

Shifting soil resource limitations and ecosystem retrogression across a three million year semi-arid substrate age gradient

Gregory S. Newman · Stephen C. Hart

Received: 25 October 2014 / Accepted: 16 March 2015 / Published online: 24 March 2015
© Springer International Publishing Switzerland 2015

Abstract The current paradigm of plant nutrient limitation during ecosystem development predicts a change from nitrogen (N) limitation when substrates are young to phosphorus (P) limitation when substrates are old. However, there are surprisingly few direct tests of this model. We evaluated this theory experimentally along a three million year semi-arid substrate age gradient using resource additions to intercanopy spaces dominated by the C4 bunchgrass *Bouteloua gracilis*. Unlike other gradients in subtropical and temperate ecosystems, soil water availability also increases strongly across this semi-arid system due to finer texture with substrate age. We found that aboveground net primary production (ANPP) of *B. gracilis* was limited by both water and N on the 55 ky substrate; not limited by N, P, or water

on the 750 ky substrate; and limited by P alone on the 3000 ky substrate. Notably, measures of foliar nutrient concentration and N:P mass ratios were unable to predict nutrient limitations in these semi-arid systems. In unamended plots, mean ANPP declined dramatically at 3000 ky compared to the younger substrate age sites, presumably due to progressive limitation by P. This decline in ANPP late in ecosystem development is consistent with a reduction in soil total carbon and N storage at this site and provides a mechanism for successional retrogression in ecosystem structure and function. Our results unify biogeochemical theory across disparate ecosystems while illustrating the important water-nutrient interactions in these semi-arid ecosystems to further define the nature of nutrient limitations in terrestrial ecosystems.

G. S. Newman · S. C. Hart
School of Forestry, Northern Arizona University,
Flagstaff, AZ, USA

Present Address:
G. S. Newman (✉)
Natural History Museum of Denmark, University of
Copenhagen, Sølvgade 83S, 1353, Copenhagen K,
Denmark
e-mail: gregory.newman@snm.ku.dk

Present Address:
S. C. Hart
Life & Environmental Sciences and Sierra Nevada
Research Institute, University of California, Merced,
CA, USA

Keywords Chronosequence · Nitrogen · N:P stoichiometry · Phosphorus · Piñon–juniper · Soil development

Introduction

Theories of ecosystem development have been largely confined to humid temperate and subtropical ecosystems and limited to tests of N and P biogeochemistry. Arid and semi-arid ecosystems are less frequently tested within these models of ecosystem structure and function and have been recently characterized as

apparent exceptions to these theories with unclear generality (Peltzer et al. 2010). Plant productivity of semi-arid ecosystems is often co-limited by water and nutrients (Hooper and Johnson 1999) and, due to the moisture-related qualities of soil development characteristic within them, the universality of long-term ecosystem development and retrogressive phases requires incorporation of semi-arid ecosystem tests.

Nitrogen (N) and phosphorus (P) availability frequently constrain biological processes in terrestrial ecosystems (Vitousek and Howarth 1991; Elser et al. 2007; Vitousek et al. 2010). The contrasting atmospheric- versus rock-derived source of N and P, respectively, to ecosystems distinguishes their availability over geologic time and contributes to the differential existence of N or P limitation across temporal and spatial scales. The Walker and Syers (1976) model of ecosystem development describes high P availability early in soil development from residual parent materials. Phosphorus availability then declines as P is both eroded from the soil and converted into biologically unavailable forms. Eventually, the ecosystem reaches a ‘terminal steady state’ of low P availability that is tightly cycled through organic forms. Concurrent with these changes, N is incorporated gradually to the ecosystem via atmospheric deposition and biological N-fixation because N is not present in most parent materials. These differences result in a theoretical shift in soil nutrient limitations to plants from N early in ecosystem development towards a progressive P limitation late in ecosystem development (Walker and Syers 1976; Vitousek 2004). This pattern of shifting N and P availability has been observed in a limited number of established long-term substrate age gradients (e.g. Crews et al. 1995; Wardle et al. 2004; Selmants and Hart 2008). Biological responses to these potential nutrient constraints have major implications for the functioning of ecosystems across the landscape and their susceptibility to environmental change. Further, several studies have concluded that multiple resource limitation probably represents the usual situation for terrestrial plants (Bloom et al. 1985; Field et al. 1992; Elser et al. 2007).

In only one instance has the theory of long-term biological shifting of N- to P-limitation been directly tested within a well-constrained model system (Vitousek and Farrington 1997; Harrington et al. 2001). Within their well-studied substrate age gradient in

Hawaii, an in situ fertilization test of this biogeochemical theory was conducted in tropical montane rain forests. Factorial additions of N and P were applied at the youngest (300 y), intermediate (20,000 y), and oldest substrates (4.1 My) to *Metrosideros polymorpha* trees and bole increment growth (Vitousek and Farrington 1997) and above-ground net primary productivity (ANPP; Harrington et al. 2001) were measured to assess nutrient limitations. Accordingly, they found that N alone limited production at the youngest site (300 y) and P alone at the oldest site (4.1 My). The intermediate site showed no significant responses to any fertilization treatment. In addition, unamended increment growth peaked at the intermediate site and declined at the oldest site (Vitousek and Farrington 1997).

Sharp declines in short-term ecosystem processes driven by long-term pedogenic changes have been characterized as ecosystem retrogression, where primary productivity, plant biomass, nutrient cycling, and soil carbon and nutrient stocks exhibit a decline phase that follows progressive and maximal biomass phases in the absence of disturbance. These changes have been associated with reductions in tree basal area, increases in tissue N-to-P (N:P) ratios, and progressive P limitation over time in forested systems (Wardle et al. 2004) and observed across nine chronosequences spanning 6–4100 ky in length in boreal, temperate, and subtropical zones (Peltzer et al. 2010). The inclusion of more arid systems has been absent in these attempts at conceptual generality.

Selmants and Hart (2008) established a three million year substrate age gradient in a series of piñon–juniper ecosystems in Arizona as a semi-arid model of long-term ecosystem development. The substrate age gradient of Arizona (SAGA) spans approximately one thousand to three million years of ecosystem development in a space-for-time substitution across four sites comprised of piñon pine (*Pinus edulis*) and one-seed juniper (*Juniperus monosperma*) woodlands. Intercanopy spaces are dominated by the bunchgrass *Bouteloua gracilis* at the three older sites and by shrubs at the youngest site. They reported a strong and predictable increase in the fine fraction of surface soil over time that was positively correlated with soil water holding capacity. Given this increase in soil water holding capacity with substrate age, we would predict that soil water limitation to biological activity might be a strong co-regulator of ecosystem

processes in semi-arid systems during ecosystem development.

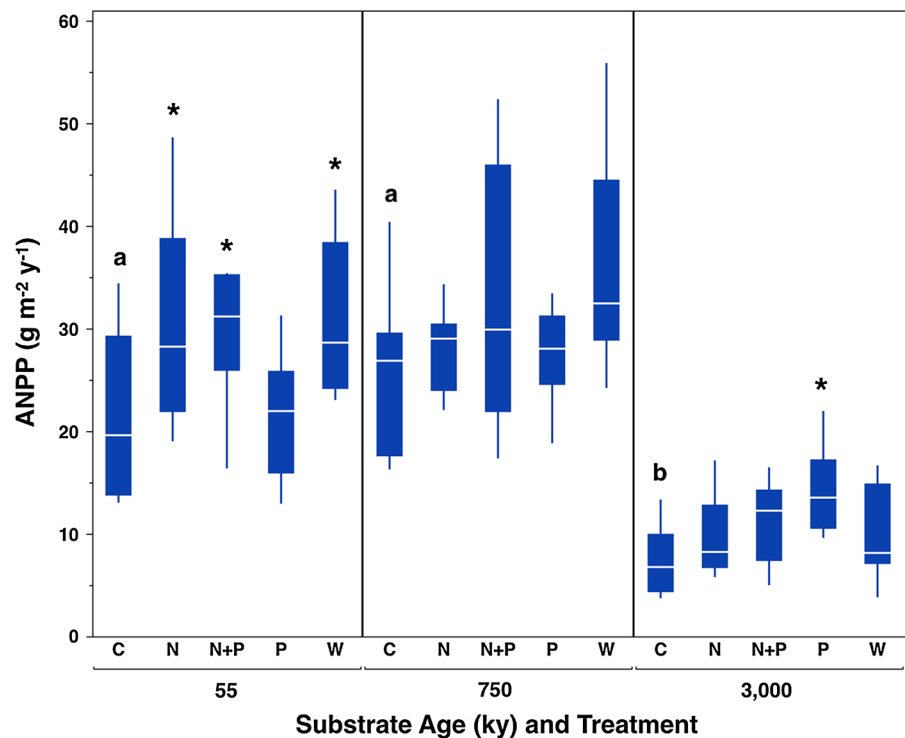
In this study, intercanopy plots at the three oldest sites were augmented with N, P, and water to assess the aboveground net primary productivity (ANPP) response of *B. gracilis* to these potentially limiting soil resources. We used this well-constrained, semi-arid model system to address the following objectives related to long-term ecosystem development: (1) test the prevailing theory from more humid systems of shifting N and P availability and limitations to plant production and assess the co-occurrence of water limitation; (2) identify the existence and extent of ecosystem retrogression associated with plant production; and (3) evaluate commonly applied foliar nutrient metrics used to assess plant nutrient limitation with a direct fertilization trial.

Methods

Our study was conducted within the San Francisco volcanic field (SFVF) in northern Arizona, an area of $\sim 5000 \text{ km}^2$ on the southwestern edge of the Colorado Plateau and north of Flagstaff, AZ (Fig. 1). This region

has undergone volcanic activity during the last \sim six million years that has migrated in an eastward direction, resulting in >600 monogenetic basaltic cinder cone vents (Tanaka et al. 1986). In accord with Jenny's (1941) factors of soil formation, four sites were established that had similar parent material, topography, present climate, and biota, but varied by substrate age: approximately 1 ky (Sunset Crater); 55 ky (O'Neill Crater); 750 ky (Red Mountain), and 3000 ky (Cedar Mountain) years (see Selmants and Hart 2008 for details). All sites are open woodlands dominated by piñon pine (*P. edulis*) and one-seed juniper (*J. monosperma*), with intercanopy spaces dominated by the C4 perennial bunchgrass *B. gracilis* at the three oldest sites and shrubs (*Fallugia paradoxa* and *Rhus trilobata*) at the youngest site. Due to these intercanopy differences at the youngest site, only the three older sites were used for this study. *B. gracilis* has a growing season coinciding with the start of the monsoon rain season in early to mid-July and culminating by late September. Mean annual temperature is $\sim 11 \text{ }^\circ\text{C}$ and mean annual precipitation is $\sim 340 \text{ mm}$, half of which falls as snow and the other half as rain during monsoonal convective thunderstorms (Sheppard et al. 2002). Growing season precipitation patterns were

Fig. 1 Aboveground net primary production (ANPP) of *Bouteloua gracilis* at three sites along the substrate age gradient of Arizona (SAGA) in unamended control (C) plots and plots amended with nitrogen (N), phosphorus (P), N+P, or water (W). Box plots denote the median (white line), upper and lower quartiles (solid boxes), and maximum and minimum values (whiskers; $n = 8$). Asterisks denote statistical treatment differences ($\alpha = 0.05$) within a site compared to the control. Different lowercase letters denote statistical differences among control plots across the three sites



considered principally important within this study because *B. gracilis* has been shown to exclusively use spring and summer rain from the upper soil horizons at other sites (Dodd et al. 1998).

Eight non-contiguous intercanopy spaces were chosen as experimental blocks (6.5 m × 3.5 m) at each of the three sites in June 2004. Within each block, five 2.25 m² plots were established and randomly assigned to one of the following treatments in a randomized complete block design: control, +water, +N, +P, and +N+P. The logistical constraints of hauling large quantities of water over long-distances to remote locations prevented us from implementing a full-factorial design. A 50-cm wide buffer and a 50-cm deep soil trench were created between all plots to remove any lateral root or mycorrhizal access to adjacent treatments. To minimize above- and below-ground influences from trees, we chose intercanopy spaces that were large enough so that surrounding trees were at least one tree height from the experimental block boundaries.

In early July 2004, just prior to the onset of monsoonal rains, water and nutrient additions (as salts) were implemented within each corresponding plot. Nitrogen was applied once at a rate of 7.5 g N m⁻² year⁻¹ as ammonium nitrate. Phosphorus was applied once at a rate of 5 g P m⁻² year⁻¹ as triple super phosphate. The relatively modest N application rate was used because 10 g N m⁻² year⁻¹ was found to cause mortality in piñon pine trees after several years of fertilization at the Sevilleta, New Mexico, Long Term Ecological Research site (M. Allen, University of California, Riverside, pers. comm.). Given our selected N application rate, the rate of P addition was chosen so that the biological N-to-P mass ratio requirement (i.e., 12–13; Güsewell 2004) was exceeded after accounting for the P-fixation capacity of these soils (Selmants and Hart 2010). Water was applied weekly at a rate of 3.6 L m⁻² a total of 10 times during the growing season for a cumulative water addition of approximately 36 mm. This resulted in an experimental water increase of 31 % over the mean 2004 growing season precipitation of 117 mm across the three sites (Selmants and Hart 2008).

Peak standing biomass was harvested at the conclusion of the growing season in early October 2004 as a measurement of ANPP (Bonham 1989). All current year *B. gracilis* biomass was clipped from a

0.5 × 1.5 m strip within each plot, dried at 70 °C for 48 h, and weighed. *B. gracilis* comprised >95 % of the intercanopy aboveground biomass (G. Newman, unpublished data). Dried plant biomass samples from each plot were ground in a Wiley Mill (A. Thomas Co., Philadelphia, PA, USA) to pass through a 40-mesh (<425 μm) screen. Total N and total P were determined using a modified micro-Kjeldahl digestion (Parkinson and Allen 1975) and analyzed on a Lachat flow-injection analyzer (Lachat Instruments, Inc., Loveland, CO, USA). We estimated aboveground uptake by multiplying aboveground biomass by nutrient concentration because aboveground biomass senesces each year.

Resource limitation was determined where ANPP response to amendment was significantly higher than the unamended control (Chapin et al. 1986). One-way ANOVA was used to test differences in ANPP and tissue nutrient concentration among treatments at each site and in the control treatments across sites. When the treatment factor was significant, the significance of within-site experimental water and nutrient additions was analyzed by multiple comparisons versus the control using a post hoc Dunnett's test. Control treatments across sites were analyzed by one-way ANOVA and differences in control variables were determined by multiple pair-wise comparisons using a post hoc Tukey HSD test. Statistical significance was set a priori at $\alpha = 0.05$. All statistical analyses were performed with JMP Pro v11 (SAS Institute Inc, Cary, NC, USA).

Results

Aboveground NPP of *B. gracilis* increased significantly with water, N, and N+P additions on the younger substrate (55 ky), stimulating ANPP by 43, 45 and 48 % above the control, respectively (Fig. 1). The intermediate (750 ky) substrate showed no significant growth response to any resource amendments. The oldest (3000 ky) substrate had statistically significant ANPP responses to only P addition (84 % above the control). Additionally, ANPP in the unamended control plots at the youngest and intermediate sites were statistically similar (21.3 and 25.8 g m⁻² year⁻¹, respectively) while ANPP at the oldest substrate (7.4 g m⁻² year⁻¹) was 71 % lower than at the intermediate substrate ($F = 15.8, p < 0.0001$; Fig. 1).

Nitrogen concentration of *B. gracilis* aboveground tissue was significantly elevated with N fertilization on both the 55 and 750 ky, but not on the 3000 ky substrate (Table 1). Surprisingly, the intermediate-aged substrate had tissues with the lowest overall N concentrations. Stimulation of aboveground P concentration was not achieved with any fertilization treatment and there was a single, anomalous decline in P concentration with N+P fertilization on the 750 ky substrate. The N:P mass ratios in aboveground *B. gracilis* tissues were generally low (3.8–18.2) and only responded to N and N+P fertilization at the two younger sites, where N:P ratios increased. There was no change in N:P on the oldest site because N and P concentrations were unchanged with fertilization.

Variation in the aboveground uptake of N was driven more by changes in N concentration on the youngest and intermediate substrates and by productivity responses on the oldest substrate, where N uptake nearly doubled despite similar N concentration (Table 1). Contrastingly, P uptake was always the result of changes in ANPP as P concentration was remarkably consistent across sites and treatments.

Discussion

We made the second known direct, in situ test of the general ecosystem theory that young substrates are biologically N-limited while older substrates become progressively P-limited, resulting in ecosystem retrogression. We found that our three million year semi-arid model system is largely consistent with tests and theory from more humid ecosystems. While these data support the generalization of this concept, the results aren't entirely expected because there are several reasons why more arid systems might not conform to the Walker and Syers (1976) model of ecosystem development: arid systems lack significant net leaching losses of nutrients, rates of weathering are slower, water availability could confound nutrient limitations, and dust inputs might provide rejuvenation of nutrients at rates that rival losses and internal transformations. In fact, Lajtha and Schlesinger (1988) did not find that a granitic chronosequence supported these patterns of P changes with soil development in the Jornada basin of the Chihuahuan desert. While this arid system was markedly short (25 ky) in age, it had

Table 1 Nitrogen (N) and phosphorus (P) properties for *Bouteloua gracilis* aboveground growth at three sites along the substrate age gradient of Arizona (SAGA)

Substrate age (ky)	Treatment	Foliar N (g kg ⁻¹)	N uptake (mg m ⁻²)	Foliar P (g kg ⁻¹)	P uptake (mg m ⁻²)	Foliar N:P
55	Control	18.5 (0.5) ^a	384 (36) ^a	1.9 (0.1)	41 (5) ^a	9.9 (0.3) ^a
	+N	21.3 (0.6)*	646 (46)*	1.9 (0.1)	58 (4)	11.4 (0.2)*
	+N+P	23.1 (0.6)*	726 (49)*	1.9 (0.1)	61 (3)*	12.1 (0.4)*
	+P	17.6 (0.6)	373 (42)	2.1 (0.1)	45 (5)	8.6 (0.4)
	+Water	16.6 (0.7)	497 (33)	1.8 (0.1)	55 (4)	9.6 (0.4)
750	Control	11.2 (0.3) ^b	283 (37) ^a	1.9 (0.1)	47 (5) ^a	6.2 (0.4) ^b
	+N	15.6 (0.7)*	439 (24)*	1.5 (0.1)	43 (3)	10.5 (0.8)*
	+N+P	14.7 (0.8)*	478 (58)*	1.3 (0.1)*	43 (6)	11.1 (0.4)*
	+P	10.0 (0.4)	277 (23)	1.9 (0.1)	52 (4)	5.4 (0.3)
	+Water	9.0 (0.5)	320 (23)	1.4 (0.1)	52 (5)	6.2 (0.3)
3000	Control	18.5 (0.7) ^a	133 (21) ^b	1.6 (0.0)	12 (2) ^b	11.7 (0.6) ^a
	+N	19.7 (0.7)	196 (21)	1.6 (0.1)	16 (2)	12.9 (0.7)
	+N+P	21.0 (0.8)	238 (17)*	1.7 (0.1)	19 (2)*	12.7 (0.5)
	+P	17.4 (0.4)	246 (19)*	1.7 (0.1)	23 (2)*	10.7 (0.3)
	+Water	18.6 (0.7)	186 (19)	1.5 (0.1)	15 (2)	13.1 (0.7)

Foliar tissue stoichiometry is presented as a mass ratio. Treatment plots (2.25 m²) were amended prior to the growing season with either 7.5 g N m⁻² as granulated ammonium nitrate, 5 g P m⁻² as triple superphosphate, both N and P, 10 weekly applications of 3.6 L water m⁻² over the course of the growing season, or an unamended control. Values are treatment means and one standard error (n = 8) given in parentheses.

* Denote statistical treatment differences ($\alpha = 0.05$) within a site compared to the control. Different lowercase letters denote statistical differences among control plots across sites

left open the question of whether these theories of ecosystem development and function are generalizable across disparate climates. Our study implies that these systems are, in fact, in accord with the generalities across climatic factors.

Across SAGA, N constraints to plant aboveground productivity on young substrates shifted to P limitation by three million years of ecosystem development. Total soil N is lower (Selmants and Hart 2008) and the sum of the biologically available surface soil P pools is greater (Selmants and Hart 2010) at 55 ky than on the two older substrates. Thus, N limitation on the 55 ky substrate is consistent with current biogeochemical theory that younger ecosystems are primarily N-limited (Vitousek and Howarth 1991; Vitousek 2004). The predominant sources of ecosystem N are atmospheric deposition and biological fixation that must accumulate over time (Chapin et al. 2002). Plants capable of forming N-fixing symbioses are rare on this gradient and not present on the youngest substrates (G. Newman and S. Hart, pers. obs.). Three million years of ecosystem development has led to extensive soil weathering and biogeochemical transformations at the oldest site. Consequently, it has the lowest total, available inorganic, and organic P concentrations in the surface soil horizon across the substrate age gradient (Selmants and Hart 2010), as predicted by Walker and Syers' (1976) model. Notably, our study system has received substantial atmospheric dust inputs based on a $\text{Sr}^{87/86}$ isotope tracer study, contributing as much as 73 % to the plant-available soil pool after three million years (Emerson 2010), yet ANPP on this oldest substrate only responded significantly to P addition.

Craine and Jackson (2010) declared that there is no evidence of a grassland where production is limited by P that is also not limited by N, presuming that species replacement (such as a shift to heaths) would occur in response to low soil P conditions. However, we found strong evidence for P-limitation at the oldest substrate across a gradient where vegetation composition remains constant across all substrate ages. Generally, grass ANPP responds more favorably to N+P additions than when N or P are applied alone (Elser et al. 2007; Craine and Jackson 2010). Further, Harpole et al. (2011) conclude that synergistic N and P limitations are common across terrestrial ecosystems, although none of our N+P treatments were different from individual N or P additions across substrate ages.

Unique to more arid ecosystems, soil moisture limitation to ANPP along our gradient was apparent early, but dissipated over time in conjunction with finer surface soil textures (Selmants and Hart 2008). The N and water treatment responses on the youngest substrate, as well as the statistically non-significant but large responses to N+P (47 %) and water (55 %) additions at the intermediate site, imply a nutrient and water co-limitation in the earlier stages of ecosystem development in this semi-arid climate. This is plausible because ANPP in arid and semi-arid ecosystems typically have large responses to water availability (Lauenroth and Sala 1992; Epstein et al. 1996) and are generally co-limited by water and N (Hooper and Johnson 1999). Despite this conjecture, the water addition treatment significantly increased mean *B. gracilis* ANPP only on the youngest substrate. We expected a high sensitivity of *B. gracilis* growth to changes in soil water availability because Dodd et al. (1998) have shown that this bunchgrass responds exclusively to spring and summer rain by increasing leaf water potential and conductance within hours and for up to 2 days following even small precipitation events (Sala and Lauenroth 1982). The clay content of the surface soils increases dramatically across our substrate age gradient and this change in soil texture corresponds with consistently higher soil volumetric water content during the growing season as substrate age increases (Selmants and Hart 2008). The older sites are consequently more capable of storing these frequent, low intensity monsoonal events via higher water holding capacity (Looney et al. 2011), which presumably act to diminish the water limitation of *B. gracilis* production at later stages of semi-arid ecosystem development. This was a short-term application of potentially limiting plant resources and results might vary with a longer experimental duration, particularly the response to water availability. Additionally, this study serves as a reminder that ecosystem comparisons of soil water and nutrient availability and/or limitation should simultaneously consider the different reactive mineral contents (e.g. clay), particularly along substrate age gradients.

The changes in soil resource limitation to *B. gracilis* ANPP along our semi-arid substrate age gradient closely followed patterns modeled from ecosystem development theory tested directly on a substrate age gradient from a humid subtropical forest in Hawai'i (Vitousek and Farrington 1997; Harrington

et al. 2001) and indirectly on a two million year Mediterranean climate dune sequence in Australia (Laliberté et al. 2012). Our Arizona semi-arid model system reached P-limitation within three million years of ecosystem development. While the Hawaii humid gradient exhibited such limitation on the oldest 4.1 million year substrate, this transition to P-limitation actually occurred sometime between the large span of 20,000 and 4.1 million years (Vitousek and Farrington 1997). It is unclear at what point either system actually reaches P-limitation and therefore difficult to compare these climatic systems since the selection of substrate ages is not objectively designed to answer this question. Despite the potential differences in factors affecting P biogeochemistry over long-term ecosystem development between arid and humid climates, our results support the generality of shifting biological N- to P-limitation during long-term ecosystem development and introduce the complexity of dissipating nutrient-water co-limitation with time as soils develop water-holding capacity.

In addition to distinct shifts in the limitations to plant ANPP during ecosystem development, the absolute magnitude of aboveground production declined dramatically with the onset of P-limitation. Mean ANPP in the unamended control plots of *B. gracilis* was 72 % lower at the three million year, P-limited site, than on the intermediate substrate. In a review of the causes, consequences, and generalities of ecosystem retrogression, Peltzer et al. (2010) suggest that retrogression is ultimately driven by the long-term decline in soil nutrient availability to plants, particularly P, as a result of pedogenic processes. They show evidence of these patterns across wide climatic, geologic, and vegetation types, although consider more arid ecosystems as unclear and possible exception to the generality of these processes. Our semi-arid study system provides further support for these patterns and clearly demonstrates that a progressive P-limitation and reduced production potential at late stages of ecosystem development also result in a large decline in plant ANPP which may be directly responsible for changes in ecosystem structure and function associated with ecosystem regression. Taken together with the data compiled from substrate age gradients in more temperate and humid ecosystems (Wardle et al. 2004; Peltzer et al. 2010), our results therefore provide evidence that a regressive phase late in ecosystem development is also apparent in semi-arid systems.

A curiosity of retrogressive phases is that ecosystem processes like ANPP are apparently unable to be alleviated with short-term resource additions. Our levels of P additions, while sufficient to induce a growth response at the P-limited site, were not able to stimulate ANPP to rates observed at the younger sites, signifying a limited capacity of these plants to respond to resource additions. There may be several explanations for this reduced production potential at the 3000 ky site despite our attempts to alleviate ANPP limitations with soil resource amendments. First, a large fraction of the P addition may have become geochemically bound in the surface soil horizon and thus unavailable for plant uptake. Although we added N and P in a 1.5–1 mass ratio in an attempt to minimize this effect, the 3000 ky substrate soil may have a sufficient P-sorption capacity to inhibit plant access to the level of P addition, which can occur through precipitation reactions with calcium in these ~pH 6 soils (Schlesinger and Bernhardt 2013). However, P sorption isotherms show that the oldest site has remarkably similar P-sorption capacity as the other two sites (Selmants and Hart 2010). Secondly, current-year aboveground growth responses of *B. gracilis* to short-term soil resource additions may be moderated by large C allocations belowground (Kaye et al. 2005), resulting in a delay in the aboveground growth response of this bunchgrass following the alleviation of resource limitation. However, this explanation also appears unlikely to fully account for the lower production potential because multiple year analyses have found that *B. gracilis* ANPP is well correlated to current growing season precipitation (Smoliak 1986) and proportionately greater aboveground than belowground responses to P availability are common (Poorter and Nagel 2000; Yuan and Chen 2012). Finally, genetic differences in *B. gracilis* populations across the substrate age gradient could also have contributed to differences in ANPP among sites, as well as to the contrasts in growth responses of *B. gracilis* to increases in soil resource availability (Seliskar et al. 2002). Phenotypic or genotypic variation in the growth efficiencies (Gleason et al. 2009), ability to produce new meristems (Bret-Harte et al. 2001), or belowground allocation (Fischer et al. 2007) could produce growth constraints in retrogressive ecosystems. While we found no differences in aboveground P efficiency across treatments and did not measure belowground production, it would be

instructive to pursue these potential genetic constraints further.

Foliar N concentrations were distinctly more malleable than P concentrations across SAGA and particularly with amendment treatments. In fact, there was no variation in P concentrations of aboveground tissue across all treatments and sites (except an anomalously low concentration with N+P fertilization at the 750 ky site) and the foliar P concentration values of *B. gracilis* within this study system are relatively high for forbs and graminoids (Thompson et al. 1997). Foliar N and P concentrations in *M. polymorpha* have exhibited substantial variation with fertilization in the Hawaiian chronosequence (Vitousek 2004). In addition, Ostertag (2010) found greater variability in foliar P than N concentrations in 13 different species after continuous, long-term fertilization, which largely drove changes in N:P ratios in both N- and P-limited ecosystems.

Differences in the patterns in foliar nutrient concentrations and responses to nutrient amendments across substrate age gradients in humid and semi-arid climates may be due to differences in the life history strategies of the plants found in these ecosystems. A lack of tissue nutrient concentration response, particularly with P, to nutrient additions in our study has also been observed in other arid and semi-arid vegetation (Drenovsky and Richards 2004; Neff et al. 2006), and in piñon and juniper foliage across the SAGA (Selmants 2007). Variation in foliar biomass and leaf area is often a more common response than plasticity in foliar nutrient concentration as an adaptation to water-limited, low nutrient environments (Aerts and Chapin 1999). Nevertheless, previous fertilization studies with *Bouteloua* spp. have reported increased aboveground tissue nutrient concentrations with both N and P additions (Hays et al. 1982; Joern and Mole 2005).

There have been multiple attempts to define a set of tissue nutrient concentration and stoichiometric values to easily diagnose plant nutrient limitation. Wassen et al. (1995) suggested that leaf N < 13–14 g kg⁻¹ and P < 0.7 g kg⁻¹ were critical levels of tissue concentration representing N- and P-limiting conditions, respectively, and Güsewell and Koerselman (2002) reported P < 1.0 g kg⁻¹ necessary for P-limitation alone in wetland plants. However, our results demonstrated both N- and P-limitation with a borderline 15.8 g kg⁻¹ N and relatively high 1.6 g kg⁻¹ P mean

concentration of aboveground biomass at the youngest and oldest site, respectively. Most popularly to date, tissue N:P mass ratios (referred to here and elsewhere) have been established that set a threshold between N-limiting (<14) and P-limiting (>16) conditions (Koerselman and Meulman 1996), although Güsewell (2004) concluded more conservatively that N:P < 10 likely indicates N-limitation while N:P > 20 likely indicates P-limitation. Once again, the low overall range of our results for tissue N:P mass ratios (9.9–11.7), driven by the relatively high and unvarying [P] for *B. gracilis* within this model system, narrowly support the identification of N-limitation at the young site. However, they do not approach even the lowest suggested thresholds for P-limitation at the oldest site as used in other tests of progressive P limitation (Wardle et al. 2004). Drenovsky and Richards (2004) tested these critical thresholds in two desert shrubs and concluded that these values are not applicable to desert ecosystems. They suggested that vegetation could exhibit P limitation to growth despite relatively high foliar P concentrations in these water-limited ecosystems. Across five grassland sites in South Africa with foliar N:P < 6, Craine et al. (2008) demonstrated N and P co-limitation via factorial fertilization. Our results concur that analysis of nutrient limitation based on foliar stoichiometry may not be applicable across species or ecosystems for reasons that might include tissue stoichiometric differences, life history traits, root-to-shoot ratios, or conservative versus inducible growth. These cases should give caution to stoichiometric tests of nutrient limitation in more arid ecosystems in the absence of further calibrations with direct fertilization studies.

The generality of a shift from N- to P-limitation of vegetation during long-term ecosystem development was confirmed in a semi-arid system. The incorporation of water co-limitation on younger substrates that disappeared with clay and water-holding capacity development is an important characteristic of this semi-arid gradient and addition to chronosequence theory. The progressive P-limitation observed at the end of this chronosequence provides a mechanism for the drastically reduced plant aboveground production and ecosystem retrogression properties seen here and in other ecosystem development gradients. Finally, we present evidence that caution should be taken when inferring plant limitation from foliar nutrient contents and suggest further comparisons with direct

fertilization are warranted to clarify where these patterns are valid and where exceptions or confounding factors may occur.

Acknowledgments This research was supported, in part, by McIntire-Stennis appropriations to NAU and the State of Arizona. K. Hess was instrumental in field and laboratory work and D. Guido provided essential laboratory assistance. We thank M. Sundqvist, T. Kolb, and two anonymous reviewers for valuable comments and D. Binkley, T. Whitham, and C. Gehring for thoughtful discussions that significantly improved the manuscript.

References

- Aerts R, Chapin FS III (1999) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Ecol Res* 30:1–67
- Bloom AJ, Chapin FS III, Mooney HA (1985) Resource limitation in plants—an economic analogy. *Annu Rev Ecol Syst* 16:363–392
- Bonham CD (1989) *Measurements of terrestrial vegetation*. Wiley, New York
- Bret-Harte MS, Shaver GR, Zoerner JP, Johnstone JF, Wagner JL, Chavez AS, Gunkelman RF, Lippert SC, Laundre JA (2001) Developmental plasticity allows *Betula nana* to dominate tundra subjected to an altered environment. *Ecol* 82:18–32
- Chapin FS III, Vitousek PM, Van Cleve K (1986) The nature of nutrient limitation in plant communities. *Am Nat* 127:48–58
- Chapin FS III, Matson PA, Mooney HA (2002) *Principles of terrestrial ecosystem ecology*. Springer, New York
- Craine JM, Jackson RD (2010) Plant nitrogen and phosphorus limitation in 98 North American grassland soils. *Plant Soil* 334:73–84
- Craine JM, Morrow C, Stock WD (2008) Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytol* 179:829–836
- Crews T, Fownes J, Herbert D, Kitayama K, Mueller-Dombois D, Riley R, Scowcroft P, Vitousek PM (1995) Changes in soil phosphorus and ecosystem dynamics across a long soil chronosequence in Hawaii. *Ecology* 76:1407–1424
- Dodd MB, Lauenroth WK, Welker JM (1998) Differential water resource use by herbaceous and woody plant life-forms in a shortgrass steppe community. *Oecologia* 117:504–512
- Drenovsky RE, Richards JH (2004) Critical N: P values: predicting nutrient deficiencies in desert shrublands. *Plant Soil* 259:59–69
- Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol Lett* 10:1135–1142
- Emerson AA (2010) *Atmospheric inputs and plant nutrient uptake along a three million year semi-arid substrate age gradient*. Dissertation, Northern Arizona University
- Epstein HE, Lauenroth WK, Burke IC, Coffin DP (1996) Ecological responses of dominant grasses along two climatic gradients in the Great Plains of the United States. *J Veg Sci* 7:777–788
- Field CB, Chapin FS III, Matson PA, Mooney HA (1992) Responses of terrestrial ecosystems to the changing atmosphere: a resource-based approach. *Annu Rev Ecol Syst* 23:201–235
- Fischer DG, Hart SC, LeRoy CJ, Whitham TG (2007) Variation in below-ground carbon fluxes along a *Populus* hybridization gradient. *New Phytol* 176:415–425
- Gleason SM, Read J, Ares A, Metcalfe DJ (2009) Phosphorus economics of tropical rainforest species and stands across soil contrasts in Queensland, Australia: understanding the effects of soil specialization and trait plasticity. *Funct Ecol* 23:1157–1166
- Güsewell S (2004) N: P ratios in terrestrial plants: variation and functional significance. *New Phytol* 164:243–266
- Güsewell S, Koerselman W (2002) Variation in nitrogen and phosphorus concentrations in wetland plants. *Perspect Ecol Evol Syst* 5:37–61
- Harpole SW, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE (2011) Nutrient co-limitation of primary producer communities. *Ecol Lett*. doi:10.1111/j.1461-0248.2011.01651.x
- Harrington RA, Fownes JH, Vitousek PM (2001) Production and resource use efficiencies in N- and P-limited tropical forests: a comparison of responses to long-term fertilization. *Ecosystem* 4:646–657
- Hays R, Reid CPP, St. John TV, Coleman DC (1982) Effects of nitrogen and phosphorus on blue grama growth and mycorrhizal infection. *Oecologia* 54:260–265
- Hooper DU, Johnson L (1999) Nitrogen limitation in dryland ecosystems: responses to geographical and temporal variation in precipitation. *Biogeochemistry* 46:247–293
- Jenny H (1941) *Factors of soil formation: a system of quantitative pedology*. Dover Publications, New York
- Joern A, Mole S (2005) The plant stress hypothesis and variable responses by blue grama grass (*Bouteloua gracilis*) to water, mineral nitrogen, and insect herbivory. *J Chem Ecol* 31:2069–2090
- Kaye JP, Hart SC, Fulé PZ, Covington WW, Moore MM, Kaye MW (2005) Initial carbon, nitrogen, and phosphorus fluxes following ponderosa pine restoration treatments. *Ecol Appl* 15:1581–1593
- Koerselman W, Meulman AFM (1996) The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *J Appl Ecol* 33:1441–1450
- Lajtha K, Schlesinger WH (1988) The biogeochemistry of phosphorus cycling and phosphorus availability along a desert soil chronosequence. *Ecology* 69:24–39
- Laliberté E, Turner BL, Costes T, Pearse SJ, Wyrwoll K, Zemunik G, Lambers H (2012) Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. *J Ecol* 100:631–642
- Lauenroth WK, Sala OE (1992) Long-term forage production of North American shortgrass steppe. *Ecol Appl* 2:397–403
- Looney CE, Sullivan BW, Kolb TE, Kane JM, Hart SC (2011) Pinyon pine (*Pinus edulis*) mortality and response to water

- addition across a three million year substrate age gradient in northern Arizona, USA. *Plant Soil* 357:89–102
- Neff JC, Reynolds R, Sanford RL Jr, Fernandez D, Lamothe P (2006) Controls of bedrock geochemistry on soil and plant nutrients in southeastern Utah. *Ecosystem* 9:879–893
- Ostertag R (2010) Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. *Plant Soil* 334:85–98
- Parkinson JA, Allen SE (1975) A wet oxidation procedure suitable for the determination of nitrogen and mineral nutrients in biological material. *Commun Soil Sci Plant Anal* 6:1–11
- Peltzer DA, Wardle DA, Allison VJ, Baisden WT, Bardgett RD, Chadwick OA, Condon LM, Parfitt RL, Porder S, Richardson SJ, Turner BL, Vitousek PM, Walker J, Walker LR (2010) Understanding ecosystem retrogression. *Ecol Monogr* 80:509–529
- Poorter H, Nagel O (2000) The role of biomass allocation in the growth response of plants to different light, CO₂, nutrients and water: a quantitative review. *Aust J Plant Physiol* 27:595–607
- Sala OE, Lauenroth WK (1982) Small rainfall events: an ecological role in semiarid regions. *Oecologia* 53:301–304
- Schlesinger WH, Bernhardt ES (2013) *Biogeochemistry: an analysis of global change*. Springer, Netherlands
- Seliskar DM, Gallagher JL, Burdick DM, Mutz LA (2002) The regulation of ecosystem functions by ecotypic variation in the dominant plant: a *Spartina alterniflora* salt-marsh case study. *J Ecol* 90:1–11
- Selmants PC (2007) Carbon, nitrogen, and phosphorus dynamics across a three million year substrate age gradient in northern Arizona, USA. PhD Dissertation, Northern Arizona University
- Selmants PC, Hart SC (2008) Substrate age and tree islands influence carbon and nitrogen dynamics across a retrogressive semiarid chronosequence. *Global Biogeochem Cycles* 22:GB1021. doi:10.1029/2007GB003062
- Selmants PC, Hart SC (2010) Phosphorus and soil development: does the Walker and Syers model apply to semiarid ecosystems? *Ecology* 91:474–484
- Sheppard PR, Comrie AC, Packtin GD, Angersbach K, Hughes MK (2002) The climate of the US Southwest. *Clim Res* 21:219–238
- Smoliak S (1986) Influence of climatic conditions on production of *Stipa-Bouteloua* prairie over a 50-year period. *J Range Manag* 39:100–103
- Tanaka KL, Shoemaker EM, Ulrich GE, Wolfe EW (1986) Migration of volcanism in the San Francisco volcanic field, Arizona. *Geol Soc Am Bull* 97:129–141
- Thompson K, Parkinson JA, Band S, Spencer RE (1997) A comparative study of leaf nutrient concentrations in a regional herbaceous flora. *New Phytol* 136:679–689
- Vitousek PM (2004) *Nutrient cycling and limitation: Hawai'i as a model system*. Princeton University Press, NJ
- Vitousek PM, Farrington H (1997) Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry* 37:63–75
- Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea: how can it occur? *Biogeochemistry* 13:87–115
- Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecol Appl* 20:5–15
- Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. *Geoderma* 15:1–19
- Wardle DA, Walker LR, Bardgett RD (2004) Ecosystem properties and forest decline in contrasting long-term chronosequences. *Science* 305:509–513
- Wassen MJ, Olde Venterink HGM, de Swart EOAM (1995) Nutrient concentrations in mire vegetation as a measure of nutrient limitation in mire ecosystems. *J Veg Sci* 6:5–16
- Yuan ZY, Chen HY (2012) A global analysis of fine root production as affected by soil nitrogen and phosphorus. *Proc R Soc B* 279:3796–3802