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Nutrient additions have direct and indirect effects on biocrust biomass in a long-term Chihuahuan Desert grassland experiment

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ABSTRACT

Anthropogenic activities have greatly affected some of the global biogeochemical cycles, including the nitrogen (N) and phosphorus (P) cycles. This alteration of nutrients has significantly impacted ecosystems around the globe. Arid ecosystems may be particularly vulnerable to changes in nutrient availability, in addition to their frequent limitation by water availability. We conducted a nutrient-fertilization experiment with an emphasis on the cover of biological soil crusts (biocrusts) in the Chihuahuan Desert. We manipulated N, P, and Potassium (K) in a full-factorial design. We visually estimated biocrust cover and fluorometrically measured chlorophyll *a* as an index of biocrust abundance. We found that there were significant interaction effects of N*P and N*K as well as significant main effects of N and P on chlorophyll *a*. Results from a path analysis suggest that both N and P had direct positive effects on aboveground vascular plant production and direct negative effects on chlorophyll *a*. There were also indirect negative effects on biocrust cover and chlorophyll *a* by vascular plant cover. Overall, nutrient additions had a direct positive effect on vascular plant cover, which in turn had a negative impact on biocrust cover. This indicates a possible mechanism for inter-taxon competition under conditions of increased nutrient availability.

1. Introduction

Anthropogenic activities have greatly affected global biogeochemical cycles for decades (Cook, 1984; Finzi et al., 2011). Generally, this has resulted in surplus nutrients (in particular, nitrogen and phosphorus) that have significantly altered ecosystems around the globe (Vitousek et al., 1997). Nitrogen (N), which is the most frequently limiting nutrient in terrestrial systems (Vitousek and Howarth, 1991), is perhaps the most altered of the biogeochemical cycles (Kanakidou et al., 2016). Furthermore, atmospheric dust inputs have increased the deposition of multiple nutrients including phosphorus (P) and potassium (K) in the U.S. southwest (Reynolds et al., 2001).

Increased N deposition may present challenges for arid organisms that are well-adapted to limited nutrients (Ochoa-Hueso et al., 2016; Reed et al., 2016). Similarly, P and K are also important and limiting essential nutrients for terrestrial ecosystem productivity in arid environments (Fay et al., 2015). Increased nutrients may be particularly problematic for biological soil crusts (biocrusts), which predominate in arid environments (Ward, 2016). Biocrusts are a community of soil-surface organisms that are composed of varying amounts of cyanobacteria, lichens, mosses, bacteria and fungi and tend to occupy interspaces between vascular plants in arid ecosystems.

Vascular plants and biocrusts are documented to have negative, neutral or positive effects on one another (Belnap et al., 2001; Havrilla et al., 2019). Vascular plant cover tends to respond positively to increased nutrient deposition (through increased N availability in the soil) and consequently have negative effects on biocrusts via resource competition (Havrilla et al., 2019). Moreover, vascular plants (and plant litter) have been documented to shade biocrusts from sunlight and inhibit biocrust growth (Dettweiler-Robinson et al., 2018).

Previous studies have focused on either the effects of nutrient deposition on vascular plant communities (e.g. Craine et al., 2008) or biocrusts (e.g. Crain et al., 2018) or the interaction of vascular plants and biocrusts in the absence of nutrient addition (Dettweiler-Robinson et al., 2018). We sought to tease apart both the direct and indirect effects of nutrient additions (a proxy for nutrient deposition) on vascular plant and biocrust communities. In this framework, the direct effects include the response of both vascular plants and biocrusts to nutrient additions,

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while the indirect effects include the response of biocrusts to both vascular plant communities (responding to increased nutrients) and their associated litter that has been reported to shade biocrusts from sunlight (Belnap et al., 2016).

The objective of this study was to measure biocrust cover (and corresponding vascular plant and litter cover) to determine if nutrient availability (N, P, and K plus micronutrients (K^+)) limits or promotes biocrust establishment and growth. We took advantage of an already established manipulative-nutrient experiment (Nutrient Network (NutNet) desert grassland). We assessed the direct and indirect effects of nutrient additions and vascular plant and litter cover on biocrusts. Plant litter has been documented to impede biocrust growth by decreasing availability of space (Bowker et al., 2005; Ochoa-Hueso et al., 2013) and light (Belnap et al., 2016).

We made the following predictions: 1) plots receiving nutrient additions will have more vascular plant cover than the control plots because N and P are known to be co-limiting in many arid grasslands (e.g., Craine et al., 2008, 2009); 2) plots receiving both of these nutrients (N and P) will have higher biocrust cover than plots receiving only one of these two nutrients (i.e., there will be an additive effect); and 3) vascular plants should outcompete biocrusts for resources in plots that received added nutrients (Havrilla et al., 2019).

2. Methods

2.1. Study area

The Sevilleta Long-term Ecological Research (LTER) site (elevation = 1600 m a.s.l.) is located in central New Mexico (about 80 km south of Albuquerque) in the northernmost region of the Chihuahuan Desert. This area is situated on alluvium (sand = 95%; Bryan-Ricketts, 2015) and the soil order is Aridisol in the Turney loamy sand series (Bryan--Ricketts, 2015). Basal soil pH averaged at a value of 8.1 in the control plots. Additional information is available from the Sevilleta LTER website (hhtp://sev.lternet.edu).

The study site is situated on McKenzie Flats (N 32°21.849, W 106°41.114). Common vascular plants include the C_4 perennial grasses black grama (*Bouteloua eriopoda*) and blue grama (*Bouteloua gracilis*). Varying amounts of light-colored cyanobacterial-dominated biocrusts are present in plant interspaces. These biocrusts likely consist of filamentous, non-nitrogen fixing cyanobacteria (Fernandes et al., 2018). The site is relatively undisturbed as livestock have been excluded since 1973 (Thomey et al., 2011) and pronghorn antelope (*Antilocapra americana*) are present but uncommon.

The climate at the Sevilleta is typical of an arid grassland with a mean annual precipitation (MAP) of 242 mm (Shi et al., 2014). The wettest months are July–September (summer monsoon months). Mean monsoon precipitation is 163 mm with a coefficient of variation of 48.5% (Knapp et al., 2015). In the year leading up to our study (May 2016–April 2017), MAP was slightly lower (221 mm) than the long-term mean (calculated using NutNet metadata). Mean annual temperature (MAT) is 13.3 mm °C (Shi et al., 2014).

2.2. Experimental design

The NutNet experiment at the Sevilleta was completely crossed and involved eight macronutrient-treatment combinations of nitrogen (N), phosphorus (P), and potassium plus micronutrients (K^+) and the control (no added nutrients) (N, P, K, NP, NK, PK, NPK and control). All three macronutrients were applied at the rate of 10 g m^{-2} yr^{-1}. The micronutrient mixture (by mass) consisted of Ca (6 g m^{-2}), Fe (17 g m^{-2}), S (12 g m^{-2}), Mg (3 g m^{-2}), Mn (2.5 g m^{-2}), Cu (1 g m^{-2}), Zn (1 g m^{-2}), B (0.1 g m^{-2}), and Mo (0.05 g m^{-2}), and was applied at 100 g m^{-2} once at the beginning of the experiment (2008) to avoid possible toxic effects (Biederman et al., 2017). All of the fertilization values were determined by the NutNet collaborators and are consistent across the NutNet sites. The macronutrients were applied annually in late June before the start of the monsoon season. In this ongoing experiment, there are 40 experimental plots, with five replicates of each nutrient treatment combination. Each 5 m × 5 m plot is separated by a 1 m buffer. All plots were first fertilized in 2008.

2.3. Percent cover estimates

We visually estimated percent cover of vascular plants (total species cover), biocrusts and litter in each of the 40 plots during the spring of 2017. Due to the slow and cumulative growth of biocrusts, the variance in precipitation over the years prior to percent cover estimates would likely have a small effect. We estimated cover using a 1 m × 1 m quadrat in four cardinal directions and one in the center for a total of five quadrats (sub-samples) per plot. We also estimated percent cover of individual vascular plant species (as opposed to total species cover). See Supplemental Table 1 for a list of all recorded vascular plant taxa. All visual estimations of both biocrusts and vascular plants were assessed by one person (L. Baldarelli) for consistency during May and June 2017 prior to the monsoon rains. To aid in percent canopy-cover estimates, the quadrat was divided into 10 cm × 10 cm squares and individual squares were totaled for each estimate. This sometimes resulted in estimates exceeding 100%, indicating an overlap of coverage (e.g. Zhou et al., 2016). To avoid edge effects, the quadrats in each of the four cardinal directions (in the corners of each plot) were located at least 1 m from the plot edge.

2.4. Environmental parameters

After percent cover values were estimated, a biocrust sample and associated bulk soil were collected within the general area where the 1 m × 1 m frame was laid at all five quadrats within each plot (n = 200 = five samples/plot x 40 plots). For each quadrat within the plot, we filled a petri dish with biocrusts from the surface of the soil to an approximate depth of 3 mm using a metal spoon. We also collected bulk soil in a metal cup (236.6 cm^3) just below where the biocrusts were sampled (within 10 cm from the soil surface) then transferred the soil to a Ziploc® bag. The specific sample collection location for each quadrat was selected to avoid disturbance and to select for the greatest biocrust coverage (excluding biocrusts beneath the canopy of vascular plants).

Because cyanobacterial biocrusts are essentially microscopic, we also used chlorophyll *a* as an index of biocrust cover (e.g. Gao et al., 2020) in addition to our visual estimates. The same area that was sampled for biocrust cover was also sampled for chlorophyll *a*. Dimethyl sulfoxide (DMSO) (7 ml) was added to each biocrust sample (1 g, five subsamples x 40 plots = 200 samples) for chlorophyll *a* determination. All samples were vortexed for 20 s after adding DMSO and for another 20 s after incubating the samples in the dark for 1 h at 65 °C to increase the efficiency of extraction (altered from Hawkes, 2001). Samples were centrifuged and finally, chlorophyll *a* was measured fluorometrically at 665 nm (altered from Hawkes, 2001). We averaged chlorophyll *a* from the five above-mentioned quadrats (sub-samples) per plot and calculated chlorophyll *a* per area (mg m^{-2}).

All bulk soil samples were air dried in the lab prior to recording measurements. The following soil parameters were measured with the uppermost layer of the bulk soil: bulk density (BD), water-holding capacity (WHC), soil respiration and soil pH. BD was calculated (prior to sieving the soil) by dividing the dry mass of the soil sample by the volume of the metal cup. We then sieved the bulk soil below the biocrust samples and the <2 mm fraction was used for the following analyses. Water-holding capacity was measured using the gravimetric protocol in Sparks (1996). Briefly, WHC was determined by subtracting the initial dry mass from the saturated soil weights and divided by the initial dry mass of the soil. Soil pH was determined using a pH electrode in a 2:1 ratio of distilled water:soil by mass (Sparks, 1996). We measured soil respiration as an indicator of soil activity via microbes by obtaining the

quantity of CO_2 released using the Solvita CO_2-Burst standard protocol (https://solvita.com/co2-burst/) (Haney et al., 2008), which requires a 24 h incubation of 40 g soil in 5 ml of distilled water.

2.5. Statistical analyses

We tested for nutrient effects on mean biocrust cover with vascular plant cover as a covariate using an analysis of covariance (ANCOVA). Vascular plant cover was used as a covariate to remove the potential effect of this variable on biocrust cover. We then used an ANCOVA to test for significant effects of nutrient additions on chlorophyll *a* with vascular plant cover as the covariate. A Levene's test was used to test for homogeneity of variance. We $\log_{10}$-transformed chlorophyll *a* data to meet the homogeneity of variance assumption. A regression was used to assess the relationship between mean biocrust cover (%) and mean vascular plant cover (%). We also regressed the mean $\log_{10}$ chlorophyll *a* on vascular plant cover (%).

To investigate whether vascular plant species richness or diversity were significantly affected by the nutrient additions we used an ANOVA. A principal component analysis (PCA), including cover data (%) for vascular plant composition, mean litter and biocrust cover values, was used to determine patterns among these different categories based on percent cover. All of the above statistical analyses were performed in SPSS version 24. Results were considered significant if $p < 0.05$.

We used path analysis to determine if biocrust cover (proxied by $\log_{10}$ chlorophyll *a*) was directly affected by nutrients or indirectly affected through nutrients by vascular plants and litter cover. Path analysis is a useful statistical approach when considering hypotheses with multiple factors, and differs from multiple regression in that the latter assumes that there are only direct effects while path analysis considers both direct and indirect effects. However, significant effects in path analyses do not necessarily suggest causality (Mitchell, 1992; Shipley, 2016). Path analysis incorporates both endogenous (predicted within the model) and exogenous variables (external to the model). Our endogenous variables included mean vascular plant cover, mean litter cover and $\log_{10}$ chlorophyll *a*. Our exogenous variables were the macronutrients (N, P, K).

For each of our path models, we estimated the variances of residuals for the endogenous variables (mean vascular plant cover, mean litter cover and $\log_{10}$ chlorophyll *a*). Both direct (nutrients) and indirect (vascular plants/litter) effects were calculated as path coefficients using standardized regression coefficients, and are presented for the final model; the greater the absolute values, the greater the effect that variable has on the other variables. However, it is possible for a path to have a standardized coefficient but not be significant. All path analyses were performed using the *lavaan* package in R version 3.6.2 (R Core Team, 2013).

Path models were compared and considered based on the Akaike Information Criterion (AIC) (Akaike, 1973; Shipley, 2016). Following the approach of Burnham et al. (2011), we calculated the ΔAIC values (the difference between the "best" model and the alternatives). Burnham et al. (2011) note that ΔAIC values < 2 are not considered different. Some researchers consider ΔAIC values ranging from 2 to 7 insufficient to reject them (e.g. Symonds and Moussalli, 2011).

We constructed four path diagrams; a model for each macronutrient (N, P, K) separately as the exogenous variable and one model including all three macronutrients (3 exogenous variables). We hypothesized that each individual nutrient would be directly associated with vascular plant cover and with chlorophyll *a* and that vascular plant cover would be either directly associated with chlorophyll *a* or indirectly associated with chlorophyll *a* through its effects on litter cover (increased vascular plant cover should increase litter cover).

We used a redundancy analysis (RDA) in PC-ORD (McCune and Mefford, 2011) to best predict the key factors explaining the differentiation among plots. This analysis included the soil variables (listed in *2.4 Environmental parameters*), nutrient additions (see *2.2 Experimental design*) and cover estimates (see *2.3 Percent cover estimates*). We used a randomization test to determine the significance of the first axis (ter Braak, 1995). Environmental variables that are considered the best predictors are shown in the figure when the correlation between the species-environmental values are greater than ±0.4 (Legendre et al., 2011).

3. Results

3.1. Biocrusts, nutrients and vascular plants

There were no significant main effects or interaction effects of nutrients on mean biocrust cover (%) (Table 1). However, the covariate, mean vascular plant cover (%), was highly significant. There was a significant negative relationship ($r = -0.70$) between mean vascular-plant cover (%) and the visual estimate of mean biocrust cover (%) ($F_{1,38} = 36.520$, $p < 0.001$) (Fig. 1a).

3.2. Biocrust/chlorophyll a, nutrients and vascular plants

There was a marginally significant positive correlation between $\log_{10}$ chlorophyll *a* and % biocrust cover ($r = 0.31$, $F = 3.981$, $p = 0.053$), indicating that $\log_{10}$ chlorophyll *a* is a reliable index of % biocrust cover. There were significant negative effects on $\log_{10}$ chlorophyll *a* of N (mean ± S.E. = 0.44 ± 0.268 mg m^{-2} $\log_{10}$ chlorophyll *a*) and P (1.32 ± 0.178 mg m^{-2} $\log_{10}$ chlorophyll *a*) compared to the control (2.28 ± 0.063 mg m^{-2}). There were also significant interaction effects of N*P and N*K (Fig. 2) on chlorophyll *a* (Table 2). Note that there was no significant effect of the covariate, vascular plant cover. We removed the covariate from the analysis, but there were no changes in the statistical output.

There was a significant negative correlation ($r = -0.48$) of mean vascular plant cover (%) on chlorophyll *a* ($F_{1,38} = 11.640$, $p = 0.002$) (Fig. 1b). We found significant positive correlations of N (control = 40.25 ± 2.380%; N added = 57.62 ± 3.501%) and P (control = 44.47 ± 3.680%; P added = 53.4 ± 3.198%) on mean vascular-plant cover (%) (Table 3).

3.3. Vascular plant species richness and species diversity

There was a significant interaction effect of N*P on vascular plant richness (ANOVA: $F_{1,32} = 4.902$; $p = 0.034$). Mean species richness of vascular plants significantly declined when P was added with N (mean species richness ± SE for N = 10.0 ± 0.89, N + P = 8.4 ± 0.81). There were no other significant interaction or main effects on mean vascular plant species richness. ($p > 0.05$). There were significant main effects of both N and P on Shannon-Wiener vascular-species diversity (H') (N main effect: $F_{1,32} = 8.083$, $p = 0.008$; P main effect: $F_{1,32} = 11.802$, $p = 0.002$). The mean ± SE for Shannon-Wiener vascular-plant species

Table 1
ANCOVA of the effects of nutrients on mean biocrust cover (%) with mean vascular plant cover (%) as a covariate. Vascular plant cover was used as a covariate to remove the potential effect on biocrust cover. Note that the effect of vascular plant cover (%) on biocrust cover (%) is highly significant. Bold font indicates a significant effect.

Source	df	Mean Square	F	p
Nitrogen	1	71.368	0.961	0.335
Phosphorus	1	247.578	3.332	0.078
Potassium	1	30.813	0.415	0.524
Nitrogen * Phosphorus	1	26.950	0.363	0.551
Nitrogen * Potassium	1	224.849	3.026	0.092
Phosphorus * Potassium	1	99.648	1.341	0.256
Nitrogen * Phosphorus * Potassium	1	0.027	0.000	0.985
Mean vascular plant cover	**1**	**1396.691**	**18.799**	**< 0.001**
Error	31	74.294		

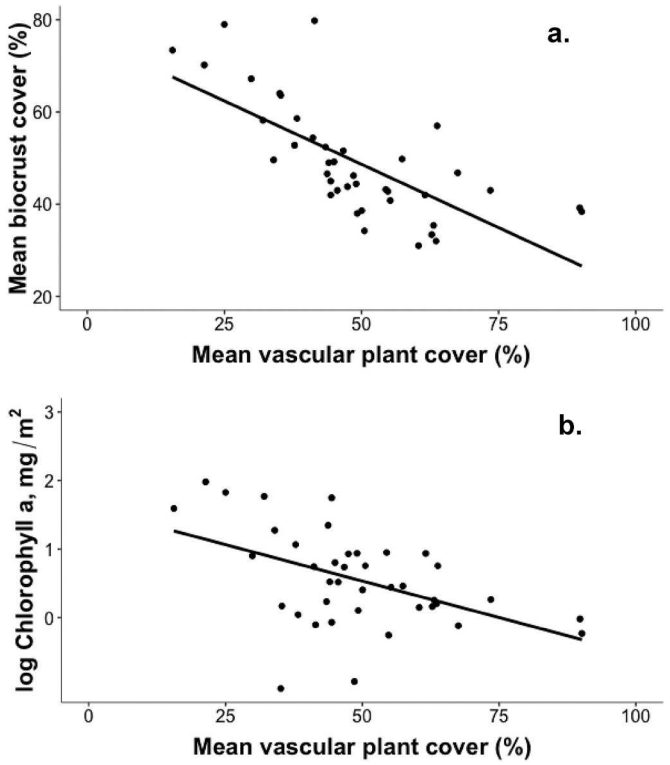

Fig. 1. There was a significant negative effect of mean vascular plant cover (%) on (a) mean biocrust cover (%) ($r = -0.70$, $F = 36.520$, $p < 0.001$, error d.f. = 38) and (b) $\log_{10}$ chlorophyll *a* ($r = -0.48$, $F = 11.640$, $p = 0.002$, error d.f. = 38).

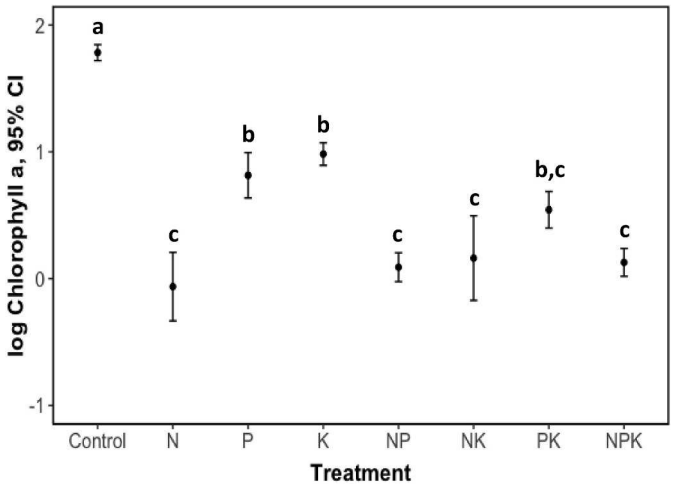

Fig. 2. The effects of nutrient fertilization on biocrust cover (indexed as $\log_{10}$ chlorophyll *a*). There were significant interaction effects between N and P on $\log_{10}$-transformed chlorophyll *a* and N and K plus micronutrients on $\log_{10}$-transformed chlorophyll *a* (Table 2). The complete control (Control) had the highest chlorophyll *a*. The different letters above the error bars indicate significant differences between treatments. Error bars represent 95% confidence intervals (CI).

diversity for the controls was 1.5 ± 0.20, for N addition 1.3 ± 0.11, and for P addition 0.7 ± 0.17. We found a marginally non-significant effect on N + P + K on species diversity ($F_{1,32} = 4.095$; $p = 0.051$).

Table 2
ANCOVA of the effects of nutrients on $\log_{10}$ transformed chlorophyll *a* (index of biocrust biomass) with mean vascular plant cover (%) as a covariate. Note the significant interaction effects of N*P and N*K. Mean vascular plant cover (%) was not significant. Bold font indicates a significant effect.

Source	df	Mean Square	F	p
Nitrogen	**1**	**7.069**	**41.378**	**< 0.001**
Phosphorus	**1**	**1.222**	**7.153**	**0.012**
otassium	1	0.436	2.553	0.120
Nitrogen * Phosphorus	**1**	**1.627**	**9.526**	**0.004**
Nitrogen * Potassium	**1**	**1.266**	**7.408**	**0.011**
Phosphorus * Potassium	1	0.108	0.633	0.432
Nitrogen * Phosphorus * Potassium	1	0.332	1.943	0.173
Mean vascular plant cover	1	0.187	1.092	0.304
Error	31	0.171		

Table 3
ANOVA of the effects of nutrient additions on vascular plant cover (%). Both N and P had a significant positive effect on vascular plant cover. Bold font indicates a significant effect.

Source	df	Mean Square	F	p
Nitrogen	**1**	**3018.906**	**18.358**	**< 0.001**
Phosphorus	**1**	**797.449**	**4.849**	**0.035**
Potassium	1	13.456	0.082	0.777
Nitrogen * Phosphorus	1	340.472	2.070	0.160
Nitrogen * Potassium	1	281.430	1.711	0.200
Phosphorus * Potassium	1	110.224	0.670	0.419
Nitrogen * Phosphorus * Potassium	1	3.660	0.022	0.882
Error	32	164.449		

3.4. Patterns of vascular plant cover and biocrusts

Biocrust cover (crust), the cover of 26 vascular plant species (Supplemental Table S1) and plant litter cover (litter) were included in the principal component analysis (PCA) for each of the 40 plots. Axis 1 explained 81.9% of the variance (eigenvalue = 163.857) while an additional 11% (eigenvalue = 22.049) of the variance was explained by axis 2. We found that biocrusts respond distinctly differently to nutrient additions compared to vascular plant species and litter in terms of % cover (Supplemental Fig. S1).

3.5. Direct and indirect effects of nutrients and vascular plants on biocrusts

The model including all of the macronutrients (N, P, K) as exogenous variables (hereafter NPK model) was the best fit for our data, having the lowest AIC value of 588.21 (Fig. 3). The K model was the next best fit with an AIC of 589.07. The N and P models yielded AIC values of 591.25 and 601.23, respectively. With the exception of the P path diagram, none of the ΔAIC values were greater than 3. Thus, all of these three diagrams (NPK, K and N) provide a similar assessment of the "best" path analysis.

A direct negative effect of N on chlorophyll *a* was highly significant (−0.69) in the NPK model (Fig. 3). Phosphorus also had a direct negative effect (−0.25) on chlorophyll *a* (Fig. 3). Both N and P also had direct positive effects on vascular plant cover (0.55 and 0.28 respectively) which indirectly, positively affected litter cover. However there were no significant paths linking vascular plant cover and/or litter cover and chlorophyll *a* (Fig. 3).

Considering the individual nutrient models, N had a direct negative effect on chlorophyll *a*, while P and K both had a direct positive effect on chlorophyll *a*. The opposite pattern was observed with nutrient effects on vascular plant cover; N had a direct positive effect on vascular plant cover, while P and K had direct negative effects on vascular plant cover. All of the models (including the NPK model) indicated an indirect negative effect of vascular plant cover on chlorophyll *a*; however this

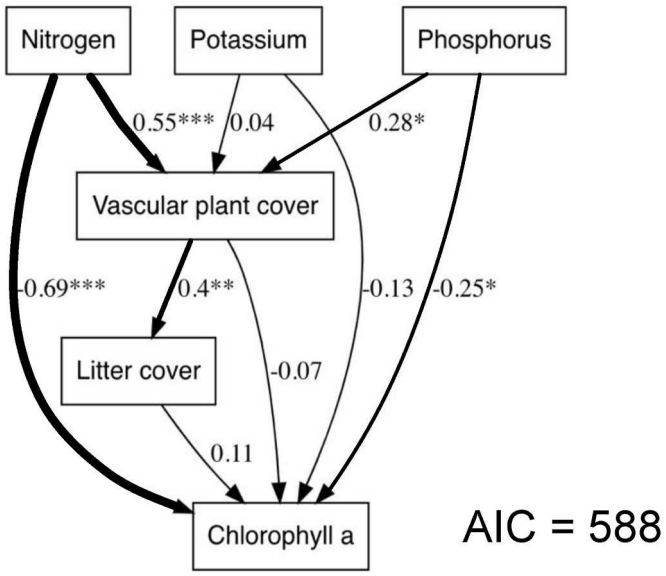

Fig. 3. Path diagram showing estimated standardized path coefficients for direct and indirect effects on chlorophyll *a*. The diagram includes nutrient additions as exogenous variables and vascular plant cover, litter cover and chlorophyll *a* as endogenous variables. Significant pathways are highlighted in bold and designated with asterisks. The thickness of the arrow indicates the estimated importance of the variable's effect on the other variables and chlorophyll *a*. This NPK path diagram includes individual direct paths from each nutrient to chlorophyll *a* and indirect paths from each nutrient through vascular plant and litter cover to chlorophyll *a*. The weightings in these analyses are standardized partial regression coefficients. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

result was only significant in the case of the P model. All models showed a direct positive effect of vascular plant cover on litter cover and indirect, nonsignificant effects of litter on chlorophyll *a* across the models.

3.6. Relationships among vascular plants, biocrusts and environmental variables

Soil variables, nutrient additions and cover estimates (including individual vascular plants, biocrusts and litter) were included in a redundancy analysis (RDA) (Supplemental Fig. S2) randomization test for the species-environment correlation for axis 1 and found a highly significant relationship ($p = 0.001$). We found that soil N, soil respiration, vascular plant cover (all RDA 1), soil P, and soil pH (RDA 2) were the most important environmental variables, differentiating the separation and clustering of particular plots (Supplemental Fig. S2). These results are consistent with those indicating that vascular plant cover was affected by N and P (Table 3).

4. Discussion

This study highlights the effect of nutrient additions on cyanobacterial-dominated biocrusts in a Chihuahuan Desert grassland. Here, we used a pre-existing nutrient fertilization experiment to determine the direct and indirect effects on a biocrust community. First we predicted that plots receiving nutrients (especially N and P) would have more vascular plant cover than the control plots. Consistent with our first hypothesis, we found significant effects of nutrients (both N and P) on our estimates of vascular plant cover. However, chlorophyll *a* values (correlated with % biocrust cover) were significantly lower in plots fertilized with N only and P only plots compared to control plots, contrary to our second prediction. This negative effect of N addition on chlorophyll *a* has been documented in other biocrust-dominated systems (e.g. Ochoa-Hueso et al., 2016; Treseder, 2008). Average N deposition rates in the western U.S. are estimated to range from 0.1 to 9 g m^{-2} yr^{-1} (Fenn et al., 2003). The majority of estimates range from 0.1 to 0.4 g m^{-2} yr^{-1} while both urban areas and agriculture areas correspond with the high-end values (Fenn et al., 2003). Our findings are consistent with another study that also found that chlorophyll *a* was negatively affected by N addition (albeit with fertilization of 3.0 g N m^{-2} yr^{-1}) (Zhou et al., 2016). However, Zhou et al. (2016) noted that their low N-deposition treatments (<3.0 g N m^{-2} yr^{-1}) had a positive effect on microbial activity, without considering the effect of vascular plants. Some proposed mechanisms for negative effects of added N on biocrust species include tissue toxicity and competition with vascular plants (Pearce et al., 2003; Van der Wal et al., 2005).

The negative effect of P on chlorophyll *a*, however, is not well supported in the literature. In fact, added P (simulating deposition) has been reported to enrich soil fertility (Belnap, 2003; Reynolds et al., 2001) and consequently support higher vascular plant cover (Madan et al., 2007). However, it is important to highlight that positive effects of deposition have focused on natural dust deposition while our study can only be interpreted as a simulation of atmospheric deposition. The average *global* estimate for atmospheric P from 1954 to 2012 was 0.019–0.14 g m^{-2} yr^{-1} and although it is likely that these values have increased over the years, it is still considerably less than the fixed amount added in our experiment as determined by the NutNet collaboration (10 g P m^{-2} yr^{-1}) (Tipping et al., 2014). Furthermore, our experimental design was originally designed to test the effects of nutrient additions on vascular plant communities, so it is possible that the simulated amounts of P benefited the vascular plant community and simultaneously had a toxic effect on chlorophyll *a*. Our NPK model supports this hypothesis, although vascular plants responded positively to added P, suggesting that some of the fertilizer P could have leached into the soil and benefited the vascular plant community. Another explanation (not necessarily mutually exclusive) could simply be that the additional P added to the plots enriched the vascular plant community sufficiently to outcompete the biocrusts for space (Bowker et al., 2005; Ochoa-Hueso et al., 2013), as seen with the N plots. We note that because vascular plants responded positively to added P in this experiment and there was a negative effect of vascular plants on biocrust cover (Fig. 1), that inter-taxon competition (biocrusts vs vascular plants) is a more likely explanation.

We also predicted that increased resource availability due to the addition of nutrients would lead to vascular plants outcompeting biocrusts. We found that vascular plant cover responded positively to both N and P (in both ANCOVA and path analyses; Fig. 3), suggesting that fertilization caused increased vascular plant production, potentially limiting space for the biocrusts. Competition between vascular plants and biocrusts in arid environments is usually considered to be low, either because the density of vascular plants is also low or biocrusts (lichen-dominated) inhibit vascular seed germination (e.g. Rosentreter et al., 2016). However, Tilman (1988) considers low-productivity environments to lead to high competition. Furthermore, Havrilla et al. (2019) showed that plant cover and richness directly reduced biocrusts as a result of resource competition. This concept of competition aligns well with the results for species composition in our PCA (Supplemental Fig. S1), where we found a distinct difference between biocrusts and vascular plants with regard to percent cover. For example, we can infer that in plots where vascular plants dominate they have outcompeted biocrusts for nutrient resources and space. Biocrusts may dominate in plots where resources are limited. Furthermore, the redundancy analysis (RDA) (Supplemental Fig. S2) revealed that the important environmental factors driving differentiation among the plots were N, P, vascular plant cover, and soil pH. There was also a strong effect of soil respiration in this RDA. A possible reason for this result is that there is a positive association of soil respiration and N on the first axis of the redundancy analysis (which explains most of the variance- McCune and Mefford, 2011; ter Braak, 1995). Soil respiration is an index of soil quality that is strongly correlated with N availability (Haney et al., 2008). There were also positive direct effects of the additions of both N

and P and vascular plant cover while biocrust cover, $\log_{10}$ chlorophyll *a* and vascular plant cover were negatively correlated (Fig. 1).

We note that our path analyses did not produce any significant paths indicating that vascular plant cover and/or litter cover significantly affected chlorophyll *a*. The most important outcome of the multi-nutrient path model (Fig. 3) is arguably that nitrogen is the main driver directly affecting both vascular plant cover as well as chlorophyll *a* values. Perhaps the lack of significant direct effects of vascular plants on chlorophyll *a* is related to seasonality and more specifically to precipitation. We conducted our study in May–June which is just before the monsoon season. Without water, vascular plants are unable to take up N and incorporate it into biomass or utilize it for physiological processes (Austin et al., 2004). In this situation, it can be suggested that N accumulated in the soil profile at higher concentrations than needed for the biocrust community. A nutrient-supplementation scenario combined with the lack of precipitation events has the potential to become saturated with N and cause detrimental effects to biocrusts (Zhou et al., 2016).

Nutrient deposition can have beneficial as well as detrimental impacts on biocrusts in arid environments (Zhou et al., 2016). Future studies should focus on determining the mechanisms underlying negative effects on biocrust cover in response to added nutrients (N and P), especially with regard to the interactions between vascular plant communities and biocrusts. For this reason, understanding the threshold for positive versus negative effects is worthwhile. Theoretical values of deposition should be used to simulate and test the effects of different scenarios of vascular plants and biocrusts in the field. This will be fundamental to understanding dryland ecosystem dynamics in the face of anthropogenic changes.

CRediT authorship contribution statement

Lauren M. Baldarelli: Conceptualization, Formal analysis, Investigation, Resources, Writing - original draft, Project administration. **Heather L. Throop:** Writing - review & editing, Supervision. **Scott L. Collins:** Methodology, Validation, Writing - review & editing. **David Ward:** Conceptualization, Formal analysis, Resources, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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