

# Reduced precipitation limits growth return on plant N in mesic grassland

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# Abstract

Grassland production is sensitive to both precipitation and plant N accumulation and utilization, such that change in one variable influences grassland response to the second variable. We investigated effects of interannual variation in precipitation on the response of community-level values of relative growth rate (RGR) to two multiplicative components of RGR, nitrogen productivity (NP; rate of change in biomass/plant N) and N concentration ([N]), in two grassland communities in Texas, USA. Communities included a diverse mixture of perennial grass and forb species and monoculture of the perennial C<sub>4</sub> grass *Panicum virgatum*. RGR and its N components were measured at the spatial scale of 7-m diameter circular patches in each community near the spring peak in mixture biomass during each of 5 years. We found that RGR varied substantially among patches and years and between communities. RGR variation was strongly correlated with variation in NP. Precipitation during the 3 months prior to RGR measurement mediated the RGR response to NP by altering the correlation between NP and [N] in both grasslands. Reduced precipitation led to more negative NP-[N] correlation coefficients, which reduced proportional change in RGR per change in NP by as much as 30% even in the absence of a precipitation effect on means of community RGR and NP. Our results highlight an under-appreciated aspect of the pervasive role of precipitation in grassland growth that was mediated via change in plant growth return on accumulated N.

## Introduction

Grassland biomass productivity frequently is limited by soil N availability (Fay et al. 2015; Stevens et al. 2015; Du et al. 2020) and ultimately, by plant N accumulation and utilization efficiency (Egan et al. 2019). However, the relationship between plant productivity and N can vary among grassland communities or years for at least three reasons. First, productivity-N relationships may vary in response to interannual variation (IAV) in precipitation. Precipitation, like plant N, influences grassland growth (e.g., Owensby et al. 1970; Lauenroth and Sala 1992; Knapp et al. 2001). N-precipitation co-limitation implies that N and water interactively regulate productivity, such that the influence of plant N on grassland growth depends on precipitation (Yahdjian et al. 2011; Wang et al. 2017). For example, low precipitation may limit growth response to additional N by limiting photosynthesis or aboveground biomass and plant light interception. Second, productivity-N relationships may differ among grassland communities if plant N levels or N utilization in growth differs among species (Del Pozo et al. 2000). Third, productivity-N relationships may vary over defined periods within a growing season because of community or interannual differences in biomass at the beginning of the measurement period. Assessing productivity as Relative Growth Rate (RGR) standardizes for initial differences in plant size or community biomass.

RGR in aboveground biomass is linked to two multiplicative components of N: aboveground biomass productivity per unit of plant N content (N productivity; NP), an index of N utilization efficiency, and aboveground plant N content per unit of plant biomass ([N]), an index of N acquisition. This simple factorization of RGR into [N] and NP derives from the understanding that plant C gain is regulated partly by plant levels of protein N that are associated with photosynthesis and that the efficiency with which

plant N is utilized in growth may vary with environmental conditions and species or community differences in N distribution among aboveground tissues. The RGR concept frequently has been applied to individual plants to determine relationships between biomass productivity and plant N (e.g., Garnier and Vancaeyzeele 1994; Wright and Westoby 2000, 2001), but rarely has been utilized at the plant community scale, and especially over multiple years that differ in precipitation, because of the difficulty of repeatedly measuring plant biomass and N content at large spatial scales.

Much of what is known about linkages between RGR and its N components comes from fertilization experiments of typically short duration with selected species or simple plant communities (e.g., der Werf et al. 1993; Wright and Westoby 2000, 2001). From these experiments, we have learned that RGR may correlate highly with NP, aboveground [N], or both (Garnier and Vancaeyzeele 1994; Baret et al. 2017; Meurer et al. 2019), NP-[N] relationships may differ among species (Del Pozo et al. 2000), and the contribution of NP to RGR may vary with N availability, particularly among inherently fast-growing species (e.g., der Werf et al. 1993). Studies with juvenile plants have demonstrated that NP and leaf [N] generally are positively correlated (Aerts and Chapin 2000). There is evidence from grass stands, however, of negative covariance between NP and plant [N] (Soussana et al. 2005). Similar results have been reported when growth was analyzed as a function of leaf properties alone. Growth per unit of leaf N and leaf [N] were negatively correlated across 28 species of woody seedlings (Wright and Westoby 2000).

We investigated precipitation effects on relationships between aboveground RGR and two N components, NP and [N], near the spring biomass peak of 5 years for two grassland communities. Communities include a planted a mixture of perennial grassland species (mixture) and monoculture of the C<sub>4</sub> grass *Panicum virgatum* L. (switchgrass).

We tested four predictions. 1) Community RGR would correlate more strongly with community NP than [N]. This prediction is based on the biological expectation that a given proportional increase in growth per unit of plant N (NP) will result in a similar proportional increase in community RGR. By contrast, RGR may not respond proportionately to a given change in community [N] if, as [N] increases, factors other than N constrain the RGR response to [N] (Wright and Westoby 2000). 2) Community NP and [N] would be inversely correlated on average among years and between communities. This prediction derives partly from the mathematical relationship between NP and [N]. The variable plant N content per unit of surface area appears in both the numerator of community [N] and denominator of NP. NP will tend to increase as [N] declines as a result. An inverse correlation between NP and [N] also is consistent with observations that NP and nitrogen use efficiency increase when N availability declines (Yuan et al. 2006; Egan et al. 2019). 3) The correlation between community NP and [N] would differ among years in response to IAV in precipitation. This prediction is based on the premise that low precipitation will reduce water availability to plants and limit photosynthetic rates or plant biomass and light interception. Reduced photosynthetic capacity and light interception should, in turn, constrain plant capacity to translate additional N into biomass such that the NP-[N] correlation will be more negative when precipitation is relatively low than higher. 4) Correlation coefficients between community NP and [N] would respond more to IAV in precipitation in mixture than switchgrass. Community-scale values of leaf dry matter content (LDMC)

vary less among years in switchgrass than mixture (Polley et al. 2020a), implying lesser IAV in relative abundances of species functional groups and greater temporal consistency in NP-[N] correlation in the switchgrass than mixture community. We tested these predictions by using remote measurements of canopy reflectance of sunlight over a 2–5 week period near the spring biomass peak during each of 5 years.

## Methods

### RGR factorization

We applied a two-component factorization of RGR in aboveground biomass at the community scale,

$$RGR = NPx [N]$$

1

where RGR is relative growth rate, the rate of change in aboveground biomass over the growth interval per unit of biomass (g biomass/g biomass/d), NP is N productivity, the rate of change in aboveground plant biomass divided by the mean value of plant N content (g biomass/g N/d; Ingestad 1979), and [N] is plant N concentration expressed as the ratio of aboveground plant N content per unit of area to aboveground biomass per unit of area (g N/g biomass). NP is a component of nitrogen use efficiency, the latter defined as the product of NP and the mean residence time of plant N (Hirose 2011). Mean values of RGR and its N components were calculated over each growth interval as follows (Hunt 1982):

$$RGR = \ln(b_2 - \ln b_1)/(t_2 - t_1)$$

2

$$NP = \left[ \left( \frac{b_2 - b_1}{t_2 - t_1} \right) x \left( \frac{\ln N_2 - \ln N_1}{N_2 - N_1} \right) \right]$$

3

$$[N] = \left( \frac{N_2}{b_2} + \frac{N_1}{b_1} \right) / 2$$

4

where b, N, and t are aboveground biomass (g/m<sup>2</sup>), aboveground N content (g/m<sup>2</sup>), and time in days (d), respectively, for final (2) and initial (1) measurement dates. RGR derived as the product of NP and mean [N] will not equate exactly to calculated values of mean RGR (Hunt 1982) but the correlation in data we analyzed was > 0.99.

Links between RGR and its N components also were investigated using ‘scaling relationships’ derived from an additive factorization of RGR (Wright and Westoby 2001), where:

$$\log RGR = \log NP + \log [N]$$

5

Slopes of log-log relationships between RGR and each of its components indicate the proportionality in change between variables. The anticipated value of 'scaling slopes' is 1.0, such that RGR changes in direct proportion to change in NP or [N]. Negative correlation between NP and [N] will reduce values of 'scaling slopes' between RGR and NP or [N] to < 1.0 and thereby reduce the fractional change in RGR per unit of change in NP or [N].

## Site

RGR and its N components were estimated during spring for mixture and switchgrass communities located at Temple, Texas, USA (31°10' N, 97°34' W) (Long-term Biomass Experiment; LTBE). Eight randomly selected stands in a former agricultural field were planted with a seed mixture containing 38 native perennial forb and grass species (Polley et al. 2020a). Remaining stands (16) were planted to a monoculture of switchgrass (cultivar Alamo). Stands were planted 6 years prior to measurements (2010). Each was 17 m wide and 137–218 m long (0.26–0.37 ha). Annual precipitation averages 905 mm (50 years record) with peaks in spring and autumn. Monthly maximum temperatures vary between 15.3 and 35.4 °C in January and August, respectively. LTBE contributes to the USDA Long-Term Agroecosystem Research (LTAR) network (<https://ltar.ars.usda.gov/>).

We calculated RGR and its N components at the spatial scale of a 7-m diameter circular patch. Thirteen patches were permanently located along the length of each mixture stand and in switchgrass stands located immediately to the south of each mixture (n = 8 stands), resulting in 104 patches per community. Mixture and switchgrass communities are not grazed or fertilized. Both communities are hayed following each growing season and both include not-seeded native and exotic species that are considered 'invaders'. Prominent invaders of mixture communities include annual grass and forb species that complete growth during spring. Included are the grass *Bromus japonicus* Thunb. ex Murray (Japanese brome) and forb *Gaillardia pulchella* Foug (Indian blanket). Prominent invaders in switchgrass include two exotic C<sub>4</sub> perennial grasses, *Panicum coloratum* L. (Klein grass) and *Sorghum halepense* (L.) Pers. (Johnson grass). Annual grasses and forbs are locally abundant where switchgrass failed to establish.

## Field measurements, variable calculation, and statistics

RGR, NP, and [N] were estimated using measurements of canopy reflectance on two dates during spring (late March–April) separated by 14–38 d in each of 5 years (2016 – 2019, 2021). Aboveground biomass reaches its spring peak in late April in mixture (Polley, Collins and Fay 2020). We measured reflectance from a rotary-wing, unmanned aerial vehicle (UAV; S1000; DJI; Shenzhen, China) using an ASD HandHeld2 spectroradiometer (spectral range of 350–1050 nm; ASD Inc., Boulder, CO, USA). We flew the GPS-guided UAV to a stationary position at 15.8 m height (25° field of view) above each patch prior to

each measurement. Three consecutive measurements of reflectance per patch were averaged for each sample. We measured reflectance within 2 h of solar noon on cloudless days and referenced measurements to a Spectralon® white reference panel at ~ 15-min intervals. Reflectance was calculated by dividing radiance reflected from the plant canopy by radiance incident on the canopy. We considered incident radiation to be the radiant flux reflected from a Spectralon® white reference panel exposed to full sunlight.

Aboveground biomass per patch (g/m<sup>2</sup>) for each sample date was calculated as an exponential function of the enhanced vegetation index (EVI) (adj. r<sup>2</sup> = 0.83, P < 0.0001; Polley et al. 2020b).

$$EVI = Gx \left( \frac{NIR - Red}{NIR + C1xRed - C2xBlue + L} \right)$$

6

where NIR, red, and blue are reflectance in near-infrared, red, and blue wavelengths, respectively, L is an adjustment for canopy background, G is a gain factor, and coefficients C1 and C2 correct for the influence of aerosols (L = 1, C1 = 6, C2 = 7.5, G = 2.5).

Community N content (g N/m<sup>2</sup>) per patch was estimated using a linear regression fit to the relationship between observed values of aboveground N content and the red edge chlorophyll index (CI<sub>red edge</sub>), where CI<sub>red edge</sub> was calculated by subtracting 1 from the ratio of reflectance in the NIR (840 nm) to reflectance in a waveband in the red edge of the spectrum (717 nm) (Gitelson et al. 2005; Li et al. 2012). The N content-CI<sub>red edge</sub> regression was developed from ground-level 'calibration' measurements of aboveground N content and reflectance. Calibration data were collected across a series of 30-cm diameter rings (n = 61) positioned in mixture and switchgrass communities to span a wide range in aboveground N content. Reflectance was measured using an ASD HandHeld2 spectroradiometer. Vegetation then was harvested to 5 cm height, dried (65 C), and weighed. Samples of dried plant material from each ring were analyzed for [N] using inductively coupled plasma atomic emission spectroscopy (Isaac and Johnson 1998). Aboveground N content (g N/ m<sup>2</sup>) per ring was calculated by multiplying biomass/m<sup>2</sup> by [N] (g N/g biomass). Linear regression explained 80% of the variance in community N content in calibration data (Supplementary Fig. 1).

We used this regression model to estimate N content at the patch scale (7-m diameter). Regression consistency in predicting N content across spatial scales was evaluated by comparing N content calculated using patch-scale reflectance to the average value of N content calculated using ground-level measurements of reflectance from eight 76-cm diameter plots randomly located in each of 16 patches. The slope of a linear regression between patch N content and the mean N content of plots per patch that was fit through the origin (Supplementary Fig. 2) did not differ significantly from 1 (t<sub>15</sub> = -1.50, P > 0.10). The regression model thus adequately predicted patch-scale N content using measurements collected at smaller spatial scale.

We used linear regression to calculate log-log scaling slopes between community RGR and its N components and to assess relationships between spatial variation in RGR in each community and year and the unique (independent) effects of each of its N components. RGR was regressed on residuals from a regression of community NP on community [N] (statistically independent effects of NP) and residuals from a regression of community [N] on community NP (independent effects of [N]). Results of simple bivariate regression of RGR on each of its N components are influenced by shared contributions of the two N components to RGR. Correlation between community NP and [N] was assessed using Pearson correlation.

Precipitation effects on NP-[N] correlation coefficients and community N were investigated by regressing mean values of Pearson correlation coefficients and community N per year on precipitation summed over the 120-day period prior to RGR measurement each year. Precipitation sums were calculated for all possible time intervals with a minimum duration of 15 days (e.g., 31–45 days) and maximum duration of 120 days (e.g., 1-120 days) each year. The precipitation value that exhibited strongest correlation (highest  $r^2$  value) with each dependent parameter was selected. Regressions were fit using SAS 9.4.

## Results

### RGR and links to NP and [N]

Spring values of community RGR at the patch scale ranged from – 29.8 to 62.4 mg/g/d among years and communities (Fig. 1). Community NP varied between – 3.6 and 6.7 g biomass/g N/d. [N] differed by as much as a factor of 3.3 among patches across years and communities. Median values of community RGR and NP were greater in switchgrass than mixture communities in 5 of 6 years.

RGR correlated more strongly with community NP than [N] (Table 1). Community RGR was highly and positively correlated with community NP (not shown; adj.  $r^2 = 0.88$ ,  $P < 0.0001$ ). Relationships between community RGR and [N] were weakly positive in 3 of 5 and 4 of 5 years for mixture and switchgrass, respectively, and not significant in remaining years.

Table 1

Relationships between community RGR (g biomass/g biomass/d), Nitrogen Productivity (NP; g biomass/g N/d), and [N] (g N/g biomass). Shown are slopes of linear regression relationships between RGR and independent effects of community NP and [N] (Independent slopes), scaling slopes of log-log relationships between RGR and community NP and [N], and statistical correlation coefficients (Pearson correlation) between NP and [N]. Adj.  $r^2$  values of regressions are shown in parentheses below slope values. NS = not significant ( $P > 0.05$ )

Community/year	Independent slopes		Pearson correlation	Scaling	
	RGR-NP	RGR-[N]	coefficients	Slopes	
			NP-[N]	RGR-NP	RGR-[N]
<b>Mixture</b>					
2016	0.0083 (0.97)	1.152 (0.08)	-0.347	0.91 (0.91)	NS
2017	0.0110 (0.99)	NS	-0.100	0.99 (0.99)	NS
2018	0.0092 (0.88)	2.092 (0.06)	-0.594	0.78 (0.91)	-0.83 (0.10)
2019	0.0080 (0.94)	2.458 (0.25)	-0.481	0.79 (0.72)	NS
2021	0.0087 (0.58)	NS	-0.356	0.92 (0.98)	-2.88 (0.19)
<b>Switchgrass</b>					
2016	0.0111 (0.77)	1.340 (0.11)	-0.826	0.70 (0.87)	-0.89 (0.21)
2017	0.0096 (0.96)	NS	-0.036	0.97 (0.96)	NS
2018	0.0099 (0.82)	2.621 (0.32)	-0.227	0.93 (0.64)	0.83 (0.25)
2019	0.0075 (0.71)	3.119 (0.16)	0.036	1.02 (0.82)	1.30 (0.21)
2021	0.0097 (0.84)	1.421 (0.08)	-0.625	0.78 (0.89)	-0.74 (0.09)

Correlation coefficients between community NP and [N] were negative in 9 of 10 year-community evaluations (Table 1), ranging from slightly positive (0.036) to strongly negative

(-0.826). RGR will change in direct proportion to change in NP when the NP-[N] correlation coefficient = 0 such that scaling slope relating RGR to NP = 1. Because the NP-[N] correlation generally was negative, however, slopes of scaling regressions between community RGR and NP were < 1.0 in 9 of 10 year-community comparisons. Across years and communities, RGR-NP scaling slopes declined linearly as NP-[N] correlation coefficients became more negative (Fig. 2). The plot of RGR-NP scaling slopes contained one outlying point. The RGR-NP response was greater than predicted by regression for mixture in the year (2021) in which RGR was most strongly negatively correlated with community [N] (Table 1). Negative correlation between community NP and [N] also altered the sign of regression slopes between community RGR and [N]. Positive relationships between RGR and statistically independent effects of [N] shifted to negative or not significant scaling slopes in 5 of 6 year-community comparisons in which NP-[N] correlation coefficients were negative (Table 1). RGR-[N] scaling slopes were positive in only 2 years and then only for switchgrass. Slopes of RGR-[N] scaling regressions, like those of RGR-NP regressions, declined in both communities as NP-[N] correlation coefficients became more negative (Fig. 2). RGR responded less to change in [N] during years in the NP-[N] correlation was strongly negative than near zero.

## Precipitation effects

NP-[N] correlation approached zero as precipitation increased in both mixture and switchgrass, although the shape of response curves differed between communities (Fig. 3). Correlation coefficients between community NP and [N] were linearly related to precipitation summed over 1–90 days prior to RGR measurements for mixture but varied as an exponential function of precipitation summed over 46–75 days prior to RGR measurements for switchgrass.

The precipitation effect on NP-[N] correlation coefficients was associated with change in community N content in mixture. Reduced precipitation reduced the mean of community N content in mixture (Fig. 4), but did not significantly affect N content in switchgrass, whether precipitation was summed over 1–90 d ( $P = 0.67$ ) or 46–75 d prior to measurements ( $P = 0.41$ ). Reduced N content in mixture was, in turn, associated with increasingly negative NP-[N] correlation coefficients (Fig. 5). Precipitation did not affect the mean of either NP or RGR in mixture ( $P = 0.92, 0.91$ ) or switchgrass ( $P = 0.60, 0.93$ ).

## Discussion

Community RGR measured near the spring peak in biomass varied substantially among years and grassland communities. RGR was strongly correlated with community NP in mixture and switchgrass. IAV in precipitation did not affect means of either community RGR or NP, but low precipitation during the 3 months prior to RGR measurement lessened scaling of RGR to NP in both simple and diverse grassland by strengthening negative correlation between community-scale values of NP and [N]. Results

demonstrate that precipitation may influence the relationship between grassland growth and plant N independently of any apparent precipitation effect on means of RGR or its N components.

## RGR and precipitation effects on RGR-NP scaling

RGR varied substantially among patches each year, among years, and between communities, likely reflecting spatial and temporal variation in abundances of species functional groups (Polley et al. 2020a). Variation in community RGR was strongly and positively correlated to variation in NP, the efficiency with which N is utilized in growth, in both mixture and switchgrass as predicted. By contrast, independent effects of [N] on RGR were weakly positive or not significant. A given proportional increase in growth rate per unit of plant N should result in a similar proportional increase in growth rate per unit of plant biomass. Studies with individual plants usually document a strong RGR-NP correlation (Wright and Westoby 2001).

Scaling of community RGR to NP depended on the sign and magnitude of correlation coefficients between community NP and [N]. NP-[N] correlation coefficients generally were negative, as may occur if greater growth per unit of N dilutes plant N by increasing aboveground biomass. As a result, RGR usually was smaller than anticipated had NP and [N] varied independently. Soussana et al. (2005) observed negative covariance between N productivity and plant [N] in grass stands. But NP need not correlate negatively with [N]. Indeed, the NP-[N] correlation for switchgrass approximated zero for two years.

Precipitation is a principal driver of grassland productivity (Sala et al. 1988; Knapp and Smith 2001) and regulated IAV in NP-[N] relationships in mixture and switchgrass communities as predicted. NP-[N] correlation coefficients became more negative as precipitation declined in both communities. Inter-annual consistency in scaling of NP to [N] would have required that growth rate for each additional unit of aboveground N be sustained among years. The observed precipitation effect on the NP-[N] correlation implies rather that precipitation mediated a tradeoff between N acquisition and use efficiency in growth. Reduced precipitation limited the growth return on additional N such that patches with relatively elevated aboveground [N] had lower NP.

There are at least two ways in which reduced precipitation could limit growth return on plant N. First, low precipitation may impose a physiological limitation on photosynthesis and growth by reducing plant water status. Higher [N] would contribute relatively little to additional growth under water limitation, leading to a negative relationship between NP and [N]. Second, low precipitation may reduce growth capacity per unit of plant N by limiting aboveground biomass and plant light interception. Negative NP-[N] correlation coefficients were associated with low levels of aboveground N content in mixture. Low N content was, in turn, associated with reduced levels of aboveground biomass (N content-biomass regression: adj.  $r^2 = 0.62$ ;  $P = 0.07$ ). Growth likely depends more on plant light interception than N content or [N] when aboveground biomass is low than high. Limits on light capture, therefore, may have

constrained the growth response to additional plant N and contributed to the precipitation-mediated tradeoff between community NP and [N].

Greatest decrease in scaling slopes in both mixture and switchgrass occurred in years in which precipitation during the 3 months prior to RGR measurements was relatively low. We detected no precipitation effect on mean values of community RGR or NP. Rather, RGR exhibited smaller proportional response to NP during relatively dry than wet years. Although precipitation did not affect means of community RGR or NP over relatively brief periods (14-38 d) in spring, we have found that spring and annual biomass production are positive functions of precipitation in both mixture and switchgrass (Polley, Collins and Fay 2020, 2022). Increased precipitation also increased the response of aboveground production to community [N] in spring in both communities (Polley, Collins and Fay 2022), implying a positive precipitation effect on community growth per unit of plant N.

## Community differences

The response of NP-[N] correlation coefficients to precipitation and link between correlation coefficients and community N content differed between mixture and switchgrass communities. NP-[N] correlation coefficients were linearly related to precipitation in mixture but exhibited an exponential increase with precipitation in switchgrass with correlation coefficients approaching zero at approximately 70 mm precipitation (46-75 d prior to measurement). The latter trend in switchgrass implies the existence of a precipitation threshold above which NP and [N] varied independently. Nitrogen effects on the scaling of RGR to NP differed even more greatly between communities. NP-[N] correlation coefficients did not vary significantly with N content in switchgrass, implying that community N dynamics exerted little effect on scaling on RGR to NP in switchgrass. Conversely, NP-[N] correlation coefficients declined from near zero as community N content declined in mixture. This trend has at least two implications. First, because negative NP-[N] correlation reduces scaling of RGR to NP, RGR was lower than anticipated at given NP value when community N content was low than high in mixture. Second, because increasingly negative NP-[N] correlation is associated with greater increase in NP per unit of decline in [N], NP increased to partially compensate for negative effects of reduced community N on mixture RGR. Greater increase in NP per unit of decline in [N] during years in which mean N content was relatively low should stabilize mean values of mixture RGR among years, consistent with observations that NP and nitrogen use efficiency increase when N availability and plant N acquisition decline (Yuan et al. 2006; Egan et al. 2019).

## Conclusions

Grassland production is sensitive to both precipitation (Sala et al. 1988; Knapp and Smith 2001) and plant N accumulation and N productivity (Fay et al. 2015; Stevens et al. 2015; Egan et al. 2019). We found that reduced precipitation lessened the proportional response of community RGR to increased NP in both simple and diverse grassland communities in the absence of precipitation effects on mean values of RGR

and NP. Results highlight an under-appreciated aspect of the pervasive role of precipitation in grassland growth that is mediated via change in the relationship between community-scale values of NP and [N].

## Declarations

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## Competing Interest

*The authors have no relevant financial or non-financial interests to disclose.*

## Author Contributions

*Conceptualization: Wayne Polley. Data collection and initial analysis: Katherine Jones and Chris Kolodziejczyk. Project and field-site management: Philip Fay. Original draft: Wayne Polley. Manuscript review and approval: All authors.*

## References

1. Aerts R, Chapin FS III (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv Eco Res* 30:1–67.
2. Baret M, Pepin S, Ward C, Pothier, D (2017) Long-term changes in stand growth dominance as related to resource acquisition and utilization in the boreal forest. *For Ecol Manag* 400:408–416. <http://dx.doi.org/10.1016/j.foreco.2017.06.026>
3. Del Pozo A, Garnier E, Aronson J (2000) Contrasted nitrogen utilization in annual C<sub>3</sub> grass and legume crops: Physiological explorations and ecological considerations. *Acta Oecol* 21:79–89.
4. Du E, Terrer C, Pellegrini AFA, Ahlström A, van Lissa CJ, Zhao X et al (2020) Global patterns of terrestrial nitrogen and phosphorus limitation. *Nat Geosci* 13:221–226. <https://doi.org/10.1038/s41561-019-0530-4>

5. Egan G, McKenzie P, Crawley M, Fornara DA (2019) Effects of grassland management on plant nitrogen use efficiency (NUE): Evidence from a long-term experiment. *Basic Appl Ecol* 41:33–43. <https://doi.org/10.1016/j.baae.2019.10.001>
6. Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET et al (2015) Grassland productivity limited by multiple nutrients. *Nat Plants* 1:15080. <https://doi.org/10.1038/NPLANTS.2015.80>
7. Garnier E, Vancaeyzeele S (1994) Carbon and nitrogen content of congeneric annual and perennial grass species: relationships with growth. *Plant Cell Environ* 17: 399–407.
8. Gitelson AA, Viña A, Ciganda V, Rundquist DC, Arkebauer TJ (2005) Remote estimation of canopy chlorophyll content in crops. *Geophys Res Lett* 32: L08403. <http://dx.doi.org/10.1029/2005GL022688>.
9. Hirose T (2011) Nitrogen use efficiency revisited. *Oecologia* 166:863–867. <https://doi.org/10.1007/s00442-011-1942-z>.
10. Hunt R (1982) *Plant growth curves: the functional approach to plant growth analysis*. Edward Arnold, London.
11. Ingestad T (1979) Nitrogen stress in birch seedlings. II. N, K, P, Ca and Mg nutrition. *Physiol Plant* 45:149–157.
12. Isaac RA, Johnson WC (1998) Elemental determination by inductively coupled plasma atomic emission spectrometry. In: Kalra YP (ed) *Handbook and reference methods for plant analysis*. CRC Press, New York, pp 165-170
13. Knapp AK, Briggs JM, Koelliker JR (2001) Frequency and extent of water limitation to primary production in a mesic temperate grassland. *Ecosystems* 4:19–28.
14. Knapp AK, Smith MD (2001) Variation among biomes in temporal dynamics of aboveground primary production. *Science* 291:481–484.
15. Lauenroth WK, Sala OE (1992) Long-term forage production of North American shortgrass steppe. *Ecol Appl* 2:397–403.
16. Li F, Mistele B, Hu Y, Yue X, Yue S, Miao Y et al (2012) Remotely estimating aerial N status of phenologically differing winter wheat cultivars grown in contrasting climatic and geographic zones in China and Germany. *Field Crops Res* 138:21–32. <http://dx.doi.org/10.1016/j.fcr.2012.09.002>
17. Meurer KHE, Bolinder MA, Andrén O, Hansson A-C, Pettersson R, Kätterer T (2019) Shoot and root production in mixed grass ley under daily fertilization and irrigation: validating the N productivity concept under field conditions. *Nutr Cycl Agroecosystems* 115: 85–99. <http://doi.org/10.1007/s10705-019-10006-3>
18. Owensby CE, Hyde RM, Anderson KL (1970) Effects of clipping and supplemental nitrogen and water on loamy upland bluestem range. *J Range Manag* 23:341–346.
19. Polley HW, Collins HP, Fay PA (2020) Biomass production and temporal stability are similar in switchgrass monoculture and diverse grassland. *Biomass Bioenergy* 142: 105758. <https://doi.org/10.1016/j.biombioe.2020.105758>

20. Polley HW, Collins HP, Fay PA (2022) Community leaf dry matter content predicts plant production in simple and diverse grassland. *Ecosphere*, accepted.
21. Polley HW, Yang C, Wilsey BJ, Fay PA (2020a) Spectrally derived values of community leaf dry matter content link shifts in grassland composition with change in biomass production. *Remote Sens Ecol Conserv* 6:344–353. <https://doi.org/10.1002/rse2.145>
22. Polley HW, Yang C, Wilsey BJ, Fay PA (2020b) Temporal stability of grassland metacommunities is regulated more by community functional traits than species diversity. *Ecosphere* 11: e03178. <https://doi.org/10.1002/ecs2.3178>
23. Sala OE, Parton WJ, Joyce LA, Lauenroth WK (1988) Primary production of the central grassland region of the United States. *Ecology* 69:40–45.
24. Soussana J-F, Teyssonneyre F, Picon-Cochard C, Dawson L (2005) A trade-off between nitrogen uptake and use increases responsiveness to elevated CO<sub>2</sub> in infrequently cut mixed C<sub>3</sub> grasses. *New Phytol* 166:217–230. <https://doi.org/10.1111/j.1469-8137.2005.01332.x>
25. Stevens CJ, Lind EM, Hautier Y, Harpole WS, Borer ET, Hobbie S., et al. (2015) Anthropogenic nitrogen deposition predicts local grassland primary production worldwide. *Ecology* 96:1459–1465.
26. van der Werf, A, van Nuenen M, Visser AJ, Lambers H (1993) Contribution of physiological and morphological plant traits to a species' competitive ability at high and low nitrogen supply - A hypothesis for inherently fast- and slow-growing monocotyledonous species. *Oecologia* 94:434–440.
27. Wang J, Knops JMH, Brassil CE, Mu C (2017) Increased productivity in wet years drives a decline in ecosystem stability with nitrogen additions in arid grasslands. *Ecology* 98:1779–1786.
28. Wright IJ, Westoby M (2000) Cross-species relationships between seedling relative growth rate, nitrogen productivity and root vs leaf function in 28 Australian woody species. *Funct Ecol* 14:97-107.
29. Wright IJ, Westoby M (2001) Understanding seedling growth relationships through specific leaf area and leaf nitrogen concentration: generalisations across growth forms and growth irradiance. *Oecologia* 127:21-29. <https://doi.org/10.1007/s004420000554>
30. Yahdjian L, Gherardi L, Sala OE (2011) Nitrogen limitation in arid-subhumid ecosystems: A meta-analysis of fertilization studies. *J Arid Environ* 75:675-680.
31. Yuan Z-Y, Li L-H, Han X-G, Chen S-P, Wang Z-W, Chen Q-S et al (2006) Nitrogen response efficiency increased monotonically with decreasing soil resource availability: A case study from a semiarid grassland in northern China. *Oecologia* 148:564–572. <https://doi.org/10.1007/s00442-006-0409-0>

## Figures

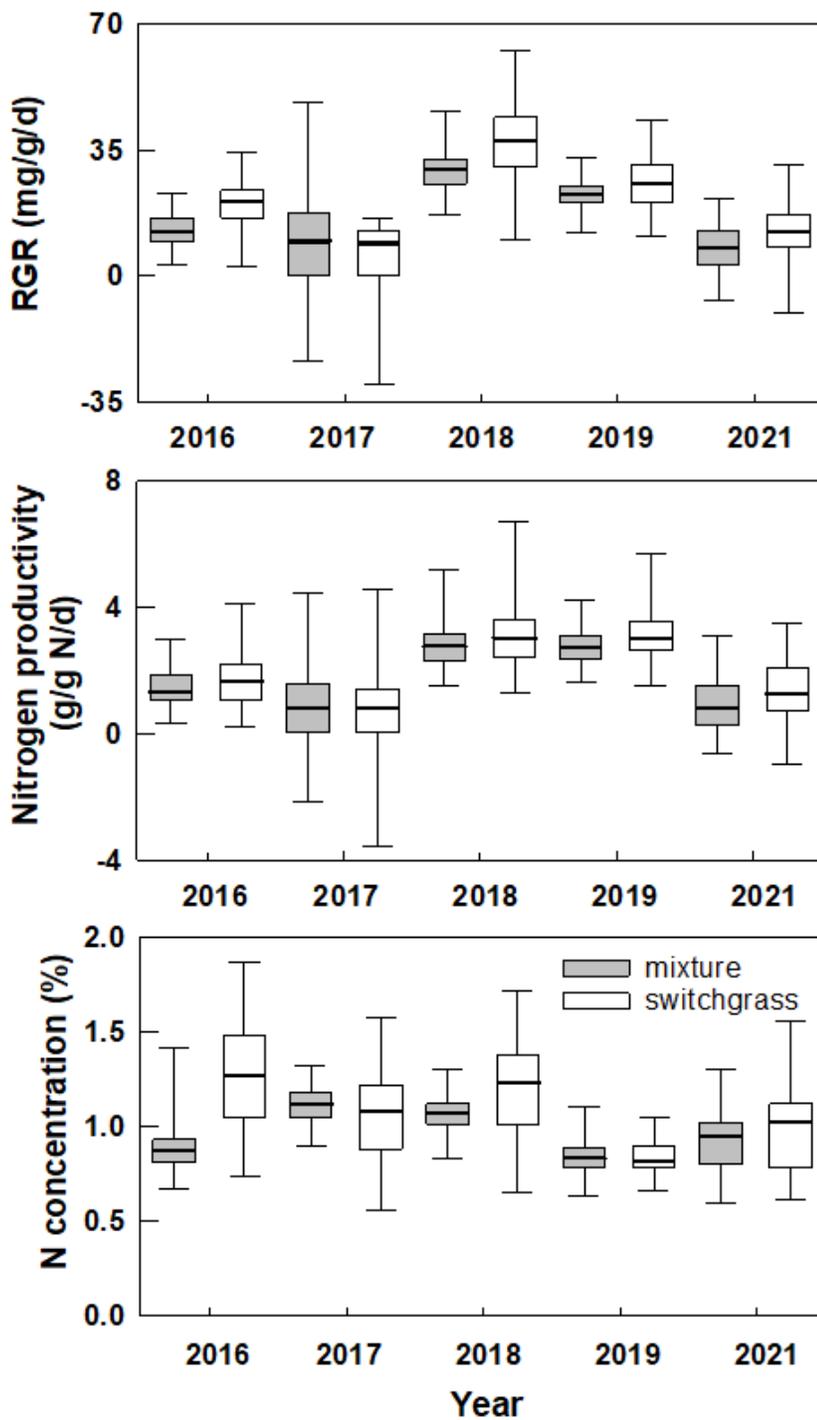


Fig. 1

Figure 1

Spatial variation in RGR, NP, and [N] in mixture and switchgrass communities. Shown are median values (bolded horizontal lines) and the first and third quartiles (boxes) of the distribution of RGR (mg biomass/g biomass/d; upper panel), Nitrogen Productivity (g biomass/g N/d; center panel) and mean [N] (%; lower panel) among 7-m diameter patches of a mixture of grassland forbs and grasses (shaded) and

switchgrass monoculture (open) during each of 5 years (n = 104). Variables were calculated over two dates in spring each year. Lines above and below boxes represent extreme values

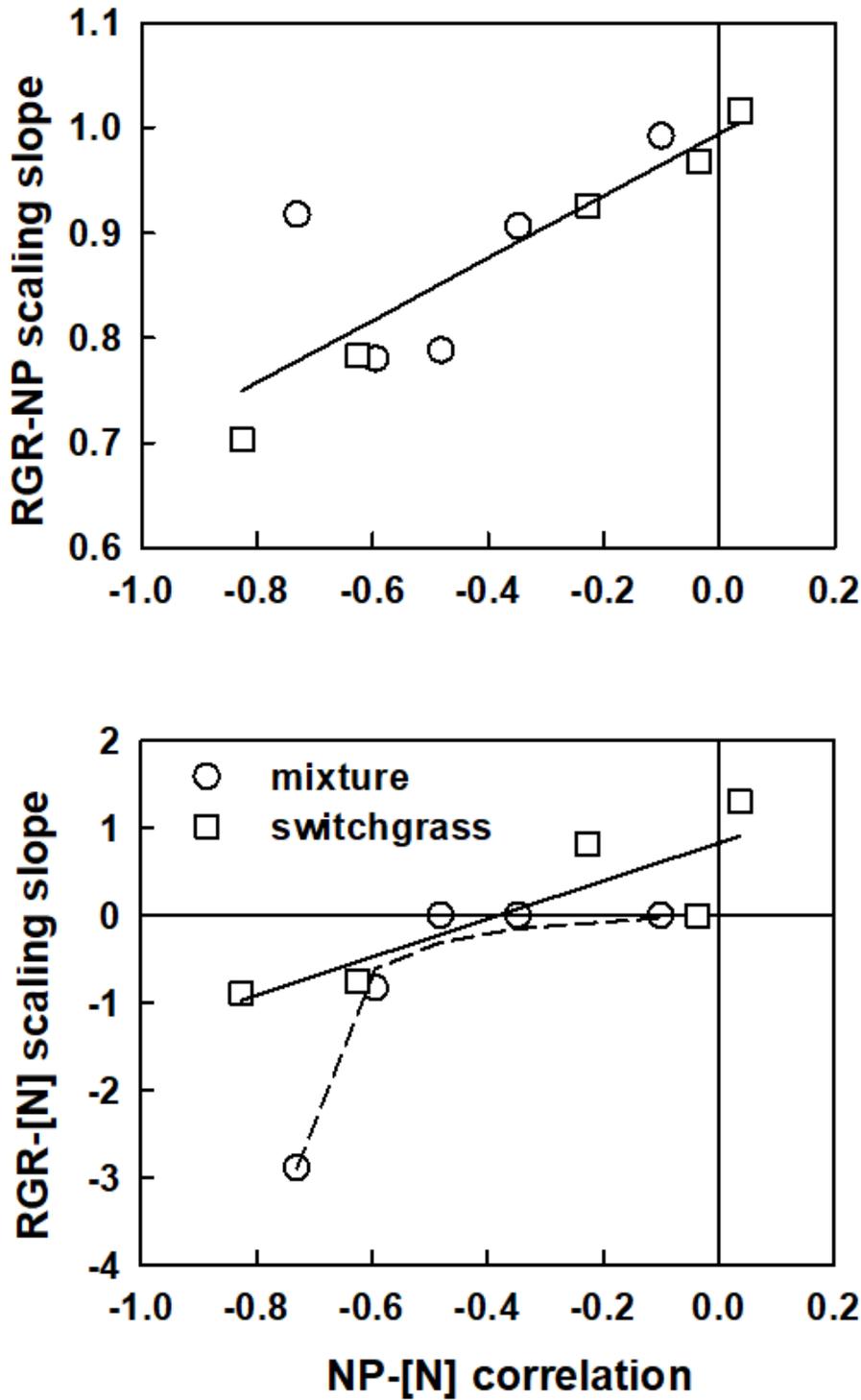


Fig. 2

Figure 2

Relationship of log-log scaling slopes between RGR and its N components (n = 104) and Pearson correlation coefficients between NP and [N]. Relationships were fit with a single regression across mixture

and switchgrass communities (upper panel; RGR-NP scaling slope =  $0.9946 + 0.2967 \times \text{correlation}$ , adj.  $r^2 = 0.68$ ,  $P = 0.0002$ ,  $n = 10$ ) or separate regressions per community (lower panel; RGR-[N] scaling slope =  $((0.2415 \times \text{correlation}) / (1 + 1.2863 \times \text{correlation}))$ , adj.  $r^2 = 0.96$ ,  $P = 0.002$  and  $0.8282 + 2.1727 \times \text{correlation}$ , adj.  $r^2 = 0.63$ ,  $P = 0.07$  for mixture and switchgrass, respectively ( $n = 5$  years). The y-axis in the upper panel does not include the origin

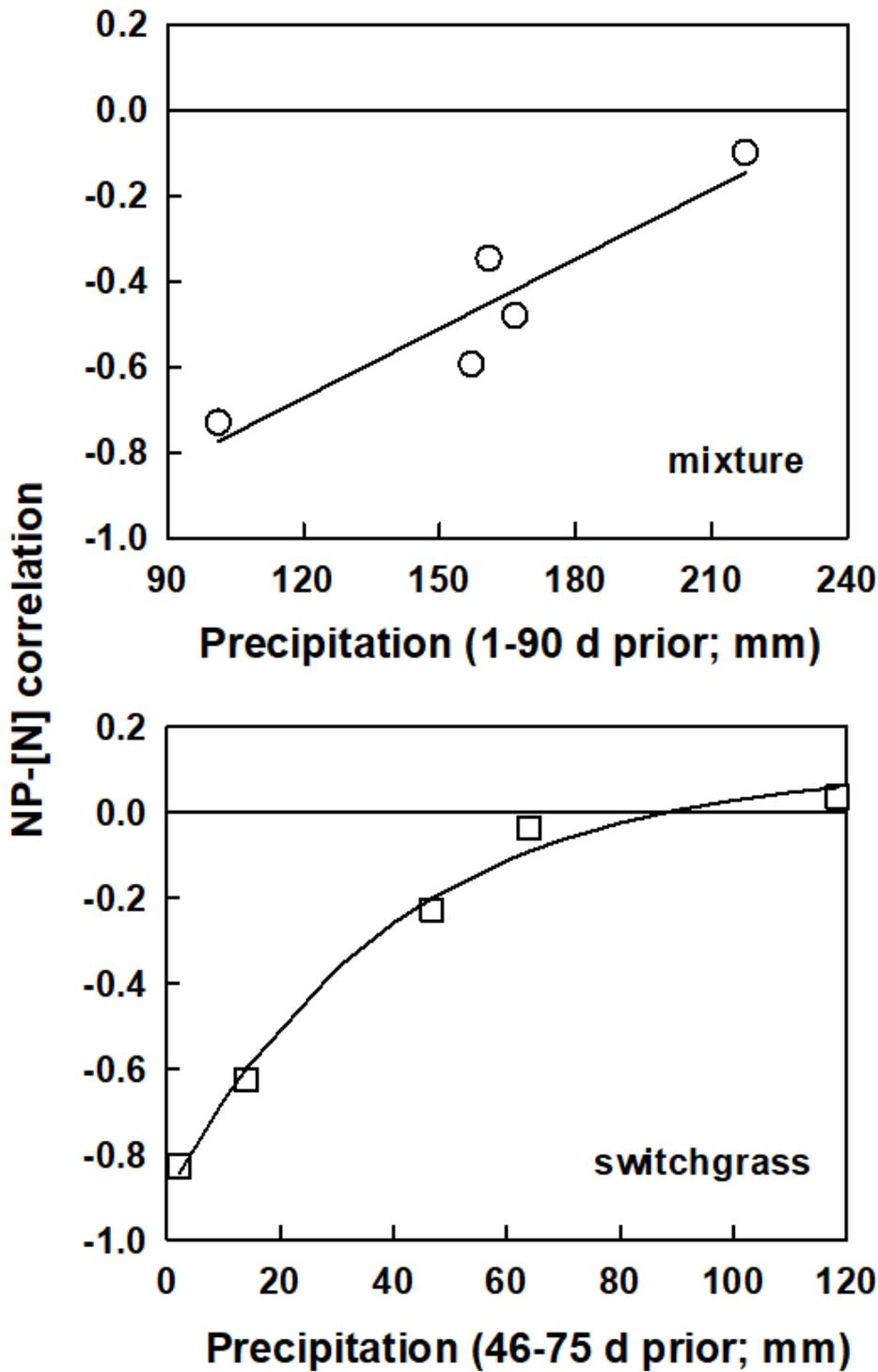


Fig. 3

Figure 3

Precipitation altered correlation between community-scale means of Nitrogen Productivity (NP) and [N] (n = 104) for mixture (upper panel) and switchgrass (lower panel). Lines represent regression fits to relationships between NP-[N] correlation coefficients and precipitation summed over 1-90 d (upper panel) or 46-75 d (lower panel) prior to the initial measurement date each year (correlation =  $-1.3206 + 0.0054 \times \text{precipitation}$ , adj.  $r^2 = 0.81$ ,  $P = 0.024$  and correlation =  $-0.8976 + 1.0066 \times (1 - \exp((-0.0252 \times \text{precipitation}))$ ), adj.  $r^2 = 0.98$ ,  $P = 0.01$  for mixture and switchgrass, respectively). The x-axis in the upper panel does not include the origin

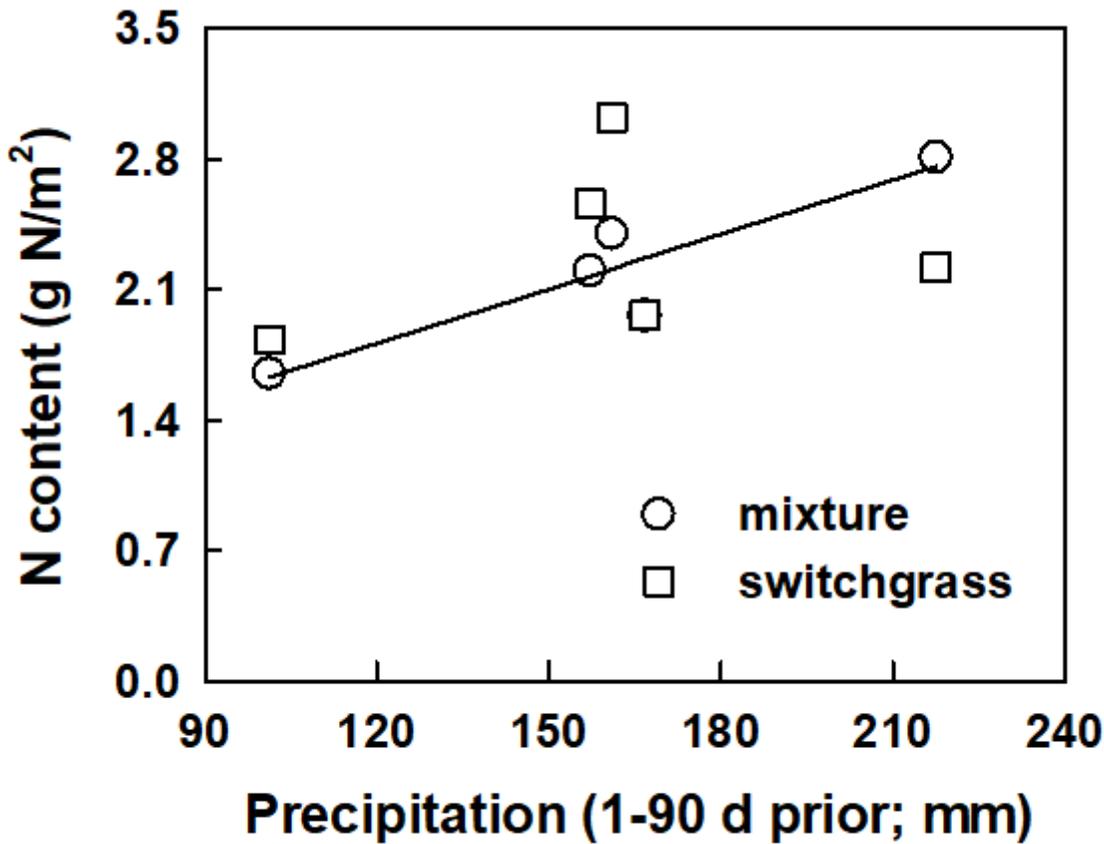


Fig. 4

Figure 4

Precipitation altered means of community N content (n = 104). Aboveground N content increased as precipitation summed over 1-90 d prior to the initial measurement date each year increased for mixture (N content =  $0.65 + 0.01 \times \text{precipitation}$ , adj.  $r^2 = 0.77$ ,  $P = 0.03$ ) but not switchgrass (0.67). Note that the x-axis does not include the origin

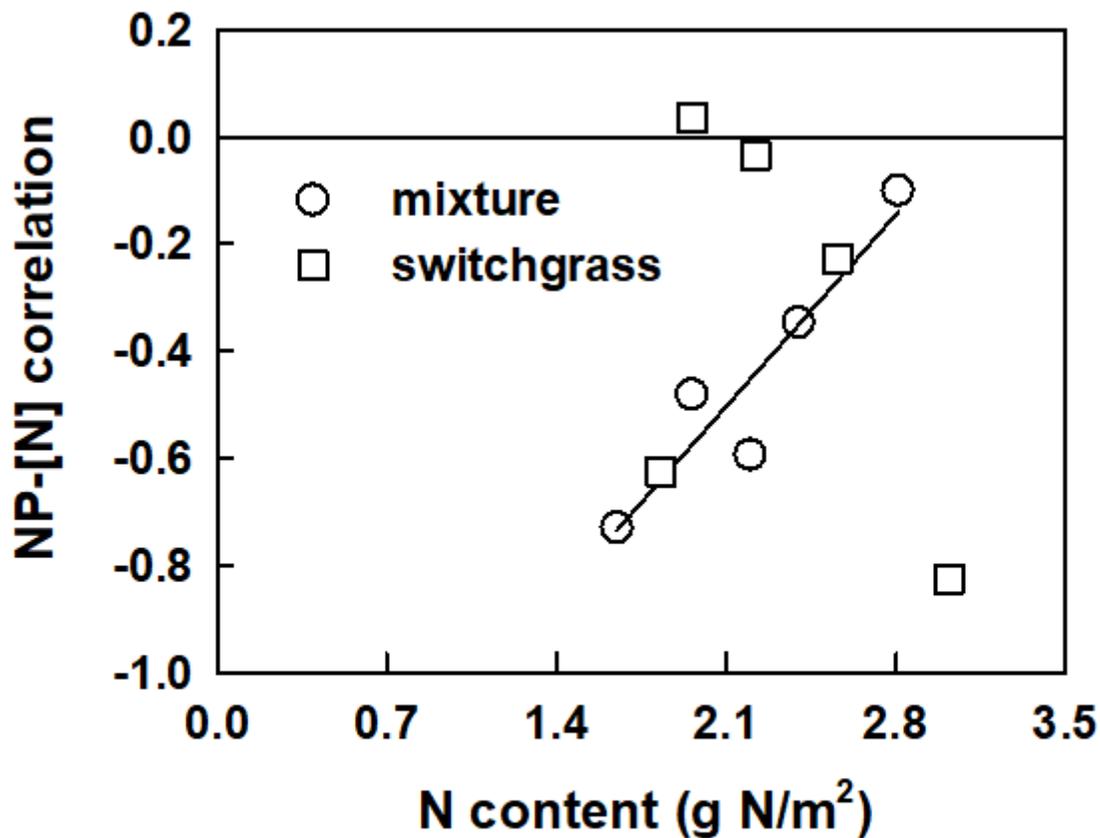


Fig. 5

Figure 5

Correlation coefficients between Nitrogen productivity (NP) and [N] varied with community N content. Pearson correlation coefficients between means of community-scale NP and aboveground [N] increased as a function of mean community N content for mixture (NP-[N] correlation =  $-1.58 + 0.51 \times \text{N content}$ , adj.  $r^2 = 0.83$ ,  $P = 0.02$ ) but not for switchgrass ( $P = 0.70$ )

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [s1.png](#)
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