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No evidence of resource limitation to aboveground growth of blue grama (*Bouteloua gracilis*) on 1 ky-old semi-arid substrate

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Abstract Biogeochemical theory and a substantial body of empirical data show that nitrogen (N), an atmospherically derived nutrient, limits plant growth on young substrates, while phosphorus (P), a rock-derived nutrient, limits plant growth on old substrates. In arid regions, water is also often a limiting resource to plant growth. We applied resource amendments of N, P, N + P, and water to blue grama (*Bouteloua gracilis*) growing on a 1 ky-old basaltic cinder substrate to test the hypothesis that N and water limit aboveground net primary production (ANPP) in a semi-arid climate, early in soil development. Contrary to our hypothesis, ANPP did not differ among treatments, suggesting that none of the resource amendments were limiting to blue grama growth. Unamended aboveground tissue N and P

concentrations were three to five times lower at the 1 ky-old site than on older (55–3000 ky-old) substrates, suggesting differences in nutrient use efficiency across the substrate age gradient.

Keywords Biogeochemical theory · Nitrogen · Phosphorus · Nutrient use efficiency · Substrate age gradient · Water

Introduction

Nitrogen (N) and phosphorus (P) are typically the most limiting nutrients to primary production in terrestrial environments (Vitousek and Howarth 1991), yet in arid regions, where water availability is also constrained, primary production is often limited by N (Ettershank et al. 1978; Fisher et al. 1988; Hooper and Johnson 1999) and water (Lauenroth et al. 1978; Yahdjian and Sala 2006; Chou et al. 2008; Robertson et al. 2009). A meta-analysis of aboveground net primary productivity (ANPP) responses to N fertilization revealed that the importance of N limitation to plant growth was enhanced as the amount of annual precipitation increased (i.e., from arid to subhumid ecosystems; Yahdjian et al. 2011), presumably by relieving water stress. Furthermore, Yahdjian et al. (2011) suggest that when soil is moist, during and following precipitation events, N limitation to plant growth may become important.

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Nutrient limitation of vegetation shifts from N limitation to P limitation as substrate age increases across both humid and arid environments (Vitousek and Farrington 1997; Newman and Hart 2015). The primary sources of these two plant essential nutrients differ. Phosphorus, a primarily rock-derived nutrient, is generally prevalent in young substrates and becomes available to plants as the parent material undergoes weathering. Over time, highly weathered substrates become depleted of P due to leaching and erosional losses, causing the ecosystem to reach a terminal steady state (Walker and Syers 1976) in the absence of significant atmospheric inputs (e.g., wind-blown dust) of P (Chadwick et al. 1999). Nitrogen enters the ecosystem via atmospheric inputs or biological N fixation, and in most parent materials N is nearly absent at the onset of ecosystem development (Walker and Syers 1976). Therefore, young terrestrial ecosystems are typically thought to be more constrained by N availability than by P availability, and this hypothesis has been supported by resource amendment studies conducted on substrate age gradients underlain with volcanic substrates (Vitousek and Farrington 1997; Newman and Hart 2015).

Relative substrate age can also be an important determinant in water limitation to plant growth in arid and semi-arid climates because, as ecosystem development progresses, soil texture varies from coarser-sized particles on younger substrates to finer-sized particles on older substrates. These soil textural changes alter soil water characteristics including: the rate of water infiltration, percolation, and soil water availability. For example, along a 1–3000 ky-old semi-arid substrate age gradient, sand-sized particles decreased, while clay content and surface water storage increased with substrate age (Selmants and Hart 2008). Furthermore, Newman and Hart (2015) demonstrated that water limitation to blue grama (*Bouteloua gracilis*, a perennial bunchgrass) occurred only at the youngest site examined (55 ky-old; Newman and Hart 2015). Consistent with biogeochemical theory, blue grama at the 55 ky-old site was also limited by N, while at the oldest, 3000 ky site blue grama was limited by P; no resource limitations were observed at the intermediate-aged site (750 ky). The youngest, 1 ky-old, site of this substrate age gradient was not included in their experiment because intercanopy spaces at this site were frequently dominated by shrubs instead of blue grama (Newman and Hart

2015). Furthermore, the presence of blue grama cover was relatively low and discontinuous compared to the three older sites. Nevertheless, the lack of inclusion of the youngest site along this substrate age gradient prevented a clear understanding of resource limitation dynamics along this semi-arid substrate age gradient.

Here, we applied N, P, and water amendments to blue grama growing on a 1 ky-old substrate and measured changes in aboveground biomass and nutrient concentrations in response to these amendments. Despite the sparse coverage of blue grama, we were able to establish 40 individual plots where blue grama was present. Our methodology followed the experimental design and resource addition protocol used previously by Newman and Hart (2015) on the three older sites of the substrate age gradient, except the patchy distribution of blue grama at the 1 ky-old site forced us to randomly assign treatments across the plots rather than use a randomized, complete-block design. We tested the following hypotheses: (1) blue grama growing on young 1 ky-old substrate dominated by coarse-textured soils would be water limited due to low soil water availability; and (2) blue grama growing on this 1 ky-old substrate would be limited by N due to relatively low soil N availability, but would not be limited by P due to comparatively high P availability.

Materials and methods

This study was conducted on a 1 ky-old substrate near Sunset Crater Volcano National Monument (35.22°N 111.32°W) by Flagstaff, Arizona. At this site, overstory vegetation consists of co-dominant piñon pine (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*), and intercanopy spaces are dominated by the shrubs Apache plume (*Fallugia paradoxa*) and skunkbrush sumac (*Rhus trilobata*). The USDA Soil Taxonomic subgroup at this site is Typic Ustorthent, and the parent material consists of volcanic cinders primarily composed of microporphyrific basalt deposited as a pyroclastic sheet (Selmants and Hart 2008; Moore and Wolfe 1987). Mean annual precipitation is 328 mm (Selmants and Hart 2008). This site represents the youngest substrate of four sites that comprise a 3 million year semi-arid substrate age gradient, the Substrate Age Gradient of Arizona (SAGA; Selmants and Hart 2008, 2010; Newman and Hart 2015;

Sullivan et al. 2015). Overstory vegetation at the three older sites is also co-dominated by piñon pine and one-seed juniper, but intercanopy spaces at these sites are dominated by the C₄ perennial bunchgrass blue grama (*Bouteloua gracilis*). As noted previously, Newman and Hart (2015) did not include this youngest substrate in their previous resource limitation study across the SAGA because of the sparse and spatially discontinuous distribution of blue grama at this site, which prevented employing the randomized, complete-block experimental design used at the other sites. However, because of the large contrasts in nutrient availability and soil water storage that occur between the youngest (1 ky) and next oldest substrate (55 ky; Selmants and Hart 2008, 2010), we built upon Newman and Hart's (2015) previous work by assessing resource limitation at the 1 ky-old site. To facilitate comparisons with resource limitations observed at the three oldest sites, we applied nutrients and water at the same rates as Newman and Hart (2015) despite the lower abundance and discontinuous distribution of blue grama in intercanopy spaces at this site. Due to the patchy nature of blue grama cover at the 1 ky-old site, we used a completely randomized experimental design.

Forty 1.5 m × 1.5 m plots of blue grama were established in a randomized design with a minimum distance of 4 m between plots. Total cover of blue grama was less than 40 % within each of the plots (mean = 18 %; SE = 1 %) compared to ~60 % within plots on the three older substrates (G. Newman, Natural History Museum of Denmark, personal communication). The percent of blue grama cover was similar among treatment plots. Each of the five treatments was randomly assigned to 8 of the 40 plots, which included: control, water addition, N addition, P addition, and N + P addition. Nutrient additions of N as ammonium nitrate (7.5 g N m⁻² year⁻¹) and phosphate as triple superphosphate (5 g P m⁻² year⁻¹) were applied once during the first week of July 2009, approximately one week prior to the initiation of the monsoon season. Newman and Hart (2015) opted to use this N application rate because a higher rate of 10 N m⁻² year⁻¹ contributed to piñon pine mortality at the Sevilleta, New Mexico, Long Term Ecological Research site (M. Allen, personal communication, University of California, Riverside). Newman and Hart (2015) selected the P application rate to meet the biological N:P mass ratio

requirement (12–13; Güsewell 2004) following consideration of the soils' P fixation capacity (Selmants and Hart 2010). At this time, weekly water additions at a rate of 3.6 L m⁻² application⁻¹ began. Over the course of the experiment, water was applied 13 times resulting in a cumulative addition of 46.8 L m⁻², equivalent to 14.3 % of mean annual precipitation (Selmants and Hart 2008) and 42.1 % of the precipitation received during the growing season in 2009 (July through October). The amount of water applied (0.36 cm per addition) in our study was selected to mimic typical monsoonal rain events received in Northern Arizona (which averaged 0.31 cm per event between August and September 2009; Sunset Crater National Monument, Station 028329, Western Regional Climate Center, www.wrcc.dri.edu). Because of the coarse texture of the soils at the 1 ky-site, most of the added water likely stayed in the soil plant-rooting zone because of the high infiltration rates of these soils and their self-mulching, which minimizes evaporation (Dregne 1976). All application rates were chosen to reflect those used in a previous resource addition study conducted at the three oldest substrates along the SAGA (Newman and Hart 2015).

Peak standing aboveground biomass of blue grama was removed by clipping all biomass within each plot at the ground surface at the end of the growing season (9 October 2009). On the date of harvest, visual estimation of the percent cover of each plot by blue grama was determined independently by the same two researchers for all plots and averaged. Percent cover did not differ significantly among treatments ($F_{(4,35)} = 0.3563$, $p = 0.84$; data not shown). Blue grama clippings were returned to the laboratory and oven dried at 70 °C for 48 h, cooled in desiccators, and then weighed. This mass divided by the sample area (2.25 m²) was used to estimate aboveground net primary productivity (ANPP; Bonham 1989). Dried plant biomass samples were ground with a Wiley Mill (A. Thomas Co., Philadelphia, PA, USA) to pass through a 40-mesh (<425 μm) screen. A micro-Kjeldahl digestion procedure (Parkinson and Allen 1975) was used to determine Total Kjeldahl N and P concentrations; NH₄⁺ and PO₄³⁻ concentrations in diluted digestates were determined on a Lachat Instruments QuickChem[®] 8000 Flow Injection Analyzer (Lachat Instruments, Loveland, Colorado, USA). Blue grama aboveground biomass senesces each year;

thus, we estimated aboveground nutrient uptake by multiplying aboveground biomass by nutrient concentration (Kaye et al. 2005).

A one-way ANOVA was used to determine if there were any significant differences among treatments for ANPP and aboveground tissue (predominately leaves) concentrations of N, P, their mass ratios, and N and P uptake. If the model was statistically significant ($\alpha < 0.05$), Tukey's HSD multiple comparisons were made among treatment means. The ANPP and N to P (N:P) mass ratios were log-transformed prior to statistical analysis to meet assumptions of normality and homogeneity of variances. All statistical analyses were performed using JMP Pro (v. 11.2.0, SAS Institute Inc. 2013).

Results

Aboveground net primary productivity did not differ significantly among treatments ($F_{(4,35)} = 0.96$, $p = 0.44$; Fig. 1). However, aboveground plant tissue concentrations of N ($F_{(4,35)} = 31.21$, $p < 0.0001$), P ($F_{(4,35)} = 11.42$, $p < 0.0001$), and the mass ratio of N to P ($F_{(4,35)} = 34.31$, $p < 0.0001$) differed significantly among treatments (Fig. 2). Amendments of N alone or in combination with P increased aboveground tissue N concentration, and amendments of P alone or in combination with N increased aboveground tissue P concentration. There was no synergistic effect when both N and P were added on either nutrient

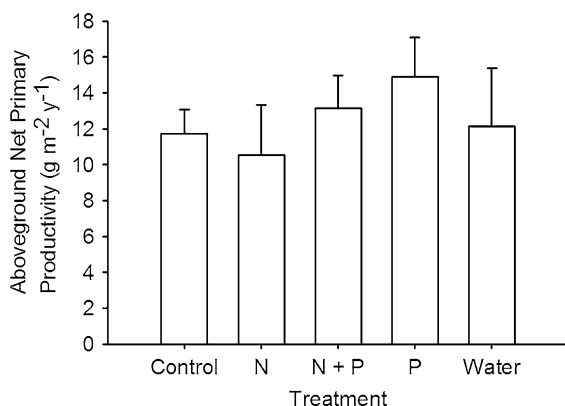


Fig. 1 Aboveground net primary productivity (ANPP, $\text{g m}^{-2} \text{y}^{-1}$) of blue grama growing on a 1 ky-old substrate in unamended (control), nitrogen (N), phosphorus (P), nitrogen and phosphorus (N + P), and water amended plots. Error bars represent one standard error of mean ($n = 8$)

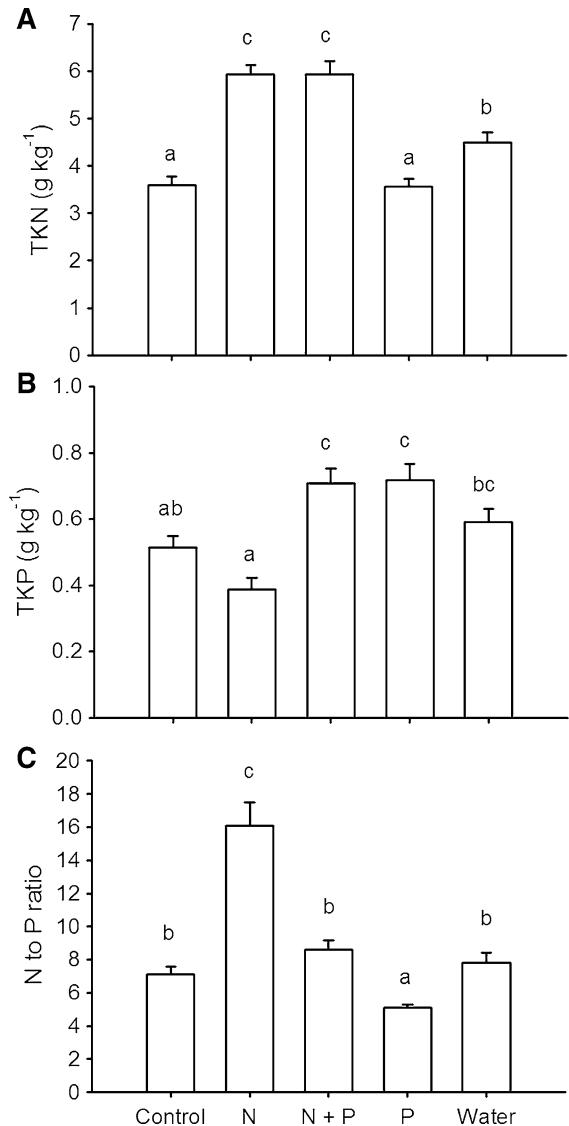


Fig. 2 Mean aboveground tissue nitrogen (N; panel A) and phosphorus (P (panel B) concentrations and N to P mass ratios (N:P; panel C) of blue grama growing on a 1 ky-old substrate in unamended (control), nitrogen (N), phosphorus (P), nitrogen and phosphorus (N + P), and water amended plots. Error bars represent one standard error of mean ($n = 8$). Different lower case letters denote statistical differences ($\alpha = 0.05$) in tissue concentrations among treatments determined by Tukey's HSD

concentration (Fig. 2a, b). The response of aboveground tissue N:P ratios followed similar patterns, increasing with N amendments, decreasing with P amendments, and remaining unchanged when both N and P were added together (Fig. 2c). Water amendment increased aboveground tissue N concentration (Fig. 2 a) but not P relative to the control; however,

mean aboveground tissue P concentration in the water-amended plots was intermediate between the control and the P and N + P amended plots (Fig. 2b).

Aboveground uptake of N did not differ significantly among treatments ($F_{(4,35)} = 1.17$, $p = 0.34$; Table 1). However, aboveground uptake of P differed significantly among treatments, with greater P uptake in the P amendment relative to the N amendment treatments, but no other treatment effects were observed ($F_{(4,35)} = 3.27$, $p = 0.02$; Table 1).

Discussion

In contrast to our hypotheses, we found no evidence of resource limitation to blue grama growing on 1 ky-old substrate in a semi-arid climate despite the low water storage capacity of the soil and low N concentrations in plants and soil relative to the older sites of the SAGA (Selmants and Hart 2008; Coble and Hart 2013). We speculate that our null results reflect the potential importance of: (1) plant co-limitation of N and water in arid ecosystems (e.g., Lauenroth et al. 1978; Cobb et al. 1997; Yahdjian et al. 2011) that is not evident from nutrient addition studies in humid ecosystem chronosequences (Vitousek and Farrington 1997); and (2) genotypic differences in blue grama at this low nutrient, high stress site limit the capacity of these plants to respond to rapid increases in resource availability (e.g., Chapin 1980).

Previous resource addition studies suggest that semi-arid plants can be co-limited by nutrients and water (Lauenroth et al. 1978; Cobb et al. 1997), and it is possible that a combination treatment of water and N

additions could have elicited a growth response in blue grama in our study. On a more developed soil (Mollisols) in the Central Plains Experimental Range (CPER) of northern Colorado, USA, Lauenroth et al. (1978) found an aboveground growth response of blue grama and other warm-season grasses to combined water and N additions after two years of amendments; a growth response to the water-only treatment occurred only after three years of amendments, and no growth response to N additions alone was observed even after five years of application. A previous experiment conducted on this 1 ky-old site found piñon pine stem growth increased only in response to a combination of water and nutrient (N, P, and potassium) amendments, which were applied for six years (Cobb et al. 1997). Furthermore, the 1 ky-old site in our study was the only SAGA site (Newman and Hart 2015) where water amendments altered aboveground blue grama tissue nutrient concentrations, suggesting resource interactions occur at the 1 ky-old site. Foliar concentrations have also been used as an indicator of nutrient limitation in lieu of nutrient addition experiments (Vitousek and Howarth 1991) with N limitation defined as $N < 13\text{--}14 \text{ g N kg}^{-1}$ (Wassen et al. 1995) or N:P ratio < 10 (Güsewell 2004), and P limitation defined as $P < 0.7 \text{ g P kg}^{-1}$ (Wassen et al. 1995) or $< 1.0 \text{ g P kg}^{-1}$ (Güsewell and Koerselman 2002) or N:P ratio > 20 (Güsewell 2004). Based on these criteria, the low aboveground (essentially foliar) tissue N (control group = 3.6 g N kg^{-1}) and P concentrations (control group = 0.51 g P kg^{-1}) observed at our 1 ky-old site fit both N and P limitation. Similarly, relative to the older unamended SAGA soils (Newman and Hart 2015) as well as other arid sites (Bowman et al. 1985; Hargrave and Seastedt 1994), blue grama aboveground tissue N and P concentrations at the 1 ky-old site are both exceptionally low (ranging from 10.2 to 18.5 g N kg^{-1} and 1.6 to 1.9 g P kg^{-1} at these other sites). Taken together, these results suggest that, despite the lack of a one-year response in ANPP, blue grama may still be resource limited; we speculate that unmeasured, water and nutrient interactions likely play a critical role in plant growth on this young 1 ky-old substrate with extremely low resource availability.

The conservative growth strategy of blue grama growing on this young, resource-poor substrate may also limit its capacity to respond to resource additions (Gleason et al. 2009). Conservative growth strategies of plants growing on resource-poor sites can limit

Table 1 Aboveground nitrogen (N) and phosphorus (P) uptake (mg m^{-2}) of blue grama following resource amendments to 1-ky old substrate

Treatment	N uptake (mg m^{-2})	P uptake (mg m^{-2})
Control	42.0 (5.0)	5.98 (0.72)a
N	65.6 (19.9)	4.27 (1.18)ab
N + P	76.0 (9.1)	9.16 (1.24)ab
P	53.8 (9.5)	10.64 (1.86)b
Water	52.1 (12.0)	7.02 (1.69)ab

Values are presented as treatment means with standard error in parentheses ($n = 8$). Where present, different lowercase letters denote statistical differences among treatments as determined by Tukey's HSD

plant growth response to nutrient additions, but tissue concentrations often increase (Chapin 1980), as was observed in this study and other fertilization studies with *Bouteloua* species (Hays et al. 1982; Joern and Mole 2005). Such luxury uptake can be advantageous in low resource environments by allowing plants to capitalize on pulses of nutrient availability (Chapin 1980). However, Newman and Hart's (2015) ability to elicit ANPP increases after one year on the other sites of the SAGA suggest that at least some genotypes of blue grama have the potential to increase growth rapidly in response to sudden increases in resource availability. We speculate that the ecotypes of blue grama growing on the low resource (except for possibly P) soils from the 1 ky-old site have a more pronounced conservative growth strategy than blue grama ecotypes at these more resource-rich sites. In support of this speculation, piñon pine trees growing on these young, stressful, volcanic soils have been shown to be genetically dissimilar to other piñon populations growing on more benign, adjacent soil types (Mopper et al. 1991). Use of similar plant genotypes to evaluate resource limitation across contrasting sites (essentially field "bioassays"; Binkley and Hart 1989) would eliminate this potential source of error in future resource-limitation studies.

There are other alternative possibilities for why we did not observe an increase in blue grama ANPP to any of our single resource additions. For instance, our resource additions may have been too low to alleviate resource limitation in these plants at the 1 ky-old site. Additionally, the plants could have been limited by another resource that we did not augment. Finally, these perennial bunchgrasses may have responded to the resource additions by increasing belowground but not aboveground biomass in the first year.

We argue that it is unlikely that our resource additions were insufficient to alleviate resource limitation for multiple reasons. First and foremost, similar rates of resource additions resulted in increases in ANPP of blue grama in the first year at the other three sites of the substrate age gradient (SAGA), even though blue grama cover (the only plant species present) was one third (18 %) of what it was at these other sites (~60 %; Newman and Hart 2015). Furthermore, although some other resource manipulation studies of blue grama in semi-arid ecosystems have used higher addition rates and have observed (after more than a single year of application)

aboveground growth responses, the resource addition rates in these studies relative to the standing aboveground biomass are fairly similar to our study. For example, although at the CPER site Lauenroth et al. (1978) more than doubled mean annual precipitation with water additions during the growing season, aboveground biomass was an order of magnitude greater (150 g m^{-2}) at their study site than at our study site. Therefore, although we only increased the growing season precipitation by 42.1 % and the annual precipitation by 14.3 %, the amount of water addition per standing aboveground plant biomass was similar in our two studies. Similarly, the N addition rate in our study ($7.5 \text{ g N m}^{-2} \text{ year}^{-1}$) was considerably higher than in the Lauenroth et al. (1978) study (~10–15 $\text{g N m}^{-2} \text{ year}^{-1}$) when expressed relative to the standing plant biomass. Furthermore, it is also unlikely that another resource besides those added was limiting to blue grama growth, resulting in luxurious uptake of N and P in the amended plots. Even though these young, basaltic soils are low in other available macro- and micro-nutrients (Cobb et al. 1997), growth limitation by these other nutrients is extremely rare in wild plants (Binkley 1986). Additionally, light availability in these sparsely covered bunchgrasses within intercanopy areas at the 1 ky-old site is essentially full-sun. Hence, we conclude that neither resource application rates nor another limiting resource are likely reasons for the lack of observed growth responses of blue grama in our study.

Short-term (one year) amendments of limiting resources may have been preferentially allocated by blue grama to belowground rather than above ground biomass production to acquire additional water or nutrients. Güsewell (2004) suggested that resource amendments may not yield an increase in biomass in the first year of a study even when a resource is limiting, particularly when plant biomass relies on previous year's growth (i.e., belowground resources or buds). Large belowground resource allocations for herbaceous bunchgrasses (including blue grama) in response to short-term increases in soil resources have been observed previously in northern Arizona (Kaye et al. 2005), and these belowground allocations could delay aboveground resource allocation. Unfortunately, just as was the case in Lauenroth et al.'s (1978) study, we do not know if any of our treatments resulted in an increase in belowground productivity. However, given that we observed ANPP increases in

blue grama to these same amendments at the other SAGA sites, we speculate that the lack of inclusion of belowground growth responses is not responsible for the null result of our treatments.

Across the SAGA, the pattern of blue grama ANPP is consistent with the concept of ecosystem retrogression, which is characterized by an increase and eventual decline in ecosystem processes (e.g., NPP, decomposition, nutrient cycling) over time scales of thousands to millions of years (Wardle et al. 2004; Peltzer et al. 2010). Retrogression has been attributed to a decline in soil nutrient availability (e.g., P) to plants over long-timescales as a result of weathering (Peltzer et al. 2010), and previous research across the SAGA supports this theory (Selmants and Hart 2008, 2010; Coble et al. 2015). For example, the oldest SAGA site has the lowest soil P concentrations (Selmants and Hart 2010) and is the only site where soil and vegetation pools are predominately supported by aeolian, rather than weathering, sources of rock-derived nutrients (Coble et al. 2015). The ANPP in blue grama unamended plots also follows a pattern of retrogression across the SAGA, with ANPP increasing across the first three sites and then declining at the oldest site (11.7, 21.3, 25.8, and 7.4 g m⁻² year⁻¹ at the 1, 55, 750, and 3000 ky-old sites, respectively; Fig. 1, Newman and Hart 2015). The ANPP was 37 % greater at the youngest (measured in 2009) than at the oldest (3000 ky-old) site (measured in 2004) despite a lower percent blue grama cover and less annual precipitation received at the youngest site (total annual precipitation at Sunset Crater National Monument: 2004 = 43.0 cm, 2009 = 31.9 cm; total growing season precipitation 2004 = 21.4 cm, 2009 = 11.1 cm; mean event size in growing season 2004 = 1.02 cm, 2009 = 0.53 cm; mean daily temperatures were similar between these two years at 7.7 °C). The taller, yet patchy distribution, of blue grama at the youngest site suggests greater resource use by individual plants, which may occur in response to low water availability on coarse textured soils, as suggested by the inverse texture hypothesis (Noy-Meir 1973). Indeed, N and P use efficiencies (sensu Berendse and Aerts 1987) of blue grama plants growing at the 1 ky-old site were 3–5 times higher than of blue grama plants growing on the older SAGA substrates or at other arid sites (Bowman et al. 1985; Hargrave and Seastedt 1994; Newman and Hart 2015). Despite substantial exogenous subsidies of rock-derived nutrients, greater

percent blue grama cover, and greater amount of precipitation received at the oldest site during our study years, soil P availability at the 3000 ky-old site appears to limit ANPP to the extent that plant production potential at 1 ky-old substrates exceeds that of older highly weathered substrates.

Our current understanding of the role of nutrient limitation in ecosystem development is derived from a limited number of studies (e.g., Vitousek and Farrington 1997; Newman and Hart 2015), conducted across a subset of the range of available substrate ages and geologic substrate types. Our study conducted at the 1-ky old site, when combined with previous research using the same suite of resource additions to soils at the three oldest sites of a semi-arid substrate age gradient, adds unanticipated complexity to extant biogeochemistry. Newman and Hart (2015) found the expected vegetation shift from N limitation to P limitation as ecosystem development progresses (Newman and Hart 2015), with water limitation occurring only at the youngest substrate (55 ky). However, our evaluation of resource limitation at the youngest substrate in the age gradient (1 ky) did not indicate N or water limitation to blue grama, a result that is inconsistent with biogeochemical theory. Although we argue that co-limitation of resources or a highly conservative growth strategy of the blue grama ecotype at this 1-ky site are the most plausible causes for the lack of any observed increase in blue grama growth, we concur with the conclusions of Sullivan et al.'s (2014) recent synthesis that field-based resource addition studies can be difficult to interpret. These authors suggest a multi-pronged approach for identifying resource limitation in terrestrial ecosystems (including indicators of soil nutrient supply, organismal indicators of nutrient limitation, and laboratory-based experiments and nutrient depletions). Our results also illustrate that field-based resource addition studies using vegetation indicators in arid environments may require full factorial experiments, be conducted over multiple years, include belowground vegetation measurements, and use similar plant genotypes within a species (field bioassays) or multiple species with contrasting life histories/plant growth strategies in order to have the greatest inferential power.

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