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Multiple resource limitation of dryland soil microbial carbon cycling on the Colorado Plateau

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Abstract

Understanding interactions among biogeochemical cycles is increasingly important as anthropogenic alterations of global climate and of carbon (C), nitrogen (N), and phosphorus (P) cycles interactively affect the Earth system. Ecosystem processes in the dryland biome, which makes up over 40% of Earth's terrestrial surface, are often distinctively sensitive to small changes in resource availability, likely because levels of many resources are low. However, data also suggest that simultaneous changes in the availability of multiple resources may be necessary to affect a response in these low-resource systems, offering an opportunity to test patterns and controls of co-limitation, serial limitation, and individual limitation in soil environments. While drylands may play a governing role in key aspects of Earth's C cycle, and while an improved understanding of resource limitation could substantially improve our forecasts of dryland responses to change, our understanding of interacting controls on soil C cycle processes remains notably poor in these dry systems. Here, we address multiple fundamental hypotheses of resource controls over ecosystem function to test how water, C, N, and P regulate soil C cycling individually and interactively in a dryland ecosystem on the Colorado Plateau. Using a series of laboratory incubations, we found that, while water, C, and N limited C cycling through serial limitation, water alone resulted in an extremely small respiratory response from target organisms, whereas water + C resulted in a dramatic increase in soil C cycling, suggesting a degree of functional co-limitation. Nitrogen additions alone resulted in no changes to soil C cycling, but when N was added in concert with water and C, N greatly increased soil C cycling rates relative to additions of water and C without N. Phosphorus additions had no effect on the C cycle either alone or synergistically. These patterns were consistent with the stoichiometry of the system and interactions among resources were surprising in ways that inform our understanding of critical theories in ecology, such as the Transient Maxima Hypothesis, supporting the suggestion that multiple resource limitation explains pulse-dynamic C cycling in drylands better than water limitation alone.

KEYWORDS
carbon-use efficiency, drylands, nitrogen, phosphorus, pulse-reserve paradigm, resource limitation, soil respiration, stoichiometry, transient maxima hypothesis

INTRODUCTION

Because of their resource scarcity, drylands represent an important end member when it comes to understanding how resources interact to regulate ecosystem function. Although drylands make up Earth's largest biome (Lal, 2019; Prăvălie, 2016; Safriel et al., 2005; Schimel, 2010), with over 40% of the terrestrial surface composed of hyperarid, arid, semiarid, and dry subhumid ecosystems, relatively few studies assess the interacting controls of water, carbon (C), and nutrients over fundamental processes regulating the C cycle in dryland ecosystems (Blett et al., 2014; Porter et al., 2005; Schaeffer & Evans, 2005). Indeed, a common assumption about drylands is that water is so limiting that other resources are less likely to play an important role (Austin, 2011). Yet where resources have been considered individually, it has been proposed that soil nitrogen (N) is a strongly limiting resource in drylands, second only to water (Hooper & Johnson, 1999), due to a combination of low litter quality, low soil organic matter, and high gaseous N loss (Barger et al., 2005; Evans et al., 2001; Schlesinger et al., 1990; Smith et al., 1994; Zaady et al., 1996). At the same time, anthropogenic N deposition has doubled the reactive N pool globally, and such large changes to the planet's N cycle have myriad consequences (Galloway et al., 2008; Vitousek et al., 2013), particularly for the terrestrial C cycle (Yue et al., 2016). Drylands are important regulators of the trend and interannual variability in the global C cycle (Ahlstrom et al., 2015; Lal, 2004; Poulter et al., 2014), and while this importance is thought to derive from the tight coupling of C and water cycles in arid regions (Ahlstrom et al., 2015; Austin et al., 2004; Delgado-Baquerizo et al., 2013), nutrient limitation may also play an important interactive yet relatively unexplored role. Thus, we suggest that an improved understanding of how resources such as water, N, and phosphorus (P) interact to control C cycling in drylands is critical in the context of improving our capacity to predict fundamental function for this vast biome.

At the core of a great deal of biogeochemical, ecological, and agricultural research is the attempt to understand how resources such as water and nutrients interact to regulate ecosystem structure and function. Liebig's 1840 Law of the Minimum, developed to improve agricultural practices, states that plant growth is not dictated by total resource availability, but by the resource in lowest supply. Building upon this, the Transient Maxima Hypothesis (Seastedt & Knapp, 1993) joined Liebig's concepts of nutrient constraints with Noy-Meir's (1973) pulse-reserve framework of how water limitation from infrequent desert precipitation events regulates ecosystem structure and function. The Transient Maxima Hypothesis suggests that ecosystem responses may reflect resource co-limitation, where a biotic response only occurs when multiple limiting resources are made available at the same time in the same space, or serial-limitation, where a biotic response to addition of primary limiting resource is synergistically enhanced by the addition of a second limiting resource (Harpole et al., 2011; Saito et al., 2008). Interactions of multiple limiting resources can lead to complex ecosystem responses, especially where soil resource heterogeneity creates "hot spots" or "hot moments" (Kuzyakov & Blagodatskaya, 2015; Leon et al., 2014; McClain et al., 2003). Beyond the absolute supply of individual resources or elements, these patterns of limitation are driven by imbalances between the ratio of elemental supply from the environment, and the much more stoichiometrically constrained elemental demand by organisms (Čapek et al., 2018; Elser et al., 2007; Reiners, 1986; Sterner & Elser, 2002, although see Camenzind et al., 2021).

While our understanding of resource limitation continues to improve (Čapek et al., 2018; Harpole et al., 2016; Zechmeister-Boltenstern et al., 2015), anthropogenic activities are rapidly altering global cycles of key resources such as water (Barnett et al., 2008; Vörösmarty & Sahagian, 2000), C (Canadell et al., 2007; Matthews et al., 2004; Schimel, 1995), N (Canfield et al., 2010; Schlesinger, 2009; Vitousek et al., 1997), and P (Mahowald et al., 2008; Ringeval et al., 2014). Shifts in temporal and spatial availability of these resources of could decouple critical interactions regulating ecosystem structure and function (McMichael et al., 2006; Patz et al., 2005; Stevens et al., 2015). Accordingly, understanding and predicting how ecosystems are regulated by interactions among and changes to the availability of limiting resources continues to represent an important frontier in global ecology.

For example, numerous lines of evidence suggest N deposition is having significant impacts on global soil heterotrophic C cycling (Yue et al., 2016). Previous meta-analyses found N addition can either reduce (Janssens et al., 2010; Ramirez et al., 2012; Treseder, 2008) or increase (Luo et al., 2016; Peng et al., 2017; Zhou et al., 2014) soil C cycling rates, and a meta-analysis by Zhou et al. (2014) showed experimental N addition increased soil respiration by 2% across all global ecosystems. However, this response varied widely among sites and, importantly, of the 295 studies, only three (~1%) represented drylands, which are likely to respond differently than other biomes due to scarce limiting resources (Yahdjian et al., 2011). Drylands are highly sensitive to intermittent pulses of low resource availability, and despite a substantial research effort and the advances that have been made, we still lack a definitive understanding of how multiple resources interact to determine fundamental dryland ecosystem function.

To address this important knowledge gap for drylands, and to use the low soil resource system as a testbed for hypotheses of multiple resource limitation, we evaluated how soil heterotrophic C cycling responded to inputs of water, C, N, and P individually and interactively on the Colorado Plateau. Previous work conducted at our study site found little detectable effect of long-term low-level N fertilization on soil biota or soil chemistry (McHugh et al., 2017; Phillips et al., 2021). Based on this lack of response, as well as previous work at a nearby site by Schaeffer and Evans (2005) that found that co-additions of C and N increased N immobilization compared to addition of N alone, we hypothesized that increases in multiple resources must co-occur for a significant response in the soil C cycle to occur. We used three distinct soil incubation experiments to evaluate responses of heterotrophic soil respiration and microbial biomass to different additions of water, C (glucose), N (ammonium nitrate), and P (potassium phosphate). These short-term incubations allowed us to evaluate the Transient Maxima Hypothesis, to explore alternative controls in drylands beyond strictly water limitation, and to test the hypothesis that multiple resources either co-limited soil microbial activity, imposed serial limitation, or affected processes independently (Figure 1a–c).

METHODS

Site description and sample collection

The study site is located in Arches National Park (38°47′ N, 109°39′ W) on the Colorado Plateau in southeastern Utah, USA. This region receives 219 mm of annual precipitation, mostly occurring as winter snow, early spring rain, and late summer monsoons, and has a mean annual temperature of 14.4°C (McHugh et al., 2017). Vegetation at the site is a mix of C_3 and C_4 bunchgrasses, annual plants, and forbs, including the native grasses *Achnatherum hymenoides*, *Pleuraphis*

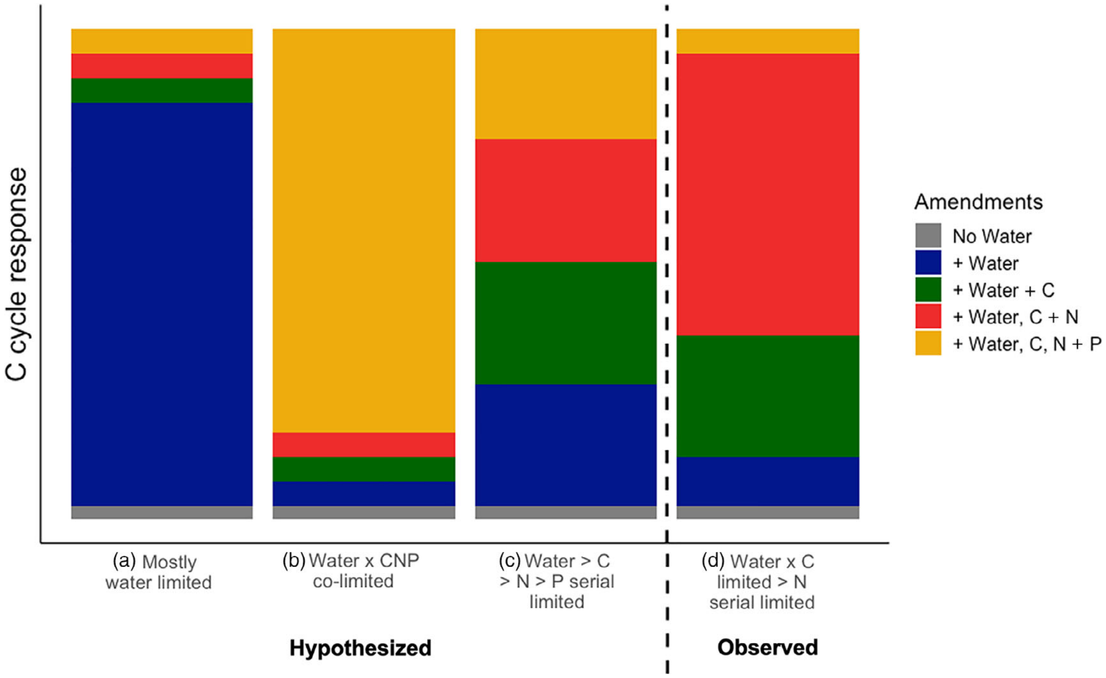

FIGURE 1 (a–c) Hypothesized and (d) observed response of soil C cycling to pulse inputs of water, C, N, and P under different scenarios of resource limitation. The *y*-axis represents the proportion of a total C cycling response driven by the resources water, C, N, and P, with each bar representing soil respiration response to a resource pulse of either water alone (blue), water + C (green), water + C and N (red), or water + C, N, and P (orange), or in the dry state (gray). In hypothesis (a), water is the only limiting resource, all other elements are available in excess of instantaneous microbial demand (in other words, microbial biomass and physiology limit the response). In (b), water and all elements co-limit the response, suggesting low, but fairly stoichiometrically balanced, levels of all elements. In (c), resources are serial-limiting in the order of water > C > N > P, suggesting soils with slightly lower C:N, and N:P than the ratio demanded by microbial biomass. Finally, in (d), water and C are functionally co-limiting, while N is the next serial limiting element in the series, and P is not limiting, suggesting that C:N is somewhat lower in the soil than demanded by microbial biomass, while P is available in excess of microbial demand; this pattern is closest to what was observed in the data. While water and C were serially limiting (i.e., water had an effect on soil C cycling C and C had no effect in the absence of water), the size of the effect of water + C than was so much larger than water alone (>2400% larger), from a C cycle perspective these resources could be viewed as functionally co-limiting

jamesii, and *Vulpia octoflora*, and the exotic invasive plants *Bromus tectorum* and *Salsola tragus*. Biological soil crusts are also present and are dominated by cyanobacteria, likely *Microcoleus* spp. (Garcia-Pichel et al., 2001). Soils are classified as aridisols (U.S. Department of Agriculture Natural Resources Conservation Service). Soil texture is a sandy loam, characterized as 71.5% sand, 15.1% silt, and 13.4% clay, with a pH of 7.99 ± 0.02 (mean ± SE), 0.40 ± 0.06% soil organic C, 0.48 ± 0.04% soil inorganic C, and 0.04 ± 0.00% soil N (McHugh et al., 2017).

Soils were collected on 14 August 2017 and again on 17 July 2018 from the upper 10 cm of the soil profile in open spaces among plant canopies after the biological soil crust layer (<1 cm depth) was removed. Samples were homogenized and passed through a 2-mm sieve to remove rocks, roots, and litter. Soils from 2017 and 2018 were both collected during similar summer drought conditions from the same experimental field location. Both soil collections had similar physical and chemical properties, plant communities, and biocrust conditions.

Soil respiration response to water, C, N, and P

We conducted two incubation experiments to investigate the influence of water, C, N, and P availability on heterotrophic soil respiration. The first experiment used a wet incubation (with soils collected from 2017) following a three-way full factorial design (Appendix S1: Figure S1a). We used three levels of labile C (0, 1, and 2 mg glucose–C ($C_6H_{12}O_6$)/g soil), five levels of N (0, 0.0625, 0.125, 0.1875, and 0.25 mg ammonium nitrate–N (NH_4NO_3)/g soil], and three levels of P (0, 0.025, and 0.05 mg monobasic potassium phosphate–P (KH_2PO_4)/g soil), for a total of 45 C:N:P levels, with the control level ($C = 0$, $N = 0$, $P = 0$ mg/g soil) receiving the same amount of deionized water as the other treatments, but no C or nutrient additions ($n = 225$). The lowest N amendment was ~50% of the cumulative total N added over 3 years of a paired field N fertilization experiment (McHugh et al., 2017). We targeted C:N:P microbial stoichiometry at each mid-level treatment ($C = 1$, $N = 0.125$, $P = 0.025$ mg/g soil) and high-level treatment ($C = 2$, $N = 0.25$, $P = 0.05$ mg/g soil) equivalent to average soil stoichiometry ($C:N = 8$, $C:P = 40$, $N:P = 5$; Cleveland & Liptzin, 2007). While the Redfield C:N:P ratios are an important framework for understanding nutrient limitation, this was not the focus of our investigation. We weighed 3 g dry-equivalent soil into 50 ml polypropylene centrifuge tubes (Fischer Scientific, Waltham, MA, USA) and then added amendments with 0.2 ml deionized H_2O/g dry soil. Control soils received only deionized H_2O. We replicated each wet amendment combination (45 total levels) five times for a total of 225 soil incubation samples. We flushed tubes with breathing air for 10 s before sealing with gas-tight caps installed with rubber septa ports for collection of air samples.

We incubated samples at room temperature (25°C) for 48 h immediately after field collection. To collect headspace gas samples at 24 and 48 h, we inserted plastic 5 ml syringes with stopcocks and non-coring side-hole needles through the rubber septa ports in each centrifuge tube. We analyzed gas samples for CO_2 concentrations on a CA-10 Carbon Analyzer (infrared gas analyzer; Sable Systems International, Las Vegas, NV, USA). We uncapped incubation samples and exposed to ambient air for 10 min before recapping between the 24 and 48 h CO_2 measurements to prevent high CO_2 conditions from developing in the centrifuge tubes. We calculated respiration as the sum of CO_2 produced per gram of soil over the 48 h incubation period.

Our second incubation experiment investigated the effect of water availability on heterotrophic soil respiration under contrasting high and low levels of $C + N + P$ (Appendix S1: Figure S1b). We used the same overall incubation approach described above. We added 3 g of dry soil (collected in 2018) to 50 ml centrifuge tubes equipped with septa ports along with either the highest combination of amendments (2 mg glucose/g dry soil; 0.25 mg ammonium nitrate/g dry soil; 0.05 mg monobasic potassium phosphate/g dry soil) or no amendments (as a control). We added amendments to soils in dry form to investigate the respiration response without water addition. We conducted the initial 24 h incubation for both the high amendment and control at field-collected (i.e., very dry summer conditions) soil water content (<2% VWC). After the initial 24 h, we added 0.2 ml H_2O/g dry soil to each sample, flushed tubes with breathing air, and sealed and incubated samples for an additional 24 h. We sampled headspace CO_2 after the 24 h dry phase and again after the 24 h wet phase and analyzed headspace gas on the Carbon Analyzer.

Because of the concern that N additions would alter the soil pH and environmental conditions, we ran a preliminary trial incubation to evaluate the interactions among C and N additions, soil pH, and soil respiration. We used the same incubation protocol described above, and measured soil pH using the slurry method immediately after the 48 h incubation. We found that NH_4NO_3 addition increased soil pH in the absence of added glucose, with no effect on soil respiration (Appendix S1: Figure S2a,b). In contrast, in the presence of added glucose, NH_4NO_3 addition decreased soil pH, which was correlated with increased soil respiration (Appendix S1:

Figure S2c,d). These results indicate that enhanced respiration combined with water likely drove the reduction in soil pH through the formation of carbonic acid or through the nitrification of ammonium, rather than soil pH directly causing a change in CO_2 evolution through the formation or dissolution of soil carbonates (Hinsinger et al., 2006). Thus, even under high N addition rates, we determined that the changes in acidity were insufficient to alter soil respiration responses.

Microbial biomass response to C, N, and P

We conducted a third incubation to determine the response of soil microbial biomass to C, N, and P amendments (Appendix S1: Figure S1c). We weighed larger subsamples (30 g dry-equivalent soil) into 250 ml glass jars. We used a subset of the same treatment levels from the previous incubation with three levels of C (0, 1, 2 mg glucose–C/g soil), and only three levels of N (0, 0.125, 0.25 mg ammonium nitrate–N/g soil), and two levels of P (0, 0.05 mg monobasic potassium phosphate–P/g soil), in full factorial combination, as well as five blank deionized water controls. We added all amendments with 0.2 ml H_2O/g dry soil. We replicated each of the 18 amendment combinations five times and ran pre- and post-fumigation measurements for a total of 180 samples. We held samples at room temperature for 48 h before fumigation and during the respiration incubation process.

We measured microbial biomass C (MBC) and N (MBN) via the liquid chloroform extraction protocol (Brooks et al., 1996; Vance et al., 1987). We immediately extracted one 10 g soil subsample and incubated another 10 g subsample for 24 h in a sealed beaker with 100 μl of liquid HPLC-grade ethanol-free chloroform before extracting. We extracted all samples with 35 ml of 0.5 mol/L K_2SO_4 on a shaker table for 1 h. We filtered the extract through Whatman no. 1 filter paper into plastic scintillation vials. We measured total organic C (TOC) and total dissolved N (TN) of the extracts on a Shimadzu TOC/TN-VCSH analyzer (Kyoto, Japan). We calculated MBC and MBN by subtracting the difference between fumigated and unfumigated samples and by using an extraction efficiency correction factor of $k_e = 0.45$ (Brookes et al., 1985).

Statistical analyses

We conducted all statistical analyses with the R statistical platform (version 3.4.3, r-project.org). We first analyzed all data via analysis of variance to determine if the observed responses varied among treatment levels (Appendix S1: Table S1). In the first and third incubation, we analyzed the response variables (respiration, MBC, MBN, TOC, and TN) response to C, N, and P via a three-way ANOVA, and comparisons among groups were made via Tukey's Honestly Significant Differences test. Percent changes were averaged across all amendment levels and presented as percent change $= 100 \times [(\text{treatment} - \text{control})/\text{control}]$. In the second dry-wet incubation, we used an unbalanced two-way ANOVA to evaluate the response of soil respiration to water addition. We log-transformed all soil respiration values before analysis. Residual variance of soil MBC and TOC pools indicated that values did not need to be transformed.

To evaluate the changes in soil C cycling as a continuous response to the amount of C, N, and P in the soil, we analyzed the three-way interactive response of total cumulative respiration, dMBC ($MBC_{final} - MBC_{init}$), and dTOC ($TOC_{initial} - TOC_{final}$) using a response surface model (RSM) analysis with the *rsm* package in R (Lenth, 2009). We used a second-order polynomial (total respiration, dTOC) or a two-way interaction (dMBC) to specify the response-surface portion of the models and used an analysis of variance to determine the contributing significant terms. We also ran response surface models for dMBN and dTN to evaluate the changes in soil N; however, we are limiting our discussion to the soil C response.

We then used the modeled response surface models to calculate soil microbial C-use efficiency (CUE) as follows. First, we predicted cumulative respiration, dMBC, and dTOC across all levels of C, N, and P, using the RSMs. We then calculated CUE two ways, $CUE_1 = (dTOC - R)/dTOC$, $CUE_2 = dMBC/(dMBC + R)$, estimated net CUE as the mean of those two calculations, and developed an RSM for the predicted CUE as a function of C, N, and P. This approach was used rather than calculating CUE directly from the data because the C, N, and P levels were not the same in the different incubations and therefore a direct calculation could not be done. We did not calculate CUE as a response to water because our experimental design did not include dMBC and dTOC under dry conditions, but it is highly unlikely that we would have been able to detect changes in either pool under dry conditions.

RESULTS

Soil respiration response to water, C, N, and P

Overall, soil heterotrophic C cycling was highly responsive to water, C, and N addition, but not responsive to P. In the first set of wet incubations, soil respiration was very low when water alone was added to soils, in the absence of any C, N, or P addition ($R = 0.0013$ μg C/g soil). With amendment additions, wet soil respiration increased significantly (1571% and 1852%) at C levels 1 and 2 (Tukey HSD, $p < 0.001$), respectively, without any N or P addition (Appendix S1: Table S1 and Figure S3). When N was added to C level 1, respiration increased 2464%, 3528%, 3322%, and 3182% at N levels 1, 2, 3, and 4 (Tukey HSD, $p < 0.001$), respectively, compared to the rates observed in the unamended control soils that still received water (Appendix S1: Table S1 and Figure S3). Soil respiration did not respond to P addition, nor to addition of N in the absence of C (Appendix S1: Table S1 and Figure S3). The RSM ($r^2 = 0.55$, $p < 0.001$) indicated that respiration increased with increasing C and N ($\beta_R = 0.08$ mg CO_2-C/mg glucose-C, and 0.34 mg CO_2-C/mg NH_4-NO_3-N), and increased with the interaction of the C and N ($\beta_R = 0.14$), but at the highest levels of C and N, respiration showed a small decrease as a function of the quadratic term of C ($\beta_R = -0.03$), and a large decrease as a function of the quadratic term of N ($\beta_R = -1.41$; Table 1, Figure 2).

Because the soil respiration response saturated with low additions of C and N, we grouped C and N into low (no addition) and high (C level 1 and 2; N level 1–4) additions. We then evaluated the interactive response to C × N, across all P levels, as well as the interactive response of dry-wet incubation soil respiration to water × CNP (i.e., the high level of all elements). In dry soils with no added amendments, soil respiration was not detectable, and did not increase with added CNP when soil was dry. Soil respiration occurred at a very low rate in response to water alone (0.000012 μg CO_2-C/g soil), but significantly increased many orders of magnitude (0.084 μg CO_2-C/g soil) in response to the coupled addition of water × CNP (Table 2, Figure 3a). Similarly, wet soil respiration increased 703% with C alone, did not respond to N alone, but increased multiplicatively with C × N by 3334% (Figure 3b). When measured across all levels of C, soil respiration increased by 17% with N addition, but did not respond to P addition (Figure 3c).

Microbial biomass response to C, N, and P

Soil MBC increased by 150% and 232% with 1 and 2 mg of C addition across all C levels, respectively, with high variability associated with the highest C amendment level (Figure 4; Appendix S1: Table S1 and Figure S4a). When compared across all N levels, MBC did not significantly increase with N or with C × N, although there was an apparent trend toward MBC increasing with C × N similar to respiration (Appendix S1: Figure S4a). The extremely high variability in MBC at high levels may have masked this effect, which was evident in the response surface analysis below. MBC was not responsive to P. Unsurprisingly, TOC increased 625% and 2245% with 1 and 2 mg of C addition (across all C levels), respectively, but decreased 35% with N addition (Table 1; Appendix S1: Figure S4c), which may have been caused by N reducing soil C via increased MBC growth and respiration. TOC was not responsive to P addition (Appendix S1: Figure S4c). Both MBN and TN unsurprisingly increased with N addition across N levels (67% and 237%, and 1734% and 3818%, respectively), but were not

TABLE 1 Response surface analysis models for total cumulative respiration (R_{TOT}), microbial biomass C (dMBC), and total extractable organic C (dTOC)

	R_{TOT}			dMBC			dTOC		
Parameter	β_R	SE	Pr(>|t|)	β_{dMBC}	SE	Pr(>|t|)	β_{dTOC}	SE	Pr(>|t|)
Intercept	−0.01	0.01	0.182	0.05	0.04	0.213	−0.20	0.08	**0.014**
C	0.08	0.01	**<0.001**				0.82	0.12	**<0.001**
N	0.34	0.10	**<0.001**				4.18	0.88	**<0.001**
P	−0.35	0.46	0.442				0.06	1.81	0.974
C × N	0.14	0.03	**<0.001**	0.52	0.17	**0.004**	1.01	0.27	**<0.001**
C × P	0.05	0.13	0.709	0.43	0.80	0.593	0.40	1.08	0.710
N × P	1.66	1.22	0.177	−5.90	6.57	0.372	−1.07	8.85	0.904
C^2	−0.03	0.00	**<0.001**				−0.36	0.05	**<0.001**
N^2	−1.41	0.34	**<0.001**				−14.89	3.00	**<0.001**
P^2	2.29	7.64	0.764						

Note: Boldface type indicates significant p values ($p \leq 0.05$).

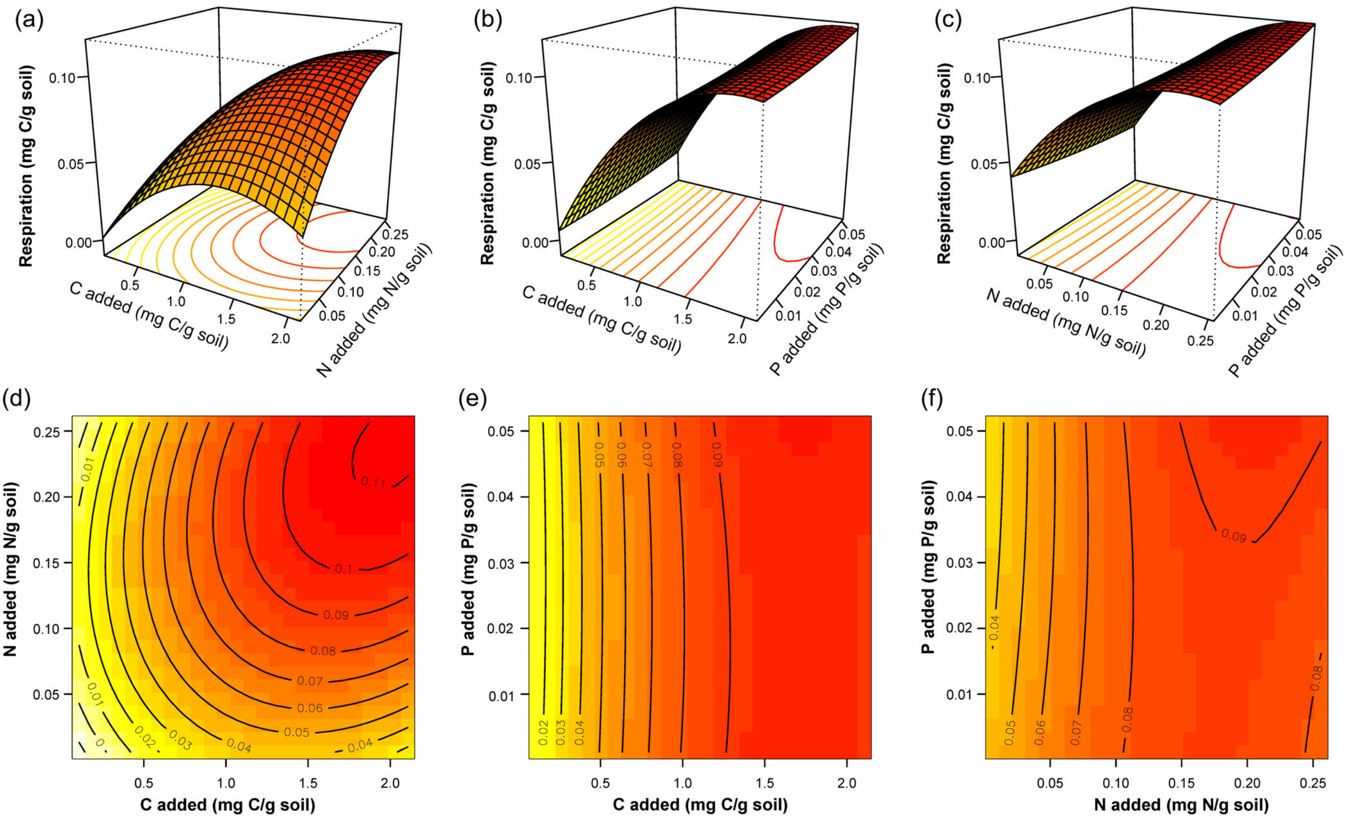

FIGURE 2 (a–c) Three-dimensional second-order response surface models for two-way amendment combinations (C, N, P, respectively) on total cumulative soil respiration (mg/g soil), indicating interactive stimulation of respiration by (a) C and N, but not (b) C and P, or (c) N and P. (d–f) Two-dimensional visualization of the same results. Yellow to red scale indicates low to high soil respiration. The topographic lines represent the two-dimensional response surface

TABLE 2 Two-way ANOVA for water and C, N, and P addition on total cumulative soil respiration (R_{TOT}; mg/g soil)

Parameter	df	F	Pr (>F)
Water	1	25.37	**<0.001**
CNP	1	15.67	**<0.001**
Water × CNP	1	15.60	**0.0013**

Note: Boldface type indicates significant p values ($p \le 0.05$).

responsive to either C or P addition (Table 1; Appendix S1: Figure S4b–d).

The RSM for the change in MBC ($\mathrm{dMBC} = \mathrm{MBC}_{\mathrm{final}} - \mathrm{MBC}_{\mathrm{initial}}$; $r^2 = 0.10$, $p = 0.019$) indicated that dMBC increased strongly as a function of the interaction between C and N (Figure 4 and Table 1), although the model fit was somewhat poor. Similarly, the RSM for the change in TOC ($\mathrm{dTOC} = \mathrm{TOC}_{\mathrm{initial}} - \mathrm{TOC}_{\mathrm{final}}$; $r^2 = 0.698$, $p < 0.001$) indicated that dTOC increased with both C and N, as well as the interaction of the two, but that the rate of increase may have declined at the highest C and N levels (Table 1 and Figure 4).

We further explored the interactive effects of C, N, and P on MBC and TOC by grouping the data into low C (no C addition) or high C (C levels 1 and 2), low N (no N addition) or high N (N levels 2 and 4; levels 1 and 3 were not used in this incubation), and low P (no P addition) or high P (P level 2) categories. MBC showed a similar response to respiration, where C alone increased MBC by 60%, N alone had no effect, and C × N resulted in the largest increase in MBC by 245% (Figure 5a). MBC with N addition (across all C levels) did not interact with P additions (Figure 5b). Soil TOC showed an inverse response to respiration and MBC, consistent with the fact that an increase in the latter two should result in a drawdown of the former (Figure 5c). Adding C increased the TOC pool by 1974%, but adding N caused a rapid reduction in the size of that pool; however, these patterns did not interact with P addition (Figure 5c,d).

Modeled soil microbial CUE ranged from 0.37 to 0.82. The RSM for CUE ($r^2 = 0.783$, $p < 0.001$) suggested that CUE decreased with added C ($p < 0.001$), increased with added N ($p < 0.001$), but was not affected by P ($p = 0.93$). CUE was strongly determined by the interactive effect of

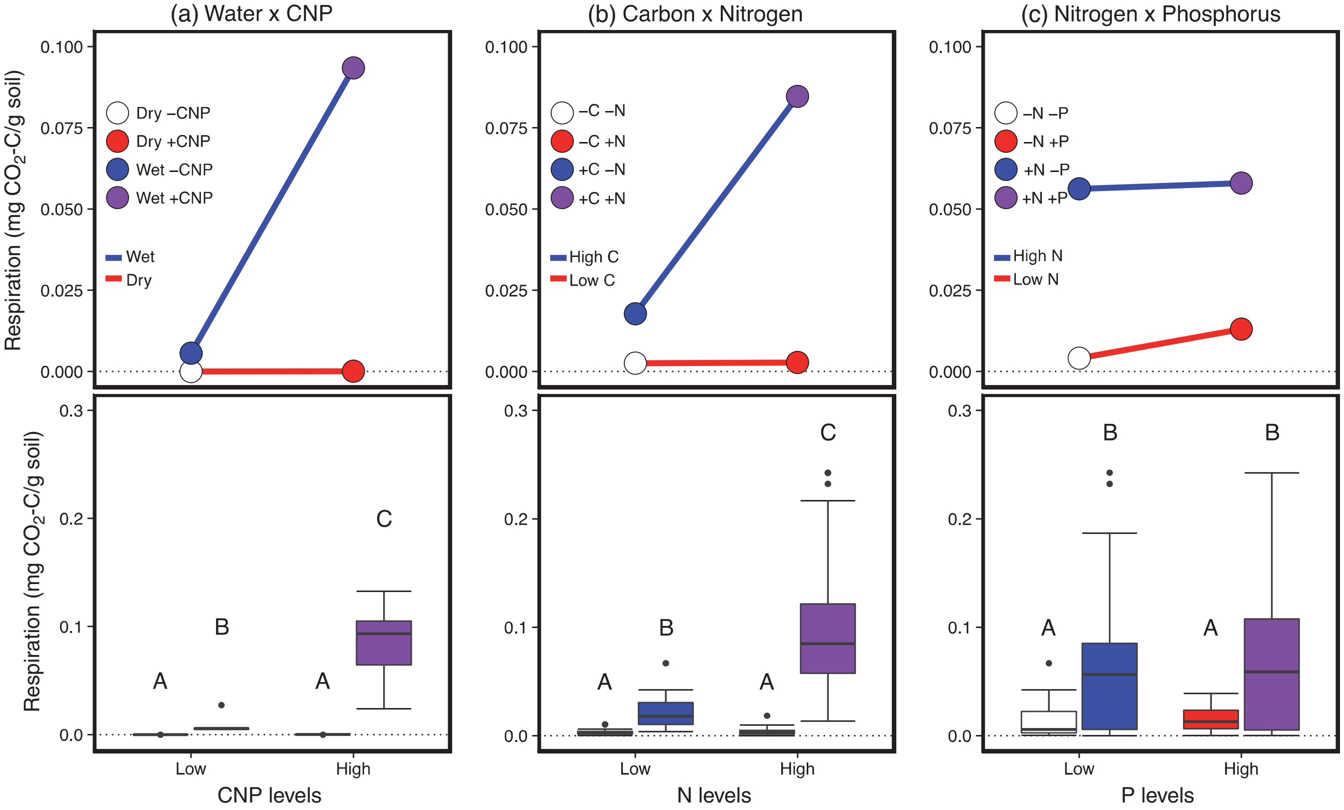

FIGURE 3 Interaction plot of soil respiration in response to (a) water × CNP, (b) C × N, and (c) N × P. Data shown are for dry or wet soils that had C and/or nutrients added (as shown by + in the legend), or that did not have C and/or nutrients added (as shown by − in the legend; e.g., −C is no C addition; +C is C addition, etc.). The upper row of panels shows the median response to the hypothesized primary (or co-) limiting resource across low and high levels of the secondary limiting resource in each group. Colored circles represent the median value for each level, and lines connect circles within the (a) wet or dry, (b) high or low C, and (c) high or low N treatments. In the lower panel, each box represents the median and first (Q1) and third (Q3) quartiles, while bars represent the minimum (Q1–1.5 × interquartile range) and maximum (i.e., Q3 + 1.3 × interquartile range); black dots represent outlier points. Panel (a) suggests functional co-limitation, indicated by the strong response to water × CNP, but very limited response to either water alone and no response to CNP alone. Panel (b) demonstrates serial limitation, indicated by the modest response to C alone, lack of response to N alone, and strong response to C × N. Panel (c) demonstrates independent limitation, indicated by the positive response to N, but lack of response to P. Different uppercase letters indicate Tukey HSD significant differences between treatment levels ($p < 0.05$)

C and N addition ($p < 0.001$; Table 1 and Figure 6). When C:N of amendments was equal to or below the average MBC:N of our system (7.6), CUE was stable around 0.74. As C:N of amendments increased above that level, and especially above a threshold of 11.7 (which is the mean MBC:N divided by the mean CUE [0.65]), CUE declined substantially. CUE showed a statistically significant decrease with C × P ($p = 0.019$) and increase with N × P ($p < 0.001$), but the magnitude of the interactive response in both cases was a change in CUE of <0.05 across the full range of added P.

DISCUSSION

Soil heterotrophic C cycling in this dryland ecosystem showed contrasting patterns of serial limitation and independent limitation, as well as evidence of "functional" co-limitation, by water, C, and N, but not P, in laboratory incubations. In particular, although the addition of water did stimulate respiration relative to dry soils, these responses were very small compared with the addition of water and C in tandem (soil respiration was 0.0091 μg C/g soil with water alone and increased >2400%, to 0.2462 μg C/g soil, when water and C were added together). It should be noted that we added "labile" C (glucose), and more recalcitrant C substrates may be more likely to have a less pronounced effect (Kuzyakov, 2010). We were surprised by such strong synergistic effects of water and C (e.g., >2400% increase when water and C were added together compared with adding water alone) because drylands are often considered so exceptionally water limited. However, our results suggest that although increasing water alone can have

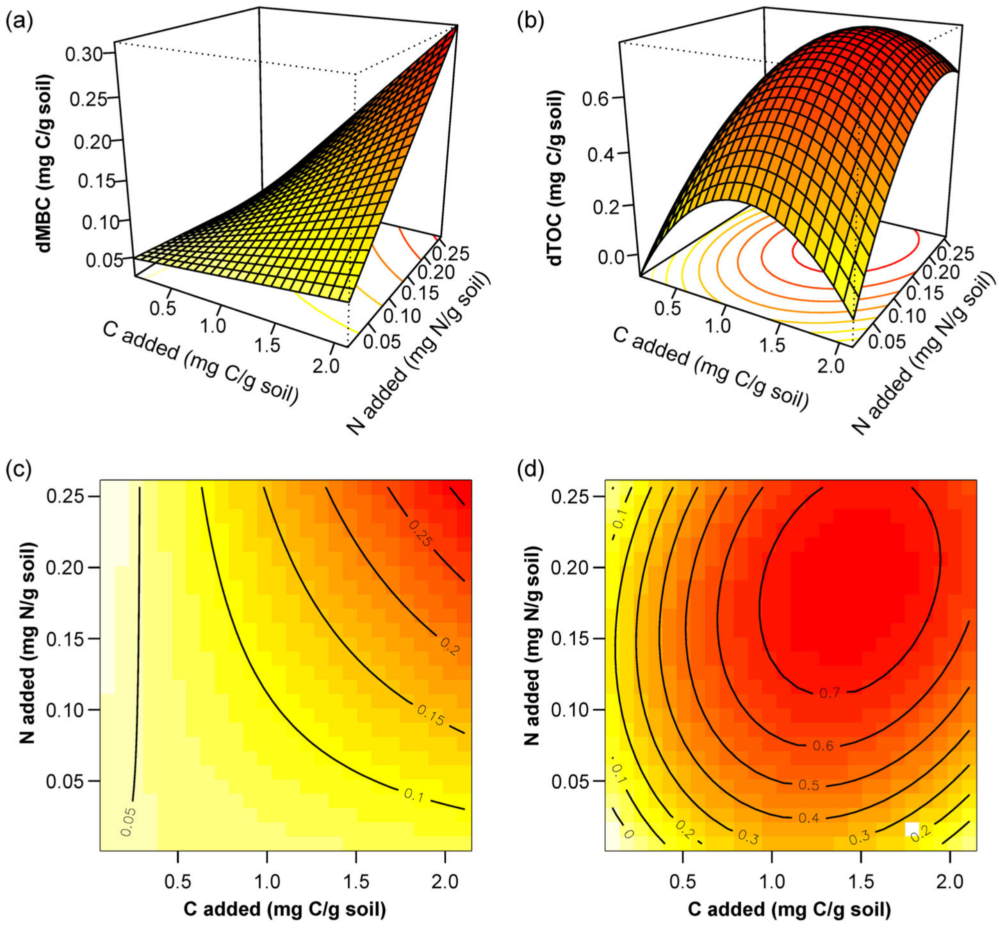

FIGURE 4 (a, b) Three-dimensional two-way interaction and second-order response surface models for (a) amendment combinations (C and N) on change in soil microbial biomass C (dMBC) and (b) total dissolved soil organic C (dTOC), indicating significant interactions between C and N addition on dMBC and dTOC. (c, d) Two-dimensional visualization of the same results. Yellow to red scale indicates low to high change in both pools. The topographic lines represent the two-dimensional response surface

effects, slight but simultaneous increases in both water and C availability can interact to regulate broad scale ecological processes. The addition of even small amounts of N in combination with water and C had much larger effects still (>2500% greater than adding water alone). Drylands are an environment where extant resource storage (e.g., soil moisture, soil organic C and N) is so low that biogeochemical cycles can experience large relative changes moment to moment with a highly responsive microbial community (Figure 1). Resource pools are often spatially variable and temporally ephemeral (Schlesinger et al., 1996), and subtle shifts in the availability of multiple nutrients may functionally co-limit heterotrophic soil activity in relation to questions of overall C exchange between dryland systems and the atmosphere. Our findings highlight the importance of investigating these small, linked changes to broaden our understanding of several critical theories in ecosystem ecology (Figure 1).

Our observed patterns fit the concepts synthesized by the Transient Maxima Hypothesis (Seastedt & Knapp, 1993) in which the periodically synchronized availability of multiple resources not only create "hot moments" of activity, but also dictate the dominant contribution to overall biotic activity. This temporal pattern likely extends to the patchy spatial distribution of resources in dryland ecosystems, where zones of resource availability are focused in "islands of fertility" beneath widely spaced perennial plants (Schlesinger et al., 1996), "mantles of fertility" of biological soil crusts in the upper mm of the soil surface (Garcia-Pichel et al., 2003; Tucker et al., 2017), and the rhizosphere around roots distributed sparsely through soil column (Jackson et al., 1996). In the rare instances in which a large precipitation pulse flushes the more concentrated C and N reserves occurring in patches and islands throughout the interstitial resource-poor bulk soil (Austin et al., 2004; Belnap et al., 2005), we would predict spatial synchronization of ecosystem

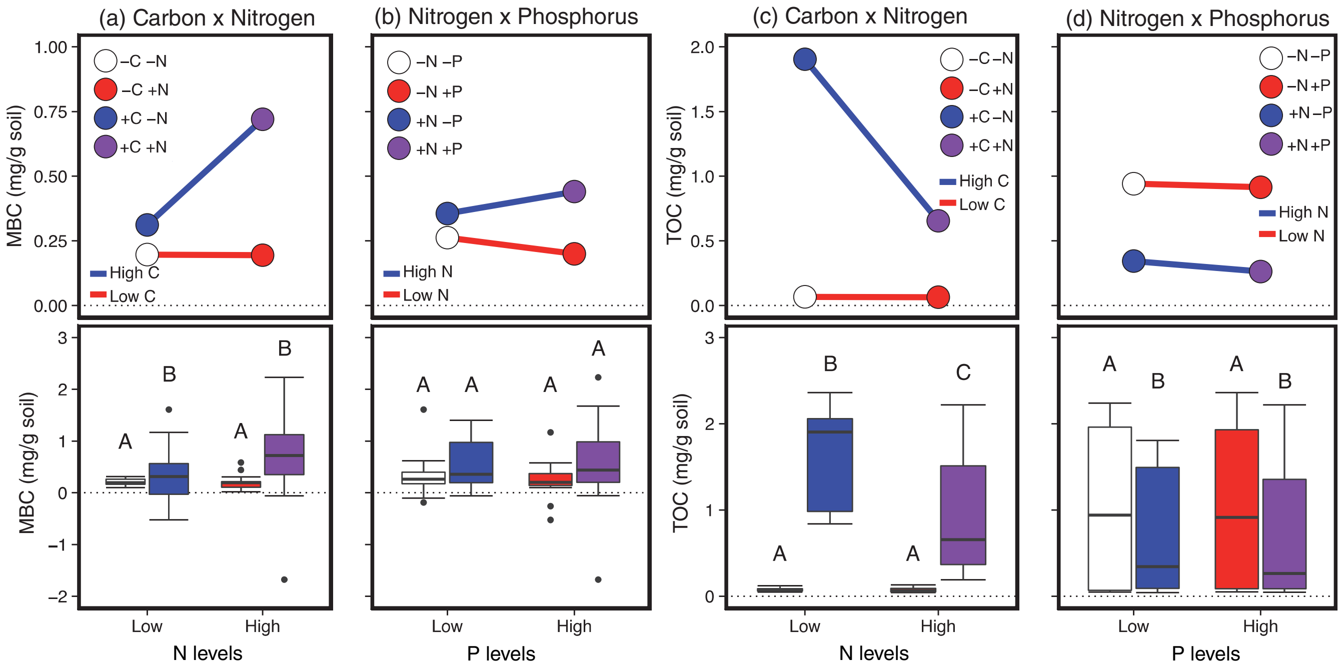

FIGURE 5 Interaction plot of soil microbial biomass C (MBC) and total dissolved soil total organic C (TOC) in response to (a, c) C × N and (b, d) N × P additions. The upper row of panels shows the median response to the hypothesized primary (or co-) limiting resource across low and high levels of the secondary limiting resource in each group. Colored circles represent the median value for each level, and lines connect circles within the (a, c) high or low C, and (b, d) high or low N treatments. In the lower panels, each box represents the median and first (Q1) and third (Q3) quartiles, while bars represent the minimum (Q1 − 1.5 × interquartile range) and maximum (i.e., Q3 + 1.3 × interquartile range); black dots represent outlier points, while different uppercase letters indicate significant differences between treatment levels ($p < 0.05$)

processes that would lead to a multiplicative increase of heterotrophic respiration, thus suggesting that the large effect of water observed in these systems is at least in part caused by water redistributing other limiting resources throughout the soil profile (Figure 1).

The transition between different limiting resources is the driving force in the shift from simultaneous co-limitation to serial limitation. A meta-analysis by Harpole et al. (2011) found that 28% of studies were simultaneously or independently co-limited, while 22% of studies demonstrated serial limitation with the addition of N and P. Studies that exhibited co-limitation had generally lower nutrient availability, suggesting a more balanced limitation of resources; while those that exhibited serial limitation were characterized by higher total nutrient availability and were more imbalanced by a single limiting resource (Harpole et al., 2011). This fits with our framework that water and C were functionally co-limiting (from a total CO_2 loss perspective), as both are notably deficient in desert soils, which have the lowest soil organic C concentrations of any biome (Jobbágy & Jackson, 2000). Based on our results, the distinction between functional co-limitation and serial limitation by water and C requires nuance. We clearly show a modest response to water addition alone, no response to CNP in the absence of water, and a response to water × C, and water × C × N. To be exact, this result indicates serial limitation by water followed by C. At the same time, it is important to acknowledge that in this instance, multiple resources are interacting to dictate overall outcomes that have aspects of functional co-limitation. The response to water alone is so small, and C alone is negligible, such that it is only with the addition of both resources that the overall effect on C cycling becomes significant.

The tight coupling of water and C availability in drylands may synergistically control soil heterotrophic C cycling in multiple ways. For instance, dryland net primary productivity (NPP; and thus C inputs into soil) is tightly coupled to water availability (Ahlstrom et al., 2015; Gherardi & Sala, 2019; Yang et al., 2008). Even so, the response of plant NPP and C cycling by soil organisms can differ depending on the seasonality and amount of precipitation (Thomey et al., 2011). Soil water availability is crucial for C (and nutrients) to diffuse from pockets of higher availability to more resource deficient parts of the soil (Davidson et al., 2012; Schimel & Bennett, 2004), which will have large effects on soil C processing (Waring et al., 2020). Given the background of

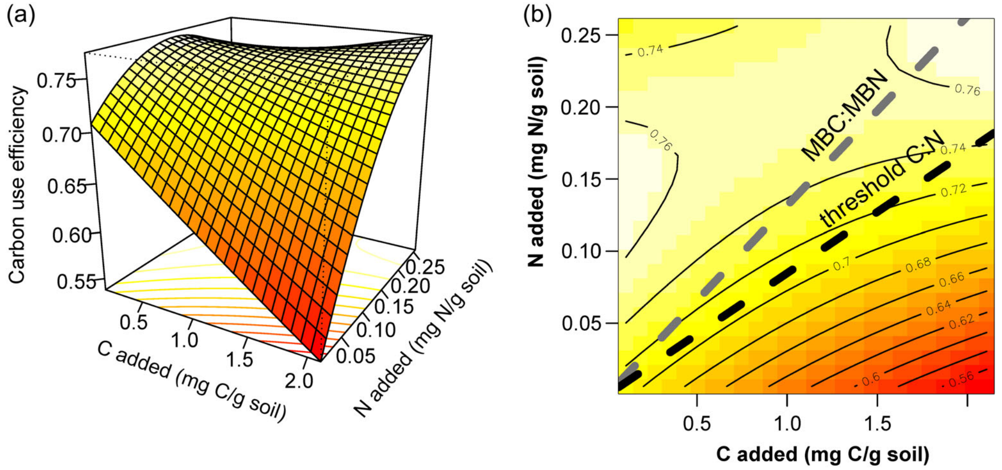

FIGURE 6 (a) Three-dimensional second-order response surface models for amendment combinations (C and N) on net C-use efficiency (CUE) calculated from response surface models of soil respiration and microbial biomass C (MBC). (b) Two-dimensional visualization of the CUE by C and N addition. Yellow to red scale indicates high to low microbial CUE. Gray dashed line indicates MBC: MBN (microbial biomass N) ratio CUE =7.6; black dashed line indicates threshold C:N ratio CUE = 11.6. Microbial C:N ratios greater than the calculated threshold suggests microbial growth is increasingly limited by N availability, while lower than that threshold, microbial biomass growth is limited by C availability. In our study system, in the absence of resource addition, soil soluble C:N is on average slightly below the threshold level, indicating limitation of microbial growth and respiration by C availability

very low organic C storage in soils in this ecosystem, a change in precipitation alone might not have much effect of on soil microbial processes unless it occurs at times that support NPP, root exudation, and/or litter decomposition. Sponseller (2007) found that soil respiration in Sonoran drylands declined over a single rainfall pulse, suggesting C limitation and that precipitation events can quickly deplete initial soil C stores. Water is often considered a proxy for multiple resources, but global changes could decouple water C cycle linkages leading to much larger synergistic or antagonistic responses than studies adding individual resources suggest.

From our data, added N could be utilized only after both water and C were available above a minimum threshold, indicating stronger water and C limitation than N limitation, which is consistent with elemental stoichiometry in our system. In general, soil microbes are thought to be C limited when the soil soluble C:N ratio is less than microbial C:N divided by microbial CUE (Čapek et al., 2018; Sinsabaugh et al., 2013, 2016). That is, when the soil C:N is below a threshold set by the demand ratio of MBC : MBN, after accounting for C respired during biomass synthesis, soil microbial biomass growth is limited by C availability. In our system, the soil soluble C:N ratio is 11.6, while the threshold C:N is 11.7 (black line in Figure 6; based on a microbial C:N ratio of 7.6 [gray line in Figure 6] and average CUE of 0.65) such that soil microbes in this system are C limited (i.e., $11.6 < 11.7$), but close to the threshold value for N limitation, which helps to explain the serial limitation by N. At substrate C:N ratios below the calculated threshold C:N ratio of 11.7, microbial CUE is relatively constant around 0.74, while at substrate C:N ratios above that level (lower right of Figure 6) CUE declines abruptly, indicating microbial growth is increasingly limited by N availability. The fact that growth of microbial biomass is C limited, even when considering the very low N concentration (0.04%) in the soil, emphasizes the importance of evaluating not only the absolute abundance of individual elements but also their relative abundances. Given the potential importance of CUE in determining the strength of the microbial C pump and subsequent soil C storage (Zhu et al., 2020) the role of C:N ratio in determining CUE may have significant effects on terrestrial C cycling.

In contrast to the findings from several global meta-analyses on the influence of N on terrestrial heterotrophic C cycling (Janssens et al., 2010; Ramirez et al., 2012; Treseder, 2008), we found that N addition enhanced soil respiration and microbial biomass. Studies that observed N inhibition suggest that the addition of N to soils often restricts or decreases microbial growth and decomposition, either by reducing microbial demand or altering the osmotic potential and soil pH causing the leaching or mobilization of limiting ions (Treseder, 2008; Vitousek et al., 1997). However, the majority of studies from the meta-analyses were conducted in temperate forest or mesic grassland systems, which may have different limiting resources and microbial responses than dryland soils. Desert systems are characterized by seasonal periods of rainfall and cooler temperatures that facilitate microbial activity, and microbial communities adapted to infrequent water pulses and nutrient inputs (Bowling et al., 2011), which may drive a stronger coupling of C and N in drylands (Delgado-Baquerizo et al., 2013; Schaeffer et al., 2003). Schaeffer et al. (2003) suggested that the co-addition of C provided the necessary substrate for microbial heterotrophs that would normally outcompete other functional groups, such as denitrifiers, for the available N, and that the increase in soil respiration occurred with an increase in microbial N immobilization and growth. The addition of both C and N resulted in a greater net increase in MBC (Table 1). Because conversion of MBC into soil organic matter is considered a main pathway of soil C stabilization (Dungait et al., 2012), this result suggests that enhanced microbial C cycling with N fertilization might promote greater input of C to organic soil pools.

Finally, despite studies showing widespread P effects on ecosystem C cycling (Cleveland et al., 2002; Elser et al., 2007; Vitousek et al., 2010) and support for both N and P limitation in many terrestrial systems (Ågren et al., 2012; Davidson et al., 2004; Marklein & Houlton, 2012; Reed et al., 2007), we found no effect of P, individual or interactive, on soil respiration or microbial biomass with any of the C:N:P combinations or treatment levels. It is possible that P could be limiting at a later point in soil microbial activity by constraining microbial ATP production or synthesis of phospholipid bilayers (Wu et al., 2010). Further, biological soil crusts may also play an important role in dryland P cycling by limiting loss via erosion and increasing bioavailability, while simultaneously utilizing soil P (Belnap, 2011). From a stoichiometric perspective, the limited response of soil C cycling to P relative to C and N is unsurprising given that extractable P levels in this system were approximately equal to extractable N levels (i.e., N:P = 1.1, compared to an average microbial N:P ratio of ~4.9 in grasslands) and the C:P ratio (9.9) is only 20% of the average C:P ratio (~47) in microbial biomass in grasslands (Cleveland & Liptzin, 2007); both facts suggest that the P supply exceeds the P demand for microbial growth. What is more surprising is that even in the presence of very high levels of water, C, and N, there was still no detectable influence of P, which suggests that P stores are able to provide enough P and that another element such as sulfur (Chapman, 1997) might be more limiting than P in this ecosystem. If the data from this site held for a wide range of sites, future work into alternative limiting elements, and whether availability of those elements is changing, may lend vital insight into dryland C cycling (Kaspari & Powers, 2016).

Understanding how the availability and limitation of multiple resources align to drive soil respiration is critical to accurately model and predict change for global dryland C cycling. Widespread anthropogenic changes are significantly impacting Earth's biogeochemical cycles, and these effects could be especially dramatic in drylands, which are particularly vulnerable to climate change (Allen et al., 2010; Diffenbaugh et al., 2008; Ferrenberg et al., 2015; Reed et al., 2012). For example, warmer temperatures and drier conditions are expected to reduce overall C cycling by reducing both soil respiration and photosynthesis, as well as subsequent C belowground inputs (Wertin et al., 2015, 2017). While atmospheric N deposition is relatively low on the Colorado Plateau (e.g., ~3 kg ha^{-1} year^{-1}) and while at this site increased N inputs did not result in significant changes to plant communities (Phillips et al., 2021), slight increases in anthropogenic N fertilization associated with agriculture and industrial emissions (Vitousek et al., 1997) can increase risk of fire in some dryland communities due to increased growth of annual grasses, invasive species, and fuel layers (Perkins, 2010; Rao et al., 2010; Seabloom et al., 2021). Further, shifts in seasonal precipitation patterns (both reduced frequency and greater intensity) and prolonged drought conditions will likely have even stronger influence driving these limiting multi-resource processes, as could the interactions among these varied global change effects. Because of expected increases in aridification and the likely cascading effects of these changes on C and nutrient cycles, we expect to see "pulsier" responses that may exceed the initial water-C threshold observed here, however, these responses will also be difficult to predict. Regardless, dryland responses to these changes will likely have global-scale effects on biogeochemical cycles (Ahlstrom et al., 2015; Delgado-Baquerizo et al., 2013; Keenan & Williams, 2018; Plaza et al., 2018), a prediction that puts emphasis the importance of understanding the complex antagonistic and synergistic dynamics of coupled limiting resources in this biome.

While these results are from a single study with soils removed from their native environment, the approach allowed us to test multiple levels of different limiting resources, which would be unfeasible in most field experiments, and highlights the importance of using conceptual experimental models to test key tenets of ecological theory. The data clearly show that, even under idealized conditions, there is strong control of resources other than water, building upon existing concepts of the controls over dryland soil respiration (Collins et al., 2008; Schwinning & Sala, 2004). Further, both temporal and spatial limitations must be considered when considering limitation of resources and how a host of global changes could affect the dryland C cycle. These results point to a need for an improved understanding of how multiple resources do or do not vary in concert and how these variations ultimately determine the amount of C moving as CO_2 from the soil to the atmosphere and vice versa. As we have demonstrated, alterations in the availability of limiting nutrients can have broader ecosystem consequences for C cycling, the soil microbial community, and fluxes of soil organic matter in an oligotrophic semiarid dryland system.

CONCLUSION

Drylands are characterized by low availability of many resources (e.g., soil moisture, soil organic C and N) and by resource pools that are often spatially variable and temporally ephemeral. This offers the opportunity to test fundamental hypotheses about coupled terrestrial biogeochemical cycles, as well as highlights the need for increased resolution in the conceptual and quantitative models used to explore and forecast dryland ecosystem function. Although understanding the patterns of soil water and seasonal precipitation is critical for dryland systems, climate change has the potential to alter or decouple the relationships between water and the availability of other resources through shifts in future climate and nutrient deposition patterns (Stevens et al., 2015; Weltzin et al., 2003). The data presented here highlight the need to improve our understanding of how C, nutrients, and water interact to influence soil efflux of CO_2 to the atmosphere, with the potential for large variation in the amount of CO_2 released depending on the concurrent spatial and temporal availability of multiple resources. Because anthropogenic activities and disturbances are simultaneously affecting these resources in arid and semiarid ecosystems worldwide and because drylands dominate key aspects of Earth's C cycle, a better understanding of how limiting resources interact to affect the dryland C cycle is critical for quantifying coupled biogeochemical cycles and effects of climate change at the global scale.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Ryan T. Choi, Colin L. Tucker, and Sasha C. Reed designed the study. Ryan T. Choi and Colin L. Tucker carried out all field and lab work. Ryan T. Choi and Colin L. Tucker performed data analysis. Ryan T. Choi and Colin L. Tucker wrote the first draft of the manuscript, and all authors contributed substantially to revisions. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

DATA AVAILABILITY STATEMENT

All data (Choi et al., 2021) are available in a U.S. Geological Survey data release, according to federal guidelines, at https://doi.org/10.5066/P9EEDPB0.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

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