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Co-limitation of resources reveals adaptations of a tropical tree to heterogeneous environments along an elevational cline

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Introduction: Plant species often exhibit significant variation in functional traits in populations along elevational gradients to cope with varying stress conditions. While plant development has been assumed to be most limited by a single resource, growing evidence suggests the potential for interactions of co-limiting resources to impact plant performance. Here, we aimed to determine how light, nitrogen, and water availability influence the growth and physiology of different populations of koa (*Acacia koa*), a tree species of concern that occurs across a large elevational gradient in tropical Hawaii, United States.

Methods: Populations from three seed sources [low (L), mid (M), and high (H) elevation] were grown in a controlled greenhouse experiment and exposed to co-limiting light, water, and nutrient (nitrogen) conditions. Light response, gas exchange, water status, resource use efficiency, nutrients and shoot non-structural carbohydrate concentrations, and growth and biomass allocation responses were quantified.

Results: We found that resource co-limitation sometimes interacted to determine responses of the measured parameters. In general, the low elevation (L) koa population was more sensitive to conditions where both moisture and nutrients were limiting, while the high elevation (H) koa population was more sensitive to conditions where either light and moisture or light and nitrogen were co-limiting. The M population performed well overall regardless of resource limitation.

Discussion: Our findings lend support to the theory that multiple resources limit growth and physiology of populations rather than the traditional view of a single resource limiting performance. Therefore, the possibility that multiple resource limitations drive population differences should be considered when developing population-based guidelines for forest and tree species restoration.

KEYWORDS

populations, resource co-limitation, resource use efficiency, acclimation, adaptation, *Acacia koa*

Introduction

Plant species often exhibit significant variation in functional traits in populations along elevational gradients. This variance might increase further in response to greater environmental heterogeneity anticipated under future climatic conditions (Begon et al., 1996; Araújo and Rahbek, 2006; Bigelow and Canham, 2007). Changes in elevation are accompanied by substantial spatial and temporal variability in resource availability such as light (quality and quantity) and edaphic conditions (nutrient and water availability), which are mostly modulated by temperature (Battaglia et al., 2000). At lower elevations in some systems, plants can take advantage of high nitrogen and water resources (Raich and Schlesinger, 1992) but have to deal with increased competition for light. Conversely, at higher elevations where soil nutrients and moisture are often limited, light is readily available (Körner et al., 1989; Körner, 2012). Plants growing in each environment will require different adaptations and acclimation strategies to cope with the various stress conditions (Somero, 2010).

Species adaptations in response to local environments can create substantial phenotypic differentiation among populations (Ghalambor et al., 2007). Intraspecific phenotypic variation can reflect micro-geographic adaptation and divergent selection (Richardson et al., 2014). Phenotypic plasticity can change trait expression rapidly within generations and may differ drastically across populations in dissimilar resource environments (West-Eberhard, 1989). One such example can be found in populations established across gradients of water availability, where the selection may favor a phenotype that has enhanced drought tolerance in the driest sites (e.g., transpiration, growth, etc.; Crispo, 2008; Paudel et al., 2021). Recent studies in various systems have established the ecological implications of intraspecific phenotypic variation, like selecting populations for species restoration based on their response to resource limitation (Matesanz et al., 2020) and adaptability to future climates (Cocozza et al., 2016). The tendency and ability of individuals or populations to acclimate is dictated by their phenotypic plasticity and specific response to various resource limitations.

The adaptive phenotypes of populations of a species influence their response to resource limitations. Traditionally according to Liebig's Law, plant performance was assumed to be limited by the single resource in the scarcest supply relative to demand; and this limitation was thought to largely determine plant responses in a specific environment. However, there is growing evidence suggesting that, most likely, more than one resource may act simultaneously and synergistically to impact plant growth (Gleeson and Tilman, 1992; Eskelinen and Harrison, 2015; Zandalinas and Mittler, 2022) indicating resource co-limitation (Zandalinas et al., 2021; Pascual et al., 2023). Plants undergo a series of structural, anatomical, and physiological adaptations to adjust for co-limiting resources (Holmgren, 2000; Quero et al., 2008), which may become increasingly important under future climatic conditions (Eskelinen and Harrison, 2015).

Site conditions for plant establishment and growth are often resource-limited by more than one factor and plants may perform poorly when additional stress is superimposed. Identifying populations within species that can better cope with co-limiting resource conditions is little studied and complex as it requires a documentation of the amount of phenotypic plasticity within and among populations (Ernesto and Ferendo, 2012). Investigations into resource co-limitation may reveal some of the mechanisms of how different populations within a species interact and adapt their phenotypes to changing environments (Craine and Dybzinski, 2013).

When exposed to resource limitation, populations adapted to resource-poor environments may exhibit more conservative phenotypic responses that further limit their growth, while the opposite response may occur in populations that are adapted to grow under sufficient resource conditions (Kobe, 1996; Fownes and Harrington, 2004). For example, in some species, high light conditions only produce smaller leaves to reduce transpiration rate in response to drought when N is not limiting (Hirose and Bazzaz, 1998), which might be the case in high elevation populations. In contrast, low light conditions in combination with sufficient N can also negatively impact a seedling's ability to cope with drought as plants under these conditions increased leaf area under drought in some studies (Körner and Diemer, 1987; Harrison and LaForgia, 2019). The positive, interactive effect of sufficient light and N should intensify in any population, mainly when soil moisture is not limiting because these resources increase photosynthesis, which accelerates plant growth (Kubiske et al., 1996; Valladares and Pearcy, 2002). Additionally, because plant growth depends on carbon availability and the balance between photosynthesis and respiration, the amount of stored non-structural carbohydrates (NSCs) in plants is posited to be an important plant trait to quantify resistance and resilience during resource limitations (Myers and Kitajima, 2007; O'Brien et al., 2017), but is rarely tested within populations and may be an important life-history strategy.

Koa (*Acacia koa* A. Gray; Fabaceae) is a forest tree species endemic to the Hawaiian Islands and grows along a considerable elevational gradient ranging from 300 to 2,100 masl. In the past, koa forests covered significant portions of the islands, but deforestation for agricultural land and urbanization caused a severe decline in the koa forest area (Wilkinson and Elevitch, 2003). Koa restoration efforts have been challenged by disease, excess soil nitrogen and water, and low light (competition) on low elevation sites and by frost, low nutrient availability, and drought on higher elevation sites (Scowcroft et al., 2004; Pejchar et al., 2005; Scowcroft et al., 2010), particularly when located on the windward side of the mountainous Hawaii Island. With climate change, these challenges may be amplified with an anticipated increase in hotter and drier conditions (Giambelluca et al., 2008; Elison Timm, 2017). This emphasizes the importance of testing the role of intraspecific variation among koa populations and its role in the co-limitation of resources (Elevitch et al., 2006), which may ultimately aid in development of seed transfer guidelines for restoration (Rose et al., 2019b).

The overall goals of this study were to test the physiological, biochemical, and morphological responses of different populations of *Acacia koa* to co-limiting conditions of light, nitrogen, and soil moisture. The koa populations were collected along a wide elevational gradient, under the assumption that these populations have undergone genetic selection to the prevailing site conditions (Gugger et al., 2018). The specific objectives were: (i) to determine if there are elevational adaptations of functional traits among populations and (ii) to characterize whether these population-level adaptations result in distinct and advantageous response strategies when exposed to co-limiting resource conditions.

Materials and methods

Seed collection, germination, and seedling establishment

We collected three open-pollinated populations of koa from natural stands along an elevation gradient on the windward side of Hawaii Island in Hawaii, United States: AKO Mix (310 masl, 19°41′14″N, 155°8′7″W), BK-Saddle5 (1,250 masl, 19°42′3.39″N, 155°14′7.22”W), and PHH (2050 masl, 19°41′12”N, 155°27′56”W). The low elevation sourced AKO Mix (L), included 20 half-sib families and was collected in 2007 near Hilo, Hawaii. The mid (M) and high (H) elevation seed sources each included 24 half-sib families that were collected in 2007 at 1250 masl and 2050 masl, respectively, on windward Mauna Kea.

Regular cloud cover, precipitation patterns, the slope of the mountains, and sharp changes in temperature play an essential role in creating differences in light, nutrient holding capacity, and moisture status, particularly on the windward side of mountainous Hawaii Island. As a result, the annual average solar radiation is 130–150 Wm^{-2}, 200–220 Wm^{-2}, and 260–280 Wm^{-2}, the average annual rainfall is 5,400–6,000 mm, 3,000–3,500 mm, and 2,000–2,500 mm, and the soil nutrient holding capacity is considered high, medium, and low for the L, M, and H sites, respectively (Giambelluca et al., 2013).[1] Average air and soil temperatures generally decline with elevation, and winter frosts occur at the H sites (Rose et al., 2020).

Seeds were transported to the lab at the Department of Forestry and Natural Resources, Purdue University (West Lafayette, IN, United States). In summer 2018, seeds were scarified using a nail clipper by making a shallow cut at the edge of each seed (just deep enough to allow water to penetrate the seed coat) and soaked overnight in warm water before sowing the next day in single-cell trays for germination under mist in the lab at 25°C. Germinants were selected for uniformity (size and developmental stage) and transplanted into pots of 6.4 cm diameter and 25.4 cm depth (D40L/D40H; Stuewe & Sons TP818, Tangent, OR, United States) filled with growing medium consisting of coarse sphagnum peat moss (60–70%), bark, and perlite (BM7; Berger, Saint-Modeste, Quebec, Canada) in a greenhouse in the Horticulture and Landscape Architecture Plant Growth Facility at Purdue University.

For the first 2 weeks after germination, seedlings from each population were irrigated every 2–3 days to saturation, and every second watering was fertigated with a 50% dilution of Petunia Special with Black Iron (20-3-19 N-P-K including micronutrients; ICL Specialty Fertilizers, Dublin, OH). Fifteen days post-germination, seedlings were transplanted to bigger pots of 6.9 cm diameter and 35.6 cm length (60 L/60H; Stuewe & Sons TP818, Tangent, OR, United States) and grown for another 2 weeks to allow seedlings to recover from potential transplant stress. During this time the watering and fertilization regimes were continued as described above. Following the transplant recovery period when at least 50% of the seedlings were > 10 ± 3.5 cm tall, seedlings were randomly assigned to different light, nitrogen (N; in the form of ammonia), and moisture treatment combinations.

Treatments and experimental design

A split-plot design was used where a light treatment was applied at the whole-plot level with two levels of light (LIGHT and SHADE; see below), while the koa populations (three populations), nitrogen (N+ and N−), and soil moisture treatments (WET and DRY) were applied randomly at the sub-plot level. To account for potential spatial differences in greenhouse conditions, two adjacent benches were set up with the above design, each with three replicate seedlings for each treatment combination. In a preliminary analysis of the response variables no significant bench effects or interactions with the treatments were found, therefore we removed this factor from further analyses and used the six seedlings as independent replicates with a total of 144 seedlings. Treatment conditions were maintained over a period of 3 months with air temperatures at ~26°C/22°C (day/night).

The high light treatment (LIGHT) provided ambient light conditions and a R:FR of 1.48, while the low light treatment (SHADE) provided about 23.5% of ambient light and a R:FR of 0.68. The SHADE treatment was achieved by using a neutral density filter (25% of FL; Model 210 0.6 ND; LEE Filters, Andover, Hampshire, United Kingdom). Based on outside weather conditions, a supplemental light source was used whenever needed to maintain photosynthetically active radiation (PAR) above 500 $\mu mol\ m^{-2}\ s^{-1}$ using sodium vapor lamps for a period of 13.5 h. Over the experimental period, photosynthetically active radiation (PAR) in the two light levels differed significantly from one another ($p < 0.05$, Supplementary Figure S1A), with the PAR in the SHADE ranging between 20 and 55% of ambient conditions.

For the nitrogen treatment, half the seedlings were watered with a solution of 150 ± 10 mg/L in the N^+ treatment level while seedlings in the N^- treatment were watered with only 50 ± 7 mg/L. The type and concentration of nitrogen in the solution were based on optimum N concentration for *Acacia koa* seedlings in nursery settings (Dumroese et al., 2011). The nitrogen treatment was applied biweekly in the first month of the experiment, and after that, it was applied at every second irrigation event. In DRY treatments, the concentration of N was increased so that nitrogen was equal per volume of applied water. Additional nitrogen was applied to all seedlings prior to the experiment since the growing substrate alone did not provide enough nutrients for establishment. Nitrogen (N^+) addition significantly increased N concentration by 67% in the irrigation water of the N^+ treatments (Supplementary Figure S1B). Although koa is leguminous, we noticed only two or three cases of nodule development in our seedlings in this study.

Substrate water content

Substrate wetness was determined using a 3-Way Soil Meter that measures soil wetness conditions from dry to moist to wet on a scale of 0 to 10 (GroundWorx, United States). Measurements were taken in pots every 2 weeks. Wet and dry treatments were based on a wetness measurement of 8 and 4, respectively. Irrigation was adjusted twice based on the size of the plants using the weighting method (initial and mid-period). Our overall goal of the water treatment was to apply irrigation to maintain relative differences between the two treatments. Although we aimed to maintain the above levels, there were some differences in substrate wetness in different light treatments. For example, low light reduces evapotranspiration under drought conditions, as should be the case under field conditions. Thus, substrate wetness varied with light treatments (Supplementary Figure S1C).

Gas exchange and light response curves

Koa exhibits heteroblasty in which leaves transition from bipinnately compound, horizontally oriented true leaves to vertically

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[1] https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/survey/

oriented phyllodes. In our study, we measured only true leaves as phyllodes did not emerge until the end of our experiment. Leaf CO_2 and H_2O exchange measurements were conducted on leaflets of young fully expanded true leaves (4th leaf from apical meristem) in five seedlings in each treatment combination under light-saturation using an infrared gas analyzer (Li-COR 6400XT, Lincoln, NE, United States) equipped with a standard broad-leaf cuvette (6 cm^2). Because the leaflets did not fully cover the 6 cm^2 LI-6400XT leaf chamber, leaf areas needed to be adjusted for the photosynthetic measurements. Leaflet size was quantified from a photograph taken of the leaf in the chamber using ImageJ (National Institutes of Health, Bethesda, Maryland) using methods of Thyroff et al. (2019).

Cuvette conditions were a leaf temperature of 25°C, 50% relative humidity, PPFD at 1200 $\mu mol\ m^{-2}\ s^{-1}$, with 10% blue light (using LED light source), a constant airflow of 400 mmol air s^{-1}, and 400 $\mu mol_{CO2}\ mol^{-1}$. Photosynthetic parameter measurements including maximum photosynthetic rate (P_{max}), stomatal conductance (g_{max}) and transpiration rate (E_{max}) were performed twice during the experimental period at end of the second and third months between 10:00 and 14:00 h across all the treatments.

In addition to spot gas-exchange measurements, photosynthesis/photosynthetic photon flux density (P_n/PPFD) response curves were developed using the same cuvette setting. Light response curves in each treatment were measured after 60 days into the treatments on four seedlings each. Light intensities were applied in a decreasing order starting at 1600, 1200, 900, 600, 400, 200, 50, and 0 $\mu mol\ m^{-2}\ s^{-1}$ for the full light exposed treatments and in increasing order for the shade seedlings as suggested in Dang (2013). During 0 $\mu mol\ m^{-2}\ s^{-1}$ measurements, we covered the cuvette and seedling with a black cloth, while the other parts of the seedling were in their respective light treatments (Dang, 2013). Light response curves were formed by plotting net photosynthesis assimilation (P_n, $\mu mol\ CO_2\ m^{-2}\ s^{-1}$) against PPFD and fitted to non-rectangular hyperbola (Thornley, 2002). Dark respiration rate (R_d) was based on light response curves. Water use efficiency (WUE_i) was calculated as the ratio of P_{max}/g_{max}, and leaf-level carbon use efficiency (CUE_l) was calculated as the ratio of $[1-(R_d/P_{max})/(1+R_d/P_{max})]$ (Limousin et al., 2015).

Leaf water potential

At the same time as the photosynthesis measurements, leaf water potential (LWP) was measured on a leaflet of each of five seedlings using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR, United States) and was collected from the same leaf that had been used for gas exchange. After determining LWP, the leaflet was used for measuring leaf nitrogen concentration.

Nitrogen and non-structural carbohydrate analyses

The area of leaves sampled for gas exchange and LWP was measured as described above and dried at 65°C for at least 48 h and weighed to calculate the leaf mass per unit area (LMA) or specific leaf area. After this, leaves were ground to analyze for total nitrogen content using a LECO CNS-2000 Analyzer (LECO Corporation, St. Joseph, MI, United States). Nitrogen concentrations (N_l) was calculated for each leaf by multiplying the total N content with leaflet biomass ($g\ g^{-1}$). Photosynthetic nitrogen-use efficiency ($PNUE_l$) was calculated as the P_n:N_l ratio.

To compare stress-tolerance mechanisms among the populations, in response to the treatments, the concentration of soluble sugars (glucose, fructose, and sucrose combined) and starch was quantified in the stems of the koa seedlings at the end of the experiment. Immediately after sampling, all samples were subject to heat shock on-site using a microwave temperature 150°C for 30 s. In the laboratory, samples were dried at 60°C until a constant weight was achieved in a drying oven. Next, the tissues were milled into a fine powder using a ball mill (Retsch, Hann, Germany) at a frequency of 25 tilts s^{-1} (~5 min). NSC analyses followed the methods of Wong (1990), modified as described in Hoch et al. (2002). Dried wood powder (12–18 mg) was extracted with 2 mL deionized water at 90°C for 10 min. An aliquot of each sample extract was taken to determine the low molecular weight carbohydrates using invertase (from baker's yeast; Sigma-Aldrich, Buchs, Switzerland) to break the sucrose into glucose and fructose. Glucose and fructose were converted into gluconate-6-phosphate using glucose hexokinase (Sigma Diagnostics, St. Louis, MO, United States) and phosphogluconate isomerase (from baker's yeast; Sigma-Aldrich). The total amount of gluconate-6-phosphate was determined as the increase in NADH+ H+ using a photometer (HR 700; Hamilton, Reno, NE, United States). To determine the starch content, the remaining extract was incubated at 40°C for 15 h with amyloglucosidase (from *Aspergillus niger*; Sigma-Aldrich) to break starch into glucose.

Non-structural carbohydrate (NSC) concentration was calculated as the total amount of glucose per sample dry mass as described above. The starch concentrations were calculated as the total NSC concentration minus the soluble sugar concentration.

Growth and biomass measurements

Height and root-collar diameter were measured on all seedlings after transplanting (initial), once a month during the experimental period, and at the end of the experiment (final). After transplanting, the seedling root collar was marked so that the diameter was measured at the same location each time. The root-collar diameter was determined by calculating the average of two digital caliper measurements perpendicular to each other. The shoot height was measured from a set point 5 mm above the cotyledon to the tip of the shoot. Height and root collar diameter growth over the experimental period were calculated as the difference between final and initial measurements for each seedling. After leaf removal, each seedling was separated into stems and roots. Root systems were carefully washed and samples were dried at 60°C until a constant mass was achieved. The total biomass (TB) was determined by summing the leaf, stem, and root mass. Leaf mass ratios (LMR), stem mass ratios (SMR), and root mass ratios (RMR) were calculated by dividing the TB. Root-to-shoot ratio (R:S) was calculated by dividing the root mass by the shoot mass (combined stem and leaf mass).

Statistical analysis

Prior to analysis, all data were checked for normality and homoscedasticity, and as a result, height growth data were transformed to satisfy the model assumptions. Data were analyzed using analysis of variance (ANOVA) with linear mixed models (lmer) in the 'lme4' package (Bates et al., 2015) for R (RStudio Team, Boston, MA, United States). Fixed effects were populations, light, nitrogen, and water. When significant treatment or interaction effects were detected, the 'emmeans' package in R (Lenth and Lenth, 2018) was used to perform *post-hoc* pairwise comparison of 'estimated marginal means'. Tukey's HSD tests were used for *post-hoc* comparisons within significant fixed-effect factors and interactions ($\alpha = 0.05$).

Results

Growth and morphology

Under conditions where light and water were *not* limiting, height growth was greatest in the L population, followed by the M and then the H population (L > M > H). When either *only* light or water was limiting, height growth was similar among the three populations; however, when both light *and* water were co-limiting, height growth was greatest in the M and L populations followed by the H population (M ≥ L > H; P × L × W, $p < 0.001$, Table 1).

Root collar diameter growth was greatest in the mid-elevation (M) population followed by the L and then the H population (M > L > H) when no resources were limiting. When only light was limiting, the order among populations in root collar diameter growth was similar in H and M and lowest in L, while when water was limiting root collar diameter growth was greatest in the H and M populations and lowest in the L population (H ≥ M > L). When both water and light were co-limiting, root diameter growth was lower among all populations, but populations retained the same order as under non-limiting conditions (M = L > H; P × L × W, $p = 0.039$, Table 1).

Under non-limiting conditions of light and water, total biomass was similar between M and L and lowest in the H population (M = L > H). When only light and only moisture were limiting total biomass was similar in H and M while lower in the L population (H = M > L). But, when both light and water were co-limiting, total biomass did not differ among populations, but total biomass improved by 15% in the L population (Figure 1; Table 1, P × L × W, $p < 0.001$).

Across all three populations, shoot mass ratio (SMR) was highest in M and lowest in the L population under non-limiting condition (M > H > L). When *only* nitrogen *or* light was limiting, the response patterns were similar among the populations; however, when light *and* nitrogen were co-limiting the H population had the lowest SMR (M > L > H) among the populations (Table 1; P × L × N, $p = 0.05$).

Photosynthesis and water relations

Depending on light and nitrogen availability, P_{max} varied among the three populations (P × L × N, $p = 0.047$, Table 2). When both were not limiting, P_{max} was higher in the high-elevation population than the low-elevation population (Figure 2, i.e., ranking H > M > L). Under light limitation, the H population experienced a much greater P_{max} reduction (>50%) in response to shade than the other populations. However, under conditions where *only* nitrogen was limiting, or when both light *and* nitrogen were co-limiting, there were no differences among populations in P_{max} compared to non-limiting conditions.

When no resources were limiting, the transpiration rate (E_{max}) among populations was highest in the H population while no difference was found between M and L (H > M = L). When *only* water or *all* three resources were limiting, there were no differences in E_{max} among the three populations; similarly, there were no differences among populations when *only* nitrogen was limiting or when light *and* water were co-limiting. However, when light and nitrogen were

TABLE 1 P-values for the analysis of variance (ANOVA) results testing the effects of population (df = 2), light (df = 1), nitrogen (df = 1), and water (df = 1) availability and their interactions on stem diameter growth (D growth), height growth (H growth), leaf mass ratio (LMR), shoot mass ratio (SMR), and root mass ratio (RMR), total biomass (TB), and root to shoot ratio (R/S ratio) in *Acacia koa* seedlings.

Parameters	H growth	D growth	TB	LMR	SMR	RMR	R/S ratios
Populations (P)	**<0.001**	**<0.001**	**0.041**	0.342	0.082	0.162	0.392
Light (L)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.076**	0.067	0.413
Nitrogen (N)	**<0.001**	**0.012**	**<0.001**	**0.012**	**<0.001**	**<0.001**	**<0.001**
Water (W)	**0.022**	0.351	**<0.001**	0.311	**<0.001**	**<0.001**	**<0.001**
P × L	0.531	0.942	**0.042**	0.422	0.054	0.431	0.691
P × N	0.821	0.311	0.891	0.941	0.771	0.151	**0.031**
L× N	0.451	**0.032**	**<0.001**	0.322	0.062	0.622	0.171
P × W	0.211	0.831	0.172	0.411	0.181	0.711	0.521
L × W	**0.021**	**0.044**	**<0.001**	**0.045**	**<0.001**	**0.022**	0.191
N × W	0.561	0.461	0.282	0.421	0.282	0.381	0.492
P × L × N	0.882	0.674	0.463	0.341	**0.045**	0.065	0.912
P × L × W	**<0.001**	**0.039**	**0.0015**	0.321	0.661	0.142	0.252
P × N × W	0.781	0.122	0.741	0.541	0.882	0.551	0.961
L × N × W	0.752	0.952	0.832	**0.031**	**0.002**	0.951	0.182
P × L × N × W	0.561	0.873	0.561	0.141	0.621	0.422	0.671

For all analysis, significant effects are in bold ($p < 0.05$, $n = 6$).

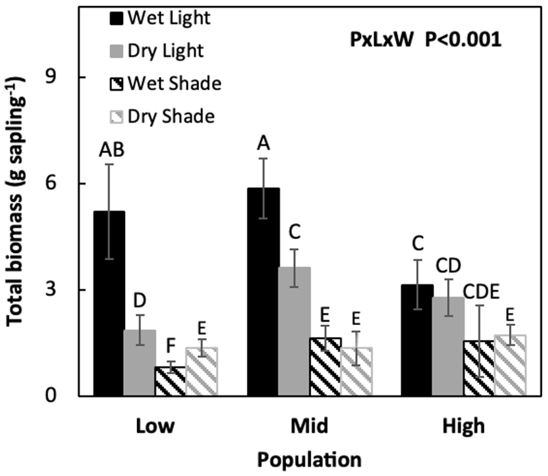

FIGURE 1
Mean (±SE) values for *Acacia koa* seedling total plant biomass for two light and water treatments of three seed sources. Different letters indicate significant differences between treatments (α = 0.05, *n* = 12).

TABLE 2 P-values for the analysis of variance testing (ANOVA) effects of populations (df = 2), light (df = 1), nitrogen (df = 1), and water (df = 1) and their interactions on *Acacia koa* seedling photosynthesis (P_{max}), transpiration rate (E_{rmax}), dark respiration rates (R_d), total leaf nitrogen (TN), photosynthetic water use efficiency (WUE_i), photosynthetic carbon use efficiency (CUE_l), and leaf water potentials (LWP) at the end of the experiment.

Parameters	P_{max}	E_{max}	R_d	WUE_l	CUE_l	LWP
Populations (P)	**0.001**	**0.034**	**0.032**	0.921	0.061	**<0.001**
Light (L)	**<0.001**	**<0.001**	**<0.001**	0.961	0.471	**<0.001**
Nitrogen (N)	0.622	**<0.001**	**0.024**	**0.041**	0.242	**0.03**
Water (W)	**<0.001**	**0.0021**	**<0.001**	0.321	**0.045**	**<0.001**
P × L	0.321	0.322	0.542	0.861	**0.023**	0.381
P × N	0.393	0.592	0.045	0.811	0.791	0.111
L× N	0.881	**0.034**	0.453	0.351	0.762	0.241
P × W	0.622	0.451	0.551	0.971	0.211	**<0.001**
L × W	**0.005**	0.122	**0.034**	0.332	0.451	**<0.001**
N × W	0.883	0.801	0.563	0.473	0.452	0.621
P × L × N	**0.047**	0.362	0.541	0.922	0.351	0.501
P × L × W	0.513	**0.035**	0.333	0.893	0.341	0.86
P × N × W	0.484	**0.043**	0.551	0.982	**0.035**	**0.031**
L × N × W	0.224	**0.045**	0.323	0.393	0.341	0.931
P × L × N × W	0.234	**0.023**	0.452	0.892	0.321	**<0.001**

For all analyses, significant effects are in bold ($p < 0.05$, $n = 6$).

co-limiting, E_{max} was highest in the L and lowest in the M population (L > H > M; P × L × N × W, $p = 0.023$, Table 2).

When nitrogen *and* water were not limiting the carbon use efficiency (CUE_l) was greatest in the M population and lowest in the H population (M > L > H). When *only* nitrogen was limited, the order changed to H > L > M, while when *only* light was limiting the order among populations changed to M > H > L. But, when light *and* nitrogen were co-limiting, population responses in CUE_l remained the same as under non-limiting conditions (P × N × W; $p = 0.035$, Figure 2; Table 2).

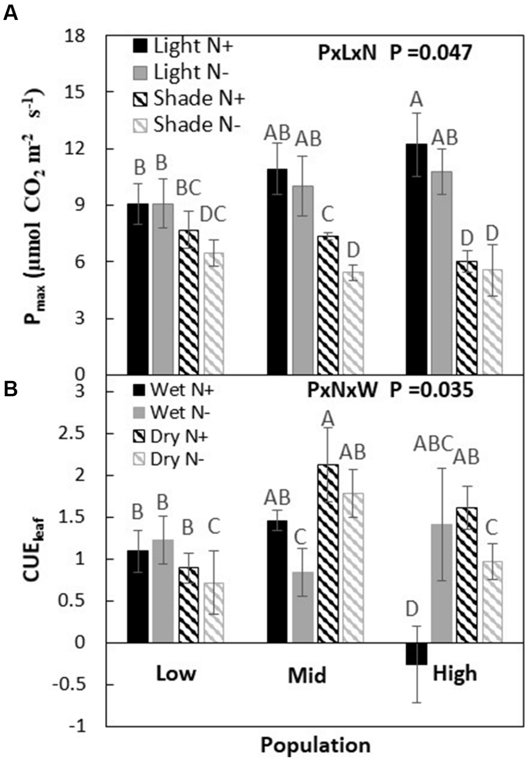

FIGURE 2
Maximum photosynthesis (P_{max}) at saturated light at two light and nitrogen **(A)** and leaf-level carbon use efficiency (CUE_l) at water and nitrogen **(B)** treatments of *Acacia koa* seedlings from the low, mid, and high elevations populations. Each data point represents the mean ± SE at α = 0.05, *n* = 12.

Leaf water potential (LWP) was the highest (less negative) in the low elevation population and more negative in the M and H populations (L > M = H) when resources were not limiting. Under drought, the H population had a higher LWP compared to the H and L population (H > M = L). When *only* nitrogen was limited, populations ranked as L = M > H. On the other hand, under light *only* or light *and* water co-limitation, the H population had a higher LWP compared to the M and L populations (H > M = L). But, when light *and* water or *all* three resources were co-limited, the order of populations was like that found under conditions where no resources were limiting (P × L × N × W, $p < 0.001$, Figure 3; Table 2).

Nitrogen and NSC Reserves

Although main effects were significant, we did not observe a strong interaction effect between populations and co-limiting resources on N_l (P × L × W, $p = 0.111$, Figures 4A,B; Table 3). Photosynthetic NUE ($PNUE_l$) was lowest in M and similar in H and L when resources were non-limiting. When *only* nitrogen was limiting, the M population had the highest $PNUE_l$ and the L population the lowest (M > H > L), with a 20, 60, and 35% increase over plants grown in non-limiting condition in M, H, and L

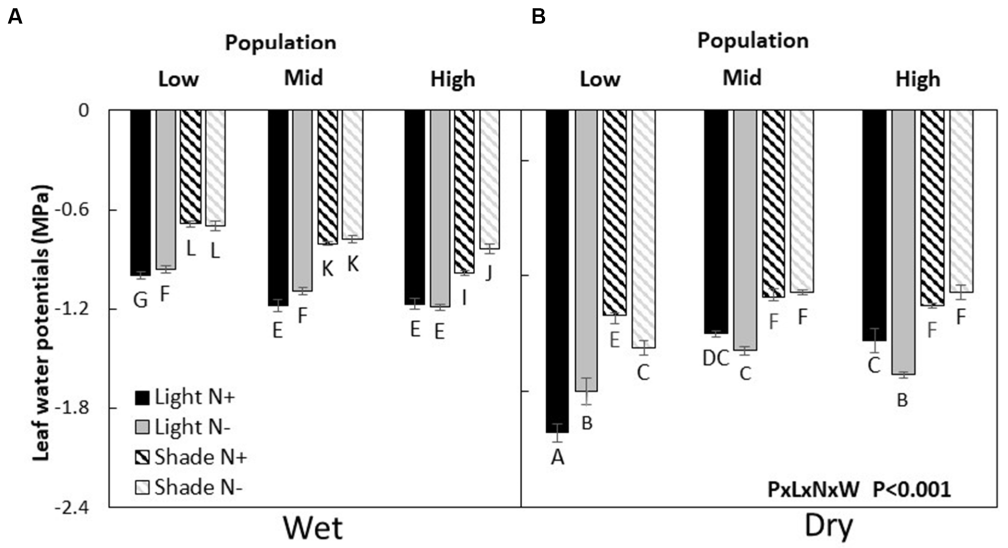

FIGURE 3
Leaf water potentials (mean ± SE) of wet **(A)** and dry **(B)** *Acacia koa* seedlings from the low, mid, and high elevations populations at two light and nitrogen treatments. Different letters indicate significant differences between treatments (α = 0.05, *n* = 6).

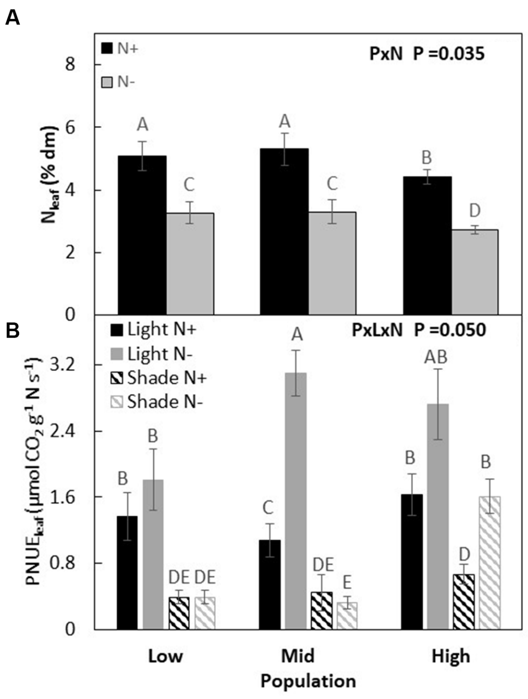

FIGURE 4
Leaf nitrogen % of dry matter of *Acacia koa* seedlings from the low, mid, and high elevations populations at two nitrogen treatments **(A)** and leaf photosynthetic nitrogen use efficiency (PNUE_l) at light and nitrogen treatments **(B)** (mean ± SE) at the end of the experiment. Different letters indicate significant differences between treatments [α = 0.05, *n* = 24 for **(A)** and *n* = 12 for **(B)**].

populations, respectively. When *only* light was limiting or when both light *and* nitrogen were co-limiting, PNUE_l was highest in the H population and lowest in the L population (H > M > L), which was similar to the order found under non-limiting conditions.

When light *and* water were not limiting, sugar concentrations were highest in the H population and lowest in the L population (H > M > L), but when *only* light was limiting sugar concentrations were highest in the M population and lowest in the H population (M > L > H). Under drought, L and M populations had similar sugar concentrations while the H population had the lowest concentration among the populations. Differences were observed between populations when both water *and* light were co-limiting (P × L × W, $p < 0.001$, Figure 5A; Table 3). When water was not limiting, starch concentrations were similar for all three populations, while under drought M had the highest starch concentrations and the L population the lowest (M > H > L), increasing by 19% in M and decreasing by 25% in the L population compared to the non-limiting conditions (P × W, $p = 0.035$, Figure 5D; Table 3).

Under non-limiting conditions of light *and* nitrogen, NSC concentrations were similar for M and L population but low for the H population (M = L < H). When *only* nitrogen was limiting or when *only* light was limiting, there were no differences between the populations. When both light *and* nitrogen were co-limiting, the H population had the lowest NSC concentrations (P × L × N, $p = 0.045$, Figure 5B; Table 3). Similarly, no differences between populations were found under non-limiting conditions of light *and* water, but when *only* light was limiting NSC was similar in M and L populations and lowest in the H population. Under drought, NSC concentrations were highest in mid and lowest in the high elevation population (M > L > H), but when both light *and* water were co-limiting, NSC were highest in the L population and lowest in the M and H populations (L > M = H; P × L × W, $p < 0.001$, Figure 5C; Table 3).

Discussion

This study provides some of the first evidence that populations of koa distributed along a steep elevational cline can differ in their physiological responses to co-limitation of two or

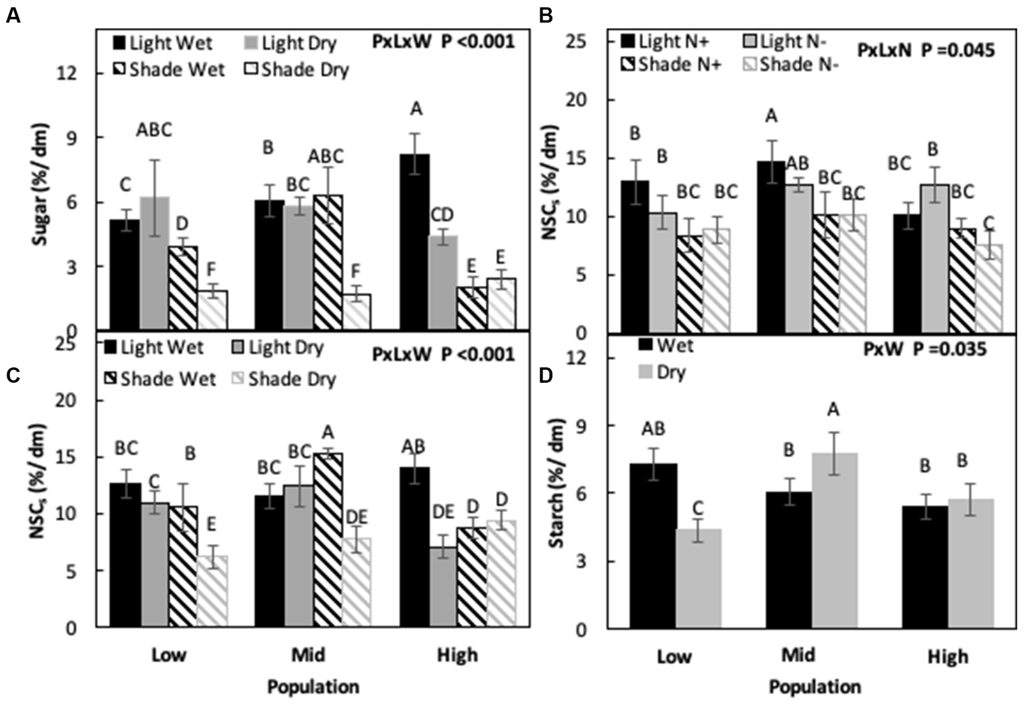

FIGURE 5
Sugar **(A)**, total non-structural carbohydrates (NSCs) concentration **(B,C)**, and starch **(D)** in *Acacia koa* stems at the end of the experiment across various combination of treatments. Different letters indicate significant differences between treatments [α = 0.05, *n* = 12 for **(A–C)** and *n* = 24 for **(D)**].

TABLE 3 P-values for the analysis of variance testing (ANOVA) effects of populations (df = 2), light (df = 1), nitrogen (df = 1), and water (df = 1) and their interactions on *Acacia koa* leaf nitrogen %, photosynthetic nitrogen use efficiency ($PNUE_l$), stem sugar, stem starch, and total non-structural carbohydrate concentrations (NSC) at the end of the experiment.

Parameters	N_l	$PNUE_l$	Sugar	Starch	NSC
Populations (P)	**0.039**	**<0.001**	0.981	0.792	0.851
Light (L)	**0.002**	**<0.001**	**<0.001**	0.392	**<0.001**
Nitrogen (N)	**<0.001**	**<0.001**	**0.046**	0.411	0.461
Water (W)	**0.034**	**<0.001**	**<0.001**	0.692	**0.026**
P × L	0.692	0.632	0.322	0.511	0.912
P × N	**0.035**	0.171	0.372	0.622	0.361
L × N	0.173	**<0.001**	0.843	0.981	0.881
P × W	0.532	0.391	0.284	**0.035**	0.311
L × W	0.193	**0.021**	0.255	0.851	0.561
N × W	0.492	0.231	0.242	0.521	0.821
P × L × N	0.93	**0.051**	0.241	0.151	**0.045**
P × L × W	0.252	0.421	**<0.001**	0.431	**<0.001**
P × N × W	0.962	0.292	0.261	0.331	0.161
L × N × W	0.182	0.283	**0.016**	**0.012**	**<0.001**
P × L × N × W	0.672	0.923	0.141	0.881	0.311

For all analyses, significant effects are in bold ($p < 0.05$, $n = 6$).

more resources. This suggests that adaptation rather than acclimatization (Skelton et al., 2021) has occurred over relatively small spatial and temporal scales. In general, the koa population from low elevation (L) was more sensitive to conditions where both moisture and nutrients were limiting, while the koa population from high elevation (H) was more sensitive to conditions where either light and moisture or light and nitrogen were co-limiting. In contrast, the mid-elevation population (M) was less responsive to either set of co-limiting conditions. While gradients of resources have been tied to the response and physiological capacity of species and communities (Clark et al., 1992; Poorter and Bongers, 2006; Wright and Sutton-Grier, 2012), our findings indicate that the responses to complex interactions among resources can also apply at the population level, thereby providing support to the notion that limitation of multiple resources (Gleeson and Tilman, 1992; Eskelinen and Harrison, 2015) can separate populations, rather than limitation by single resources.

Population differences in response to single resources limitation have been shown for physiological responses for a range of species (Körner and Diemer, 1987; Abrams, 1990; Paudel et al., 2021; Skelton et al., 2021), but their effects when two or more resources are co-limiting have not been explored in detail. Some of the physiological variables in our study provide further support that response to colimitation of resources may vary at the population level. For instance, while the high elevation population showed a much greater reduction for P_{max} (>50%) in response to shade than the other populations, P_{max} of the low and mid populations was more sensitive to co-limitation of light and nitrogen (Figure 3; Table 2). Similarly, leaf carbon and nitrogen use efficiency (CUE_l and $PNUE_l$) responses to co-limitation of nitrogen and water varied between the populations (Figures 2, 4; Table 2). The contrast in the response of gas exchange to drought and shade may have occurred because the interaction between light and drought on leaves differed depending on whether plants were adapted to high-light or low-light environments (Niinemets et al., 2004).

Leaf water potential (LWP) showed similar population-level interactions in response to co-occurring resource limitations. Under wet conditions, the L population maintained the highest LWPs, even when N was co-limiting. Under drought, however, the L population had the lowest resistance to drought when light was co-limiting. This response might reflect koa's adaptation for gap regeneration in the denser forest canopies found at low elevations (Engelbrecht et al., 2007; Rose et al., 2019a). The low elevation population occurs naturally under conditions of high water and nutrient resource availability, and plants growing under such conditions tend to have large leaf area (Markesteijn and Poorter, 2009), which is accompanied by the increases in vulnerability to water deficit under high light. At the species level, the colimitation of light and moisture has been shown to directly reduce photosynthesis and transpiration rates, reducing potential carbon gain, and negatively affecting osmotic regulation and water use efficiency (WUE_i; Aranda et al., 2007).

Although not as noticeable as the physiological variables, the colimitation of resources had differing effects on the allocation of carbon to growth and reserves among the koa populations. When light and water were colimiting, it appears that the H population allocated more to growth *and* reserves than the L and M populations, while these differences in reserve allocation among populations were not noticeable when only water *or* light was limiting. When light and moisture were co-limiting, NSC concentrations were higher in the stems of the H population (by 25%) compared to the L and M populations (Figure 5). This might relate to the need for the high elevation population to adapt to environmental conditions in a colder climate including tolerance to frost, which requires specific responses such as osmotic adjustments and cavitation repair (Plavcová and Jansen, 2015; Hartmann and Trumbore, 2016), while lower elevation populations may lack potential plasticity in this response. Although this could be the driver for the response, differences in stem NSCs concentration could also be a result of source vs. sink limitation. The L population had low photosynthetic rates in combination with a relatively high allocation to growth (source limitation) when under water and light stress (Figure 5D), while the H population had relatively high photosynthesis combined with reduced growth under the same conditions (sink limitation).

Separating sink and source limitation has been a longstanding focus for the investigation of NSC dynamics in plants in response to stress, exploring whether the environmental stress conditions constrain growth directly (sink limitation) or indirectly by restricting photosynthesis, subsequently limiting growth (source limitation; Körner, 2003, Muller et al., 2011). This has led to a continuing debate on whether stresses cause either a carbon (C) surplus indirectly since growth is more sensitive to a stress than photosynthesis (Boyer, 1970; Muller et al., 2011; Woodruff and Meinzer, 2011; Hoch, 2015) or whether the allocation to reserves may be a direct adaptation to tolerating stresses (Sala et al., 2012; Wiley and Helliker, 2012; Dietze et al., 2014). While stress-induced increases of NSC have been observed across tree species and biomes (Galvez et al., 2011; Fajardo and Piper, 2021; Signori-Müller et al., 2021), we did not observe increases in NSC concentration in this study. This was possibly because of the indeterminate growth strategies of this tropical species combined with the fact that the applied stress treatments were not intended to be severe, allowing seedlings to continue to grow in all treatments, therefore reducing the potential impact of sink limitations. Interestingly, the reduction in growth allocation in the H population was less relative to the change observed in the L and M populations, which, when combined with a smaller decrease in reserves, might indicate that the H population preferentially allocated more C to reserve storage compared to the other two populations. Investigating the differences in NSC dynamics among the koa populations was not a major aim of this study; however, studies exploring NSC responses to stress within different populations of a single species are rare (Blumstein et al., 2020, 2022). Although our data are too limited to explore these dynamics in detail, the observed responses appear to support an allocation dynamic based on sink limitations when seedlings and populations were exposed to a single stressor that mainly affected growth allocation among populations. On the other hand, when exposed to two or more colimiting stressors, differences between the koa populations in growth and reserve allocation patterns were revealed. Observations such as these should provide interesting and novel insights into this debate and may create a new avenue to explore the relationships between growth and reserve allocation using different populations within a species when exposed to stressors.

Overall, growth and biomass allocation patterns were much less sensitive to stress than the physiological responses, most likely a response to the limited severity of our experimental conditions. However, the lower responsiveness of growth and biomass traits to colimiting resources could also have been the result of a relatively short experimental period, indicating that more time may be needed for such morphological responses to become expressed compared to physiological responses that can adjust much more rapidly (Craine and Dybzinski, 2013). Thus, we suggest that future studies evaluate responses over a longer-time frame, as well as conduct experiments in the field within the natural range of koa. Field studies would allow for investigation of other important aspects of koa ecology, such as the potential effects of N fixing rhizobium, differences in air and soil temperatures among elevation gradients and associated interactions with native soils, as well as koa performance beyond the juvenile stage.

Conclusion

For successful establishment in marginal and heterogenous environments, plants must often overcome multiple limitations of resources. Our findings with koa, a tropical species that occurs across wide elevation gradients in highly heterogeneous environments, have provided new insight toward understanding the complex, interacting effects of co-limiting resources at the population level. Overall, the mid-elevation population showed the greatest plasticity of growth and physiology in the face of multiple limiting resources. This evidence suggests that while populations can adapt to resource colimitation by increasing resource use efficiency to maintain plant growth, there are also differences between populations that point to specific adaptations of populations to the growing conditions. This finding is especially interesting given that koa colonized and evolved relatively recently on Hawaii Island compared to the long-term evolution of many other forest tree species (Gugger et al., 2018). Our results also lend support to population-based management strategies (e.g., climate change adaptability, seed transfer for reforestation). Finally, our findings provide important insights that can be used to help guide future opportunities for testing co-limitation of resources across a wide range of populations. The responses we found were mainly physiological in nature, likely due to the short-term nature of the study, suggesting that future research should verify that such responses can lead to corresponding effects on growth over longer time frames.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

IP developed the original ideas, implemented the study, conducted data analysis, interpreted and visualized the results, and wrote the manuscript. KR contributed to the methods, interpretation of the results, and edited and revised the manuscript. SL interpreted and visualized the results and edited and revised the manuscript. DJ supervised the study and edited and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1225778/full#supplementary-material

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