Landscape variation in defense traits along gradients of multiple resources in a tropical savanna plant

Mohanbabu, N.; Veldhuis, M.P.; Jung, D.; Ritchie, M.E.

Citation

Mohanbabu, N., Veldhuis, M. P., Jung, D., & Ritchie, M. E. (2023). Landscape variation in defense traits along gradients of multiple resources in a tropical savanna plant. *Oikos*, *2023*(9). doi:10.1111/oik.09909

OIKOS

Research article

Landscape variation in defense traits along gradients of multiple resources in a tropical savanna plant

Neha Mohanbabu[1], **Michiel P. Veldhuis**[2], **Dana Jung**[1] **and Mark E. Ritchie**[1]

[1]Department of Biology, Syracuse University, Syracuse, NY, USA
[2]Institute of Environmental Sciences, Leiden University, Leiden, the Netherlands

Correspondence: Neha Mohanbabu (nehamohanbabu@gmail.com)

Oikos
2023: e09909
doi: 10.1111/oik.09909
Subject Editor: Robin Pakeman
Editor-in-Chief:
Gerlinde De Deyn
Accepted 27 April 2023

Many plant species are widely distributed and consequently are exposed to multiple abiotic factors and diverse herbivores, each of which may distinctly affect the magnitude of different defense traits. Alternative theories for optimal allocation to plant defense traits predict both positive and negative associations between magnitude of defense and resource availability. These predictions may apply even within species. This suggests potential for a single species' patterns of association of defense traits and resources to vary with both the type of defense and identity of resource, but relatively few studies have explored intraspecific variation in multiple defense traits along several resource gradients simultaneously. In order to address this gap, especially in an ecosytem dominated by large mammalian herbivores, we assessed relationships between multiple resources (rainfall, soil N, and soil P) and plant defense traits (prickle density, phenolics, and lignin content) using a widely distributed tropical savanna herb, *Solanum incanum*, growing in naturally occuring resource gradients within the Serengeti National Park. We found substantial intraspecific variation in all three defense traits across sites (n = 43). Variation in prickle density was positively associated with rainfall and soil P, but not soil N. In contrast to prickle density, phenolics and lignin were uncorrelated with all three resource gradients. This independent association of soil P with a carbon-based defense, prickle density, suggests potential for resources that are not components of defenses to influence allocation to defense traits. Such influence may reflect association between resource and herbivore abundance and/or preference. These varied patterns in resource–defense associations further emphasize the tremendous variation in anti-herbivore traits which may be influenced by different plant resources and highlight the need to consider multiple resource gradients in understanding evolution of plant traits.

Keywords: intraspecific variation, mammal herbivory, multiple resources, phosphorus, physical defense

NORDIC SOCIETY OIKOS

www.oikosjournal.org

Introduction

Plants invest in a variety of anti-herbivore defenses such as physical (Hanley et al. 2007, Barton 2016), chemical (Levin 1976, Bennett and Wallsgrove 1994) and mutualistic defenses (Heil and McKey 2003). Even within species, there is a significant amount of variation in the type and quantity of defense due to plasticity and local adaptation and some of this variation may be driven by plants' access to resources (Castillo et al. 2013, Abdala-Roberts et al. 2016, Lynn and Fridley 2019). Plant resource availability may influence the costs and benefits of allocating resources to defense traits rather than plant growth and reproduction (Coley et al. 1985, Herms and Mattson 1992, Stamp 2003, Endara and Coley 2011, Hahn and Maron 2016). Such cost-benefit relationships can be further modified by risks of tissue loss from different types of herbivores (insect versus mammal, generalist versus specialist) and intensity of herbivory (Carmona et al. 2011, Perkovich and Ward 2022).

Resources may influence defense traits in unexpected ways if plant growth and herbivory risk are limited or driven by different resources. Several studies have shown that plants and herbivores can be simultaneously limited by multiple resources such as water/light, nitrogen (N), and phosphorus (P) (Elser et al. 2007, Harpole et al. 2011, Sperfeld et al. 2012). But previous hypotheses (Hahn and Maron 2016, Koffel et al. 2018) largely assume that plant and herbivore abundance are limited by the same resource (or that herbivores prefer plant tissue rich in nutrients limiting to plants in that system such as N), in which case plants have more resources to allocate to defense and face higher herbivory risks at higher resource availability. In contrast, the herbivory risk might be associated with availability of resources that do not strongly limit plant growth such as when plants may be most limited by N or P and herbivores may be most limited by sodium (Na) (Kaspari et al. 2017, Borer et al. 2019, Welti et al. 2019, Kaspari 2020). Thus, allocation to plant defenses may respond to different resources in distinct ways depending on resources limiting plant growth and herbivore abundance/preference. Additionally, resources that are not major structural components of defenses such as P or Na may also influence allocation to defense indirectly. However, these different possibilities have received little attention as studies on variation in defense traits along multiple resource gradients are relatively rare. Given the growing evidence of the importance of resources other than carbon (C) and N to both plants and herbivores (Fay 2015, Kaspari et al. 2017, Prather et al. 2018, Borer et al. 2019), it is crucial to understand their impact on plant defense traits.

The association of defense traits with resource gradients may vary depending on the identity of the resource. Even though most plant defense theories (Feeny 1976, Coley et al. 1985, Herms and Mattson 1992, Hahn and Maron 2016, Koffel et al. 2018) treat resources generically (but see Bryant et al. 1983), they may offer a broad framework to understand patterns in resource–defense relationships. Some plant defense theories have predicted that even within species, defenses should decrease with increasing resources, especially if the plant is adapted to a physiologically stressful condition (Hahn and Maron 2016). Alternatively, plant defenses may be positively associated with resources, especially if allocation to defenses is indirectly mediated by high herbivory pressure at resource rich sites (Hahn and Maron 2016). Evidence from systems with predominantly insect herbivores provides some support to both the predictions (Hahn and Maron 2016, Abdala-Roberts et al. 2016, Moreira et al. 2018, Hahn et al. 2019). The contrasting patterns for the intraspecific resource–defense relationships highlight the importance of the type of defense and identity of resource and herbivores for understanding these patterns. Moreover, plants subjected to intense mammalian herbivory are relatively poorly studied and might not show the same patterns as plants subjected to insect herbivory. Consequently, there is a clear need to explore intraspecific relationships between the magnitude of different plant defense traits and availability of different resources.

Plant defense response to mammalian herbivory may differ from that of the more heavily researched response to insect herbivory (Carmona et al. 2011). Firstly, chemical defenses may be less effective against mammalian herbivores that consume larger quantities and diverse group of plants, thus diluting the impact of any single chemical (Mattson 1980). Secondly, mammalian consumption of whole or large portions of an individual plant in a single bite poses a different set of challenges to plants than invertebrate herbivory (Sanson 2006) and possibly results in different types of anti-herbivore strategies, such as avoidance through divaricate branching or structural defenses such as prickles (Hanley et al. 2007, Tomlinson et al. 2016, Coverdale et al. 2019, Wigley et al. 2019). Unlike most chemical defenses, resources allocated to physical defenses cannot be metabolically reverted to resources for future plant growth and reproduction. Thirdly, the resources, predation risk and environmental conditions (e.g. temperature) that determine insect herbivore abundances (or herbivory intensity) and defenses may be different than those of mammalian herbivores. These factors may inherently affect the cost–benefit ratio of allocation to defenses and consequently may influence the 'dominant' type of defense depending on the identities of herbivores (Perkovich and Ward 2022) and resources but have rarely been tested for ecosystems with mammalian herbivory.

In this paper, we studied intraspecific variation in three types of C-based defenses: prickle density, phenolic content, and lignin content, at 43 sites for a widely distributed perennial herbaceous plant species, *Solanum incanum.* These sites spanned substantial natural gradients of rainfall (a proxy for water availability) and total soil N and P (Ruess and Seagle 1994, Anderson et al. 2007) in the Serengeti National Park, Tanzania which also features a wide range of mammalian herbivory intensity across the landscape. We assessed associations between the different plant defense traits and plant resources.

Material and methods

Field site

The Serengeti National Park in northern Tanzania is among the last remaining contiguous grassland savanna ecosystems in the world. It is home to a highly diverse group of resident and migratory large mammalian herbivores including wildebeest *Connochaetes taurinus*, plains zebra *Equus quagga*, Thomson's and Grant's gazelles *Eudorcas thomsonii*, *Nanger soemmerringii*, impala *Aepyceros melampus*, eland *Taurotragus oryx*, elephants *Loxodonta africana*, topi *Damaliscus lunatus* and Coke's hartebeest *Alcelaphus buselaphus* (Sinclair and Norton-Griffiths 1979, McNaughton 1985). These herbivores often exert intense herbivory pressure with plant losses as high as 60–90% of the annual aboveground biomass (McNaughton 1985) with likely minimal loss to insect herbivores as in other savanna ecosystems (Davies et al. 2016). More recent research has also shown that variation in herbivory intensity is associated with resource gradients such that herbivory intensity is negatively associated with rainfall, positively associated with P availability to plants and unassociated with N availability to plants (Mohanbabu and Ritchie 2022) in the park. These associations are a result of resource supplies influencing herbivory intensity as the patterns hold even with nutrients from fenced plots (i.e. no herbivory) indicating that nutrient recycling from herbivory, if any, were weak (Mohanbabu and Ritchie 2022). The inference is further supported by the fact that the underlying variation in soil N and P in the Serengeti has been attributed to volcanic ash deposits in the southeast to highly leached granite-derived sands in the north (Anderson and Talbot 1965, Sinclair et al. 2008).

Study organism

Solanum incanum (hereafter *Solanum*), is a pan-African and pan-Asian herbaceous plant which is consumed by several browser and mixed-feeding herbivore species such as impala, elephant, gazelles and eland (based on available data from other members of *Solanum* genus from East Africa (Kartzinel et al. 2015, Coverdale et al. 2019). Even within the Serengeti, *Solanum* is widely distributed, naturally occurring in a variety of habitats, and invests in both physical and chemical defenses. Their stems have prickles which can vary drastically in density and is likely inducible by herbivory similar to other *Solanum* species in the region (Coverdale et al. 2019). Additionally, it produces both phenolics and alkaloids, the latter of which is more prominently present in reproductive parts such as flowers and fruits (Al Sinani and Eltayeb 2017). Therefore, we measure only leaf phenolic content. In terms of the leaf structural content, like other plants, they also invest in structural carbon such as lignin and cellulose which may also confer some anti-herbivore properties due to increased leaf toughness and reduced palatability.

Study design

From 2000 to 2002, several sites (n = 102) across the national park were identified to form a network of vegetation survey plots; the trans-Serengeti plots (Anderson et al. 2007). These plots encompass much of the substantial spatial variation in rainfall (700–1125 mm), total soil N (0.2–3.7 mg g^{-1}) and total soil P (0.007–0.53 mg g^{-1}) across Serengeti National Park (Ruess and Seagle 1994, Anderson et al. 2007). As can be expected in natural systems, some of the gradients are correlated: soil P and rainfall are negatively associated, and soil P and soil N are positively associated (Supporting information). We account for this correlation statistically by using all three resources in the same regression, but only experimental manipulations can result in truly uncorrelated predictors.

Plots were sampled by randomly dropping points in 10 × 10 km grids and choosing one accessible point per grid such that the plot network includes a wide range of variation in abiotic factors. In 2018, we sampled 61 of these plots of which *Solanum* was present in 43 of them (Fig. 1), only slightly less than the abundant grasses, *Digitaria macroblephara* (at 50 sites) and *Themeda triandra* (at 47 sites). At each site, we sampled five randomly selected individuals of *Solanum* that were at least 5 m apart. For each individual, we recorded prickle density as the number of prickles on a 5 cm length of stem at approx. 5 cm from the base and we also collected five fully expanded mature leaves which were air-dried at 45°C and shipped to Syracuse University, USA for further analyses. We collected soil cores at each site to characterize soil nutrient availability. Total soil N and P were estimated at the Sokoine University of Agriculture, Tanzania using Kjeldahl method (Carter and Gregorich 2007) and persulfate digestion method (Carter and Gregorich 2007), respectively. We use a 10 year average for rainfall at these sites that was extracted from the Climate Hazard Group InfraRed Precipitation with Station data (CHIRPS) database (Funk et al. 2015) which provides high resolution precipitation estimates based on both long-term climate averages and weather station data.

Chemical defense traits

We estimated total foliar phenolic content using the Folin-Ciocalteu assay (Ainsworth and Gillespie 2007). Briefly, 5 mg of the dried and ground leaf sample per individual was homogenized in 2 ml of ice-cold 95% v/v methanol and centrifuged at 13 000g for 5 min at room temperature after 48 h of incubation in the dark. One hundred µl of the supernatant was diluted with 100 µl of distilled water and 200 µl of 10% (v/v) Folin-Ciocalteu reagent (Sigma Aldrich) was added and mixed thoroughly. After 2 min, 800 µl of 700 mM sodium carbonate solution was added to each tube and the reaction mixture was incubated at room temperature for 20 min. One ml of the reaction mixture was transferred to a cuvette and absorbance at 765 nm was recorded for blanks, gallic acid standards and samples. Phenolic content was calculated as gallic acid equivalents based on the standard curve.

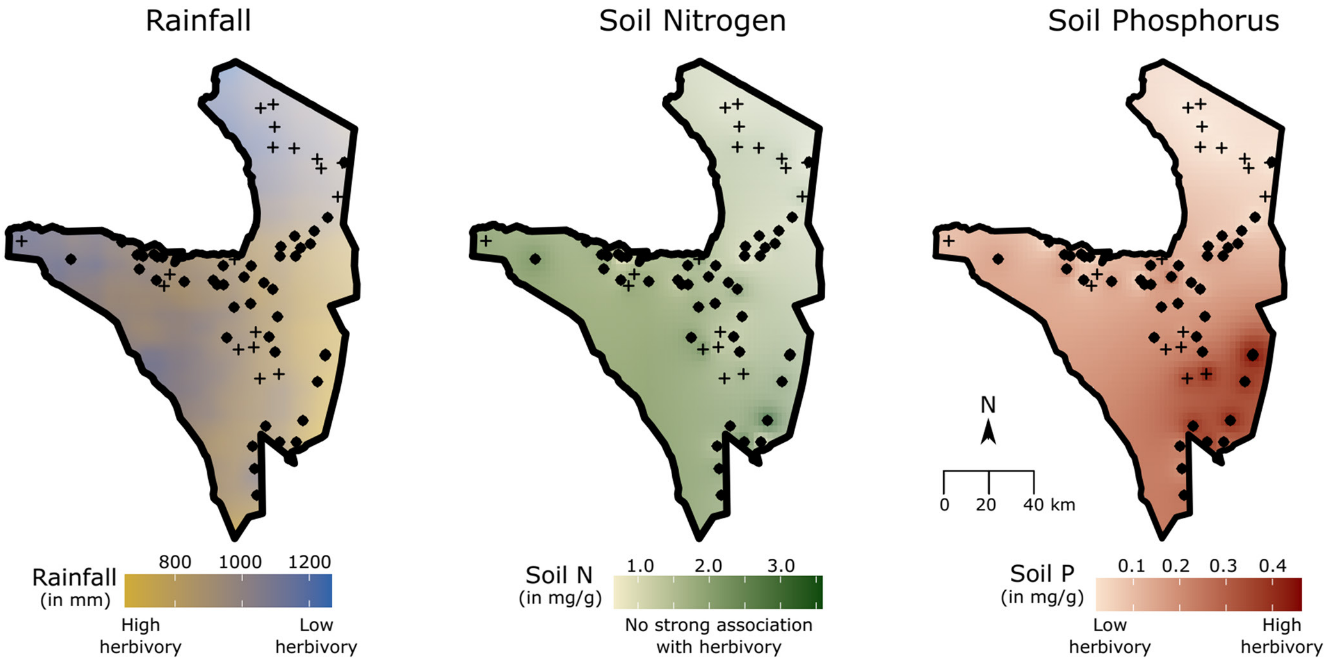

Figure 1. Illustration of the different resource gradients – rainfall, total soil N, and total soil P- across the Serengeti National Park. '+' indicate all 61 sites sampled and dots indicate sites with *Solanum incanum*. The associations of herbivory intensity with the different resource availabilities to plants are based on previous research from the national park (Mohanbabu and Ritchie 2022).

Although *Solanum* may also produce alkaloids, they disintegrate quickly unless stored at −80°C, which limited our ability to quantify alkaloids from air-dried samples.

We estimated structural lignin content of *Solanum* leaves using a multi-step sequential digestion with ANKOM 200 Fiber Analyzer. The leaf samples for each site were pooled and ~ 0.5 g of the dried leaf material was sealed in a special 25 µ porous bags from ANKOM. The bags were weighed and sequentially treated with neutral detergent solution at 100°C for 75 min, acid detergent solution at 100°C for 60 min and 98% sulfuric acid at room temperature for 3 h. Between each step, the bags were washed with hot water and acetone to remove any remaining detergents, dried at 105°C overnight and weighed. Finally, the filter bags were ashed at 500°C for 330 min in a muffle furnace. The difference in the weights between concentrated sulfuric acid treatment and ashing were used to estimate the percentage of lignin per dry mass of plant tissue.

Statistical analysis

All the data analyses were run in R (www.r-project.org).

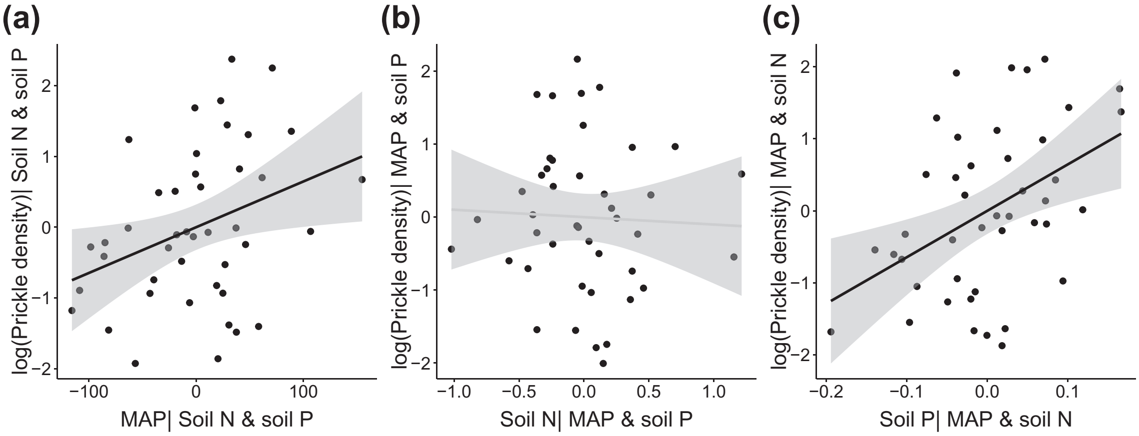

Figure 2. Variation in prickle density along gradients of (a) rainfall, (b) soil N, and (c) soil P represented as partial residual graphs, i.e. covariation due to other variables in the model are already accounted for. In the figure, each dot is the residual of log transformed mean prickle density at a site, dark lines represent significant relationships and grey lines represent insignificant relationships based on univariate models, and grey areas represent the 95% confidence intervals.

Variation in defense traits

As sites (n = 43) are the experimental units in this study, we averaged data for the five individuals per site for all traits except lignin, for which samples were already pooled at the site level before ANKOM analyses as the procedure required larger amounts of plant tissue. To explore variation in defense traits along gradients of resource availability, we ran linear models using 'lm()' with a given defense trait as the dependent variable and rainfall, total soil N and P as the independent variables. Including interaction terms did not improve the models and therefore, we leave them from the analyses. Such a model allowed us to account for correlations among the measured environmental variables. As all three resources are in the model, and 'lm()' uses type III sums of squares, the effect of each variable is calculated after accounting for the effects of other variables in the model. For the traits for which we have data for within site variation, we also ran mixed models using 'lmerTest' package (Kuznetsova et al. 2017) including 'Site' as a random effect and the environmental variables as the fixed effects and found that the inference does not change (Supporting information). Finally, all models satisfied the assumptions of normality, homoscedasticity, and low multicollinearity (we use VIF < 3 as the cut-off similar to Zuur et al. 2010 as completely uncorrelated predictors are hard to find in natural systems).

For the purposes of visualizing associations of defense traits with different resources, we present partial residual plots (Fig. 2, 3) of variation in both dependent (log(prickle density + 1)) and independent (rainfall, total soil N and soil P) variables once the covariance between the remaining independent variables is accounted for.

Results

All three defense traits showed substantial intraspecific variation: prickle density ranged from no prickles (undefended) to ~ 72 prickles per 5 cm, and total phenolic and structural lignin content varied by about a factor of three, ranging between 1.75 and 8.77 nmol of gallic acid equivalents/mg of sample, and 4.57–13.43%, respectively. Differences between individuals within a site accounted for some of the variability (41% for prickle density and ~ 11% for phenolic content), but this variability did not change patterns at the landscape scale (Supporting information).

Resources explained some of the variation in defense traits. Prickle density increased with both rainfall (0.46 (0.20), p = 0.03) (Fig. 2a, Table 1) and soil P (0.78(0.26), p = 0.004) (Fig. 2c, Table 1), but did not change with soil N (−0.06 (0.22), p = 0.78) (Fig. 2b, Table 1). In contrast, total

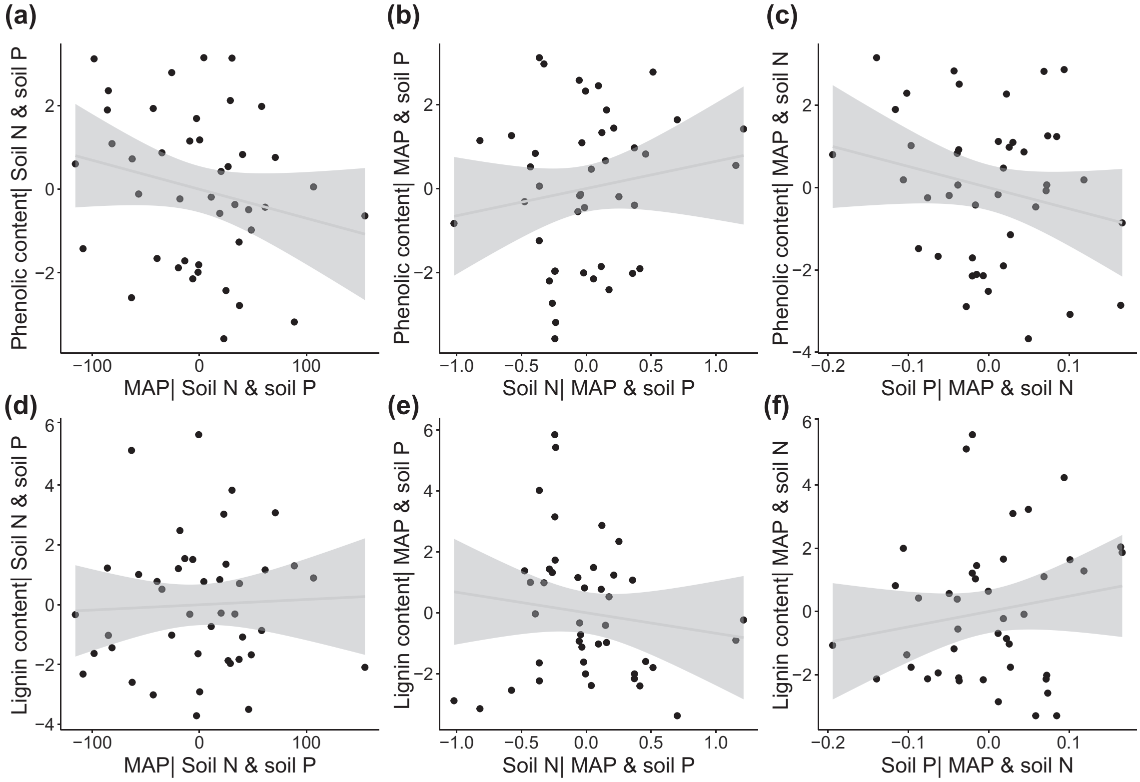

Figure 3. Variation in phenolic and lignin contents along gradients of (a, d) rainfall, (b, e) soil N, and (c, f) soil P represented as partial residual graphs, i.e. covariation due to other variables in the model are already accounted for. In the figure, each dot is the residual of phenolic or lignin content at a site, dark lines represent significant relationships and grey lines represent insignificant relationships based on univariate models, and grey areas represent the 95% confidence intervals.

Table 1. Summary of the linear models, associated coefficients (and standard error) and adjusted R^2 for each of the defense traits prickle density, total phenolic content and lignin. Bold values indicate $p < 0.05$.

Factors	Prickle density	Total phenolic content	Lignin
MAP	**0.46(0.20)**	−0.50(0.35)	0.13(0.43)
Soil N	−0.06(0.22)	0.39(0.39)	−0.40(0.48)
Soil P	**0.78(0.26)**	−0.63(0.44)	0.59(0.54)
R^2	0.23	−0.01	−0.04

phenolic content (Fig. 3a–c, Table 1) and lignin (Fig. 3d–f, Table 1) were uncorrelated with rainfall and soil resource gradients.

Discussion

We explored patterns of intraspecific variation in defense traits along multiple resource gradients in a plant species that can experience substantial herbivory by large mammals. We found that prickle density was positively associated with both rainfall and total soil P in the system (Fig. 2a, c). To our knowledge, this is the first-time total soil P has been shown to be associated with a physical defense trait even though P is not a major structural component of largely carbon-based defense traits. One explanation for this association is the potential for resources to influence herbivores and their associated risk of plant damage (Hahn and Maron 2016, Koffel et al. 2018). Interestingly, contrary to our expectation, prickles were positively associated with rainfall even when herbivory intensity at this site decreases along this gradient (Mohanbabu and Ritchie 2022). Lastly, our results for phenolics and lignin contrast with most prior research, which generally shows positive associations between climate factors (temperature and precipitation) or N supply and chemical defenses (Moreira et al. 2015, Hahn and Maron 2016, Abdala-Roberts et al. 2016, Hahn et al. 2019). Thus, our work highlights the potential for associations of various types of defense traits to three common resources that limit plant growth.

Our results on the positive association between soil P and prickle density (Fig. 2c) adds to the evidence from earlier research on insect herbivores showing that P supply may affect anti-herbivore defenses (Cuevas-Reyes et al. 2004, 2011, De Long et al. 2016, Graff et al. 2020). However, the proximate mechanisms still remain unclear. A potential explanation is that resource-rich sites may support higher herbivore biomass and herbivory intensity causing the plants to allocate more to defenses (Hahn and Maron 2016). As plant P supply in the Serengeti is positively associated with herbivory intensity at the landscape scale (Fig. 1) (Mohanbabu and Ritchie 2022), there is some support for this explanation. Furthermore, resources may also affect allocation to defense even in the absence of resource-herbivory intensity associations. An alternate mechanism could be elevated rates of photosynthesis and C assimilation at higher soil P resulting in C allocation towards a C-based physical defense, such as prickle density (Herms and Mattson 1992). More work is needed to elucidate mechanisms by which P may affect defense allocation.

The positive association between prickle density and rainfall (Fig. 2a) is unlikely to have been affected by herbivory risk since herbivory intensity in the Serengeti is negatively associated with rainfall (Mohanbabu and Ritchie 2022). In contrast to herbivory, rainfall, productivity and plant C:N ratios are positively associated in grasslands (Sinclair 1975, McNaughton 1985, Holdo et al. 2009). Increasing rainfall may allow for higher rates of CO_2 uptake relative to N- or P-based biochemical machinery (Olff et al. 2002). If plants assimilate additional C above what is needed for growth at high rainfall sites, that C may be allocated to C-based structural defense at relatively lower costs (Herms and Mattson 1992). This expectation and result are different from some of the earlier studies that have linked spinescence to aridity in interspecific comparisons from arid sites (Milton 1991) but more recent evidence suggest that spinescence may not be strongly or generally associated with climate (Anest et al. 2021). Regardless, we find the opposite pattern than expected for an intraspecific comparison for a relatively wetter region. An intriguing possibility is that across a broad water availability gradient, resource-defense associations may be non-linear, that is, negative over low ranges of precipitation and positive over higher ranges.

The general lack of variation in lignin and phenolics with resource supplies may indicate that those traits may not be as strongly influenced by mammalian herbivory as are prickles. Even in other systems, phenolics has been shown to be negatively (Wallis et al. 2011, Moreira et al. 2018), positively or neutrally associated with resource availability (Moreno and Bertiller 2012, Abdala-Roberts et al. 2016, Wang et al. 2016). Similar variation in positive versus negative associations have been reported for intraspecific variation in lignin as well: positive (Castro-Díez et al. 1997, Wang et al. 2016, Moreira et al. 2018), no change (Castro-Díez et al. 1997, Moreno and Bertiller 2012), and negative associations (Wallis et al. 2011). The wide range of patterns in phenolics and lignin might result from several mechanisms. Firstly, both phenolics and lignin may be responding to insect herbivory or microbial pathogens, and abundances of insects and pathogens in savannas may be low compared to mammalian herbivores (Davies et al. 2016). Secondly, both phenolics and lignin are leaf-level carbon-based defenses and may get allocated to labile (total phenolics) or structural (lignin) carbon related to the leaf economics, plant relative growth rate and leaf lifespan, with inadvertent covarying consequences for plant defenses (Coley et al. 1985). Despite both defense traits exhibiting no association with plant resources, phenolics were indeed negatively associated with lignin (Pearson correlation coefficient = −0.37, p = 0.02, Supporting information), which suggests a potential trade-off for plants in allocating carbon to these different compounds. Finally, the two defenses may contribute to plant fitness in ways not necessarily related to defense, such as when phenolics protect against light damage (Isah 2019, Erb and Kliebenstein 2020) and lignin provides structural support and extends leaf lifespan (Kitajima et al. 2012).

Our work adds to a limited number of field studies on associations of defenses with multiple resources in the presence of mammalian herbivores. Our field survey limits our power of inference and field experiments that manipulate different resource levels and herbivory are likely necessary to determine cause–effect relationships of resources and herbivory risk on defense traits. Although our analysis is limited to a single species, *S. incanum* which exhibits multiple types of defenses, is common in an unusually wide variety of environments (i.e. was present at 43 of the 61 sites) and is consumed by multiple mammalian herbivore species (based on available data from other members of *Solanum* genus from East Africa (Kartzinel et al. 2015, Coverdale et al. 2019) is ideal for such studies. Even though our work may easily be extended to a vast majority of species in Solanaceae that invest in different types of defenses, future work on different plant families will help evaluate any generality of our results. Additionally, *Solanum* also allocates to other defenses such as glycoalkaloids and trichomes which we were unable to measure but they contribute to the overall costs and benefits of defenses. Finally, it is possible that the herbivores imposing potential damage and defense traits are responding to the supply of a different resource we did not measure, such as Na or Ca. However, concentrations of these elements in soil and/or plant tissue show low correlation with that of herbivory intensity in the Serengeti (Mohanbabu and Ritchie 2022). Regardless, variation in defense traits along gradients of other resources such as Na and Ca are poorly studied and warrant attention in future research.

In conclusion, we find substantial intraspecific variation in defense traits of *Solanum incanum*, especially prickle density, that is associated with variation in multiple environmental resources. Interestingly, prickle density was positively associated with soil P suggesting a potential for influence of resources other than C and N on plant defenses. In contrast, a threefold variation in phenolic and lignin content was unexplained by variation in multiple resources. Our work indicates that future research should consider both different types of defenses and resources in order to better understand the complex link between resource supply and allocation to defenses.

Acknowledgements – We would like to thank Emilian Mayemba, Jim Frederickson and Toni Hoenders for assistance in the field and the editors for their feedback on an earlier version of this manuscript. Special thanks to TAWIRI, COSTECH and TANAPA for allowing us to carry out research at Serengeti National Park.

Funding – This study was supported by NSF grant DEB 1557085.

Permits – Permits were obtained from TAWIRI, COSTECH (no. 2017-315-NA-2014-268), and TANAPA (TNP/HQ/C.10/13) for field work in the Serengeti National Park, Tanzania.

Author contributions

Neha Mohanbabu: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Project administration (lead); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Michiel P. Veldhuis**: Formal analysis (supporting); Validation (supporting); Writing – review and editing (supporting). **Dana Jung**: Methodology (supporting). **Mark E. Ritchie**: Conceptualization (equal); Formal analysis (supporting); Funding acquisition (lead); Investigation (supporting); Methodology (supporting); Resources (lead); Supervision (lead); Writing – review and editing (supporting).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.905qfttr1 (Mohanbabu et al. 2023).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Abdala-Roberts, L., Rasmann, S., Berny-Mier Y Terán, J. C., Covelo, F., Glauser, G. and Moreira, X. 2016. Biotic and abiotic factors associated with altitudinal variation in plant traits and herbivory in a dominant oak species. – Am. J. Bot. 103: 2070–2078.

Ainsworth, E. A. and Gillespie, K. M. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. – Nat. Protoc. 2: 875–877.

Al Sinani, S. S. S. and Eltayeb, E. A. 2017. The steroidal glycoalkaloids solamargine and solasonine in *Solanum* plants. – S. Afr. J. Bot. 112: 253–269.

Anderson, G. D. and Talbot, L. M. 1965. Soil factors affecting the distribution of the grassland types and their utilization by wild animals on the Serengeti plains, Tanganyika. – J. Ecol. 53: 33–56.

Anderson, T. M., Metzger, K. L. and McNaughton, S. J. 2007. Multi-scale analysis of plant species richness in Serengeti grasslands. – J. Biogeogr. 34: 313–323.

Anest, A., Charles-Dominique, T., Maurin, O., Millan, M., Edelin, C. and Tomlinson, K. W. 2021. Evolving the structure: climatic and developmental constraints on the evolution of plant architecture. A case study in *Euphorbia*. – New Phytol. 231: 1278–1295.

Barton, K. E. 2016. Tougher and thornier: general patterns in the induction of physical defence traits. – Funct. Ecol. 30: 181–187.

Bennett, R. N. and Wallsgrove, R. M. 1994. Secondary metabolites in plant defence mechanisms. – New Phytol. 127: 617–633.

Borer, E. T., Lind, E. M., Firn, J., Seabloom, E. W., Anderson, T. M., Bakker, E. S., Biederman, L., La Pierre, K. J., MacDougall, A. S., Moore, J. L., Risch, A. C., Schutz, M. and Stevens, C. J. 2019. More salt, please: global patterns, responses and impacts of foliar sodium in grasslands. – Ecol. Lett. 22: 1136–1144.

Bryant, J. P., Chapin, F. S. and Klein, D. R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. – Oikos 40: 357–368.

Carmona, D., Lajeunesse, M. J. and Johnson, M. T. J. 2011. Plant traits that predict resistance to herbivores. – Funct. Ecol. 25: 358–367.

Carter, M. R. and Gregorich, E. G. 2007. Soil sampling and methods of analysis. – CRC Press.

Castillo, G., Cruz, L. L., Hernández-Cumplido, J., Oyama, K., Flores-Ortiz, C. M., Fornoni, J., Valverde, P. L. and Núñez-Farfán, J. 2013. Geographic association and temporal variation of chemical and physical defense and leaf damage in *Datura stramonium*. – Ecol. Res. 28: 663–672.

Castro-Díez, P., Villar-Salvador, P., Pérez-Rontomé, C., Maestro-Martínez, M. and Montserrat-Martí, G. 1997. Leaf morphology and leaf chemical composition in three *Quercus* (Fagaceae) species along a rainfall gradient in NE Spain. – Trends Ecol. Evol. 11: 127–134.

Coley, P. D., Bryant, J. P. and Chapin, F. S. 1985. Resource availability and plant antiherbivore defense. – Science 230: 895–899.

Coverdale, T. C., McGeary, I. J., O'Connell, R. D., Palmer, T. M., Goheen, J. R., Sankaran, M., Augustine, D. J., Ford, A. T. and Pringle, R. M. 2019. Strong but opposing effects of associational resistance and susceptibility on defense phenotype in an African savanna plant. – Oikos 128: 1772–1782.

Cuevas-Reyes, P., Quesada, M., Siebe, C. and Oyama, K. 2004. Spatial patterns of herbivory by gall-forming insects: a test of the soil fertility hypothesis in a Mexican tropical dry forest. – Oikos 107: 181–189.

Cuevas-Reyes, P., De Oliveira-Ker, F. T., Fernandes, G. W. and Bustamante, M. 2011. Abundance of gall-inducing insect species in sclerophyllous savanna: understanding the importance of soil fertility using an experimental approach. – J. Trop. Ecol. 27: 631–640.

Davies, A. B., van Rensburg, B. J., Robertson, M. P., Levick, S. R., Asner, G. P. and Parr, C. L. 2016. Seasonal variation in the relative dominance of herbivore guilds in an African savanna. – Ecology 97: 1618–1624.

De Long, J. R., Sundqvist, M. K., Gundale, M. J., Giesler, R. and Wardle, D. A. 2016. Effects of elevation and nitrogen and phosphorus fertilization on plant defence compounds in subarctic tundra heath vegetation. – Funct. Ecol. 30: 314–325.

Elser, J. J., Bracken, M. E., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B. and Smith, J. E. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – Ecol. Lett. 10: 1135–1142.

Endara, M.-J. and Coley, P. D. 2011. The resource availability hypothesis revisited: a meta-analysis. – Funct. Ecol. 25: 389–398.

Erb, M. and Kliebenstein, D. J. 2020. Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy1[OPEN]. – Plant Physiol. 184: 39–52.

Fay, P. A. 2015. Grassland productivity limited by multiple nutrients. – Nat. Plants 1: 15080.

Feeny, P. 1976. Plant apparency and chemical defense. – In: Wallace, J. W. and Mansell, R. L. (eds.), Biochemical interaction between plants and insects. Springer, pp. 1–40.

Funk, C., Peterson, P., Landsfeld, M., Pedreros, D., Verdin, J., Shukla, S., Husak, G., Rowland, J., Harrison, L., Hoell, A. and Michaelsen, J. 2015. The climate hazards infrared precipitation with stations – a new environmental record for monitoring extremes. – Sci. Data 2: 150066.

Graff, P., Gundel, P. E., Salvat, A., Cristos, D. and Chaneton, E. J. 2020. Protection offered by leaf fungal endophytes to an invasive species against native herbivores depends on soil nutrients. – J. Ecol. 108: 1592–1604.

Hahn, P. G. and Maron, J. L. 2016. A framework for predicting intraspecific variation in plant defense. – Trends Ecol. Evol. 31: 646–656.

Hahn, P. G., Agrawal, A. A., Sussman, K. I. and Maron, J. L. 2019. Population variation, environmental gradients, and the evolutionary ecology of plant defense against herbivory. – Am. Nat. 193: 20–34.

Hanley, M. E., Lamont, B. B., Fairbanks, M. M. and Rafferty, C. M. 2007. Plant structural traits and their role in anti-herbivore defence. – Perspect. Plant Ecol. Evol. Syst. 8: 157–178.

Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E., Elser, J. J., Gruner, D. S., Hillebrand, H., Shurin, J. B. and Smith, J. E. 2011. Nutrient co-limitation of primary producer communities. – Ecol. Lett. 14: 852–862.

Heil, M. and McKey, D. 2003. Protective ant–plant interactions as model systems in ecological and evolutionary research. – Annu. Rev. Ecol. Evol. Syst. 34: 425–553.

Herms, D. A. and Mattson, W. J. 1992. The dilemma of plants : to grow or defend. – Q. Rev. Biol. 67: 283–335.

Holdo, R. M., Holt, R. D. and Fryxell, J. M. 2009. Opposing rainfall and plant nutritional gradients best explain the wildebeest migration in the Serengeti. – Am. Nat. 173: 431–445.

Isah, T. 2019. Stress and defense responses in plant secondary metabolites production. – Biol. Res. 52: 39.

Kartzinel, T. R., Chen, P. A., Coverdale, T. C., Erickson, D. L., Kress, W. J., Kuzmina, M. L., Rubenstein, D. I., Wang, W. and Pringle, R. M. 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. – Proc. Natl Acad. Sci. USA 112: 8019–8024.

Kaspari, M. 2020. The seventh macronutrient: how sodium shortfall ramifies through populations, food webs and ecosystems. – Ecol. Lett. 23: 1153–1168.

Kaspari, M., Roeder, K. A., Benson, B., Weiser, M. D. and Sanders, N. J. 2017. Sodium co-limits and catalyzes macronutrients in a prairie food web. – Ecology 98: 315–320.

Kitajima, K., Llorens, A. M., Stefanescu, C., Timchenko, M. V., Lucas, P. W. and Wright, S. J. 2012. How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. – New Phytol. 195: 640–652.

Koffel, T., Daufresne, T., Massol, F. and Klausmeier, C. A. 2018. Plant strategies along resource gradients. – Am. Nat. 192: 360–378.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. 2017. lmerTest Package: tests in linear mixed effects models. – J. Stat. Softw. 82: 1–26.

Levin, D. A. 1976. The chemical defenses of plants to pathogens and herbivores. – Annu. Rev. Ecol. Syst. 7: 121–159.

Lynn, J. S. and Fridley, J. D. 2019. Geographic patterns of plant–herbivore interactions are driven by soil fertility. – J. Plant Ecol. 12: 653–661.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen content. – Annu. Rev. Ecol. Syst. 11: 119–161.

McNaughton, S. J. 1985. Ecology of a grazing ecosystem : the Serengeti. – Ecol. Monogr. 55: 259–294.

Milton, S. J. 1991. Plant spinescence in arid southern Africa: does moisture mediate selection by mammals? – Oecologia 87: 279–287.

Mohanbabu, N. and Ritchie, M. E. 2022. Large herbivore impact on plant biomass along multiple resource gradients in the Serengeti. – J. Ecol. 110: 1537–1547.

Mohanbabu, N., Veldhuis, M. P., Jung, D. and Ritchie, M. E. 2023. Data from: Landscape variation in defense traits along gradients of multiple resources in a tropical savanna plant. – Dryad Digital Repository, https://doi.org/10.5061/dryad.905qfttr1.

Moreira, X., Abdala-Roberts, L., Parra-Tabla, V. and Mooney, K. A. 2015. Latitudinal variation in herbivory: influences of climatic drivers, herbivore identity and natural enemies. – Oikos 124: 1444–1452.

Moreira, X., Castagneyrol, B., Abdala-Roberts, L., Berny-Mier y Teran, J. C., Timmermans, B. G. H., Bruun, H. H., Covelo, F., Glauser, G., Rasmann, S. and Tack, A. J. M. 2018. Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory. – Ecography 41: 1124–1134.

Moreno, L. and Bertiller, M. B. 2012. Variation of morphological and chemical traits of perennial grasses in arid ecosystems. Are these patterns influenced by the relative abundance of shrubs? – Acta Oecol. 41: 39–45.

Olff, H., Ritchie, M. E. and Prins, H. H. 2002. Global environmental controls of diversity in large herbivores. – Nature 415: 901–904.

Perkovich, C. and Ward, D. 2022. Differentiated plant defense strategies: herbivore community dynamics affect plant–herbivore interactions. – Ecosphere 13: e3935.

Prather, C. M., Laws, A. N., Cuellar, J. F., Reihart, R. W., Gawkins, K. M. and Pennings, S. C. 2018. Seeking salt: herbivorous prairie insects can be co-limited by macronutrients and sodium. – Ecol. Lett. 21: 1467–1476.

Ruess, R. W. and Seagle, S. W. 1994. Landscape patterns in soil microbial processes in the Serengeti National Park, Tanzania. – Ecology 75: 892–904.

Sanson, G. 2006. The biomechanics of browsing and grazing. – Am. J. Bot. 93: 1531–1545.

Sinclair, A. R. E. 1975. The resource limitation of trophic levels in tropical grassland ecosystems. – J. Anim. Ecol. 44: 497–520.

Sinclair, A. R. E. and Norton-Griffiths, M. 1979. Serengeti: dynamics of an ecosystem. – Univ. of Chicago Press.

Sinclair, A. R. E., Packer, C., Mduma, S. A. R. and Fryxel, J. M. 2008. Serengeti III : human impacts on ecosystem dynamics. – Univ. of Chicago Press.

Sperfeld, E., Martin-Creuzburg, D. and Wacker, A. 2012. Multiple resource limitation theory applied to herbivorous consumers: Liebig's minimum rule vs interactive co-limitation. – Ecol. Lett. 15: 142–150.

Stamp, N. 2003. Out of the quagmire of plant defense hypotheses. – Q. Rev. Biol. 78: 23–55.

Tomlinson, K. W., van Langevelde, F., Ward, D., Prins, H. H. T., de Bie, S., Vosman, B., Sampaio, E. V. S. B. and Sterck, F. J. 2016. Defence against vertebrate herbivores trades off into architectural and low nutrient strategies amongst savanna Fabaceae species. – Oikos 125: 126–136.

Wallis, C. M., Huber, D. P. and Lewis, K. J. 2011. Ecosystem, location, and climate effects on foliar secondary metabolites of lodgepole pine populations from central British Columbia. – J. Chem. Ecol. 37: 607–621.

Wang, X. F., Liu, J. F., Gao, W. Q., Deng, Y. P., Ni, Y. Y., Xiao, Y. H., Kang, F. F., Wang, Q., Lei, J. P. and Jiang, Z. P. 2016. Defense pattern of Chinese cork oak across latitudinal gradients: influences of ontogeny, herbivory, climate and soil nutrients. – Sci. Rep. 6: 27269.

Welti, E. A. R., Sanders, N. J., de Beurs, K. M. and Kaspari, M. 2019. A distributed experiment demonstrates widespread sodium limitation in grassland food webs. – Ecology 100: e02600.

Wigley, B. J., Coetsee, C., Augustine, D. J., Ratnam, J., Hattas, D. and Sankaran, M. 2019. A thorny issue: woody plant defence and growth in an East African savanna. – J. Ecol. 107: 1839–1851.

Zuur, A. F., Ieno, E. N. and Elphick, C. S. 2010. A protocol for data exploration to avoid common statistical problems. – Methods Ecol. Evol. 1: 3–14.