and data collection (including management of assistants, see acknowledgements) 95%, coordination of sample processing and analysis 80%, data analysis 100%, creation of figures 100%, writing first draft of the manuscript 100%, revision of manuscript 80%, corresponding author.

Author contributions: BMJE and JS conceived the idea. BMJE, JS and MK specified the study design. The experiment was coordinated and carried out by MK with the support of two assistants and additional helpers (see acknowledgements). Chemical analyses were coordinated by BMJE, JS and MK and carried out by the Biogeochemistry Laboratory at the Smithsonian Tropical Research Institute and the BayCEER Laboratories of Analytical Chemistry. The data were analysed, and figures and tables created by MK with input from BMJE and JS. MK wrote the first draft of the manuscript, and revised it with input from BMJE and JS. MK is corresponding author.

Manuscript 3

Title: Plasticity of plant silicon and nitrogen concentrations in response to water regimes varies across temperate grassland species

Authors: Marius Klotz, Jörg Schaller, Heike Feldhaar, Jürgen Dengler, Gerhard Gebauer, Gregor Aas, Anita Weissflog, Bettina M. J. Engelbrecht

Status: Published in Functional Ecology

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Contribution MK (in %): Study design 0%, implementation of experiments and data collection 0%, coordination of sample processing and analysis 0%, data analysis 100%, creation of figures 100%, writing first draft of the manuscript 100%, revision of manuscript 80%, corresponding author.

Author contributions: BMJE, JD and GA conceived the idea and designed the study with input from JS, GG, HF and AW. The experiment was coordinated by JD and AW, and sample analyses coordinated by JS, GG and BMJE. Student helpers supported the implementation of the experiment (see acknowledgements). Chemical analyses were carried out by the BayCEER Laboratories of Analytical Chemistry and Isotope Biogeochemistry at the University of Bayreuth. The data were analysed, and figures and tables created by MK with input from BMJE and JS. MK wrote the first draft of the manuscript, and revised it with input mainly from BMJE and JS, and also the remaining co-authors. MK is corresponding author.

VI Full publication list

- Dengler, J., Becker, T., Conradi, T., Dolnik, C., Heindl-Tenhunen, B., Jensen, K., Kaufmann, J., Klotz, M., Kurzböck, C., Lampe, P., Langer, N., Marquardt, B., Putfarken, D., Rahmlow, M., Schuhmacher, O. and Went, J. 2017. GrassVeg.DE – die neue kollaborative Vegetationsdatenbank für alle Offenlandhabitats Deutschlands. - *Tuexenia* 37: 447–455.
- Klotz, M., Schaller, J., Kurze, S. and Engelbrecht, B. M. J. 2021. Variation of foliar silicon concentrations in temperate forbs: effects of soil silicon, phylogeny and habitat. - *Oecologia* 196: 977–987.
- Klotz, M., Schaller, J., Feldhaar, H., Dengler, J., Gebauer, G., Aas, G., Weissflog, A. and Engelbrecht, B. M. J. 2022. Plasticity of plant silicon and nitrogen concentrations in response to water regimes varies across temperate grassland species. - *Functional Ecology* 36: 3211–3222.
- Klotz, M., Schaller, J. and Engelbrecht, B. M. J. 2023. Effects of plant-available soil silicon on seedling growth and foliar nutrient status across tropical tree species. - *Oikos*: e10030.
- Klotz, M., Schaller, J. and Engelbrecht, B. M. J. 2023. Silicon-based anti-herbivore defense in tropical tree seedlings. - *Frontiers in Plant Science* 14: 1250868.

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VIII Eidesstattliche Versicherung und Erklärung

(§ 9 Satz 2 Nr. 3 PromO BayNAT)

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