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TESIS DOCTORAL

Biotic and abiotic determinants of the sex ratio in a monoecious shrub in a dry ecosystem

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A mis padres, mis hermanos, mis sobrinos, a Karla y Pedro

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RESUMEN

Antecedentes

Las plantas para sobrevivir, crecer y reproducirse han desarrollado a lo largo de su evolución adaptaciones que les permite soportar cambios ambientales en varias escalas espaciales y temporales. La asignación de recursos entre la supervivencia, crecimiento y reproducción depende de las adaptaciones de cada especie y de las condiciones ambientales donde éstas crecen. Cuando los recursos como el agua o nutrientes son limitados, las plantas adaptan su fisiología o morfología redistribuyendo los recursos para su supervivencia, crecimiento y reproducción. Si las limitaciones de agua y nutrientes se vuelven más extremas, se generan condiciones de estrés para la planta y podría ocurrir competencia o facilitación por los recursos. Estas interacciones pueden generar heterogeneidad ambiental provocando una agregación local con otros individuos de su misma especie y con las de otras especies. La disponibilidad de recursos generalmente está representada por el tamaño de la planta, el cual puede influir directamente en la reproducción y crecimiento. Plantas más grandes invierten proporcionalmente más en la reproducción, por tanto, los costos de reproducción podrían aumentar con el tamaño de la planta. El tamaño de la planta también puede ser influido por la densidad dentro de un vecindario vegetal, y consecuentemente mediar la producción de flores femeninas en especies unisexuales. El cambio climático antropogénico, las perturbaciones por el pastoreo y la deposición de nutrientes proveniente de la producción agrícola están imponiendo nuevas fuerzas selectivas en las poblaciones de plantas. Las plantas si no responden a estos cambios adaptándose a nuevas condiciones ambientales, correrían el riesgo de desaparecer.

Objetivos

En esta tesis doctoral se examinan factores que influyen en la expresión sexual y reproducción de especies de *Croton,* un arbusto clave para el funcionamiento del matorral seco interandino en Loja, Ecuador. El objetivo principal fue contribuir al conocimiento de factores bióticos y abióticos determinantes de la variación en la reproducción de *Croton,* y su vulnerabilidad frente al cambio global

En el primer capítulo evaluamos si la variación entre años y entre sitios, el tamaño de la planta, la densidad de plantas y el área del dosel de vecinos conespecíficos influyeron en la expresión y agregación espacial de la feminidad de *Croton.* Nuestros resultados indican que sitios con diferente elevación, tamaño de la planta y cobertura vegetal influyeron en la expresión sexual de *Croton* y en la agregación de la feminidad. Se encontraron patrones agregados de feminidad en más parcelas en el sitio con menor elevación.

En el segundo capítulo realizamos un experimento para evaluar cómo la variación en temperatura y humedad a lo largo de un gradiente de elevación, la adición de Nitrógeno (N) y Fósforo (P), y el pastoreo influyen en la sincronía de la floración y reproducción de *Croton*. Los resultados revelaron que plantas de mayor tamaño, sitios con temperaturas más altas y una mayor disponibilidad de nitrógeno aumentaron la sincronía reproductiva en *Croton*. El aumento de la sincronía reproductiva, los sitios con mayor temperatura y la adición de N y P incrementaron la producción de frutos y la germinación de semillas de *Croton*. Estos resultados sugieren que una reducción en la sincronía de floración podría afectar el éxito reproductivo de *Croton*.

En el tercer capítulo evaluamos los efectos de la variación del clima de acuerdo con la elevación, la exclusión del pastoreo y las adiciones de nitrógeno y fósforo sobre la supervivencia de plántulas y la supervivencia, crecimiento y fructificación de plantas adultas de *Croton*, así como sobre la composición y estructura de las comunidades de especies vegetales asociadas con *Croton*. Los resultados indicaron que la exclusión del pastoreo mejoró la supervivencia de las plantas adultas de *Croton* en las tres elevaciones. La exclusión del pastoreo y la adición de N y P aumentaron el crecimiento de *Croton* en las elevaciones Baja y Media. El reclutamiento y la supervivencia de plántulas de *Croton* disminuyeron conforme se alejaban del dosel de las plantas adultas. La cubierta de *Croton* tuvo una relación positiva con la abundancia y diversidad de plantas.

En el cuarto capítulo evaluamos el efecto de la elevación sobre las características morfológicas, el aislamiento reproductivo y la limitación de polinizadores en *Croton*. Los rasgos morfológicos como el grosor de la hoja, el volumen de la planta, la longitud de la inflorescencia y el número de inflorescencias fueron diferentes entre plantas en diferentes elevaciones. Evidenciamos un aislamiento reproductivo incipiente entre las elevaciones Alta y Baja. Los experimentos de polinización dentro de cada elevación mostraron una limitación de polinizadores en *Croton* en la Elevación Alta.

Metodología

Nuestro estudio se realizó en un valle interandino del Cantón Catamayo, Provincia de Loja, Ecuador, en la Depresión Andina, una región entre el norte de Perú y el sur de Ecuador. Este matorral seco interandino experimenta una temperatura media anual de 24,8°C, con una precipitación media anual de 317 mm y una estación seca prolongada (entre siete y ocho meses). En este valle, los arbustos dominantes de *Croton* se distribuyen de forma continua entre los 1100 y los 2080 m de altitud (Espinosa et al. 2019). Los parches de *Croton* forman una matriz que incluye vegetación espinosa y xerófila escasa dominada por árboles y arbustos perennes. *Croton* es un arbusto monoico de 0,5 a 1,5 m de altura que habita en una amplia variedad de hábitats, en su mayoría semiáridos y, a menudo, en vegetación secundaria. Las plantas alcanzan la madurez reproductiva en 2 o 3 años y pueden sobrevivir durante varios años.

En el primer capítulo, para determinar si la variación entre años y entre sitios, el tamaño de la planta, la densidad de plantas y el área del dosel de vecinos conespecíficos influyen en la expresión y agregación espacial de la feminidad de *Croton*, georreferenciamos en dos sitios (1.700 y 1.400 m s.n.m.) en cinco parcelas de 10 × 10 m, dentro de cada sitio, la posición de cada planta reproductora de *Croton* durante la primera parte de la época de floración en dos años, y medimos su altura, largo y ancho. El índice de feminidad de cada planta se determinó por el número de yemas y flores femeninas y masculinas. Se determinó la densidad de plantas para cada parcela, junto con el número de vecinos y el área de dosel sumada de vecinos conespecíficos (a 1,0, 2,0 y 2,5 m de radio, y las cinco plantas más cercanas) de cada planta focal. Para evaluar la influencia del año, sitio, tamaño de la planta, densidad y los doseles de los vecinos conespecíficos sobre la feminidad, se utilizaron modelos de efectos mixtos (GLMM). El patrón espacial de la feminidad de las plantas se caracterizó con la función K. Para facilitar la interpretación visual, utilizamos la función L. Todas las funciones se estimaron hasta 2,5 m de radio. Evaluamos el ajuste general de cada modelo con una prueba de bondad de ajuste utilizando el estadístico u.

En el segundo capítulo, para evaluar cómo la variación en temperatura y humedad a lo largo de un gradiente de elevación, la adición de Nitrógeno (N) y Fósforo (P), y el pastoreo influyen en la sincronía de la floración y reproducción de *Croton*, en tres elevaciones, establecimos parcelas con pastoreo y exclusión de pastoreo, combinadas con cuatro tratamientos de nutrientes: control y adición de nitrógeno (N), fósforo (P) solo y en combinación. Registramos la floración femenina y masculina de *Croton* mensualmente desde septiembre de 2017 hasta agosto de 2019 y calculamos un índice de sincronía de floración. También estimamos el número de frutos, cuajado, peso de semilla y su germinación. Usamos modelos binomiales y lineales de efectos mixtos para evaluar si el volumen de la planta, el año, la elevación, la exclusión del pastoreo y la adición de nutrientes influyen en la sincronía de floración. Usamos un modelo lineal generalizado con una distribución binomial para evaluar la influencia de la sincronía en la probabilidad de geminación. Para evaluar si el año, la elevación y la adición de nutrientes influyeron en la probabilidad de depredación de semillas usamos modelos binomiales y lineales.

En el tercer capítulo, para estimar los efectos de la variación del clima de acuerdo con la elevación, la exclusión del pastoreo y las adiciones de nitrógeno y fósforo sobre la supervivencia de plántulas y la supervivencia, crecimiento y fructificación de plantas adultas de *Croton*, así como sobre la composición y estructura de las comunidades de especies vegetales asociadas con *Croton*, en tres elevaciones, establecimos parcelas con pastoreo y exclusión de pastoreo, combinadas con cuatro tratamientos de nutrientes: control y adición de nitrógeno (N), fósforo (P) solo y en combinación. Medimos el reclutamiento y la supervivencia de las plántulas de *Croton*, así como la supervivencia, el crecimiento, la fructificación de los adultos de *Croton* y la composición y estructura de las plantas vecinas. Usamos modelos lineales generalizados para evaluar el crecimiento de las plantas, la supervivencia y la supervivencia de las plántulas en función de la elevación, la exclusión del pastoreo, la adición de nutrientes (N y P) y para el análisis de interacciones bidireccionales. Utilizamos modelos lineales generalizados para evaluar la influencia de la cobertura de *Croton* en el número total de tallos en pie de todas las especies y la riqueza de especies. Para evaluar la influencia de la cobertura de *Croton* en la diversidad de Shannon utilizamos un modelo lineal.

En el cuarto capítulo, para determinar el efecto de la elevación sobre las características morfológicas, el aislamiento reproductivo y la limitación de polinizadores en *Croton*, medimos los rasgos morfológicos de *Croton* en tres elevaciones y realizamos cruces experimentales de polinización entre y dentro de cada población en diferentes elevaciones para evaluar el grado de aislamiento reproductivo y limitación de polinizadores. Usamos modelos de efectos mixtos lineales generalizados para evaluar la variación del grosor de la hoja en función del área foliar específica y la elevación. La inclusión del volumen de la planta y el área foliar específica como variables de estado nos permitió dar cuenta del efecto de la variación en el tamaño de la planta y la hoja entre los individuos. Calculamos el aislamiento reproductivo medio para cada uno de los pares de cruces por elevación. Consideramos evidencia clara de aislamiento reproductivo cuando los cruces con el índice de aislamiento reproductivo tenían un valor positivo y su barra de intervalo de confianza no se superponía a cero.

Resultados

Para el primer capítulo, nuestros resultados indican que sitios con diferente elevación, tamaño de la planta y cobertura vegetal influyeron en la expresión sexual de *Croton* y en la agregación de la feminidad. Se encontraron patrones agregados de feminidad en más parcelas en el sitio con menor elevación.

Para el segundo capítulo, los resultados revelaron que plantas de mayor tamaño, sitios con temperaturas más altas y una mayor disponibilidad de nitrógeno aumentaron la sincronía reproductiva en *Croton*. El aumento de la sincronía reproductiva, los sitios con mayor temperatura y la adición de N y P incrementaron la producción de frutos y la germinación de semillas de *Croton*. Estos resultados sugieren que una reducción en la sincronía de floración podría afectar el éxito reproductivo de *Croton*.

Para el tercer capítulo, los resultados indicaron que la exclusión del pastoreo mejoró la supervivencia de las plantas adultas de *Croton* en las tres elevaciones. La exclusión del pastoreo y la adición de N y P aumentaron el crecimiento de *Croton* en las elevaciones Baja y Media. El reclutamiento y la supervivencia de plántulas de *Croton* disminuyeron conforme se alejaban del dosel de las plantas adultas. La cubierta de *Croton* tuvo una relación positiva con la abundancia y diversidad de plantas.

Para el cuarto capítulo, los resultados mostraron que los rasgos morfológicos como el grosor de la hoja, el volumen de la planta, la longitud de la inflorescencia y el número de inflorescencias fueron diferentes entre plantas en diferentes elevaciones. Evidenciamos un aislamiento reproductivo incipiente entre las elevaciones Alta y Baja. Los experimentos de polinización dentro de cada elevación mostraron una limitación de polinizadores en *Croton* en la Elevación Alta.

Conclusiones

1. Nuestro estudio mostro cómo factores como sitios con diferente elevación, el tamaño de la planta y la cobertura vegetal pueden influir en la expresión de la feminidad y en la agregación espacial de la feminidad plantas monoicas.
2. Condiciones climáticas contrastantes en el gradiente de elevación presentaron variación morfológica y aislamiento reproductivo incipiente en *Croton*. Los experimentos de polinización dentro de cada elevación sugieren la posibilidad de una limitación de polinizadores en *Croton* en la Elevación Alta.
3. Plantas más grandes en las elevaciones Baja y Media, y aquellas en sitios con mayor disponibilidad de nitrógeno tuvieron mayor sincronía reproductiva en *Croton*. El aumento de la sincronía reproductiva y la adición de N y P se asoció con un incremento en la producción de frutos y la probabilidad de germinación de *Croton*.
4. El incremento en la cobertura de *Croton* puede aumentar la abundancia y diversidad de plantas en el matorral seco interandino. La presencia de *Croton* parece favorecer la continuidad de individuos conspecíficos y heteroespecíficos.

INTRODUCCIÓN GENERAL

La reproducción es una tasa vital fundamental (Shivanna y Tandon 2014, Bogdziewicz 2022). Las plantas para sobrevivir, crecer y reproducirse han desarrollado a lo largo de su evolución adaptaciones que les permite soportar cambios ambientales en varias escalas espaciales y temporales (Fontanari et al. 2022, Hangartner et al. 2022). La asignación de recursos entre la supervivencia, crecimiento y reproducción depende de las adaptaciones de cada especie y de las condiciones ambientales donde éstas crecen (Obeso 2002, Matesanz et al. 2010). El costo para la reproducción puede ser mayor en hábitats donde hay una baja disponibilidad de recursos (agua o nutrientes; Euler et al. 2012, Varga y Soulsbury 2020) o donde existe algún tipo de estrés biótico sobre la planta (herbivoría o competencia con otros individuos; Graff et al. 2013, Rahmanian et al. 2021).

La disponibilidad de agua en el suelo depende de la variación en precipitación y temperatura (Moore y Lauenroth 2017, Peguero-Pina et al. 2020). Incrementos de temperatura aumentan las tasas de transpiración de las plantas y la evaporación del suelo (Siyum 2020, Guauque-Mellado et al. 2022). Para reducir la pérdida de agua las plantas adaptan su fisiología o morfología (forma, tamaño, grosor o desprendimiento de hojas) cambiando sus tasas de fotosíntesis y respiración (Chaves et al 2002, Basave-Villalobos et al. 2022). Estos cambios fisiológicos o morfológicos de las plantas en respuesta a la disponibilidad de agua pueden ocurrir como plasticidad fenotípica (Liberati et al. 2021, Wang & Callaway 2021). Las diferencias también pueden últimamente resultar en cambios evolutivos que les permiten persistir en ambientes secos o xéricos (Ramírez-Valiente y Cavender-Bares 2017, Ding et al. 2021).

Los nutrientes del suelo constituyen un recurso esencial para la supervivencia, el crecimiento y la reproducción de las plantas (Milla et al. 2005, Westerband et al. 2015). La disponibilidad de elementos esenciales como el nitrógeno y fósforo pueden estar limitada debido al clima y a la actividad biológica (Peñuelas et al. 2013, Maestre et al. 2016). La absorción de nutrientes por la planta depende de su disponibilidad y de la demanda por la planta (Marleau et al. 2011, Ellsworth y Sternberg 2019). Las plantas con demandas elevadas de nutrientes crecen débilmente en ambientes con un contenido bajo de estos, mientras que las plantas adaptadas a ambientes con un contenido bajo de nutrientes presentan tasas más bajas de crecimiento (Penuelas et al. 2020, Hernández-Cáceres et al. 2021). Cuando las limitaciones de agua y nutrientes se vuelven más extremas, se generan condiciones de estrés para la planta y puede aumentar la competencia entre individuos por los recursos (Butterfield et al. 2015, Pajares et al 2018).

Aunque las plantas son percibidas como consumidoras y competidoras por los recursos, también pueden cambiar la distribución de los recursos abióticos y la dinámica biótica favoreciendo a otras especies (McIntire y Fajardo 2014). Cuando las condiciones ambientales son más estresantes, algunas plantas pueden crear condiciones ambientales favorables para otras plantas bajo su dosel, ofreciéndoles sombra, humedad y nutrientes (Tewksbury y Lloyd 2001, Briscoe et al 2022). Estas interacciones pueden generar heterogeneidad ambiental provocando una agregación local con otros individuos de su misma especie y con las de otras especies (Lara-Romero et al. 2017, Shin et al. 2017). Como resultado la biodiversidad puede aumentar con la presencia de especies facilitadoras a través de la modificación de los nichos disponibles (Hulshof et al. 2013, McIntire y Fajardo 2014).

Los recursos obtenidos por la planta generalmente se dimensionan de acuerdo con su tamaño, el cual puede influir directamente en la reproducción y crecimiento (Horvitz y Schemske 2002, Bonser y Aarssen 2009). Los costos reproductivos debido al tamaño pueden variar entre individuos (Lacey 1986). Las plantas más grandes invierten proporcionalmente más en la reproducción, por tanto, los costos de reproducción podrían aumentar con el tamaño de la planta (Obeso 2002). Las plantas de mayor tamaño generalmente presentan una floración más temprana y extensa (Valencia et al. 2016, Rafferty et al. 2016). Además, pueden producir flores más grandes, más vistosas o coloridas, características relevantes para atraer polinizadores (Givnish 1980, Cortés-Flores et al. 2017). El tamaño de la planta también puede ser influido por la densidad dentro de un vecindario vegetal (beneficios de sombra, agua, nutrientes; Horvitz y Schemske 2002, Obeso 2002, Ward et al. 2018), y consecuentemente mediar la producción de flores femeninas en especies unisexuales (Lázaro y Méndez 2007, Wang et al. 2019). El cambio climático antropogénico, el pastoreo y la deposición de nutrientes proveniente de la producción agrícola está imponiendo una nueva selección en las poblaciones de plantas en los ecosistemas (Bellard et al. 2012, Leizeaga et al. 2022). Estas poblaciones corren el riesgo de desaparecer si no responden a estos cambios, adaptándose a nuevas condiciones ambientales mediante su plasticidad fenotípica (Matesanz et al. 2010, Sardans et al. 2021).

Las actividades humanas están alterando la atmósfera y los sustratos de muchas formas. El aumento de CO_2 en la atmósfera incrementa la temperatura global y como consecuencia están cambiando los patrones anuales de precipitación (Ochoa-Hueso et al. 2014, Allen et al. 2017). La deposición actual de nitrógeno (N) antropogénico global supera todas las fuentes naturales de fijación de N lo que puede alterar los balances d N:P (nitrógeno y fósforo) en el suelo (Güsewell 2004, Penuelas et al. 2020). El pastoreo reduce la densidad de la cobertura vegetal, el crecimiento y la reproducción de las plantas (Mothershead y Marquis 2000, Riginos y Hoffman 2003). Estos cambios globales en el clima, en la química atmosférica y en el uso del suelo pueden alterar el funcionamiento de los ecosistemas al cambiar la disponibilidad de recursos para los organismos (Binkley 2005, Bhadouria et al. 2016, Leizeaga et al. 2022).

Nuestro estudio se realizó en un valle interandino del Cantón Catamayo, Provincia de Loja, Ecuador, en la Depresión Andina (Richter et al. 2009), una región entre el norte de Perú y el sur de Ecuador (Richter y Moreira-Muñoz 2005, Peters et al. 2014, Quintana et al. 2017).

Este matorral seco interandino experimenta una temperatura media anual de 24,8°C, con una precipitación media anual de 317 mm y una estación seca prolongada donde el 57% de la precipitación anual se presenta entre febrero y mayo (Espinosa et al. 2019). En este valle, los arbustos dominantes de *Croton* se distribuyen de forma continua entre los 1100 y los 2080 m de altitud (Espinosa et al. 2019). *Croton* varía en agregación espacial que aumenta a lo largo del gradiente de elevación y disminuye con los niveles de presión de pastoreo (Espinosa et al. 2013). Los parches de *Croton* forman una matriz que incluye vegetación espinosa y xerófila escasa dominada por árboles perennes como *Vachellia macracantha* (Humb. & Bonpl. ex. Willd.), *Bursera graveolens* (Kunth), *Colicodendron scabridum* (Kunth) y arbustos perennes como *Lantana canescens* (Kunth), *Agave americana* (L.), *Ipomoea carnea* (Jacq.) y *Opuntia quitensis* (Ulloa y Jørgensen 1995; Espinosa et al. 2013; Aguirre et al. 2017).

La taxonomía de *Croton* en nuestra área de estudio sigue siendo problemática y optamos por ser cautos en su clasificación y referirnos a ella como *Croton* (Euphorbiaceae). *Croton* es un arbusto monoico de 0,5 a 1,5 m de altura que habita en 1una amplia variedad de hábitats, en su mayoría semiáridos y, a menudo, en vegetación secundaria (Ulloa y Jørgensen 1995; Van Ee et al. 2011). Las plantas alcanzan la madurez reproductiva en 2 o 3 años y pueden sobrevivir durante varios años (Espinosa et al. 2019).

Esta disertación está enfocada a ampliar la comprensión de los efectos de factores bióticos y abióticos sobre la expresión sexual, reproducción, supervivencia y crecimiento de *Croton* sp., un arbusto clave en el funcionamiento del matorral seco interandino del sur de Ecuador.

OBJETIVOS:

- En el **primer capítulo**, investigamos la influencia de factores que afectan la expresión sexual del arbusto monoico, *Croton* en un matorral estacionalmente seco. Específicamente, evaluamos si la variación entre años y entre sitios con diferente elevación, el tamaño de la planta, la densidad de plantas reproductivas y la cobertura vegetal de *Croton* influyen (a) en la proporción de flores femeninas y masculinas, y (b) en la variación espacial de estas proporciones.
- En el **segundo capítulo** realizamos un experimento para evaluar cómo la variación de la temperatura y la humedad en un gradiente de elevación, la adición de N y P y el pastoreo influyen en la sincronía de la floración y la reproducción de *Croton*.
- En el **tercer capítulo**, evaluamos cómo la variación de la temperatura y la humedad en un gradiente de elevación, la exclusión del pastoreo y la adición de nitrógeno y fósforo afectan la supervivencia, el crecimiento, la reproducción y el reclutamiento de plántulas y adultos de *Croton* y la diversidad vegetal asociada.
- En el **cuarto capítulo** evaluamos los niveles de divergencia morfológica y aislamiento reproductivo de *Croton* entre poblaciones en un gradiente de elevación.

Referencias

Aguirre, Z. (2017). Biodiversidad de la provincia de Loja, Ecuador. *Arnaldoa*, *24*(2), 523–542. https://doi.org/10.22497/arnaldoa.242.24206

Allen, K., Dupuy, J. M., Gei, M. G., Hulshof, C., Medvigy, D., Pizano, C., Salgado-Negret, B., Smith, C. M., Trierweiler, A., Bloem, S. J. V., Waring, B. G., Xu, X., & Powers, J. S. (2017). Will seasonally dry tropical forests be sensitive or resistant to future changes in rainfall regimes? *Environmental Research Letters*, *12*(2), 023001. https://doi.org/10.1088/1748-9326/aa5968

Basave-Villalobos, E., Cetina-Alcalá, V. M., Conde-Martínez, V., López-López, M. Á., Trejo, C., & Ramírez-Herrera, C. (2022). Morpho-physiological responses of two multipurpose species from the tropical dry forest to contrasting light levels: Implications for their nursery and field management. *Plants*, *11*(8), 1042. https://doi.org/10.3390/plants11081042

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., & Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*(4), 365–377. https://doi.org/10.1111/j.1461-0248.2011.01736.x

Bhadouria, R., Singh, R., Srivastava, P., & Raghubanshi, A. S. (2016). Understanding the ecology of tree-seedling growth in dry tropical environment: a management perspective. *Energy, Ecology and Environment*, *1*(5), 296–309. https://doi.org/10.1007/s40974-016-0038-3

Binkley, D. (2005). *NATO Science Series IV: Earth and Environmental Sciences*. https://doi.org/10.1007/1-4020-3447-4_8

Bogdziewicz, M. (2022). How will global change affect plant reproduction? A framework for mast seeding trends. *New Phytologist*, *234*(1), 14–20. https://doi.org/10.1111/nph.17682

Bonser, S. P., & Aarssen, L. W. (2009). Interpreting reproductive allometry: Individual strategies of allocation explain size-dependent reproduction in plant populations. *Perspectives in Plant Ecology, Evolution and Systematics*, *11*(1), 31–40. https://doi.org/10.1016/j.ppees.2008.10.003

Briscoe, N. J., Morris, S. D., Mathewson, P. D., Buckley, L. B., Jusup, M., Levy, O., Maclean, I. M. D., Pincebourde, S., Riddell, E. A., Roberts, J. A., Schouten, R., Sears, M. W., & Kearney, M. R. (2022). Mechanistic forecasts of species responses to climate change: the promise of biophysical ecology. *arXiv*. https://doi.org/10.48550/arxiv.2210.16552

Butterfield, B. J., Bradford, J. B., Armas, C., Prieto, I., & Pugnaire, F. I. (2015). Does the stress-gradient hypothesis hold water? Disentangling spatial and temporal variation in plant effects on soil moisture in dryland systems. *Functional Ecology*, *30*(1), 10–19. https://doi.org/10.1111/1365-2435.12592

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., Carvalho, I., Faria, T., & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of Botany*, *89*(7), 907–916. https://doi.org/10.1093/aob/mcf105

Cortés-Flores, J., Hernández-Esquivel, K. B., González-Rodríguez, A., & Ibarra-Manríquez, G. (2017). Flowering phenology, growth forms, and pollination syndromes in tropical dry forest species: Influence of phylogeny and abiotic factors. *American Journal of Botany*, *104*(1), 39–49. https://doi.org/10.3732/ajb.1600305

Ding, J., Travers, S. K., & Eldridge, D. J. (2021). Grow wider canopies or thicker stems: Variable response of woody plants to increasing dryness. *Global Ecology and Biogeography*, *30*(1), 183–195. https://doi.org/10.1111/geb.13212

Ellsworth, P. Z., & Sternberg, L. S. L. (2019). Linking soil nutrient availability, fine root production and turnover, and species composition in a seasonally dry plant community. *Plant and Soil*, *442*(1–2), 49–63. https://doi.org/10.1007/s11104-019-04153-3

Espinosa, C. I., Luzuriaga, A. L., Cruz, M. de la, & Escudero, A. (2013). Climate and grazing control nurse effects in an Ecuadorian dry shrubby community. *Journal of Tropical Ecology*, *30*(1), 23–32. https://doi.org/10.1017/s0266467413000692

Espinosa, C. I., Vélez-Mora, D. P., Ramón, P., Gusmán-Montalván, E., Duncan, D. H., & Quintana-Ascencio, P. F. (2019). Intraspecific interactions affect the spatial pattern of a dominant shrub in a semiarid shrubland: A prospective approach. *Population Ecology*, *61*(2), 217–226. https://doi.org/10.1002/1438-390x.1018

Euler, T. von, Ågren, J., & Ehrlén, J. (2012). Floral display and habitat quality affect cost of reproduction in *Primula farinosa*. *Oikos*, *121*(9), 1400–1407. https://doi.org/10.1111/j.1600-0706.2012.20433.x

Fontanari, J. F., Matos, M., & Santos, M. (2022). Local adaptation, phenotypic plasticity, and species coexistence. *arXiv*. https://doi.org/10.48550/arxiv.2205.00044

Givnish, T. J. (1980). Ecological constraints on the evolution of breeding systems in seed plants: dioecy and dispersal in gymnosperms. *Evolution*, *34*(5), 959. https://doi.org/10.2307/2408001

Graff, P., Rositano, F., & Aguiar, M. R. (2013). Changes in sex ratios of a dioecious grass with grazing intensity: The interplay between gender traits, neighbour interactions and spatial patterns. *Journal of Ecology*, *101*(5), 1146–1157. https://doi.org/10.1111/1365-2745.12114

Guauque-Mellado, D., Rodrigues, A., Terra, M., Mantovani, V., Yanagi, S., Diotto, A., & Mello, C. de. (2022). Evapotranspiration under drought conditions: The case study of a

Seasonally Dry Atlantic Forest. *Atmosphere*, *13*(6), 871. https://doi.org/10.3390/atmos13060871

Güsewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, *164*(2), 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

Hangartner, S., Sgrò, C. M., Connallon, T., & Booksmythe, I. (2022). Sexual dimorphism in phenotypic plasticity and persistence under environmental change: An extension of theory and meta-analysis of current data. *Ecology Letters*. https://doi.org/10.1111/ele.14005

Hernández-Cáceres, D., Stokes, A., Angeles-Alvarez, G., Abadie, J., Anthelme, F., Bounous, M., Freschet, G. T., Roumet, C., Weemstra, M., Merino-Martín, L., & Reverchon, F. (2021). Vegetation alters how soil properties and climate influence microbial activity and functional diversity in rhizosphere and bulk soil along an elevation gradient. *Soil Biology and Biochemistry*, 108485. https://doi.org/10.1016/j.soilbio.2021.108485

Horvitz, C. C., & Schemske, D. W. (2002). Effects of plant size, leaf herbivory, local competition and fruit production on survival, growth and future reproduction of a neotropical herb. *Journal of Ecology*, *90*(2), 279–290. https://doi.org/10.1046/j.1365-2745.2001.00660.x

Hulshof, C. M., Violle, C., Spasojevic, M. J., McGill, B., Damschen, E., Harrison, S., & Enquist, B. J. (2013). Intra-specific and inter-specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. *Journal of Vegetation Science*, *24*(5), 921–931. https://doi.org/10.1111/jvs.12041

Lacey, E. P. (1986). Onset of reproduction in plants: Size-versus age-dependency. *Trends in Ecology & Evolution*, *1*(3), 72–75. https://doi.org/10.1016/0169-5347(86)90021-2

Lara-Romero, C., Gusmán-M, E., Ramón, P., Vélez-Mora, D., & Espinosa, C. I. (2017). Does size matter? Ontogenetic responses of an Andean shrub to conspecific density-dependence. *Perspectives in Plant Ecology, Evolution and Systematics*, *25*, 59–67. https://doi.org/10.1016/j.ppees.2017.02.002

Lázaro, A., & Méndez, M. (2007). Variation in sexual expression in the monoecious shrub *Buxus balearica* at different scales. *Plant Biology*, *9*(6), 736–744. https://doi.org/10.1055/s-2007-965249

Leizeaga, A., Duran, C., Hicks, L. C., Sandén, H., Wondie, M., & Rousk, J. (2022). Using a tropical elevation gradient to evaluate the impact of land-use intensity and forest restoration on the microbial use of organic matter under climate change. *Global Biogeochemical Cycles*, *36*(4). https://doi.org/10.1029/2021gb007109

Liberati, D., Guidolotti, G., Dato, G. de, & Angelis, P. D. (2021). Enhancement of ecosystem carbon uptake in a dry shrubland under moderate warming: the role of nitrogen-driven changes in plant morphology. *Global Change Biology*. https://doi.org/10.1111/gcb.15823

Maestre, F. T., Eldridge, D. J., Soliveres, S., Kéfi, S., Delgado-Baquerizo, M., Bowker, M. A., García-Palacios, P., Gaitán, J., Gallardo, A., Lázaro, R., & Berdugo, M. (2016). Structure and functioning of dryland ecosystems in a changing world. *Annual Review of Ecology, Evolution, and Systematics*, *47*(1), 1–23. https://doi.org/10.1146/annurev-ecolsys-121415-032311

Marleau, J. N., Jin, Y., Bishop, J. G., Fagan, W. F., & Lewis, M. A. (2011). A stoichiometric model of early plant primary succession. *The American Naturalist*, *177*(2), 233–245. https://doi.org/10.1086/658066

Matesanz, S., Gianoli, E., & Valladares, F. (2010). Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences*, *1206*(1), 35–55. https://doi.org/10.1111/j.1749-6632.2010.05704.x

McIntire, E. J. B., & Fajardo, A. (2014). Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, *201*(2), 403–416. https://doi.org/10.1111/nph.12478

Milla, R., Castro-Díez, P., Maestro-Martínez, M., & Montserrat-Martí, G. (2005). Relationships between phenology and the remobilization of nitrogen, phosphorus and potassium in branches of eight Mediterranean evergreens. *New Phytologist*, *168*(1), 167–178. https://doi.org/10.1111/j.1469-8137.2005.01477.x

Moore, L. M., & Lauenroth, W. K. (2017). Differential effects of temperature and precipitation on early- vs. late-flowering species. *Ecosphere*, *8*(5), e01819. https://doi.org/10.1002/ecs2.1819

Mothershead, K., & Marquis, R. J. (2000). Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology*, *81*(1), 30–40. https://doi.org/10.1890/0012-9658(2000)081[0030:fiohti]2.0.co;2

Obeso, J. R. (2002). The costs of reproduction in plants. *New Phytologist*, *155*(3), 321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x

Ochoa-Hueso, R., Bell, M. D., & Manrique, E. (2014). Impacts of increased nitrogen deposition and altered precipitation regimes on soil fertility and functioning in semiarid Mediterranean shrublands. *Journal of Arid Environments*, *104*, 106–115. https://doi.org/10.1016/j.jaridenv.2014.01.020

Pajares, S., Campo, J., Bohannan, B. J. M., & Etchevers, J. D. (2018). Environmental controls on soil microbial communities in a Seasonally Dry Tropical Forest. *Applied and Environmental Microbiology*, *84*(17). https://doi.org/10.1128/aem.00342-18

Peguero-Pina, J. J., Vilagrosa, A., Alonso-Forn, D., Ferrio, J. P., Sancho-Knapik, D., & Gil-Pelegrín, E. (2020). Living in drylands: Functional adaptations of trees and shrubs to cope with high temperatures and water scarcity. *Forests*, *11*(10), 1028. https://doi.org/10.3390/f11101028

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., Velde, M. van der, Bopp, L., Boucher, O., Godderis, Y., Hinsinger, P., Llusia, J., Nardin, E., Vicca, S., Obersteiner, M., & Janssens, I. A. (2013). Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications*, *4*(1), 2934. https://doi.org/10.1038/ncomms3934

Penuelas, J., Janssens, I. A., Ciais, P., Obersteiner, M., & Sardans, J. (2020). Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Global Change Biology*, *26*(4), 1962–1985. https://doi.org/10.1111/gcb.14981

Peters, T., Braeuning, A., Muenchow, J., & Richter, M. (2014). An ecological paradox: high species diversity and low position of the upper forest line in the Andean Depression. *Ecology and Evolution*, *4*(11), 2134–2145. https://doi.org/10.1002/ece3.1078

Quintana, C., Pennington, R. T., Ulloa, C. U., & Balslev, H. (2017). Biogeographic barriers in the Andes: Is the Amotape Huancabamba zone a dispersal barrier for Dry Forest plants?1. *Annals of the Missouri Botanical Garden*, *102*(3), 542–550. https://doi.org/10.3417/d-17-00003a

Rafferty, N. E., Bertelsen, C. D., & Bronstein, J. L. (2016). Later flowering is associated with a compressed flowering season and reduced reproductive output in an early season floral resource. *Oikos*, *125*(6), 821–828. https://doi.org/10.1111/oik.02573

Rahmanian, S., Ejtehadi, H., Farzam, M., Hejda, M., Memariani, F., & Pyšek, P. (2021). Does the intensive grazing and aridity change the relations between the dominant shrub

Artemisia kopetdaghensis and plants under its canopies? *Ecology and Evolution*, *11*(20), 14115–14124. https://doi.org/10.1002/ece3.8124

Ramírez-Valiente, J. A., & Cavender-Bares, J. (2017). Evolutionary trade-offs between drought resistance mechanisms across a precipitation gradient in a seasonally dry tropical oak (*Quercus oleoides*). *Tree Physiology*, *37*(7), 889–901. https://doi.org/10.1093/treephys/tpx040

Richter, M., & Moreira-Muñoz, A. (2005). Heterogeneidad climática y diversidad de la vegetación en el sur de Ecuador: Un método de fitoindicación. *Revista Peruana de Biología*, *12*(2), 217-238. https://doi.org/10.15381/rpb.v12i2.2395

Richter, M., Diertl, K.-H., Emck, P., Peters, T., & Beck, E. (2009). Reasons for an outstanding plant diversity in the tropical Andes of Southern Ecuador. *Landscape Online*, *12*(1), 1–35. https://doi.org/10.3097/lo.200912

Riginos, C., & Hoffman, M. T. (2003). Changes in population biology of two succulent shrubs along a grazing gradient. *Journal of Applied Ecology*, *40*(4), 615–625. https://doi.org/10.1046/j.1365-2664.2003.00826.x

Sardans, J., Janssens, I. A., Ciais, P., Obersteiner, M., & Peñuelas, J. (2021). Recent advances and future research in ecological stoichiometry. *Perspectives in Plant Ecology, Evolution and Systematics*, *50*, 125611. https://doi.org/10.1016/j.ppees.2021.125611

Shin, S., Lee, S. G., & Kang, H. (2017). Spatial distribution patterns of old-growth forest of dioecious tree *Torreya nucifera* in rocky Gotjawal terrain of Jeju Island, South Korea. *Journal of Ecology and Environment*, *41*(1), 31. https://doi.org/10.1186/s41610-017-0050-3

Shivanna, K. R., & Tandon, R. (2014). *Reproductive ecology of flowering plants: A Manual*. https://doi.org/10.1007/978-81-322-2003-9_2

Siyum, Z. G. (2020). Tropical dry forest dynamics in the context of climate change: Syntheses of drivers, gaps, and management perspectives. *Ecological Processes*, *9*(1), 25. https://doi.org/10.1186/s13717-020-00229-6

Tewksbury, J. J., & Lloyd, J. D. (2001). Positive interactions under nurse-plants: spatial scale, stress gradients and benefactor size. *Oecologia*, *127*(3), 425–434. https://doi.org/10.1007/s004420000614

Ulloa, C., & Jørgensen, P. (1995). *Árboles y arbustos de los Andes del Ecuador*. (2nd ed). Abya-Yala.

Valencia, E., Méndez, M., Saavedra, N., & Maestre, F. T. (2016). Plant size and leaf area influence phenological and reproductive responses to warming in semiarid Mediterranean species. *Perspectives in Plant Ecology, Evolution and Systematics*, *21*, 31–40. https://doi.org/10.1016/j.ppees.2016.05.003

Van Ee, B. W., Riina, R., & Berry, P. E. (2011). A revised infrageneric classification and molecular phylogeny of New World *Croton* (Euphorbiaceae). *TAXON*, *60*(3), 791–823. https://doi.org/10.1002/tax.603013

Varga, S., & Soulsbury, C. D. (2020). Environmental stressors affect sex ratios in sexually dimorphic plant sexual systems. *Plant Biology*, *22*(5), 890–898. https://doi.org/10.1111/plb.13125

Wang, X., Huang, L., Gichira, A. W., & Wang, X. (2019). The effects of density on size-dependent gender plasticity in the monoecious species *Sagittaria potamogetifolia* (Alismataceae). *Saudi Journal of Biological Sciences*, *26*(2), 413–420. https://doi.org/10.1016/j.sjbs.2018.11.014

Wang, S., & Callaway, R. M. (2021). Plasticity in response to plant–plant interactions and water availability. *Ecology*, e03361. https://doi.org/10.1002/ecy.3361

Ward, D., Trinogga, J., Wiegand, K., Toit, J. du, Okubamichael, D., Reinsch, S., & Schleicher, J. (2018). Large shrubs increase soil nutrients in a semi-arid savanna. *Geoderma*, *310*, 153–162. https://doi.org/10.1016/j.geoderma.2017.09.023

Westerband, A., Doviak, M., Quay-Velzquez, G. L., & Medeiros, J. S. (2015). Aspect reduces soil moisture and tree cover, but not nitrogen mineralization or grass cover, in semiarid pinyon-juniper woodlands of the Southwestern United States. *The Southwestern Naturalist*, *60*(1), 21–29. https://doi.org/10.1894/fmo-18.1

CAPÍTULO 1

Environmental drivers of femaleness of an inter-Andean monoecious shrub

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Abstract

Hetero-and conspecific interactions, nutrient availability, climate, habitat heterogeneity, and disturbances can generate variation and spatial patterns of femaleness in plants. We assessed whether year, site, plant size, plant density, and canopy area of conspecific neighbors influenced the expression and spatial aggregation of femaleness in *Croton* aff. *wagneri,* a monoecious shrub from dry shrublands of the inter-Andean valleys in Ecuador. We georeferenced in two sites (1,700 and 1,400 m.a.s.l) in five 10 × 10 m plots, within each site, the position of each *Croton* reproductive plant during first part of flowering season in two years, and measured their height, length, and width. The femaleness index of each plant was determined by the number of female and male buds and flowers. Plant density was determined for each plot, along with the number of neighbors and the summed canopy area of conspecific neighbors (at 1.0, 2.0, and 2.5 m radius, and the five closest plants) from each focal plant. *Croton´s* femaleness at the lower elevation site was greater than at the higher elevation site and increased with plant size and with canopy of the closest five neighbors. Soil at the lower elevation site had higher temperatures and lower water content. Aggregate patterns of femaleness were found in more plots at the lower elevation site. Our results indicate that location, plant size, and canopies of conspecific neighbors of *Croton* can affect femaleness and its aggregation and support the hypothesis that femaleness can be influenced by facilitative interactions.

Abstract in Spanish is available with online material.

KEYWORDS

Croton, Ecuador, Loja, monoecy, neighbors, plant size, sexual expression

INTRODUCTION

Sexual expression in flowering plants is diverse. Species range from having individuals with perfect flowers only (hermaphroditism) to having individuals with female flowers and individuals with male flowers (dioecy; Barrett, 2002). There are polymorphic systems with different combinations of breeding system within (e.g., monoecy) or between individuals in different species (Barrett & Hough, 2013; Elzinga & Varga, 2017). Variation of sexual expression within and between populations can significantly influence evolutionary processes as well as individual fitness and variation in reproduction along environmental gradients (e.g., Calviño & Galetto, 2010; Lázaro & Méndez, 2007; Wolfe, 1998; Yakimowski, & Barrett, 2014). Hence, identifying environmental conditions and mechanisms through which sexual expression changes are essential in order to understand the evolution of plants (Barrett, 2002; Charlesworth, 2013; Delph, 2003).

A major component of plant reproduction is its individual spatial distribution (Bleher, Oberrath, & Böhning-Gaese, 2002; Suzuki, 2000). Factors that can generate plant spatial patterns include hetero-and conspecific interactions (Espinosa et al., 2019; Nanami, Kawaguchi, & Yamakura, 2005), nutritional requirements (Marques, Fernandes, Reis, & Assunção, 2002; Nanami, Kawaguchi, & Yamakura, 2011), environmental heterogeneity (Zuo, Zhao, Zhao, et al., 2008), breeding systems (Bleher, Oberrath, & Böhning-Gaese, 2002), and disturbances (Rayburn, & Monaco, 2011; Wolf, 2005). The influence of some of these factors on the spatial pattern of dioecious species has allowed us to understand how male and female function are distributed in natural populations (Obeso, 2002; Rayburn, & Monaco, 2011; Wolf, 2005). However, it has been scarcely evaluated if these same factors can influence spatial pattern of femaleness in monoecious species (Lázaro, & Méndez, 2007; Torices, & Méndez, 2010).

In dioecious species, differences in reproductive cost between sexes may result in differential fitness between sexes across environmental gradients. Such differences can subsequently generate spatial segregation of sexes (Bierzychudek, & Eckhart, 1988; Nuñez, Nuñez, & Kitzberger, 2008). In other words, due to high reproductive cost, females may occupy more favorable conditions with respect to spatial heterogeneity (Garbarino, Weisberg, Bagnara, & Urbinati, 2015), water availability (Ortiz, Arista, & Talavera, 2002), and/or soil fertility (Lawton, & Cothran, 2000). For example, in *Valeriana edulis* increased elevation was associated with increased water availability and female frequency, but aridification caused male frequency to increase upslope at 175 meters per decade (Petry et al., 2016). However, as far as we know, there are few studies that have addressed differential inversion in male and female function of monoecious species in environmental gradients (Aizen, & Kenigsten, 1990).

Variability in plant size can help explain sexual phenotypic expression in species with different reproductive systems (e.g., Clay, 1993; Dorken, & Barrett, 2003; Klinkhamer, de Jong, & Metz, 1997). Both in dioecious and monoecious species, herbaceous and animal-pollinated plants allocation to female function (i.e., ovules and seeds) generally increases with plant size (Bickel & Freeman 1993; Kavanagh et al. 2011; Klinkhamer, de Jong, & Metz, 1997; Obeso, 2002; Sarkissian, Barrett, & Harder 2001), while in woody and wind-pollinated plants this relationship is reversed (Bickel, & Freeman, 1993; Ganeshaiah, & Shaanker, 1991; Obeso, 2002; Pickup, & Barrett, 2011). Plant size, however, usually covaries with conspecific density (Silvertown, & Charlesworth, 2009; Weiner, 1988), and it has been suggested that both plant size and local density may influence sexual expression (Weiner, 1988). These relationships have only recently begun to be explored in monoecious species (Wang et al. 2019); therefore, it is important to continue contributing to the understanding of these interactions in monoecious species.

In southern Ecuador, the Andes Mountain range forms a complicated topography with high mountains and valleys shaping the inter-Andean region (Richter 2003). In the inter-Andean region, moisture in clouds coming from the Pacific Ocean is more rarely released as rain in its central valleys but contributes with higher wetness in the eastern sides of the mountain range as rain shadows (Harden, 2006, Young, Young, & Josse, 2011). The existence of multiple dry and wet areas has generated different microenvironments (Quintana, Girardello, Barfod, & Balslev, 2016; Richter & Moreira- Muñoz 2005). This environmental heterogeneity encourages interacting effects between topography and climate (Ramón et al. 2016). *Croton* aff. *wagneri* occurs in this habitat in Ecuador and is distributed throughout the inter-Andean region. Its main threats are fire, introduction of some non-native species and grazing (León-Yánez et al. 2019). We expect that *Croton* aff. *wagneri* will exhibit variability in the number of male and female flowers among plants in this ecosystem in response to local stress gradients of temperature and moisture.

We investigated the influence of factors potentially affecting sexual expression of a monoecious shrub, *Croton* aff. *wagneri*, a dominant shrub in the inter-Andean dry scrub. We refer to sexual expression of individual plants as the ratio of male and female flowers that each individual presents. Specifically, we assessed whether year, site, plant size, reproductive plants density, and summed canopies of conspecific neighbors influence (a) the sexual expression of femaleness, and (b) the spatial aggregation of femaleness.

METHODS

Study species

Croton aff. *wagnerii* (hereafter *Croton*) is a monoecious shrub in the Euphorbiaceae family that inhabits a wide variety of habitats, mostly semi-arid and often in secondary vegetation (van Ee, Riina, & Berry, 2011). This shrub can reach 1.5 m in height. It has stellate indumentum leaves with entire margins and palmate venation (van Ee, Riina, & Berry, 2011). *Croton* plants reach reproductive maturity in 2–3 years and can survive for several years (Espinosa et al., 2019). Their inflorescences are terminal with flowers unisexual; male flowers have 12 to 14 stamens; female flowers have sepals and petals reduced; styles are bifid for a total of six stigmatic tips (van Ee, Riina, & Berry, 2011). In the inflorescence of this genus, the pistillate flowers are in the lower part and the staminate flowers in the upper part (Domínguez, & Bullock, 1989; van Ee, Riina, & Berry, 2011). Flowering of *Croton* in the study area begins in November and ends in April and exhibits variability in number of male and female flowers between and within plants in this ecosystem (Vélez-Mora et al. 2020, unpublished data). Flowers of *Croton* are visited by flies, large and small bees, syrphids and other insects with greater variety and abundance at lower elevations (Vélez-Mora, personal observation).

Our study was performed at Hacienda Alamala in dry shrublands characteristic of southern Ecuadorian inter-Andean valleys, in Catamayo, Loja Province, Ecuador. Local climate is hot (24.8°C mean annual temperature) and dry, with an extended dry season (317 mm mean annual precipitation, 57% of it occurring between February and May) (Espinosa et al., 2019; Figure S1a). Water deficit (precipitation less than double the temperature) was prevalent 10 months a year (Espinosa, Luzuriaga, de la Cruz, Montero, & Escudero, 2013; Espinosa et al., 2019; Figure S1a). The local shrubland vegetation is dominated by *Croton*. *Croton* varies in spatial aggregation along the elevation gradient and with grazing pressure levels (Espinosa, Luzuriaga, de la Cruz, Montero, & Escudero, 2013). Patches of *Croton* form a matrix including sparse xerophytic and spiny vegetation codominated by perennials such as *Lantana canescens* Kunth, *Vachellia macracantha* (Humb. & Bonpl. ex. Willd.) Seigler & Ebinger, Bursera graveolens (Kunth) Triana & Planch, Colicodendron scabridum (Kunth) Seem. and *Opuntia quitensis* F.A.C. Weber (Espinosa, Luzuriaga, de la Cruz, Montero, & Escudero, 2013; Sierra, 1999; Ulloa, & Jørgensen, 1995). Along this environmental gradient, there is variation in air and soil temperature, moisture and soil nutrient composition (Espinosa, Luzuriaga, de la Cruz, Montero, & Escudero, 2013). The topography is rugged with moderately steep slopes (Espinosa et al., 2019; Figure S1). The study sites are inhabited by small herds of wild donkeys and horses (Espinosa et al., 2019; personal observation). During the winter season livestock load increases with cows and goats, especially at low elevation areas (Young, Young, & Josse, 2011, personal observation).

Study sites and data collection

Sites were chosen based on accessibility, vegetation, and slope as examples of plant associations near the extremes of the local elevation gradient. One site was located at 1,700 a. s. l. and identified as the higher elevation site (3°58′07.90″ S, 79°25′19.71″ W), and the other at 1,400 a.s.l., identified as the lower elevation site (3°59′40.15″ S, 79°26′31.29″ W; map with location of the sites in Figure S2). At each site and within a 70 × 50 m plot, five subplots of 10 × 10 m were established in 2013 and 2016. The subplots were distributed as follows: four in the corners and one in the center with respect to the larger plot. In each plot, the spatial location of the main stem of each reproductive plant was tagged and georeferenced using a Leica FlexLine TS02 total station (Leica, Wetzlar-Germany) during the flowering season (November–December) in both 2013 and 2016. We consider a reproductive plant to be one that had female and male buds and/ or female and male flowers. During the winter season, September 2018 to March 2019, HOBO data loggers S-TMB-M006 and S-SMC-M005 (Onset, USA) were used to measure soil temperature and moisture at a depth of 10 cm, with a sampling interval of 5 min at each site which recorded 66,932 and 48,931 soil temperature and moisture samples for higher elevation site and lower elevation site, respectively.

Plant attributes

To estimate plant size, plant height as well as length and width of canopy of each sampled plant were measured. Plant size was considered as the volume of an elliptical cylinder that is consistent with the shape of *Croton*. This was calculated with the formula:

$$V = \left(\frac{a}{2}\right)\left(\frac{b}{2}\right) \cdot \pi \cdot h \qquad (1)$$

Where a is the length, b is the width of the canopy and h is the height of the plant. The volume of each plant was transformed to natural logarithm.

We also calculated the canopy of each plant as follows:

$$C = \left(\frac{a}{2}\right)\left(\frac{b}{2}\right) \cdot \pi \qquad (2)$$

Where a is the length, b is the width of the canopy of the plant. For analysis, canopy data were transformed to natural logarithm.

Reproductive plant density and neighborhood composition

In November and December of 2013 and 2016, reproductive individuals were selected and counted in each 10 × 10 m subplot at the two sites with different elevation. This time interval includes at least one-third of the annual flowering of our focal species which depends on the extent of the regional rainy season. Plant density was calculated as the number of reproductive individuals in the 10 × 10 m plots (10 density values were obtained for each year). To assess influence of specific neighborhood over femaleness, we generated estimates of neighborhood composition at different distances from each focal plant with *markstat* function from R package "spatstat" v 1.57-1 (Baddeley, Rubak, & Turner, 2015). Calculated variables were as follows:

(a) Number of neighbors that were within 1.0, 2.0, and 2.5 m radius (maximum radius); (b) Sum of volumes of conspecific neighbors that were at 1.0, 2.0, and 2.5 m radius (maximum radius); (c) Sum of canopies of neighbors that were 1.0, 2.0, and 2.5 m (maximum radius), and (d) Sum of volumes and canopies of the five closest neighbors.

Phenotypic femaleness index (Gi)

During the flowering season (November - December) in both 2013 and 2016, five inflorescences were randomly selected on each plant of each 10 × 10 m plot in both sites (1,145 plants and 5,725 total inflorescences). Then, number of female and male buds and number of female and male flowers of each inflorescence were counted in situ and an average of female and male flowers per inflorescence was obtained from each plant. The standardized phenotypic femaleness index (G_i) of each plant was used to estimate the sexual expression of *Croton,* according to Lloyd (1980) as follows:

$$G_i = \frac{o_i}{o_i + p_i(\sum o_i \,/ \sum p_i)} \qquad (3)$$

where o_i is the number of female flowers of the inflorescence and p_i is the number of male flowers of the inflorescence. The index is standardized by $\sum o_i \,/ \sum p_i$ representing the ratio of the total female flower number to the total male flower number of all individuals for both 2013 and 2016. A value of G_i of 1 represents plants that produce only ovules and 0 plants that produce only pollen (see also Sarkissian, Barrett, & Harder, 2001).

Data analysis

To evaluate the influence of year, site, plant size, density, and canopies of conspecific neighbors over femaleness, mixed-effect models (GLMM) were used with the functions of the *lme* R package (linear mixed-effects models; Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2019). Year, site, and reproductive plant density were considered as fixed factors and plot as random factor.

We measured the correlation among all independent variables using the Pearson Correlation coefficient. We avoided multicollinearity by discarding variables which had high pairwise correlation greater than 0.6 or −0.6 with other independent variables (Zar, 2013). Our final data set included the following independent variables: year (2013, 2016), site (higher and lower elevation site), plant size (logarithm of volume), reproductive plant density (number of reproductive individuals per plot), sum of canopies of five closest neighbors. Akaike information criterion (AIC) was used to evaluate variable combinations (Burnham, & Anderson, 2002). All analyses were performed on the statistical platform R (R Core Team, 2019).

Spatial analysis

We evaluated differences in reproductive plant density between years and sites using a generalized linear model with Poisson error distribution for number of individuals. The spatial pattern of plants was characterized with the K-function (Ripley, 1976). For a homogeneous point pattern, K-function is defined by the intensity parameter λ (density), hence $\lambda K(r)$ is the expected number of points within a circle of radius r around an arbitrary point. To facilitate visual interpretation, we used the L-function (Besag, 1977), that is, square root transformed K ($L(r) = [K(r)/\pi]^{1/2} - r$). Homogeneous K-functions were estimated using Ripley's isotropic edge correction (Ripley, 1978). All the functions were estimated up to 2.5 m (i.e., $r_{max} = 2.5$ m). To evaluate the significance of the spatial null model, 95% simulation envelopes were computed from 399 Monte Carlo simulations of each model. We evaluated the overall fit of each model with a goodness-of-fit test using the u statistic (Diggle, Besag, & Gleaves, 1976; Loosmore, & Ford 2006). We performed spatial analyses using the R package "spatstat" v 1.57-1 (Baddeley, Rubak, & Turner, 2015).

We used a one-way ANOVA-like method (Ramón et al. 2016) to test for differences in spatial distribution of femaleness between sites. We computed differences between groups setting $w(r) = R^{2,}$ according to Diggle (2003). The significance of the computed statistic was evaluated with 1,000 bootstrap resamplings to analyze replicated point patterns with the R package "replicatedpp2w" (Ramón et al. 2016).

RESULTS

In the flowering season of 2013, we found a total of 275 reproductive plants at the higher elevation site (total of individuals of five plots) and 186 at the lower elevation site while in the flowering season of 2016 we found 350 reproductive plants at the higher elevation site and 334 at the lower elevation site. Average reproductive plant density was 55.0 individuals/ 100 m^2 (SE = 1.92) at the higher elevation site and 37.2 individuals/100 m^2 (SE = 9.41) at the lower elevation site, respectively, in 2013. Average reproductive plant density was 70.0 individuals/100 m^2 (SE = 8.71) at the higher elevation site and 66.8 individuals/100 m^2 (SE = 8.02) at the lower elevation site in 2016. We found clear differences in *Croton* reproductive plant density between years (51.3 ± 3 SE plants in 2013 and 73.6 ± 7 in 2016) but did not find clear differences between sites (Table 1).

Table 1. Results of a GLM evaluating plant density as a function of year and site.

Variation source	Estimate	SE	t value	p-value
Site at 1,700 m (reference)	-14911.95	5107.79	-2.92	**0.0096**
Year	7.433	2.54	2.93	**0.0093**
Site at 1,400 m	-10.50	7.61	-1.38	0.1901

Average monthly soil temperature between September 2018 and March 2019 was 30.44 ± 0.01°C at the lower elevation site and 25.93 ± 0.01 at the higher elevation site (Figure 1). Monthly average of soil water content was 0.093 ± 0.0002 m3/m3 at the lower elevation site and 0.136 ± 0.0002 m^3/m^3 at the higher elevation site. Soil water content loss after a rain was faster at the lower elevation site (Figure 1, right plot).

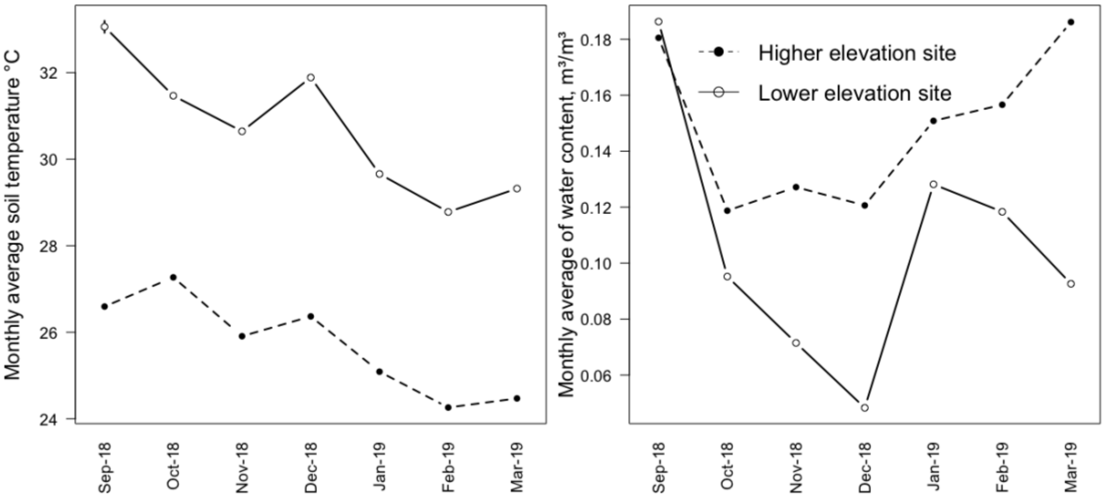

Figure 1. Monthly average temperature (left) and monthly average of water content (right) in soil in winter season from September 2018 to March 19 at Site at 1,700 m (broken line) and Site at 1,400 m (continuous line)

Femaleness of *Croton* as a function of plant size and conspecific neighborhoods

The two most likely models for femaleness (*Gi*) variation of *Croton* included (a) site and sum of canopies of five closest neighbors, and (b) site, plant size, and plot-level density as predictors (see Table S2). Femaleness at the lower elevation site was greater than at the higher elevation site (coefficient = 0.29 ± SE 0.0481; Table 2). A positive relationship was found between femaleness and plant size (volume log transformed) of *Croton* in the two sites; femaleness increased as plant size increased, that is, per unit increase in plant size there was an average of 0.01 increase in femaleness (0.012 ± SE =0.0036; Table 2, Figure 2). There was an interaction between the effect of site and that of canopy area of the five closest neighbors on femaleness. We observed that the canopy area of the five closest neighbors had a positive effect on *Croton* femaleness at the low elevation site, but this effect disappeared at the high elevation site (Table 3, Figure 3; Table S2).

Table 2. Effects of site, plant size (log volume cm^3) and density on femaleness of *Croton* evaluated with a linear mixed-effects models fit by REML. SE: standard error. df: degrees of freedom.

Fixed effects	**Value**	**SE**	**df**	**t-value**	**p-value**
Site at 1,700 m (reference)	0.2989	0.0481	998	6.2213	**0.0000**
Site at 1,400 m	0.0380	0.0076	143	4.9957	**0.0000**
Plant size	0.0127	0.0036	143	3.5477	**0.0005**
Density	0.0001	0.0002	143	0.7804	0.4365

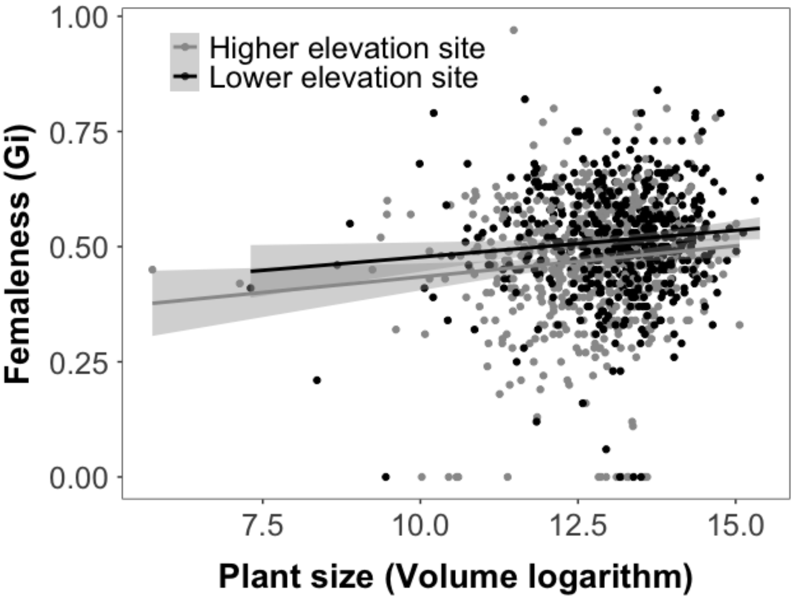

Figure 2. Scatterplot showing the relationship between femaleness (*GI*) and plant size (volume logarithm) of *Croton* at the higher elevation site (1,700 m) and at the lower elevation site (1,400 m).

Spatial patterns of femaleness between years and sites

The results of Ripley's K-function in 2013 at the higher elevation site (1,700 a.s.l.) indicated a uniform spatial pattern of femaleness of *Croton* in two plots in ranges between 0–0.5 m and 0.5–1 m (Figure 4). In the other plots, femaleness had a random pattern (see Table S1). At the lower elevation site (1,400 a.s.l.), femaleness had an aggregated spatial pattern on a plot in ranges between 0.5–1 m and 1–2.5 m (Figure 4). In the other plots, femaleness had a random pattern (see Table S1).

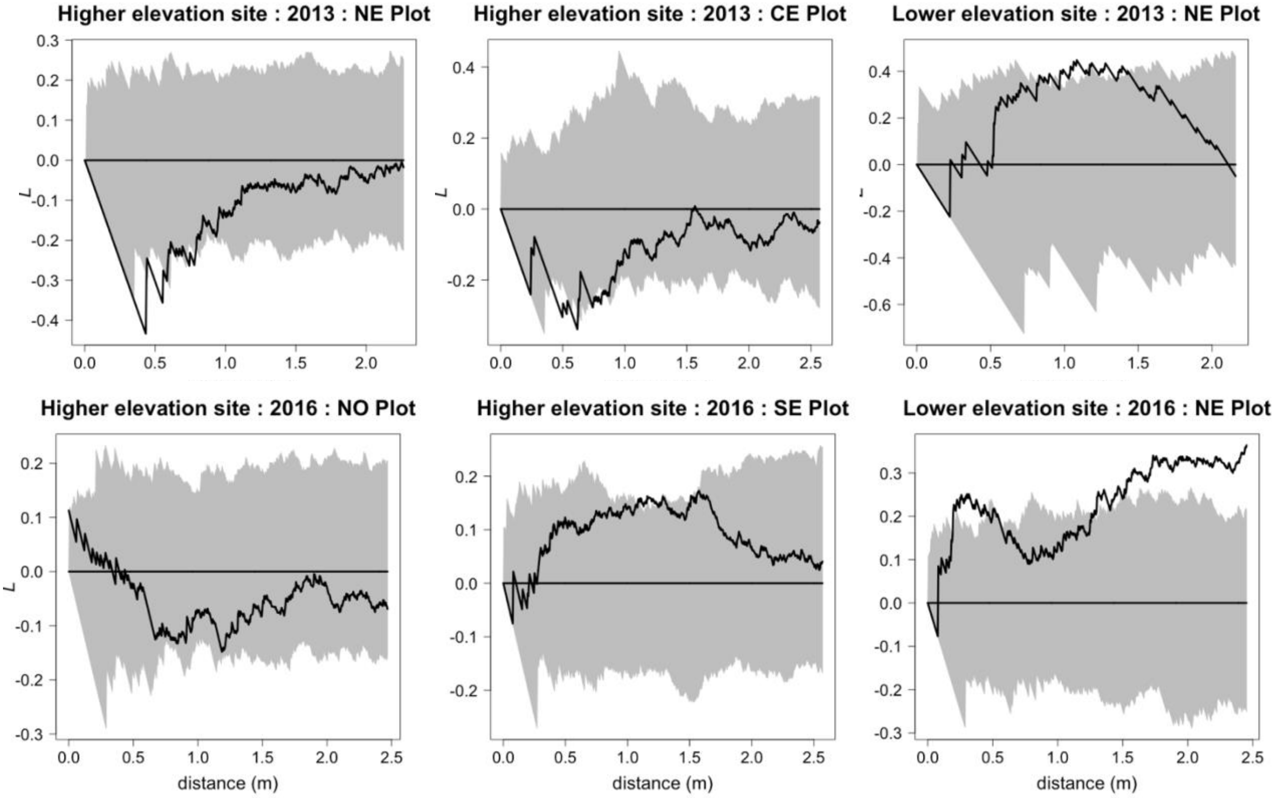

Figure 4. Spatial distribution of femaleness in *Croton* study plots. Patterns of femaleness of *Croton* in 2013 (top) and in 2016 (bottom) determined by Ripley's K-Function (estimated L) at higher elevation site (1,700 m) and at lower elevation site (1,400 m). NE, NO, SE and CE mean plots northeast, northwest, southeast, southwest and center, respectively, regarding the larger plot. The grey area represents the confidence envelope that limits the area determining the random pattern; the continuous lines above the envelope represent the aggregated spatial pattern and, below, the uniform pattern of femaleness of *Croton* in the plots studied. Note that y axes show different scales for clarity. Plots that had a defined spatial pattern are shown.

In 2016 at the higher elevation site femaleness of *Croton* showed uniformity in a plot in a range between 0.5–1 m and aggregation in a plot in range between 1–1.5 m (Figure 4). The other plots showed a random pattern of femaleness (see Table S1). At the lower elevation site, femaleness showed an aggregated spatial pattern on a plot in ranges between 0–0.5 m and 1–2.5 m (Figure 4). The other plots showed a random pattern of femaleness (see Table S1).

Evaluation of spatial patterns of femaleness between sites

We did not find clear grouping or repulsion differences between sites in 2013 (BTSS = 2,527.95, *p* = .26, Figure 5a) nor in 2016 (BTSS = 1,026.06, *p* = .47, Figure 5b). By contrast, comparing the averaged K-functions between 2013 and 2016, clear differences were found between years for both at the higher elevation site (Wilcoxon signed-rank test V = 5, p < .0001) and at the lower elevation site (Wilcoxon signed-rank test V = 93, *p* < .0001).

Table 3. Effect of interaction between site and sum of canopies of the five closest neighbors on femaleness of *Croton* evaluated with a linear mixed-effects models fit by REML. SE: standard error. df: degrees of freedom.

Fixed effects	**Value**	**SE**	**df**	**t-value**	**p-value**
Site at 1,700 m (reference)	0.5274	0.0973	998	5.4211	**0.0000**
Site at 1,400 m	-0.2176	0.1479	143	-1.4709	0.1435
Sum of canopies of 5 closest neighbors	-0.0013	0.0022	143	-0.5829	0.5609
Site at 1400 m × sum of canopies of 5 closest neighbors	0.0060	0.0034	143	1.7619	0.0802

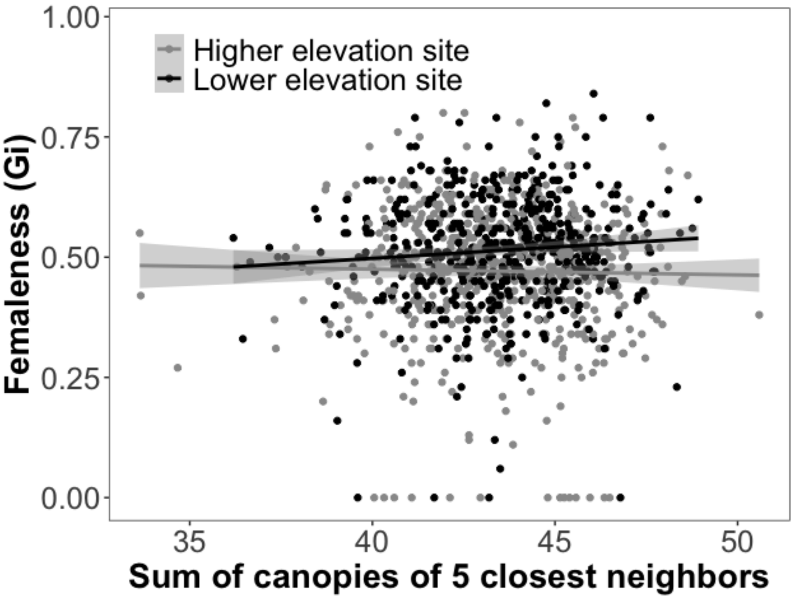

Figure 3. Scatterplot showing the relationship between femaleness (*GI*) and sum of canopies (Canopy logarithm) of *Croton* at the higher elevation site (1,700 m) and at the lower elevation site (1,400 m).

DISCUSSION

Site, plant size, and canopies of conspecific neighbors of *Croton*

Our results indicated higher femaleness in *Croton* at the lower elevation site, located at 1,400 a.s.l., than at the higher elevation site, located at 1,700 a.s.l. Because our study lacked replication for elevation the effects of this variable were confounded with those of the sites and any interpretation of these data on the consequences of elevation on femaleness of *Croton* should be considered as tentative pending its corroboration in future studies. Lower elevation was associated with a greater presence of female individuals in *Juniperus communis* subsp. *alpina* in the Sierra Nevada in southeastern Spain (Ortiz, Arista, & Talavera, 2002; from 2,100 to 2,700 a.s.l.), *Taxus baccata* in the Marche region, central Italy (Garbarino, Weisberg, Bagnara, & Urbinati, 2015; from 450 to 770 a.s.l.), and *Valeriana edulis* in the Rocky Mountains of Colorado, USA (Petry, Soule, Iler, et al., 2016; from 2000 to 3,790 a.s.l.). We also found that larger plants had greater femaleness in both sites studied. In tropical mountain ecosystems, there is greater availability of nutrients at lower elevations (Homeier, Breckle, Günter, Rollenbeck, & Leuschner, 2010; Soethe, Wilcke, Homeier, Lehmann, & Engels, 2008). Increase of nutrients at lower elevations could contribute to higher growth and investment of resources to femaleness in *Croton* (Calviño, & Galetto, 2010; Klinkhamer, de Jong, & Metz, 1997). In monoecious species, this relationship has been less studied possibly because it may be more practical to evaluate spatial segregation of clearly female individuals into dioecious species (Aizen, & Kenigsten, 1990; Bleher, Oberrath, & Böhning-Gaese, 2002).

Conspecific neighboring canopy area influenced *Croton's* femaleness. Similar results were found in *Croton* at the same elevations when evaluating the effect of conspecific density but over total flower production (male flowers and female flowers) in *Croton* (Lara-Romero, Gusmán-M, Ramón, Vélez-Mora, & Espinosa, 2017). Although there was no clear difference in density between sites, influence of canopies of conspecific neighbors was positive at the lower elevation site favoring femaleness in *Croton*. Shrub canopy can improve microenvironmental conditions, accumulation of organic matter, nutrients in soil (Moro, Pugnaire, Haase, & Puigdefábregas, 1997), and improve water availability (Holmgren, Scheffer, & Huston, 1997; Sagar, Pandey, & Singh, 2012) making the climatic conditions at the lower elevation site (high temperatures and low water content) more favorable for growth and expression of femaleness in *Croton* (Butterfield, Bradford, Armas, Prieto, & Pugnaire, 2016; Holmgren, Scheffer, & Huston, 1997; Lara-Romero et al. 2016; Lara-Romero, Gusmán-M, Ramón, Vélez-Mora, & Espinosa, 2017; Pugnaire, & Luque 2001). *Croton* is a dominant shrub and constitutes a nurse species that contributes to regulate the functioning of the dry inter-Andean shrubland community (Espinosa, Luzuriaga, de la Cruz, & Escudero, 2014). Absolute conspecific density should be considered in subsequent work since a positive relationship between *Croton* density and femaleness could improve supply of water and nutrients to invest in reproductive functions. Although negative effects due to density-dependent mortality caused by seed predation and fungal attack would also have to be assessed (Cascante, Quesada, Lobo, & Fuchs, 2002; Fujimori, Samejima, Kenta, et al., 2006). The heterospecific density of this species also could influence its own femaleness. Similarly, in alpine plant communities from Europe, North and South America, Asia, and a sub-Antarctic island it was found that greater richness among the facilitating species increased the reproductive performance of the nurse species (Schöb, Michalet, Cavieres, et al., 2014).

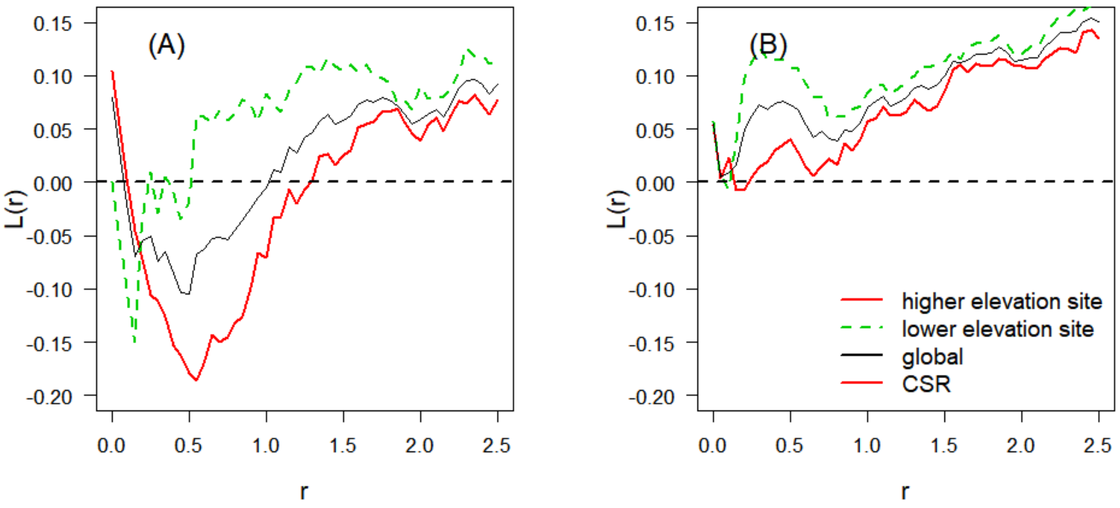

Figure 5. Averaged *K(r)* functions for each of the site (transformed as $L(r) = \sqrt{K(r)}/\pi - r$ to facilitate interpretation). CSR: expected value of complete spatial randomness, Global: overall average *K(r)* function.

Spatial patterns of femaleness in *Croton*

Aggregation of *Croton* femaleness was observed in some plots at the lower elevation site (1,400 a.s.l.). A study developed in a tropical forest in Panama where past and current spatial patterns of a community of 298 different species of shrubs, understory, mid-canopy, and top-canopy trees were explored suggested that the monoecious reproductive system could also be a variable associated with the aggregation of species (Flügge, Olhede, & Murrell, 2012). The spatial patterns found at the lower elevation site supports the hypothesis that the expression of femaleness in monoecious species could be favored by conspecific facilitation processes (Garbarino, Weisberg, Bagnara, & Urbinati, 2015; Ortiz, Arista, & Talavera, 2002; Soliveres, Maestre, Berdugo, & Allan, 2015). Uniform or random spatial patterns of femaleness in *Croton* found in other plots could be due to changes in survival that affect density between years causing conspecific interactions to change direction and become negative or neutral (Bruno, Stachowicz, & Bertness, 2003; Ludwig, Dawson, Prins, Berendse, & de Kroon, 2004; Tielbörger, & Kadmon, 2000). It is possible that if we had performed a spatial survey from a grid as suggested by Wiegand, Kissling, Cipriotti, & Aguiar (2006), we would have had more possibilities of finding spatial patterns in the remaining plots considering size and especially shape of each plant.

Spatial analysis of *Croton* in a previous study revealed that aggregation was greater at high altitude and inhibition at short scales (<1 m; Ramón et al. 2016). Increased grazing at high altitudes and higher rainfall contrasts between elevations with climate change can favor larger differences in *Croton's* survival between altitudes and consequently increase differences in density between their populations (Anderegg, Anderegg, Sherman, & Karp, 2012; Bai, Ma, Zhang, Su, & Leng, 2019; Gilfedder, & Kirkpatrick, 1994; Jonasson, Medrano, & Flexas, 1997). This may indicate that the benefits of positive interactions may be more contrasting between elevations.

CONCLUSIONS AND LIMITATIONS

Our study shows how factors such as site, plant size, and neighborhood canopies can influence the expression of femaleness and the generation of spatial aggregation of femaleness in a monoecious shrub of an inter-Andean dry scrub. We present evidence that the expression of femaleness in monoecious species can be influenced by the same factors that influence the spatial segregation of female individuals in dioecious species. We point out that spatial patterns found at the lower elevation site, where higher soil temperatures and lower moisture content occurred, support the hypothesis that femaleness in monoecious species can be favored by interactions of conspecific facilitation. We also highlight the importance of continually evaluating hetero- and conspecific interactions to ensure the persistence of *Croton* in the dry inter-Andean scrubland in southern Ecuador.

We focus on the early part of *Croton* flowering season and additional work will be necessary to complete the description of the spatial distribution of femaleness of this species. Our fieldwork was carried out between November-December in 2013 and 2016. When we began this work, there was no information available on flowering behavior of our focal species. Later, in 2017-2019, we documented its flowering phenology and observed that flowers were available during a longer interval (September to April; Figure S3). We also observed that the variation in beginning and in extent of *Croton* flowering depended on the schedule and intensity of rains with earlier starts in some years. As with many researchers, the timing and frequency of our field activity are strongly constrained by budgets and academic schedules. During 2013 and 2016, we were able to find plenty of flowers allowing us to characterize femaleness during the study interval. However, future studies should be implemented to assess the whole flowering annual season.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

Vélez-Mora involved in conceptualization, project administration, data collection and curation, analysis, funding acquisition, supervision, writing and editing. Ramón involved in analysis, writing and editing. Vallejo involved in data collection and curation, writing and editing. Romero involved in data collection and curation, writing and editing. Duncan involved in conceptualization, writing and editing. Quintana-Ascencio involved in supervision, analysis, methodology, writing and editing.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi. org/10.5061/dryad.x69p8 czfx (Vélez-Mora et al. 2020).

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REFERENCES

Aizen, M. A., & Kenigsten, A. (1990). Floral sex ratios in scrub oak *(Quercus ilicifolia*) vary with microtopography and stem height. *Canadian Journal of Botany*, 68(6), 1364-1368. https://doi.org/10.1139/b90-174

Anderegg, W. R. L., Anderegg, L. D. L., Sherman, C., & Karp, D. S. (2012). Effects of widespread drought-induced aspen mortality on understory plants. *Conservation Biology*, 26(6), 1082–1090. https://doi.org/10.1111/j.1523-1739.2012.01913.x

Baddeley, A., Rubak, E., & Turner, R. (2015). *Spatial point patterns: methodology and applications with R*. London, UK: Chapman and Hall/CRC Press.

Bai, Q., Ma, Z., Zhang, Y., Su, S., & Leng, P. (2019). The sex expression and sex determining mechanism in *Pistacia* species. *Breeding Science*, 69, 205–214. https://doi.org/10.1270/jsbbs.18167

Barrett, S. C. H. (2002). The evolution of plant sexual diversity. *Nature Reviews. Genetics*, 3(4), 274–284. https://doi.org/10.1038/nrg776

Barrett, S. C., & Hough, J. (2013). Sexual dimorphism in flowering plants. *Journal of Experimental Botany*, 64(1), 67–82. https://doi.org/10.1093/ jxb/ers308

Besag, J. (1977). Contribution to the discussion on Dr Ripley's paper. *Journal of the Royal Statistical Society*, 39, 193–195.

Bierzychudek, P., & Eckhart, V. (1988). Spatial segregation of the sexes of dioecious plants. *The American Naturalist*, 132(1), 34–43. https://doi.org/10.1086/284836

Bickel, A. M., & Freeman, D. C. (1993). Effects of pollen vector and plant geometry on floral sex ratio in monoecious plants. *American Midland Naturalist*, 130(2), 239–247. https://doi.org/10.2307/2426124

Bleher, B., Oberrath, R., & Böhning-Gaese, K. (2002). Seed dispersal, breeding system, tree density and the spatial pattern of trees-a simulation approach. *Basic and Applied Ecology*, 3, 115–123. https://doi.org/10.1078/1439-1791-00088

Bruno, J. F., Stachowicz, J. J., & Bertness, M. D. (2003). Inclusion of facilitation into ecological theory. *Trends in Ecology & Evolution*, 18(3), 119–125. https://doi.org/10.1016/S0169-5347(02)00045-9

Burnham, K. P., & Anderson, D. R. (2002). *A practical information-theoretic approach. Model selection and multimodel inference* (2nd ed.). New York, NY: Springer.

Butterfield, B. J., Bradford, J. B., Armas, C., Prieto, I., & Pugnaire, F. I. (2016). Does the stress-gradient hypothesis hold water? Disentangling spatial and temporal variation in plant effects on soil moisture in dryland systems. *Functional Ecology*, 30(1), 10–19. https://doi.org/10.1111/1365-2435.12592

Calviño, A., & Galetto, L. (2010). Variation in sexual expression in relation to plant height and local density in the andromonoecious shrub *Caesalpinia gilliesii* (Fabaceae). *Plant Ecology*, 209(1), 37–45. https://doi.org/10.1007/s11258-009-9717-x

Cascante, A., Quesada, M., Lobo, J. J., & Fuchs, E. A. (2002). Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree *Samanea saman*. *Conservation Biology*, 16(1), 137–147. https://doi.org/10.1046/j.1523-1739.2002.00317.x

Clay, K. (1993). Size-dependent gender change in green dragon (*Arisaema dracontium*; Araceae). *American Journal of Botany*, 80(7), 769–777. https://doi.org/10.1002/j.1537-2197.1993.tb152 93.x

Charlesworth, D. (2013). Plant sex chromosome evolution. *Journal of Experimental Botany*, 64(2), 405–420. https://doi.org/10.1093/jxb/ ers322

Delph, L. (2003). Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. *Evolution and Development*, 5(1), 34–39. https://doi.org/10.1046/j.1525-142X.2003.03006.x

Diggle, P. J., Besag, J., & Gleaves, J. T. (1976). Statistical analysis of spatial point patterns by means of distance methods. *Biometrics*, 32, 659– 667. https://doi.org/10.2307/2529754

Diggle, P. J. (2003). *Statistical analysis of spatial point patterns* (2nd ed.). London, UK: Arnold.

Dorken, M. E., & Barrett, S. C. (2003). Life-history differentiation and the maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution*, 57(9), 1973–1988. https://doi.org/10.1111/j.0014-3820.2003.tb003 78.x

Domínguez, C. A., & Bullock, S. H. (1989). La reproducción de *Croton suberosus* (Euphorbiaceae) en luz y sombra. *Revista de Biología Tropical*, 37(1), 1–9.

Elzinga, J. A., & Varga, S. (2017). Prolonged stigma and flower lifespan in females of the gynodioecious plant *Geranium sylvaticum*. Flora, 226, 72–81. https://doi.org/10.1016/j.flora.2016.11.007

Espinosa, C. I., Luzuriaga, A. L., de la Cruz, M., Montero, M., & Escudero, A. (2013). Co-occurring grazing and climate stressors have different effects on the total seed bank when compared to the persistent seed bank. *Journal of Vegetation Science*, 24(6), 1098-1107. https://doi.org/10.1111/jvs.12043

Espinosa, C. I., Luzuriaga, A. L., de la Cruz, M., & Escudero, A. (2014). Climate and grazing control nurse effects in an Ecuadorian dry shrubby community. *Journal of Tropical Ecology*, 30(1), 23–32. https://doi.org/10.1017/S0266 46741 3000692

Espinosa, C. I., Vélez-Mora, D. P., Ramón, P., Gusmán-Montalván, E., Duncan, D. H., & Quintana-Ascencio, P. F. (2019). Intraspecific interactions affect the spatial pattern of a dominant shrub in a semiarid shrubland: A prospective approach. *Population Ecology*, 61(2), 217–226. https://doi.org/10.1002/1438-390X.1018

Flügge, A. J., Olhede, S. C., & Murrell, D. J. (2012). The memory of spatial patterns: Changes in local abundance and aggregation in a tropical forest. *Ecology*, 93(7), 1540-1549. https://doi.org/10.1890/11-1004.1

Fujimori, N., Samejima, H., Kenta, T., Ichie, T., Shibata, M., Iida, S., & Nakashizuka, T. (2006). Reproductive success and distance to conspecific adults in the sparsely distributed tree *Kalopanax pictus*. *Journal of Plant Research*, 119(3), 195–203. https://doi.org/10.1007/s1026 5-006-0268-0

Ganeshaiah, K. N., & Shaanker, R. U. (1991). Floral sex ratios in monoecious species-Why are trees more male-biased than herbs? *Current Science*, 60(5), 319–321.

Garbarino, M., Weisberg, P. J., Bagnara, L., & Urbinati, C. (2015). Sexrelated spatial segregation along environmental gradients in the dioecious conifer, *Taxus baccata*. *Forest Ecology and Management*, 358, 122–129. https://doi.org/10.1016/j.foreco.2015.09.009

Gilfedder, L., & Kirkpatrick, J. B. (1994). Climate, grazing and disturbance, and the population dynamics of *Leucochrysum albicans* at Ross, *Tasmania. Australian Journal of Botany*, 42(4), 417–430. https://doi.org/10.1071/BT994 0417

Harden, C. (2006). Human impacts on headwater fluvial systems in the northern and central Andes. *Geomorphology*, 79(3–4), 249–263. https://doi.org/10.1016/j.geomorph.2006.06.021

Holmgren, M., Scheffer, M., & Huston, M. A. (1997). The interplay of facilitation and competition in plant communities. *Ecology*, 78(7), 1966–1975.

Homeier, J., Breckle, S. W., Günter, S., Rollenbeck, R. T., & Leuschner, C. (2010). Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich Ecuadorian montane rain forest. *Biotropica*, 42(2), 140–148. https://doi.org/10.1111/j.1744-7429.2009.00547.x

Jonasson, S., Medrano, H., & Flexas, J. (1997). Variation in leaf longevity of *Pistacia lentiscus* and its relationship to sex and drought stress inferred from leaf $\delta^{13}C$. *Functional Ecology*, 11(3), 282–289. https://doi.org/10.1046/j.1365-2435.1997.00090.x

Kavanagh, P. H., Lehnebach, C. A., Shea, M. J., & Burns, K. C. (2011). Allometry of sexual size dimorphism in dioecious plants: Do plants obey Rensch's rule? *The American Naturalist*, 178(5), 596–601. https://doi.org/10.1086/662175

Klinkhamer, P. G., de Jong, T. J., & Metz, H. (1997). Sex and size in cosexual plants. *Trends in Ecology & Evolution*, 12, 260–265. https://doi.org/10.1016/S0169-5347(97)01078-1

Lara-Romero, C., de la Cruz, M., Escribano-Ávila, G., García-Fernández, A., & Iriondo, J. M. (2016). What causes conspecific plant aggregation? Disentangling the role of dispersal, habitat heterogeneity and plant–plant interactions. *Oikos*, 125(9), 1304–1313. https://doi.org/10.1111/oik.03099

Lara-Romero, C., Gusmán-M, E., Ramón, P., Vélez-Mora, D., & Espinosa, C. I. (2017). Does size matter? Ontogenetic responses of an Andean shrub to conspecific density-dependence. *Perspectives in Plant Ecology, Evolution and Systematics*, 25, 59–67. https://doi.org/10.1016/j.ppees.2017.02.002

Lawton, R. O., & Cothran, P. (2000). Factors influencing reproductive activity of *Juniperus* virginiana in the Tennessee Valley. *Journal of the Torrey Botanical Society*, 127(4), 271–279. https://doi.org/10.2307/3088645

Lázaro, A., & Méndez, M. (2007). Variation in sexual expression in the monoecious shrub *Buxus balearica* at different scales. *Plant Biology*, 9(6), 736–744. https://doi.org/10.1055/s-2007-965249

León-Yánez, S., Valencia, R., Pitmam, N., Endara, L., Ulloa, C., & Navarrete, H. (Eds). (2019). *Libro Rojo de Plantas Endémicas del Ecuador*. Retrieved from https://bioweb.bio/flora web/librorojo

Lloyd, D. G. (1980). Sexual strategies in plants III. A quantitative method for describing the gender of plants. *New Zealand Journal of Botany*, 18(1), 103–108.

Loosmore, N. B., & Ford, E. D. (2006). Statistical inference using the G or K point pattern spatial statistics. *Ecology*, 87(8), 1925–1931.

Ludwig, F., Dawson, T. E., Prins, H. H. T., Berendse, F., & de Kroon, H. (2004). Below-ground competition between trees and grasses may overwhelm the facilitative effects of hydraulic lift. *Ecology Letters*, 7(8), 623–631. https://doi.org/10.1111/j.1461-0248.2004.00615.x

Marques, A., Fernandes, G., Reis, I., & Assunção, R. (2002). Distribution of adult male and female *Baccharis concinna* (Asteraceae) in the Rupestrian fields of Serra Do Cipó, Brazil. *Plant Biology*, 4(1), 94–103. https://doi.org/10.1055/s-2002-20441

Moro, M. J., Pugnaire, F. I., Haase, P., & Puigdefábregas, J. (1997). Effect of the canopy of *Retama sphaerocarpa* on its understorey in a semiarid environment. *Functional Ecology*, 11(4), 425–431. https://doi.org/10.1046/j.1365-2435.1997.00106.x

Nanami, S., Kawaguchi, H., & Yamakura, T. (2005). Sex ratio and gender-dependent neighboring effects in Podocarpus nagi, a dioecious tree. *Plant Ecology*, 177(2), 209–222. https://doi.org/10.1007/s11258-005-2210-2

Nanami, S., Kawaguchi, H., & Yamakura, T. (2011). Spatial pattern formation and relative importance of intra- and interspecific competition in codominant tree species, *Podocarpus nagi* and *Neolitsea aciculate*. *Ecological Research*, 26(37), 46. https://doi.org/10.1007/s1128 4-010-0750-y

Nuñez, C. I., Nuñez, M. A., & Kitzberger, T. (2008). Sex-related spatial segregation and growth in a dioecious conifer along environmental gradients in northwestern Patagonia. *Ecoscience*, 15(1), 73–80.

Obeso, J. R. (2002). The costs of reproduction in plants. *New Phytologist*, 155(3), 321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x

Ortiz, P. L., Arista, M., & Talavera, S. (2002). Sex ratio and reproductive effort in the dioecious *Juniperus communis* subsp. *alpina* (Suter) Čelak. (Cupressaceae) along an altitudinal gradient. *Annals of Botany*, 89(2), 205–211. https://doi.org/10.1093/aob/mcf028

Pickup, M., & Barrett, S. C. H. (2011). Reversal of height dimorphism promotes pollen and seed dispersal in a wind-pollinated dioecious plant. *Biology Letters*, 8(2), 245–248. https://doi.org/10.1098/rsbl.2011.0950

Petry, W. K., Soule, J. D., Iler, A. M., Chicas-Mosier, A., Inouye, D. W., Miller, T. E., & Mooney, K. A. (2016). Sex-specific responses to climate change in plants alter population sex ratio and performance. *Science*, 353(6294), 69–71. https://doi.org/10.1126/science.aaf2588

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team. (2019). nlme: *Linear and nonlinear mixed effects models*. R package version 3.1-139.

Pugnaire, F. I., & Luque, M. T. (2001). Changes in plant interactions along a gradient of environmental stress. *Oikos*, 93(1), 42–49. https://doi.org/10.1034/j.1600-0706.2001.930104.x

Quintana, C., Girardello, M., Barfod, A. S., & Balslev, H. (2016). Diversity patterns, environmental drivers and changes in vegetation composition in dry inter-Andean valleys. *Journal of Plant Ecology*, 10(3), 461– 475. https://doi.org/10.1093/jpe/rtw036

R Core Team. (2019). R: *A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Ramón, P., de la Cruz, M., Chacón-Labella, J., & Escudero, A. (2016). A new non-parametric method for analyzing replicated point patterns in ecology. *Ecography*, 39(11), 1109–1117. https://doi.org/10.1111/ecog.01848

Rayburn, A. P., & Monaco, T. A. (2011). Linking plant spatial patterns and ecological processes in grazed Great Basin plant communities. *Rangeland Ecology & Management*, 64(3), 276–282. https://doi.org/10.2111/REM-D-10-00130.1

Richter, M. (2003). Using epiphytes and soil temperatures for eco-climatic interpretations in southern Ecuador (Der Nutzen von funktionalen Pflanzentypen und Bodentemperaturen für klimaökologische Interpretationen in Süd-Ecuador). *Erdkunde*, 57(3), 161–181. https://doi.org/10.3112/erdku nde.2003.03.01

Richter, M., & Moreira-Muñoz, A. (2005). Heterogeneidad climática y diversidad de la vegetación en el sur de Ecuador: Un método de fitoindicación. *Revista Peruana de Biología*, 12(2), 217–238.

Ripley, B. D. (1976). The second-order analysis of stationary point processes. *Journal of Applied Probability*, 13(2), 255–266. https://doi.org/10.2307/3212829

Ripley, B. D. (1978). Spectral analysis and the analysis of pattern in plant communities. *The Journal of Ecology*, 66, 965–981. https://doi.org/10.2307/2259308

Sagar, R., Pandey, A., & Singh, J. S. (2012). Composition, species diversity, and biomass of the herbaceous community in dry tropical forest of northern India in relation to soil moisture and light intensity. *The Environmentalist*, 32(4), 485–493. https://doi.org/10.1007/s10669-012-9414-5

Soethe, N., Wilcke, W., Homeier, J., Lehmann, J., & Engels, C. (2008). Plant growth along the altitudinal gradient-role of plant nutritional status, fine root activity, and soil properties. E. Beck J. Bendix I. Kottke F. Makeschin & R. Mosandl *In Gradients in a tropical mountain ecosystem of Ecuador* (pp. 259–266). Berlin, Heidelberg, Germany: Springer.

Sarkissian, T. S., Barrett, S. C., & Harder, L. D. (2001). Gender variation in *Sagittaria latifolia* (Alismataceae): Is size all that matters? *Ecology*, 82(2), 360–373. https://doi.org/10.2307/2679865

Schöb, C., Michalet, R., Cavieres, L. A., Pugnaire, F. I., Brooker, R. W., Butterfield, B. J., … Al Hayek, P. (2014). A global analysis of bidirectional interactions in alpine plant communities shows facilitators experiencing strong reciprocal fitness costs. *New Phytologist*, 202(1), 95–105. https://doi.org/10.1111/nph.12641

Sierra, R. (1999). Propuesta preliminar de un sistema de clasificación de vegetación para el Ecuador Continental: proyecto INEFAN/GEFBIRF y EcoCiencia. In *Propuesta preliminar de un sistema de clasificación de vegetación para el Ecuador Continental*: proyecto INEFAN/GEF-BIRF y EcoCiencia.

Silvertown, J., & Charlesworth, D. (2009). *Introduction to plant population biology*, Oxford, United Kingdom: John Wiley & Sons.

Soliveres, S., Maestre, F. T., Berdugo, M., & Allan, E. (2015). A missing link between facilitation and plant species coexistence: nurses benefit generally rare species more than common ones. *Journal of Ecology*, 103(5), 1183–1189. https://doi.org/10.1111/1365-2745.12447

Suzuki, A. (2000). Patterns of vegetative growth and reproduction in relation to branch orders: The plant as a spatially structured population. *Trees*, 14(6), 329–333. https://doi.org/10.1007/s0046 80050226

Tielbörger, K., & Kadmon, R. (2000). Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology*, 81(6), 1544–1553. https://doi.org/10.2307/177305

Torices, R., & Méndez, M. (2010). Influence of inflorescence size on sexual expression and female reproductive success in a monoecious species. *Plant Biology*, 13, 78–85. https://doi.org/10.1111/j.1438-8677.2009.00292.x

Ulloa, C., & Jørgensen, P. M. (1995). *Arboles y arbustos de los Andes del Ecuador* (p. 264). Aarhus, Denmark: Aarhus University.

van Ee, B. W., Riina, R., & Berry, P. E. (2011). A revised infrageneric classification and molecular phylogeny of New World *Croton* (Euphorbiaceae). *Taxon*, 60(3), 791–823. https://doi.org/10.1002/tax.603013

Vélez-Mora, D., Ramón, P., Vallejo, C., Romero, A., Duncan, D., & Quintana-Ascencio, P. F. (2020). Data from: Environmental drivers of femaleness of an inter-Andean monoecious shrub. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.x69p8czfx

Weiner, J. (1988). The influence of competition on plant reproduction. In J. Lovett, & L. Lovett (Eds.), *Plant reproductive ecology*: *Patterns and strategies* (pp. 228–245). Oxford, UK: Oxford University Press.

Wiegand, T., Kissling, W. D., Cipriotti, P. A., & Aguiar, M. R. (2006). Extending point pattern analysis for objects of finite size and irregular shape. *Journal of Ecology*, 94(4), 825–837. https://doi.org/10.1111/j.1365-2745.2006.01113.x

Wolfe, L. M. (1998). Regulation of sex expression in desert and Mediterranean populations of an andromonoecious plant (*Gagea chlorantha*, Liliaceae). *Israel Journal of Plant Sciences*, 46(1), 17–25. https://doi.org/10.1080/07929978.1998.10676703

Wolf, A. (2005). Fifty year record of change in tree spatial patterns within a mixed deciduous forest. *Forest Ecology and Management*, 215(1–3), 212–223. https://doi.org/10.1016/j.foreco.2005.05.021

Wang, X., Huang, L., Gichira, A. W., & Wang, X. (2019). The effects of density on size-dependent gender plasticity in the monoecious species *Sagittaria potamogetifolia*

(Alismataceae). *Saudi Journal of Biological Sciences*, 26(2), 413–420. https://doi.org/10.1016/j.sjbs.2018.11.014

Yakimowski, S. B., & Barrett, S. C. H. (2014). Variation and evolution of sex ratios at the northern range limit of a sexually polymorphic plant. *Journal of Evolutionary Biology*, 27(7), 1454–1466. https://doi.org/10.1111/jeb.12322

Young, B., Young, K. R., & Josse, C. (2011). Vulnerability of tropical Andean ecosystems to climate change. *In Climate change and biodiversity in the tropical Andes*. SCOPE, IAI (pp. 170–181).

Zar, J. H. (2013). *Biostatistical analysis: Pearson new international edition*, London, United Kingdom: Pearson Higher Ed.

Zuo, X., Zhao, H., Zhao, X., Zhang, T., Guo, Y., Wang, S., & Drake, S. (2008). Spatial pattern and heterogeneity of soil properties in sand dunes under grazing and restoration in Horqin Sandy Land, Northern China. *Soil and Tillage Research*, 99(2), 202–212. https://doi.org/10.1016/j.still.2008.02.008

SUPPORTINGIN FORMATION

Additional supporting information may be found online in the Supporting Information section.

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SUPPORTING INFORMATION

Environmental drivers of femaleness of an inter-Andean monoecious shrub

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Figure S1. Topography of the study site.

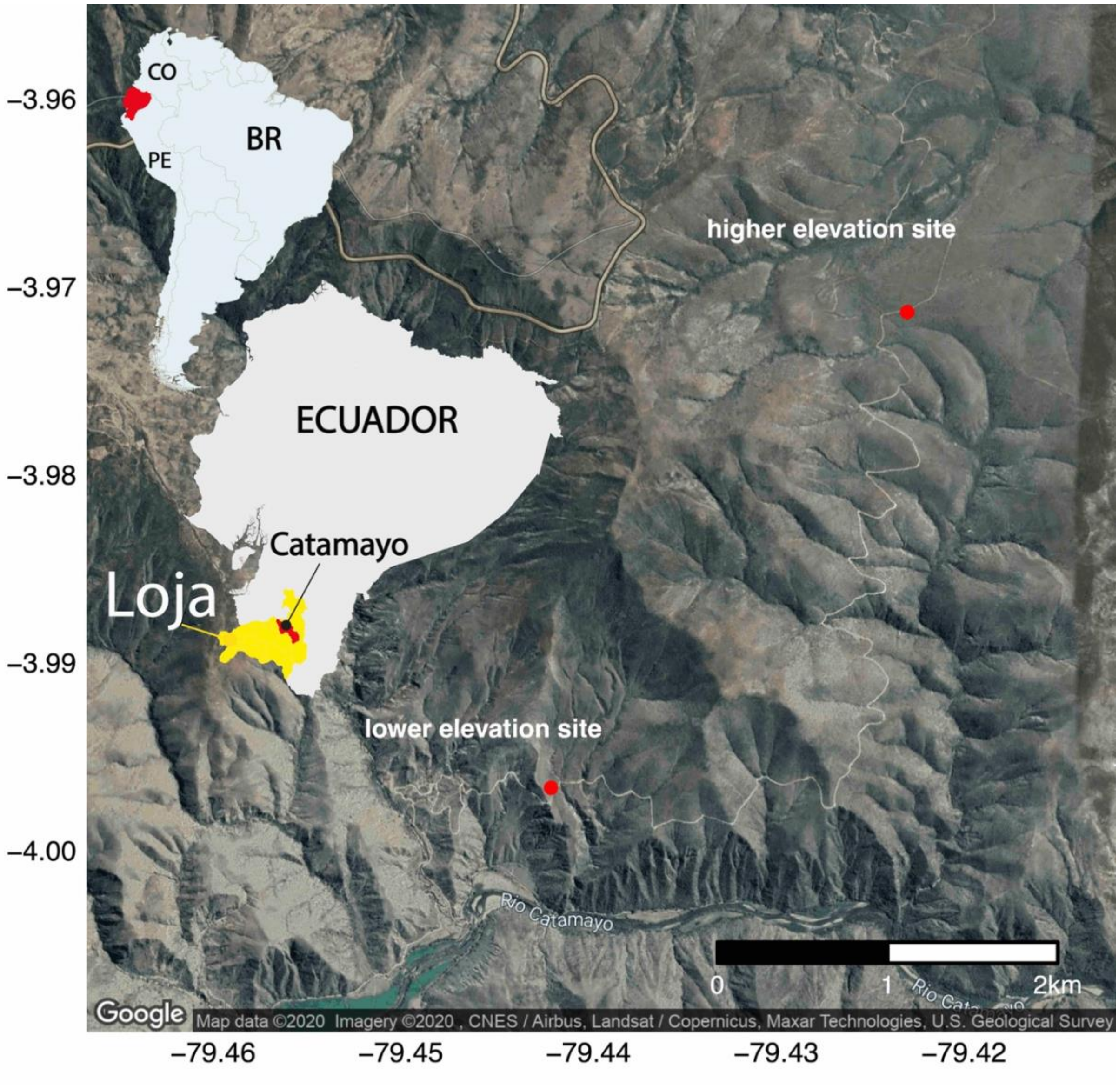

Figure S2. Location of the study sites of *Croton* in the inter-Andean dry shrub at south of Ecuador and northwest of South America.

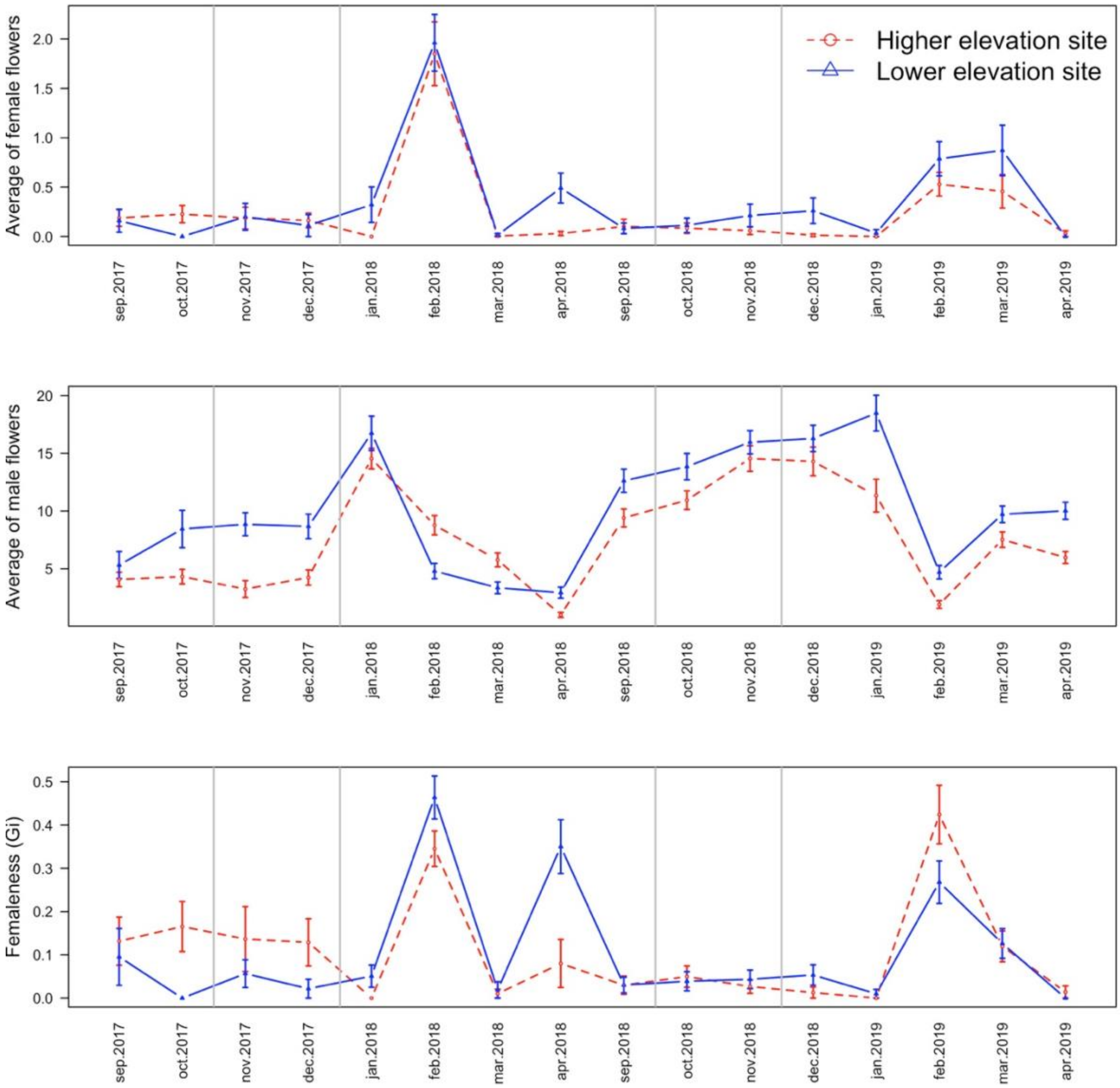

Figure S3. Average of female flowers and male flowers, and femininity index (Gi) from September 2017 to April 2019 are shown exclusively for *Croton* flowering season. Gray vertical lines indicate sampling months of this study.

Table S1. Evaluation of overall fit of each spatial model by a goodness-of-fit for each year, site and plot. Values indicate the probability of the model being consistent with a random pattern; those in bold had probability lower than 0.05 consistent with evidence for the alternative hypothesis of clear spatial pattern. Radii were evaluated from 0 to 0.5, from 0.5 to 1 m and from 1 to 2.5 m. The sign + means positive interaction or association and the sign – means negative interaction or repulsion.

		radii evaluated			
Year – Site	**Plot**	0 - 0.5 m	0.5 - 1 m	1 - 2.5 m	**Interaction**
2013 – Higher elevation site	Northeast	**<0.001**	**<0.001**	0.50	-
	Northwest	**0.04**	0.05	0.60	-
	Southeast	0.45	0.62	0.74	
	Southwest	**0.01**	0.09	0.65	-
	Central	**0.01**	**<0.001**	0.36	-
2013 – Lower elevation site	Northeast	0.87	**0.01**	**0.01**	+
	Northwest	0.06	0.41	0.66	
	Southeast	0.69	0.97	0.35	
	Southwest	0.17	0.06	0.05	
	Central	0.25	0.25	0.25	
2016 – Higher elevation site	Northeast	0.61	0.55	**0.07**	
	Northwest	0.40	0.10	0.28	
	Southeast	0.23	**0.01**	**0.01**	+
	Southwest	0.38	0.46	0.89	
	Central	0.82	0.18	0.17	
2016 – Lower elevation site	Northeast	**<0.001**	**0.01**	**<0.001**	+

Northwest	0.18	0.20	0.07
Southeast	0.12	0.10	0.63
Southwest	0.97	0.15	0.61
Central	0.13	0.82	0.38

Table S2. Set of linear models fitted assessing levels of femaleness (*Gi*) of *Croton* as a function of year, site, plant size, plant density and plant canopy cover. Year: consecutive years in which sampling was performed (2013 and 2016). Site: site at 1700 a.s.l. and site at 1400 a.s.l. Plant size: natural logarithm of plant volume. Density: number of reproductive individuals per plot. Plant canopy cover: sum of canopies of the five closest neighbors. dAIC: Difference between AIC value of each model versus value of model with the lowest value of AIC. The models were placed according to the ascending value of AIC as an indicator of model likelihood. df: Degrees of freedom. Weight: Weight for each model.

Model	**dAIC**	**df**	**weight**
Site + sum of canopies of 5 closest neighbors	0	5	0.818
Site + plant size + density	3.3	6	0.154
Site × sum of canopies of 5 closest neighbors	8.4	6	0.012
Site + sum of canopies of the 5 closest neighbors + year	9.4	6	0.007
Null model	9.8	3	0.006
Site + plant size + density + year	12.5	7	0.002
Site + plant size + density + sum of canopies of 5 closest neighbors + year	25.4	8	<0.001

CAPÍTULO 2

Plant size, elevation, and nutrients affect reproductive synchrony, which increases reproduction in a key shrub of the inter-Andean dry shrubland

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(Manuscript in preparation)

SUMMARY

Flowering synchrony can be affected by moisture, nutrient availability, and plant size. The changes in degree of flowering synchrony in turn can influence reproductive success. We performed an experiment to evaluate how variation in temperature and moisture along an elevation gradient, N and P addition, and grazing influence flowering synchrony and reproduction of *Croton*, a key shrub in the inter-Andean dry shrubland. At three elevations, we installed grazing and exclusion plots, combined with four nutrient treatments: control, and addition of nitrogen (N), phosphorous (P) alone and in combination. We recorded female and male flowering of *Croton* monthly from September 2017 to August 2019 and calculated a flowering synchrony index. We also obtained number of fruits, fruit set, seed weight and their germination. We show that larger plants, higher temperatures, and greater nitrogen availability increase reproductive synchrony on *Croton*. Increase in reproductive synchrony, increase in temperature and addition of N and P improve production of fruits and germination of *Croton* seeds. These results suggest that a reduction in flowering synchrony could affect reproductive success of *Croton*.

INTRODUCTION

Reproductive synchrony is defined as the temporal overlap of organisms with their potential mates and resources for reproduction (Donnelly et al. 2011, Pires et al. 2013, Fisogni et al. 2022). It is identified as a tendency of individuals to carry out some stage of the reproductive cycle simultaneously with other members of the population (Li & Yang 2018). In plants, flowering synchrony is the result of a complex interactions between several environmental factors, such as variation in moisture, and nutrient availability (Ausín et al. 2005, Brearley et al. 2007, Lesica & Kittelson 2010, Wilczek et al. 2010, Moore & Lauenroth 2017), as well as intrinsic factors such as plant size (Pires et al. 2013). These are phenological responses reflecting individual variation to changing variables determining temporal patterns of flowering and fruiting. Most studies on factors affecting reproductive synchrony in plants have been develop in dioecious species (Elzinga et al. 2007, Forrest & Miller-Rushing 2010) with more limited information on the mechanisms driving variation reproductive synchrony of monoecious species (Bronstein et al. 1990, Wang et al. 2012, Chmura et al. 2019).

Changes in flowering timing and/or the degree of synchrony in flowering could influence reproductive success (Kudo 2006; Elzinga et al. 2007; Hall et al. 2018). Synchronous flower displays attract more pollinators and promote outcrossing by maximizing the number of potential mates (Marquis 1988, Kudo 1993, Ollerton & Díaz 1999). Therefore, synchrony can increase the probability of fruit production (Bolmgren & Eriksson 2015, Rodríguez-Pérez & Traveset 2016, Hall et al. 2018), increase masting to satiate seed predators (microlepidoptera and dipteran larvae) before dispersal and reduce seed loss (Augspurger 1981, Crone & Lesica 2004, Bogdziewicz et al. 2020, 2021), and perhaps, increase the probability of germination due to effective pollen exchange between different individuals of the population. However, there is no clear evidence regarding how reproductive synchrony could influence seed germination.

Plant size is associated with environmental conditions and plant age (Waller 1988). Larger individuals may flower earlier (Ollerton & Lack 1998, Munguía-Rosas et al. 2011), produce more flowers and have higher fecundity (Weiner & Thomas 1986, Herrera 1993, Kato & Hiura 1999), and have larger and heavier seeds (Silvertown 1989, Cornelissen 1999). One explanation for this is that larger individuals accumulate and dispose of more internal resources for reproduction (Wright & van Schaik 1994). Synchrony level and seed production were lower in smaller plants in *Sorbus aucuparia*, *Pinus pinea*, five *Quercus* species, and three *Chionochloa* species from various countries (Bogdziewicz et al. 2020). Fruit set increased with plant size in *Juglans regia* in Sichuan Province, southwest China (Cao et al. 2020). We therefore considered that plant size is a good predictor of plant status and is closely related to some reproductive variables (Bustamante & Búrquez 2008, Pires et al. 2013, Boucher et al. 2017, Vélez-Mora et al. 2020, 2021, 2022).

Factors such as soil moisture (Tadey 2020), grazing (Norton & Reid 2009), and nutrient supply (Liu et al. 2017) also affect plant flowering. In tropical dry ecosystems, seasonal water limitation can be severe, promoting synchronous reproduction during the rainy season (Borchert et al. 2004, Singh & Kushwaha 2006) and reducing water stress in reproductive adults (van Schaik et al 1993). Rapid initiation of reproduction following rainfall may come at the expense of allocation to reproductive or somatic tissue across the entire growing season (Cohen 1976, Rathcke & Lacey 1985, Lasky et al. 2016). Trampling or browsing of livestock decreases plant density and cover (Riginos & Hoffman 2003, Metzger et al. 2005, Tadey 2006), reduces growth (Vélez-Mora et al. 2022), affects plant architecture, delays, or constrains sexual reproduction through less intense flowering (Freeman et al. 2003, Mothershead & Marquis 2000, Tadey 2020). The availability of phosphorus (P) from the soil facilitates the absorption of nitrogen (N) by plants (Ren et al 2016, Su et al. 2021) stimulating their growth (Xia & Wan 2008, Cao et al. 2011, Zhang et al. 2013) and reproduction (Smith et al., 2012, Xia & Wan, 2013, Xi et al. 2015, Su et al. 2021), although it could also speed up or delay flowering times (Cleland et al. 2006, Liu et al. 2017) causing asynchronous flowering (Rafferty & Ives, 2011). Despite the importance of these factors in plant reproduction, we did not find in the available literature any direct evaluations of the combined effect of soil moisture, grazing, and nutrient availability on reproductive synchrony in plants from seasonally dry ecosystems.

In the last decade we have observed an intensification of agricultural and livestock activities in the dry inter-Andean shrublands (Quintana et al. 2019, Vélez-Mora et al. 2022). These activities have altered the N:P balance on soils through aerosol and direct deposition (Mahowald & Carslaw 2022) and diverted water from the few local streams (Minga-León et al. 2018, Arteaga et al. 2020). Simultaneously, the frequency of extremely dry years and the annual number of rainless days have also increased. Climate change, grazing, and nutrient addition are widespread threats to species persistence and sustainable community functioning in dry ecosystems (Ulrich et al. 2014, Smith et al. 2016, Maestre et al. 2021) and they seem to be magnified in mountainous areas such as inter-Andean valleys (Coppus 2003, Zehetner 2006, Murgueitio 2011).

We performed an experiment to evaluate how variation in temperature and moisture along a narrow elevation gradient (300 m), N and P addition, and grazing influence flowering synchrony and reproduction of *Croton*, a key shrub in the inter-Andean dry shrubland in southern Ecuador. Previous studies showed that grazing increased the facilitating effects of *Croton* (Espinosa et al. 2019, Vélez-Mora et al. 2022). Soil N and P stocks and N and P deposition were also shown to vary with elevation influencing *Croton* vital rates and population abundance (Vélez-Mora et al. 2022). We predicted that: (1) higher moisture and lower temperature conditions, absence of grazing, higher availability of nitrogen and phosphorus, and larger plant sizes will increase reproductive synchrony in *Croton*; and (2) a higher reproductive synchrony will increase the number of fruits, fruit set, seed predation, and the probability of germination in *Croton* seeds.

METHODS

Study site

Our study was performed at an inter-Andean valley of the Catamayo Canton, Loja Province, Ecuador in the Andean Depression (Richter et al. 2009), a region between northern Perú and southern Ecuador (Richter and Moreira-Muñoz 2005; Peters et al. 2014; Quintana et al. 2017) (Fig. S10). This dry inter-Andean shrubland experiences an average annual temperature of 24.8 °C, with an average annual precipitation of 317 mm and a prolonged dry season where 57% of the annual precipitation occurs between February and May (Espinosa et al. 2019). In this valley, the dominant *Croton* shrubs are distributed continuously between 1100 and 2080 m of elevation (Espinosa et al. 2019). *Croton* varies in spatial aggregation that increases along the elevation gradient and decreases with grazing pressure levels (Espinosa et al. 2013). Patches of *Croton* form a matrix including sparse xerophytic and spiny vegetation co-dominated by tree perennials such as, *Vachellia macracantha* (Humb. & Bonpl. ex. Willd.), *Bursera graveolens* (Kunth), *Colicodendron scabridum* (Kunth), and shrubby perennials such as *Lantana canescens* (Kunth), *Agave americana* (L.), *Ipomoea carnea* (Jacq.) and *Opuntia quitensis* (Ulloa and Jørgensen 1995; Espinosa et al. 2013; Aguirre et al. 2017).

We chose three elevations: 1700 m a.s.l. (high elevation; 3°58’07.90’’ S, 79°25’19.71’’ W), 1550 m a.s.l. (medium elevation; 3°59'20.09" S, 79°25'28.64’’ W) and 1400 m a.s.l. (low elevation; 3° 59’40.15’’S, 79°26’31.29’’ W). Along this environmental gradient, there is variation in soil temperature, moisture, and soil nutrient composition. Total N concentration in the soil is lower at the low and medium elevations than at the high elevation, although N deposition is higher at the low elevation (Vélez-Mora et al. 2022). Total P concentration is higher at the low elevation compared to the medium and high elevations, both in the soil and deposited (Vélez-Mora et al. 2022). Soil water content is lower at the lower elevation and soil temperature is higher at the low elevation (Vélez-Mora et al. 2022). Precipitation in winter (between November and March) is similar between these elevations and percentage of soil sand decrease with elevation (Vélez-Mora et al. 2022).

At the regional level, dry weather and erosion at low elevations has led to the formation of Entisols characterized by sandy, stony, and rocky formations generating shallow soils (Winckell et al. 1997; Moreno et al. 2018). At higher elevations, wetter conditions promoted development of clayey soils classified as Vertisols (Soil Survey Staff 2006; Moreno et al. 2018). The topography is rugged with moderately steep slopes (Espinosa et al. 2019). These sites are visited by small herds of feral donkeys and horses. In recent years we have witnessed increasing goat and cattle herds in the area. According to local shepherds, cattle occasionally consume *Croton*. We also observed that livestock trample seedlings, small plants and damage the branches of *Croton* plants, mainly in winter (Vélez-Mora et al. 2022).

Study species

The taxonomy of *Croton* in our study area remains problematic and we opted to be cautious on its classification and refer to it as *Croton* (Euphorbiaceae). *Croton* is a 0.5 - 1.5 m tall monoecious shrub that inhabits a wide variety of habitats, mostly semi-arid and often in secondary vegetation (Ulloa and Jørgensen 1995, van Ee et al. 2011; Figure 2b). Plants reach reproductive maturity in 2–3 years and can survive for several years (Espinosa et al. 2019). Recent studies indicate clear morphological variation in leaves and inflorescences among forms at different elevations and suggest that there is incipient reproductive isolation between local populations of *Croton* occurring at local elevation extremes (Vélez-Mora et al. 2021).

Experimental design and treatments

In July 2015, we initiated a manipulative field experiment along an elevation gradient to evaluate the effects of temperature and moisture variation, grazing, and N and P addition to the soil on flowering synchrony of *Croton* and how this synchrony influences fruit and seed set and germination probabilities. Average annual temperature (°C) at the high elevation is 20.68, at the medium elevation 21.90 and at the low elevation 22.87 (Vélez-Mora et al. 2022). In winter rains generally start in October and end in April (Espinosa et al. 2019, Vélez-Mora et al. 2022). As we observed that winter rains generally start in October and end in April and decided, therefore, to measure precipitation in the period of likely greatest rainfall from November 2017 to March 2018. At each elevation, we obtained a weekly rainfall average during this period and summed these weekly averages to obtain monthly precipitation. Average precipitation during this winter period is 49.12 mm for the high elevation, 47.25 for the medium elevation and 46.25 for the low elevation (Vélez-Mora et al. 2022).

Two pairs of 10 m × 10 m plots (at least 100 m apart) were established at each elevation. In each pair, one plot was excluded and the other not excluded from grazing. In total, we established 12 plots. Plot assignment was established randomly. Plots excluded from grazing were fenced with four lines of barbed wire at a maximum height of 1.5 m in July 2015, while those in which grazing was allowed had only wooden posts placed at the four corners. On 18 March 2017, 10 March 2018, and 19 April 2019, N and P were applied to subplots within each plot (Fig. S9). Nutrients were applied depending on the previous availability of these nutrients in the soil of each subplot. One replicate of each treatment (control, N, P and N+P) was applied per plot. (Calculations and exact amounts applied of N and P by subplot are shown in Vélez-Mora 2022). On average, 346.81 g of urea (N) and 506.24 g of triple superphosphate (P) were applied per treated subplot at the high elevation; 382.20 g and 503.76 g at the medium elevation, and 391.05 g and 467.24 g at the low elevation. Also, a subplot without nutrient addition was assigned as control treatment in each plot. In steep areas, this was positioned at the top end of a plot to minimize nutrient cross-contamination from other subplots.

Croton flowering synchrony

We recorded *Croton* flowering monthly from September 2017 to August 2019 (two years). We randomly chose five plants per nutrient subplot and on each plant, we randomly chose five inflorescences to count (1) the number of female buds, (2) female and (3) male flowers, and (4) fruits per inflorescence. At the onset of winter (between December and January), when inflorescence withered, we chose a new group of five inflorescences.

We calculated flowering synchrony using an index that quantifies overlap of phenophase intensity (Freitas and Bolmgren 2008):

$$d_i = \frac{1}{T_i}\frac{1}{N-1}\sum_{j=1}^{N}\sum_{t=1}^{T}\sqrt{f_{i,t}\, f_{j,t}}\ , j \neq i$$

Where, *d* is the level of synchronization for individual *i* in relation to the population of censured individuals *j*. For our case, *i* is an individual with female flowers and *j* is an individual with male flowers. This synchrony index ranges from 0 (no synchrony) to 1 (perfect synchrony; Freitas and Bolmgren 2008). Details of the calculation methods are available in Freitas and Bolmgren (2008). We calculated synchrony index for the five individuals monitored within each nutrient treatment subplot, which were within grazed and ungrazed plots, for each elevation and for each year.

Flowering overlap estimates

We estimated the overlap coefficients and evaluated variation of *Croton* female and male flowering at each elevation. We used the data of nutrient control treatment with a circular distribution and implemented the tests in R package *overlap* (see Ridout and Linkie 2009). Probability density function of the flowering distribution assumes that a plant is equally likely to be found at all times when it is in flower. We then performed a two-step process to quantify the degree of overlap between male and female flowering at each elevation. First, we converted the survey dates to days of the year (1–365) and then to radians, to consider the year as a circular continuum and apply circular statistics (Stagemeier et al. 2020). We then estimated each flowering distribution separately by fitting a nonparametric von Mises kernel density function (see Fisogni et al. 2022). Second, we compute a measure of overlap between the two estimated distributions. The overlap coefficient (Δ) ranges from 0 (no overlap) to 1 (complete overlap) and corresponds to the area shared by the two features being compared (Fig. 1). We use Δ_1 due to low sample size (see Ridout and Linkie 2009). The estimators vary in the concentration parameter (*c*) of the kernel density, with $c = 1.25$ for Δ_1 (Ridout and Linkie 2009).

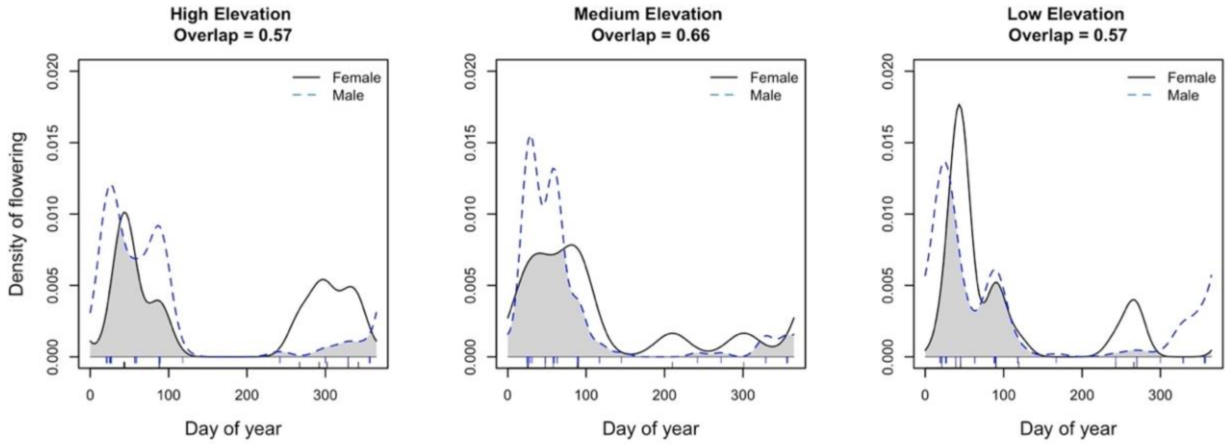

Figure 1. Examples of kernel densities and overlap coefficients showing elevation pairs with female and male flowering overlap (shaded gray area). The overlap coefficient ranges from 0 (no overlap) to 1 (complete overlap). The rug plots at the base of the fitted density curves show the actual flowering dates for each elevation throughout the year. Day of year 0 corresponds to January 1.

Fruits per infructescence and fruit set

We averaged number of fruits among the five inflorescences per plant to estimate fruit yield per year, elevation, grazing exclusion, and nutrient addition treatment. To get fruit set per plant, we divided the maximum number of fruits by the maximum number of female flowers of each inflorescence. Then we averaged fruit set among all the inflorescences (n = 15 to 20 inflorescences per plant) of each individual.

Seed weight

In April 2018 and April 2019, we selected five plants with at least one immature infructescence from each subplot with nutrient addition and their respective control treatment. As *Croton* dispersal syndrome is ballistic (Griz and Machado 2001), we covered them with tulle bags to avoid losing them. We identified with a label each chosen immature infructescence of each plant to collect all seeds. Not always there were enough plants with infructescences available, and some subplot samples may represent fewer plants than five. In May 2018 and May 2019, we collected in a paper bag the ripe fruits from the labeled infructescences in each plot.

In June 2018 and June 2019, respectively, we counted the number of fruits per labeled infructescence, extracted and separated seeds with signs of herbivory (such as holes in the seed coat) and mixed all seeds from the five infructescences from a plant. Between February and March 2020, we haphazardly selected 10 seeds per plant, weighed them (AGZN220 Torbal analytical balance with capacity and resolution of 220g x 0.0001g) and measured their length, width, and depth, and estimated seed volume with the formula for a spheroid (volume = $4/3 \cdot \pi \cdot$ length $\cdot$ width $\cdot$ depth; Riefler et al. 2005).

Seed germination

In September 2020, we set in a germination trial a random sample of 10 seeds per plant collected in May 2018. Similarly, in October 2020, we set in a similar trail a random sample of 10 seeds per plant collected in May 2019. The chosen seeds were individually imbibed for 24 hours in 1.5 ml microtube with distilled water. We disinfected the seeds with 70% alcohol for two minutes, rinsed them three times with sterile distilled water, and planted them in Petri dishes with two layers of paper moistened with sterilized distilled water. The seeds of each plant were placed in independent dishes. We checked the Petri dishes for two months, every day to observe possible germination and every two or three days the paper was with watered with sterilized distilled water. We considered germination when we observed the cotyledon or root primordia.

Data analysis

We divided the analysis of synchrony in two parts. First, we used a binomial model to evaluate the probability of synchrony; here we considered synchrony values greater than zero as 1 and values equal to zero as 0. Then, we evaluated, using a linear model, what factors affected the strength of synchrony. For the later, we only included synchrony values greater than zero. This linear mixed-effects model variation of synchrony (>0; square root transformed) as a function of plant volume (logarithmic transformed), year, elevation, grazing exclusion, and nutrient addition. We assessed with a linear mixed model the variation on average number of fruits per infructescence as a function of plant volume, synchrony level, year and elevation as fixed factors and plant as random factor. We used a generalized linear model with a binomial distribution to evaluate influence of synchrony on probability of gemination. In this model we used only synchrony values greater than zero. Because the small sample size, we did not include in this model factors such as year, elevation, grazing exclusion, nutrient addition treatment and seed weight. To evaluate germination per year, elevation, grazing exclusion, and nutrient addition, we applied a chi-square test for each factor where 0 was no germination and 1 was germination. We used a linear model and a linear mixed-effects model to assess the variation on seed weight (square root transformed) as a function of plant volume (logarithmic transformed), year, elevation, grazing exclusion, and nutrient addition and plant as a random factor. To assess seed predation, we divided analysis into two parts. First, we used a binomial model to assess whether year, elevation, grazing, and nutrient addition influenced predation probability. Here we consider predation values greater than zero as 1 and values equal to zero as 0. Then, using a linear model, we evaluated whether elevation and nutrient addition influenced seed predation (square root). For the latter, we only include synchrony values greater than zero. The most parsimonious models, with or without random effects, were identified with AIC and its relative weight of evidence (Burnham and Anderson, 2002; AIC tables can be found in supplementary material).

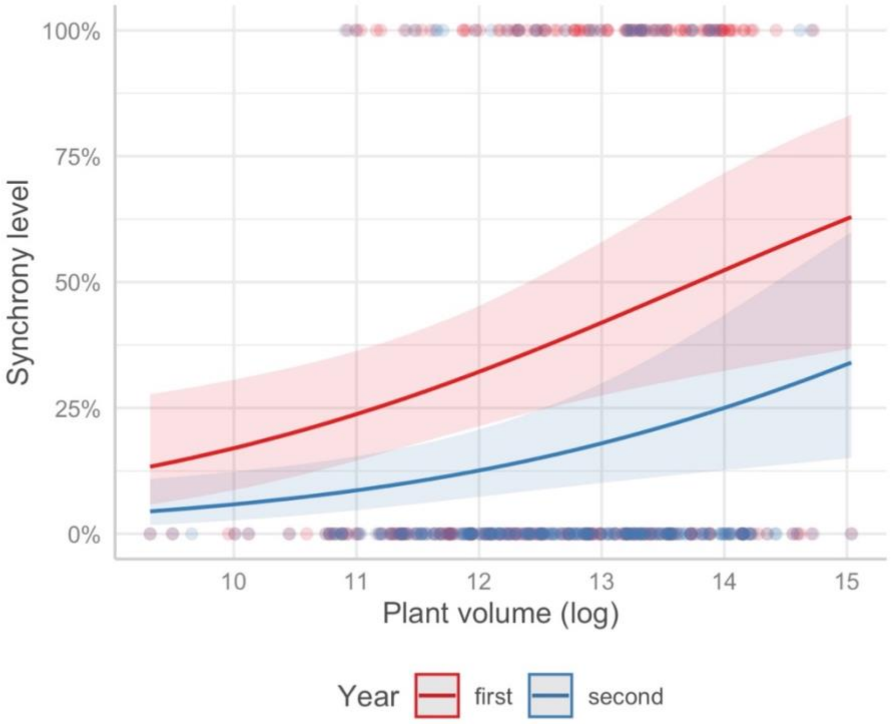

Figure 2. Flowering synchrony in relation to plant volume (plant size) between the two years of study. Original observations are shown with dots around the trend lines.

RESULTS

Croton flowering synchrony

Flowering synchrony increased with plant size and was lower in the second than in the first year of study (Fig. 2, Fig. S1). Flowering synchrony varied with year of study, elevation, and nutrient addition (Fig 3, Fig. S2). We used the average of logarithm of plant volume (12.88) to standardize these results and facilitate the description of comparisons between treatments. Flowering synchrony was lower in the second year of study (0.25 ± Std. Error [SE] 0.02 for the first year and 0.20 ± SE 0.19 for the second year respectively). Flowering synchrony at the Medium and Low elevation was higher than in High Elevation (Fig 3, Fig. S2). Flowering synchrony increased with addition of N (0.37 ± SE 0.03; Fig 3, Fig. S2). We do not have evidence that grazing exclusion influences flowering synchrony.

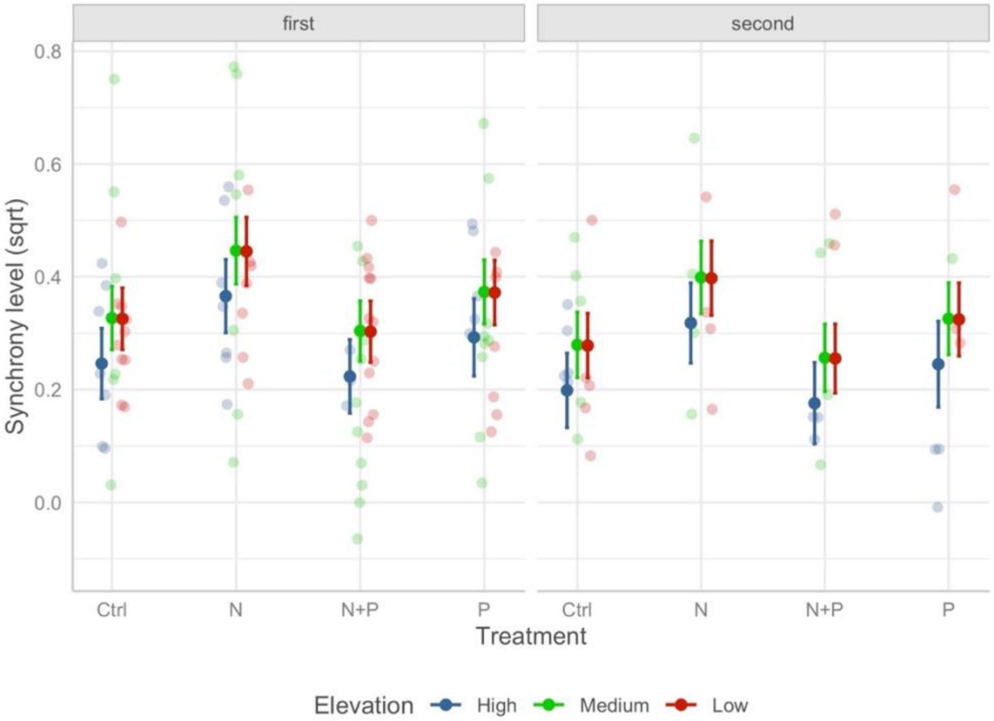

Figure 3. Flowering synchrony level per year, nutrient addition, and elevation. Error bars correspond to 95% confidence intervals. Original observations are shown with dots around the error bars.

Fruits per infructescence and fruit set

Flowering synchrony, elevation and addition of nutrients influenced the average number of fruits per infructescence of *Croton* (Fig. 4; Fig. S3). Flowering synchrony increased the average number of fruits per infructescence (Fig. 4). We used the average of logarithm of plant volume (12.98) and the flowering synchrony (0.30) to standardize by the average number of fruits per infructescence. In control plots at Low Elevation the average number of fruits per infructescence was higher than in the other elevations (1.45 ± SE 0.17) but increased more at Medium Elevation with the addition of N+P (1.84 ± SE 0.27; Fig. 4, Fig. S3). The average number of fruits per infructescence decreased at Low Elevation with the addition of P (1.33 ± SE 0.24; Fig. 4, Fig. S3). We do not have evidence that year of study or grazing exclusion influenced the average number of fruits per infructescence. The addition of N+P also influenced fruit set and was higher in the second year (Fig. 5, Fig. S4).

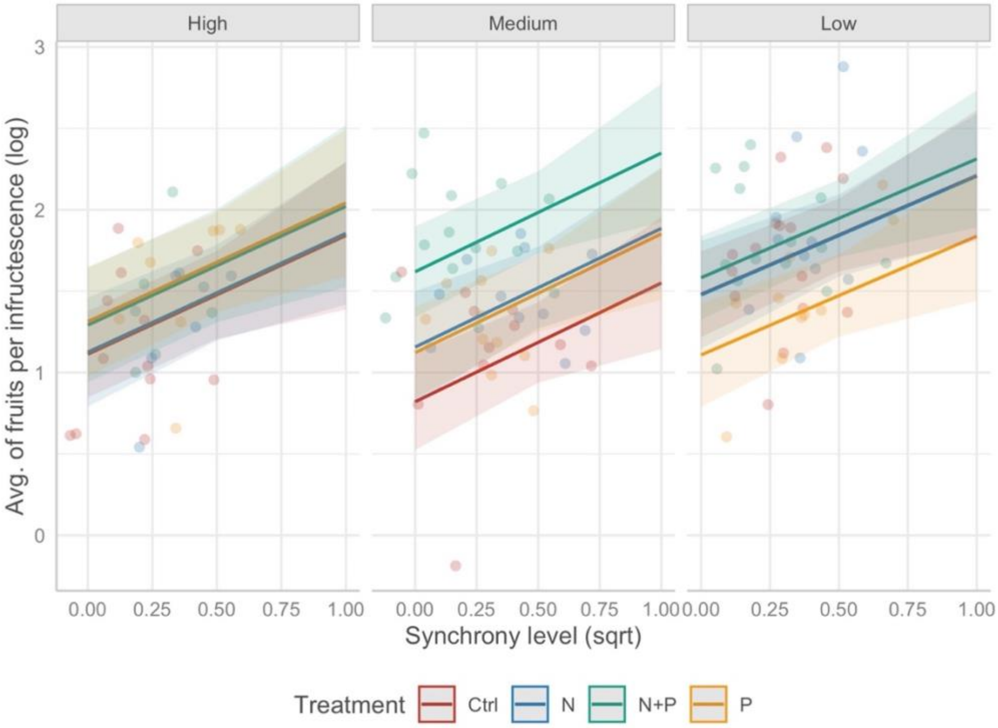

Figure 4. Variation of the average number of fruits per inflorescence according to flowering synchrony level, elevation, and nutrient addition. Original observations are shown with dots around the trend lines.

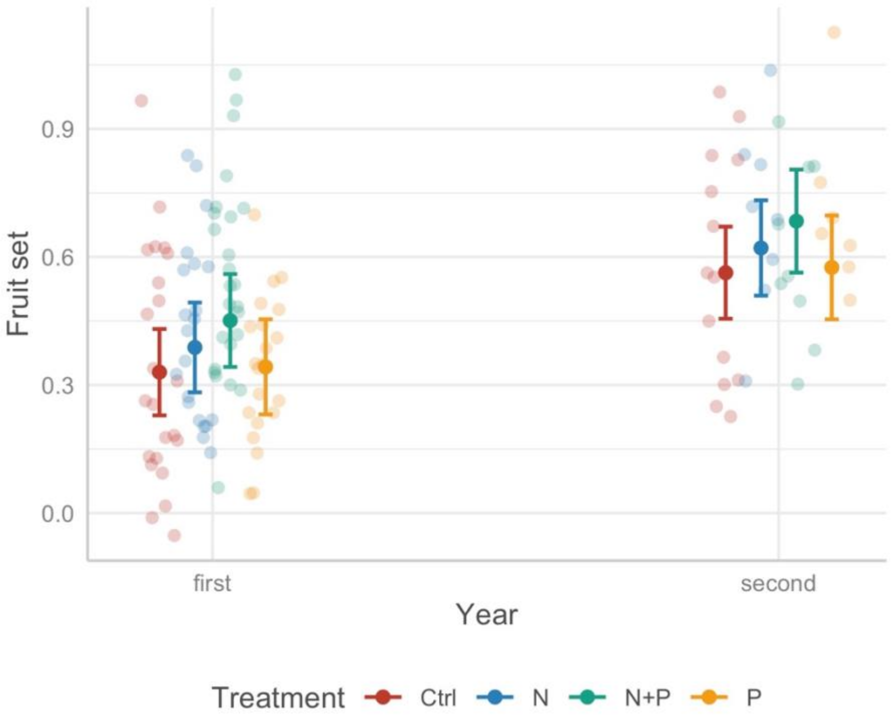

Figure 5. Variation of fruit set per year and nutrient addition. Error bars correspond to 95% confidence intervals. Original observations are shown with dots around the error bars.

Germination

Croton germination probability increased with rising flowering synchrony (Fig. 6; Fig. S5). Germination was higher with the addition of P ($X^2 = 16.51$, p = 0.0008; Fig. 7).

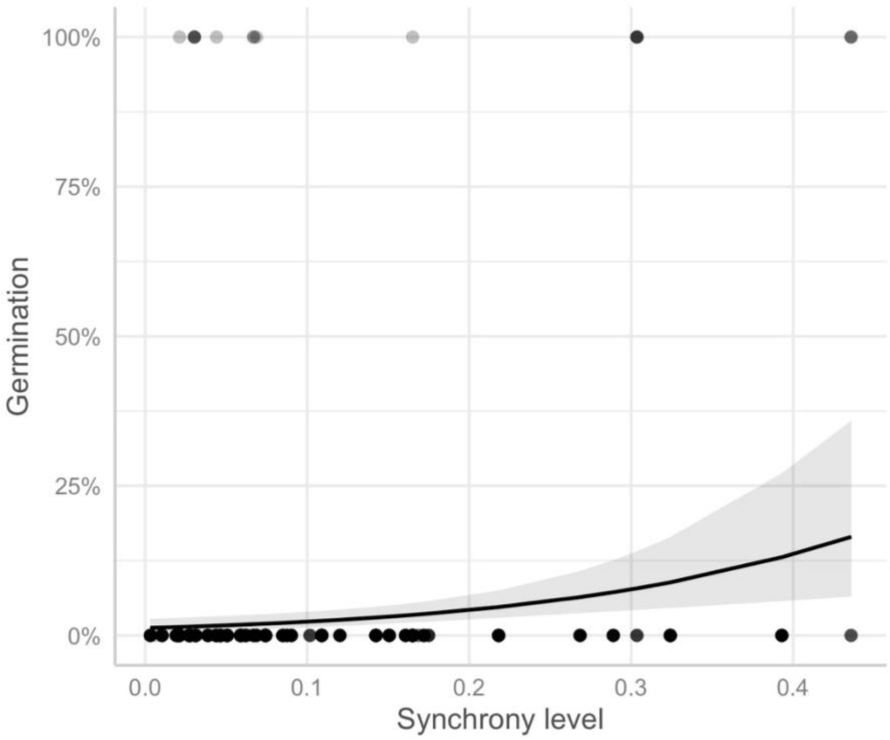

Figure 6. Variation of the germination probabilities according to flowering synchrony level. Original observations are shown with dots around the trend lines.

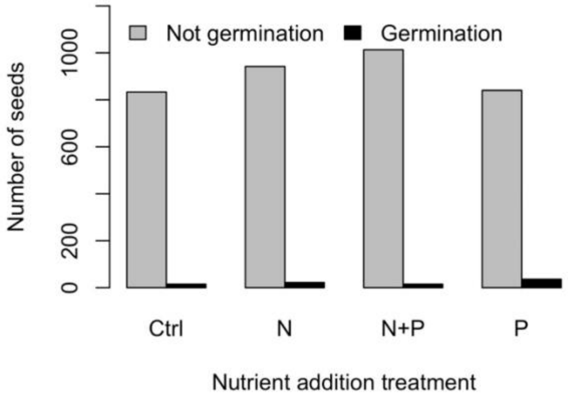

Figure 7. Effect of nutrient addition on *Croton* seed germination. According to chi square test, germination was higher with the addition of P ($X^2 = 16.51$, p = 0.0008).

Seed weight

Year, elevation, and addition of nutrients influenced *Croton* seeds weight (Fig. 8; Fig. Fig. S6). We used the average of logarithm of plant volume (13.22) to standardize by seed weight. Seed weight at Low Elevation was higher (1.01 ± SE 0.02) than at Medium and High elevations (0.96 ± SE 0.02 and 0.98 ± SE 0.05, respectively; Fig. S6). At Medium Elevation, seed weight with addition of N+P was higher (0.99 ± SE 0.08) than with the addition of N (0.98 ± SE 0.04; Fig. 8; Fig. S6). Seed weight was lower in the second year (0.97 ± SE 0.01) than in the first year of study (0.98 ± SE 0.05; Fig. 8; Fig. S6).

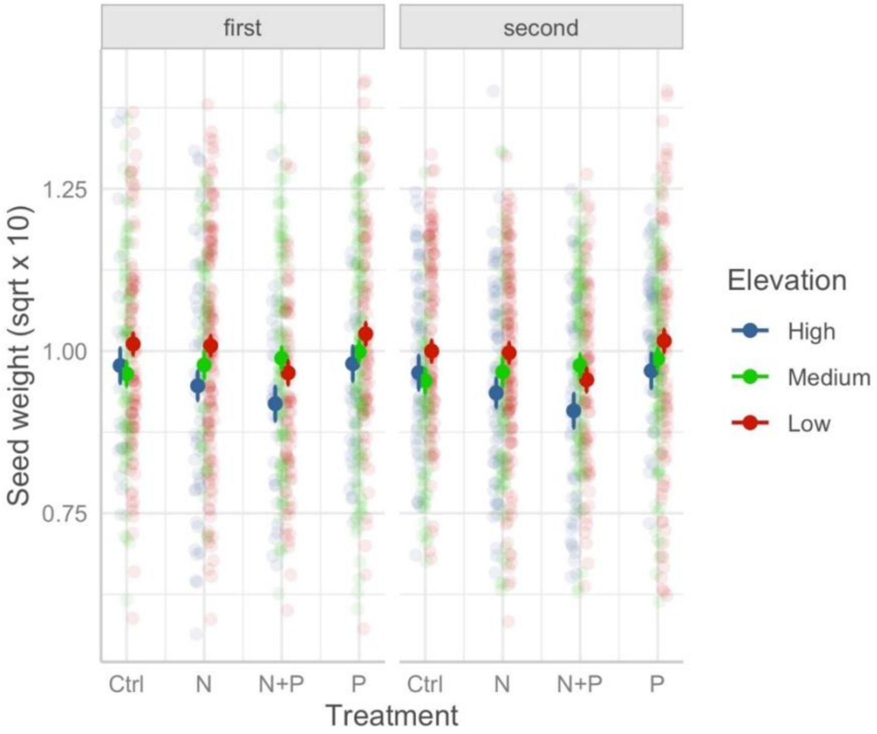

Figure 8. Seed weight per year, nutrient addition, and elevation. Error bars correspond to 95% confidence intervals. Original observations are shown with dots around the error bars.

Seed predation

Year, flowering synchrony (Fig. 9; Fig. S7) and elevation (Fig. 10; Fig. S8) influenced *Croton* seed predation. We used the average flowering synchrony (0.04) to standardize for seed predation (square root). Seed predation at Low (0.52 ± SE 0.05) and Medium (0.45 ± SE 0.06) elevations was lower than at the High Elevation (0.70 ± SE 0.06).

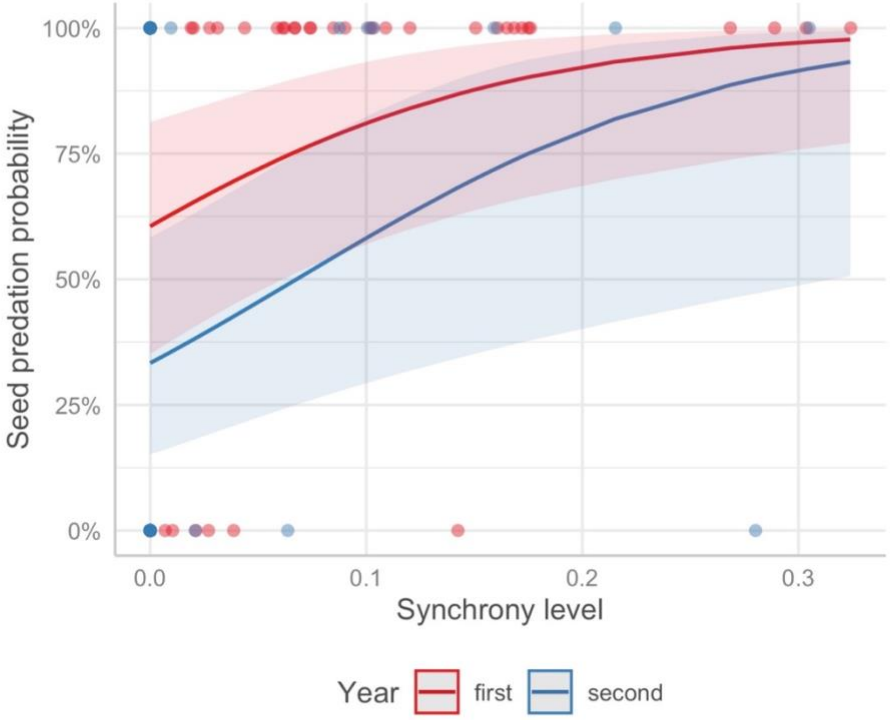

Figure 9. Seed predation probability in relation to flowering synchrony level between the two years of study. Original observations are shown with dots around the trend lines.

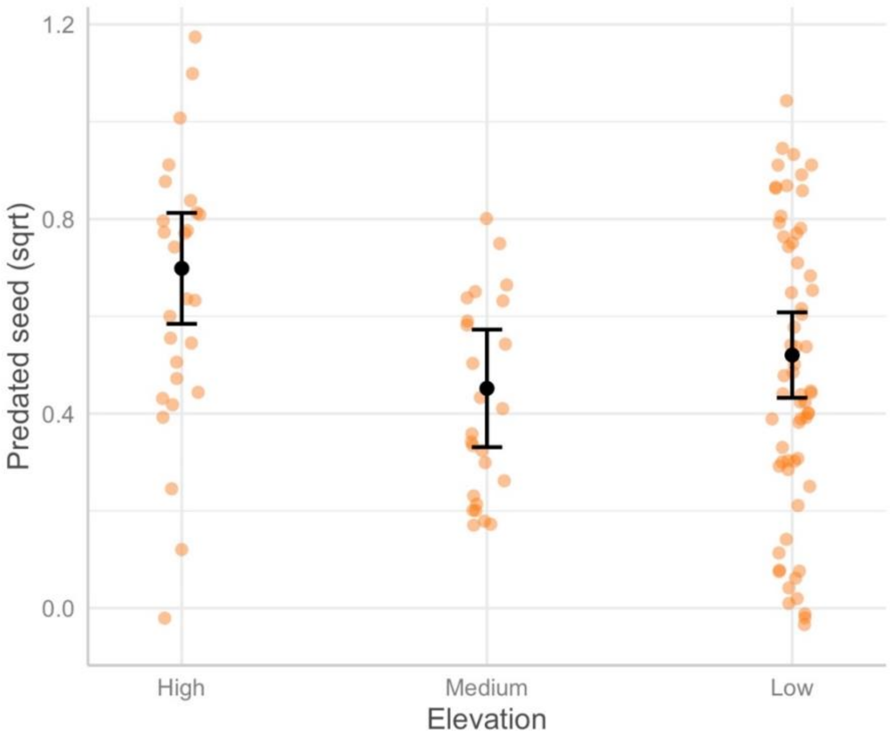

Figure 10. Variation of seed predation per elevation. Error bars correspond to 95% confidence intervals. Original observations are shown with dots around the error bars.

DISCUSION

Our findings indicate that plant size, variation in temperature, and moisture associated with elevation, and nutrients influence *Croton* flowering synchrony in inter-Andean dry shrubland. Flowering synchrony in turn influences the reproductive success of *Croton*. Because flowering synchrony also facilitates gene flow through pollination, reducing the observed flowering phenology overlap will likely directly affect plant fitness (Ghazoul 2006, Ison et al. 2014, Hall et al 2018, Rivest et al 2021, Fisogni et al. 2022). At the same time, reduced flower availability or asynchronous flowering over time is likely to affect pollinator fitness (Kaluza et al. 2018, Schenk et al. 2018, Slominski & Burkle 2021), and seasonally dry ecosystems are probably to ultimately feel the consequences of altered processes such as nutrient cycle (Forrest & Miller-Rushing 2010, Delgado-Baquerizo et al. 2013, Li et al. 2022).

Effect of plant size, elevation, and nutrients on flowering synchrony

Flowering, fruiting and even germination require a nutrient supply (Rathcke & Lacey 1985, Muñoz et al. 2004). Therefore, resource availability and ability of a plant to assimilate and allocate these resources influence flowering (Rathcke & Lacey 1985, Obeso 2002). Plants of some species flower only after they have accumulated a minimum level of resources, often represented by plan size (Rathcke & Lacey 1985, Obeso 2002, Bogdziewicz et al. 2020). Large plants that have accumulated resources in growing season can have an earlier, longer, and more abundant flowering (Augspurger 1980, Primack 1980, Rathcke & Lacey 1985, Bogdziewicz et al. 2020). This allows for greater overlap of male and female function between cosexual individuals, increasing reproductive synchrony (Smith & Bronstein 1996, Fisogni et al 2022). In seasonally dry ecosystems, interannual rainfall and soil moisture promote both increased plant size (growth) and flowering (Daubenmire 1972, Opler et al. 1980, Whigham et al. 1990, Borchert 1992, 2004, García-Cervigón et al. 2020). In our study there was an increase in flowering synchrony with *Croton* plant size. Flowering time is restricted by the need for sufficient time for vegetative growth and accumulation of resources for flowering (Vélez-Mora et al. 2022). Thus, the larger *Croton* plants flowered more intensely in the rainy season, increasing overlap for mating among individuals (Lacey 1986, Houle 2002, Ollerton & Lack 1998, but see Vélez-Mora et al 2022). It is important to mention that *Croton* has a certain degree of self-compatibility through geitonogamy, therefore, we cannot guarantee that fruit and seed formation is a product of pollen exchange between different individuals (Vélez-Mora et al. 2021). Although production of fruits and seeds is greater when there is an interbreeding among individuals (Vélez-Mora et al. 2021). Knowing paternity of seeds through a genetic study could allow us to know effective degree of interbreeding among individuals in synchrony (Llaurens et al. 2008, Rocha et al. 2018).

Plants were larger plants at the Low and Medium elevations than in the High elevation. In the sites with larger plants, N increased flowering synchrony. The short rainy season plus the incidence of high temperatures (22℃ at the Medium Elevation and 23℃ at the Low Elevation, Vélez-Mora et al. 2022) raise evapotranspiration and reduce the availability of soil water, accelerating the use of water and nutrients by part of the plants for growth and reproduction (Craine et al. 2012, Abel et al. 2021, Sohel et al. 2022). Several studies carried out in tropical forests have shown the interactive effect of precipitation and temperature on organic matter decomposition and net primary productivity (Rustad et al. 2001, Taylor et al. 2017, Lie et al. 2019). A meta-analysis showed that experimentally induced warming increases net N mineralization rates of the upper organic soil horizon by a mean of 46% at 12 sites (Rustad et al. 2001). The observed increase in net N mineralization is consistent with results of other studies where there are positive relationships between temperature and net N mineralization (Gonçalves & Carlyle 1994, MacDonald et al. 1995, Butler et al. 2012, Gong et al. 2015, Liu et al. 2022). Because N is generally considered one of the keys limiting nutrients in terrestrial ecosystems (Vitousek et al. 1997), a warming-induced increase in internal inorganic N production could stimulate plant growth and flowering of the ecosystem in sites with limited N (Rustad et al. 2001, Petraglia et al. 2014, Taylor et al. 2017, Rivero-Villar et al. 2021). In cooler environments (< 20°C) such as high elevations, excess rainfall reduces both organic matter decomposition and net primary productivity (Schuur 2003; Del Grosso et al. 2008). As an example, almost half of tree species in dry forest of Costa Rica and Mexico have seasonal flowering cycles of about a month and a half long (Bullock & Solis-Magallanes 1990), compared to longer flowering cycles of more than five months in wet forest (Frankie et al. 1974, Peters 2011). These processes are widely known for tropical wet forests, but little known for seasonally dry tropical forests which also need further attention (Powers et al. 2015, Rivero-Villar et al. 2021).

In N-rich environments, particularly those high-elevation sites affected by atmospheric N deposition (e.g., Vélez-Mora et al. 2022), a warming-induced increase in internal N production could drive or exacerbate N saturation conditions, where N input would equal or exceed the capacity of an ecosystem to assimilate added N (Aber et al. 1989, 1998). Some studies have shown warming-induced increases in soil leachate and N runoff (Joslin & Wolfe 1993, Lukewille & Wright 1997). Symptoms of N saturation could include nutrient imbalances in plant tissues, increased gaseous N loss (as NO and N_2O), and increased leaching of N from soils with a consequent decrease in surface water quality associated with these dry ecosystems (Vitousek et al. al. 1997, Fenn et al. 1998).

Effect of flowering synchrony on fruit production and germination

Greater overlap in flowering increases the probabilities of pollen/gene exchange through pollination influencing plant fitness through fruit formation and seed germination (Ghazoul 2006, Morales and Traveset 2008, Ison et al 2014, Gleiser et al 2018, Hall et al 2018, Kehrberger and Holzschuh 2019, Rivest et al 2021). Both flowering and pollinators adapt to synchronize with changes in moisture and temperature (Bolmgren, 1998; Inouye et al., 2003). Therefore, climate- and nutrient-imposed changes in flowering phenology can decouple plant-pollinator synchrony, endangering plant reproduction (Elzinga et al., 2007). In our study, addition of N and P at the Medium Elevation increased fruit production, while addition of P at the Low Elevation reduced it. An extra supply of P can be detrimental in sites where the concentration of P is high (Vélez-Mora et al. 2022). With increased root uptake and P translocation to shoots, excess P tends to accumulate in older leaves, leading to P toxicity (Dong et al. 1998, Aung et al. 2006). Increased concentration of P inside older leaves also leads to more uptake of N which delays/decreases the formation of reproductive organs (Malhotra et al. 2018). The N:P ratio increases with increasing temperature and with proximity to the equator (Reich & Oleksyn 2004) and it is likely that *Croton* plants are adapted to this nutrient to invest in their reproductive functions as we saw in the production of fruits of the control treatment at the Low Elevation (Fig. 4). N:P ratios are influenced by global change (Güsewell 2004), increased deposition of N or P in the form of aerosols could endanger the reproduction of this species (Delgado-Baquerizo et al. 2013, Xia & Wan 2013, Wang et al. 2016, Vélez-Mora et al. 2022, Mahowald & Carslaw 2022).

Seed predation and flowering synchrony

Flowering synchrony can have several benefits. Flowering synchrony helps attract pollinators and seed dispersers by mass display effect, to satiate seed predators, and to promote outcrossing by maximizing the number of potential mates (Rathcke & Lacey 1985, Marquis 1988, O 'Neil 1997, Ollerton & Diaz 1999). An experimental study in *Hybanthus prunifolius* showed that synchronous flowering enhanced the attraction of pollinators (bees) and helped satiate seed predators prior to dispersal (Microlepidoptera and Diptera larvae; Augspurger 1981). *Croton* seed predation was lower at Medium and Low elevations. This difference in *Croton* seed predation could be due to two phenological strategies to confront seed predators: (1) timing of flowering, early or late (Pilson 2000) and (2) masting, synchronized and massive production of fruits and seeds (Kelly and Sork 2002, Mendoza et al. 2015). Early flowering generally reduces seed predation relative to late flowering (Augspurger 1981, Pettersson 1994, Mahoro 2002). As an example, early flowering in *Polemonium foliosissimum* enhanced pollination, but directional selection for early flowering was offset by increased risk of seed predation (Zimmerman 1980). Masting is an adaptation that promotes increased production of large and heavy seeds (van Schaik et al. 1993). This allows seed predators to satisfy their needs as soon as possible to prevent further seed loss (Janzen 1974, Herrera 1991). *Croton* probably uses a strategy similar to masting to avoid seed predation, since fruit production (Fig. 4) and seed weight (Fig. 8) were higher at Medium and Low elevations, which had less seed predation (Fig. 10). This phenological phenomenon is not well understood and it is necessary to evaluate in greater detail the effect of the timing of flowering and the synchronized and massive production of fruits and seeds in *Croton*.

CONCLUSIONS

Larger plants, higher temperatures at low and medium elevations, and higher nitrogen availability increase reproductive synchrony in *Croton*. Increase in reproductive synchrony, increase in temperature at the Low Elevation and addition of N and P at the Medium Elevation improve production of *Croton* fruits, although increasing of flowering synchrony increases probabilities of seed predation, especially at the High Elevation. Flowering synchrony also increases germination in *Croton* seeds. Since temperatures and precipitation are predicted to continue to change globally in the future (Feng et al. 2013, Pau et al. 2013), we can expect an exacerbation of these effects at high elevations. Although precipitation patterns may have interactive variation dependent on elevation and temperature, increased variation in precipitation (Klos et al. 2014, Dannenberg et al. 2019), nutrient deposition (Basto et al. 2015) and probably grazing (Erb et al. 2018), will intensify the observed patterns in the future. Reduced flowering synchrony can directly affect the reproductive success of *Croton* and cause adverse effects on pollinators and seed predators, which could alter ecological balance of the inter-Andean dry shrubland (Blüthgen & Klein 2011, Fisogni et al. 2022, Vélez-Mora et al 2022). Additional studies of changes in long-term flowering synchrony at the community level across elevation gradients at other inter-Andean shrubland sites are needed to better understand the global impacts of climate changes on plant communities and ecosystems. In particular, provision of data on biotic interactions between plants and their pollinators would allow exploration of the ecological consequences of facilitation, resource use, and ultimately fitness, and how these relate to changes in phenological synchrony patterns.

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REFERENCES

Abel, C., Horion, S., Tagesson, T., Keersmaecker, W. D., Seddon, A. W. R., Abdi, A. M., & Fensholt, R. (2021). The human–environment nexus and vegetation–rainfall sensitivity in tropical drylands. *Nature Sustainability*, *4*(1), 25–32. https://doi.org/10.1038/s41893-020-00597-z

Aber, J. D., Nadelhoffer, K. J., Steudler, P., & Melillo, J. M. (1989). Nitrogen Saturation in Northern Forest Ecosystems. *BioScience*, *39*(6), 378–386. https://doi.org/10.2307/1311067

Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., McNulty, S., Currie, W., Rustad, L., & Fernandez, I. (1998). Nitrogen Saturation in Temperate Forest Ecosystems Hypotheses revisited. *BioScience*, *48*(11), 921–934. https://doi.org/10.2307/1313296

Aung, K., Lin, S.-I., Wu, C.-C., Huang, Y.-T., Su, C., & Chiou, T.-J. (2006). *pho2*, a Phosphate Overaccumulator, Is Caused by a Nonsense Mutation in a MicroRNA399 Target Gene. *Plant Physiology*, *141*(3), 1000–1011. https://doi.org/10.1104/pp.106.078063

Augspurger, C. K. (1980). Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. *Evolution*, *34*(3), 475–488. https://doi.org/10.1111/j.1558-5646.1980.tb04837.x

Augspurger, C. K. (1981). Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators in *Hybanthus prunifolius* (Violaceae). *Ecology*, *62*(3), 775–788. https://doi.org/10.2307/1937745

Basto, S., Thompson, K., Phoenix, G., Sloan, V., Leake, J., & Rees, M. (2015). Long-term nitrogen deposition depletes grassland seed banks. *Nature Communications*, *6*(1), 6185. https://doi.org/10.1038/ncomms7185

Blüthgen, N., & Klein, A.-M. (2011). Functional complementarity and specialisation: The role of biodiversity in plant–pollinator interactions. *Basic and Applied Ecology*, *12*(4), 282–291. https://doi.org/10.1016/j.baae.2010.11.001

Bogdziewicz, M., Szymkowiak, J., Calama, R., Crone, E. E., Espelta, J. M., Lesica, P., Marino, S., Steele, M. A., Tenhumberg, B., Tyre, A., Żywiec, M., & Kelly, D. (2020). Does masting scale with plant size? High reproductive variability and low synchrony in small and unproductive individuals. *Annals of Botany*, *126*(5), 971–979. https://doi.org/10.1093/aob/mcaa118

Bogdziewicz, M., Szymkowiak, J., Tanentzap, A. J., Calama, R., Marino, S., Steele, M. A., Seget, B., Piechnik, Ł., & Żywiec, M. (2021). Seed predation selects for reproductive variability and synchrony in perennial plants. *New Phytologist*, *229*(4), 2357–2364. https://doi.org/10.1111/nph.16835

Bolmgren, K., & Eriksson, O. (2015). Are mismatches the norm? Timing of flowering, fruiting, dispersal and germination and their fitness effects in Frangula alnus (Rhamnaceae). *Oikos*, *124*(5), 639–648. https://doi.org/10.1111/oik.01491

Borchert, R. (1992). Computer Simulation of Tree Growth Periodicity and Climatic Hydroperiodicity in Tropical Forests. *Biotropica*, *24*(3), 385. https://doi.org/10.2307/2388608

Borchert, R., Meyer, S. A., Felger, R. S., & Porter-Bolland, L. (2004). Environmental control of flowering periodicity in Costa Rican and Mexican tropical dry forests. *Global Ecology and Biogeography*, *13*(5), 409–425. https://doi.org/10.1111/j.1466-822x.2004.00111.x

Boucher, F. C., Verboom, G. A., Musker, S., & Ellis, A. G. (2017). Plant size: a key determinant of diversification? *New Phytologist*, *216*(1), 24–31. https://doi.org/10.1111/nph.14697

Bustamante, E., & Búrquez, A. (2008). Effects of plant size and weather on the flowering phenology of the organ pipe cactus (*Stenocereus thurberi*). *Annals of Botany*, *102*(6), 1019–1030. https://doi.org/10.1093/aob/mcn194

Butler, S. M., Melillo, J. M., Johnson, J. E., Mohan, J., Steudler, P. A., Lux, H., Burrows, E., Smith, R. M., Vario, C. L., Scott, L., Hill, T. D., Aponte, N., & Bowles, F. (2012). Soil warming alters nitrogen cycling in a New England forest: implications for ecosystem function and structure. *Oecologia*, *168*(3), 819–828. https://doi.org/10.1007/s00442-011-2133-7

Bronstein, J. L., Gouyon, P., Gliddon, C., Kjellberg, F., & Michaloud, G. (1990). The ecological consequences of flowering asynchrony in monoecious figs: a simulation study. *Ecology*, *71*(6), 2145–2156. https://doi.org/10.2307/1938628

Bullock, S. H., & Solis-Magallanes, J. A. (1990). Phenology of canopy trees of a tropical deciduous forest in Mexico. *Biotropica*, *22*(1), 22. https://doi.org/10.2307/2388716

Burnham, K. P., & Anderson, D. R. (2002). A practical information-theoretic approach. *Model Selection and Multimodel Inference, 2nd Ed. Springer, New York*, *2*.

Cao, C., Jiang, S., Ying, Z., Zhang, F., & Han, X. (2011). Spatial variability of soil nutrients and microbiological properties after the establishment of leguminous shrub *Caragana* *microphylla* Lam. plantation on sand dune in the Horqin Sandy Land of Northeast China. *Ecological Engineering*, *37*(10), 1467–1475. https://doi.org/10.1016/j.ecoleng.2011.03.012

Cao, G., Li, R., Li, L., Zeng, H., & Wang, J. (2020). Gender specialization and factors affecting fruit set of the wind-pollinated heterodichogamous *Juglans regia*. *Plant Species Biology*, *35*(2), 138–146. https://doi.org/10.1111/1442-1984.12268

Cleland, E. E., Chiariello, N. R., Loarie, S. R., Mooney, H. A., & Field, C. B. (2006). Diverse responses of phenology to global changes in a grassland ecosystem. *Proceedings of the National Academy of Sciences*, *103*(37), 13740–13744. https://doi.org/10.1073/pnas.0600815103

Cohen, D. (1976). The Optimal Timing of Reproduction. *The American Naturalist*, *110*(975), 801–807. https://doi.org/10.1086/283103

Cornelissen, J. H. C. (1999). A triangular relationship between leaf size and seed size among woody species: allometry, ontogeny, ecology and taxonomy. *Oecologia*, *118*(2), 248–255. https://doi.org/10.1007/s004420050725

Craine, J. M., Wolkovich, E. M., Towne, E. G., & Kembel, S. W. (2012). Flowering phenology as a functional trait in a tallgrass prairie. *New Phytologist*, *193*(3), 673–682. https://doi.org/10.1111/j.1469-8137.2011.03953.x

Crone, E. E., & Lesica, P. (2004). Causes of synchronous flowering in *Astragalus scaphoides*, an iteroparous perennial plant. *Ecology*, *85*(7), 1944–1954. https://doi.org/10.1890/03-0256

Daubenmire, R. (1972). Phenology and other characteristics of tropical semi-deciduous forest in north-western Costa Rica. *The Journal of Ecology*, *60*(1), 147. https://doi.org/10.2307/2258048

Delgado-Baquerizo, M., Maestre, F. T., Gallardo, A., Bowker, M. A., Wallenstein, M. D., Quero, J. L., Ochoa, V., Gozalo, B., García-Gómez, M., Soliveres, S., García-Palacios, P., Berdugo, M., Valencia, E., Escolar, C., Arredondo, T., Barraza-Zepeda, C., Bran, D., Carreira, J. A., Chaieb, M., … Zaady, E. (2013). Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature*, *502*(7473), 672–676. https://doi.org/10.1038/nature12670

Del Grosso, S., Parton, W., Stohlgren, T., Zheng, D., Bachelet, D., Prince, S., Hibbard, K., & Olson, R. (2008). Global potential net primary production predicted from vegetation class, precipitation, and temperature. *Ecology*, *89*(8), 2117–2126. https://doi.org/10.1890/07-0850.1

Donnelly, S. E., Lortie, C. J., & Aarssen, L. W. (1998). Pollination in *Verbascum thapsus* (Scrophulariaceae): the advantage of being tall. *American Journal of Botany*, *85*(11), 1618–1625. https://doi.org/10.2307/2446490

Dong, B., Rengel, Z., & Delhaize, E. (1998). Uptake and translocation of phosphate by *pho2* mutant and wild-type seedlings of *Arabidopsis thaliana*. *Planta*, *205*(2), 251–256. https://doi.org/10.1007/s004250050318

Donnelly, A., Caffarra, A., & O'Neill, B. F. (2011). A review of climate-driven mismatches between interdependent phenophases in terrestrial and aquatic ecosystems. *International Journal of Biometeorology*, *55*(6), 805–817. https://doi.org/10.1007/s00484-011-0426-5

Elzinga, J. A., Atlan, A., Biere, A., Gigord, L., Weis, A. E., & Bernasconi, G. (2007). Time after time: flowering phenology and biotic interactions. *Trends in Ecology & Evolution*, *22*(8), 432–439. https://doi.org/10.1016/j.tree.2007.05.006

Erb, K.-H., Kastner, T., Plutzar, C., Bais, A. L. S., Carvalhais, N., Fetzel, T., Gingrich, S., Haberl, H., Lauk, C., Niedertscheider, M., Pongratz, J., Thurner, M., & Luyssaert, S.

(2018). Unexpectedly large impact of forest management and grazing on global vegetation biomass. *Nature*, *553*(7686), 73–76. https://doi.org/10.1038/nature25138

Feng, X., Porporato, A., & Rodriguez-Iturbe, I. (2013). Changes in rainfall seasonality in the tropics. *Nature Climate Change*, *3*(9), 811–815. https://doi.org/10.1038/nclimate1907

Frankie, G. W., Baker, H. G., & Opler, P. A. (1974). Comparative phenological studies of trees in tropical wet and dry forests in the lowlands of Costa Rica. *The Journal of Ecology*, *62*(3), 881. https://doi.org/10.2307/2258961

Freeman, R. S., Brody, A. K., & Neefus, C. D. (2003). Flowering phenology and compensation for herbivory in *Ipomopsis aggregata*. *Oecologia*, *136*(3), 394–401. https://doi.org/10.1007/s00442-003-1276-6

Fisogni, A., Manincor, N. de, Bertelsen, C. D., & Rafferty, N. E. (2022). Long-term changes in flowering synchrony reflect climatic changes across an elevational gradient. *Ecography*, *2022*(2). https://doi.org/10.1111/ecog.06050

Forrest, J., & Miller-Rushing, A. J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1555), 3101–3112. https://doi.org/10.1098/rstb.2010.0145

García-Cervigón, A. I., Camarero, J. J., Cueva, E., Espinosa, C. I., & Escudero, A. (2020). Climate seasonality and tree growth strategies in a tropical dry forest. *Journal of Vegetation Science*, *31*(2), 266–280. https://doi.org/10.1111/jvs.12840

Ghazoul, J. (2006). Floral diversity and the facilitation of pollination. *Journal of Ecology*, *94*(2), 295–304. https://doi.org/10.1111/j.1365-2745.2006.01098.x

Gonçalves, J. L. M., & Carlyle, J. C. (1994). Modelling the influence of moisture and temperature on net nitrogen mineralization in a forested sandy soil. *Soil Biology and Biochemistry*, *26*(11), 1557–1564. https://doi.org/10.1016/0038-0717(94)90098-1

Gong, S., Zhang, T., Guo, R., Cao, H., Shi, L., Guo, J., & Sun, W. (2015). Response of soil enzyme activity to warming and nitrogen addition in a meadow steppe. *Soil Research*, *53*(3), 242–252. https://doi.org/10.1071/sr14140

Griz, L. M. S., & Machado, I. C. S. (2001). Fruiting phenology and seed dispersal syndromes in caatinga, a tropical dry forest in the northeast of Brazil. *Journal of Tropical Ecology*, *17*(2), 303–321. https://doi.org/10.1017/s0266467401001201

Güsewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, *164*(2), 243–266. https://doi.org/10.1111/j.1469-8137.2004.01192.x

Hall, E. S., Piedrahita, L. R., Kendziorski, G., Waddle, E., Doak, D. F., & Peterson, M. L. (2018). Climate and synchrony with conspecifics determine the effects of flowering phenology on reproductive success in *Silene acaulis*. *Arctic, Antarctic, and Alpine Research*, *50*(1), e1548866. https://doi.org/10.1080/15230430.2018.1548866

Herrera, J. (1991). Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Lamiaceae). *American Journal of Botany*, *78*(6), 789–794. https://doi.org/10.1002/j.1537-2197.1991.tb14480.x

Herrera, C. M. (1993). Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecological Monographs*, *63*(3), 251–275. https://doi.org/10.2307/2937101

Houle, G. (2002). The advantage of early flowering in the spring ephemeral annual plant *Floerkea proserpinacoides*. *New Phytologist*, *154*(3), 689–694. https://doi.org/10.1046/j.1469-8137.2002.00418.x

Ison, J. L., Wagenius, S., Reitz, D., & Ashley, M. V. (2014). Mating between *Echinacea angustifolia* (Asteraceae) individuals increases with their flowering synchrony and spatial proximity. *American Journal of Botany*, *101*(1), 180–189. https://doi.org/10.3732/ajb.1300065

Janzen, D. H. (1974). Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica*, *6*(2), 69. https://doi.org/10.2307/2989823

Joslin, J. D., & Wolfe, M. H. (1993). Temperature increase accelerates nitrate release from high-elevation red spruce soils. *Canadian Journal of Forest Research*, *23*(4), 756–759. https://doi.org/10.1139/x93-099

Kaluza, B. F., Wallace, H. M., Heard, T. A., Minden, V., Klein, A., & Leonhardt, S. D. (2018). Social bees are fitter in more biodiverse environments. *Scientific Reports*, *8*(1), 12353. https://doi.org/10.1038/s41598-018-30126-0

Kato, E., & Hiura, T. (1999). Fruit set in *Styrax obassia* (Styracaceae): the effect of light availability, display size, and local floral density. *American Journal of Botany*, *86*(4), 495–501. https://doi.org/10.2307/2656810

Kelly, D., & Sork, V. L. (2002). Mast seeding in perennial plants: why, how, here? *Annual Review of Ecology and Systematics*, *33*(1), 427–447. https://doi.org/10.1146/annurev.ecolsys.33.020602.095433

Kudo, G. (1993). Relationship between flowering time and fruit set of the entomophilous alpine shrub, Rhododendron aureum (Ericaceae), inhabiting snow patches. *American Journal of Botany*, *80*(11), 1300–1304. https://doi.org/10.1002/j.1537-2197.1993.tb15368.x

Kudo, G. (2006). Flowering phenologies of animal-pollinated plants: Reproductive strategies and agents of selection. In L. D. Harder, & S. C. H. Barrett (Eds.), Ecology and evolution of flowers (pp. 139–158). Oxford University Press.

Lacey, E. P. (1986). Onset of reproduction in plants: Size-versus age-dependency. *Trends in Ecology & Evolution*, *1*(3), 72–75. https://doi.org/10.1016/0169-5347(86)90021-2

Lasky, J. R., Uriarte, M., & Muscarella, R. (2016). Synchrony, compensatory dynamics, and the functional trait basis of phenological diversity in a tropical dry forest tree community:

effects of rainfall seasonality. *Environmental Research Letters*, *11*(11), 115003. https://doi.org/10.1088/1748-9326/11/11/115003

Li, H., Jin, X., & Yang, Y. (2018). Consequences of asynchronous heading in a perennial bunchgrass (*Elymus excelsus*). *Scientific Reports*, *8*(1), 3323. https://doi.org/10.1038/s41598-018-21759-2

Li, C., Yu, H., Xu, Y., Zhu, W., Wang, P., & Huang, J. (2022). Close linkages between leaf functional traits and soil and leaf C:N:P stoichiometry under altered precipitation in a desert steppe in northwestern China. *Plant Ecology*, *223*(4), 407–421. https://doi.org/10.1007/s11258-021-01218-1

Lie, Z., Lin, W., Huang, W., Fang, X., Huang, C., Wu, T., Chu, G., Liu, S., Meng, Z., Zhou, G., & Liu, J. (2019). Warming changes soil N and P supplies in model tropical forests. *Biology and Fertility of Soils*, *55*(7), 751–763. https://doi.org/10.1007/s00374-019-01382-7

Liu, Y., Miao, R., Chen, A., Miao, Y., Liu, Y., & Wu, X. (2017). Effects of nitrogen addition and mowing on reproductive phenology of three early-flowering forb species in a Tibetan alpine meadow. *Ecological Engineering*, *99*, 119–125. https://doi.org/10.1016/j.ecoleng.2016.11.033

Liu, Y., Men, M., Peng, Z., Houx, J. H., & Peng, Y. (2022). Nitrogen availability determines ecosystem productivity in response to climate warming. *Ecology*, e3823. https://doi.org/10.1002/ecy.3823

Llaurens, V., Castric, V., Austerlitz, F., & Vekemans, X. (2008). High paternal diversity in the self-incompatible herb *Arabidopsis halleri* despite clonal reproduction and spatially restricted pollen dispersal. *Molecular Ecology*, *17*(6), 1577–1588. https://doi.org/10.1111/j.1365-294x.2007.03683.x

Lükewille, A., & Wright, R. (1997). Experimentally increased soil temperature causes release of nitrogen at a boreal forest catchment in southern Norway. *Global Change Biology*, *3*(1), 13–21. https://doi.org/10.1046/j.1365-2486.1997.00088.x

MacDonald, N. W., Zak, D. R., & Pregitzer, K. S. (1995). Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. *Soil Science Society of America Journal*, *59*(1), 233–240. https://doi.org/10.2136/sssaj1995.03615995005900010036x

Mahowald, N., & Carslaw, K. S. (2022). Aerosol in the Earth system. In *Aerosols and Climate* (pp. 53-99). Elsevier.

Malhotra, H., Sharma, S., & Pandey, R. (2018). Phosphorus nutrition: plant growth in response to deficiency and excess. In Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak B., eds. *Plant nutrients and abiotic stress tolerance* Springer, Singapore. pp. 171–190. https://doi.org/10.1007/978-981-10-9044-8_7

Mahoro, S. (2002). Individual flowering schedule, fruit set, and flower and seed predation in *Vaccinium hirtum* Thunb. (Ericaceae). *Canadian Journal of Botany*, *80*(1), 82–92. https://doi.org/10.1139/b01-136

Marquis, R. J. (1988). Phenological variation in the neotropical understory shrub *Piper arielanum*: causes and consequences. *Ecology*, *69*(5), 1552–1565. https://doi.org/10.2307/1941653

Mendoza, I., Martin, G., Caubère, A., Châtelet, P., Hardy, I., Jouard, S., & Forget, P.-M. (2015). Does masting result in frugivore satiation? A test with *Manilkara* trees in French Guiana. *Journal of Tropical Ecology*, *31*(6), 553–556. https://doi.org/10.1017/s0266467415000425

Metzger, K. L., Coughenour, M. B., Reich, R. M., & Boone, R. B. (2005). Effects of seasonal grazing on plant species diversity and vegetation structure in a semi-arid ecosystem.

Journal of Arid Environments, *61*(1), 147–160. https://doi.org/10.1016/j.jaridenv.2004.07.019

Mothershead, K., & Marquis, R. J. (2000). Fitness impacts of herbivory through indirect effects on plant–pollinator interactions in *Oenothera macrocarpa*. *Ecology*, *81*(1), 30–40. https://doi.org/10.1890/0012-9658(2000)081[0030:fiohti]2.0.co;2

Munguía-Rosas, M. A., Ollerton, J., Parra-Tabla, V., & De-Nova, J. A. (2011). Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured: Phenotypic selection on flowering phenology. *Ecology Letters*, *14*(5), 511–521. https://doi.org/10.1111/j.1461-0248.2011.01601.x

Muñoz, A. A., Celedon-Neghme, C., Cavieres, L. A., & Arroyo, M. T. K. (2004). Bottom-up effects of nutrient availability on flower production, pollinator visitation, and seed output in a high-Andean shrub. *Oecologia*, *143*(1), 126–135. https://doi.org/10.1007/s00442-004-1780-3

Norton, D. A., & Reid, N. (2009). Sheep grazing reduces Hieracium pilosella flowering. *New Zealand Journal of Agricultural Research*, *52*(2), 129–131. https://doi.org/10.1080/00288230909510497

Obeso, J. R. (2002). The costs of reproduction in plants. *New Phytologist*, *155*(3), 321–348. https://doi.org/10.1046/j.1469-8137.2002.00477.x

Ollerton, J., & Lack, A. (1998). Relationships between flowering phenology, plant size and reproductive success in shape *Lotus corniculatus* (Fabaceae). *Plant Ecology*, *139*(1), 35–47. https://doi.org/10.1023/a:1009798320049

Ollerton, J., & Diaz, A. (1999). Evidence for stabilising selection acting on flowering time in Arum maculatum (Araceae): the influence of phylogeny on adaptation. *Oecologia*, *119*(3), 340–348. https://doi.org/10.1007/s004420050794

O’Neil, P. (1997). Natural Selection on Genetically Correlated Phenological Characters in Lythrum salicaria L. (Lythraceae). *Evolution*, *51*(1), 267. https://doi.org/10.2307/2410980

Opler, P. A., Frankie, G. W., & Baker, H. G. (1980). Comparative Phenological Studies of Treelet and Shrub Species in Tropical Wet and Dry Forests in the Lowlands of Costa Rica. *The Journal of Ecology*, *68*(1), 167. https://doi.org/10.2307/2259250

Pau, S., Wolkovich, E. M., Cook, B. I., Nytch, C. J., Regetz, J., Zimmerman, J. K., & Wright, S. J. (2013). Clouds and temperature drive dynamic changes in tropical flower production. *Nature Climate Change*, *3*(9), 838–842. https://doi.org/10.1038/nclimate1934

Petraglia, A., Tomaselli, M., Mondoni, A., Brancaleoni, L., & Carbognani, M. (2014). Effects of nitrogen and phosphorus supply on growth and flowering phenology of the snowbed forb *Gnaphalium supinum* L. *Flora - Morphology, Distribution, Functional Ecology of Plants*, *209*(5–6), 271–278. https://doi.org/10.1016/j.flora.2014.03.005

Peters, C.M. (2011). Economic Botany and Management Potential of Neotropical Seasonally Dry Forests. In: Dirzo, R., Young, H.S., Mooney, H.A., Ceballos, G., eds. *Seasonally Dry Tropical Forests: Ecology and Conservation*. Island Press: Washington, DC. pp. 239–257.

Pettersson, M. W. (1994). Large plant size counteracts early seed predation during the extended flowering season of a *Silene uniflora* (Caryophyllaceae) population. *Ecography*, *17*(3), 264–271. https://doi.org/10.1111/j.1600-0587.1994.tb00102.x

Pilson, D. (2000). Herbivory and natural selection on flowering phenology in wild sunflower, Helianthus annuus. *Oecologia*, *122*(1), 72–82. https://doi.org/10.1007/pl00008838

Pettersson, M. W. (1994). Large plant size counteracts early seed predation during the extended flowering season of a Silene uniflora (Caryophyllaceae) population. *Ecography*, *17*(3), 264–271. https://doi.org/10.1111/j.1600-0587.1994.tb00102.x

Pires, J. P. de A., Silva, A. G. da, & Freitas, L. (2013). Plant size, flowering synchrony and edge effects: What, how and where they affect the reproductive success of a Neotropical tree species. *Austral Ecology*, *39*(3), 328–336. https://doi.org/10.1111/aec.12082

Primack, R. B. (1980). Variation in the phenology of natural populations of montane shrubs in New Zealand. *The Journal of Ecology*, *68*(3), 849. https://doi.org/10.2307/2259460

Rafferty, N. E., & Ives, A. R. (2011). Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecology Letters*, *14*(1), 69–74. https://doi.org/10.1111/j.1461-0248.2010.01557.x

Rathcke, B., & Lacey, E. P. (1985). Phenological patterns of terrestrial plants. *Annual Review of Ecology and Systematics*, *16*(1), 179–214. https://doi.org/10.1146/annurev.es.16.110185.001143

Reich, P. B., & Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences*, *101*(30), 11001–11006. https://doi.org/10.1073/pnas.0403588101

Ren, F., Song, W., Chen, L., Mi, Z., Zhang, Z., Zhu, W., Zhou, H., Cao, G., & He, J.-S. (2016). Phosphorus does not alleviate the negative effect of nitrogen enrichment on legume performance in an alpine grassland. *Journal of Plant Ecology*, rtw089. https://doi.org/10.1093/jpe/rtw089

Riefler, M., Novak, O., Strnad, M., & Schmülling, T. (2005). Arabidopsis Cytokinin Receptor Mutants Reveal Functions in Shoot Growth, Leaf Senescence, Seed Size, Germination, Root Development, and Cytokinin Metabolism. *The Plant Cell*, *18*(1), 40–54. https://doi.org/10.1105/tpc.105.037796

Riginos, C., & Hoffman, M. T. (2003). Changes in population biology of two succulent shrubs along a grazing gradient. *Journal of Applied Ecology*, *40*(4), 615–625. https://doi.org/10.1046/j.1365-2664.2003.00826.x

Rivest, S., Lajoie, G., Watts, D. A., & Vellend, M. (2021). Earlier spring reduces potential for gene flow via reduced flowering synchrony across an elevational gradient. *American Journal of Botany*, *108*(3), 538–545. https://doi.org/10.1002/ajb2.1627

Rocha, O. J., Gómez, C., Hamrick, J. L., Trapnell, D. W., Smouse, P. E., & Macaya, G. (2018). Reproductive consequences of variation in flowering phenology in the dry forest tree *Enterolobium cyclocarpum* in Guanacaste, Costa Rica. *American Journal of Botany*, *105*(12), 2037–2050. https://doi.org/10.1002/ajb2.1205

Rodríguez-Pérez, J., & Traveset, A. (2016). Effects of flowering phenology and synchrony on the reproductive success of a long-flowering shrub. *AoB Plants*, *8*, plw007. https://doi.org/10.1093/aobpla/plw007

Rustad, L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., Cornelissen, J., & Gurevitch, J. Gcte, N. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*(4), 543–562. https://doi.org/10.1007/s004420000544

Schenk, M., Krauss, J., & Holzschuh, A. (2018). Desynchronizations in bee–plant interactions cause severe fitness losses in solitary bees. *Journal of Animal Ecology*, *87*(1), 139–149. https://doi.org/10.1111/1365-2656.12694

Schuur, E. A. G. (2003). Productivity and global climate revisited: the sensitivity of tropical forest growth to precipitation. *Ecology*, *84*(5), 1165–1170. https://doi.org/10.1890/0012-9658(2003)084[1165:pagcrt]2.0.co;2

Silvertown, J. (1989). The paradox of seed size and adaptation. *Trends in Ecology & Evolution*, *4*(1), 24–26. https://doi.org/10.1016/0169-5347(89)90013-x

Singh, K. P., & Kushwaha, C. P. (2006). Diversity of flowering and fruiting phenology of trees in a tropical deciduous forest in India. *Annals of Botany*, *97*(2), 265–276. https://doi.org/10.1093/aob/mcj028

Slominski, A. H., & Burkle, L. A. (2021). Asynchrony between solitary bee emergence and flower availability reduces flower visitation rate and may affect offspring size. *Basic and Applied Ecology*, *56*, 345–357. https://doi.org/10.1016/j.baae.2021.08.003

Smith, C. M., & Bronstein, J. L. (1996). Site variation in reproductive synchrony in three neotropical figs. *Journal of Biogeography*, *23*(4), 477–486. https://doi.org/10.1111/j.1365-2699.1996.tb00009.x

Smith, J. G., Sconiers, W., Spasojevic, M. J., Ashton, I. W., & Suding, K. N. (2012). Phenological Changes in Alpine Plants in Response to Increased Snowpack, Temperature, and Nitrogen. *Arctic, Antarctic, and Alpine Research*, *44*(1), 135–142. https://doi.org/10.1657/1938-4246-44.1.135

Sohel, Md. S. I. (2022). Systematic review and meta-analysis reveals functional traits and climate are good predictors of tropical tree water use. *Trees, Forests and People*, *8*, 100226. https://doi.org/10.1016/j.tfp.2022.100226

Su, L., Liu, M., You, C., Guo, Q., Hu, Z., Yang, Z., & Li, G. (2021). Nitrogen and phosphorus addition differentially enhance seed production of dominant species in a temperate steppe. *Ecology and Evolution*, *11*(21), 15020–15029. https://doi.org/10.1002/ece3.8185

Tadey, M. (2006). Grazing without grasses: Effects of introduced livestock on plant community composition in an arid environment in northern Patagonia. *Applied Vegetation Science*, *9*(1), 109–116. https://doi.org/10.1658/1402-2001(2006)9[109:gwgeoi]2.0.co;2

Tadey, M. (2020). Reshaping phenology: Grazing has stronger effects than climate on flowering and fruiting phenology in desert plants. *Perspectives in Plant Ecology, Evolution and Systematics*, *42*, 125501. https://doi.org/10.1016/j.ppees.2019.125501

Taylor, P. G., Cleveland, C. C., Wieder, W. R., Sullivan, B. W., Doughty, C. E., Dobrowski, S. Z., & Townsend, A. R. (2017). Temperature and rainfall interact to control carbon cycling in tropical forests. *Ecology Letters*, *20*(6), 779–788. https://doi.org/10.1111/ele.12765 van Schaik, C. P., Terborgh, J. W., & Wright, S. J. (1993). The phenology of tropical forests: adaptive significance and consequences for primary consumers*. *Annual Review of Ecology and Systematics*, *24*(1), 353–377. https://doi.org/10.1146/annurev.es.24.110193.002033

Vélez-Mora, D., Ramón, P., Vallejo, C., Romero, A., Duncan, D., & Quintana-Ascencio, P. F. (2021). Environmental drivers of femaleness of an inter-Andean monoecious shrub. *Biotropica*, *53*(1), 17–27. https://doi.org/10.1111/btp.12839

Vélez-Mora, D. P., Trigueros-Alatorre, K., & Quintana-Ascencio, P. F. (2021). Evidence of Morphological Divergence and Reproductive Isolation in a Narrow Elevation Gradient. *Evolutionary Biology*, *48*(3), 321–334. https://doi.org/10.1007/s11692-021-09541-1

Vélez-Mora, D. P., Gusmán, E., Espinosa, C. I., & Quintana-Ascencio, P. F. (2022). Changes in climate, grazing pressure and nutrient inputs affect the structural integrity and functioning of Andean shrublands. *Plant Ecology & Diversity*. https://doi.org/10.1080/17550874.2022.2122753

Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W., Schlesinger, W. H., & Tilman, D. G. (1997). Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications*, *7*(3), 737–750. https://doi.org/10.1890/1051-0761(1997)007[0737:haotgn]2.0.co;2

Waller D. M. (1988) Plant morphology and reproduction. In J. L. Doust & L. L. Doust (Eds.), *Plant Reproductive Ecology: Patterns and Strategies* pp. 203–27. Oxford University Press.

Wang, X., Zhou, W., Lu, J., Wang, H., Xiao, C., Xia, J., & Liu, G. (2012). Effects of Population Size on Synchronous Display of Female and Male Flowers and Reproductive Output in Two Monoecious *Sagittaria* Species. *PLoS ONE*, *7*(10), e48731. https://doi.org/10.1371/journal.pone.0048731

Wang, Y., Li, L., Zhou, D., & Weiner, J. (2016). The allometry of reproductive allocation in a *Chloris virgata* population in response to simulated atmospheric nitrogen deposition. *Basic and Applied Ecology*, *17*(5), 388–395. https://doi.org/10.1016/j.baae.2016.01.004

Weiner, J., & Thomas, S. C. (1986). Size Variability and Competition in Plant Monocultures. *Oikos*, *47*(2), 211. https://doi.org/10.2307/3566048

Whigham, D. F., Zugasty Towle, P., Cabrera Cano, E. F., O'Neill, J. P., & Ley, E. (1990). Effect of annual variation in precipitation on growth and litter production in a tropical dry forest in the Yucatan of Mexico. *Tropical Ecology*, *31*(2), 23-34.

Wright, S. J., & van Schaik, C. P. (1994). Light and the Phenology of Tropical Trees. *The American Naturalist*, *143*(1), 192–199. https://doi.org/10.1086/285600

Xi, Y., Zhang, T., Zhang, Y., Zhu, J., Zhang, G., & Jiang, Y. (2015). Nitrogen Addition Alters the Phenology of a Dominant Alpine Plant in Northern Tibet. *Arctic, Antarctic, and Alpine Research*, *47*(3), 511–518. https://doi.org/10.1657/aaar0014-054

Xia, J., & Wan, S. (2008). Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist*, *179*(2), 428–439. https://doi.org/10.1111/j.1469-8137.2008.02488.x

Xia, J., & Wan, S. (2013). Independent effects of warming and nitrogen addition on plant phenology in the Inner Mongolian steppe. *Annals of Botany*, *111*(6), 1207–1217. https://doi.org/10.1093/aob/mct079

Zhang, Y., Cao, C., Han, X., & Jiang, S. (2013). Soil nutrient and microbiological property recoveries via native shrub and semi-shrub plantations on moving sand dunes in Northeast China. *Ecological Engineering*, *53*, 1–5. https://doi.org/10.1016/j.ecoleng.2013.01.012

Zimmerman, M. (1980). Reproduction in *Polemonium*: pre-dispersal seed predation. *Ecology*, *61*(3), 502–506. https://doi.org/10.2307/1937415

Supplemental material

Plant size, elevation, and nutrients affect reproductive synchrony, which increases reproduction in a key shrub of the inter-Andean dry

Binomial model of synchrony

glm (sync ~ logvol + Elevation + Exclusion + Treatment + Year, family=binomial)

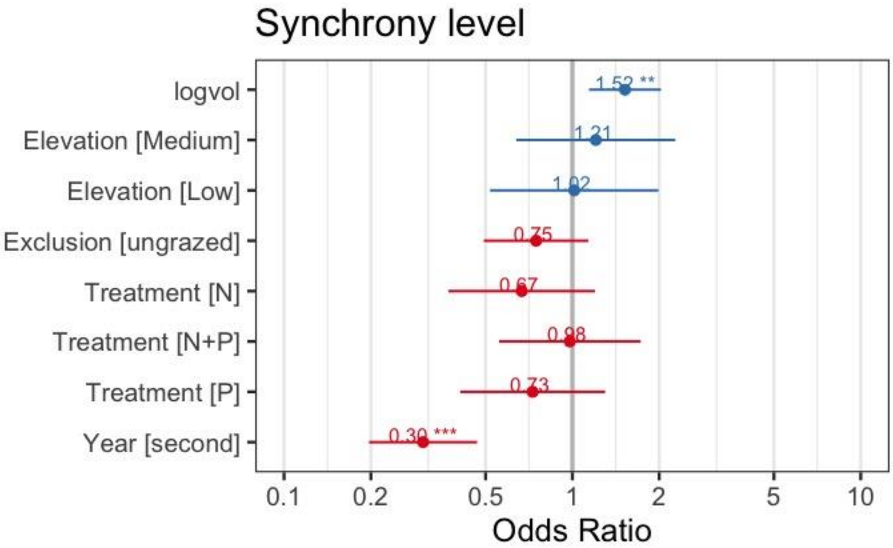

Figure S1. Plot of odds ratios (95% confidence interval) for plant volume (logvol), elevation, grazing, nutrient addition, and year for the binomial model. Odds ratios greater or less than 1 indicate that there is an effect of either plant volume, elevation, grazing, nutrient addition, or year on flowering synchrony level.

Lineal model of synchrony

lm (sq.sync ~ logvol + Elevation + Exclusion + Treatment + Year)

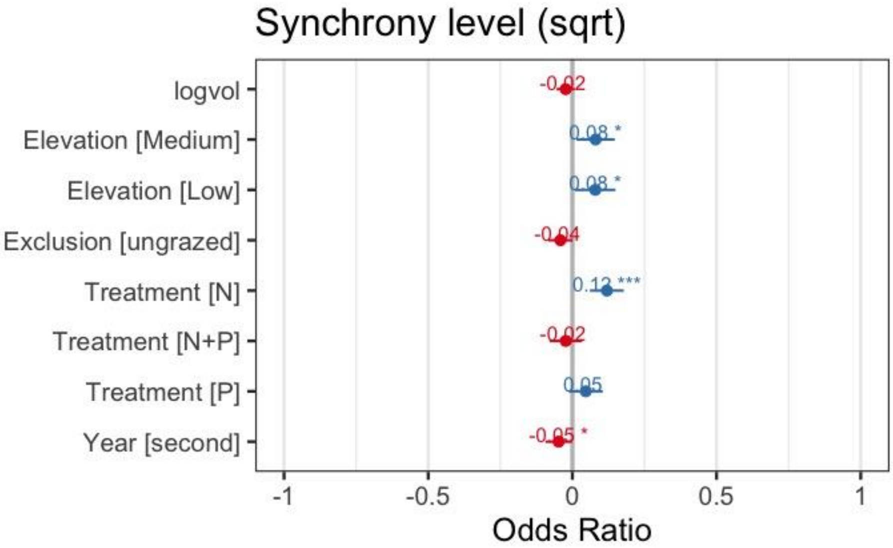

Figure S2. Plot of odds ratios (95% confidence interval) for plant volume (logvol), elevation, grazing, nutrient addition, and year for the linear model. Odds ratios greater or less than 0 indicate that there is an effect of either plant volume, elevation, grazing, nutrient addition, or year on flowering synchrony level.

Linear mixed-effect model for fruits per infrutecence

lme (log.avg.fr ~ logvol + sync + Elevation * Treatment + Exclusion + Year, random=~1|Plant, method = "ML")

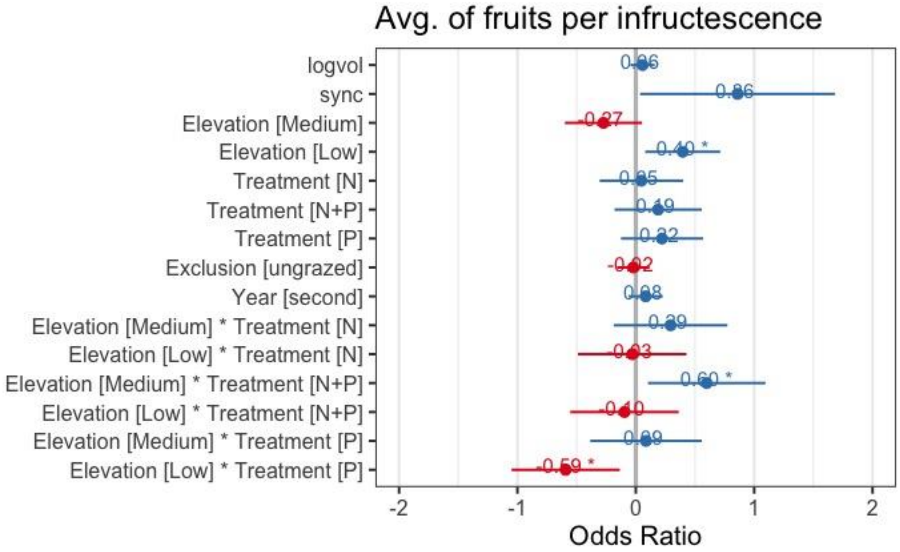

Figure S3. Plot of odds ratios (95% confidence interval) for plant volume (logvol), flowering synchrony level (sync), elevation, nutrient addition, grazing, and interaction between elevation and nutrient addition for the mixed linear model. Odds ratios greater or less than 0 indicate that there is an effect of either plant volume, flowering synchrony level, elevation, grazing or nutrient addition, on average of fruits per infructescence.

Linear model for fruit set

lm (fruit.set ~ logvol + sq.sync + Elevation + Exclusion + Treatment + Year)

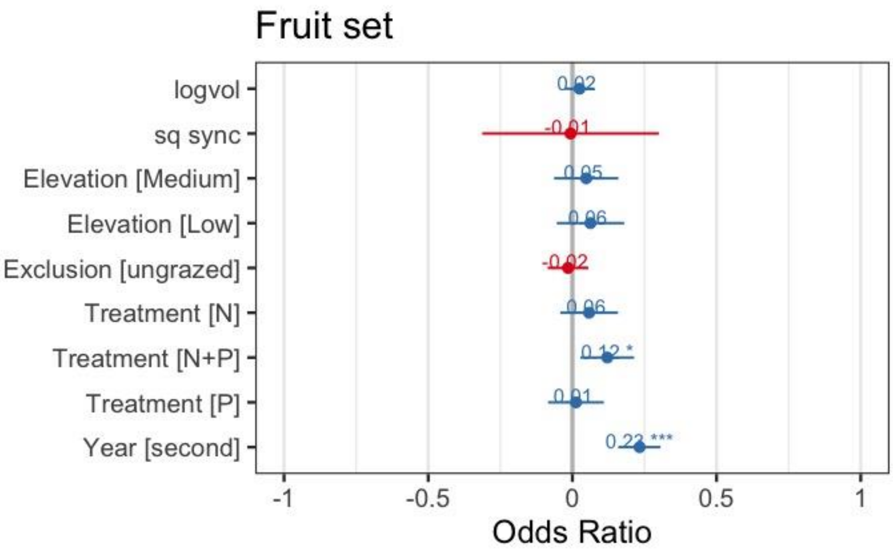

Figure S4. Plot of odds ratios (95% confidence interval) for plant volume (logvol), flowering synchrony level (sq sync, square root), elevation, nutrient addition, and year for the linear model. Odds ratios greater or less than 0 indicate that there is an effect of either plant volume, flowering synchrony level, elevation, or nutrient addition, on fruit set.

Binomial model for germination

glm (germ ~ sync, family=binomial)

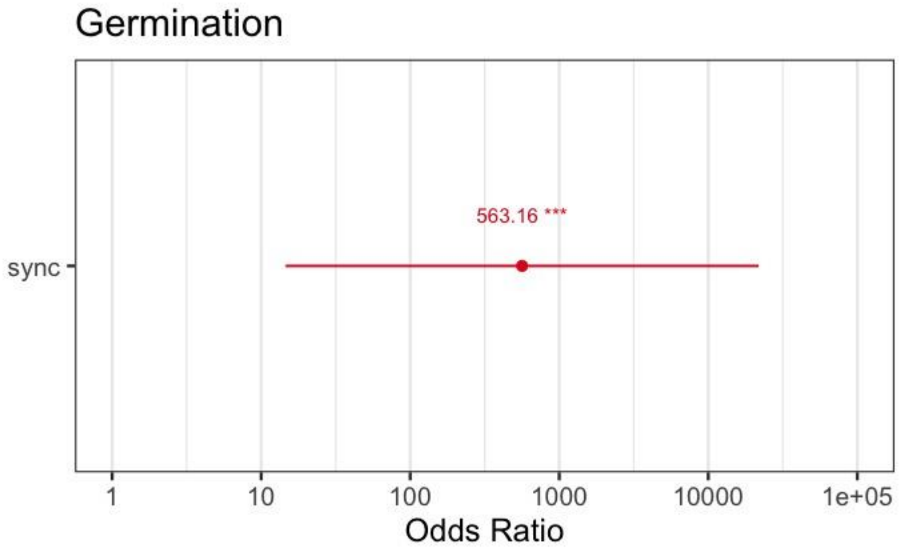

Figure S5. Plot of odds ratios (95% confidence interval) for synchrony flowering level for the binomial model. Odds ratios greater or less than 1 indicate that there is an effect of synchrony flowering level on seed germination.

Linear model for seed

lm (sq.weight * 10 ~ logvol + Year + Elevation * Treatment + Exclusion)

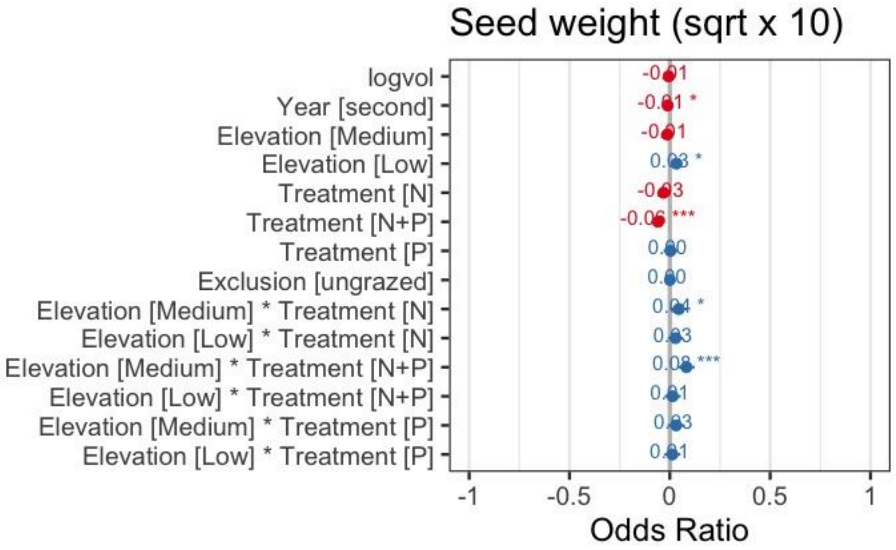

Figure S6. Plot of odds ratios (95% confidence interval) for plant volume (logvol), year, elevation, nutrient addition, grazing, and interaction between elevation and nutrient addition for the mixed linear model. Odds ratios greater or less than 0 indicate that there is an effect of either plant volume, year, elevation, nutrient addition, grazing, or a combined effect between elevation and nutrient addition on seed weight.

Binomial model of seed predation

glm (pred ~ logvol + sync + Elevation + Exclusion + Treatment + Year, family = binomial)

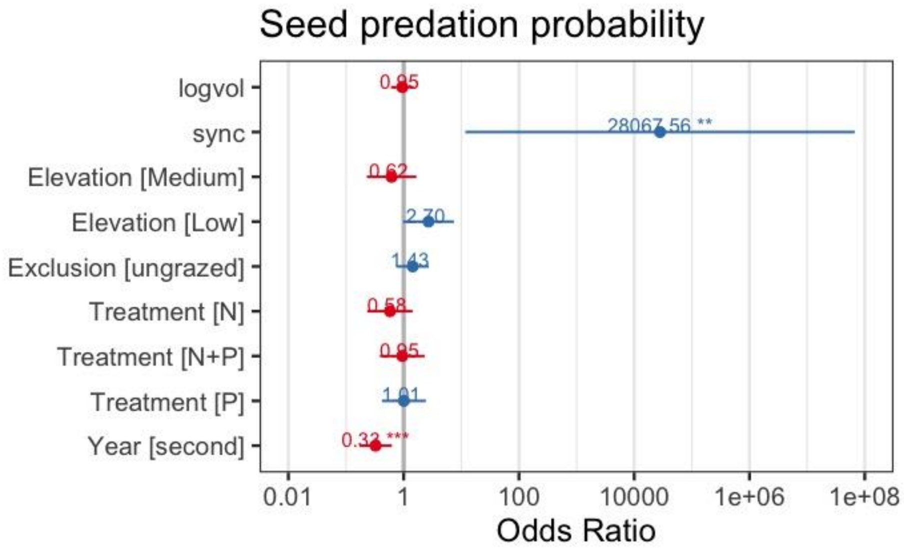

Figure S7. Plot of odds ratios (95% confidence interval) for plant volume (logvol), flowering synchrony level, elevation, grazing, nutrient addition, and year for the binomial model. Odds ratios greater or less than 1 indicate that there is an effect of either plant volume, flowering synchrony level, elevation, grazing, nutrient addition, or year on seed predation.

Lineal model for seed predation

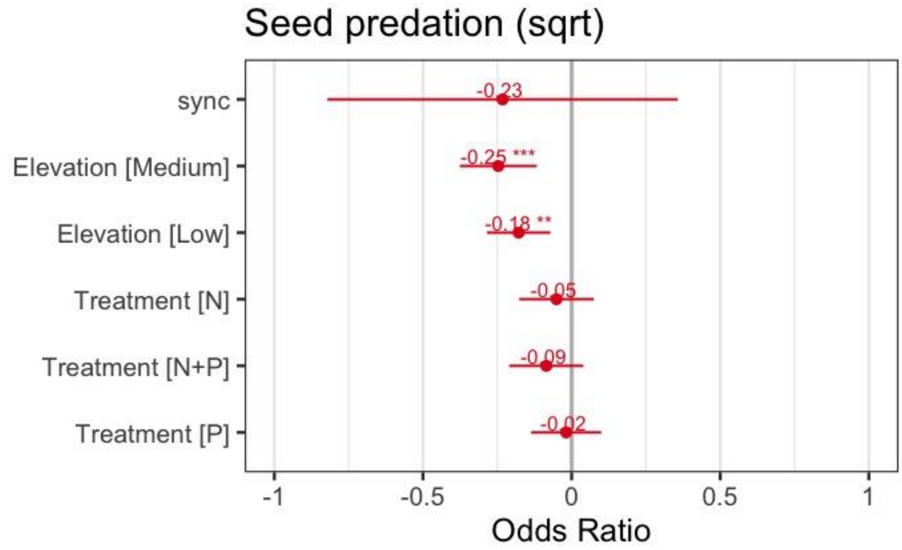

Figure S8. Plot of odds ratios (95% confidence interval) for flowering synchrony level, elevation, and nut rient addition for the linear model. Odds ratios greater or less than 0 indicate that there is an effect of flo wering synchrony level, elevation, or nutrient addition on seed predation.

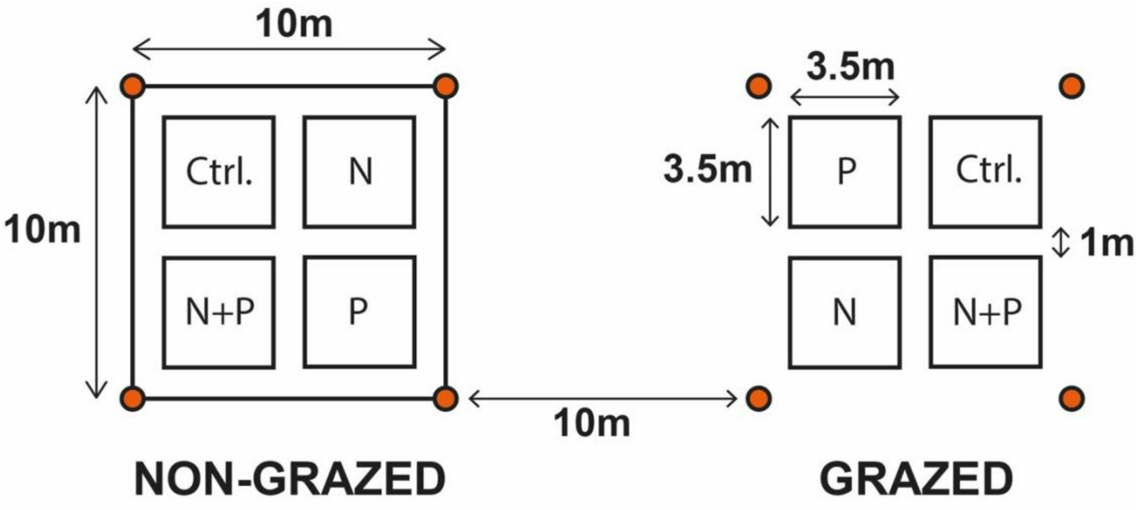

Figure S9. Design of plots excluded and not excluded from grazing.

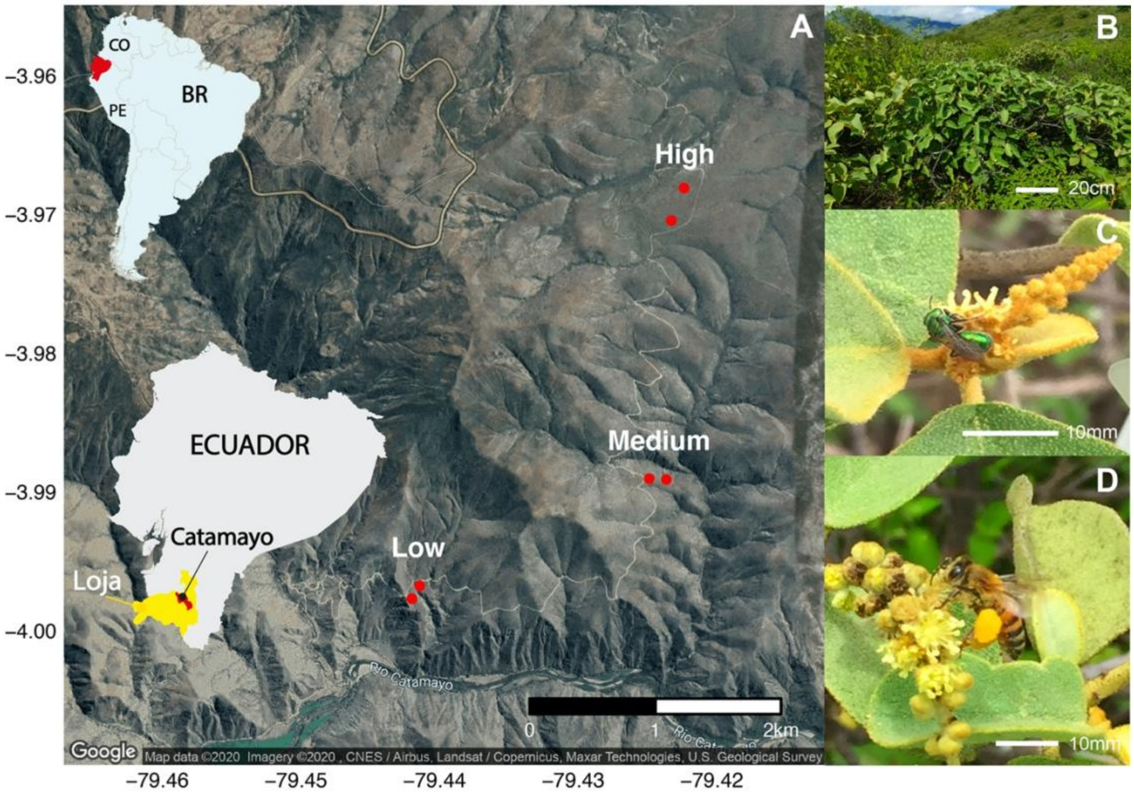

Figure S10. A: Map of Ecuador showing the locations of studied sites colored red at each elevation. B: Study species. C: A species of wasp visiting female Croton flowers. D: A bee visiting male *Croton* flowers.

CAPÍTULO 3

Changes in climate, grazing pressure and nutrient inputs affect the structural integrity and functioning of Andean shrublands

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ABSTRACT

Background: Changes in climate and the intensity of agriculture expansion can alter plant population dynamics and community composition and structure of dry shrublands.

Aims: We tested how temperature and moisture along an elevation gradient, grazing, and nutrient addition in soil affected demographic attributes of *Croton* shrubs and the composition and structure of plant species in an inter-Andean dry shrubland.

Methods: At three elevations, we installed grazing and exclusion plots, combined with four nutrient treatments: control, and addition of nitrogen (N), phosphorous (P) alone and in combination. We measured recruitment and survival of *Croton* seedlings, as well as survival, growth, fruiting of adult *Croton* and the composition and structure of neighbouring plants.

Results: Grazing exclusion improved adult survival of *Croton* at all three elevations. Grazing exclusion and addition of N and P increased adult growth of *Croton* at low and medium elevations. *Croton* seedling recruitment and survival decreased with distance to adult plants. The cover of *Croton* had a positive relationship with plant abundance and diversity.

Conclusions: Temperature, moisture, grazing and nutrient addition can alter the demography and cover of *Croton*, as well as the composition and structure of its neighbouring plants threatening the functioning of the inter-Andean dry shrubland.

Introduction

Climate change, grazing and excessive nitrogen (N) and phosphorus (P) input can alter the structure and functioning of drylands around the world where water availability is a limiting resource (Westerband et al. 2015; Silva et al. 2021). In these environments, temperature increases, and moisture decreases at lower elevations (Richter and Moreira-Munoz 2005; Vélez-Mora et al. 2021). Changes in temperature and moisture along elevation gradients in dry ecosystems are not the only drivers that contribute to plant growth and reproduction, but also grazing (Eldridge et al. 2016), soil overfertilization (Wassen et al. 2021) and the unpredictability of precipitation (Maestre et al. 2005; Ding and Eldridge 2021) may have important effects.

Positive interactions between facilitating (or nurse) plants and their facilitated plants during the drought season often increase survival, growth, and reproduction among plants (see Maestre et al. 2009; Cavieres and Badano 2010; Erfanzadeh et al. 2021). Moisture, nutrients, and cover (shading) in these environments are usually more accessible under adult plant canopies (nurse effect), favouring seedling survival and growth (Soliveres et al. 2010; Espinosa et al. 2019; Maestre et al. 2021). Favourable temperature and moisture conditions below facilitating plant canopies can promote plant associations (Scanlon et al. 2007; Howard et al. 2012) likely driving plant diversity (McIntire and Fajardo 2014; Mihoč et al. 2016; Erfanzadeh et al. 2021) and functioning (Maestre et al. 2016, 2021) in these semi-arid ecosystems. Existing studies suggest that the effects of plant–plant interactions tend to become more positive for biomass and biodiversity in ecosystems dominated by facilitating shrubs when aridity increases (Mazía et al. 2016; Rey et al. 2016; Berdugo et al. 2019; Erfanzadeh et al. 2021). A synthesis of community-level studies has found that more than 25% of species in drylands are spatially associated with facilitating plants (Soliveres and Maestre 2014). This illustrates the importance of understanding how plant interactions promote maintenance of plant diversity in extreme environments and how plant communities are affected by human activities that reduce plant biomass and cover such as livestock grazing (Maestre et al. 2016; Wu et al. 2021).

Grazing is a driver of changes in composition and diversity of plant communities (Augustine and McNaughton 1998; Kutt et al. 2012; Eldridge et al. 2013). It directly influences the structure of a plant community by reducing plant growth (Daryanto et al. 2013; Eldridge et al. 2016) and in inter-Andean ecosystems has been shown to strongly affect both plant diversity and plant growth (Espinosa et al. 2013). Grazing also affects cycling and storage of N and P in the soil. In semi-arid grasslands in northern China, N storage (in aerial biomass, litter, roots, and soil) increased significantly due to grazing exclusion (He et al. 2008; Li et al. 2012), while in the northern highlands of Ethiopia, P storage in soil increased between 26% and 39% when grazing was excluded (Mekuria and Aynekulu 2013).

Changes in N and P inputs, mainly due to human activity, can have large effects on nutrient cycling within tropical ecosystems (Galloway et al. 2004; Elser 2011; Bejarano-Castillo et al. 2015; Craine et al. 2018). This nutrient imbalance occurs due to N deposition after burning, grazing and agricultural production (Jaramillo and Murray-Tortarolo 2019), and P loss through leaching and runoff because of heavy rains (Raghubanshi 1991; Rundel and Boonpragob 1995; Singh et al. 1997; García-Oliva et al. 2011; Hou et al. 2018). These changes in soil nutrients affect the primary productivity of the ecosystem (Peñuelas et al. 2013; Yuan and Chen 2015) and can also cause substantial alterations in biodiversity and the functioning of ecosystems (Carnicer et al. 2015). Despite its importance, our knowledge about the dynamics of N and P in tropical dryland ecosystems remains scarce (Waring et al. 2019).

Tropical dryland ecosystems are home to a wide diversity of species. They cover ca. 42% of tropical forests (Murphy and Lugo 1986) and contain 20% of global plant diversity hotspots (Davies et al. 2012; Maestre et al. 2021). These drylands play a key role in regulating global cycles of N (Tian et al. 2020), P (Delgado-Baquerizo et al. 2013) and water (Wang et al. 2012) and their plant communities provide various and important ecosystem services (Siyum 2020). Despite their enormous social and ecological importance, tropical dryland ecosystems are the most vulnerable and least protected ecosystems on Earth (Portillo-Quintero and Sánchez-Azofeifa 2010; Stan and Sanchez-Azofeifa 2019). Consequently, urgent action is required to halt their decline (Yao et al. 2020; Maestre et al. 2021).

The inter-Andean dry valleys are present in Colombia, Ecuador, Perú, and Bolivia (Pennington and Ratter 2006; Wood 2006; Linares-Palomino et al. 2011). In these ecosystems, shrubs represent a large portion of the diversity (43%; 12% are endemic), followed by herbs (36%) and trees (20%) (Quintana et al. 2016). Their main threats are extensive grazing (Iñiguez 2004), invasive grasses (Tapia- Armijos et al. 2015), nutrient deposition by burned biomass (Rollenbeck et al. 2013), high levels of human pressure (Tapia-Armijos et al. 2017) and climate change (Herzog et al. 2011; Cisneros et al. 2021). In these ecosystems, cattle forage mostly grasses along elevation gradients (Iñiguez 2004; Espinosa et al. 2013) while goats forage mainly at low elevations where there is greater abundance of shrubs and leguminous trees (Iñiguez 2004).

This study developed from previous research on the population ecology and reproductive biology of *Croton* shrubs (a complex of interbreeding hybrids taxonomically unresolved) and on their interactions with other species in inter-Andean communities in Ecuador (Espinosa et al. 2013, 2019; Lara-Romero et al. 2017; Vélez-Mora et al. 2020, 2021). This research recognised the importance of changes in elevation on these communities and indicated the relevance of grazing pressure and nutrient input resulting from increasing agricultural development in neighbouring areas (Espinosa et al. 2013, 2019; Solano et al. 2018; Vélez-Mora et al. 2021). Here, we examine the demography of *Croton* in an inter- Andean dry valley to investigate experimentally the effects of current environmental and anthropogenic changes in such regions on dryland shrub communities. We present results of an experiment established in 2015 at different elevations involving grazing exclusion and nutrient additions to evaluate hypotheses derived from initial observational studies to better understand the consequences of anthropogenic changes on *Croton* and associated plants.

Croton is a dominant member of plant communities in inter-Andean regions of Ecuador (León-Yánez et al. 2011). Our study was conducted in the Andean Depression (Richter et al. 2009), a region between northern Perú and southern Ecuador (Richter and Moreira-Munoz 2005; Peters et al. 2014; Quintana et al. 2017). We utilised elevational changes in temperature and moisture as surrogates of potential current changes in climate (Sundqvist et al. 2013) across a narrow elevation range of 300 m (Vélez-Mora et al. 2020). Previous studies showed that grazing increased the facilitating effects of *Croton* on other species around 1550 m and reduced diversity and plant cover around 1950 m (Espinosa et al. 2013). They further showed that the facilitating effects of *Croton* canopy improved survival of conspecific seedlings (Espinosa et al. 2019) and that plant density and plant size of *Croton* increased seedling and adult survival and reproductive performance, while growth decreased due to intraspecific competition (Lara-Romero et al. 2017). Soil nitrogen stocks were also shown to increase with elevation (Solano et al. 2018). Based on these findings, we hypothesised that (1) under higher moisture and lower temperature conditions, without grazing and with higher availability of nitrogen and phosphorus, seedling and adult plant survival, growth and *Croton* fruit yield will increase; and (2) a reduction of density and cover of *Croton*, as a consequence of grazing and resource (moisture and nutrients) changes with elevation, will reduce the facilitating effect of *Croton* and consequently the richness and diversity of associated plants. To test these hypotheses, we evaluated the effects of climate variation in accordance with elevation, grazing exclusion, and nitrogen and phosphorus additions (1) on survival of seedlings and adult *Croton* plants, (2) on growth and fruiting of *Croton* plants, and (3) on the composition and structure of their associated plant species.

Materials and methods

The dry shrubland of the inter-Andean valleys of southern Ecuador

Several abiotic factors in the inter-Andean ecosystems provide stressful conditions for organisms. Seasonal and highly variable air and soil temperature extremes during the day, coupled with unpredictable and limited precipitation (Figure 1g and figure A1 in Espinosa et al. 2019) favour xeromorphic adaptations (Figure 2c, d). In addition, shallow and nutrient poor soils (Figure 1a, c and h) on very steep slopes (Figure 2a) exposed to erosion limit resources for growth and reproduction during restricted times when moisture is available (Arteaga et al. 2020). Furthermore, recent rising temperatures, increasing pressure from livestock (Figure S1) and reduction of water availability diverted for agriculture and human consumption have imposed further limitations on growth and reproduction (Fries et al. 2020).

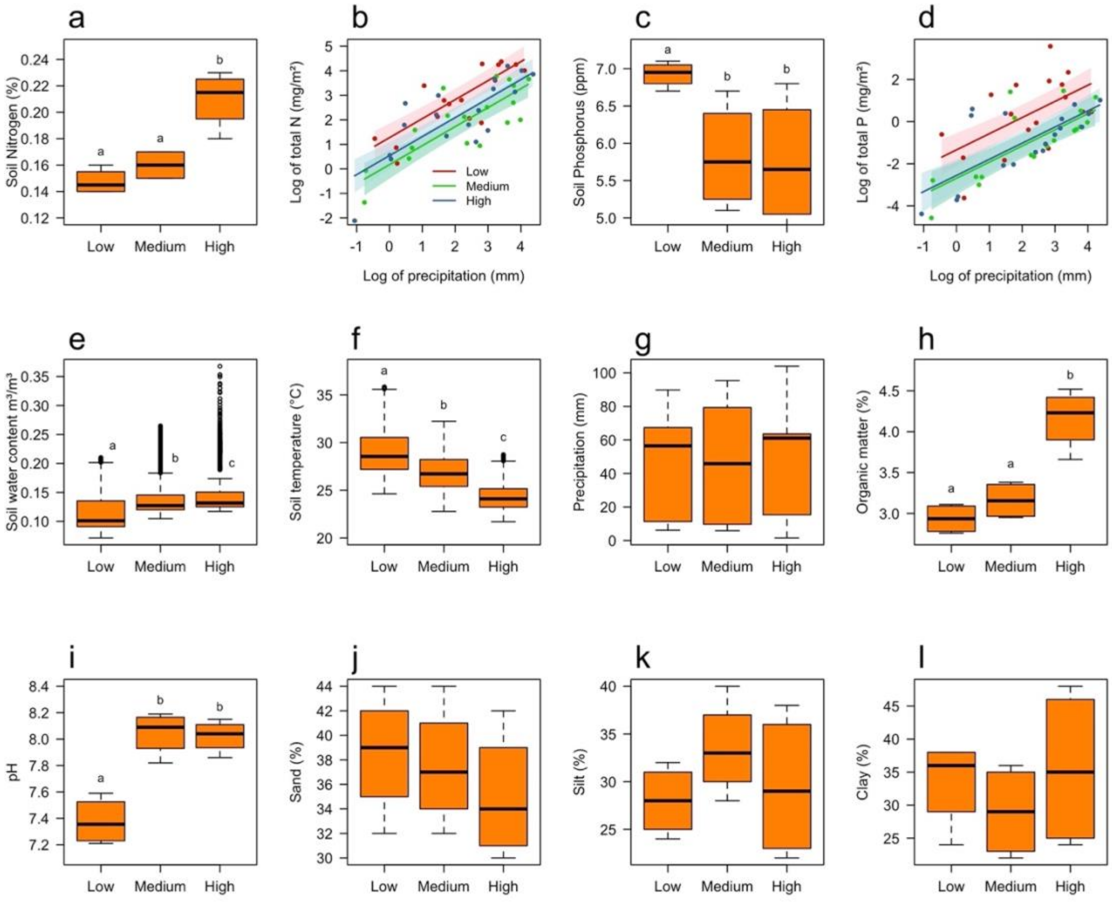

Figure 1. Summary plots of soil variables, climate, and N and P deposition in the inter-Andean valley of Catamayo, Loja Province, Ecuador at elevations of 1400 m a.s.l. (Low), 1550 m (Medium), and 1700 m (High). a-c: concentrations of N and P from soil. b-d: wet deposition of N and P; observed values are indicated by different coloured points for each elevation as well as the polygons with confidence intervals to 95%. e-f: soil water content and temperature; g: precipitation during the rainy season November 2017 - March 2018; h-l: percent of organic matter content, pH, percent of sand, silt, and clay content of soil samples. Letters on box plots indicate clear differences between elevations.

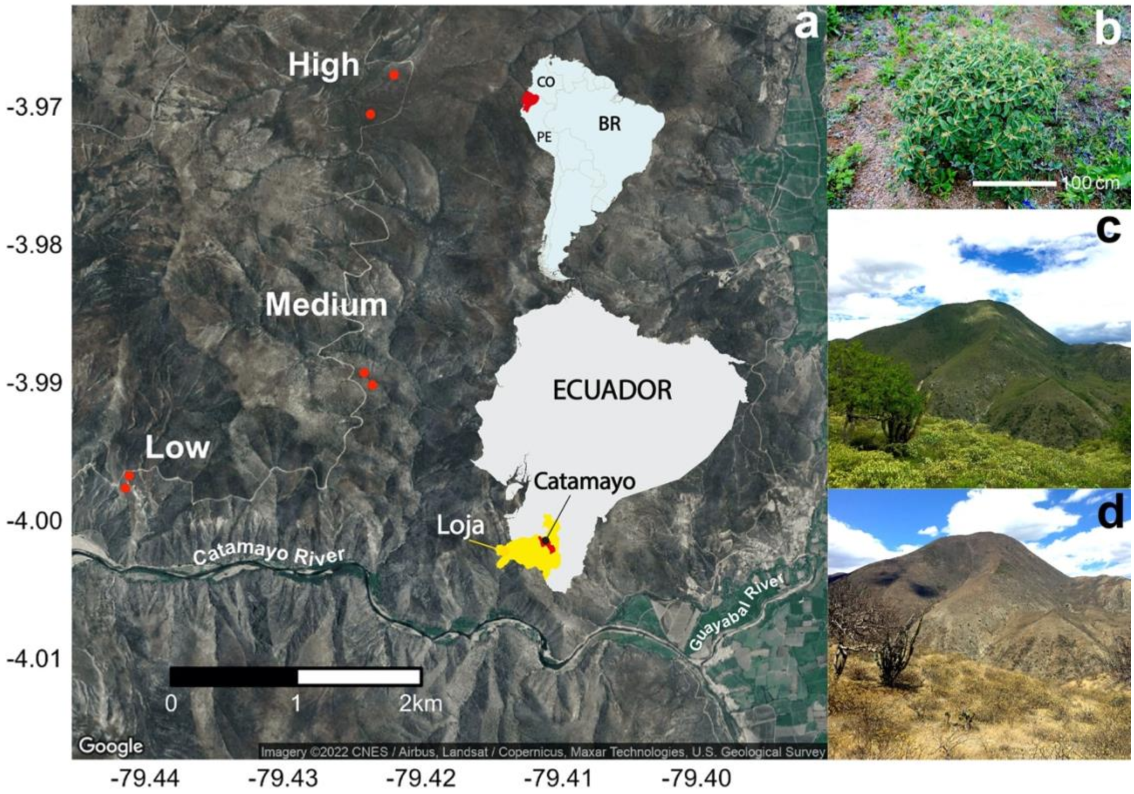

Figure 2. a: Paired grazed and grazing excluded study sites (red dots in satellite image) in the inter-Andean valley of Catamayo, Loja Province, Ecuador (inset) at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high). The green areas at the right edge of the image are sugarcane plantations; b: *Croton* sp., the focal study species with its greenish-brown inflorescences; c: panoramic view near the site at 1400 m, April 2019; d: view of the same location in August 2019 during the dry season. The dry and leafless trees and shrubs reveal some cacti.

Study site

Our study was performed at an inter-Andean valley of the Catamayo Canton, Loja Province, Ecuador (Figure 2). This dry inter-Andean shrubland experiences an average annual temperature of 24.8°C, with an average annual precipitation of 317 mm and a prolonged dry season where 57% of the annual precipitation occurs between February and May (Espinosa et al. 2019; data from City Airport in Catamayo 2011–2015 located 6 km from our study site). In this valley, the dominant *Croton* shrubs are distributed continuously between 1100 and 2080 m of elevation

(Espinosa et al. 2019). *Croton* varies in spatial aggregation that increases along the elevation gradient and decreases with grazing pressure levels (Espinosa et al. 2013). Patches of *Croton* form a matrix including sparse xerophytic and spiny vegetation co-dominated by tree perennials such as, *Vachellia macracantha* (Humb. & Bonpl. ex. Willd.), *Bursera graveolens* (Kunth), *Colicodendron scabridum* (Kunth), and shrubby perennials such as *Lantana canescens* (Kunth), *Agave americana* (L.), *Ipomoea carnea* (Jacq.) and *Opuntia quitensis* (Ulloa and Jørgensen 1995; Espinosa et al. 2013; Aguirre Mendoza et al. 2017).

We chose three elevations: 1700 m a.s.l. (high elevation; 3°58'07.90'' S, 79°25'19.71'' W), 1550 m a.s.l. (medium elevation; 3°59'20.09''+S, 79°25'28.64'' W) and 1400 m a.s.l. (low elevation; 3° 59'40.15''S, 79°26'31.29'' W). Along this environmental gradient, there was variation in soil temperature, moisture, and soil nutrient composition. We used generalised linear models with Gaussian distribution to evaluate the variation of total N (%) and total P (ppm) in the soil (%), precipitation (mm), organic matter (%), sand (%), and clay (%), soil water content ($m^3/m^{3),}$ soil temperature (°C), and soil pH and silt (%) as a function of elevation (data were logarithmically transformed when necessary to meet model assumptions). We used a linear model to evaluate the variation of N (mg/ m^2) and P (mg/m^2) deposition as a function of precipitation per elevation.

Total N concentration in the soil was lower at the low and medium elevations than at the high elevation (Figure 1a), although N deposition by rainfall was higher at the low elevation (Figure 1b). Total P concentration was higher at the low elevation compared to the medium and high elevations, both in the soil and deposited by rain (Figure 1c, d). Soil water content (recorded in winter between January and February 2019) was lower at the lower elevation (Figure 1e). Soil temperature (also recorded in winter between January and February 2019) was higher at the low elevation (Figure 1f). There was more organic matter at the high elevation (Figure 1h) and soil pH was more alkaline at the medium and high elevations than in the low elevation (Figure 1i).

Precipitation in winter (between November 2017 and March 2018) was similar between these elevations (Figure 1g and

Figure S2) as well as percentage of silt and clay in soil (Figure 1k, l). Percentage of soil sand decreased with elevation (Figure 1j).

At the regional level, dry weather and erosion at low elevations has led to the formation of Entisols characterised by sandy, stony, and rocky formations generating shallow, soils (Winckell et al. 1997; Moreno et al. 2018). At higher elevations, wetter climate has promoted development of clayey soils classified as Vertisols (Soil Survey Staff 2006; Moreno et al. 2018). Our soil analyses showed loam, clay, and clay-loam soils along the elevation gradient (Tables S1B and S2B). The topography is rugged with moderately steep slopes (Espinosa et al. 2019). These sites are visited by small herds of feral donkeys and horses. In recent years we have witnessed increasing goat and cattle herds in the area. According to local shepherds, cattle occasionally consume *Croton*. We also observed that livestock trample seedlings, small plants and damage the branches of *Croton* plants, mainly in winter (Figure S1).

At highest elevation (1700 m of elevation), maize is grown in association with pastures (Moreno et al. 2018), while at lowest elevations (1200 m of elevation), there is intense agricultural activity only in areas irrigated by the Catamayo River (Figure 2). The warm, tropical valleys are suitable for short-cycle crops such as sugarcane, banana, tomato, cucumber, pepper, zarandaja, beans and cassava, and perennials such as coffee and fruit trees (Moreno et al. 2018; Chamba-Morales et al. 2019). The Catamayo valley, located on flat land of recent alluvial sediments, accommodates the large *Ingenio Monterrey Azucarera Lojana* CA – MALCA and sugarcane fields irrigated with water from the rivers Catamayo and Guayabal (Winckell et al. 1997; Moreno et al. 2018; Figure 2a). Sugarcane cultivation covers an area of 2,172 hectares in the Catamayo Canton (Ochoa-Cueva et al. 2022). The extensive annual burning of sugarcane likely mobilises nutrients across the elevation gradient. Approximately five years ago, moringa (*Moringa oleifera*) cultivation was established near to our study site (Vélez-Mora personal observation).

Study species

The taxonomy of *Croton* in our study area remains problematic and we opted to be cautious on its classification and refer to it as *Croton* (Euphorbiaceae). *Croton* is a 0.5–1.5 m tall monoecious shrub that inhabits a wide variety of habitats, mostly semi-arid and often in secondary vegetation (Ulloa and Jørgensen 1995; Van Ee et al. 2011; Figure 2b). Plants reach reproductive maturity in 2–3 years and can survive for several years (Espinosa et al. 2019). Recent studies indicate clear morphological variation in leaves and inflorescences among forms at different elevations and suggest that there is incipient reproductive isolation between local populations of *Croton* occurring at local elevation extremes (Vélez-Mora et al. 2021).

Experimental design and treatments

In July 2015, we initiated a field experiment along an elevation gradient to evaluate the effects of climate (temperature and moisture) variation, grazing, and N and P addition to the soil on *Croton* survival, growth, fruit yield, seedling survival, and on associated plant diversity. Average annual temperature (°C) at the high elevation was 20.68 (standard error [SE] = 0.01), at the medium elevation 21.90 (SE = 0.01) and at the low elevation 22.87 (SE = 0.01). We observed that winter rains generally start in October and end in April (see Figure A1 in Espinosa et al. 2019) and decided, therefore, to measure precipitation in the period of likely greatest rainfall from November 2017 to March 2018. At each elevation, we obtained a weekly rainfall average during this period and summed these weekly averages to obtain monthly precipitation (Figure S2). Average precipitation during this winter period was 49.12 mm (SE = 12.38) for the high elevation, 47.25 (SE = 17.99) for the medium elevation and 46.25 (SE = 16.24) for the low elevation.

Two pairs of 10 m × 10 m plots (at least 100 m apart) were established at each elevation. In each pair, one plot was excluded and the other not excluded from grazing. In total, we established 12 plots. Plot assignment was established randomly. Plots excluded from grazing were fenced with four lines of barbed wire at a maximum height of 1.5 m in July 2015, while those in which grazing was allowed had only wooden posts placed at the four corners. Wire was replaced when damage was observed, and all old posts and wire were replaced by new material in February 2019. For the nutrient addition treatments, subplots of 3.5 m × 3.5 m were established within the 10 m × 10 m plots, separated from each other by 1 m (see Figure S3). On 18 March 2017, 10 March 2018, and 19 April 2019, N and P were applied to subplots within each plot. Nutrients were applied depending on the previous availability of these nutrients in the soil of each subplot. One replicate of each treatment (control, N, P and N+P) was applied per plot. (Calculations and exact amounts applied of N and P by subplot are shown in Tables S1 and S2). On average, 346.81 g of urea (N) and 506.24 g of triple superphosphate (P) were applied per treated subplot at the high elevation; 382.20 g and 503.76 g at the medium elevation, and 391.05 g and 467.24 g at the low elevation. Also, a subplot without nutrient addition was assigned as control treatment in each plot. In steep areas, this was positioned at the top end of a plot to minimise nutrient cross-contamination from other subplots. We considered that a 10 m × 10 m plot size as suitable for the experiment due to high density of *Croton* across the elevation gradient (20.75 plants on average ±1.55 SE per plot).

Survival, growth, fruit yield by inflorescence and seedling survival

We recorded in July 2015, shortly before plots were fenced, the height, length, and canopy width of each *Croton* plant greater than 15 cm in height to estimate their volume (see Vélez-Mora et al. 2020). In July 2019, the same measurements were repeated, and live and dead plants were recorded to evaluate the survival of plants initially recorded in July 2015. Plant size (canopy length, canopy width, and plant height as in Vélez-Mora et al. 2020) was recorded in July 2017 and July 2019. We used these measurements to estimate *Croton* growth. Sample sizes for each demographic variable are in Table S3.

We recorded *Croton* fruit yield monthly from September 2017 to September 2019. We randomly chose five plants per nutrient subplot and on each plant, we randomly chose five inflorescences to count the number of fruits per inflorescence. When fruits had dispersed or fell and the inflorescence withered, we chose another group of five inflorescences after the onset of winter (between December and January). Fruit yield per inflorescence was estimated as the maximum number of fruits counted in any month during each annual reproductive season. We considered number of fruits produced per inflorescence on a plant as an indicator of the reproductive condition of the plant (Data in Espinosa et al. 2019 can be used to estimate the total number of fruits per plant).

During August and November 2019, February 2020, and March 2021, we recorded the number of *Croton* seedlings recruited in each subplot at each elevation, noting their status (dead/alive), height, and distance (cm) to their nearest (supposed) maternal plant.

Vegetation sampling

In June 20 May 201617, and June 2021, we recorded and taxonomically identified every plant with stems >10 cm in each plot at each elevation at the end of the rainy season. From these records, we calculated the total number of standing stems of all species, species richness, and the Shannon diversity index per plot.

Data analysis

We used generalised linear models to evaluate plant growth, survival, and seedling survival variation as a function of elevation, grazing exclusion, nutrient addition (N and P), and for the analysis of two-way interactions. The logarithm of plant volume was considered as the most informative plant size variable (see Vélez-Mora et al. 2020) and its initial value was included as a covariable in these models if relevant (expressed as linear or quadratic terms). We used a logit link and a binomial distribution to evaluate *Croton* adult and seedling survival and used normal errors and the identity link to evaluate

plant growth. Random effects by replicated site by elevation were negligible and we dropped these variables from the models. A generalised linear mixed-effects model (*lme4* R package; Bates et al. 2015) with a logit link and a binomial distribution was used to evaluate fruiting probability per inflorescence as a function of plant volume and elevation (plant volume and elevation as fixed factors and plants as random factors). We used a generalised linear mixed model with negative binomial errors and a logarithmic link to evaluate fruit yield by inflorescence (annual maximum number of fruits) as a function of plant volume and the interaction between elevation with nutrient treatments (plant volume, elevation, and nutrient treatments as fixed factors and plants as random factors). Generalised linear models with Poisson distribution were used to assess the influence of *Croton* cover on number of total standing stems of all species and species richness (*vegan* R package; Oksanen et al. 2020) in 2016 and in 2021. Cover was summarised as the sum of canopies of each *Croton* individual (see Vélez-Mora et al. 2020) per plot. To assess the influence of *Croton* cover on Shannon diversity (vegan R package) in 2016 and in 2021, we used a linear model. For this model we used the logarithm of Shannon diversity. All analyses were performed in version 4.1.1 of R software (R Core Team 2021).

Results

Effects of elevation, grazing exclusion and N and P addition on survival, growth, and fruit yield

Elevation, grazing, nutrient addition to the soil, and plant volume affected survival, growth, and fruit yield of adult *Croton* individuals. Survival increased with plant volume (Figure 3, Table S4).

We used the average of logarithm of plant volume from 2015 (11.66) to standardise by size survival probabilities and facilitate the description of comparisons among treatments. At each elevation, low, medium, and high, *Croton* survival increased in non-grazed plots without addition of nutrients (low: 0.93 ± SE 0.59; medium: 0.97 ± SE 0.68 and high: 0.91 ± SE 0.53 elevations) compared to control grazed plots (0.86 ± SE 0.80; Figure 3). At low elevation, survival decreased (0.53 ± SE 0.53; Figure 3) in non-grazed plots with added P, compared to the control. At medium elevation, there was a reduction in survival (0.79 ± SE 1.08) in non-grazed plots with added of N+P, compared to control non-grazed plots with no added nutrients (0.97 ± SE 0.68; Figure 3; Figure S4). At high elevation, we observed the lowest survival (0.42 ± SE 0.53; Figure 3; Figure S4) in grazed plots (control treatment).

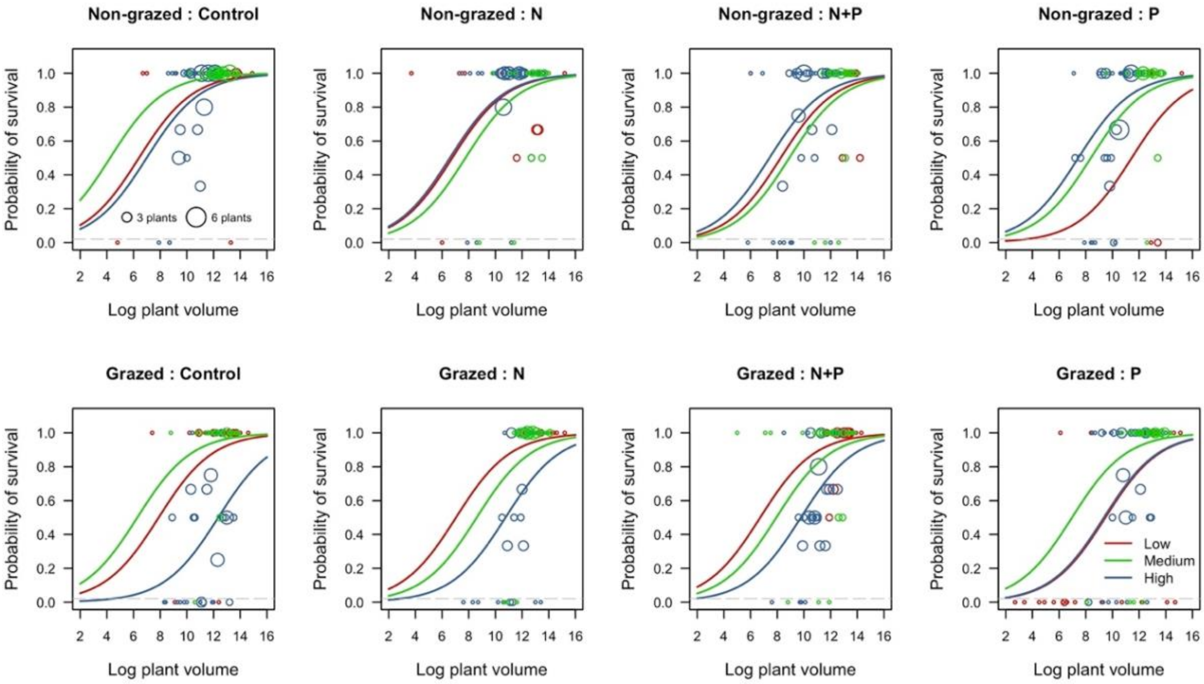

Figure 3. Plant volume, elevation, and the interaction between elevation, grazing exclusion, and nutrients on the survival of *Croton* plants in the inter-Andean valley of Catamayo, Loja Province, Ecuador at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high). Solid lines are trend lines for a binomial fit of plant survival probability by elevation, grazing/no grazing, and

nutrients according to plant volume. Coloured empty circles indicate the number of plants (see top left panel for legend) included in a binned treatment category. Grey horizontal dashed line is a reference (model intercept = 0.02) to distinguish survival among the three elevations, and between exclusion and nutrient additions. The R^2 value of survival model was 0.21.

Croton growth increased and then decreased with plant volume (Figure 4, Table S5). We used the average of logarithm of plant volume from 2015 (145.06; only surviving plants) to standardise growth. At each elevation, low, medium, and high, *Croton* growth increased in the non-grazed plots with added N+P (low: 12.85 ± SE 0.15; medium: 12.69 ± SE 0.15; and high: 12.07 ± SE 0.15 elevations) compared to controls (Figure 4). However, at high elevation, growth decreased with only P addition in non-grazed plots (11.40 ± SE 0.16; Figure 4; Figure S5).

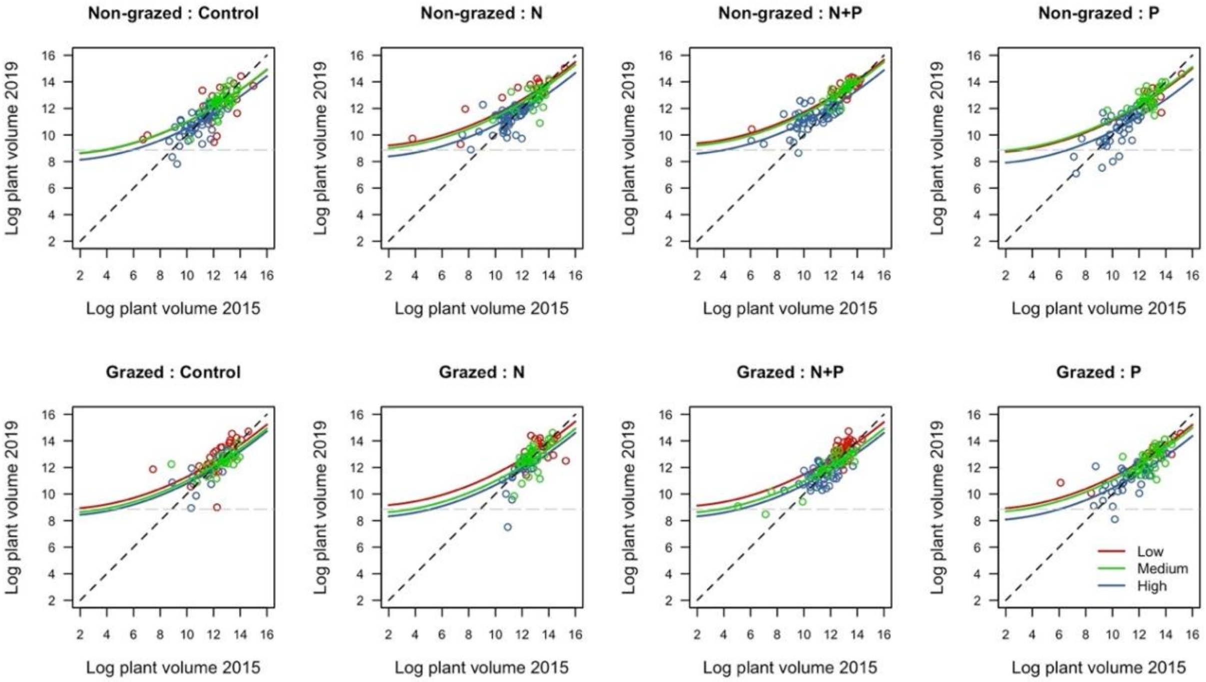

Figure 4. The relationship between *Croton* plant volume in 2015 and in 2019 in the inter-Andean valley of Catamayo, Loja Province, Ecuador at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high). Solid lines are trend lines for a quadratic fit showing plant growth

for elevation, grazing exclusion, and nutrient addition between initial (2015) and final plant volume (2019). Circles represent each plant surveyed. Plants (open circles) above dashed diagonal line indicate growth, and plants below indicate decrease. Grey horizontal dashed line is a reference (model intercept = 8.87) to distinguish growth among the three elevations, between grazing and exclusion, and between nutrient additions. The R^2 value of growth model was 0.70. Elevation and interaction between exclusion and nutrients, and elevations and nutrients were related to the growth of *Croton* plants.

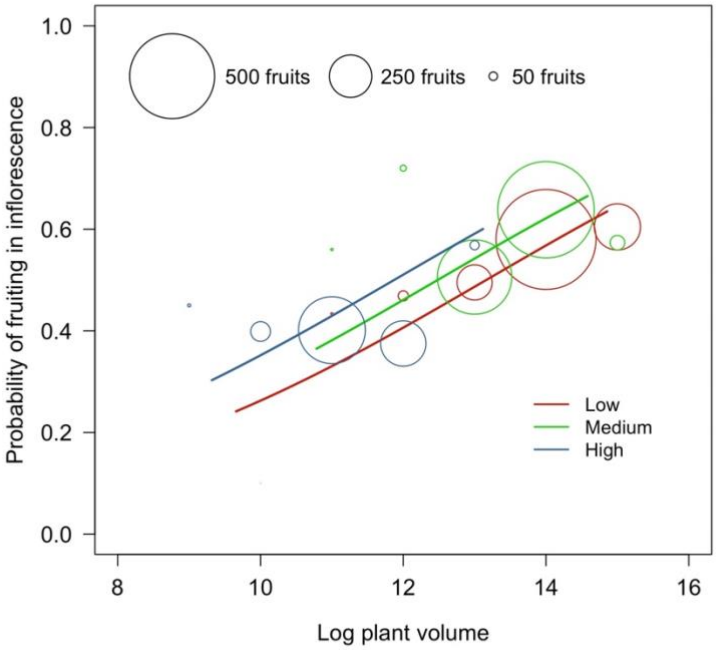

Figure 5. The relationship between plant volume and the probability of fruit yield per inflorescence of *Croton* by elevation in the inter-Andean valley of Catamayo, Loja Province, Ecuador at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high). Solid lines are trend lines for a binomial fit of probability of fruiting per inflorescence per elevation by plant volume. Circles are the number of fruits observed by inflorescences per elevation. The R^2 value of probability of the fruiting model was 0.22.

Fruiting probability of *Croton* increased with plant volume (Figure 5, Table S6) and was not influence by elevation (Figure S6). *Croton* fruit yield also increased with plant volume (Figure 6). We used the average of plant volume from 2019 (12.88) to standardise fruit yield. At low elevation, fruit yield was highest in plots with added N (8.79 ± SE 0.10 Figure 6). At medium elevation, fruit yield increased with added N+P (7.39 ± SE 0.14; Figure 6, Table S7), and at high elevation fruit yield was lower with N addition (4.63 ± SE 0.14) compared to plots without nutrients addition (control treatment; Figure 6; Figure S7).

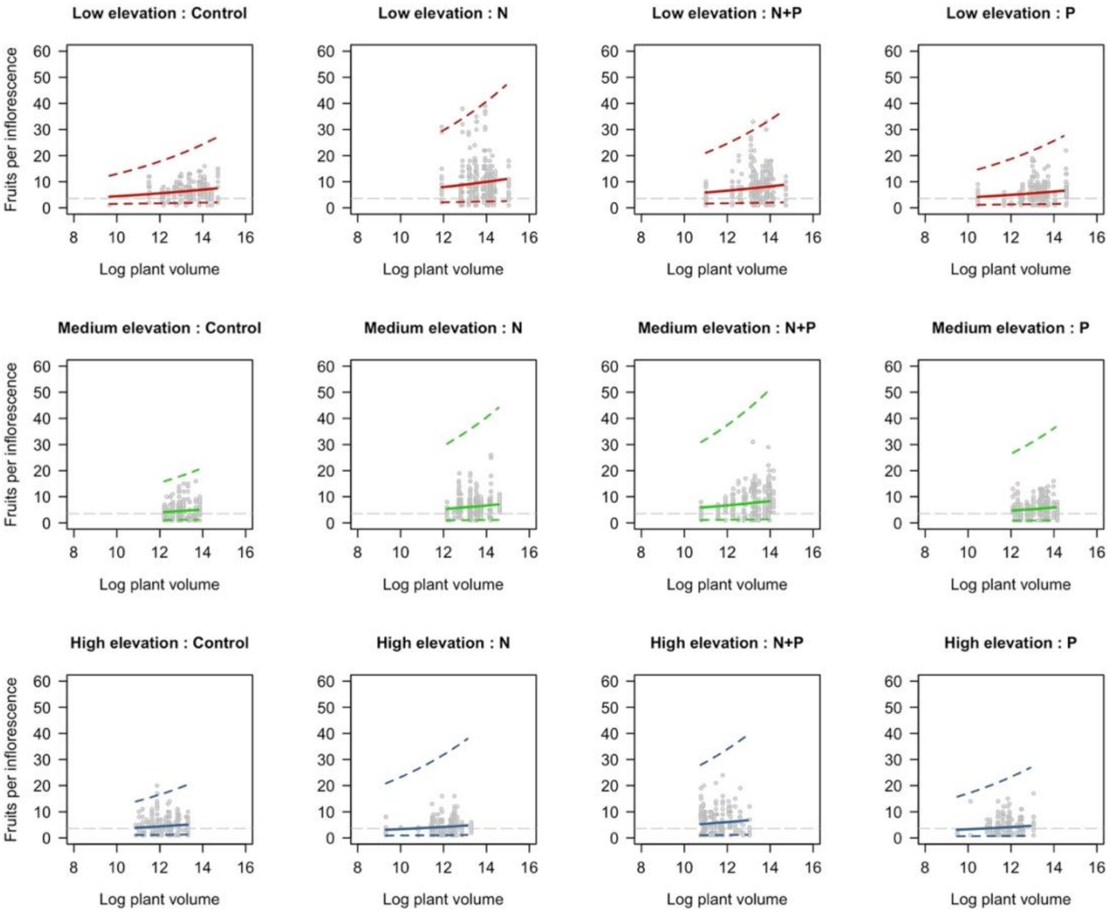

Figure 6. The relationship between plant volume and fruit yield per inflorescence as a function of elevation and nutrient addition in *Croton* sp. in the inter-Andean valley of Catamayo, Loja Province, Ecuador at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high).

Solid lines are trend lines for a negative binomial fit of fruit yield per inflorescence for elevation, grazing exclusion, and added nutrients according to plant volume. Grey circles represent annual maximum number of fruits per inflorescence. Dashed lines are 95% confidence intervals. Horizontal grey dashed line is a reference (model intercept = 3.57, with plant volume = 8) to facilitate distinguishing fruit yield (number of fruits) among elevations, and between nutrients. The R^2 value of fruit yield model was 0.30.

Effect of canopy on seedling survival

Croton seedling survival decreased with distance from the closest supposed maternal plant (Figure 7, Table S8). The effect of elevation on *Croton* seedling survival was inconclusive, but there was weak evidence of lower survival at low elevation (Figure 7; Figure S8). We did not have enough data to evaluate the effects of nutrient addition on seedling survival.

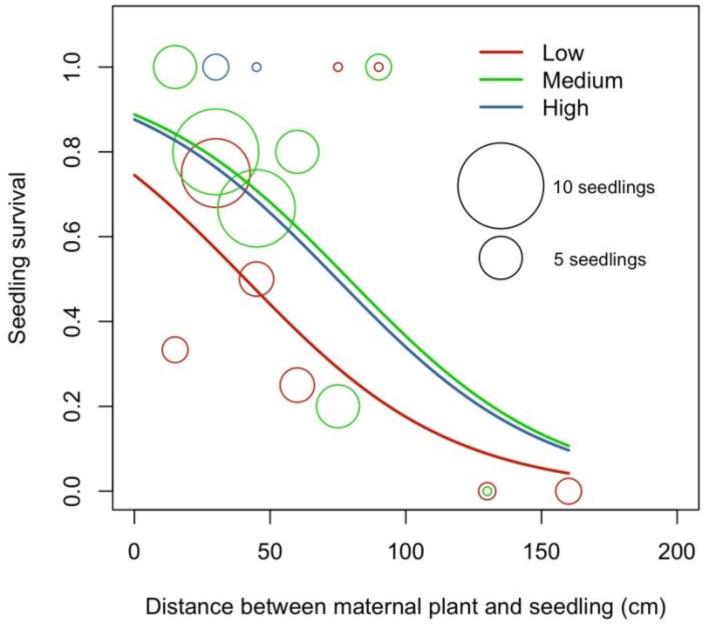

Figure 7. The relationship between the distance between seedlings and their maternal plants of *Croton* sp. in the inter-Andean valley of Catamayo, Loja Province, Ecuador at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high). Solid lines are trend lines for a binomial fit

of seedling survival probability for elevation by distance between maternal plant and seedling. Circles are the number of seedlings per elevation. The R^2 value of this model was 0.17.

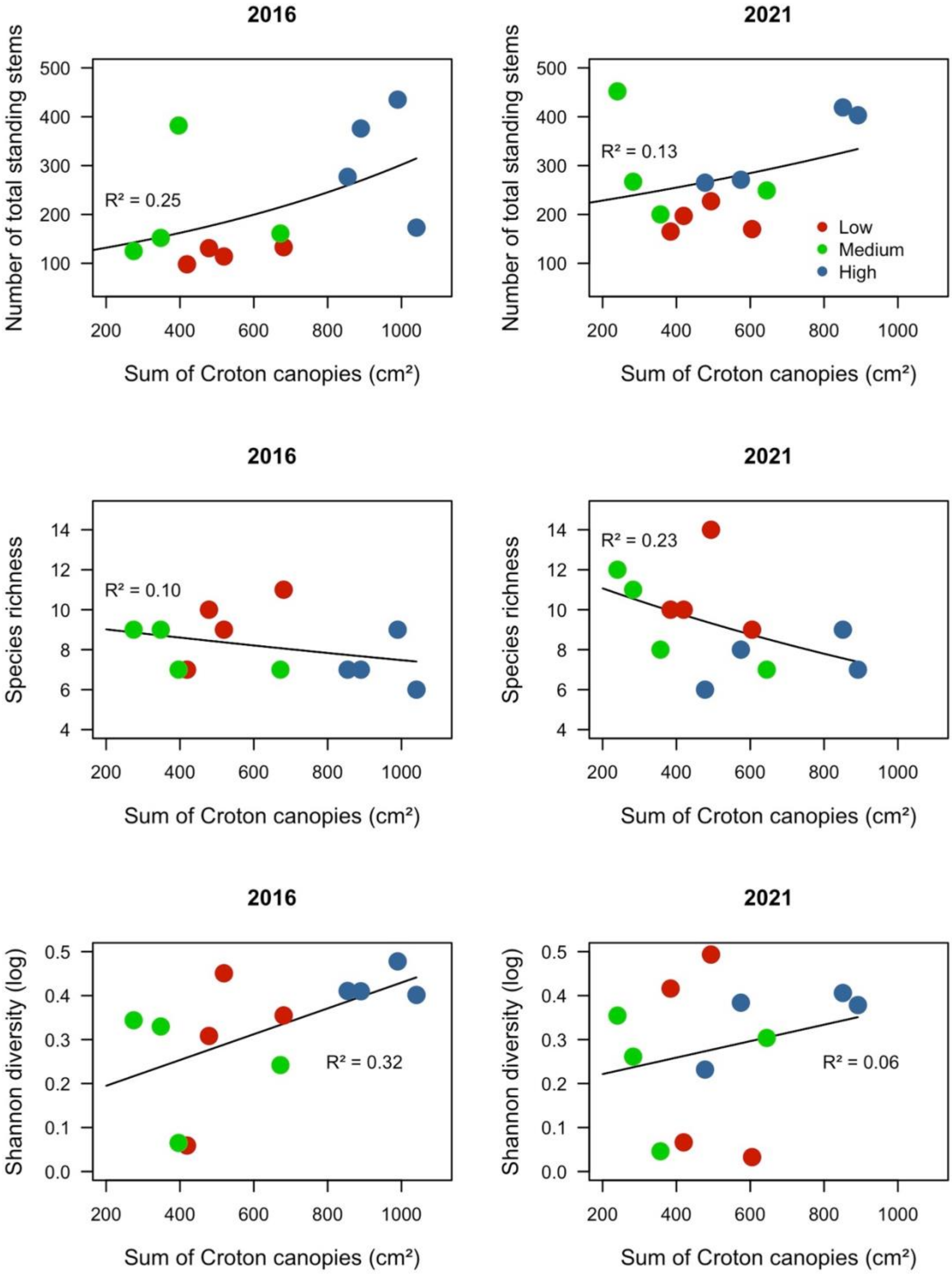

Figure 8. The number of total standing stems (abundance), species richness and Shannon diversity in 2016 and 2021 as a function of *Croton* canopy cover in the inter-Andean valley of Catamayo,

Loja Province, Ecuador at elevations of 1400 m a.s.l. (low), 1550 m (medium), and 1700 m (high). Solid lines are trend lines for a Poisson fit for both number of total standing stems of all species and for species richness and a linear fit for Shannon diversity. There was no clear evidence of association between richness and *Croton* canopy cover canopy in 2016 and 2021, nor for Shannon diversity in 2021.

Effect of Croton cover on abundance, richness, and diversity

There was a clear positive relationship between the sum of *Croton* canopies per subplot and total number of standing stems in both evaluation years, 2016 and 2021 (Figure 8, Table S9). In 2021, there was a higher number of total standing stems recorded. No clear relationship between the sum of *Croton* canopies and species richness was evident in either 2016 or 2021. However, in 2016 there was a clear positive relationship between the sum of *Croton* canopies and Shannon diversity (Figure 8). We documented a clear reduction in *Croton* cover in 2019 especially at high elevations (Figure S9).

Discussion

During the last decade we have observed an intensification of agricultural and livestock activities (cows and goats) in our study area (Leon-Velarde et al. 2000; Quintana et al. 2019). These activities have increased the nutrient load of soils and diverted water from the few local streams (Minga-León et al. 2018; Arteaga et al. 2020). Simultaneously, the frequency of extremely dry years and annual number of days without rain have also increased. Climate change, grazing and nutrient addition are a widespread threat for species persistence and sustainable community functioning in dry ecosystems (Ulrich et al. 2014; Smith et al. 2016; Maestre et al. 2021) and appear to be magnified in mountain areas such as the inter-Andean valleys (Coppus et al. 2003; Zehetner and Miller 2006; Murgueitio et al. 2011). Our experimental work showed how variation in climate (temperature and moisture) along a narrow elevation gradient, the availability of N and P, and grazing influenced overall species interactions and abundance, as well as survival, growth, and fruit yield of *Croton*, a key species of the inter-Andean dry shrubland.

There is evidence that native ungulates were more abundant and widespread in South America in the past. Thus, it is likely that the reintroduction of large ungulates after the arrival of European colonisers may have restored some ecological functions lost after the extinction of the American megafauna in the late Pleistocene (Lundgren et al. 2020). There is also indirect evidence that ungulates play an important ecological and evolutionary role in many communities (Janzen 1984, 1986), especially those containing grasses or short-cycle plants (Belsky et al. 1993; Archibald et al. 2019). We do not know how important the past presence of ungulates in the region we studied may have been. Given the seasonality of local weather and lack of continuous vegetation cover, we suspect that the local presence of ungulates was relatively scarce. However, a previous study comparing grazed and ungrazed sites in the same area found evidence of negative effects of grazing on plant cover and interactions (Espinosa et al. 2013).

Our study suggests that grazing is a critical stressor of *Croton* demography. Although consumption of *Croton* by livestock in the region is relatively low, the tendency of cows and goats to browse pods and young shoots of acacias in winter (Iñiguez 2004; Espinosa et al. 2013), in addition to consuming grass in the area in recent years, could cause trampling of shrubs and seedlings, affecting *Croton* persistence especially at high elevations. *Croton* survival may have benefited by grazing exclusion, since without fences and with smaller *Croton* plants at the high elevation (Vélez-Mora et al. 2021), it is easier for livestock to roam for food at this elevation. As in other dry ecosystems, reduction of livestock may improve *Croton* plant survival. In western New South Wales, Australia, grazing by sheep and rabbits (non-native herbivores), and kangaroos (native herbivores) increased mortality of four perennial grasses (Grice and Barchia 1992). For these species (*Aristida browniana, Enneapogon avenaceus, Monachather paradoxa and Panicum laevinode*), mortality rates were higher with grazing, regardless of whether this was caused by native or non-native herbivores. In semiarid woodlands in eastern Australia, recent grazing by livestock (sheep, goats and cattle), and rabbits similarly affected survival of young shrubs, mainly *Eremophila longifolia* (Travers et al. 2019). Since shrubs in dry ecosystems regenerate relatively slowly, grazing has the potential to alter such systems over the long term (Dawson and Ellis 1996). Therefore, a decrease in survival of *Croton* due to grazing may severely alter their population structure and consequently reduce vegetation cover that protects their congeners and other species of the dry inter-Andean shrub systems (Espinosa et al. 2013, 2019).

Our study also revealed that soil nutrient concentrations affect *Croton* survival at different elevations. At the low elevation, plants in ungrazed plots to which P was added had lower survival, compared to control plots. Addition of N+P at the medium elevation also reduced survival compared to controls. These reductions in survival at low and medium elevations in ungrazed plots but with nutrient addition can, at least partially, be attributed to increases in plant competition. At the low elevation, average interspecific density in ungrazed plots without added nutrients (control treatment) was 75 individuals in 2021 but rose to 94 individuals with P added. However, at the medium elevation, such changes in density were not evident in ungrazed plots to which both N and P were added. Background availability of N at low and medium elevations was lower compared to that at the high elevation while availability of P was higher at low elevations. Addition of N and P to ungrazed plots could have increased interspecific density (especially at the low elevation), generating a decrease in *Croton* survival. The continuous contribution of N through volatilisation and runoff of fertilisers from nearby crops (Moreno et al. 2018; Larios-González et al. 2021), cattle excrement (Scharpenseel et al. 1996), ash deposition from sugarcane burning (Granli and Bøckman 1995; Jaramillo 2011; Guamán et al. 2012), and the almost absence of organic matter and low water availability at the low elevation (Figure 1e–h, respectively) could threaten *Croton* survival at this elevation (Gallego-Zamorano et al. 2022). Other studies have also reported negative effects of interspecific

density on shrub survival after nutrient addition. For example, in the Cape Floristic Region, South Africa, NPK fertiliser increased weed density, decreasing the survival of shrubs (Holmes 2001) that are sensitive to high P levels (Stock and Allsopp 1992).

In terms of the effects on growth, addition of N +P without grazing improved *Croton* growth at all elevations, whereas the addition of P decreased growth at the higher elevation. This suggests that grazing exclusion and availability of both P and soil water (Schulte-Uebbing et al. 2021) can improve growth of *Croton* if the effects of nutrients do not conflict with a potential increase in interspecific density. Further study should better answer how nutrient addition (artificially or by natural deposition) can promote growth among species in this ecosystem (Xu et al. 2021). Fertilisation could support growth of other species, such as grasses that could be limiting *Croton* growth, particularly at high elevations. In recent years, we have observed an increase in density of grasses surrounding the *Croton* shrubs at high elevations (Figures S10, S11 and S12). At this elevation, there are low concentrations of P in the soil (Figure 1c). An increase in P availability in soil can favour growth of certain grass species (Wassen et al. 2005; Siddique et al. 2010; Daws et al. 2021). Adaptation to low P availability can be considered a stress-tolerant strategy that gives certain plant species, such as *Croton*, a competitive advantage over other species. Therefore, to promote *Croton* growth it is essential to consider a balanced supply of N and P according to the needs at each elevation together with controlled grazing.

Croton fruit yield improved at the low elevation with addition of N and at medium elevation by addition of N and P. Low elevation had lower concentrations of N (Figure 1a) and higher concentrations of P in the soil compared to concentrations at high and medium elevations (Figure 1c). Balancing the availability of soil N and P is important at these elevations to improve fruit yield and seed quality (see Kramer and Kozlowski 1979; Drenovsky and Richards 2005). We have observed at the low and medium elevations that fruit yield and seed size are relatively high without N and P application (Vélez-Mora et al. 2021). Perhaps N and P application would be necessary for improved production and seed quality and to ensure establishment and survival of *Croton* seedlings, or perhaps the main limitation is the availability of soil water (Figure 1e; Trindade et al. 2020). This question could be answered with experiments of balanced addition of N and P at each elevation, exclusion of long-term grazing, and water availability. In the Great Basin Desert in western North America, addition of N and water balanced their availability in relation to P, improving seed production and quality in the native shrub *Sarcobatus vermiculatus* (Drenovsky and Richards 2005). At Northeast Normal University, Jilin Province, China, *Chloris virgata* increased the reproductive production (mass of seeds produced) with a mediate level of N (2.5 g m^{-2}) (Wang et al. 2016). Our study stresses the importance of balancing levels of N and P for adequate yield of *Croton* fruits in sites co-limited by these two nutrients.

We confirmed that the survival of *Croton* seedlings decreases as they shift away from the canopy of their maternal or facilitator plant. *Croton* canopy is critical for seedling establishment and recruitment in extreme environments due to less evaporation from soil by shade promoting density-dependent community plant associations (Lara-Romero et al. 2017; Jonge et al. 2021). We further suspect that drivers, such as grazing, intensification of drought at low elevations and disproportionate enrichment of nutrients, can change plant composition (Siddique et al. 2010; Espinosa et al. 2019; Daws et al. 2021). These drivers can also jeopardise facilitation offered by *Croton* canopy and consequently reduce recruitment of both *Croton* and other species. Long-term experiments are necessary to understand the interactive effects (climate change, nutrients, and grazing) on recruitment regimes and seedling establishment in this and other dry tropical environments (see Bhadouria et al. 2016, 2017, 2020).

Conclusions

Croton cover is essential to promote abundance and plant diversity in the inter-Andean dry shrubland. The presence of *Croton* in this dry shrubland not only guarantees permanence of *Croton* species, but also of the plant community. The positive interaction between *Croton* and their facilitated species should be considered in restoration and conservation plans to ensure the functioning and services that this ecosystem provides, especially at high elevations. Four years from starting our study, we observed a general reduction in *Croton* cover at high elevations (Figure S11). We suspect that the main causes of this reduction were extensive grazing (cows and goats), addition of nutrients (N and P) and increased abundance of herbaceous plants. Trampling and excrement from livestock alter the physical and chemical properties of soil, moisture, and nitrogen content (Pulido et al. 2018; Zhan et al. 2020), affecting the composition and diversity of plant communities (Koerner et al. 2018; Lyseng et al. 2018). In addition, an increase of N through atmospheric deposition, which further favours the growth of nitrophilous grasses, causes competitive exclusion of key ecosystem species such as *Croton* (Bobbink et al. 1998; Figure S12).

The above changes, along with climate change, can degrade the structural attributes of the inter-Andean shrubland preventing these communities from providing critical environmental services, such as the provision of water and control of erosion. We are convinced that wise landscape planning and the search for alternative productive options to grazing goats and cows, and small-scale commercial crops, should be a priority for the local society. Further research aimed at identifying the limits to ecological and social resilience and resistance of communities in these ecosystems will help in the development of more environmentally compatible human use of resources in these regions.

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References

Aguirre Mendoza Z, Aguirre Mendoza N, Muñoz Ch J. 2017. Biodiversidad de la provincia de Loja, Ecuador. Arnaldoa. 24(2):523–542.

Archibald S, Hempson GP, Lehmann C. 2019. A unified framework for plant life-history strategies shaped by fire and herbivory. New Phytol. 224(4):1490–1503. doi:10.1111/nph.15986.

Arteaga J, Ochoa P, Fries A, Boll J. 2020. Identification of priority areas for integrated management of semiarid watersheds in the ecuadorian andes. JAWRA J Am Water Resour Assoc. 56(2):270–282. doi:10.1111/1752-1688.12837.

Augustine DJ, McNaughton SJ. 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. J Wildl Manage. 62(4):1165. doi:10.2307/3801981.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw. 67(1):1–48.

Bejarano-Castillo M, Campo J, Roa-Fuentes LL. 2015. Effects of increased nitrogen availability on C and N cycles in tropical forests: a meta-analysis. PLoS ONE. 10(12):e0144253. doi:10.1371/journal.pone.0144253.

Belsky AJ, Carson WP, Jensen CL, Fox GA. 1993. Overcompensation by plants: herbivore optimization or red herring? Null. 7(1):109–121. doi:10.1007/bf01237737.

Berdugo M, Maestre FT, Kéfi S, Gross N, Bagousse-Pinguet YL, Soliveres S. 2019. Aridity preferences alter the relative importance of abiotic and biotic drivers on plant species abundance in global drylands. J Ecol. 107(1):190–202. doi:10.1111/13652745.13006.

Bhadouria R, Singh R, Srivastava P, Raghubanshi AS. 2016. Understanding the ecology of tree-seedling growth in dry tropical environment: a management perspective. Energy, Ecol Environ. 1(5):296–309. doi:10.1007/s40974-016-0038-3.

Bhadouria R, Srivastava P, Singh R, Tripathi S, Singh H, Raghubanshi AS. 2017. Tree seedling establishment in dry tropics: an urgent need of interaction studies. Environ Syst Decis. 37(1):88–100. doi:10.1007/s10669-017-9625-x.

Bhadouria R, Srivastava P, Singh R, Tripathi S, Verma P, Raghubanshi AS. 2020. Effects of grass competition on tree seedlings growth under different light and nutrient availability conditions in tropical dry forests in India. Ecol Res. 35(5):807–818. doi:10.1111/1440-1703.12131.

Bobbink R, Hornung M, Roelofs JGM. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. J Ecol. 86 (5):717-738. doi:10.1046/j.1365-2745.1998.8650717.x.

Carnicer J, Sardans J, Stefanescu C, Ubach A, Bartrons M, Asensio D, Peñuelas J. 2015. Global biodiversity, stoichiometry and ecosystem function responses to humaninduced C–N–P imbalances. J Plant Physiol. 172:82–91. doi:10.1016/j.jplph.2014.07.022.

Cavieres LA, Badano EI. 2010. Consequences of facilitation on species diversity in terrestrial plant communities. In: Pugnaire FI, editor. Positive plant interactions and community dynamics. Boca Raton, FL, US: CRC Press; pp. 39–57.

Chamba-Morales MD, Lapo-Paredes LE, Vásquez ER. 2019. La Agricultura familiar campesina en el cantón Catamayo, provincia de Loja. Cedamaz. 9(2):66–74.

Cisneros CMG, Heringer G, Domen YSM, Sánchez LR, Meira-Neto JAA. 2021. The environmental filtering and the conservation of tropical dry forests in mountains in a global change scenario. Biodivers Conserv. 30 (10):2689–2705. doi:10.1007/s10531-021-02215-6.

Coppus R, Imeson AC, Sevink J. 2003. Identification, distribution and characteristics of erosion sensitive areas in three different Central Andean ecosystems. Catena. 51(3– 4):315–328. doi:10.1016/s0341-8162(02)00169-8.

Craine JM, Elmore AJ, Wang L, Aranibar J, Bauters M, Boeckx P, Crowley BE, Dawes MA, Delzon S, Fajardo A, Fang Y, Fujiyoshi L, Gray A, Guerrieri R, Gundale MJ, Hawke DJ, Hietz P, Jonard M, Kearsley E, Kenzo T, and Makarov M et al,. 2018. Isotopic evidence for oligotrophication of terrestrial ecosystems. Nat Ecol Evol. 2(11):1735–1744. doi:10.1038/s41559-018-0694-0.

Daryanto S, Eldridge DJ, Throop HL. 2013. Managing semi-arid woodlands for carbon storage: grazing and shrub effects on above- and belowground carbon. Agric Ecosyst Environ. 169:1–11. doi:10.1016/j.agee.2013.02.001.

Davies J, Poulsen L, Schulte-Herbrüggen B, Mackinnon K, Crawhall N, Henwood WD, Dudley N, Smith J, Gudka M. 2012. Conserving Dryland biodiversity. Nairobi, Kenya: Global Drylands Initiative, IUCN.

Dawson TJ, Ellis BA. 1996. Diets of mammalian herbivores in Australian arid, hilly shrublands: seasonal effects on overlap between euros (hill kangaroos), sheep and feral goats, and on dietary niche breadths and electivities. J Arid Environ. 34(4):491–506. doi:10.1006/jare.1996.0127.

Daws MI, Walters SJ, Harris RJ, Tibbett M, Grigg AH, Morald TK, Hobbs RJ, Standish RJ. 2021. Nutrient enrichment diminishes plant diversity and density, and alters long-term ecological trajectories, in a biodiverse forest restoration. Ecol Eng. 165:106222. doi:10.1016/j.ecoleng.2021.106222.

Delgado-Baquerizo M, Maestre FT, Gallardo A, Bowker MA, Wallenstein MD, Quero JL, Ochoa V, Gozalo B, García-Gómez M, Soliveres S, García-Palacios P, Berdugo M, Valencia E, Escolar C, Arredondo T, Barraza-Zepeda C, Bran D, Carreira JA, Chaieb M, Zaady E. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature. 502(7473):672–676. doi:10.1038/nature12670.

Ding J, Eldridge DJ. 2021. Community-level responses to increasing dryness vary with plant growth form across an extensive aridity gradient. J Biogeogr. 48(7):1788–1796. doi:10.1111/jbi.14114.

Drenovsky RE, Richards JH. 2005. Nitrogen addition increases fecundity in the desert shrub *Sarcobatus vermiculatus*. Oecologia. 143(3):349–356. doi:10.1007/s00442-004-1821-y.

Eldridge DJ, Poore AGB, Ruiz-Colmenero M, Letnic M, Soliveres S. 2016. Ecosystem structure, function, and composition in rangelands are negatively affected by livestock grazing. Ecol Appl. 26(4):1273–1283. doi:10.1890/15-1234.

Eldridge DJ, Soliveres S, Bowker MA, Val J. 2013. Grazing dampens the positive effects of shrub encroachment on ecosystem functions in a semi-arid woodland. J Appl Ecol. 50(4):1028–1038. doi:10.1111/1365-2664.12105.

Elser JJ. 2011. A World awash with nitrogen. Science. 334(6062):1504–1505. doi:10.1126/science.1215567.

Erfanzadeh R, Yazdani M, Arani AM. 2021. Effect of different shrub species on their sub-canopy soil and vegetation properties in semiarid regions. Land Degrad Dev. 32 (11):3236–3247. doi:10.1002/ldr.3977.

Espinosa CI, Luzuriaga AL, de la Cruz M, Escudero A. 2013. Climate and grazing control nurse effects in an Ecuadorian dry shrubby community. J Trop Ecology. 30(1):23–32. doi:10.1017/s0266467413000692.

Espinosa CI, Vélez-Mora DP, Ramón P, Gusmán-Montalván E, Duncan DH, Quintana-

Ascencio PF. 2019. Intraspecific interactions affect the spatial pattern of a dominant shrub in a semiarid shrubland: a prospective approach. Popul Ecol. 61(2):217–226. doi:10.1002/1438-390x.1018.

Fries A, Silva K, Pucha-Cofrep F, Oñate-Valdivieso F, Ochoa-Cueva P. 2020. Water balance and soil moisture deficit of different vegetation units under semiarid conditions in the andes of southern Ecuador. Climate. 8(2):30. doi:10.3390/cli8020030.

Gallego-Zamorano J, Huijbregts MAJ, Schipper AM. 2022. Changes in plant species richness due to land use and nitrogen deposition across the globe. Null. 28(4):745–755. doi:10.1111/ddi.13476.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, et al. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry. 70(2):153–226. doi:10.1007/s10533-004-0370-0.

García-Oliva F, Jaramillo VJ. 2011. Impact of anthropogenic transformation of seasonally dry tropical forests on ecosystem biogeochemical processes. In: Dirzo R, Young HS, Mooney HA, Ceballos G, eds. Seasonally dry tropical forests. Washington, DC: Island Press. pp. 159–172.

Granli T, Bøckman OC. 1995. Nitrous oxide (N2O) emissions from soils in warm climates. Fert Res. 42(1):159–163.

Grice AC, Barchia I. 1992. Does grazing reduce survival of indigenous perennial grasses of the semi-arid woodlands of western new south wales? Aust J Ecol. 17(2):195–205. doi:10.1111/j.1442-9993.1992.tb00798.x.

Guamán R, Pardo C, Paz C. 2012. *La contaminación ambiental y su impacto en la salud de los moradores del barrio la algarrobera del cantón Catamayo* [Undergraduate's thesis]. Universidad Nacional de Loja]. Dspace. https://dspace.unl.edu.ec/jspui/handle/123456789/20028

Herzog SK, Martínez R, Jørgensen PM, Tiessen H. 2011. Climate change and biodiversity in the tropical Andes. Paris: InterAmerican Institute for Global Change Research (IAI) and Scientific Committee on Problems of the Environment (SCOPE).

He N, Yu Q, Wu L, Wang Y, Han X. 2008. Carbon and nitrogen store and storage potential as affected by land-use in a *Leymus chinensis* grassland of northern China. Soil Biol Biochem. 40(12):2952–2959. doi:10.1016/j.soilbio.2008.08.018.

Holmes PM. 2001. Shrubland restoration following woody alien invasion and mining: effects of topsoil depth, seed source, and fertilizer addition. Restor Ecol. 9(1):71–84. doi:10.1046/j.1526-100x.2001.009001071.x.

Hou E, Chen C, Luo Y, Zhou G, Kuang Y, Zhang Y, Heenan M, Lu X, Wen D. 2018. Effects of climate on soil phosphorus cycle and availability in natural terrestrial ecosystems. Glob Chang Biol. 24(8):3344–3356. doi:10.1111/gcb.14093.

Howard KSC, Eldridge DJ, Soliveres S. 2012. Positive effects of shrubs on plant species diversity do not change along a gradient in grazing pressure in an arid shrubland. Basic Appl Ecol. 13(2):159–168. doi:10.1016/j.baae.2012.02.008.

Iñiguez L. 2004. Goats in resource-poor systems in the dry environments of West Asia, Central Asia and the Inter-Andean valleys. Small Ruminant Res. 51(2):137–144. doi:10.1016/j.smallrumres.2003.08.014.

Janzen DH. 1984. Dispersal of small seeds by big herbivores: foliage is the fruit. Am Nat. 123(3):338–353. doi:10.1086/ 284208.

Janzen DH. 1986. Chihuahuan desert nopaleras: defaunated big mammal vegetation. Annu Rev Ecol Syst. 17(1):595–636. doi:10.1146/annurev.es.17.110186.003115.

Jaramillo P (2011). *Insuficiencia normativa para regular conductas atentatorias contra el medio ambiente* [Undergraduate's thesis, Universidad Nacional de Loja]. Dspace. https://dspace.unl.edu.ec/jspui/handle/123456789/20001

Jaramillo VJ, Murray-Tortarolo GN. 2019. Chapter 7 Tropical dry forest soils: global change and local-scale consequences for soil biogeochemical processes. Dev Soil Sci. 36:109–130. doi:10.1016/b978-0-444-63998-1.00007-0.

Jonge MMJ, Benítez-López A, Hennekens S, Santini L, Huijbregts MAJ, Schipper AM, Gill J. 2021. Conditional love? Co-occurrence patterns of drought-sensitive species in European grasslands are consistent with the stress-gradient hypothesis. Global Ecol Biogeogr. 30(8):1609–1620. doi:10.1111/geb.13323.

Koerner SE, Smith MD, Burkepile DE, Hanan NP, Avolio ML, Collins SL . . . Zelikova TJ, Lemoine NP, Forrestel EJ, Eby S, Thompson DI. 2018. Change in dominance determines herbivore effects on plant biodiversity. Nat Ecol Evol. 2(12):1925–1932. doi:10.1038/s41559-018-0696-y.

Kramer PJ, Kozlowski TT. 1979. Physiology of woody plants. New York: Academic Press.

Kutt AS, Vanderduys EP, Perry JJ, Perkins GC, Kemp JE, Bateman BL, Kanowski J, Jensen R. 2012. Signals of change in tropical savanna woodland vertebrate fauna 5 years after cessation of livestock grazing. Wildl Res. 39(5):386–396. doi:10.1071/wr11137.

Lara-Romero C, Gusmán-M E, Ramón P, Vélez-Mora D, Espinosa CI. 2017. Does size matter? Ontogenetic responses of an Andean shrub to conspecific density-dependence. Perspect Plant Ecol Evol Syst. 25:59–67. doi:10.1016/j.ppees.2017.02.002.

Larios-González RC, Centeno LG, Ríos MJ, Del Espinoza CSA, Salazar JRC. 2021. Nitrogen losses due to volatilization from two nitrogenous sources and two application methods. Siembra. 8(2):e2475. doi:10.29166/ siembra.v8i2.2475.

Leon-Velarde C, Quiroz R, Zorogastúa P, Tapia M (2000). Sustainability concerns of livestock-based livelihoods in the Andes. *In Contribution of livestock to mountain livelihoods. Proceedings of the International Symposium on livestock in mountain/highland production systems: research and development challenges into the next millennium, Pokhara, Nepal*, 7-10 December, 1999 (pp. 183–202). International Centre for Integrated Mountain Development.

León-Yánez S, Valencia R, Pitman N, Endara L, Ulloa Ulloa C, Navarrete H. 2011. Libro Rojo de las Plantas Endémicas del Ecuador. Quito: Herbario QCA.

Linares-Palomino R, Oliveira-Filho AT, Pennington RT. 2011. Neotropical seasonally dry forests: diversity, endemism, and biogeography of woody plants. In: Bullock S, Mooney H, Medina E, eds. Seasonally dry tropical forests. Washington, DC: Island Press. pp. 3–21.

Li Y, Zhou X, Brandle JR, Zhang T, Chen Y, Han J. 2012. Temporal progress in improving carbon and nitrogen storage by grazing exclosure practice in a degraded land area of China's Horqin Sandy Grassland. Agric Ecosyst Environ. 159:55–61. doi:10.1016/j.agee.2012.06.024.

Lundgren EJ, Ramp D, Rowan J, Middleton O, Schowanek SD, Sanisidro O, Carroll SP, Davis M, Sandom CJ, Svenning J-C, et al. 2020. Introduced herbivores restore Late Pleistocene ecological functions. Proc Natl Acad Sci. 117 (14):7871–7878. doi:10.1073/pnas.1915769117.

Lyseng MP, Bork EW, Hewins DB, Alexander MJ, Carlyle CN, Chang SX, Willms WD. 2018. Long-Term grazing impacts on vegetation diversity, composition, and exotic species presence across an aridity gradient in northern temperate grasslands. Plant Ecol. 219(6):649–663. doi:10.1007/s11258-018-0824-4.

Maestre FT, Benito BM, Berdugo M, Concostrina-Zubiri L, Delgado-Baquerizo M, Eldridge DJ, Guirado E, Gross N, Kéfi S, Bagousse-Pinguet YL, et al. 2021. Biogeography of global drylands. New Phytol. 231(2):540–558. doi:10. 1111/nph.17395.

Maestre FT, Callaway RM, Valladares F, Lortie CJ. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. J Ecol. 97(2):199–205. doi:10.1111/j.1365-2745.2008.01476.x.

Maestre FT, Eldridge DJ, Soliveres S, Kéfi S, Delgado-Baquerizo M, Bowker MA, García-Palacios P, Gaitán J, Gallardo A, Lázaro R, et al. 2016. Structure and functioning of dryland ecosystems in a changing world. Annu Rev Ecol Evol Syst. 47(1):1–23. doi:10.1146/annurev-ecolsys-121415-032311.

Maestre FT, Valladares F, Reynolds JF. 2005. Is the change of plant–plant interactions with abiotic stress predictable? A meta-analysis of field results in arid environments. J Ecol. 93(4):748–757. doi:10.1111/j.1365-2745.2005. 01017.x.

Mazía N, Moyano J, Perez L, Aguiar S, Garibaldi LA, Schlichter T. 2016. The sign and magnitude of tree–grass interaction along a global environmental gradient. Global Ecol Biogeogr. 25(12):1510–1519. doi:10.1111/ geb.12518.

McIntire EJB, Fajardo A. 2014. Facilitation as a ubiquitous driver of biodiversity. New Phytol. 201(2):403–416. doi:10.1111/nph.12478.

Mekuria W, Aynekulu E. 2013. Exclosure land management for restoration of the soils in degraded communal grazing lands in northern Ethiopia. Land Degrad Dev. 24 (6):528–538. doi:10.1002/ldr.1146.

Mihoč MAK, Giménez-Benavides L, Pescador DS, Sánchez AM, Cavieres LA, Escudero A. 2016. Soil under nurse plants is always better than outside: a survey on soil amelioration by a complete guild of nurse plants across a long environmental gradient. Plant Soil. 408(1–2):31–41. doi:10.1007/s11104-016-2908-z.

Minga-León S, Gómez-Albores MA, Bâ KM, Balcázar L, Manzano-Solís LR, Cuervo-Robayo AP, Mastachi-Loza CA. 2018. Estimation of water yield in the hydrographic basins of southern Ecuador. Hydrol Earth Syst Sci Discuss. 2018:1–18. doi:10.5194/hess-2018-529.

Moreno J, Yerovi F, Herrera M, Yánez D, Espinosa J. 2018. Soils from the Highlands. In: Espinosa J, Moreno J, Bernal G, eds. The Soils of Ecuador. World Soils Book Series: Springer; pp. 79–111.

Murgueitio E, Calle Z, Uribe F, Calle A, Solorio B. 2011. Native trees and shrubs for the productive rehabilitation of tropical cattle ranching lands. For Ecol Manage. 261 (10):1654–1663. doi:10.1016/j.foreco.2010.09.027.

Murphy PG, Lugo AE. 1986. Ecology of tropical dry forest. Annu Rev Ecol Syst. 17(1):67–88. doi:10.1146/annurev.es. 17.110186.000435.

Ochoa-Cueva PA, Arteaga J, Arévalo AP, Kolok AS. 2022. A potential pesticides exposure index (PPEI) for developing countries: applied in a transboundary basin. Integr Environ Assess Manag. 18(1):187–197. doi:10.1002/ieam.4470.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O’Hara RB, Simpson GL, Solymos P, et al. 2020. Vegan. Community Ecol Package. 2:5–7. https://CRAN.R-project.org/package= vegan.

Pennington RT, Ratter JA. 2006. Neotropical savannas and seasonally dry forests: plant diversity, biogeography, and conservation. Abingdon: Taylor & Francis.

Peñuelas J, Poulter B, Sardans J, Ciais P, van der Velde M, Bopp L, Boucher O, Godderis Y, Hinsinger P, Llusia J, et al. 2013. Human-Induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nat Commun. 4(1):2934. doi:10.1038/ ncomms3934.

Peters T, Braeuning A, Muenchow J, Richter M. 2014. An ecological paradox: high species diversity and low position of the upper forest line in the Andean Depression. Ecol Evol. 4(11):2134–2145. doi:10.1002/ece3.1078.

Portillo-Quintero CA, Sánchez-Azofeifa GA. 2010. Extent and conservation of tropical dry forests in the Americas. Biol Conserv. 143(1):144–155. doi:10.1016/j.biocon.2009. 09.020.

Pulido M, Schnabel S, Lavado Contador JF, Lozano-Parra J, González F. 2018. The impact of heavy grazing on soil quality and pasture production in rangelands of SW Spain. Land Degrad Dev. 29(2):219–230. doi:10.1002/ ldr.2501.

Quintana C, Girardello M, Balslev H. 2019. Balancing plant conservation and agricultural production in the ecuadorian dry Inter-Andean Valleys. PeerJ. 7:e6207. doi:10. 7717/peerj.6207.

Quintana C, Girardello M, Barfod AS, Balslev H. 2016. Diversity patterns, environmental drivers and changes in vegetation composition in dry inter-Andean valleys. J Plant Ecol. 10(3):461–475. doi:10.1093/jpe/rtw036.

Quintana C, Pennington RT, Ulloa CU, Balslev H. 2017. Biogeographic Barriers in the Andes: Is the Amotape— Huancabamba zone a dispersal barrier for dry forest plants? 1. Ann Mo Bot Gard. 102(3):542–550. doi:10. 3417/d-17-00003a.

Raghubanshi AS. 1991. Dynamics of soil biomass C, N, and P in a dry tropical forest in India. Biol Fertil Soils. 12(1):55–59.

R Core Team. 2021. R: a language and environment for statistical computing. URL. Vienna, Austria: R Foundation for Statistical Computing. https://www. R-project.org/

Rey PJ, Alcántara JM, Manzaneda AJ, Sánchez-Lafuente AM. 2016. Facilitation contributes to Mediterranean woody plant diversity but does not shape the diversity– productivity relationship along aridity gradients. New Phytol. 211(2):464–476. doi:10.1111/nph.13916.

Richter M, Diertl K-H, Emck P, Peters T, Beck E. 2009. Reasons for an outstanding plant diversity in the tropical Andes of Southern Ecuador. Landscape Online. 12(1):1–35. doi:10.3097/lo.200912.

Richter M, Moreira-Munoz A. 2005. Heterogeneidad climática y diversidad de la vegetación en el sur de Ecuador: un método de fitoindicación. Revista Peruana de Biología. 12(2):217–238. doi:10.15381/rpb.v12i2.2395.

Rollenbeck R, Otte I, Fabian P, Wilcke W, Pucha D Bräuning A, Bendix J. 2013. Current and future variations of nutrient depositions and influences on tree growth. In: Bendix J, Beck E, Bräuning A, Makeschin F, Mosandl R, Scheu S, Wilcke W, eds. Ecosystem Services, Biodiversity and Environmental Change in a Tropical Mountain Ecosystem of South Ecuador. Berlin, Heidelberg: Springer. pp. 287–296.

Rundel PW, Boonpragob K. 1995. Dry forest ecosystems of Thailand. In: Bullock SH, Mooney HA, Medina E eds. Seasonally Dry Tropical Forests. Cambridge University Press, Cambridge. pp. 93–123.

Scanlon TM, Caylor KK, Levin SA, Rodriguez-Iturbe I. 2007. Positive feedbacks promote power-law clustering of Kalahari vegetation. Nature. 449(7159):209–212. doi:10.1038/nature06060.

Scharpenseel HW, Pfeiffer EM, Wiechmann H. 1996. Land use related nitrogen and global change. In: Ahmad, N, ed. Nitrogen Economy in Tropical Soils. Dordrecht: Springer. pp. 389–398.

Schulte-Uebbing LF, Ros GH, Vries WD. 2021. Experimental evidence shows minor contribution of nitrogen deposition to global forest carbon sequestration. Glob Chang Biol. doi:10.1111/gcb.15960.

Siddique I, Vieira ICG, Schmidt S, Lamb D, Carvalho CJR, Figueiredo RDO, Blomberg S, Davidson EA. 2010. Nitrogen and phosphorus additions negatively affect tree species diversity in tropical forest regrowth trajectories. Ecology. 91(7):2121–2131. doi:10.1890/09-0636.1.

Silva JO, Souza-Silva H, Rodrigues PMS, Cuevas-Reyes P, Espírito-Santo MM. 2021. Soil resource availability, plant defense, and herbivory along a successional gradient in a tropical dry forest. Plant Ecol. 222(5):625–637. doi:10.1007/s11258-021-01131-7.

Singh JS, Singh S, Raghubanshi AS, Singh S, Kashyap AK, Reddy VS. 1997. Effect of soil nitrogen, carbon and moisture on methane uptake by dry tropical forest soils. Plant Soil. 196(1):115–121.

Siyum ZG. 2020. Tropical dry forest dynamics in the context of climate change: syntheses of drivers, gaps, and management perspectives. Ecol Processes. 9(1):25. doi:10. 1186/s13717-020-00229-6.

Smith P, House JI, Bustamante M, Sobocká J, Harper R, Pan G, West PC, Clark JM, Adhya T, Rumpel C, Paustian K, Kuikman P, Cotrufo MF, Elliott JA, McDowell R, Griffiths RI, Asakawa S, Bondeau A, Jain AK, Pugh TAM. 2016. Global change pressures on soils from land use and management. Glob Chang Biol. 22(3):1008–1028. doi:10.1111/gcb.13068.

Soil Survey Staff. 2006. Natural Resources Conservation Service, Agriculture Department, eds. Keys to soil taxonomy. 10th edn ed. Washington: Government Printing Office.

Solano M, Ramón P, Gusmán-M E, Burneo JI, Quichimbo P, Jiménez L. 2018. Efecto del gradiente altitudinal sobre las reservas decarbono y nitrógeno del suelo en un matorral seco en Ecuador. Ecosistemas. 27(3):116–122.

Soliveres S, DeSoto L, Maestre FT, Olano JM. 2010. Spatio-Temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. J PPEES Sources. 12(3):227–234. doi:10. 1016/j.ppees.2010.02.003.

Soliveres S, Maestre FT. 2014. Plant–plant interactions, environmental gradients and plant diversity: a global synthesis of community-level studies. Perspect Plant Ecol Evol Syst. 16(4):154–163. doi:10.1016/j.ppees.2014. 04.001.

Stan K, Sanchez-Azofeifa A. 2019. Tropical dry forest diversity, climatic response, and resilience in a changing climate. Forests. 10(5):443. doi:10.3390/f10050443.

Stock WD, Allsopp N. 1992. Functional perspective of ecosystems. In: Cowling RM, ed. The ecology of fynbos. Nutrients, fire and diversity. Cape Town: Oxford University Press; pp. 241–259.

Sundqvist MK, Sanders NJ, Wardle DA. 2013. Community and ecosystem responses to elevational gradients: processes, mechanisms, and insights for global change. Annu Rev Ecol Evol Syst. 44(1):261–280. doi:10.1146/ annurev-ecolsys-110512-135750.

Tapia-Armijos MF, Homeier J, Espinosa CI, Leuschner C, de la Cruz M. 2015. Deforestation and forest fragmentation in South Ecuador since the 1970s – losing a hotspot of biodiversity. PLoS ONE. 10(9):e0133701–18. doi:10.1371/ journal.pone.0133701.

Tapia-Armijos MF, Homeier J, Munt DD. 2017. Spatio-Temporal analysis of the human footprint in South Ecuador: influence of human pressure on ecosystems and effectiveness of protected areas. Appl Geogr. 78(C):22–32. doi:10.1016/j.apgeog.2016.10.007.

Tian H, Xu R, Canadell JG, Thompson RL, Winiwarter W, Suntharalingam P, Davidson EA, Ciais P, Jackson RB, Janssens-Maenhout G, Prather MJ, Regnier P, Pan N, Pan S, Peters GP, Shi H, Tubiello FN, Zaehle S, Zhou F, Yao Y. 2020. A comprehensive quantification of global nitrous oxide sources and sinks. Nature. 586(7828):248–256. doi:10.1038/s41586-020-2780-0.

Travers SK, Eldridge DJ, Val J, Oliver I. 2019. Rabbits and livestock grazing alter the structure and composition of mid-storey plants in a wooded dryland. Agric Ecosyst Environ. 277:53–60. doi:10.1016/j.agee.2019.03.003.

Trindade DPF, Sfair JC, Paula AS, Barros MF, Tabarelli M. 2020. Water availability mediates functional shifts across ontogenetic stages in a regenerating seasonally dry tropical forest. J Veg Sci. 31(6):1088–1099. doi:10.1111/jvs. 12896.

Ulloa C, Jørgensen P. 1995. Árboles y arbustos de los Andes del Ecuador. 2nd Edn, vol. 30. Aarhus: Aarhus Universitetsforlag.

Ulrich W, Soliveres S, Maestre FT, Gotelli NJ, Quero JL, Delgado-Baquerizo M, Bowker MA, Eldridge DJ, Ochoa V, Gozalo B, Valencia E, Berdugo M, Escolar C, García-Gómez M, Escudero A, Prina A, Alfonso G, Arredondo T, Bran D, Zaady E. 2014. Climate and soil attributes determine plant species turnover in global drylands. J Biogeogr. 41(12):2307–2319. doi:10.1111/jbi. 12377.

Van Ee BW, Riina R, Berry PE. 2011. A revised infrageneric classification and molecular phylogeny of New World *Croton* (Euphorbiaceae). Taxon. 60(3):791–823. doi:10. 1002/tax.603013.

Vélez-Mora D, Ramón P, Vallejo C, Romero A, Duncan D, Quintana-Ascencio PF. 2020. Environmental drivers of femaleness of an inter-Andean monoecious shrub. Biotropica. 53(1):17–27. doi:10.1111/btp.12839.

Vélez-Mora DP, Trigueros-Alatorre K, Quintana-Ascencio PF. 2021. Evidence of morphological divergence and reproductive isolation in a narrow elevation gradient. Evol Biol. 1–14. doi:10.1007/s11692-021-09541-1.

Wang L, D'Odorico P, Evans JP, Eldridge DJ, McCabe MF, Caylor KK, King EG. 2012. Dryland ecohydrology and climate change: critical issues and technical advances. Hydrol Earth Syst Sci. 16(8):2585–2603. doi:10.5194/ hess-16-2585-2012.

Wang Y, Li L, Zhou D, Weiner J. 2016. The allometry of reproductive allocation in a *Chloris virgata* population in response to simulated atmospheric nitrogen deposition. Basic Appl Ecol. 17(5):388–395. doi:10.1016/j.baae.2016. 01.004.

Waring BG, Pérez-Aviles D, Murray JG, Powers JS. 2019. Plant community responses to stand-level nutrient fertilization in a secondary tropical dry forest. Ecology. 100 (6):e02691. doi:10.1002/ecy.2691.

Wassen MJ, Schrader J, van Dijk J, Eppinga MB. 2021. Phosphorus fertilization is eradicating the niche of northern Eurasia's threatened plant species. Nat Ecol Evol. 5 (1):67–73. doi:10.1038/s41559-020-01323-w.

Wassen MJ, Venterink HO, Lapshina ED, Tanneberger F. 2005. Endangered plants persist under phosphorus limitation. Nature. 437(7058):547–550. doi:10.1038/ nature03950.

Westerband A, Doviak M, Quay-Velzquez GL, Medeiros JS. 2015. Aspect reduces soil moisture and tree cover, but not nitrogen mineralization or grass cover, in semiarid pinyon-juniper woodlands of the Southwestern United States. Southwest Nat. 60(1):21–29. doi:10.1894/fmo-18.1.

Winckell A, Zebrowski C, Sourdat M. 1997. Los paisajes naturales del Ecuador. In: Centro Ecuatoriano de Investigación Geográfica, Instituto Panamericano de Geografía e Historia (Sección Ecuador), Instituto Francés de Investigación Científica para el Desarrollo en Cooperación, eds. Geografía Básica del Ecuador. Quito: Instituto Geográfico Militar; pp. 417.

Wood JR. 2006. Inter-Andean dry valleys of Bolivia–Floristic affinities and patterns of endemism: insights from acanthaceae, asclepiadaceae and labiatae. In: Pennington RT, Lewis GP, Ratter JA, eds. Neotropical savannas and seasonally dry forests. Abingdon: Taylor & Francis. pp. 243–256.

Wu B, Zeng H, Lü N, Wang Y, Fu B, Xu Z. 2021. Essential dryland ecosystem variables. Curr Opin Environ Sustainability. 48:68–76. doi:10.1016/j.cosust.2020.10. 007.

Xu M, Zhu Y, Zhang S, Feng Y, Zhang W, Han X. 2021. Global scaling the leaf nitrogen and phosphorus resorption of woody species: revisiting some commonly held views. Sci Total Environ. 788:147807. doi:10.1016/j.scitotenv.2021.147807.

Yao J, Liu H, Huang J, Gao Z, Wang G, Li D, Yu H, Chen X. 2020. Accelerated dryland expansion regulates future variability in dryland gross primary production. Nat Commun. 11(1):1665. doi:10.1038/s41467-020- 15515-2.

Yuan ZY, Chen HYH. 2015. Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. Nat Clim Chang. 5(5):465–469. doi:10.1038/ nclimate2549.

Zehetner F, Miller WP. 2006. Soil variations along a climatic gradient in an Andean agro-ecosystem. Geoderma. 137(1–2):126–134. doi:10.1016/j.geoderma.2006.07.005.

Zhan T, Zhang Z, Sun J, Liu M, Zhang X, Peng F, Tsunekawa A, Zhou H, Gou X, Fu S. 2020. Meta-Analysis demonstrating that moderate grazing can improve the soil quality across China’s grassland ecosystems. Appl Soil Ecol. 147:103438. doi:10.1016/j.apsoil.2019.103438.

Supplemental Material

Changes in climate, grazing pressure and nutrients threaten integrity and functioning of Andean shrublands
Diego P. Vélez Mora, Elizabeth Gusmán, Carlos Iván Espinosa, Pedro F. Quintana-Ascencio.

Table S1A. Elements for the calculation of Nitrogen doses.

Nutrient	Symbol	Units	Source
Nitrogen	N%	kg ha^{-1}	Equation 1
Nitrogen	N_{eff}	kg ha^{-1}	Equation 2, using reference N = 70%: High concentration according to laboratory parameters.
Nitrogen available	N_a	kg ha^{-1}	Equation 3, Availability of N at 50%
Apparent density of soil	ad	g/cm^3	0.1
Urea	U	kg ha^{-1}	Equation 4, urea with 45% nitrogen

Equation 1

$$N\ (kg\ ha^{-1}) = \frac{N\%\ \times 100 \times (ad\ \times 10000 \times 0.1)}{1000}$$

Equation 2

$$N_{eff}(kg\ ha^{-1}) = \frac{0.7\ \times 100 \times (ad \times 10000 \times 0.1)}{1000}$$

Equation 3

$$N_a(kg\ ha^{-1}) = \frac{N_{eff}(kg\ ha^{-1}) - N(kg\ ha^{-1})}{0.5}$$

Equation 4

$$U\ (kg\ ha^{-1}) = \frac{N_a(kg\ ha^{-1})}{0.45}$$

Equation 5

$$U\ (g/m^2) = \left(\frac{U\ (kg\ ha^{-1})}{10000}\right) \times 1000$$

Equation 6

$$U\ (g/subplot) = U(g/m^2)\ \times\ 12.25\ m^2$$

Table S1B. Procedure for calculating the doses of N using urea, from the soil analysis of each plot and elevation. Exclusion (Excl): Non-grazed = NG, Grazed = G.

Elevation	Plot	Excl	Soil textural	N%	N (kg ha^{-1})	N (kg ha^{-1})	N (kg ha^{-1})	Urea (kg ha^{-1})	Urea (g/m^2)	g/subplot (g/12.25 m^2)
		Equations	Eq. 1	Eq. 2		Eq. 3	Eq. 4	Eq. 5	Eq. 6	
High	P1	NG	loam	0.18	23.40	91	135.20	300.44	30.04	368.04
High	P2	G	clay	0.23	29.90	91	122.20	271.56	27.16	332.66
High	P3	G	loam	0.21	27.30	91	127.40	283.11	28.31	346.81
High	P4	NG	clay	0.22	28.60	91	124.80	277.33	27.73	339.73
Medium	P5	G	clay loam	0.17	22.10	91	137.80	306.22	30.62	375.12
Medium	P6	NG	loam	0.15	19.50	91	143.00	317.78	31.78	389.28
Medium	P7	G	clay loam	0.15	19.50	91	143.00	317.78	31.78	389.28
Medium	P8	NG	loam	0.17	22.10	91	137.80	306.22	30.62	375.12
Low	P9	G	clay loam	0.16	20.80	91	140.40	312.00	31.20	382.20
Low	P10	NG	loam	0.14	18.20	91	145.60	323.56	32.36	396.36
Low	P11	G	clay loam	0.14	18.20	91	145.60	323.56	32.36	396.36
Low	P12	NG	clay loam	0.15	19.50	91	143.00	317.78	31.78	389.28

Final column shows the doses of urea applied to each subplot.

Table S2A. Elements for the calculation of Phosphorous doses.

Nutrient	Symbol	Units	Source
P ppm	P	ppm	Equation 1
Phosphorus	P_{eff}	kg ha^{-1}	Equation 2, using reference P = 21%: High concentration according to laboratory parameters.
Phosphorus available	P_a	kg ha^{-1}	Equation 3, Availability of N at 25%
Apparent density of soil	ad	g/cm^3	0.1
Triple superphosphate	TSP	kg ha^{-1}	Equation 4, triple superphosphate with 44% P_2O_5

Equation 1

$$P_2O_5\ (kg\ ha^{-1}) = \frac{2.293\ \times P\ ppm \times (ad\ \times 10000 \times 0.1)}{1000}$$

Equation 2

$$P_2O_{5_{eff}}(kg\ ha^{-1}) = \frac{2.293\ \times 21 \times (ad \times 10000 \times 0.1)}{1000}$$

Equation 3

$$P_2O_{5_a}(kg\ ha^{-1}) = \frac{P_2O_{5_{eff}}(kg\ ha^{-1}) - P_2O_5(kg\ ha^{-1})}{0.25}$$

Equation 4

$$STP\ (kg\ ha^{-1}) = \frac{P_2O_{5_a}(kg\ ha^{-1})}{0.44}$$

Equation 5

$$STP\ (g/m^2) = \left(\frac{STP\ (kg\ ha^{-1})}{10000}\right) \times 1000$$

Equation 6

$$STP\ (g/subplot) = STP\ (g/m^2)\ \times\ 12.25\ m^2$$

Table S2B. Procedure for calculating the doses of P using triple superphosphate, from the soil analysis of each plot and elevation. Exclusion (Excl): Non-grazed = NG, Grazed = G.

Elevation	Plot	Excl	Soil textural	P ppm	P_2O_5 (kg ha^{-1})	P_2O_5 (kg ha^{-1})	P_2O_5 (kg ha^{-1})	STP (kg ha^{-1})	STP (g/m^2)	g/subpt (g/12.25 m^2)
			Equations		Eq. 1	Eq. 2	Eq. 3	Eq. 4	Eq. 5	Eq. 6
High	P1	NG	loam	6.8	20.27	62.60	169.32	384.81	38.48	471.39
High	P2	G	clay	6.1	18.18	62.60	177.66	403.78	40.38	494.63
High	P3	G	loam	4.9	14.61	62.60	191.97	436.30	43.63	534.46
High	P4	NG	clay	5.2	15.50	62.60	188.39	428.17	42.82	524.50
Medium	P5	G	clay loam	6.7	19.97	62.60	170.51	387.52	38.75	474.71
Medium	P6	NG	loam	5.4	16.10	62.60	186.01	422.75	42.27	517.86
Medium	P7	G	clay loam	5.1	15.20	62.60	189.59	430.88	43.09	527.82
Medium	P8	NG	loam	6.1	18.18	62.60	177.66	403.78	40.38	494.63
Low	P9	G	clay loam	6.7	19.97	62.60	170.51	387.52	38.75	474.71
Low	P10	NG	loam	7	20.87	62.60	166.93	379.39	37.94	464.75
Low	P11	G	clay loam	6.9	20.57	62.60	168.12	382.10	38.21	468.07
Low	P12	NG	clay loam	7.1	21.16	62.60	165.74	376.68	37.67	461.43

Final column shows the doses of urea applied to each subplot.

Table S3. Sample sizes by treatment combinations

Elevation	Nutrient treatment	Exclusion	Plant survival	Plant growth	Fruit yield per inflorescence	Seedling survival
		Sample size	Number of plants evaluated	Number of plants evaluated	*Number of evaluated inflorescences	Number of seedlings evaluated
Low	Control	Grazed	n = 34	n = 30	n = 149	n = 73
Low	Control	Non-grazed	n = 22	n = 19	n = 112	n = 25
Low	N	Grazed	n = 19	n = 19	n = 168	n = 77
Low	N	Non-grazed	n = 21	n = 17	n = 154	n = 37
Low	N+P	Grazed	n = 40	n = 38	n = 152	n = 49
Low	N+P	Non-grazed	n = 25	n = 22	n = 170	n = 12
Low	P	Grazed	n = 35	n = 20	n = 120	n = 79
Low	P	Non-grazed	n = 14	n = 11	n = 131	n = 10
Medium	Control	Grazed	n = 26	n = 24	n = 113	n = 23
Medium	Control	Non-grazed	n = 34	n = 34	n = 123	n = 36
Medium	N	Grazed	n = 37	n = 34	n = 134	n = 26
Medium	N	Non-grazed	n = 24	n = 20	n = 176	n = 54
Medium	N+P	Grazed	n = 31	n = 26	n = 176	n = 14
Medium	N+P	Non-grazed	n = 27	n = 23	n = 126	n = 34
Medium	P	Grazed	n = 38	n = 34	n = 168	n = 8
Medium	P	Non-grazed	n = 31	n = 29	n = 126	n = 15
High	Control	Grazed	n = 51	n = 21	n = 103	n = 5
High	Control	Non-grazed	n = 71	n = 61	n = 113	n = 2
High	N	Grazed	n = 43	n = 23	n = 106	n = 7
High	N	Non-grazed	n = 71	n = 66	n = 98	n = 25
High	N+P	Grazed	n = 68	n = 44	n = 118	n = 3
High	N+P	Non-grazed	n = 67	n = 53	n = 105	n = 6
High	P	Grazed	n = 60	n = 45	n = 107	n = 2
High	P	Non-grazed	n = 65	n = 48	n = 99	n = 1

*We also use this information to evaluate probability of fruiting.

Table S4. Summary of the generalized linear model:
Survival ~ Log plan volume 2015 + Treatment + Exclusion + Elevation + Treatment:Exclusion + Treatment:Elevation + Exclusion:Elevation

Source of variation	**Estimate**	**Std. Error**	**z value**	**p**
Low Elevation (Intercept) Control (Intercept) Grazed (Intercept)	-3.87	0.80	-4.85	**<0.001**
Log plant volume 2015	0.49	0.06	8.42	**<0.001**
N	0.41	0.85	0.49	0.627
N+P	0.57	0.71	0.80	0.425
P	-0.78	0.61	-1.28	0.202
Non-grazed	0.73	0.59	1.23	0.217
Medium Elevation	0.79	0.88	0.89	0.373
High Elevation	-2.19	0.53	-4.11	**0.000**
N : Non-grazed	-0.59	0.59	-1.00	0.317
N+P : Non-grazed	-1.48	0.53	-2.78	**0.005**
P : Non-grazed	-1.69	0.53	-3.18	**0.001**
N : Medium Elevation	-1.55	1.16	-1.34	0.181
N+P : Medium Elevation	-1.38	1.08	-1.28	0.200
P : Medium Elevation	0.44	1.04	0.42	0.673
N : High Elevation	0.37	0.86	0.43	0.666
N+P : High Elevation	0.70	0.75	0.93	0.355
P : High Elevation	2.25	0.66	3.38	**0.001**
Non-grazed : Medium Elevation	0.28	0.68	0.41	0.683
Non-grazed : High Elevation	1.91	0.53	3.59	**0.000**

R^2 = 0.21.

Table S5. Summary of linear model: Log plant volume 2019 ~ Log plant volume 2015 + Log plant volume^2 2015 + Treatment + Exclusion + Elevation + Treatment:Exclusion + Treatment:Elevation + Exclusion:Elevation.

Source of variation	**Estimate**	**Std. Error**	**t value**	**p**
Low Elevation (Intercept) Control (Intercept) Grazed (Intercept)	8.87	0.86	10.32	**<0.001**
Log plant volume 2015	-0.02	0.16	-0.10	0.919
Log plant volume^2 2015	0.03	0.01	3.56	**0.000**
N	0.23	0.18	1.30	0.196
N+P	0.18	0.15	1.22	0.225
P	-0.01	0.18	-0.07	0.942
Non-grazed	-0.33	0.15	-2.22	**0.027**
Medium Elevation	-0.30	0.16	-1.88	0.060
High Elevation	-0.50	0.16	-3.17	**0.002**
N : Non-grazed	0.37	0.16	2.24	**0.025**
N+P : Non-grazed	0.57	0.15	3.70	**0.000**
P : Non-grazed	0.12	0.16	0.80	0.426
N : Medium Elevation	-0.23	0.21	-1.10	0.272
N+P : Medium Elevation	-0.19	0.20	-0.97	0.333
P : Medium Elevation	0.07	0.21	0.34	0.732
N : High Elevation	-0.34	0.20	-1.69	0.091
N+P : High Elevation	-0.30	0.18	-1.63	0.104
P : High Elevation	-0.34	0.21	-1.64	0.102
Non-grazed : Medium Elevation	0.33	0.15	2.19	**0.029**
Non-grazed : High Elevation	0.02	0.14	0.16	0.870

$R^2 = 0.70$.

Table S6. Summary of generalized linear mixed-effects model with the binomial distribution: Fruiting Probability ~ Log plant volume + Elevation + (1|Plant)

Fixed effects:	Estimate	Std. Error	z value	p
(Intercept)	-4.08	1.20	-3.40	**0.001**
Log plant volume	0.33	0.09	3.63	**0.000**
High Elevation	-0.22	0.22	-1.03	0.302
Medium Elevation	0.20	0.18	1.10	0.273

$R^2 = 0.22$.

Table S7. Summary of generalized linear mixed-effects model with negative binomial distribution: Maximum number of fruits ~ Log plant volume + Elevation * Treatment + (1|Plant)

Fixed effects:	**Estimate**	**Std. Error**	**z value**	**p**
Low Elevation (Intercept) Control (Intercept) Grazed (Intercept)	0.38	0.37	1.02	0.308
Log plant volume	0.11	0.03	4.03	**0.000**
Medium Elevation	-0.31	0.10	-3.08	**0.002**
High Elevation	-0.24	0.10	-2.32	**0.020**
N	0.36	0.10	3.76	**0.000**
N+P	0.17	0.10	1.73	0.084
P	-0.11	0.10	-1.11	0.266
Medium Elevation: N	-0.09	0.14	-0.66	0.509
High Elevation : N	-0.40	0.14	-2.81	**0.005**
Medium Elevation : N+P	0.33	0.14	2.34	**0.019**
High Elevation : N+P	0.16	0.14	1.13	0.260
Medium Elevation : P	0.25	0.14	1.77	0.076
High Elevation : P	0.05	0.14	0.36	0.716

$R^2 = 0.30$.

Table S8. Summary of the generalized linear model: Seedling survival ~ Distance to maternal plant + Elevation

Source of variation	**Estimate**	**Std. Error**	**z value**	**p**
Low Elevation (Intercept)	1.07	0.60	1.78	0.076
Distance between maternal plant and seedling	-0.03	0.01	-2.54	**0.011**
High Elevation	0.88	1.22	0.73	0.467
Medium Elevation	1.00	0.57	1.74	0.082

$R^2 = 0.17$.

Table S9. Linear models and generalized linear models to evaluate influence of the sum of *Croton* canopies on abundance, richness, and plant diversity

Source of variation	**Estimate**	**Std. Error**	**z value**	**p**
Abundance 2016				
Number of total standing stems 2016 (Intercept)	4.675	0.058	80.98	**<0.001**
Sum of Croton canopies 2015 (cm^2)	0.001	0.000	13.29	**<0.001**
Abundance 2021				
Number of total standing stems 2021 (Intercept)	5.322	0.050	106.87	**<0.001**
Sum of Croton canopies 2019 (cm^2)	0.001	0.000	6.36	**<0.001**
Richness 2016				
Species richness 2016 (Intercept)	2.246	0.269	8.35	**<0.001**
Sum of Croton canopies 2015 (cm^2)	0.000	0.000	-0.58	0.563
Richness 2021				
Species richness 2021 (Intercept)	2.521	0.265	9.51	**<0.001**
Sum of Croton canopies 2019 (cm^2)	-0.001	0.000	-1.17	0.241
Diversity 2016				
Shannon diversity 2016 (Intercept)	1.143	0.118	9.64	**<0.001**
Sum of Croton canopies 2015 (cm^2)	0.000	0.000	2.25	**0.048**
Diversity 2021				
Shannon diversity 2021 (Intercept)	1.213	0.166	7.30	**<0.001**
Sum of Croton canopies 2019 (cm^2)	0.000	0.000	0.81	0.436

Supplemental Figures

Figure S1. Presence of cattle among the vegetation of the High Elevation in winter.

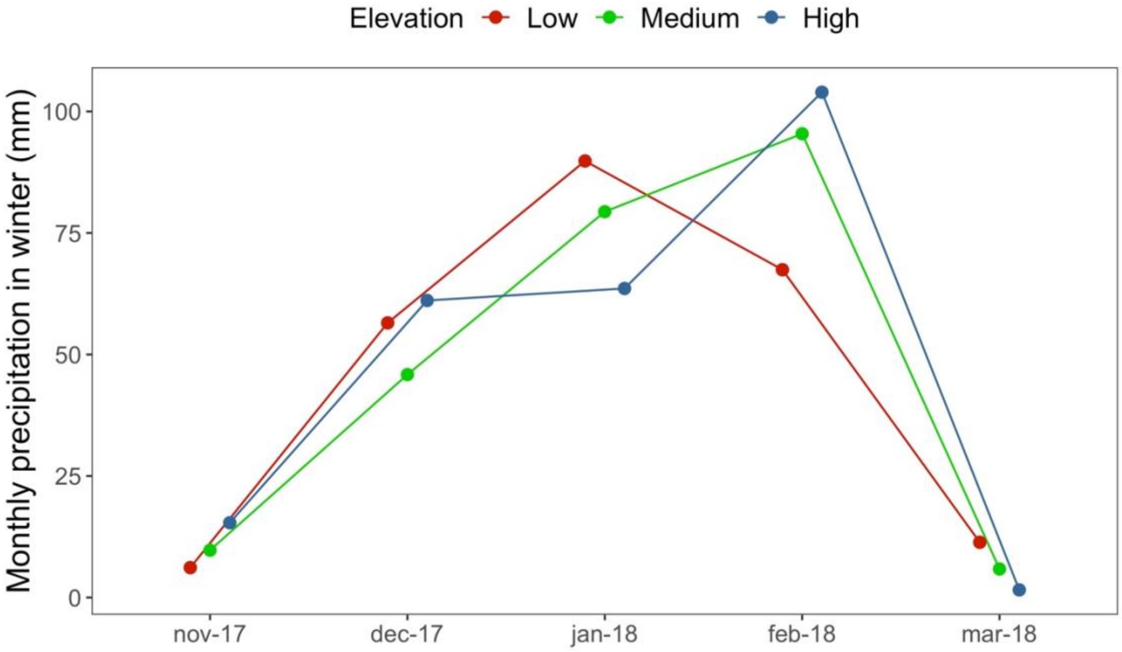

Figure S2. Monthly rainfall (mm) of the winter period 2017 - 2018 of study site.

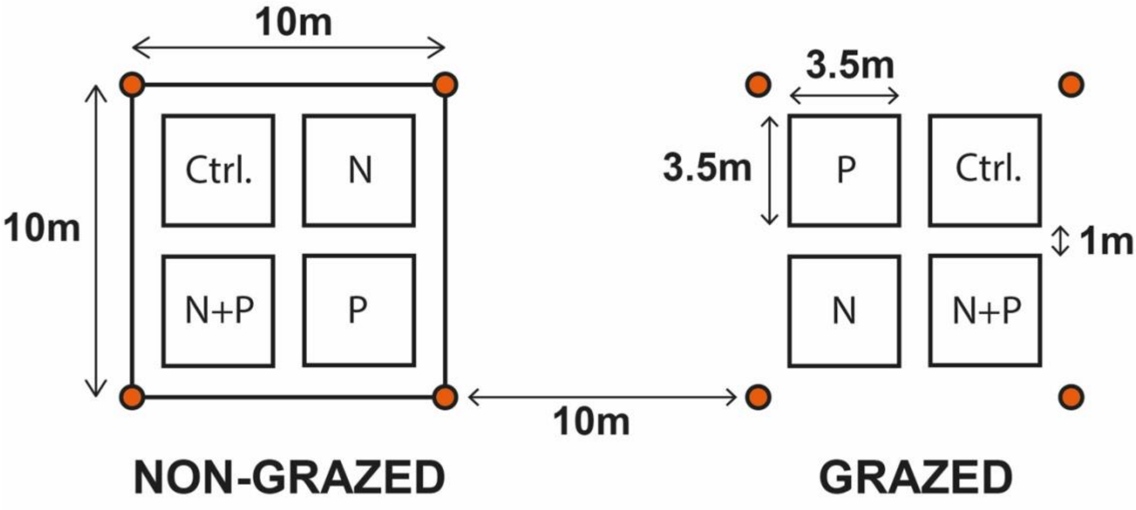

Figure S3. Design of plots excluded and not excluded from grazing.

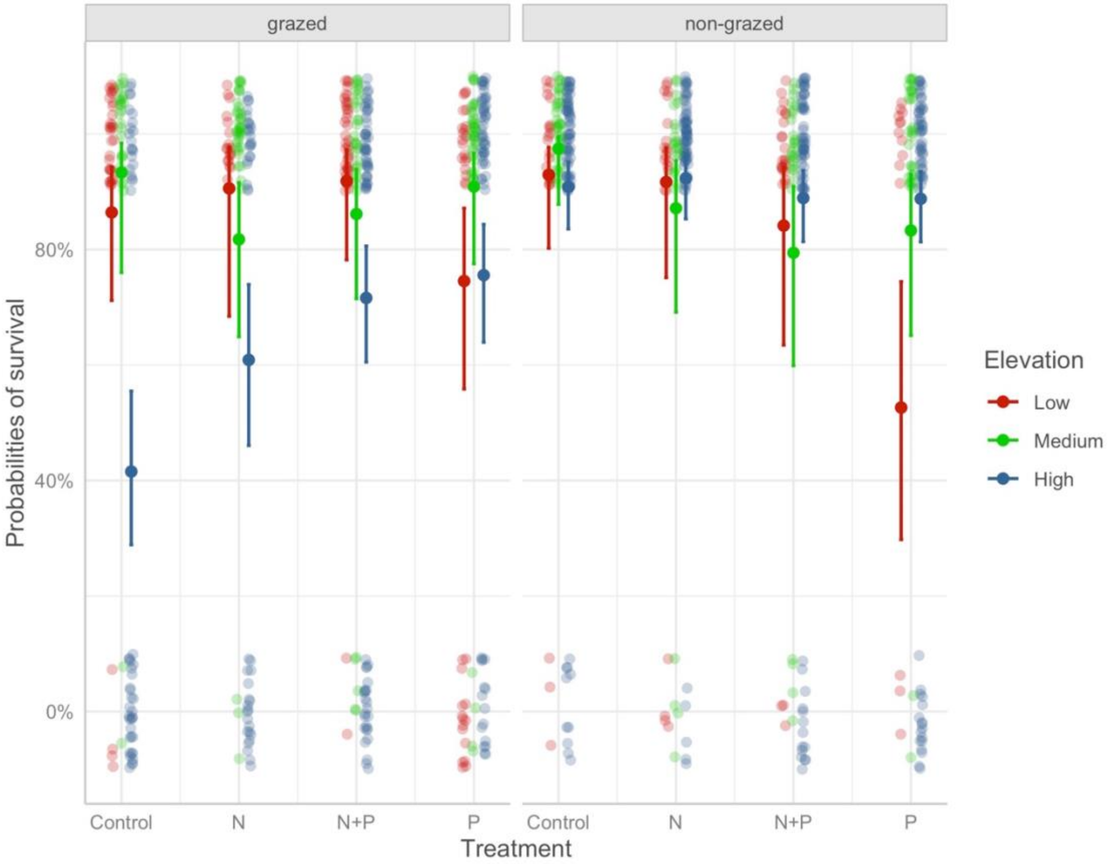

Figure S4. Probabilities of survival by nutrients by grazing exclusion and elevation. Data were derived from generalized linear models with binomial distribution that incorporate statistical interactions between elevation, grazing exclusion, adding of N and P. Error bars correspond to 95% confidence intervals. Original observations of survival (1 = 100%) and mortality (0 = 0%) are shown with points top and bottom, respectively. All estimates were adjusted for average of logarithm of plant volume 2015 (11.66).

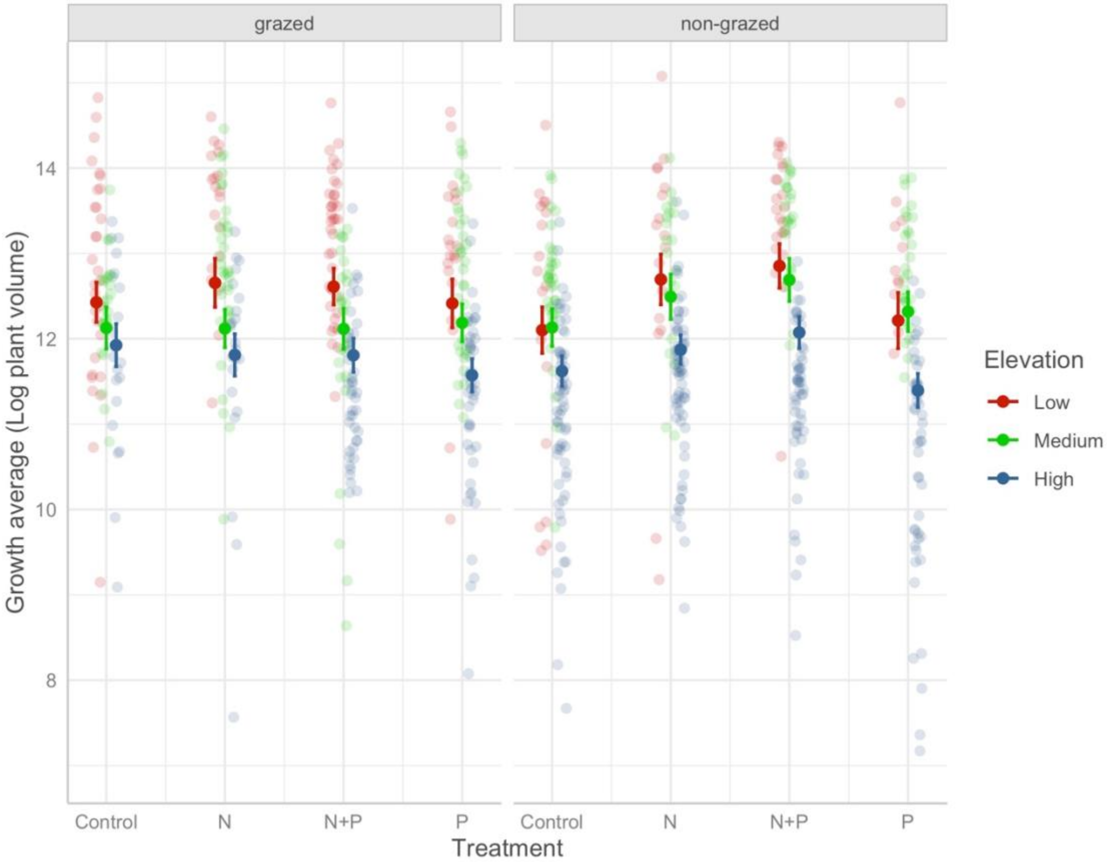

Figure S5. Growth average by nutrients by grazing exclusion and elevation. Data were derived from linear models that incorporate statistical interactions between elevation, grazing exclusion, adding of N and P. Error bars correspond to 95% confidence intervals. Original observations are shown with dots around error bars. All estimates were adjusted for average of logarithm of plant volume (11.95) and average of logarithm of plant volume 2015 squared (145.06).

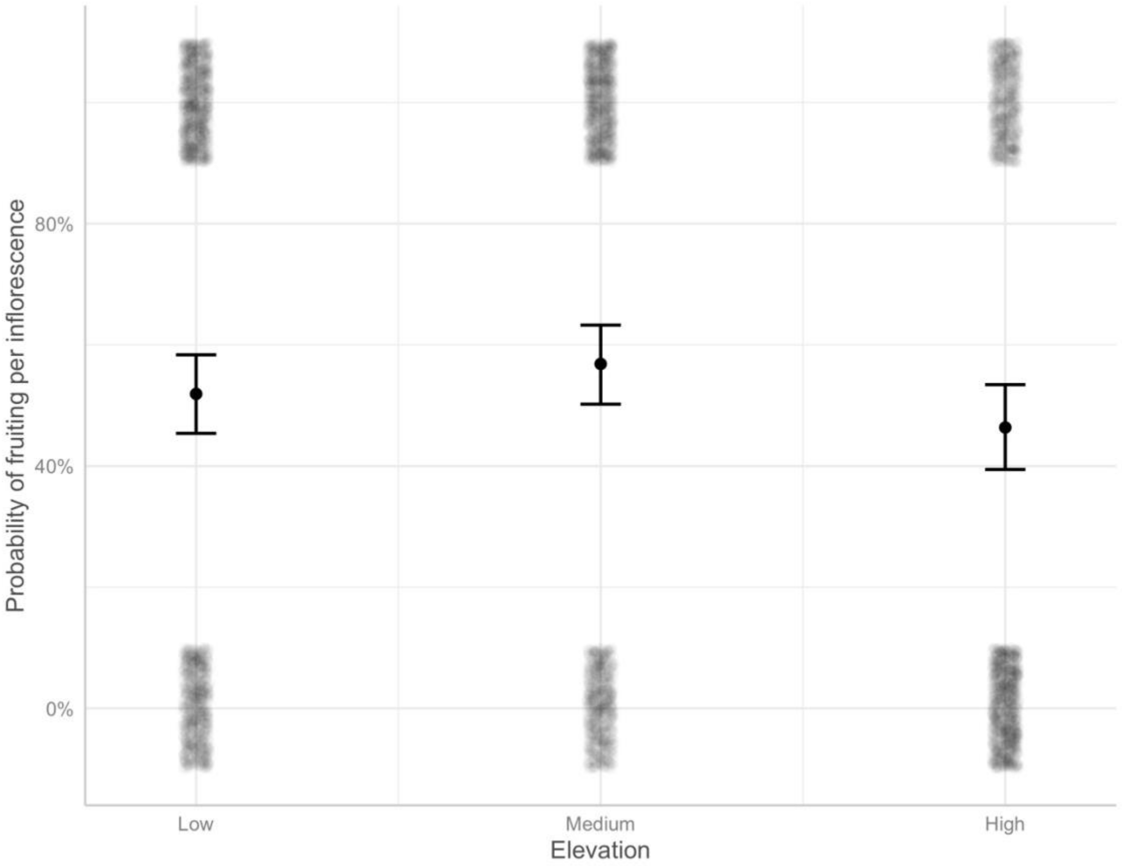

Figure S6. Probability of fruiting per inflorescences by elevation. Data were derived from binomial generalized linear mixed models incorporating statistical interactions between elevation. Error bars correspond to 95% confidence intervals. Original observations of fruiting (1 = 100%) and not fruiting (0 = 0%) are shown with points top and bottom, respectively. All estimates were adjusted for average of logarithm of plant volume (12.72).

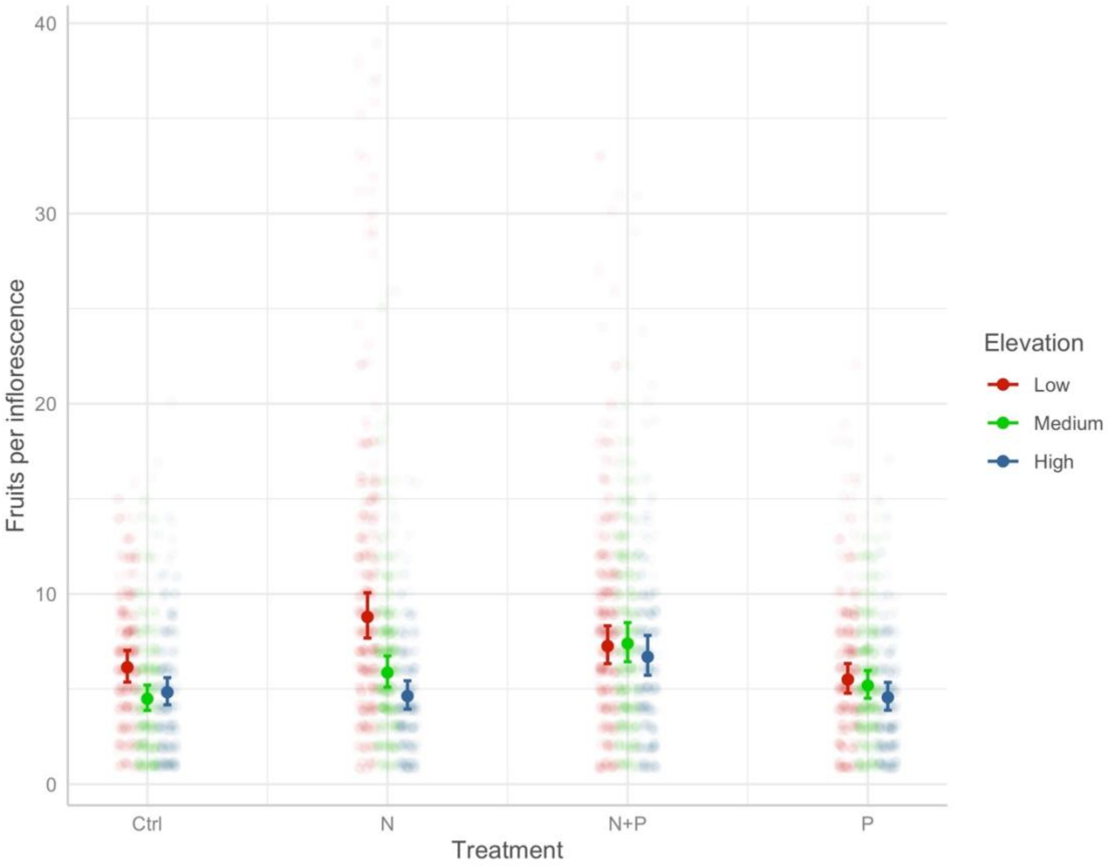

Figure S7. Fruit yield per inflorescences by nutrients and elevation. Data were derived from generalized linear mixed-effects model incorporating statistical interactions between elevation, and nutrient addition (N and P). Error bars correspond to 95% confidence intervals. Original observations are shown with dots around error bars. All estimates were adjusted for average of logarithm of plant volume (for 12.88).

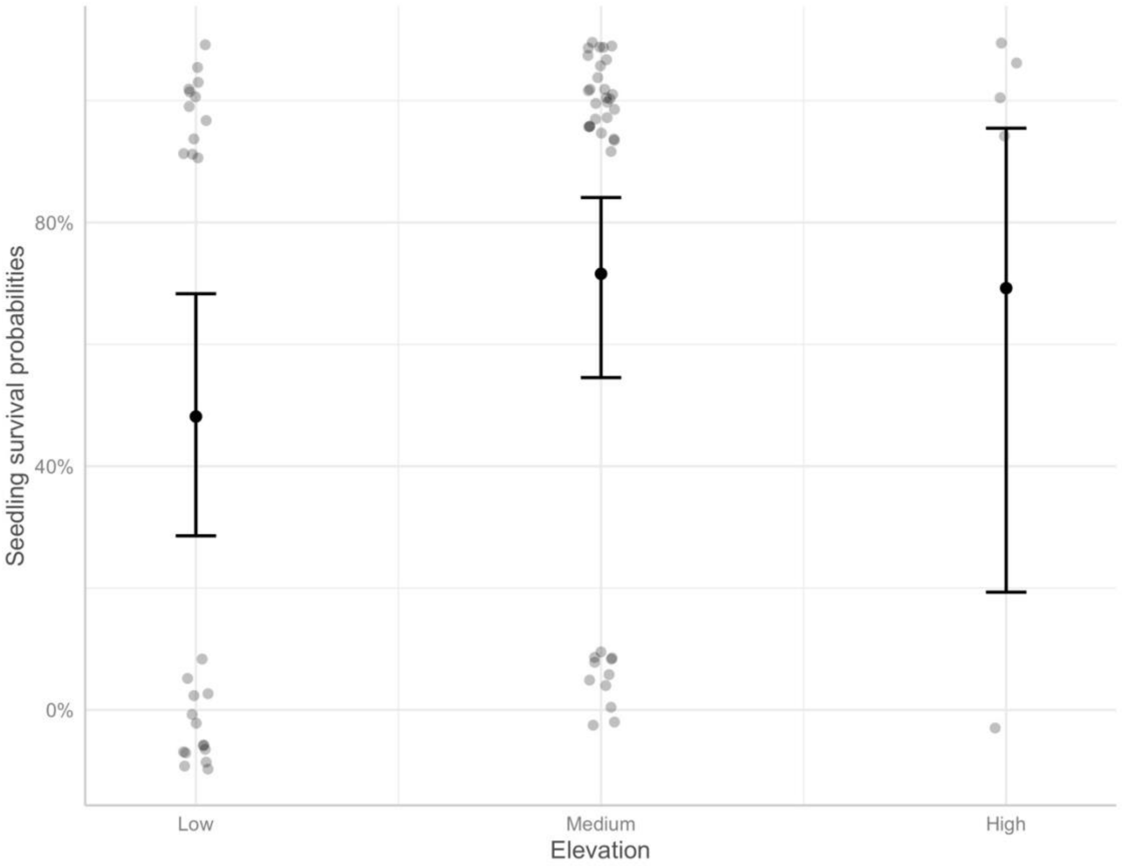

Figure S8. Seedling survival probabilities by elevation. Data were derived from generalized linear models with binomial distribution. Error bars correspond to 95% confidence intervals. Original observations of survival (1 = 100%) and mortality (0 = 0%) are shown with points top and bottom, respectively. Estimates were adjusted for average distance between seedling and maternal plant (43.72).

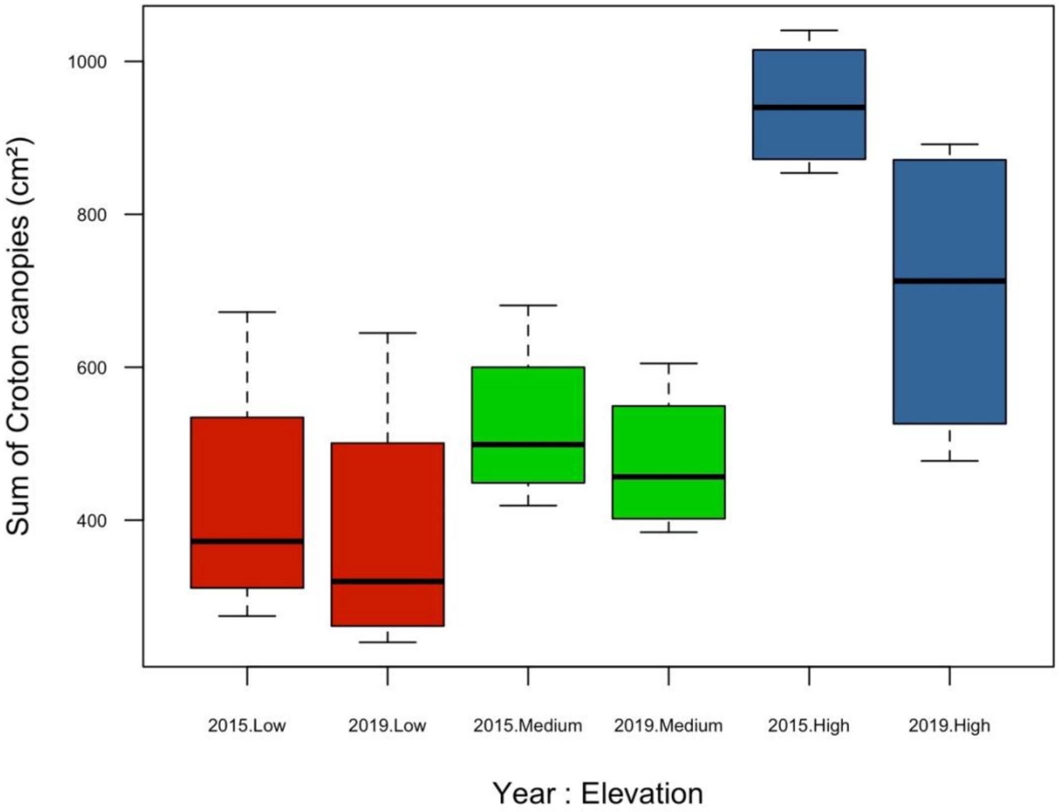

Figure S9. We show decrease of plant cover of *Croton* at the High Elevation between 2015 and 2019.

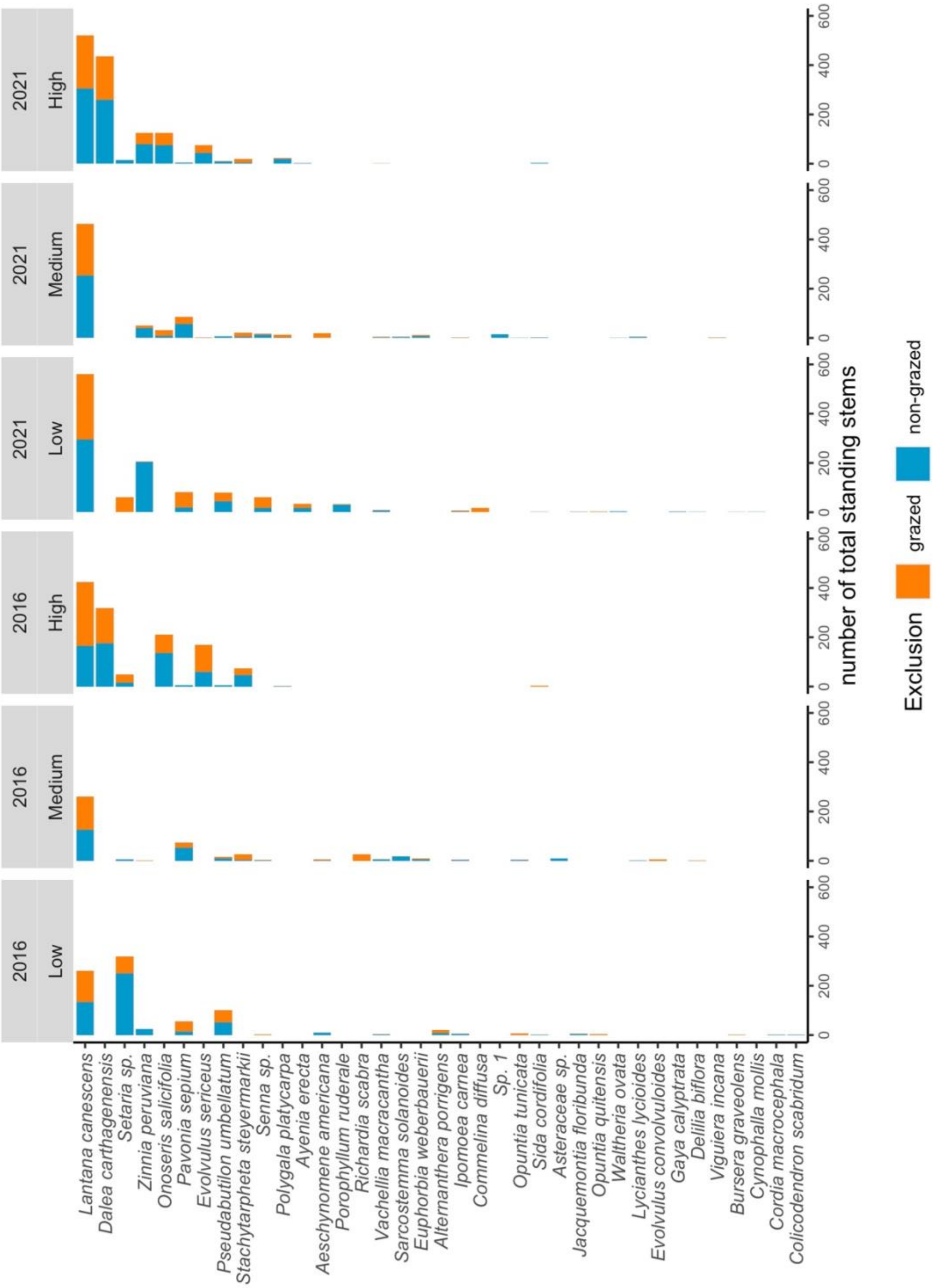

Figure S10. List of species from study site registered in 2016 and 2021. We show the species by year, by elevation and by grazing exclusion.

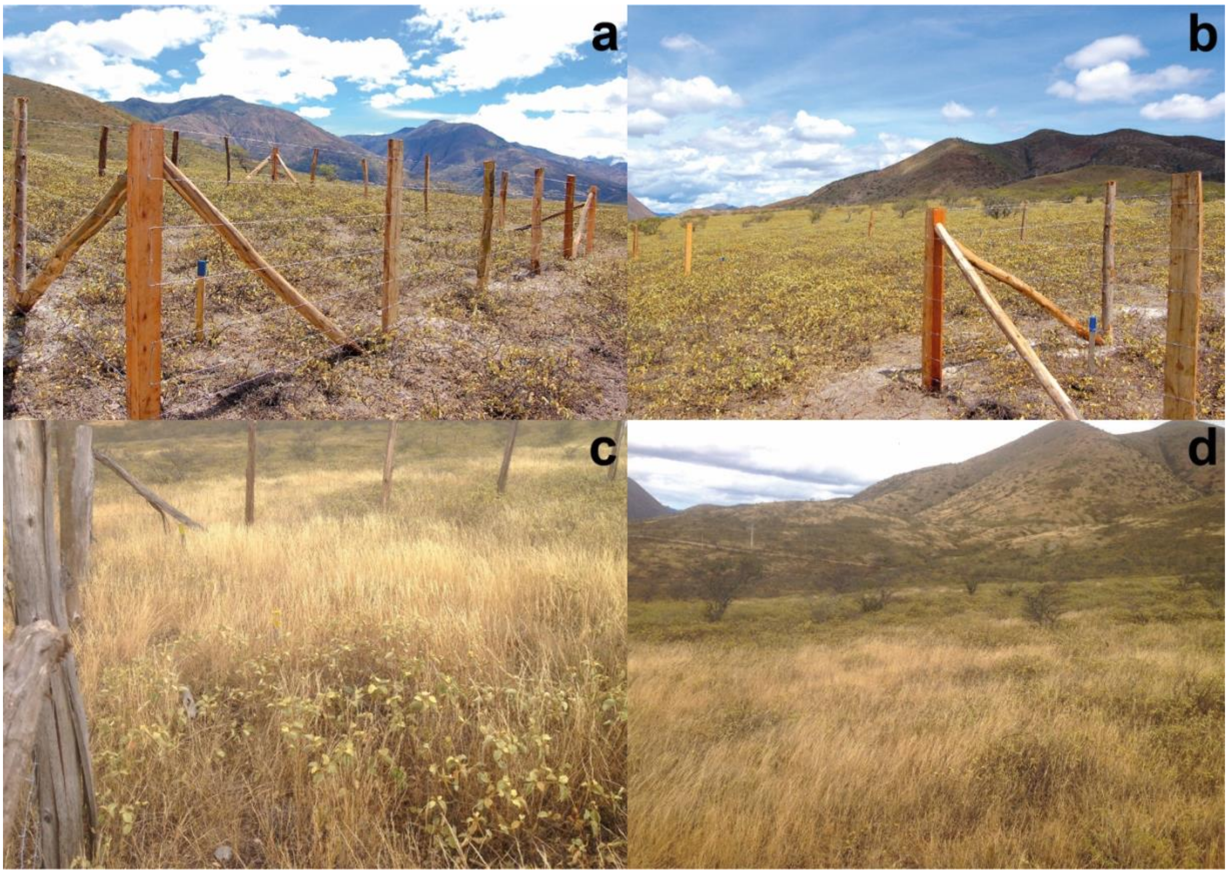

Figure S11. a: Plots without presence of grasses yet at the High Elevation, year 2015. b: Panoramic view at the High Elevation, year 2015. c: Grasses around *Croton* in the study plot (grazing exclusion) at the High Elevation, year 2019. d: Panoramic view with dominant grasses covers at the High Elevation, year 2019. Image captured near to the High Elevation plots.

Figure S12. Grass growth around adult plants and seedlings of *Croton* in winter. White labels indicate *Croton* seedlings.

CAPÍTULO 4

Evidence of morphological divergence and reproductive isolation in a narrow elevation gradient

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Abstract

Elevation gradients generate different environmental conditions. This environmental differentiation can influence morphological adaptation, habitat isolation, reproductive isolation, and pollinator limitation in plants. Habitat differentiation and isolation often act first on phenotypic traits and then on genotype variation, causing genetic divergences between populations. We evaluated the effect of elevation on morphological traits, reproductive isolation, and pollinator limitation in *Croton* aff. *wagneri* in dry shrublands of inter-Andean valleys in Ecuador. We measured morphological traits of *Croton* at three elevations and carried out experimental pollination crosses between and within each population at different elevations to assess the degree of reproductive isolation and pollinator limitation. Morphological traits such as leaf thickness, plant volume, inflorescence length and inflorescence number were dissimilar between plants in different elevations. There was evidence of incipient reproductive isolation between plants in populations at the highest and the lowest studied elevations. Pollination experiments within each elevation showed a limitation of pollinators in *Croton* in the highest elevation. Intrinsic barriers to pollen dispersal and ecological divergence can produce reproductive incompatibilities between individuals with different traits along the *Croton* elevation gradient.

Keywords *Croton* · Elevation gradient · Inter-Andean shrubland · Morphological divergence · Pollen limitation · Reproductive isolation

Introduction

Speciation involves the evolution of reproductive isolation barriers. Reproductive isolation entails interruption of gene flow between populations of a species (Alix et al., 2017; White et al., 2020). Evolution of reproductive isolation barriers in plants originate by processes such as sexual and pollinator isolation (McKinnon et al., 2004; Rundle et al., 2005), gametic isolation (McCartney & Lessios, 2004; Ramsey et al., 2003) and habitat isolation (Matute et al., 2009; Rojo et al., 2020). Habitat isolation dissociates populations exposing them to novel environments in which they must adapt to persist (Noble et al., 2019). The direction of adaptive change depends on the phenotypic and genetic variation that is exposed to natural selection (Noble et al., 2019). Because selection acts on phenotypes, not directly on genotypes or genes, new traits can originate by environmental induction, as well as mutation, and then undergo selection and genetic accommodation (Levis & Pfennig, 2020). These processes enhance morphological variation and genetic structuring between populations because of new genetic rearrangements that can cause reproductive incompatibility (Draghi & Whitlock, 2012; Radersma et al., 2020). As an example, in the Galapagos the cyclical phenomenon of "El Niño" causes intense rains followed by periods of drought (Snell & Rea, 1999). These cyclical phenomena cause a prolonged change in habitat and in seed size (Grant & Grant, 1996). Variation in seed size is associated with the bimodal size of beaks of "El Garrapatero" (*Geospiza fortis*; Huber et al., 2007). The G. *fortis* population has large-beaked individuals that prefer large seeds and small-beaked individuals that prefer small seeds (Grant & Grant, 1996). This morphological divergence of beak size in G. *fortis* reveals both restrictions in gene flow between two morphs as well as two distinct gene pools showing a widely accepted speciation pattern in many taxa (Grant, 1999; Huber et al., 2007; Schluter, 2000). Such phenotypic plasticity could facilitate speciation through morphological divergence between populations (Gomez-Mestre & Buchholz, 2006; Jiang et al., 2019; Rundle & Nosil, 2005).

Environmental changes often trigger gene flow restriction, phenotypic and population divergence (Minelli, 2016; Pfennig & McGee, 2010; Shaw & Mullen, 2011). Phenotypic divergence produced through selection and reproductive isolation can generate ecologically differentiated adaptive populations that could result in the generation of new species (Adams & Huntingford, 2004; Mallet, 2008). Once a phenotype is expressed in a population, to support local environmental changes through physiological tolerance (phenotypic plasticity), selection can favor the expression of these traits through genetic adaptation (Chevin et al., 2010; Pfennig et al., 2010; Sun et al., 2020). Several studies of adaptive traits in plants suggest that genetic adaptation is omnipresent due to clinal variation in phenotypes and genotypes between populations (Depardieu et al 2021; Kremer et al., 2014; Pais et al., 2017). In *Vitis vinifera*, the variation of adaptive traits of their leaves (plants with large leaves inhabit hot and humid climates, and plants with small leaves inhabit cold and dry climates) showed high heritability (Chitwood et al., 2014). Divergent populations of *Cornus florida* showed evidence of local genetic adaptation at various loci under selection that express traits of foliar osmotic potential for adaptation to drought (Pais et al., 2017). These studies suggest that plasticity is an evolutionary source for plant populations to adapt to environmental change (Radersma et al., 2020). Adaptations of populations to new environmental conditions can be become genetically fixed (Corl et al., 2010; Levis & Pfennig, 2020). This process can accumulate genetic differences between populations and contribute to reproductive isolation (Alix et al., 2017; Cardona et al., 2020; Pfennig et al., 2010). It is necessary to understand how processes that generate phenotypic variation interact with natural selection to explain and predict evolutionary paths (Uller et al., 2020).

Variation in reproductive morphology and pollinators plays an important role in plant species isolation with elevation. Two Andean species of *Polylepis* decreased their number of inflorescences in an elevation gradient between 3500 to 4100 m above sea level (m a.s.l.; Cierjacks et al., 2008). In the Helan Mountain Range, China, inflorescence size increased with elevation in insect-pollinated plants on a gradient between 1300 to 3100 m a.s.l. (Zhu et al., 2009). Inflorescence size is key to pollination of flowers that exhibit differences in their sexual expression (Harder & Prusinkiewicz, 2013). Increased number of open flowers in an inflorescence can promote more pollinator visits and greater reproductive success (Gurung et al., 2019; Harder & Prusinkiewicz, 2013).

Several studies have demonstrated the effect of elevation over pollination interactions (Gugerli, 1998; Ramos-Jiliberto et al., 2010; Zhao & Wang, 2015). Often, pollinator availability is low at higher altitudes, so populations at these elevations tend to develop self-compatibility (Alonso, 2005; Arroyo et al., 2017; Gugerli, 1998). An understanding of the influence of environmental heterogeneity on phenotypic and genotypic adaptation, pollination systems and sexual expression is essential to comprehend isolation and speciation processes (Matesanz et al., 2020; Minelli, 2016; Olito et al., 2018; Pélabon et al., 2011).

In tropical mountainous regions temperature and humidity vary according to elevation gradients (Apaza-Quevedo et al., 2015). Elevation generates morphological variations in plants allowing them to adapt locally (Scheepens et al., 2010). Some of the adaptations that plants develop in response to changes in temperature and moisture in elevation gradients are specific leaf area (SLA; Cruz-Nicolás et al., 2020; Jian et al., 2009), leaf thickness (LT; Scheepens et al., 2010), plant size (Badr et al., 2017), inflorescence length (Wang et al., 2019; Wu et al., 2016) and number of inflorescences (Quilot-Turion et al., 2013). In the Jura region and the Alps, populations of *Campanula* *thyrsoides showed* phenotypic plasticity in both SLA and LT, and large genetic variation across an elevation gradient between 1600 to 2200 ma.s.l. (Scheepens et al., 2010). In Egypt, populations of *Achillea fragrantissima* growing at high altitudes had larger plant size and greater number of total and polymorphic ISSR markers compared to populations growing at low elevations in more arid sites (Badr et al., 2017). *In Glycine max* (soybean) and *Penstemon centranthifolius* inflorescence length was determined as a hereditary trait (Mitchell & Shaw, 1993; Wang et al., 2019). These environment-induced phenotypic variations could also be influenced by genetic effects due to local adaptation or genetic drift (Scheepens et al., 2010).

In mountainous regions in the Andes, the Alps and Tibet, there has been evidence of pollinator limitation at high elevations (e.g., Alonso, 2005; Arroyo et al., 2017; Ramos-Jiliberto et al., 2010; Zhao & Wang, 2015). However, there is a paucity of studies evaluating the effect of elevation gradients on pollen limitation (Levis & Pfennig, 2020). These processes are essential to understand selection forces favoring plant speciation along elevation gradients (Cardona et al., 2020; Matesanz et al., 2020; Sun et al., 2020).

We evaluated morphological divergence and reproductive isolation on *Croton* aff. *wagneri* populations at an elevation gradient in the Andes of Loja, Ecuador. *Croton* aff. *wagneri* is a dominant member of plant communities occurring in inter-Andean regions of Ecuador (León-Yánez et al., 2011). Our study occurred in the lowest zone of the Andes called the Andean Depression (Richter et al., 2009). In this zone, elevation gradients cause abrupt changes in moisture and temperature, generating different microenvironments (Quintana et al., 2017; Richter & Moreira-Muñoz, 2005). Our study comprised a narrow elevation range of 300 m (Vélez- Mora et al., 2020). We hypothesize that the variation of moisture and temperature throughout this elevation gradient influence plant morphological traits (specific leaf area and leaf thickness) and reproductive isolation between *Croton* aff. *wagneri* populations located at different elevations. Our research objectives were: (1) Determine morphological divergence of *Croton* aff. *wagneri* at different elevations; (2) Evaluate reproductive isolation of *Croton* aff. *wagneri* at different elevations, and (3) Assess pollen limitation at each elevation. We refer to pollen limitation as limited pollen receipt resulting from insufficient visits by pollenbearing animals (Willmer, 2011). We measured vegetative and reproductive trait variation among populations at three different elevations and performed experimental pollination crosses among and within elevations. We measured fitness components such as fruit set, and seed set associated with the different crosses as indicators of reproductive isolation and pollinator limitation.

Methods

Study Species

Croton aff. *wagneri* (Euphorbiaceae, hereafter *Croton*) is a 0.5—1.5 m tall monoecious shrub (Ulloa & Jørgensen, 1995; Fig. 1B). *Croton* has axillary or terminal inflorescences with small unisexual flowers (Ulloa & Jørgensen, 1995). It has pubescent female flowers located at the base of the inflorescence and male flowers at the top (Ulloa & Jørgensen, 1995; Fig. 1C and D). Male Croton flowers alternate occasionally in the middle with a few female flowers (Ulloa & Jørgensen, 1995; Webster, 1993). Number of flowers per inflorescence can vary between 10 and 60 male flowers and between 4 and 16 female flowers (Vélez-Mora unpublished data). Female *Croton* flowers are sessile or united with short pedicels (Ulloa & Jørgensen, 1995). The female flower calyx has five segments attached at the base with petals and glands generally absent (Ulloa & Jørgensen, 1995). The gynoecium consists of a compound pistil (van Ee et al., 2011). The ovary has three locules with one seminal primordium per locule (Ulloa & Jørgensen, 1995; van Ee et al., 2011). Styles are bifid and are 3 to 6 mm long (Ulloa & Jørgensen, 1995; Webster, 1993; personal observation). There may be two to three male flowers that are supported by a bract with five sepals attached at the base and five reduced petals (Ulloa & Jørgensen, 1995). Number of stamens varies from 10 to 20 and the filaments are free (Ulloa & Jørgensen, 1995; Webster, 1993). Stamens are 3 to 6 mm long (personal observation). *Croton* is likely pollinated by a variety of nectarseeking pollinator species, particularly those belonging to the Hymenoptera and Diptera insect orders (Webster, 2014). Average number of fruits per inflorescence is 4.85 + SE 0.04 (Vélez-Mora unpublished data). Most seed dispersal occurs within a few meters (Espinosa et al., 2019; Jara-Guerrero et al., 2015). The female flowers bloom before the male flowers, possessing a well-established dichogamy avoiding geitonogamy in the same inflorescence (Domínguez & Bullock, 1989; Webster, 2014). However, pollination between male and female flowers from different inflorescences in the same plant can occur (c.f. Domínguez & Bullock, 1989).

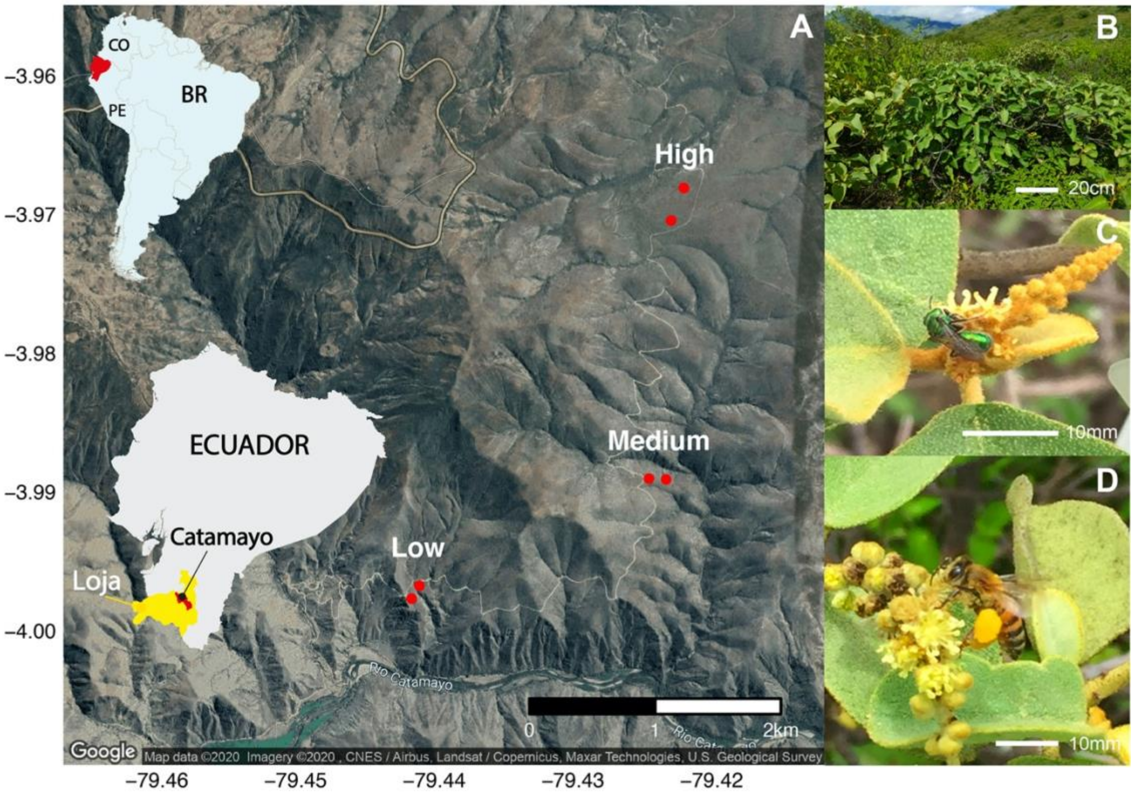

Fig. 1. A: Map of Ecuador showing the locations of studied sites colored red at each elevation. B: Study species. C: A species of wasp visiting female *Croton* flowers. D: A bee visiting male *Croton* flowers.

Study Site

Our study was performed at an inter-Andean valley at Catamayo, Loja Province, Ecuador. This valley extends into the basin of the Río Catamayo from the riverbed at 1100 m a.s.l. to approximately 2700 m a.s.l. at the local highest point. In this valley Croton is distributed continuously between 1100 and 2080 m of elevation (Leal, 2015; personal observation). *Croton* shrubs are conspicuous elements in a sparse xerophytic and spiny vegetation (Fig. 1B). Plant species surrounding *Croton* are perennials such as *Lantana canescens* Kunth, *Vachellia macracantha* (Humb. & Bonpl. ex. Willd.) Seigler & Ebinger, *Bursera graveolens* (Kunth) Triana y Planch., *Colicodendron scabridum* (Kunth) Seem. and *Opuntia quitensis* F.A.C. Weber (Espinosa et al., 2013, 2019; Sierra, 1999; Ulloa & Jørgensen, 1995). The topography is rugged with moderately steep slopes (Fig. 1A). Ungulates (donkeys, cows, goats) wander on the property (Espinosa et al., 2013). We have not seen these animals browsing on *Croton*, and local herders confirmed that these animals seldom feed on it, but we have observed livestock trample seedlings and small plants and damaging plant branches. This ecosystem experiences hot (24.8 °C mean annual temperature) and dry weather, with an extended dry season (317 mm mean annual precipitation, 57% of it occurring between February and May) (Espinosa et al., 2019; Figure S1A). Water deficit is prevalent 10 months a year (Espinosa et al., 2019; Figure S1A).

We selected six sites, two per elevation, at three elevations within the Hacienda Alamala: 1700 m a.s.l. identified as High Elevation (3° 58′ 07.90″ S, 79° 25′ 19.71″ W), 1550 m a.s.l. identified as Medium Elevation (3° 59′ 20.09″ S, 79° 25′ 28.64″ W) and 1400 m a.s.l. identified as Low Elevation (3° 59′ 40.15″ S, 79° 26′ 31.29″ W; Fig. 1A). Distances among sites in different elevations were greater than 2.5 km (but less than 6 km) while sites within the same elevation were at least 100 m apart (but less than 300 m apart).

Morphological Traits of Croton Plants Between Elevations

To evaluate morphological traits of *Croton* we selected haphazardly breeding plants at two sites at each elevation. We selected eight plants per site for a total of 48 plants. We randomly collected four leaves per plant (n = 192 leaves). To measure leaf area, we used a CI-202 Portable Laser Area Meter (CID Bio-Science Inc, Camas, Washington, USA). Fresh weight of these leaves was obtained 1 h after collection. Leaves were dried for 42 h in a forced air oven (DFO-36, MRC ltd., United Kingdom). Fresh weight and dry weight of leaves were measured on an analytical scale (OHAUS PA84C, Analytical Pioneer, China). Specific leaf area (SLA; $cm^2\ g^{-1}$), leaf dry matter content (LDMC; $mg\ g^{-1}$) and leaf thickness (LT; μm) were calculated with the following equations (Vile et al., 2005):

$$SLA = \frac{LA}{LD}$$

$$LDMC = \frac{LD}{1-(LW)}$$

$$LT = \frac{1}{(SLA \times LDMC)}$$

Where *LA* is leaf area (cm^2), *LD* is leaf dry mass (g) and *LW* is leaf water content (g). We characterized each plant by their volume (see Vélez-Mora et al., 2020), number of inflorescences and average inflorescence length. We evaluated plant volume as a measure of resource availability (Shivanna & Tandon, 2014) and number of inflorescences and average inflorescence length as a measure of reproductive effort (Harper & Ogden, 1970). When plants had ~ 100 inflorescences, we counted all inflorescences on the plant. In some individuals the number of inflorescences was estimated because they were too numerous (from January to March; Vélez-Mora 2019, unpublished data). We divided plant canopy into four quadrants, selected one at random, counted the number of inflorescences and multiplied it by four. For inflorescence length, we measured five inflorescences randomly per plant.

We used generalized linear mixed effect models (using R package *nlme*; Pinheiro et al., 2020) to evaluate leaf thickness variation as a function of specific leaf area (Supplementary Information Table S1) and elevation (elevation and specific leaf area as fixed factors and sites and plants as random factors). We evaluated variation on number of inflorescences (transformed with logarithms) and inflorescence length as a function of plant volume (transformed with logarithms) and elevation (plant volume and elevation as fixed factors and sites and plants as a random factor). Hierarchically, we evaluated the random effects of plants within sites and sites within elevations. The inclusion of plant volume and specific leaf area as state variables allowed us to account for the effect of variation in plant and leaf size among individuals. We identified the most likely model for each set using small sample Akaike criteria (AICc; Burnham & Anderson, 2002; see Supplementary Information Tables S1-S9). For inflorescence length, there were no clear differences between the two most likely models. We choose the model with the effects of elevation and volume because it had the highest r-squared among the set and it was consistent with previous findings (Supplementary Information Tables S7). All analyses were performed in version 3.6.2 of R software (R Core Team, 2019).

Study Site Micro-Climate

During our field season between 1 and 28 Feb. 2019, we monitored at each elevation air temperature and humidity, soil temperature and moisture at a depth of 10 cm, and photosynthetic light, all with a sampling interval of five minutes, using HOBO data loggers S-TMB-M006, S-SMC-M005, and S-LIA-M003 (Onset, Bourne, Massachusetts, USA). We built an ordination regularized discriminant analysis (RDA; *rda* function in package vegan of R Core Team, 2019; Oksanen et al., 2019) to describe the combined association of these variables with elevation in our sites. Regularized discriminant analysis ordination included the mean and the standard deviation of daily air temperature, daily soil temperature, relative air humidity, photosynthetic active radiation, soil moisture and dew point (Supplementary Information Fig. S1 and Fig. S2). We measured with Spearman correlations (*cor* function; stats package of R Core Team, 2019) the association of these scores with the studied leaf attributes to identify possible different selection pressures across elevations.

Reciprocal Pollinations Between Elevations to Assess Reproductive Isolation

We performed reciprocal hand pollinations between the High, Medium, and Low elevations. Pollinations began on 7 Feb. 2019, during the species' peak bloom. Two sites were selected per elevation and eight breeding plants were chosen at each site (total 48 plants). One day before the first pollination, inflorescences with pre-anthesis female buds were bagged to ensure that they were not pollinated. Reciprocal pollinations consisted of hand pollinations between elevations and bagging of the inflorescences after being pollinated. Reciprocal pollinations were contrasted with cross pollination within each elevation. This treatment consisted of bagging and hand pollination with pollen from other plants of the same population. In the first attempt, one day after bagging, two trials of hand pollination were carried out per plant and inflorescence: one in the morning (between 700 and 1100 h) and another in the afternoon (between 1400 and 1800 h) in the same inflorescence. Additional pollinations were attempted on the fourth day for those flowers that were not in anthesis before. A third and last pollination was repeated after 15 days for remaining flowers that did not open before. No female flowers remained available in the chosen inflorescences after this final attempt. A total of 144 pollination trials were carried out (eight plants × two sites × three elevations × two pollen provenances from respective elevation + crosses within elevation). The experimental unit was the inflorescence for each treatment.

For hand pollination, fresh inflorescences with male flowers at each site were collected one hour before each pollination attempt. Inflorescences were kept in a cooler in plastic bags. Three male flowers with pollen available from different donor plants were used. To check availability of pollen, anthers were rubbed against a clean piece of black cloth. Anthers of each male flower were gently rubbed one by one over stigmas of all open female flowers (Supplementary Information Fig. S3). After 36 days we collected the fruits of the reciprocal pollinations. Not all the fruits reached full maturity because we had to collect them before livestock could damage the bags from the treatments. We counted number of aborted fruits, number of fruits developed and number of seeds for each fruit. We consider as aborted fruits scars left on the inflorescences by female reproductive structures that fell before developing (see Domínguez & Bullock, 1989). Total fruits were the sum of aborted fruits and developed fruits in the inflorescence. Fruit set of each treatment was calculated as proportion of fruits developed throughout the inflorescence. Seed set for each treatment was calculated as proportion of seeds developed in all fruits of the entire inflorescence. We calculated the reference potential number of seeds assuming three seeds per fruit. Reproductive isolation (RI) was calculated following Sobel and Chen (2014). The reproductive isolation for fruit set and seed set was calculated as:

$$RI \;= 1 - 2 \times \left(\frac{X_{CBE}}{X_{CWE} \; + \; X_{CBE}} \right)$$

Where X_{CBE} is the value of fruit and seed set of the reciprocal crosses between elevations and X_{CWE} is the value of fruit and seed set of the crosses within each elevation. Reproductive isolation values range from 1 (complete isolation) to -1 (complete disassortative mating). RI = 0 indicates random mating (Sobel & Chen, 2014; Ramírez-Aguirre et al., 2019). We considered for the analysis of reproductive isolation only plants greater than 400,000 cm^3 (41 large plants of a total of 48) because we observed that smaller plants have reduced fertility (mean of large plant fruits 5.1 ± 0.66 SE and small plants 2.9 ± 0.98 SE; Espinosa et al., 2019; see Supplementary Information Fig. S4). We calculated the mean RI (and its 95% confidence interval) for each of the pairs of crosses per elevation. We considered clear evidence of reproductive isolation when crosses with the reproductive isolation index (RI) had a positive value and its confidence interval bar did not overlap zero.

Pollen Limitation Within Each Elevation

We carried out four pollination treatments to estimate pollen limitation: (1) Crosses within elevation (also incorporated in reproductive isolation analysis); (2) Handgeitonogamous pollination: bagging and manual pollination with pollen of the same plant; (3) Open pollination: not bagging and no manual pollination, and (4) Control: bagged flowers to eliminate access of animal pollinators. These treatments were carried out in parallel with reciprocal pollination between elevations for reproductive isolation, so they were carried out under the same protocol. Inflorescences of open pollination treatment were bagged six days after last pollination to protect developing fruits. A total of 144 trials were conducted excluding treatment of crosses within elevation (eight plants × two sites × three elevations × three treatments). The experimental unit was the inflorescence for each treatment. Plants for the pollination treatments were different from the plants used in the measurement of leaves so as not to influence their reproductive investment. For the treatments that involved bagging, 4 × 6" pollination bags of nonwoven transparent polypropylene fabric were used (Carolina Biological Supply Company, Burlington, North Carolina). We also only considered plants larger than 400,000 cm^3 to analyze pollen limitation. We compared the four pollination treatments using a non-parametric paired Wilcoxon test.

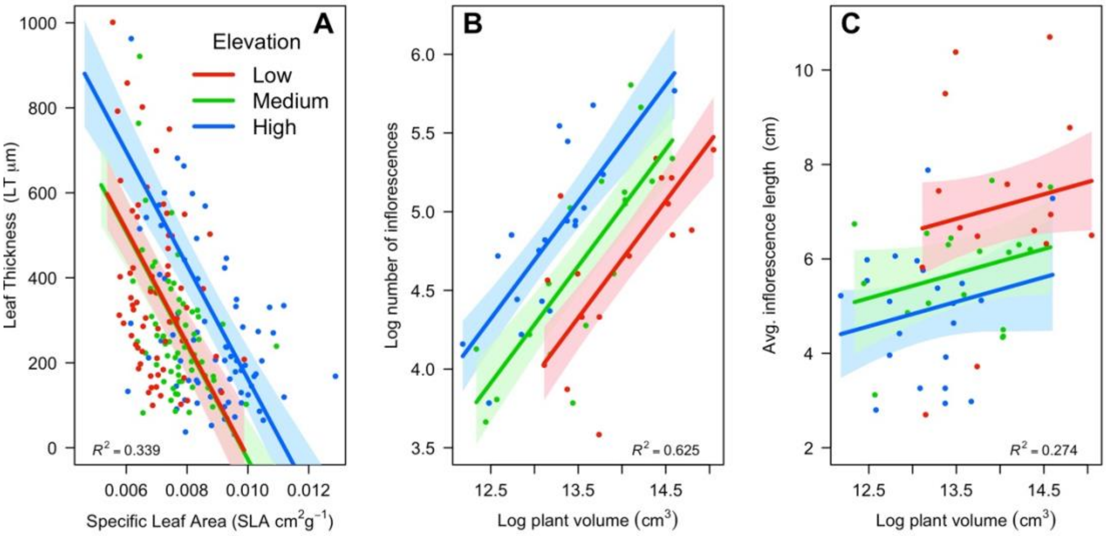

Fig. 2. Variation of morphological traits of *Croton* according to elevation. A: Leaf thickness increased according to specific leaf area and was higher in the High Elevation. B: Number of inflorescences increased according to plant volume and was higher in the High Elevation. C: Average inflorescence length increased according to plant volume and was higher in the Low Elevation.

Results

Morphological Traits of Croton Plants Between Elevations

Leaf thickness decreased with specific leaf area (Fig. 2A). Leaf thickness was higher in the High Elevation compared to the other two elevations (Fig. 2A, Supplementary Information Table S3). Number of inflorescences and inflorescence length increased with plant volume (Fig. 2B and C). Number of inflorescences was different between elevations and was higher in High Elevation (Fig. 2B). Inflorescence length was greater in the Low Elevation compared to the High Elevation (Fig. 2C). We did not observe differences in inflorescence length between Medium Elevation and High Elevation or between Medium Elevation and Low Elevation.

Climate

The regularized discriminant analysis differentiated the elevations with the climate variables studied. The percentage of the variance explained by the first axis (PC1) was 93% and the percentage explained by the second axis (PC2) was 7%. The first axis (PC1—Fig. 3) clearly separated the three elevations while the second axis (PC2) clearly separated the Low Elevation from the Medium Elevation and the High Elevation that were like each other in this axis. In the first axis, the High Elevation had the lowest scores associated with high average soil and air humidity and high soil water content variation but low values for all the other environmental variables. In the first axis, the Low Elevation had intermediate scores and the Medium Elevation the high scores. In the second axis, the Low Elevation had the highest scores, while the High Elevation and Medium Elevation had lower scores. High scores in the second axis were associated with more variable air and soil humidity, higher temperatures, and higher dew points. Change in leaf thickness with elevation, adjusted by leaf area (coefficients for elevation in Supplementary Information Table S1) was associated with the first axis of the environmental regularized discriminant analysis (Spearman correlation = -1).

Reciprocal Pollinations Between Elevations to Assess Reproductive Isolation

We used fruit and seed set variation of reciprocal pollinations between elevations contrasted with cross pollinations within each elevation to calculate reproductive isolation (RI). Using fruit set we recognized reproductive isolation between Low Elevation (as pollen donors) and High Elevation plants (as pollen receptors) and between Medium Elevation (as pollen donors) and High Elevation plants (as pollen receptors; Fig. 4). Using seed set, the results were consistent with those of fruit set but less clear (Fig. 4). We did not find clear evidence of reproductive isolation or disassortative mating of plants in any other cross between populations.

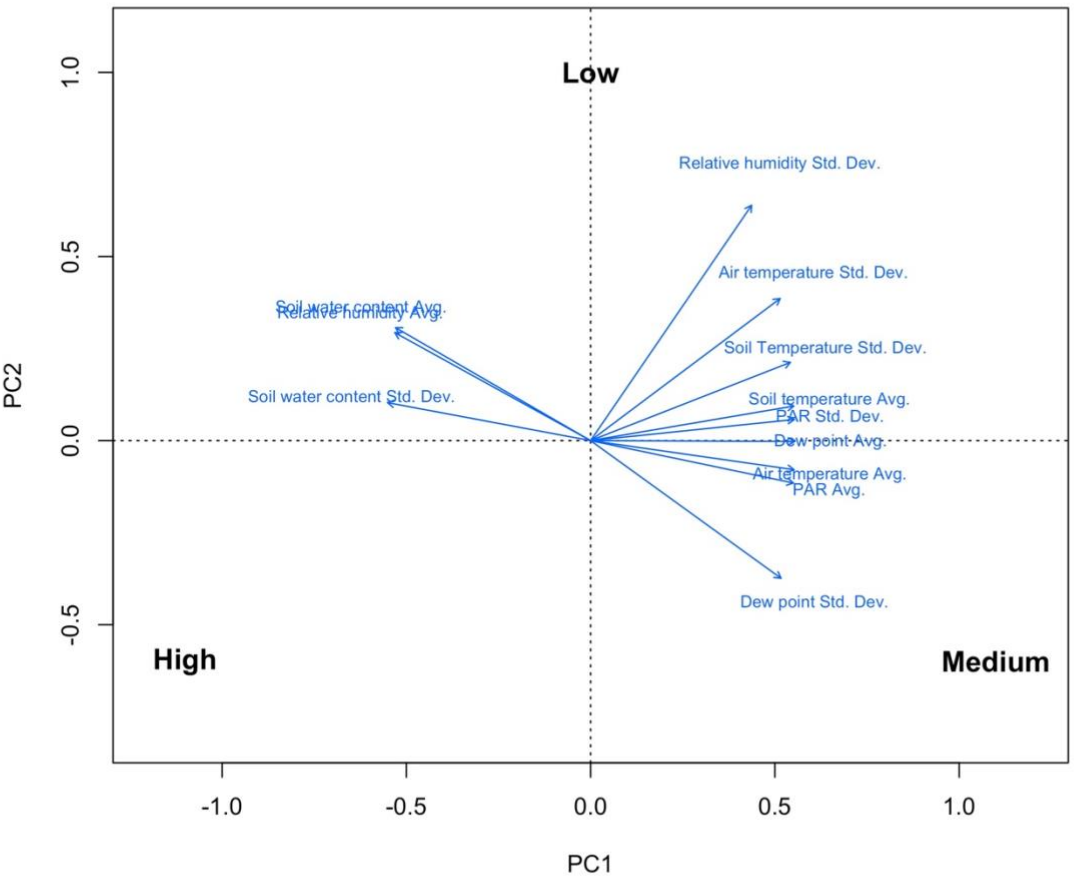

Fig. 3. The regularized discriminant analysis differentiated the elevations with the climate variables studied. The first axis (PC1 explained 93% of the variance) clearly separated the three elevations while the second axis (PC2 explained 7% of the variance) clearly separated the Low Elevation from the Medium Elevation and the High Elevation that were like each other in this axis.

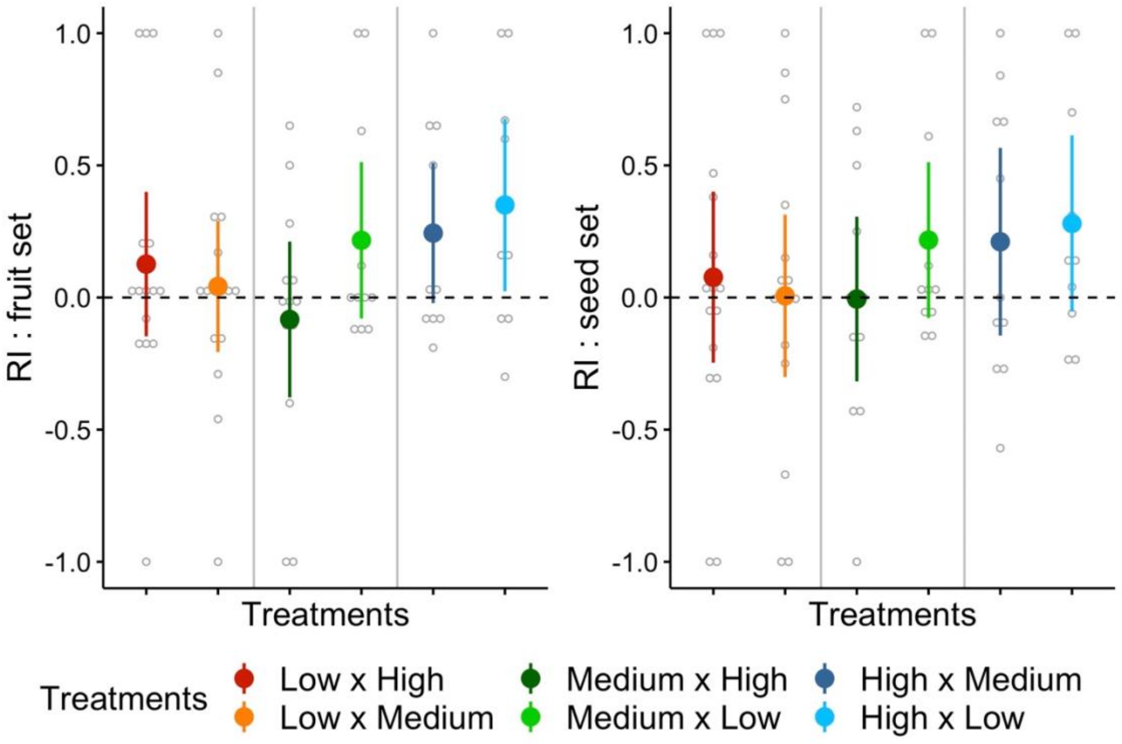

Fig. 4. Reciprocal crosses between elevations to determine reproductive isolation (RI) by fruit and seed set of *Croton*. The mean and the confidence intervals indicated reproductive isolation between Low Elevation plants and High Elevation plants according to fruit set.

Pollen Limitation Within Each Elevation

Fruit and seed set with open pollination were clearly higher at Low Elevation compared to the other two elevations and treatments (Fig. 5). Fruit and seed set with open pollination were higher compared to control treatment at Low and Medium elevations (Fig. 5). Fruit and seed set were clearly higher with crosses within and handgeitonogamy compared to control treatment at Medium Elevation (Fig. 5). Fruit and seed set did not differ between the treatments at High Elevation, except between the treatments within and control by seed set (Fig. 5).

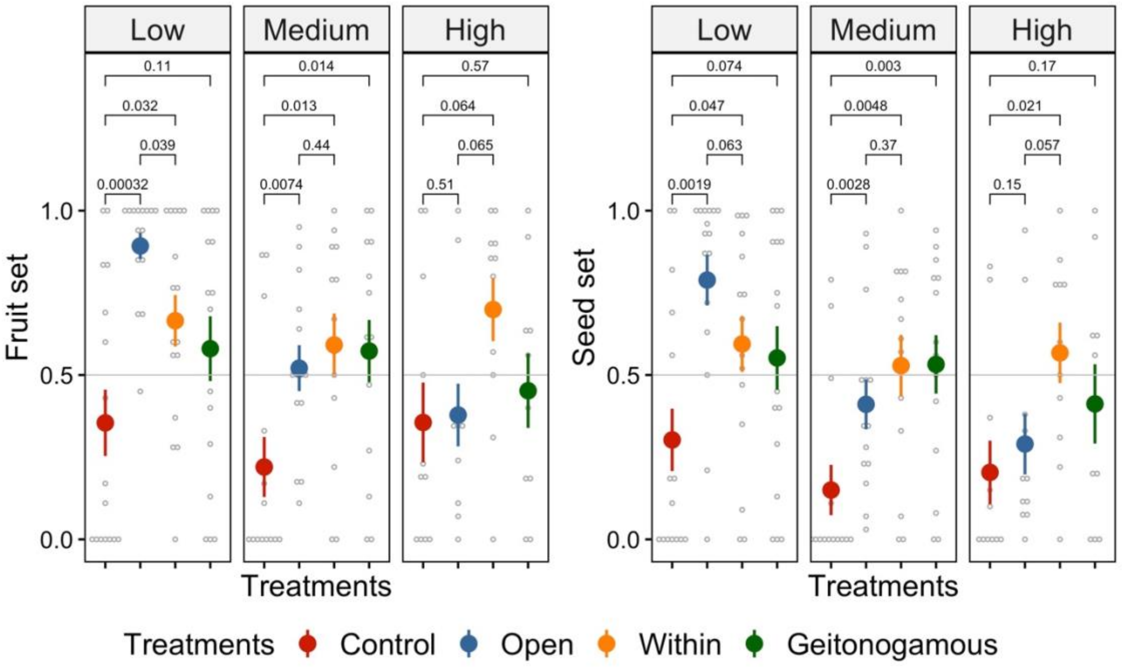

Fig. 5. Fruit and seed set by treatment within each elevation. We compared differences among the four pollination treatments using a non-parametric paired Wilcoxon test after an Analysis of variance. Fruit and seed set with open pollination were higher at Low Elevation compared to the other two elevations and treatments. Fruit and seed set was marginally different between within crosses and open bag treatment at High Elevation.

Discussion

Our data contribute to a better understanding of the interaction between elevation, morphological trait variation, reproductive isolation, and pollinator limitation among *Croton* in the southern Ecuadorian Andes. Individual morphological trait variation of Croton plants was concordant with reproductive isolation between individuals of populations at different elevations.

Morphological Traits of *Croton* Plants Between Elevations

Our results evidenced morphological divergences between *Croton* populations at different elevations. The plants at the High Elevation produced a greater number of inflorescences. Their leaves were thicker and with a greater specific leaf area. However, plants at the High Elevation had less volume, and the inflorescence length was shorter. Higher inflorescence production suggests that plants possess sufficient resources to invest in flower and seed production and prioritize reproduction instead of growth (Fabbro & Körner, 2004). Allocation of resources to reproductive structures in alpine plant species was three times greater at high elevations than at low elevations (Fabbro & Körner, 2004). High number of *Croton* flowers at the High Elevation could be associated with a higher availability of nitrogen and organic matter existing at this elevation (Wright et al., 2004; Supplementary Information Fig. S5). In addition, in the High Elevation resources for the reproductive function are in greater proportion for production of male *Croton* flowers, the most economical reproductive function (Velez-Mora et al., 2020). In the high tropical mountains, climate is cold and dry, and air and soil temperatures decrease with increasing elevation (Domic & Capriles, 2009). Under these conditions, plants produce thick leaves to counteract drought and mechanical stress caused by the wind (Pérez et al., 2020; Vogel, 2009). Several studies indicate reduction of specific leaf area with increasing elevation to withstand unfavorable environmental conditions (Apaza-Quevedo et al., 2015; Jian et al., 2009; Scheepens et al., 2010). Our results showed an opposite relationship in *Croton*, with a lower specific leaf area at Low Elevation probably due to higher soil water stress. In *Viola maculata* in the Central Andes of Chile, for example, the specific leaf area and stomatal conductance increased with elevation, reducing water stress (Seguí et al., 2018). This relationship is consistent with specific leaf area and soil moisture content data in *Croton* (Supplementary Information Fig. S1). This relationship suggests that *Croton* can develop differential responses to the environment at relatively short distances, either by phenotypic plasticity or by genetic adaptations.

Leaf thickness and specific leaf area generally tend to evolve separately from plant size (Pérez et al., 2020). Reduction in plant size at high elevations serves as self-shading where plants adhere to the ground to decouple their climate from the surrounding environment, prevent desiccation, and accumulate heat in the plant canopy (Hallik et al., 2009). Inflorescence length also shortens with increasing elevation in alpine environments (Fabbro & Körner, 2004). Shortening of inflorescences at high elevations creates a warm environment that could substitute for the advantage of large plants to attract the most pollinators (Donnelly et al., 1998). These strategies can increase seed set and attractiveness to pollinators (Fabbro & Körner, 2004).

Reciprocal Pollinations Between Elevations to Assess Reproductive Isolation

Our results indicated an incipient reproductive isolation between *Croton* populations at the most extreme studied elevations in this relatively short gradient. Environmental heterogeneity generated by elevation can lead to local adaptation in plants (Chapman et al., 2016; Cordell et al., 1998). Recent studies have suggested that phenotypic plasticity may signal genetic divergence between populations (Caetano et al., 2020; Walter et al., 2020). The *Achillea fragrantissima* populations that grew at high altitudes differed from the rest by being larger and presenting a greater number of ISSR markers in Egypt (elevation range between 132 and 1154 m; Badr et al., 2017). A genetic study on *Croton* populations in the same geographic area as ours but at different elevations and using AFLP markers provided evidence of clear genetic differentiation between high and low elevation populations (Leal, 2015). *Croton* populations of each elevation still maintained a partial compatibility with each other in agreement with our results.

Pollen Limitation Within Each Elevation

Open pollination produced higher fruit and seed set at Low Elevation and similar values as other treatments (except bagged flowers) in Medium Elevation. This did not occur at High Elevation where fruit and seed set were higher with hand pollination with pollen from neighboring plants compared to the other treatments, including open pollination. The lower fruit and seed set at High Elevation in open pollination offers us indirect evidence of limitation of pollinators in *Croton*. Our study did not evaluate diversity of pollinators associated with *Croton* pollination. However, several studies have shown decreases in abundance and diversity of pollinators as elevation increases consistent with our preliminary observations in this region (Arroyo et al., 2006; Ramos-Jiliberto et al., 2010; Torres-Díaz et al., 2011). In two subspecies of *Campanula spatulata,* insect visitation rate decreased with elevation on Mount Olympos (elevation range 400—2200 m.a.s.l; Blionis & Vokou, 2002). In the same study, plant size, flower size and number of flowers differed between elevations indicating reproductive isolation and morphological divergence in these subspecies (Blionis & Vokou, 2002). Low visit rates of pollinators at High Elevation were consistent with low output of fruit and seed of *Croton* at this elevation. Scarcity or ineffectiveness of pollinators to ensure pollen dispersal at high elevations and limited dispersal of *Croton* seeds (Espinosa et al., 2019) could restrict gene exchange between populations at different elevations (Cardona et al., 2020; van der Niet et al., 2014).

Intrinsic barriers to gene flow and ecological divergence produce reproductive incompatibilities between ecotypes (Schliewen et al., 2001; Walter et al., 2018, 2020). There were clear differences in temperature and moisture between high and low elevation in our study site. These climatic conditions could generate a strong selection for both plants and pollinators (Cardona et al., 2020; Halbritter et al., 2018). Several studies show how insect composition and environmental conditions change along elevation in narrow geographic spaces in the Andes (Arroyo et al., 2017; Hall, 2005; Medina et al., 2002; Pyrcz, 2004; Ramos-Jiliberto et al., 2010). Change in composition of insects could generate a restriction of gene flow between elevations and strengthen local adaptation of *Croton* at each elevation (Peakall & Whitehead, 2014; van der Niet et al., 2014). Local adaptation could help *Croton* plants to adjust in response to their habitat and pollination environments (Dai et al., 2017). Our findings are consistent with other studies where ecological adaptations (SLA for example according to Scheepens et al., 2010) in combination with reproductive isolation could lead to a divergence of *Croton* populations in parapatry (Itino & Hirao, 2016; Nosil, 2012; Walter et al., 2020). Low gene flow through reduced dispersal of pollen and seeds (Espinosa et al., 2019) could be causing reproductive isolation between populations of *Croton* at high and low elevations. Abrupt changes in temperature and humidity along elevation gradient could be selective forces that disrupt pollinator movement between elevations (Bridle & Vines, 2007; Halbritter et al., 2018; Lenormand, 2002). This could reinforce genetic isolation barriers for *Croton* in High Elevation populations probably leading to divergence and local adaptation at each elevation (White et al., 2020). Elevation gradients offer an interesting opportunity to study adaptive traits under strong selection pressure and homogenizing effect of gene flow (Gonzalo-Turpin & Hazard, 2009; Halbritter et al., 2018; Sexton et al., 2011). In these regions of the Andes there is a great diversity of species, many of them endemic with restricted elevation which will benefit from a better understanding of their population ecology and evolution (c.f. Herzog et al., 2011; Homeier et al., 2010; Josse et al., 2009; Quintana et al., 2017).

Limitations

In this study, it was not possible to determine differences in other fitness components between pollination treatments and elevations since fruits were collected before their complete development to prevent cows and goats in the study site from damaging the experimental bags. However, independent information indicated that Low Elevation seeds (0.0090 ± SE = 0.00013 g; n = 716) were larger and heavier compared to Medium Elevation seeds (0.0066 ± SE = 0.00032 g; n = 111) and High Elevation (0.0064 ± SE = 0.00019 g; n = 330; unpublished data from Vélez-Mora). Future work should directly evaluate composition and visitation rates of pollinators in this elevation gradient. Likewise, experiments based on reciprocal transplants of plants or experiments in common garden conditions should be carried out to evaluate genotypic and phenotypic differences between populations to reaffirm our interpretations.

Conclusion

Our study provides observational and experimental evidence for simultaneous variation of plant morphological traits and incipient reproductive isolation of *Croton* in a narrow elevation gradient with contrasting climatic conditions. Lower number of fruits and seeds of the High Elevation population compared to the Low Elevation population and pollination experiments within each elevation evidenced a limitation of pollinators in *Croton* in the High elevation population. Intrinsic barriers to pollen and seed dispersal, and ecological divergence may produce reproductive incompatibilities between individuals with different traits along the *Croton* elevation gradient.

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Author's contributions DPVM and PFQA conceived the study and design. DPVM, KTA and PFQA collected the samples and measurements. DPVM carried out the pollinations. PFQA conducted the analyses. DPVM and PFQA wrote the draft of the manuscript. All authors edited and reviewed the final version of the manuscript.

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Availability of data and material If this paper is accepted our data will be deposited in Dryad Digital Repository. However, they will be available for Evolutionary Biology and for peer reviewers if required.

Code availability Code will be sent to the reviewers when they require it.

Compliance with ethical standards

Conflict of interest: The authors have no conflict of interest to declare.

References

Adams, C. E., & Huntingford, F. A. (2004). Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. *Biological Journal of the Linnean Society*, *81*(4), 611–618.

Alix, K., Gérard, P. R., Schwarzacher, T., & Heslop-Harrison, J. S. P. (2017). Polyploidy and interspecific hybridization: Partners for adaptation, speciation and evolution in plants. *Annals of Botany*, *120*(2), 183–194.

Alonso, C. (2005). Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*. *American Journal of Botany*, *92*(8), 1264–1269.

Apaza-Quevedo, A., Lippok, D., Hensen, I., Schleuning, M., & Both, S. (2015). Elevation, topography, and edge effects drive functional composition of woody plant species in tropical Montane forests. *Biotropica*, *47*(4), 449–458.

Arroyo, M. T. K., Muñoz, M. S., Henríquez, C., Till-Bottraud, I., & Pérez, F. (2006). Erratic pollination, high selfing levels and their correlates and consequences in an altitudinally widespread above-tree-line species in the high Andes of Chile. *Acta Oecologica*, *30*(2), 248–257.

Arroyo, M. T. K., Pacheco, D. A., & Dudley, L. S. (2017). Functional role of long-lived flowers in preventing pollen limitation in a high elevation outcrossing species. *AoB Plants*, *9*(6), 195–212.

Badr, A., El-Shazly, H. H., Ahmed, H. I. S., Hamouda, M., El-Khateeb, E., & Sakr, M. (2017). Genetic diversity of *Achillea fragrantissima* in Egypt inferred from phenotypic variations and ISSR markers associated with traits of plant size and seed yield. *Plant Genetic Resources*, *15*(3), 239–247.

Blionis, G. J., & Vokou, D. (2002). Structural and functional divergence of *Campanula spatulata* subspecies on Mt Olympos (Greece). *Plant Systematics and Evolution*, *232*(1), 89–105.

Bridle, J. R., & Vines, T. H. (2007). Limits to evolution at range margins: When and why does adaptation fail? *Trends in Ecology & Evolution*, *22*(3), 140–147.

Burnham, K. P., & Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information-theoretic approach* (2nd Edn.). Springer.

Caetano, R. A., Sanchéz, S., Costa, C. L. N., & de Aguiar, M. A. M. (2020). Sympatric speciation based on pure assortative mating. *Journal of Physics A: Mathematical and Theoretical*, *53*(15), 155601.

Cardona, J., Lara, C., & Ornelas, J. F. (2020). Pollinator divergence and pollination isolation between hybrids with different floral color and morphology in two sympatric *Penstemon* species. *Scientific Reports, 10*(1), 8126.

Chapman, M. A., Hiscock, S. J., & Filatov, D. A. (2016). The genomic bases of morphological divergence and reproductive isolation driven by ecological speciation in *Senecio* (Asteraceae). *Journal of Evolutionary Biology*, *29*(1), 98–113.

Chevin, L.-M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biology*, *8*(4), e1000357.

Chitwood, D. H., Ranjan, A., Martinez, C. C., Headland, L. R., Thiem, T., Kumar, R., Covington, M. F., Hatcher, T., Naylor, D. T., Zimmerman, S., Downs, N., Raymundo, N., Buckler, E. S., Maloof, J. N., Aradhya, M., Prins, B., Li, L., Myles, S., & Sinha, N. R. (2014). A modern ampelography: a genetic basis for leaf shape and venation patterning in grape. *Plant Physiology*, *164*(1), 259–272.

Cierjacks, A., Rühr, N. K., Wesche, K., & Hensen, I. (2008). Effects of altitude and livestock on the regeneration of two tree line forming *Polylepis* species in Ecuador. *Plant Ecology*, *194*(2), 207–221.

Cordell, S., Goldstein, G., Mueller-Dombois, D., Webb, D., & Vitousek, P. M. (1998). Physiological and morphological variation in *Metrosideros polymorpha*, a dominant Hawaiian tree species, along an altitudinal gradient: the role of phenotypic plasticity. *Oecologia*, *113*(2), 188–196.

Corl, A., Davis, A. R., Kuchta, S. R., & Sinervo, B. (2010). Selective loss of polymorphic mating types is associated with rapid phenotypic evolution during morphic speciation. *Proceedings of the National Academy of Sciences*, *107*(9), 4254–4259.

Cruz-Nicolás, J., Giles-Pérez, G., González-Linares, E., Múgica-Gallart, J., Lira-Noriega, A., Gernandt, D. S., Eguiarte, L. E., & Jaramillo-Correa, J. P. (2020). Contrasting evolutionary processes drive morphological and genetic differentiation in a subtropical fir (*Abies*, Pinaceae) species complex. *Botanical Journal of the Linnean Society, 192*(2), 401–420.

Dai, W., Kadiori, E. L., Wang, Q., & Yang, C. (2017). Pollen limitation, plasticity in floral traits, and mixed mating system in an alpine plant *Pedicularis siphonantha* (Orobanchaceae) from different altitudes. *Journal of Systematics and Evolution*, *55*(3), 192–199.

Depardieu, C., Gérardi, S., Nadeau, S., Parent, G. J., Mackay, J., Lenz, P., Lamothe, M., Girardin, M. P., Bousquet, J., & Isabel, N. (2021). Connecting tree-ring phenotypes, genetic associations and transcriptomics to decipher the genomic architecture of drought adaptation in a widespread conifer. *Molecular Ecology*. https://doi.org/10.1111/mec.15846

Domic, A. I., & Capriles, J. M. (2009). Allometry and effects of extreme elevation on growth velocity of the Andean tree *Polylepis tarapacana* Philippi (Rosaceae). *Plant Ecology*, *205*(2), 223–234.

Domínguez, C. A., & Bullock, S. H. (1989). La reproducción de *Croton suberosus* (Euphorbiaceae) en luz y sombra. *Revista De Biología Tropical*, *37*(1), 1–9.

Donnelly, S. E., Lortie, C. J., & Aarssen, L. W. (1998). Pollination in *Verbascum thapsus* (Scrophulariaceae): The advantage of being tall. *American Journal of Botany*, *85*(11), 1618–1625.

Draghi, J. A., & Whitlock, M. C. (2012). Phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability along the major axis of environmental variation. *Evolution*, *66*(9), 2891–2902.

Espinosa, C. I., Luzuriaga, A. L., de la Cruz, M., Montero, M., & Escudero, A. (2013). Co-occurring grazing and climate stressors have different effects on the total seed bank when compared to the persistent seed bank. *Journal of Vegetation Science*, *24(*6), 1098–1107.

Espinosa, C. I., Vélez-Mora, D. P., Ramón, P., Gusmán-Montalván, E., Duncan, D. H., & Quintana-Ascencio, P. F. (2019). Intraspecific interactions affect the spatial pattern of a dominant shrub in a semiarid shrubland: A prospective approach. *Population Ecology*, *61*(2), 217–226.

Fabbro, T., & Körner, C. (2004). Altitudinal differences in flower traits and reproductive allocation. *Flora—Morphology, Distribution, Functional Ecology of Plants*, *199*(1), 70–81.

Fenster, C. B. (1995). Mirror image flowers and their effect on outcrossing rate in *Chamaecrista fasciculata* (Leguminosae). *American Journal of Botany*, *82*(1), 46–50.

Gomez-Mestre, I., & Buchholz, D. R. (2006). Developmental plasticity mirrors differences among taxa in spadefoot toads linking plasticity and diversity. *Proceedings of the National Academy of Sciences*, *103*(50), 19021–19026.

Gonzalo-Turpin, H., & Hazard, L. (2009). Local adaptation occurs along altitudinal gradient despite the existence of gene flow in the alpine plant species Festuca *eskia*. *Journal of Ecology*, *97*(4), 742–751.

Grant, B. R., & Grant, P. R. (1996). High survival of Darwin's finch hybrids: Effects of beak morphology and diets. *Ecology*, *77*(2), 500–509.

Grant, P. R. (1999). *Ecology and evolution of Darwin's finches*. Princeton University Press.

Gugerli, F. (1998). Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia*, *114*(1), 60–66.

Gurung, S., Pradhan, A., & Chettri, A. (2019). Pollination in an endemic and threatened monoecious herb *Begonia satrapis* CB Clarke (Begoniaceae) in the eastern Himalaya, India. *Journal of Threatened Taxa*, *11*(10), 14328–14333.

Halbritter, A. H., Fior, S., Keller, I., Billeter, R., Edwards, P. J., Holderegger, R., Karrenberg, S., Pluess, A. R., Widmer, A., & Alexander, J. M. (2018). Trait differentiation and adaptation of plants along elevation gradients. *Journal of Evolutionary Biology*, *31*(6), 784–800.

Hall, J. P. (2005). Montane speciation patterns in *Ithomiola* butterflies (Lepidoptera: Riodinidae): are they consistently moving up in the world? *Proceedings of the Royal Society b: Biological Sciences*, *272*(1580), 2457–2466.

Hallik, L., Niinemets, Ü., & Wright, I. J. (2009). Are species shade and drought tolerance reflected in leaf-level structural and functional differentiation in Northern Hemisphere temperate woody flora? *New Phytologist, 184*(1), 257–274.

Harder, L. D., & Prusinkiewicz, P. (2013). The interplay between inflorescence development and function as the crucible of architectural diversity. *Annals of Botany, 112*(8), 1477–1493.

Harper, J. L., & Ogden, J. (1970). The reproductive strategy of higher plants: I. The concept of strategy with special reference to *Senecio vulgaris* L. *The Journal of Ecology, 58*(3), 681–698.

Herzog, S. K., Martínez, R., Jørgensen, P. M., & Tiessen, H. (2011). *Climate change and biodiversity in the tropical Andes*. Inter-American Institute for Global Change Research (IAI) and Scientific Committee on Problems of the Environment (SCOPE).

Homeier, J., Breckle, S., Günter, S., Rollenbeck, R. T., & Leuschner, C. (2010). Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species-rich Ecuadorian montane rain forest. *Biotropica*, *42*(2), 140–148.

Huber, S. K., León, L. F. D., Hendry, A. P., Bermingham, E., & Podos, J. (2007). Reproductive isolation of sympatric morphs in a population of Darwin's finches. *Proceedings of the Royal Society B*: *Biological Sciences*, *274*(1619), 1709–1714.

Itino, T., & Hirao, A. S. (2016). Plant genetic diversity and plant– pollinator interactions along altitudinal gradients. In *Structure and function of mountain ecosystems in Japan* (pp. 63–88). Springer.

Jara-Guerrero, A., De la Cruz, M., Espinosa, C. I., Méndez, M., & Escudero, A. (2015). Does spatial heterogeneity blur the signature of dispersal syndromes on spatial patterns of woody species? A test in a tropical dry forest. *Oikos*, *124*(10), 1360–1366.

Jian, Q., Keming, M., & Yuxin, Z. (2009). Leaf-trait relationships of *Quercus liaotungensis* along an altitudinal gradient in Dongling Mountain, Beijing. *Ecological Research*, *24*(6), 1243–1250.

Jiang, S., Luo, M.-X., Gao, R.-H., Zhang, W., Yang, Y.-Z., Li, Y.-J., & Liao, P.-C. (2019). Isolation-by-environment as a driver of genetic differentiation among populations of the only broadleaved evergreen shrub *Ammopiptanthus mongolicus* in Asian temperate deserts. *Scientific Reports, 9*, 12008.

Josse, C., Cuesta, F., Navarro, G., Barrena, V., Cabrera, E., Moreno, E. C., Ferreira, W., Peralvo, M., Saito, J., & Tovar, A. (2009). *Ecosistemas de los Andes del Norte y Centro*. Universidad de los Andes.

Kremer, A., Potts, B. M., & Delzon, S. (2014). Genetic divergence in forest trees: understanding the consequences of climate change. *Functional Ecology*, *28*(1), 22–36.

Leal, M.C. (2015). *Cambios en las características morfológicas y genéticas de Croton sp. en un gradiente altitudinal en matorral seco*. Undergraduate Thesis, Universidad Técnica Particular de Loja, Ecuador.

Lenormand, T. (2002). Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, *17*(4), 183–189.

León-Yánez, S., Valencia, R., Pitmam, N., Endara, L., Ulloa, C., & Navarrete, H. (2011). *Libro rojo de plantas endémicas del Ecuador: Croton wagneri*. Pontificia Universidad Católica del Ecuador.

Levis, N. A., & Pfennig, D.W. (2020). Phenotypic plasticity and the origins of novelty. In Levine, H., Jolly, M. K., Kulkarni, P., & Nanjundiah, V. (Eds.*), Phenotypic switching: Implications in biology and medicine* (pp. 443–458). Academic Press.

Mallet, J. (2008). Hybridization, ecological races and the nature of species: Empirical evidence for the ease of speciation. *Philosophical Transactions of the Royal Society b: Biological Sciences*, *363*(1506), 2971–2986.

Matesanz, S., Ramos-Muñoz, M., Blanco-Sánchez, M., & Escudero, A. (2020). High differentiation in functional traits but similar phenotypic plasticity in populations of a soil specialist along a climatic gradient. *Annals of Botany*, *125*(6), 969–980.

Matute, D. R., Novak, C. J., & Coyne, J. A. (2009). Temperature-based extrinsic reproductive isolation in two species of *Drosophila. Evolution*, *63*(3), 595–612.

McCartney, M. A., & Lessios, H. A. (2004). Adaptive evolution of sperm bindin tracks egg incompatibility in neotropical sea urchins of the genus *Echinometra. Molecular Biology and Evolution*, *21*(4), 732–745.

McKinnon, J. S., Mori, S., Blackman, B. K., David, L., Kingsley, D. M., Jamieson, L., Chou, J., & Schluter, D. (2004). Evidence for ecology's role in speciation. *Nature*, *429*(6989), 294–298.

Medina, C. A., Escobar, F., & Kattan, G. H. (2002). Diversity and habitat use of dung beetles in a restored Andean landscape. *Biotropica*, *34*(1), 181–187.

Minelli, A. (2016). Species diversity vs. morphological disparity in the light of evolutionary developmental biology. *Annals of Botany*, *117*(5), 781–794.

Mitchell, R. J., & Shaw, R. G. (1993). Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): Clones and crosses. *Heredity*, *71*(2), 185–192.

Noble, D. W. A., Radersma, R., & Uller, T. (2019). Plastic responses to novel environments are biased towards phenotype dimensions with high additive genetic variation. *Proceedings of the National Academy of Sciences*, *116*(27), 13452–13461.

Nosil, P. (2012). *Ecological speciation*. Oxford University Press.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O´Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). vegan: Community ecology package. R package version 2.5-7 https://cran.r- project.org/web/packages/vegan/index.html

Olito, C., Abbott, J. K., & Jordan, C. Y. (2018). The interaction between sex-specific selection and local adaptation in species without separate sexes. *Philosophical Transactions of the Royal Society B, 373(*1757), 20170426.

Pais, A. L., Whetten, R. W., & Xiang, Q. Y. (2017). Ecological genomics of local adaptation in *Cornus florida* L. by genotyping by sequencing. *Ecology and Evolution*, *7*(1), 441–465.

Peakall, R., & Whitehead, M. R. (2014). Floral odour chemistry defines species boundaries and underpins strong reproductive isolation in sexually deceptive orchids. *Annals of Botany, 113*(2), 341–355.

Pélabon, C., Armbruster, W. S., & Hansen, T. F. (2011). Experimental evidence for the Berg hypothesis: Vegetative traits are more sensitive than pollination traits to environmental variation. *Functional Ecology, 25*(1), 247–257.

Pérez, F., Lavandero, N., Ossa, C. G., Hinojosa, L. F., Jara-Arancio, P., & Arroyo, M. T. K. (2020). Divergence in plant traits and increased modularity underlie repeated transitions between low and high elevations in the Andean genus *Leucheria. Frontiers in Plant Science, 11*, 714.

Pfennig, D. W., & McGee, M. (2010). Resource polyphenism increases species richness: A test of the hypothesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1540), 577–591.

Pfennig, D. W., Wund, M. A., Snell-Rood, E. C., Cruickshank, T., Schlichting, C. D., & Moczek, A. P. (2010). Phenotypic plasticity's impacts on diversification and speciation. *Trends in Ecology & Evolution*, *25*(8), 459–467.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., & R Core Team (2020). nlme: Linear and nonlinear mixed effects models. R package version 3.1 https://cran.r-project.org/web/packages/nlme/index.html

Pyrcz, T. W. (2004). Pronophiline butterflies of the highlands of Chachapoyas in northern Peru: faunal survey, diversity and distribution patterns (Lepidoptera, Nymphalidae, Satyrinae). *Genus*, *15*(4), 455–622.

Quilot-Turion, B., Leppälä, J., Leinonen, P. H., Waldmann, P., Savolainen, O., & Kuittinen, H. (2013). Genetic changes in flowering and morphology in response to adaptation to a high-latitude environment in *Arabidopsis lyrata*. *Annals of Botany*, *111*(5), 957–968.

Quintana, C., Girardello, M., Barfod, A. S., & Balslev, H. (2017). Diversity patterns, environmental drivers and changes in vegetation composition in dry inter-Andean valleys. *Journal of Plant Ecology*, *10*(3), 461–475.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/

Radersma, R., Noble, D. W. A., & Uller, T. (2020). Plasticity leaves a phenotypic signature during local adaptation. *Evolution Letters*, *4*(4), 360–370.

Ramírez-Aguirre, E., Martén-Rodríguez, S., Quesada-Avila, G., Quesada, M., Martínez-Díaz, Y., Oyama, K., & Espinosa-García, F. J. (2019). Reproductive isolation among three sympatric *Achimenes* species: pre- and post-pollination components. *American Journal of Botany*, *106*(7), 1021–1031.

Ramos-Jiliberto, R., Domínguez, D., Espinoza, C., López, G., Valdovinos, F. S., Bustamante, R. O., & Medel, R. (2010). Topological change of Andean plant–pollinator networks along an altitudinal gradient. *Ecological Complexity*, *7*(1), 86–90.

Ramsey, J., Bradshaw, H. D., & Schemske, D. W. (2003). Components of reproductive isolation between the monkeyflowers *Mimulus lewisii* and *M. cardinalis* (Phrymaceae). *Evolution*, *57*(7), 1520–1534.

Richter, M., Diertl, K.-H., Emck, P., Peters, T., & Beck, E. (2009). Reasons for an outstanding plant diversity in the tropical Andes of Southern Ecuador. *Landscape Online*, *12*(1), 1–35.

Richter, M., & Moreira-Muñoz, A. (2005). Heterogeneidad climática y diversidad de la vegetación en el sur de Ecuador: un método de fitoindicación. *Revista Peruana De Biología*, *12*(2), 217–238.

Rojo, J. H., Fernández, D. A., Figueroa, D. E., & Boy, C. C. (2020). Phenotypic and genetic differentiation between diadromous and landlocked puyen *Galaxias maculatus*. *Journal of Fish Biology*, *96*(4), 956–967.

Rundle, H. D., Chenoweth, S. F., Doughty, P., & Blows, M. W. (2005). Divergent selection and the evolution of signal traits and mating preferences. *PLoS Biology*, *3*(11), e368.

Rundle, H. D., & Nosil, P. (2005). Ecological speciation. *Ecology Letters*, *8*(3), 336–352.

Scheepens, J. F., Frei, E. S., & Stöcklin, J. (2010). Genotypic and environmental variation in specific leaf area in a widespread Alpine plant after transplantation to different altitudes. *Oecologia*, *164*(1), 141–150.

Schliewen, U., Rassmann, K., Markmann, M., Markert, J., Kocher, T., & Tautz, D. (2001). Genetic and ecological divergence of a monophyletic cichlid species pair under fully sympatric conditions in Lake Ejagham, Cameroon. *Molecular Ecology*, *10*(6), 1471–1488.

Schluter, D. (2000). *The ecology of adaptive radiation*. Oxford University Press.

Seguí, J., Lázaro, A., Traveset, A., Salgado-Luarte, C., & Gianoli, E. (2018). Phenotypic and reproductive responses of an Andean violet to environmental variation across an elevational gradient. *Alpine Botany*, *128*(1), 59–69.

Sexton, J. P., Strauss, S. Y., & Rice, K. J. (2011). Gene flow increases fitness at the warm edge of a species' range. *Proceedings of the National Academy of Sciences*, *108*(28), 11704–11709.

Shaw, K. L., & Mullen, S. P. (2011). Genes versus phenotypes in the study of speciation. *Genetica*, *139*(5), 649–661.

Shivanna, K. R., & Tandon, R. (2014). *Reproductive ecology of flowering plants*: *A manual*. Springer.

Sierra, R. (1999). *Propuesta preliminar de un sistema de clasificación de vegetación para el Ecuador continental*. Proyecto INEFAN/GEF-BIRF y EcoCiencia.

Snell, H., & Rea, S. (1999). The 1997–98 El Niño in Galápagos: Can 34 years of data estimate 120 years of pattern? *Noticias De Galápagos*, *60*, 111–120.

Sobel, J. M., & Chen, G. F. (2014). Unification of methods for estimating the strength of reproductive isolation. *Evolution*, *68*(5), 1511–1522.

Sun, S. J., Catherall, A. M., Pascoal, S., Jarrett, B. J. M., Miller, S. E., Sheehan, M. J., & Kilner, R. M. (2020). Rapid local adaptation linked with phenotypic plasticity. *Evolution Letters*, *4*(4), 345–359.

Torres-Díaz, C., Gómez-González, S., Stotz, G. C., Torres-Morales, P., Paredes, B., Pérez-Millaqueo, M., & Gianoli, E. (2011). Extremely long-lived stigmas allow extended cross-pollination opportunities in a high Andean plant. *PLoS ONE*, *6*(5), e19497.

Uller, T., Feiner, N., Radersma, R., Jackson, I. S., & Rago, A. (2020). Developmental plasticity and evolutionary explanations. *Evolution & Development*, *22*(1–2), 47–55.

Ulloa, C., & Jørgensen, P. (1995). *Árboles y arbustos de los Andes del Ecuador* (2nd Edn). Abya-Yala.

Van der Niet, T., Peakall, R., & Johnson, S. D. (2014). Pollinator-driven ecological speciation in plants: new evidence and future perspectives. *Annals of Botany*, *113*(2), 199–212.

van Ee, B. W., Riina, R., & Berry, P. E. (2011). A revised infrageneric classification and molecular phylogeny of new world *Croton* (Euphorbiaceae). *Taxon*, *60*(3), 791–823.

Vélez-Mora, D., Ramón, P., Vallejo, C., Romero, A., Duncan, D., & Quintana-Ascencio, P. F. (2020). Environmental drivers of femaleness of an inter-Andean monoecious shrub. *Biotropica*, *53*(1), 17–27.

Vile, D., Garnier, E., Shipley, B., Laurent, G., Navas, M. L., Roumet, C., Lavorel, S., Díaz, S., Hodgson, J. G., Lloret, F., Midgley, G. F., Poorter, H., Rutherford, M. C., Wilson, P. J., & Wright, I. J. (2005). Specific leaf area and dry matter content estimate

thickness in laminar leaves. *Annals of Botany*, *96*(6), 1129–1136.

Vogel, S. (2009). Leaves in the lowest and highest winds: temperature, force and shape. *New Phytologist*, *183*(1), 13–26.

Walter, G. M., Aguirre, J. D., Blows, M. W., & Ortiz-Barrientos, D. (2018). Evolution of genetic variance during adaptive radiation. *The American Naturalist*, *191*(4), E108–E128.

Walter, G. M., Abbott, R. J., Brennan, A. C., Bridle, J. R., Chapman, M., Clark, J., Filatov, D., Nevado, B., Ortiz-Barrientos, D., & Hiscock, S. J. (2020). *Senecio* as a model system for integrating studies of genotype, phenotype and fitness. *New Phytologist*, *226*(2), 326–344.

Wang, J., Zhao, X., Wang, W., Qu, Y., Teng, W., Qiu, L., Zheng, H., Han, Y., & Li, W. (2019). Genome-wide association study of inflorescence length of cultivated soybean based on the highthroughout single-nucleotide markers. *Molecular Genetics and Genomics*, *294*(3), 607–620.

Webster, G. L. (1993). A provisional synopsis of the sections of the genus *Croton (Euphorbiaceae)*. *Taxon*, *42*(4), 793–823.

Webster, G. L. (2014). Euphorbiaceae. In Kubitzki K. (Ed), *Flowering plants*. *Eudicots* (pp. 51–216). Springer.

White, N. J., Snook, R. R., & Eyres, I. (2020). The past and future of experimental speciation. *Trends in Ecology & Evolution*, *35*(1), 10–21.

Willmer, P. (2011). *Pollination and floral ecology*. Princeton University Press.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., Flexas, J., Garnier, E., Groom, P. K., Gulias, J., Hikosaka, K., Lamont, B. B., Lee, T., Lee, W., Lusk, C., & Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821–827.

Wu, X., Li, Y., Shi, Y., Song, Y., Zhang, D., Li, C., Buckler, E. S., Li, Y., Zhang, Z., & Wang, T. (2016). Joint-linkage mapping and GWAS reveal extensive genetic loci that regulate male inflorescence size in maize. *Plant Biotechnology Journal*, *14*(7), 1551–1562.

Zhao, Z. G., & Wang, Y. K. (2015). Selection by pollinators on floral traits in generalized Trollius ranunculoides (Ranunculaceae) along altitudinal gradients. *PLOS ONE*, *10*(2), e0118299.

Zhu, Y., Jiang, Y., Liu, Q., Kang, M., Spehn, E. M., & Körner, C. (2009). Elevational trends of biodiversity and plant traits do not converge—A test in the Helan Range, NW China. *Plant Ecology*, *205*(2), 273–283.

Supplementary Information

Title: Evidence of morphological divergence and reproductive isolation in a narrow elevation gradient

Journal: Evolutionary Biology

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Table S1. AICc compare the models for Leaf thickness

Model	**dAICc**	**df**	**weight**
Leaf thickness ~ Specific leaf area + Elevation	0	7	0.67
*Leaf thickness ~ Specific leaf area × Elevation	1.4	9	0.33
Leaf thickness ~ Elevation	75.4	6	<0.001

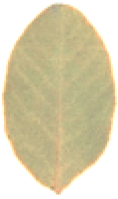
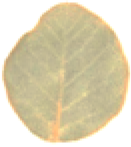
*Leaf thickness ~ Specific leaf area + Elevation + Specific leaf area × Elevation. The dAICc column show the relative difference in AICc between the best-supported model and other models. The weight column contains the Akaike weights, which sum up to one and are a measure of proportionality for each model.

Table S2. ANOVA of the model Leaf thickness ~ Specific leaf area + Elevation

Source of variation	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	155	285.04	**<0.001**
Specific leaf area	1	155	79.06	**<0.001**
Elevation	2	3	8.59	0.057

numDF: Degrees of freedom of numerator; denDF: Degrees of freedom of denominator

Table S3. Summary of model Leaf thickness ~ Elevation + Specific leaf area.

Source of variation	**Effect size**	**SE**	**DF**	**t-value**	**p value**
High Elevation (reference) 	1499.80	124.35	155	12.06	**<0.001**
Medium Elevation 	-188.96	49.96	3	-3.78	**0.032**
Low Elevation 	-183.83	54.05	3	-3.40	**0.042**
Specific Leaf Area (cm^{2}g^{-1})	-133822.44	13937.06	155	-9.60	**<0.001**

The elevation and specific leaf area clearly influenced leaf thickness. The AIC value for this model was 2847.897. In this model we evaluate random effects. The first one was plants within sites equal to 101.7, the second one was sites equal to 0.02, and residual of equal 201.4. The value of R^2m was 0.339 and R^2c was 0.473. R^2m indicates the variance explained only by fixed effects and R^2c indicates the variance explained by fixed and random effects (Nakagawa and Schielzeth 2013).

Table S4. AICc compare the models for Number of inflorescences (logarithm transform)

Model	**dAICc**	**df**	**weight**
Number of inflorescences ~ Elevation + Log plan volume	0	7	0.967
*Number of Inflorescences ~ Elevation × Log plan volume	6.8	9	0.033
Number of inflorescences ~ Elevation	42.9	6	<0.001

*Number of inflorescences ~ Elevation + Log plan volume + Elevation × Log plan volume.

Table S5. ANOVA of the model Number of inflorescences ~ Elevation + Log plan volume

Source of variation	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	49	9007.21	<0.0001
Log plan volume	1	49	51.52	<0.0001
Elevation	2	3	13.82	0.0306

Table S6. Summary of model Number of inflorescences ~ Elevation + Log plan volume. In this model we evaluate random effects.

Source of variation	**Effect size**	**SE**	**DF**	**t-value**	**p value**
Low Elevation (reference)	-5.71	1.18	49	-4.84	**<0.001**
Log plan volume	0.74	0.08	49	8.83	**<0.001**
High Elevation	0.74	0.14	3	5.19	**0.014**
Medium Elevation	0.33	0.13	3	2.46	0.091

The elevation and plant volume clearly influenced number of inflorescences. The AIC value for this model was 70.889. In this model we evaluate random effects. The first one was plants within sites equal to 0.37, the second one was sites equal to 0.000008, and residual of equal 0.002. The value of R^2m was 0.59 and R^2c was 0.999.

Table S7. AICc compare the models for inflorescence length

Source of variation	**dAICc**	**df**	**weight**
Inflorescences lenght ~ Elevation	0	6	0.53
Inflorescences lenght ~ Elevation + Log plan volume	0.8	7	0.36
*Inflorescences lenght ~ Elevation × Log plan volume	3.2	9	0.11

*Inflorescences lenght ~ Elevation + Log plan volume + Elevation × Log plan volume

Table S8. ANOVA of the model Inflorescences length ~ Elevation + Log plan volume

Source of variation	**numDF**	**denDF**	**F-value**	**p-value**
(Intercept)	1	49	780.8979	**<0.0001**
Log plan volume	1	49	11.7771	**0.0012**
Elevation	2	3	4.5160	0.1245

Table S9. Summary of model Inflorescence length ~ Elevation + Log plan volume

Source of variation	**Effect size**	**SE**	**DF**	**t-value**	**p value**
(Intercept)	-0.17	4.91	49	-0.03	0.973
Log plan volume	0.52	0.35	49	1.49	0.144
High Elevation	-1.76	0.59	3	-2.98	0.059
Medium Elevation	-1.16	0.55	3	-2.09	0.128

The elevation influenced inflorescence length. The AIC value for this model was 219.046. In this model we evaluate random effects. The first one was plants within sites equal to 1.552, the second one was sites equal to 0.0006, and residual of equal 0.02. The value of R^2m was 0.274 and R^2c was 0.999.

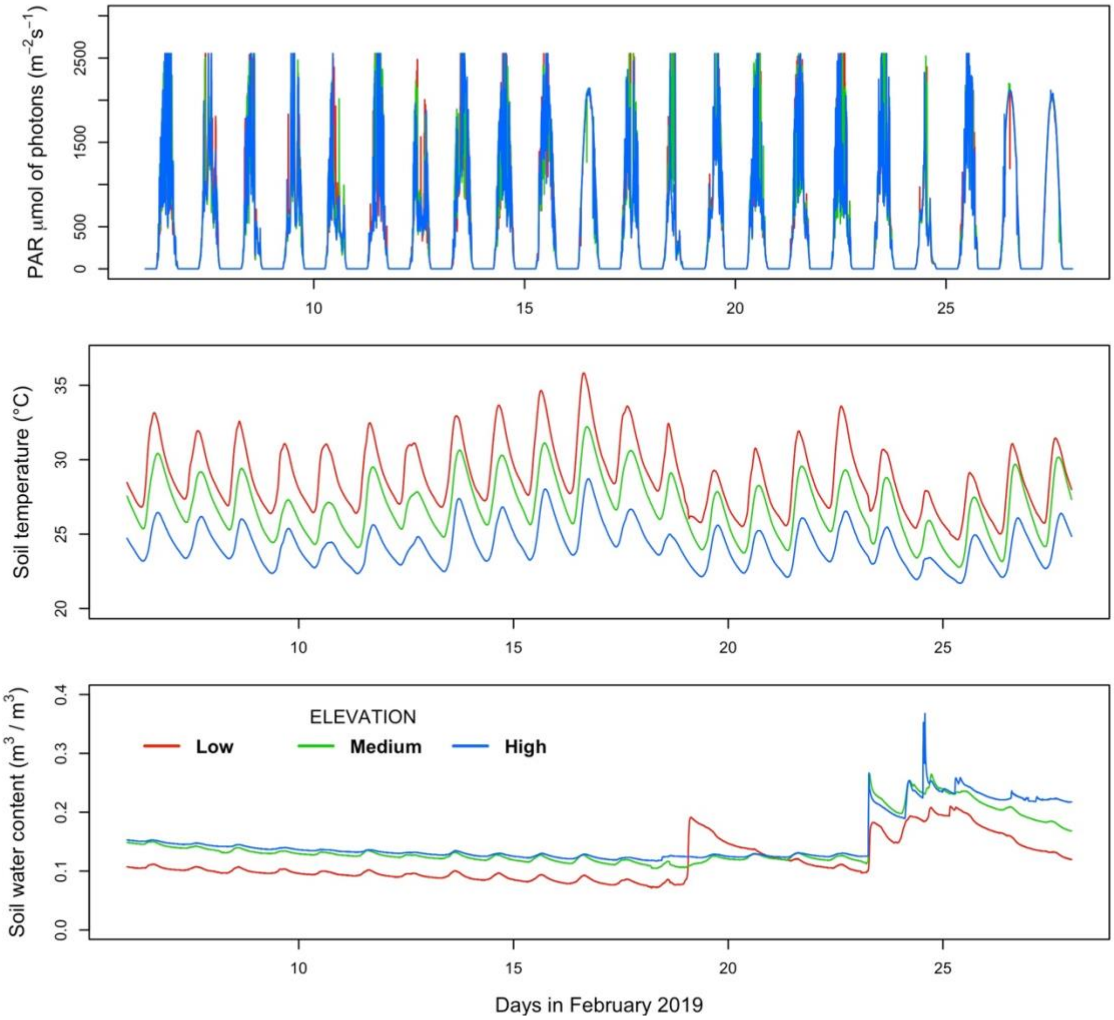


Figure S1. Variation between elevations of photosynthetically active radiation, soil temperature, and soil water content. The observations correspond to days between February 1 and 28, 2019. At Low Elevation the soil temperature is high, and the water content is low.

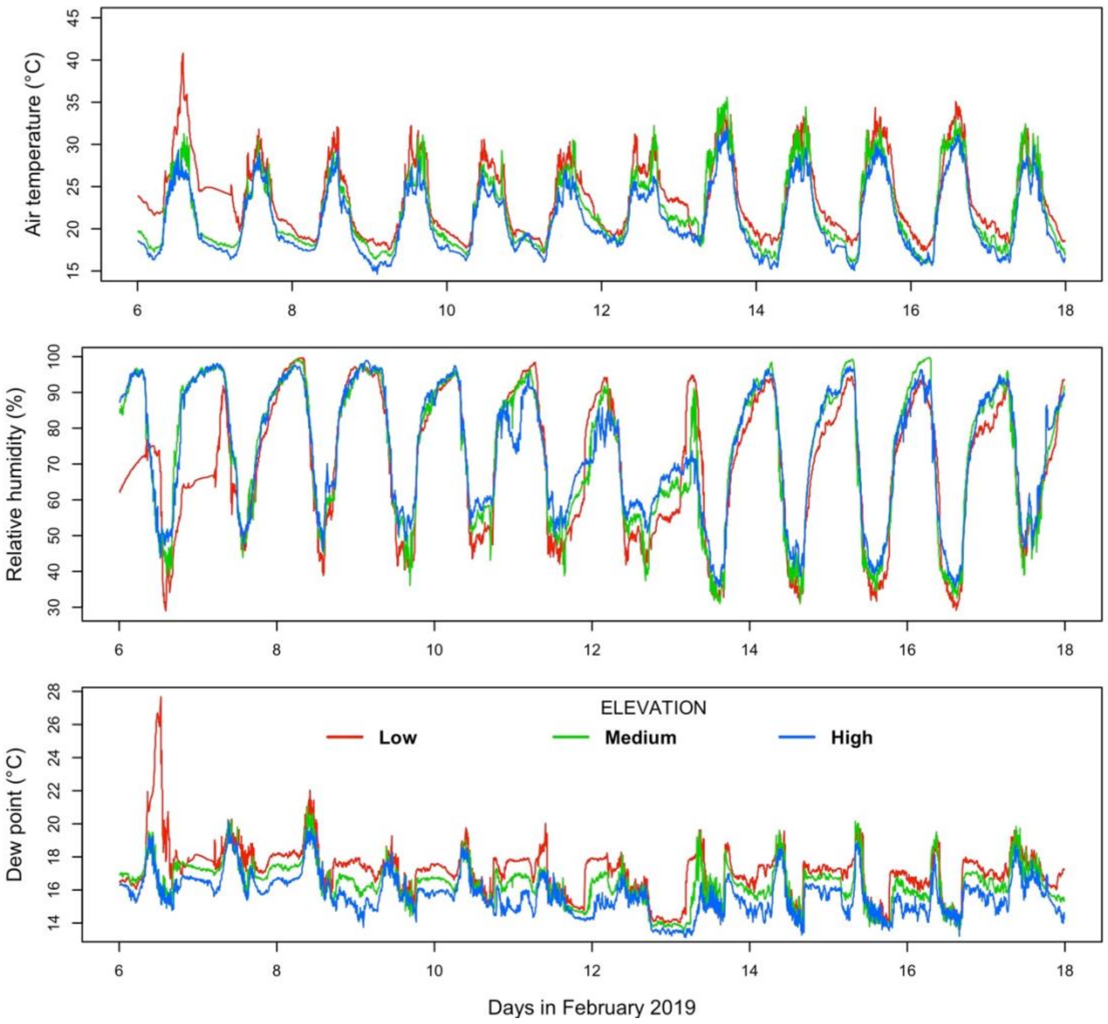


Figure S2. Variation between elevations of dew point, relative humidity, and air temperature. The observations correspond to days between February 1 and 28, 2019. When selecting observations from sunrise (600) to sunset (1800) figure shows the observations from February 6 to 18. At Low Elevation dew point is high.

Figure S3. Pollinating *Croton*. Cotton gloves were used due to the intense solar radiation of study site.

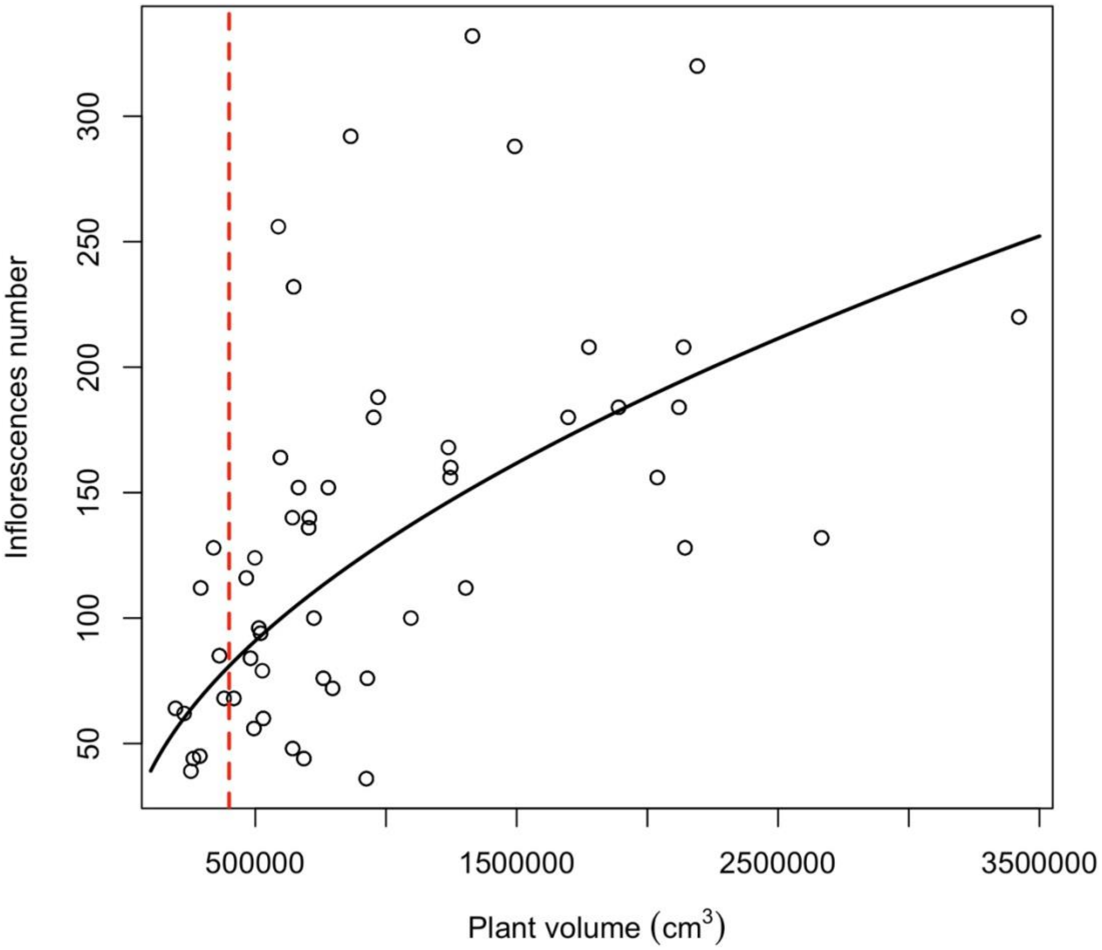


Figure S4. Inflorescences number as a function of plant volume. Open circles after the dashed red line (400000 cm^3) show larger individuals with a greater inflorescences number and a larger plant size, suggesting that they are reproductive plants (Espinosa, Vélez-Mora et al. 2019).

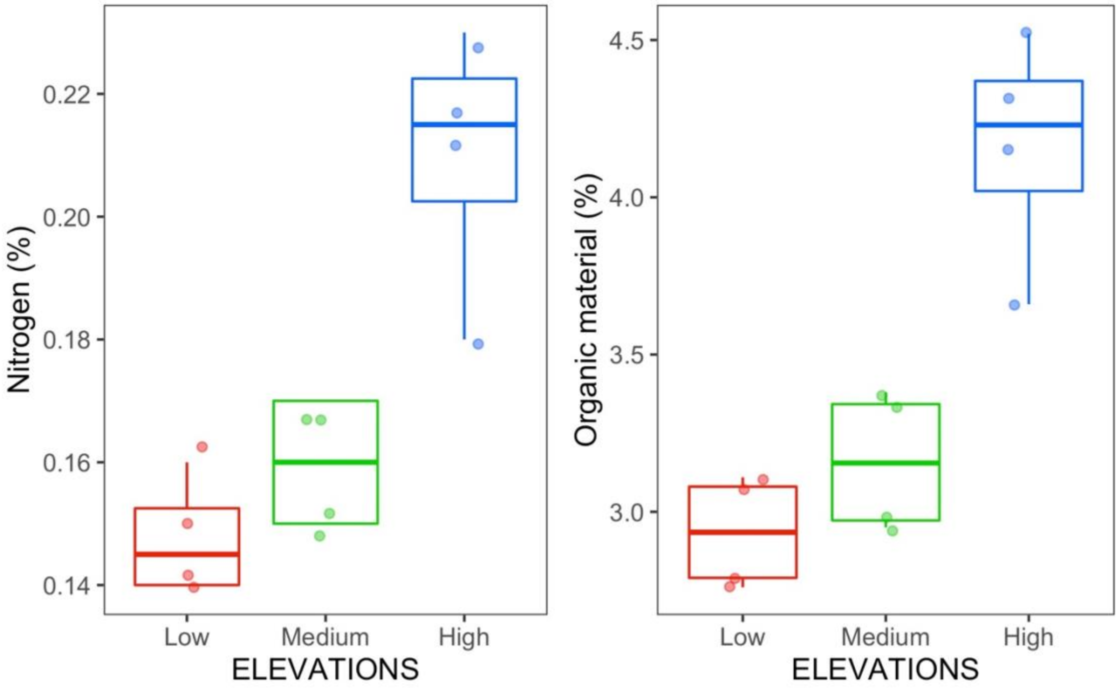


Figure S5. The differences of nitrogen (%) and organic matter (%) between elevations are shown. The High Elevation clearly has a high percentage of nitrogen and organic matter compared to the other two elevations. For soil analyzes, four samples were collected per elevation. Each soil sample is the combination of four soil subsamples collected at each elevation.

DISCUSIÓN GENERAL

Esta disertación aporta información sobre los determinantes bióticos y abióticos de la expresión sexual del arbusto monoico *Croton*, la variación espacial de su expresión sexual, sincronía reproductiva, supervivencia, crecimiento, reproducción, reclutamiento de plántulas, y la diversidad vegetal asociada a *Croton*. También contribuye al conocimiento sobre procesos de divergencia morfológica y aislamiento reproductivo a lo largo de gradientes de elevación en un matorral seco interandino al sur de Ecuador.

La variación de temperatura y humedad, el tamaño de la planta y la ocurrencia de vecinos conespecíficos tienen una influencia en la producción de flores femeninas en especies unisexuales. En el Capítulo 1 mostramos cómo los sitios con diferente elevación, el tamaño de la planta y la cobertura vegetal influyeron en la expresión sexual de *Croton* y en la agregación de la feminidad. La facilitación de la cobertura vegetal conespecífica podría promover patrones de agregación de feminidad en especies monoicas en sitios con temperaturas más moderadas en el suelo y con menor contenido de humedad. El dosel arbustivo puede mejorar las condiciones microambientales, la acumulación de materia orgánica, los nutrientes en el suelo (Moro, Pugnaire, Haase y Puigdefábregas, 1997) y mejorar la disponibilidad de agua (Holmgren, Scheffer y Houston, 1997, Sagar, Pandey y Singh, 2012) haciendo que estas condiciones en los vecindarios de conespecíficos sean más favorables para la producción y desarrollo de flores femeninas en *Croton* (Butterfield, Bradford, Armas, Prieto y Pugnaire, 2016; Holmgren, Scheffer y Houston, 1997; Lara-Romero et al. 2016; Lara-Romero et al. 2017; Pugnaire, y Luque 2001). Debido a la variación de condiciones de temperatura y humedad entre elevaciones, las plantas dependen de plasticidad fenotípica para adaptarse a posibles cambios extremos en estos ambientes. Estas adaptaciones podrían establecerse evolutivamente en la población provocando una diferenciación genética entre poblaciones.

Los gradientes de elevación generan diferentes condiciones ambientales. Esta diferenciación ambiental provoca selección divergente originando cambios adaptativos plásticos y probablemente un aislamiento reproductivo. En el Capítulo 4 observamos una variación en los rasgos morfológicos vegetativos y reproductivos de *Croton* y un aislamiento reproductivo incipiente entre poblaciones a lo largo del gradiente de elevación. Nuestros resultados mostraron menor área foliar específica en la Elevación Baja probablemente debido a un mayor estrés hídrico del suelo. Otros estudios muestran tendencias similares en donde el área foliar específica y la conductancia estomática aumentan con la elevación para reducir el estrés hídrico (p. ej. *Viola maculata*; Seguí et al. 2018).

La producción de frutos y semillas fue diferente entre elevaciones sugiriendo una posible limitación de polinizadores en *Croton*, especialmente en la Elevación Alta. En este estudio, no evaluamos la diversidad de polinizadores asociados con la polinización de *Croton*, sin embargo, otros estudios han mostrado una reducción en la abundancia y diversidad de polinizadores cuando aumenta la elevación en la región andina (Arroyo et al. 2006, Ramos-Jiliberto et al. 2010, Torres-Díaz et al., 2011). La posible escasez o ineficacia de los polinizadores para garantizar la dispersión del polen en elevaciones altas y la dispersión limitada de semillas de *Croton* (Espinosa et al., 2019) podría restringir el intercambio de genes entre poblaciones a diferentes elevaciones (Cardona et al., 2020; van der Niet et al., 2014). Las diferencias en temperatura y humedad entre elevaciones podrían generar selección tanto de plantas como de polinizadores (Cardona et al., 2020; Halbritter et al., 2018). El cambio en la composición de los insectos podría también ocasionar una restricción del flujo de genes entre elevaciones y favorecer la adaptación local de *Croton* en cada elevación (Peakall & Whitehead, 2014; van der Niet et al., 2014).

La variación de temperatura y humedad a lo largo de un gradiente de elevación impone importantes restricciones para el desarrollo de poblaciones vegetales en ecosistemas estacionalmente secos. Otros factores como la disponibilidad de nutrientes y el pastoreo también influyen en la reproducción, supervivencia y crecimiento vegetal. La variación de temperatura y humedad del suelo, los nutrientes y el tamaño de la planta pueden desacoplar el tiempo de floración entre parejas potenciales en especies unisexuales, produciendo flores femeninas y masculinas entre plantas en diferentes tiempos. El solapamiento temporal de organismos con sus parejas potenciales y recursos para la reproducción es conocido como sincronía floral o reproductiva. En el Capítulo 2 documentamos que las plantas de *Croton* de mayor tamaño, en la elevación más baja (que presentó las temperaturas más altas y los sitios con mayor disponibilidad de nitrógeno) aumentaron la sincronía reproductiva. El aumento de la sincronía reproductiva, de temperatura y de N y P incrementaron la producción de frutos y la probabilidad de germinación de *Croton*. Estos resultados coinciden con otros estudios que indican que la floración y los polinizadores pueden sincronizarse con los cambios de humedad y temperatura (Bolmgren, 1998; Inouye et al., 2003). Los cambios impuestos por el clima y los nutrientes en la fenología floral también pueden desacoplar la sincronía planta-polinizador, reduciendo potencialmente la producción de semillas (Elzinga et al., 2007). Estos resultados sugieren que una reducción en la sincronía de floración podría afectar el éxito reproductivo de *Croton*.

La variación de temperatura y humedad del suelo, los nutrientes, el pastoreo y la interacción de facilitación planta-planta influyen en la demografía vegetal. En Capítulo 3 comprobamos que cambios en temperatura, humedad, niveles de pastoreo y disponibilidad de nutrientes pueden alterar la demografía de *Croton*. La exclusión del pastoreo benefició la supervivencia de *Croton*, particularmente en la Elevación Alta donde la presencia de animales se ha incrementado en los últimos años. La reducción del ganado en estas áreas probablemente pueda mejorar la supervivencia de las plantas de *Croton*. Las plantas de *Croton* en áreas sujetas a la adición de P y N+P sin pastoreo en las elevaciones Baja y Media, tuvieron menor supervivencia con respecto a las áreas de control. La adición de N y P en estas parcelas sin pastoreo puede haber aumentado la densidad de otras especies, principalmente pastos que pueden haber disminuido la supervivencia de *Croton*. Estos resultados indican que los efectos de pastoreo en el matorral seco interandino están condicionados por otros factores como el pastoreo y la presencia de otras especies que ocurren concomitantemente. El aporte continuo de N a través de la volatilización y escorrentía de fertilizantes de cultivos cercanos (Moreno et al. 2018; Larios-González et al. 2021), excrementos de ganado (Scharpenseel et al. 1996), deposición de cenizas por quema de caña de azúcar (Jaramillo 2011; Guamán et al. 2012), y la casi ausencia de materia orgánica y la baja disponibilidad de agua en la Elevación Baja parecen afectar la supervivencia de *Croton* en esta elevación (Gallego-Zamorano et al. 2022).

La adición de N+P en sitios sin pastoreo mejoró el crecimiento de *Croton* en todas las elevaciones. Esto sugiere que la exclusión del pastoreo, la disponibilidad de nutrientes y de agua del suelo (Schulte Uebbing et al. 2021) pueden mejorar el crecimiento de *Croton* si los efectos de los nutrientes no entran en conflicto con un aumento en la densidad de otras especies vegetales. En la Elevación Alta, el aumento en la disponibilidad de P en el suelo favoreció el crecimiento de pastos probablemente perjudicando el crecimiento de *Croton* (Daws et al. 2021). Para promover el crecimiento de *Croton* es fundamental considerar un suministro balanceado de N y P de acuerdo con las necesidades de cada elevación junto con un pastoreo controlado.

La producción de frutos en *Croton* se incrementó en la Elevación Baja con la adición de N y en la Elevación Media con la adición de N y P. Equilibrar la disponibilidad de N y P del suelo puede ser importante en esta elevación para incrementar la producción frutos y semillas de esta especie como se ha mostrado en otras especies (ver Kramer y Kozlowski 1979; Drenovsky y Richards 2005).

Nuestros datos corroboran que la supervivencia de las plántulas de *Croton* disminuye a medida que se alejan del dosel de las plantas facilitadoras. La presencia de suficiente dosel de *Croton* parece ser fundamental para el establecimiento y reclutamiento de plántulas en estos ambientes xéricos (Lara-Romero et al. 2017, Jonge et al. 2021). La disminución en la supervivencia de *Croton* debido al pastoreo puede alterar severamente su estructura poblacional y, en consecuencia, reducir la cobertura vegetal que protege a sus congéneres y otras especies de los sistemas arbustivos secos interandinos (Espinosa et al. 2013, 2019).

Recomendaciones

Nuestros resultados nos permiten advertir que los efectos combinados del cambio climático, la alteración del equilibrio Carbono-Nitrógeno-Fósforo (debido al aumento en la deposición de nitrógeno) y el pastoreo afectan la densidad y reproducción de una especie clave para el mantenimiento de la biodiversidad y funcionamiento del matorral seco interandino al sur de Ecuador. Es necesario trabajar en el desarrollo de herramientas conceptuales y de manejo que nos ayuden a comprender la relación a largo plazo entre el clima, el pastoreo, actividades agrícolas y la dinámica del matorral seco interandino, para que contribuyan a la conservación, educación y al desarrollo económico de los habitantes de estas regiones.

A pesar de su importancia, los ecosistemas secos se encuentran entre los ecosistemas más amenazados del mundo y menos estudiados, lo que podría ponerlos en mayor riesgo frente a los bosques húmedos (Janzen 1988, Miles et al. 2006, Portillo-Quintero y Sánchez-Azofeifa 2010, Aide et al. 2012, Gillespie et al. 2012). Los bienes y servicios que brindan los ecosistemas secos son significativamente diferentes de los de los bosques húmedos, por lo que requieren diferentes enfoques de manejo y conservación (Wunder 2001; Makonda y Gillah 2007; Gumbo et al. 2010). En Ecuador, el matorral seco interandino, con el 5% de superficie, casi no está representado en el sistema estatal de áreas protegidas (Ron 2020) y se ha convertido en un ecosistema amenazado y fragmentado (Cabrera 2002, Aguirre-Mendoza y Kvist 2005), donde las especies están aisladas y tienen pocas oportunidades de migrar. Por lo tanto, una prioridad de conservación en Ecuador debería ser la creación de áreas protegidas que incluyan remanentes significativos de matorral seco interandino (DRYFLOR et al. 2016, Quintana et al. 2016). Si la degradación continúa, se producirán pérdidas considerables de biodiversidad, incluidos algunos de los linajes más antiguos típicos de estos bosques (Särkinen et al. 2012) y los servicios ecosistémicos (Quintana et al. 2016).

Un enfoque con múltiples agentes es una opción viable para atender los problemas multidimensionales y heterogéneos en este ecosistema. Un consorcio entre universidades, organismos gubernamentales y no gubernamentales debe contribuir a la investigación y formulación de políticas y leyes para la conservación de estos ecosistemas secos. Aunque promulgar políticas prudentes por sí solo no es una solución para todos los problemas, los gobiernos también deben interesarse en reconocer los ecosistemas secos como una opción de medios de vida viable e invertir en su gestión sostenible (Bekele y Girmay 2014). Para llamar la atención de los responsables políticos y los agentes de desarrollo, se requiere una fuerte evidencia empírica de los beneficios económicos y la importancia ambiental de estos ecosistemas (Siyum 2020). Por lo tanto, es necesario continuar con investigación para la gestión sostenible del matorral seco interandino.

CONCLUSIONES GENERALES

1. Nuestro estudio mostro cómo factores como sitios con diferente elevación, el tamaño de la planta y la cobertura vegetal pueden influir en la expresión de la feminidad y en la agregación espacial de la feminidad plantas monoicas. La expresión de la feminidad en especies monoicas puede verse influenciada por los mismos factores que influyen en la segregación espacial de plantas femeninas en especies dioicas.
2. Señalamos que los patrones espaciales encontrados en el sitio de menor elevación, donde la temperatura del suelo es más alta y con menor contenido de humedad, respaldan la hipótesis de que la feminidad en especies monoicas puede verse favorecida por interacciones de facilitación conespecífica.
3. Observamos variación morfológica y aislamiento reproductivo incipiente en *Croton* en condiciones climáticas contrastantes en el gradiente de elevación.
4. Los experimentos de polinización dentro de cada elevación sugieren la posibilidad de una limitación de polinizadores en *Croton* en la Elevación Alta. La Elevación Alta presentó menor número de frutos y semillas en comparación con la Elevación Baja.
5. Las plantas más grandes en las elevaciones Baja y Media, y aquellas en sitios con mayor disponibilidad de nitrógeno tuvieron mayor sincronía reproductiva en *Croton*.
6. El aumento de la sincronía reproductiva en la Elevación Baja y la adición de N y P en la Elevación Media se asoció con un incremento en la producción de frutos y la probabilidad de germinación de *Croton*, aunque el aumento de la sincronía de floración también se asoció con incrementos en la probabilidad de depredación de semillas, especialmente en la Elevación Alta.
7. El incremento en la cobertura de *Croton* puede aumentar la abundancia y diversidad de plantas en el matorral seco interandino. La presencia de *Croton* en este matorral seco no solo parece favorecer la continuidad de individuos conspecíficos, sino

también a otros miembros de la comunidad vegetal. La interacción positiva entre *Croton* con otras especies debe considerarse en planes de restauración y conservación para garantizar el funcionamiento y los servicios que brinda este ecosistema.