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ECOSYSTEM ECOLOGY – ORIGINAL RESEARCH

Tight coupling of leaf area index to canopy nitrogen and phosphorus across heterogeneous tallgrass prairie communities

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Abstract Nitrogen (N) and phosphorus (P) are limiting nutrients for many plant communities worldwide. Foliar N and P along with leaf area are among the most important controls on photosynthesis and hence productivity. However, foliar N and P are typically assessed as species level traits, whereas productivity is often measured at the community scale. Here, we compared the community-level traits of leaf area index (LAI) to total foliar nitrogen (TFN) and total foliar phosphorus (TFP) across nearly three orders of magnitude LAI in grazed and ungrazed tallgrass prairie in north-eastern Kansas, USA. LAI was strongly correlated with both TFN and TFP across communities, and also within plant functional types (grass, forb, woody, and sedge) and grazing treatments (bison or cattle, and ungrazed). Across almost the entire range of LAI values and contrasting communities, TFN:TFP ratios indicated co-limitation by N and P in almost all communities; this may further indicate a community scale trend of an optimal N and P allocation per unit leaf area for growth. Previously, results from the arctic showed similar tight relationships between LAI:TFN, suggesting N is supplied to canopies to maximize photosynthesis per unit leaf area. This tight coupling between LAI, N, and P in tallgrass prairie suggests a process of optimal allocation of N and P, wherein LAI remains similarly constrained by N and P despite differences in species composition, grazing, and canopy density.

Keywords Grazers · Co-limitation · Grassland · Fire · Nutrients

Introduction

In many terrestrial ecosystems, primary production is limited by nutrients (Aerts and Chapin 2000; Elser et al. 2007), with limitation by nitrogen (N), phosphorus (P), or co-limitation by both N and P common in grasslands worldwide (Fay et al. 2015). Both N and P play central roles in photosynthesis and cellular function (Reich et al. 2009; Liu et al. 2012; Walker et al. 2014). Primary production often reflects variability in N and P availability across multiple scales of measurement (i.e., individual to landscape), as well as the tight relationship between nutrient uptake and photosynthesis (Schimel et al. 1991; Reich et al. 2009; Quesada et al. 2012; Walker et al. 2014; Stevens et al. 2015; Koller et al. 2016). Foliar N and P concentrations are typically measured as species-level traits, creating a disconnect when inferring community-level (canopy) growth and productivity from measurements of individual plant-level N and P concentrations. Because canopy area and foliar N and P play central roles in a number of key ecosystem functions (including productivity, decomposition, and hence carbon and nutrient cycling), an improved understanding linking

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canopy leaf area and N and P distributions will facilitate predictions of ecosystem productivity and other ecological functions. It may also improve our ability to accurately model C cycling across ecosystems and predict how ecosystems will respond to ongoing, large-scale changes in global N and P inputs (Steffen et al. 2015).

Soil N availability is considered as a key limiting factor for plant growth and productivity in the tallgrass prairie of North America (Turner et al. 1997). Nonetheless, tallgrass prairie can also exhibit co-limitation by P wherein P addition alone does not alter biomass, but additions of N and P in concert results in greater productivity than N-addition alone (Fay et al. 2015). Soil N and P availability vary spatially and temporally according to legacies of grazing and burning, as well as edaphic properties, topography, community composition, and human inputs (Seastedt et al. 1991; Schimel et al. 1991; Ajwa et al. 1998; Avolio et al. 2014; Fay et al. 2015). Large grazers impact the spatial distribution of N and P through selective grazing preferences and patchy nutrient additions across the landscape (Johnson and Matchett 2001; Raynor et al. 2015). Burning generally decreases available soil N and P concentrations and impacts foliar N concentrations in the vegetative canopy (Seastedt 1988; Seastedt et al. 1991; Blair 1997). Soil nutrient availability also varies as a function of topography, with N availability and ANPP generally increasing downslope (Schimel et al. 1991). In turn, variations in N and P availability by burning, grazing, and topography create strong spatial variability in annual net primary productivity (ANPP) (Koerner and Collins 2014).

Canopy development and size are often described using the metric LAI (leaf area index: leaf area per unit ground area). LAI represents the one-sided leaf surface area available for photosynthesis, and variability in this metric strongly influences key ecosystem functions such as productivity and transpiration (Street et al. 2012). LAI can increase with greater N and P supply, and consequently alter species composition of communities and reduce biodiversity (Bobbink 1991; Suding et al. 2005; Borer et al. 2014). With increased N, reduced biodiversity can result from more nitrophilous species developing large canopies that block light to the understory (Bobbink 1991; Hautier et al. 2009). Because changes in LAI can reflect changes in ecosystem dynamics, a more detailed investigation is warranted to investigate the linkages among LAI, N, and P among communities and the extent to which these relationships remain consistent or change in response to landscape heterogeneity.

To date, such research on canopy nutrient scaling has only been conducted in Arctic tundra (Williams and Rastetter 1999; van Wijk et al. 2005). In tundra, Williams and Rastetter (1999) and van Wijk et al. (2005) observed a tight linear coupling between canopy nitrogen content (total foliar nitrogen; TFN) and LAI. Critically, this relationship held across contrasting tundra vegetation types in multiple Arctic locations (Street et al. 2012) and also held at the individual shoot level (Koller et al. 2016). This relationship likely reflects a combination of community assembly and species plasticity that effectively maximizes uptake of carbon per unit foliar nitrogen at the community level via species sorting and allocation of N to the canopy to maximize photosynthesis (van Wijk et al. 2005). The tight LAI-TFN relationship in the Arctic explains why a considerable 80 % of variation in gross primary productivity (gross carbon gain through photosynthesis, GPP) can be predicted from LAI alone, irrespective of vegetation type (Shaver et al. 2007; Street et al. 2007). These relationships have vastly simplified the upscaling of GPP in Arctic tundra since LAI can be remotely estimated from hand-held, aircraft and satellite sensors. Similar benefits may also arise for other ecosystems if tight coupling between LAI and canopy N occurs. This may also apply to LAI-TFP (total foliar phosphorus) relationships in P-limited or co-NP limited systems found in many grasslands (Fay et al. 2015). Furthermore, in herbaceous plant communities where the canopy makes up the majority of above ground biomass, such relationships may allow estimates of aboveground stocks of N and P from measurements of LAI, further providing data needed for process-based modeling.

Here, we investigated the relationships between LAI, canopy N and P (i.e., TFN and TFP) over a growing season across more than two orders of magnitude LAI and three distinct landscape types (ungrazed, bison-grazed, and cattle-grazed) in tallgrass prairie. We included variability in grazing and seasonality because most grasslands experience seasonal growth cycles, and ungulate grazing is one of the most profound and widespread driving forces of grassland ecosystems (Milchunas et al. 1988). We hypothesized (1) strong coupling between LAI: TFN, similar to relationships shown in Arctic tundra (Williams and Rastetter 1999; van Wijk et al. 2005; Street et al. 2012). While the relationship between canopy P (TFP) and LAI has not been explored previously, given that N and P are typically correlated and experimental manipulations of tallgrass prairie exhibit additive responses to N and P addition (Avolio et al. 2014; Fay et al. 2015), we hypothesized that (2) LAI and TFP would also be positively correlated, with any change in the TFN: TFP ratio with increasing LAI indicating a shift in N or P limitation with increasing productivity. Similar to observations reported in tundra, we hypothesized that (3) LAI: TFN and LAI: TFP would vary by plant growth form (of forbs, sedges, woody shrubs, and grasses), with (4) lower TFN per unit LAI in grasses than that of forbs (Taylor et al. 2010). The hypothesized differentiation between grasses and forbs was presumed because C_4 grass species constitute the majority of cover and productivity in the tallgrass prairie, and these species usually have lower N requirements than C_3 forbs (Turner and Knapp 1996). Finally, we hypothesized that (5) grazing by bison and cattle would increase TFN and TFP per unit LAI through increased rates of nutrient cycling or by altering community composition toward nitrophilous species through selective foraging.

Methods

Study site

The study was performed at the Konza Prairie Biological Station (KPBS), a native tallgrass prairie located near Manhattan, KS USA (39°05′N, 96°35′W). KPBS has a rich floristic diversity with over 550 vascular plant species documented in its ~25 km^2 area (Towne 2002) of which a few C_4 grass species are responsible for most of the annual aboveground productivity (Knapp et al. 1998). KPBS experiences a mid-continental climate, with mean monthly maximum temperatures ranging from 4.65 °C in January to 32.62 °C in July (1982–2011 mean, Konza Headquarters weather station). Average annual precipitation is 843 mm, with ~70 % occurring between April and September. During the year of study (2011), the climate was warmer (36.82 °C July) and slightly drier (814 mm year^{-1}) than the long-term average.

KPBS is divided into watersheds varying in presence or absence of grazers (bison or cattle) and time interval between burning treatments (1, 2, 4, 20 years). A bison herd of approximately 280 animals have access to 10 adjacent watersheds with varying fire frequencies, totaling ~980 hectares. A cattle herd of approximately 26 cow-calf pairs have access to 4 adjacent watersheds on the southeastern-most section of KPBS, totaling ~313 hectares. Cattle are present on site from May–October while bison are present year-round.

KPBS has a weathered topographic landscape of varying chert and limestone layers. Upland locations have rocky, thin-soil layers (<30 cm) typically in the Florence soil series, while lowland locations are less rocky and have deep soils (>200 cm) typically in the Tully soil series. Primarily as a function of soil depth, roots of vegetation in uplands tend to be shallower and experience more frequent and extreme reductions in volumetric soil water content (Abrams and Hulbert 1987; Nippert and Knapp 2007; Nippert et al. 2011).

Vegetation sampling

Vegetation was sampled in three watersheds at KPBS within 1.5 km of each other. Sampling occurred in locations burned with an annual fire frequency, because this is currently one of the most common fire frequencies in grasslands of the region (Ratajczak et al. 2016). The three watersheds varied according to grazing conditions—watershed N1B is grazed by bison, C1B is grazed by cattle, and 1D has not been grazed since the 1970’s. Watersheds N1B and C1B have been burned annually since 1988 and comprise 120.6 and 21.6 ha, respectively. Watershed 1D has been burned annually since 1978 and is 41.5 ha. All prescribed burning occurred in the spring of each year.

Sampling occurred in upland topographic positions on 5 dates, at approximately 14-day intervals from 07-Jun-2011 to 27-Jul-2011. On each round of sampling, two 0.1 m^2 quadrats were randomly sampled within three plots per watershed and total aboveground vegetation was harvested. Quadrats within plots were randomly located within each sampling period, approximately 5–10 m apart. Plots within watersheds were separated by 60–80 m, and replicates within plots were at least 10 m apart. Leaf samples were stored at 4 °C until processing. All brown tissue was discarded, and remaining green vegetation was separated according to species.

Leaf area index (LAI)

Following sorting by species, one-sided projected leaf area from each quadrat was measured using a LI-3200 leaf area meter (Li-COR Biosciences, Lincoln, NE, USA) following van Wijk et al. (2005). Total leaf area per quadrat was calculated as the sum of the leaf area of each species present. Leaf area index (LAI) was derived by dividing total leaf area by the ground area sampled and reported in units of m^2 leaf area per m^2 ground area.

Total foliar nitrogen (TFN)

Samples constituting leaf biomass of individual species within a quadrat were dried at 60 °C for 48 h, weighed, and ground. Subsamples (3–5 mg) were analyzed for percent nitrogen with an elemental analyzer (FlashEA 1112, Thermo Fisher Scientific). Total nitrogen fraction per species was calculated by multiplying N content ($\%N/100$) by biomass (g). TFN was calculated at the quadrat-level per plot as the cumulative species sum of the N fractions per ground area (g m^{-2}).

$$\text{TFN} = \sum_{i}^{R} N_i \times B_i, \tag{1}$$

where R is the number of species in a plot, N refers to the proportion of biomass as nitrogen for species i (g of Nitrogen/g of total biomass), and B is the biomass of species i (g biomass).

Total foliar phosphorus (TFP)

Total P content per species was analyzed following Kjeldahl acid digestion (Allen 1989) with colorimetric P determination (adapted from Murphy and Riley 1962) using a CECIL CE 1020 spectrophotometer (Spectronic, Leeds, UK). TFP was determined on one quadrat per plot, and calculated similarly to TFN as the cumulative species sum of the P fractions per ground area (g m^{-2})

$$\text{TFN} = \sum_{i}^{R} P_i \times B_i. \qquad (2)$$

TFP data for time period 5 in the cattle grazing treatment were lost during sample preparation.

Statistical analysis

We used linear mixed-effects ANOVA to assess the relationship between the response variables LAI, TFN, and TFP to the fixed effects ‘grazing type’, ‘plant functional type’, and ‘period’. Plant functional types were designated as ‘grasses’, ‘forbs’, ‘woody’, and ‘sedges’ because these classifications resulted in the broadest representation of the species sampled. The ‘grasses’ category includes both C_4 and C_3 species, but C_3 species were very uncommon (accounting for <6 % by frequency and <1 % by dry biomass of all grass individuals encountered). The interaction term between ‘plant functional type’ and ‘period’ was not assessed because not all plant functional types were present in all periods measured. The random effect structure of the models for LAI and TFN included a random intercept with the measured replicate nested within plot. For TFP data, the random effects included the intercept and the plot of measurement within watersheds.

We used linear regression to assess the relationships between LAI: TFN; LAI: TFP; and TFP: TFN across all time periods. Separate regression analyses were performed by plant functional types (forb, grass, woody shrub, and sedge) and by grazing contrasts (bison, cattle, ungrazed). To test for significance among these categorical variables (plant functional types and grazing contrasts), we used ANCOVA to compare regression slopes and intercepts. For all analyses involving TFP, corresponding LAI and TFN values were derived from quadrat ‘A’; as TFP data were only measured using vegetation from this sample location. All analyses were performed using the ‘nlme’ package in R (R Core Team 2013).

Results

The study sampled several orders of magnitude range in LAI (from 4.16 to <0.01 $m^2\ m^{-2}$), TFN (7.08–0.41 g m^{-2}), and TFP (0.33 to <0.001 g m^{-2}). Leaf area index (LAI) varied significantly ($P < 0.05$) by each of the main effects (grazing type, plant functional type, and period) as well as the interactions ‘sample period’ * ‘grazing treatment’ and ‘plant functional type’ * ‘grazing treatment’ (Fig. 1, Online Appendix 1—Table 1). TFN and TFP varied significantly ($P < 0.05$) by plant functional type* grazing treatment as well as the main effects plant functional type, grazing treatment, and the intercept (Online Appendix 1—Tables 2, 3, respectively). Neither TFN nor TFP was influenced by sample period in this analysis.

LAI had a statistically significant positive relationship with TFN ($y = 1.43x + 0.67$; $r^2 = 0.74$) and with TFP ($y = 0.11x + 0.04$; $r^2 = 0.63$) (Fig. 2). The amount of variance explained and the slope of the relationship between LAI and TFN differed significantly ($P < 0.001$) between plant functional types (Fig. 3, Online Appendix 2). Woody plants had the greatest slope ($y = 2.35x + 0.13$; $r^2 = 0.86$), followed by forbs ($y = 1.67x + 0.17$; $r^2 = 0.90$) and sedges ($y = 1.62x + 0.00$; $r^2 = 0.92$), then grasses ($y = 1.24x + 0.49$; $r^2 = 0.75$). Grasses had the lowest slope between LAI: TFN, illustrating lower amounts of foliar N with increasing canopy size compared to the other plant types (Fig. 3a). LAI: TFP had similar slopes by plant functional type ($P = 0.979$), but the intercepts varied significantly ($P = 0.019$) (Online Appendix 2). Grasses had the highest y-intercept between LAI: TFP ($y = 0.12x + 0.03$; $r^2 = 0.62$). For the forbs, woody, and sedge plants, the regression analyses produced y-intercepts of zero (forb: $y = 0.12x + 0.00$; $r^2 = 0.80$) (woody: $y = 0.13x + 0.00$; $r^2 = 0.83$) (sedge: $y = 0.16x + 0.00$; $r^2 = 0.86$) (Fig. 3b).

Similar to differences by plant functional types, the relationship between LAI: TFN varied significantly among grazing treatments ($P < 0.001$) (Fig. 4a, c, e). The fit of the relationship was strongest in grazed (Fig. 4a—Bison: $y = 1.45x + 0.91$ $r^2 = 0.87$) (Fig. 4c—Cattle: $y = 2.34x + 0.15$ $r^2 = 0.83$) versus ungrazed areas (Fig. 4e—$y = 1.00x + 1.01$ $r^2 = 0.54$) and had steeper slopes, illustrating that plant canopies in grazed prairie have greater N per unit increase in leaf area than canopies of ungrazed prairie. The relationship between LAI: TFP was similar to LAI: TFN, with strong positive correlations among grazing types (Fig. 4b, d, f), but the slopes and intercepts did not vary significantly among grazing treatments ($P > 0.05$, Online Appendix 2).

TFN and TFP were also significantly correlated ($y = 11.25x + 0.31$; $r^2 = 0.73$) (Fig. 5). The canopy N:P ratio (TFN: TFP) for all but 4 quadrats sampled had values between 20:1 and 10:1, suggesting that communities were primarily co–N:P limited (Fig. 5, based on the co-N:P limitation range of Güsewell 2004). The relationship between TFN and TFP exhibited statistically significant differences between functional groups ($P < 0.001$) (Fig. 6, Online

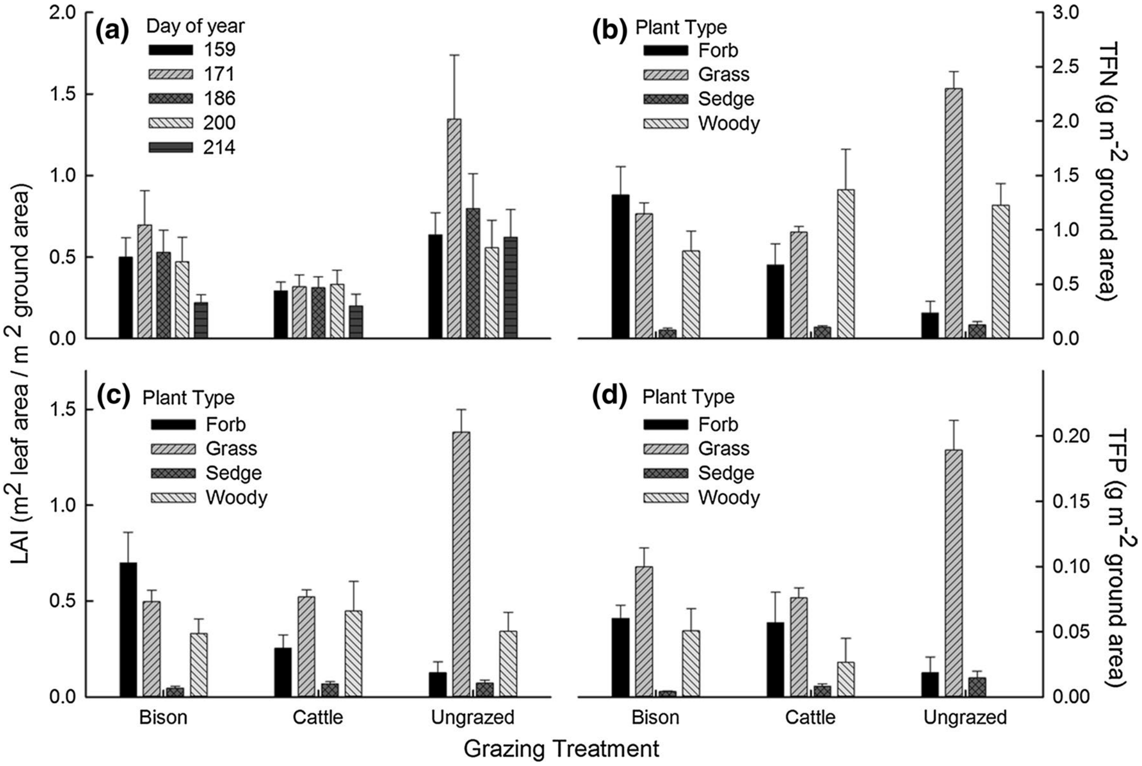


Fig. 1 Bar plots reflect significant statistical interactions for Grazing Treatments reported in Online Appendix 1, Tables 1–3. The mean ± 1 SE are shown for **a**, **c** LAI, **b** TFN, and **d** TFP

Appendix 2) and grazing treatments ($P < 0.001$) (Fig. 7, Online Appendix 2). Forbs and woody plants had more canopy nitrogen per unit phosphorus than grasses (Fig. 6). Sedges did not span a wide enough range of TFN and TFP values to make meaningful comparisons to other plant functional groups. The bison and cattle grazing treatments had similar relationships between TFN and TFP, with more canopy nitrogen per unit canopy phosphorus than the ungrazed treatment (Fig. 7).

Discussion

In agreement with our primary hypotheses (1 and 2), this study demonstrated consistent linear relationships between LAI: TFN and LAI: TFP across the study area. These relationships remained statistically strong across plant functional types and grazing treatments, in agreement with hypothesis 3. The correlation between LAI: TFN and LAI: TFP suggests that as plant canopies increase in size in tallgrass prairie, N and P foliar allocation follow similar constraints. Furthermore, treatments across the landscape (i.e., grazing treatment) or plant functional type likely account for the residuals from the underlying fundamental relationship. In addition, this study revealed tight coupling between TFN: TFP across more than two orders of magnitude in tallgrass prairie LAI. These relationships suggest a stoichiometric allocation of N and P to the canopy to maximize C uptake and productivity per unit of these limiting nutrients, as well as N and P co-limitation across communities of varying productivity and species composition.

LAI had a strong statistical relationship with TFN and TFP across all plant functional types examined—grasses, sedges, forbs, and woody shrubs (Fig. 3). The delineation of functional plant types in our study was used to account for variation in nutritional requirements that are well documented to exist among growth forms. In particular, we hypothesized (4) that the lower N requirements of C_4 grass species would result in the grasses having lower TFN per canopy area than forbs and woody species (Turner and Knapp 1996; Taylor et al. 2010). Our results supported this hypothesis with grasses having the lowest slope for LAI: TFN compared to the other plant functional groups, and showing that grasses can produce larger canopies for relatively less N investment compared to the other functional groups (Fig. 3a). This response may partially explain why C_4 grasses make up increasingly greater proportions of the total biomass in more productive locations (Turner and Knapp 1996; Nippert et al. 2011), since they can maintain greater LAI (hence light capture and shading of other

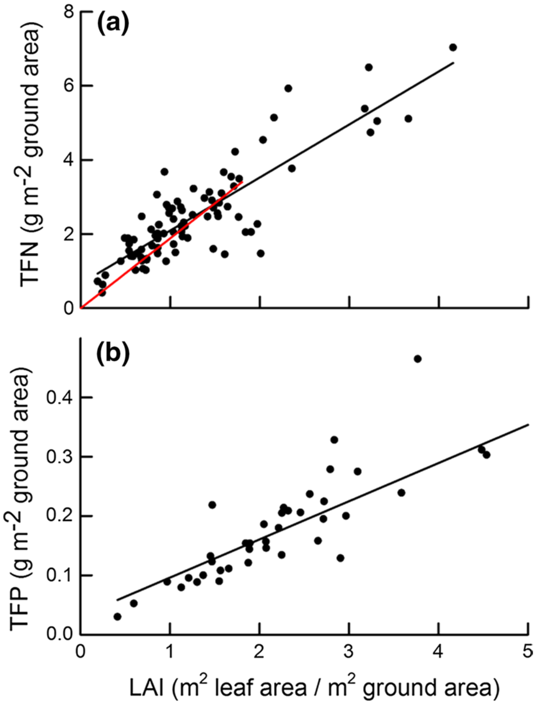


Fig. 2 Leaf area index (LAI; m^2 leaf area m^{-2} ground area) vs. **a** total foliar nitrogen (TFN; g m^{-2} ground area) and **b** total foliar phosphorus (TFP; g m^{-2} ground area) as measured bi-weekly throughout the growing season in annually burned tallgrass prairie. *Solid red line* depicts LAI: TFN reported for arctic tundra in van Wijk et al. (2005). For **a** $y = 1.43x + 0.67$, $r^2 = 0.74$, $n = 87$. For **b** $y = 0.11x + 0.04$, $r^2 = 0.63$, $n = 42$ (color figure online)

species) per unit investment of N compared to other functional types.

Interestingly, the relationship between LAI and TFP was also statistically strong, supporting hypothesis 2 (Fig. 2b). Foliar P plays an important role in photosynthesis (albeit of less direct importance than foliar N), with lower leaf P associated with reduced photosynthetic capacity ($A_{\max}$) and reduced sensitivity of $A_{\max}$ and V_{cmax} (maximum rate of carboxylation) to leaf N (Reich et al. 2009; Walker et al. 2014). As canopy size increases and contains greater amounts of N, the amount of P in the canopy increases, likely to maintain optimal rates of photosynthesis (and hence productivity) from these potentially limiting nutrients. While the forbs, sedges, and woody functional groups had similar allocation of canopy P per unit LAI, grasses tended to have more canopy P than the other functional groups per unit LAI (Fig. 3b). This result may represent a greater demand for P in the canopies of C_4 grasses, or alternatively, the lower demand for N compared to other functional groups may allow production of canopies with relatively high P content (essentially luxuriant P uptake).

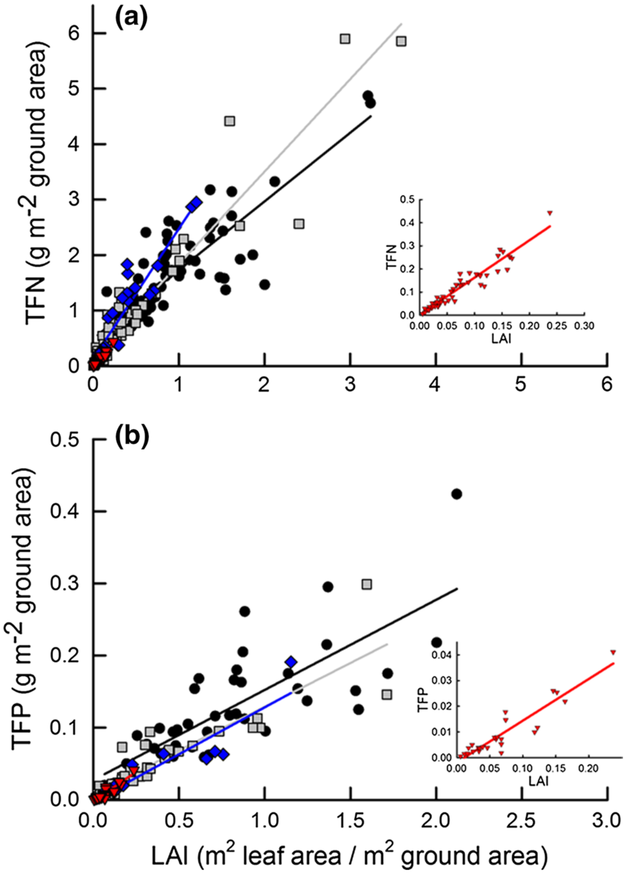
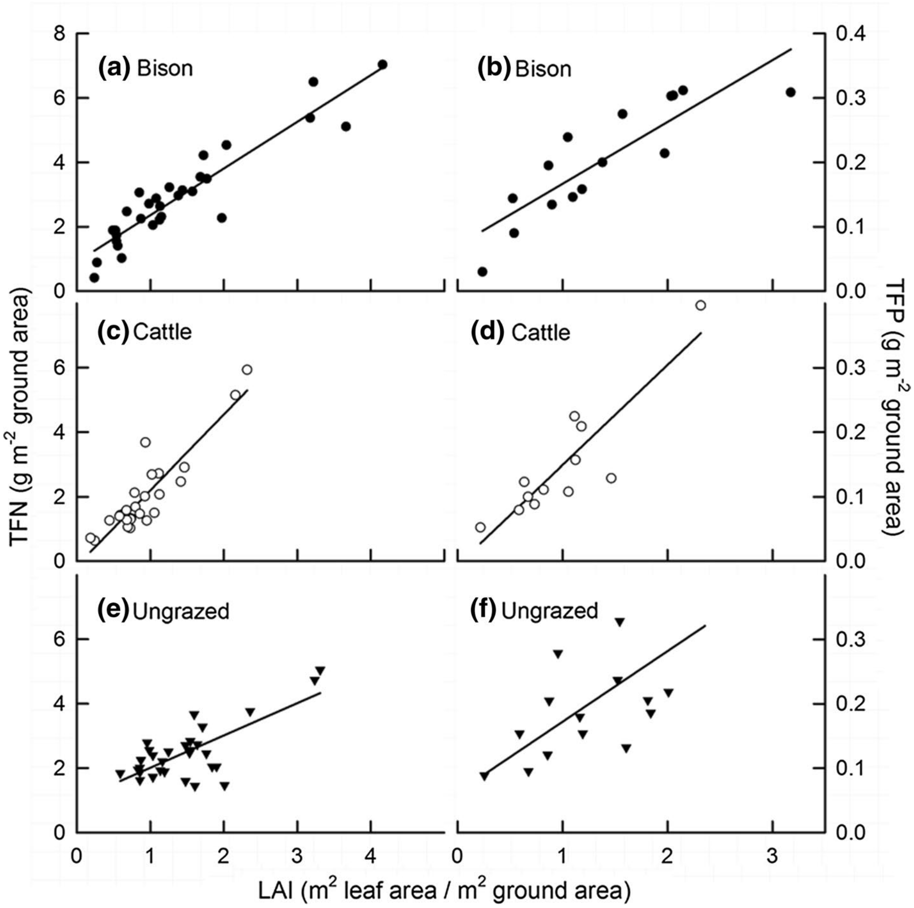


Fig. 3 The correlation between **a** LAI and TFN and **b** LAI and TFP for four plant functional groups in the tallgrass prairie: grass (*filled circle*), forb (*gray square*), woody (*blue diamond*), and sedge (*red triangle*). Relationships for sedges are also shown as an *inset figure* in both *panels* for increased resolution. Fit statistics for each functional group include: *panel* **a** grass: $y = 1.24x + 0.49$, $r^2 = 0.75$, $n = 87$; forb: $y = 1.67x + 0.17$, $r^2 = 0.90$, $n = 61$; woody: $y = 2.35x + 0.13$, $r^2 = 0.86$, $n = 24$; sedge: $y = 1.62x + 0.00$, $r^2 = 0.92$, $n = 52$; and *panel* **b** grass: $y = 0.12x + 0.03$, $r^2 = 0.62$, $n = 42$; forb: $y = 0.12x + 0.00$, $r^2 = 0.80$, $n = 34$; woody: $y = 0.13x + 0.00$, $r^2 = 0.83$, $n = 13$; sedge: $y = 0.16x + 0.00$, $r^2 = 0.86$, $n = 25$ (color figure online)

As the LAI: TFN/TFP relationships remained strong across plant types, they also remained strong across grazing treatments including bison, cattle, and non-grazed; this result is consistent with hypothesis 5. In the tallgrass prairie, community composition varies greatly over space due to grazing and burning disturbances, and topography (Hartnett et al. 1996; Fuhlendorf and Engle 2004; Collins and Calabrese 2012; Koerner and Collins 2014). Bison and cattle alter community composition and increase species diversity by selectively removing grasses over forbs (Damhoureyeh and Harnett 1997). Further, these ungulates create spatial patchiness in canopy area and plant type composition by grazing more frequently in certain areas over others (Hartnett et al. 1996; Raynor et al. 2015).

Using the N:P ratios of Güsewell (2004), we found that nearly all locations sampled fell within the range indicative

Fig. 4 The correlation between LAI: TFN (*panel* **a**, **c**, **e**—*left y-axis*) and LAI: TFP (*panel* **b**, **d**, **f**—*right y-axis*) for the three grazing treatments. Fit statistics for each grazing treatment include: **a** bison-grazed: $y = 1.45x + 0.91$, $r^2 = 0.87$, $n = 30$; **b** bison-grazed: $y = 0.07x + 0.09$, $r^2 = 0.74$, $n = 15$; **c** cattle-grazed: $y = 2.34x - 0.15$, $r^2 = 0.83$, $n = 27$; **d** cattle-grazed: $y = 0.16x + 0.00$, $r^2 = 0.80$, $n = 12$; **e** ungrazed: $y = 1.00x + 1.01$, $r^2 = 0.54$, $n = 30$; **f** ungrazed: $y = 0.11x + 0.06$, $r^2 = 0.44$, $n = 15$



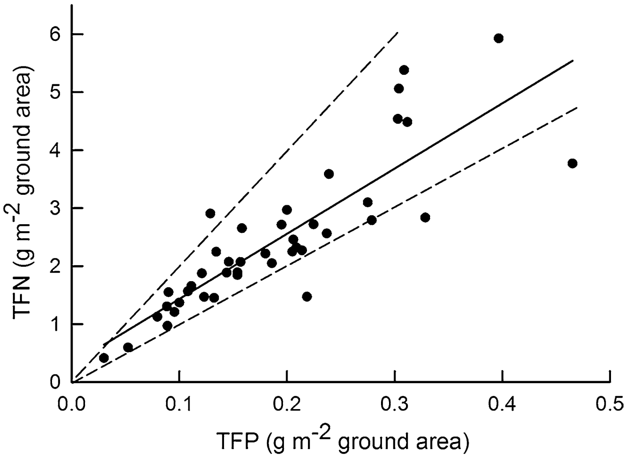


Fig. 5 The correlation between TFP and TFN as measured bi-weekly throughout the growing season in annually burned tallgrass prairie ($y = 11.25x + 0.31$, $r^2 = 0.73$, $n = 42$). *Dashed lines* depict the region defined by N:P co-limitation according to Güsewell (2004) (N:P of 20:1 to 10:1, respectively)

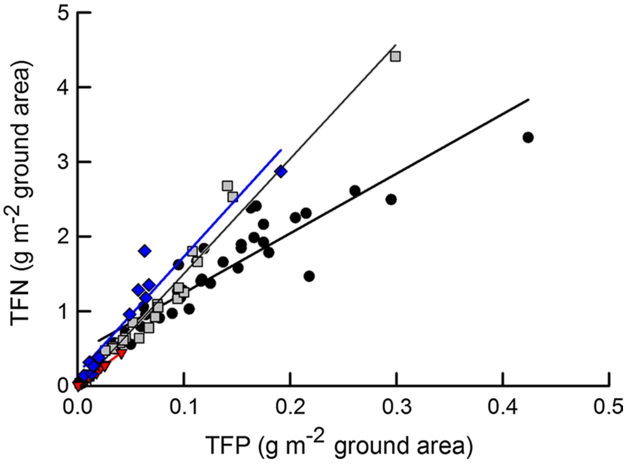


Fig. 6 The correlation between TFP and TFN for each of the four plant function types: grass (*filled circle*), forb (*gray square*), woody (*blue diamond*), and sedge (*red triangle*). Fit statistics for each functional group are: grass: $y = 7.99x + 0.44$, $r^2 = 0.84$, $n = 42$; forb: $y = 15.38x - 0.03$, $r^2 = 0.97$, $n = 28$; woody: $y = 15.65x + 0.17$, $r^2 = 0.91$, $n = 13$; sedge: $y = 10.56x + 0.01$, $r^2 = 0.98$, $n = 25$ (color figure online)

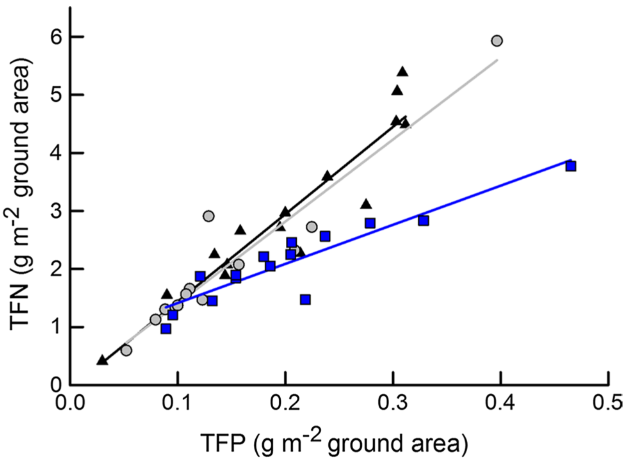


Fig. 7 The correlation between TFP and TFN for the three grazing treatments: bison-grazed (*filled triangle*), cattle-grazed (*gray circle*), and ungrazed (*blue square*). Fit statistics for each grazing treatment are: bison: $y = 15.04x - 0.07$, $r$statistically strong, supporting hypothesis $= 0.89$, $n = 15$; cattle: $y = 14.13x + 0.00$, $r^2 = 0.90$, $n = 12$; ungrazed: $y = 6.74x = 0.74$, $r^2 = 0.84$, $n = 15$ (color figure online)

of growth co-limited by N and P (Fig. 5). The result that nearly all sampled canopies had TFN: TFP ratios between 10 and 20 across the full range of LAIs sampled indicates a remarkably consistent stoichiometric ratio between N and P (Fig. 5), despite large differences in LAI (Fig. 2). The Güsewell (2004) range for co-NP limitation is broader than some ranges proposed by others (e.g., Koerselman and Meuleman 1996). However, a recent global analysis in grassland communities showed co-limitation to be more common than previously thought (Fay et al. 2015). This suggests that past narrow N:P ratio ranges may have mistakenly predicted single nutrient limitation in some communities that were co-limited. Furthermore, a narrow range of N:P ratios supports co-limitation since it suggests the two nutrients are taken up in consistent ratios, which would be expected if both nutrients were in equal demand.

Despite varying N:P ratios among individual plants and functional groups, the narrow range of N:P ratios at the community level (Fig. 5) suggests community assembly may occur to maintain co-NP limitation. Such a mechanism would help explain the narrow range of N:P ratios across the diverse range of sites sampled. Such a mechanism within a community would maximize the use of both nutrients (N and P) whereby some species require more N (typically more N-limited and have a lower N:P ratio) and other species within the community require more P (typically more P-limited and have a higher N: P). In this scenario, competition for the limiting nutrient would be reduced since the limiting nutrient varies among coexisting species within a community. However, the evidence for this proposed mechanism is not strong across the full range of LAIs sampled. When assessed at the level of plant functional types, woody plants, forbs (and sedges as far as the data allow) stay well inside co-NP limitation as LAI increases. However, grasses illustrate greater N limitation with increasing LAI, and the most productive (and high LAI) communities within this ecosystem have greater proportions of C_4 grass species (Nippert et al. 2011). Thus, no evidence exists in this ecosystem that communities assemble to maintain co-NP limitation in high productivity locations. However, in the low productivity (low LAI) communities which represent the most nutrient-limited locations, it is possible that the community (canopy) assembles to be co-NP limited. In this scenario, woody plants are marginally more P-limited, sedges are marginally more N-limited, and grasses and forbs are co-NP limited (Fig. 6). In these low-nutrient sites, species co-existence may be facilitated via a mix of species with varying N or P-limitations. This idea is conceptually similar to resource partitioning (McKane et al. 2002), but here resource competition is reduced through species having different limiting nutrients, rather than through species partitioning the same single limiting nutrient or water. Further evidence for the existence of such a mechanism is required.

While we argue that N:P co-limitation is apparent across all three grazing treatments, there are likely differences in N and P limitation between treatments that contribute to the formation of different communities. Watersheds grazed by bison and cattle exhibited higher TFN per unit LAI, relative to the ungrazed treatment (Fig. 4). Ungulate grazers in the tallgrass prairie increase soil N concentration and availability with their urine and feces, which may provide plants with higher amounts of N from the soil (Johnson and Matchett 2001). Indeed, TFN in the bison-grazed treatment was greater on average and covered a much wider range of values, corroborating the ideas that ungulates increase nitrogen cycling rates overall, while simultaneously increasing the spatial heterogeneity of nitrogen availability (Towne et al. 2005). This increase in overall nitrogen should, on average, favor more nitrophilous plant types (Fig. 1b). However, ungulates also return P to the ecosystem via excrement (Cech et al. 2010). The shift in grazed TFN: TFP ratios toward values expected for P-limitation (Fig. 7), suggests that grazers could be expediting N return to soils and plants more than they are increasing return rates of P. Selective grazing behavior might also explain some of the relative changes in TFN and TFP (Fig. 7). In this study, grasses had lower TFN values and higher TFP values per unit LAI (Fig. 3), and therefore consumption of grasses by grazers could alter community-scale patterns toward greater P-limitation. Because ungulate grazers are a natural presence in the tallgrass prairie and have a long co-evolutionary history with fire and the herbaceous community, understanding the effect of grazers on the relationship between TFN and LAI contributes to an improved knowledge of how nutrients limit canopy growth in grasslands and savannas (Hobbs et al. 1991; Anderson et al. 2006; Cech et al. 2010).

The strong correlations between TFN and LAI shown here parallel a relationship previously observed in Arctic tundra where TFN was tightly coupled to LAI across plant communities (van Wijk et al. 2005; Street et al. 2012). The similarity of this relationship across the stark contrast of mesic prairie and multiple Arctic tundra communities indicates that a correlative relationship between N per unit LAI may occur across a wider range of plant communities and ecosystem types. The coefficient of determination was moderately higher in Arctic tundra (van Wijk et al. 2005), but unlike in the tundra, variability between LAI: TFN did not increase at higher values in tallgrass prairie (Fig. 2a). In addition, the magnitude of LAI and TFN values recorded in tallgrass prairie were nearly double compared to Arctic tundra (Fig. 2a). Although these two systems have varying requirements for canopy growth, similarities among sites suggest a key role of TFN on community development. For tallgrass prairie specifically, we show that allocation of P to canopies can follow a relationship similarly constrained across a wide range of species, landscape treatments, and productivity. Further insight into basic ecological theory may be possible if an emergent relationship between LAI, TFN, and TFP extends to other community types, given the frequent role of N and P as limiting nutrients for many ecosystems (Walker et al. 2014; Fay et al. 2015) and the central role that foliar N and P play in photosynthesis, canopy development, and productivity.

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