Understanding nutrient dynamics in an African savanna: local biotic interactions outweigh a major regional rainfall gradient

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Summary

1. Nutrient availability in terrestrial ecosystems has been found to vary along regional climatic and soil gradients and drive variation in plant community composition and vegetation structure. However, more local biotic feedbacks also affect nutrient availability, but their importance in determining vegetation structure relative to regional drivers is yet unclear.

2. Mesic African savannas form a transition zone between the dry grasslands with relatively low nitrogen availability (indicated by low plant N:P ratios) and the wet woodlands of the continent characterized by relatively low phosphorous availability (high plant N:P ratios). They host strong feedback mechanisms of both vegetation and consumers, where large grazers can create short grazing lawns that alternate over short distances with tall fire-dominated bunch grasslands, and patches dominated by woody species with abundant macrodetritivores. Here, we test if such local biotic interactions can overrule regional, landscape-level drivers of plant nutrient availability.

3. In a South African savanna, we find that plant N:P ratios, N and P resorption efficiencies and proiciencies, were indeed much stronger affected by small-scale biotic-created heterogeneity than by a regional rainfall gradient (530–830 mm yr−1). Furthermore, we observe that differences in N:P ratios between vegetation structural types are not caused by simple differences in inherent plant traits.

4. This suggests that availability of N and P to plants is strongly contingent on the local biotic interplay between different vegetation structural types and within-system feedbacks linked to each vegetation type. The regional rainfall gradient did affect nutrient availability, but mainly through its effect on the relative distribution of the different vegetation structural types thus setting the range of possible outcomes of biotic interactions.

5. Synthesis. Regional and global studies that model savanna carbon and nutrient cycling only on the basis of regional gradients in soil and climatic conditions may insufficiently capture the dominant ecosystem processes involved.

Key-words: biotic feedback, determinants of plant community diversity and structure, ecological stoichiometry, fire, herbivore, Hluhluwe-iMfolozi Park, N:P ratio, nitrogen, nutrient cycling, nutrient limitation, phosphorus

Introduction

The notion that regional differences in environmental conditions determine vegetation and ecosystem structure and functioning has been (and still is) a cornerstone of both classic ecology (Holdridge 1947) and modern earth system studies (Reid et al. 2010). Specifically, the use of environmental gradients across landscapes have shown great utility in identifying key environmental determinants (e.g. nutrient availability, soil moisture, pH, salinity) of community structure (Stephenson & Stephenson 1949; Whittaker & Niering 1975) and for species distribution modelling (Elith et al. 2006). However, new concepts that emerged over the last two decades, such as ecosystem engineering (Jones, Lawton & Shachak 1994), consumer-driven-nutrient cycling (Elser & Urabe 1999), plant-soil feedback (Blomqvist et al. 2000), herbivore-
soil feedback (Veldhuis et al. 2014) and ecological stoichiometry (Sterner & Elser 2002) have changed perspectives towards within-ecosystem feedbacks. Under these concepts, communities actually co-determine local abiotic conditions instead of passively responding to them (Hess & Austin 2014). Such mechanisms raise questions on cause and effect in the correlations between environmental conditions and ecological community structures.

Tropical savanna ecosystems are especially interesting in this context since their structure and functioning is firstly known to respond to major regional environmental gradients of water and soil properties (Bell 1982; Sankaran et al. 2005; Sankaran, Ratnam & Hanan 2008), but also host strong feedback mechanisms involving vegetation processes (Belsky et al. 1989; Scholes & Archer 1997; Holdo & Mack 2014) and consumers like herbivores (McNaughton, Banyikwa & McNaughton 1997; Veldhuis et al. 2014) and fire (Bond & Keeley 2005; Bond, Woodward & Midgley 2005). Furthermore, savannas form the transition zone between tropical drylands, with relatively low nitrogen availability (as indicated by low plant N:P ratios), and tropical rain forests, with relatively low phosphorous availability (high plant N:P ratios) (Reich & Oleksyn 2004; Cech et al. 2008; Hirota et al. 2011). Therefore, the question arises to what extent this transition from low to high foliar N:P ratios linearly follows the rainfall gradient or whether within-ecosystem feedback mechanisms couple nutrient availability from external environmental conditions along (sections of) this rainfall gradient. We investigate the transition in relative nutrient availability across gradients of both rainfall and soil phosphorus in four different vegetation structural types (i.e. grazing lawns, bunch grassland, broad-leaved and fine-leaved woodlands) that have been shown to be associated with different pathways of nutrient cycling and hence nutrient availability to plants.

The availability of nutrients to plants can be studied using plant-based methods, like N:P ratios, foliar nutrient concentrations and resorption efficiencies. Increasing foliar N:P ratios are interpreted as relatively greater availability of N relative to P and vice versa, which has been calibrated using fertilizer application experiments (Koerselman & Meuleman 1996; Aerts & Chapin 2000; Tessier & Raynal 2003; Gusewell 2004). For temperate grasslands and wetlands, an N:P ratio >16 indicates P-limitation on a community level, while an N:P ratio <14 is indicative of N-limitation (Koerselman & Meuleman 1996). Such critical ratios are now well-rooted in the theory of ecological stoichiometry (Sterner & Elser 2002) and have strong experimental support (Sardans, Rivas-Ubach & Penuelas 2012). However, such well-studied thresholds for nutrient limitations in African savanna ecosystems have not yet been established (Gusewell & Koerselman 2002; Cech et al. 2008; Craine, Morrow & Stock 2008), calling for more work on plant stoichiometry in this habitat.

In response to nutrient availability plants show adaptive traits that can be studied to gain insight in the plants ability to conserve nutrients to counteract the high risk of nutrient loss (Berendse, Oudhof & Bol 1987). One of the most important processes of nutrient conservation is nutrient resorption from senescing leaves, storage and subsequent remobilization, that reduces plants dependence on nutrient supply (Chapin & Kedrowski 1983; Aerts 1996; Killingbeck 1996; Aerts & Chapin 2000; Franklin & Agren 2002; McGroddy, Daufresne & Hedin 2004). Resorption efficiency (RE), the proportion of nutrients resorbed from the total amount present prior to senescence, tends to decrease relatively with an increasing amount of green leaf nutrient status (Kobe, Lepczyk & Iyer 2005). Also, plant individuals that grow under nutrient-poor conditions have higher RE (Ralhan & Singh 1987; Killingbeck 1996). In addition, resorption proficiency (RP), the level to which nutrient concentrations are reduced in senesced leaves, has been argued to better reflect resorption success than RE (Killingbeck 1996; Wright & Westoboy 2003). These residual nutrient concentrations in senesced leaves are not calculated using the green leaf nutrient concentrations (as RE) and directly represent the nutrient losses per unit biomass due to leaf abscission.

Here, we investigate the transitions in nutrient availability for plants in African savannas across a rainfall gradient. We first identify relative distributions of four different vegetation structural types across a rainfall gradient to investigate if and how rainfall affected overall plant community structure. We then determine N:P ratios, nutrient resorption efficiencies and proficiencies on seven sites that differ in amount of rainfall as indicators of the relative availability of nutrients. We hypothesize that when rainfall is a direct driver of plant nutrient availability, then N:P ratios would increase with rainfall irrespective of vegetation structural types (Fig. 1a). Alternatively, when local feedback mechanisms mostly determine nutrient availability, we expect that N:P ratios differ between vegetation structural types but do not vary across a rainfall gradient (Fig. 1b). Last, we investigate the possibility of inherent differences between species in optimal N:P ratio in a greenhouse experiment.

Materials and methods

STUDY SITE

The field work for this study was conducted in Hluhluwe-iMfolozi Park (HiP), a 900 km² game reserve in Kwa-Zulu Natal, South-Africa. The altitude of the park increases from 90 m in the South to 580 m in the North. Seasonal mean temperatures range from 13 °C (July) to 35 °C (January). Mean annual rainfall ranges from approximately 500 mm in the South to 900 mm in the North and is positively correlated with altitude. Most rainfall falls between October and March (Skowano et al. 1999; Balfour & Howison 2002). Vegetation structural types range from open grasslands to closed Acacia and broad-leaved woodlands (Whately & Porter 1983). The vegetation is characterized by a high heterogeneity with short grazing lawns ranging between a few square meters to a couple of hectares that alternate with tall grasslands dominated by bunch/tussock-forming species. The park hosts a variety and relatively high biomass of large herbivores (Waldram, Bond & Stock 2008; Cromsigt, Prins & Ollif 2009).
we only sampled individuals that were not growing mixed with other green leaves of an individual were picked (Aerts & Chapin 2000) and broad-leaved (woody areas were classified into fine-leaved and broad-leaved areas using supervised Landsat-TM mapping (Meyer 1999). Similarly, the grass cover was further divided into lawn and bunch grass vegetation maps to reduce noise as a result of edge effects. Completely dominated by either vegetation structural type based on the simulation. Also, for the percentage cover of riverine forest and all grid cells that overlapped with areas within 50 m from the 250 m MOD44B Collection 5 product (Townshend et al. 2011). This product gives percentage canopy cover derived from Moderate Resolution Imaging Spectroradiometer (MODIS) satellite measurement of canopy reflectance. A park wide grid was generated overlaying the MODIS pixels (250 × 250 m) using ArcGIS Desktop 10.2 (ESRI 2011). Grass cover (%) was calculated as 100 – % tree cover. Subsequently, the grass cover was further divided into lawn and bunch grass areas using supervised Landsat-TM mapping (Meyer 1999). Similarly, woody areas were classified into fine-leaved (Acacia, Dichrostachys) and broad-leaved (Celtis, Harpephyllum, Euclea, Olea, Spirostachys, Commiphora, Combretum) dominance based on digitized vegetation maps of woody vegetation communities (Whatley & Porter 1983). The percentages of each vegetation structural type within each grid cell were then calculated using the Spatial Statistics Tools in ArcGIS. We omitted riverine forest and all grid cells that overlapped with areas within 50 m from major rivers to reduce the effect of local water and nutrient accumulation. Also, for the percentage cover of fine-leaved and broad-leaved woody vegetation structural types we only included grid cells that were completely dominated by either vegetation structural type based on the vegetation maps to reduce noise as a result of edge effects.

PLANT COLLECTION AND NUTRIENT CONTENT

We chose seven sites that span the rainfall gradient (see Table S1 in Supporting Information for additional site information), but were relatively similar in soil texture (median particle size: 0.05–0.15 mm), to avoid very clayey or sandy sites, and test between site variations in nutrient limitations. At each site we sampled green leaves in March and the beginning of April 2013 and senesced leaves still attached to the plant from the end of May till June 2013. For each vegetation structural type (lawn grasses, bunch grasses, fine-leaved and broad-leaved woody species) a pooled sample of about 5 g dry weight was sampled for each of the most abundant species by randomly walking through a 50 × 50 m area. For grasses, only the top three intact and green leaves of an individual were picked (Aerts & Chapin 2000) and we only sampled individuals that were not growing mixed with other vegetation structural types. For woody species we only sampled sun-exposed leaves and had no visible signs of herbivory or discoloration. Leaf samples were dried at 70 °C for 48 h in a stove, ground (Foss Cyclotec grinder, 2 mm sieve), stored in small plastic pill bottles and shipped to the University of Groningen. Leaf carbon (% C) and nitrogen (% N) content were estimated using a Bruker near-infrared spectrophotometer (NIR, Ettlingen) using a multivariate calibration of foliar samples measured both on the NIR and CHNS EA1110 elemental analyser (Carlo-Erba Instruments, Milan, Italy). Cross-validation showed that these NIR-predicted C and N concentrations are highly accurate ($R^2 = 95.7$ for N, $R^2 = 92.9$ for C, $N = 1759$). To determine leaf P concentrations, 0.5 g ground leaf sample was destructed with 8 ml 65% HNO3 in a Teflon lined glass tube by pressurized microwave digestion using a CEM discover SPD (CEM Corporation, Matthews, NC, USA). Subsequently, P concentrations were measured using a continuous flow-analyser (Technicon AutoAnalyzer II, Breda, the Netherlands), with molybden and vitamin C colorization at 820 nm.

SOIL EXCHANGEABLE PHOSPHORUS

Soil cores (0–10 cm depth, 5.5 cm diameter) were taken at each site in lawn grass, bunch grass and below woody individuals in triplicate, dried at 105 °C for 48 h and transported to the University of Groningen. Approximately 2.5 g of each ground soil sample was measured out two times and 50 ml of 5% HCl solution was added. Afterwards, the suspension was filtered over a phosphate pore filter and the extract was diluted at least 10 times. Subsequently, P concentrations were measured using a continuous flow-analyser (Technicon AutoAnalyzer II), with molybdenum and vitamin C colorization at 882 nm. These estimates of extractable soil P concentrations were subsequently used as a measure of plant available P.

RAINFALL

Rain gauge data from 17 weather stations across the park were used to create a rainfall map for HiP (mostly on different locations than the study plots), containing rainfall data from 2001 to 2007 (Veldhuis
et al. 2014). For both the vegetation cover estimates and the seven sample sites spatial coordinates were used to extract interpolated estimates of rainfall from the map.

GREENHOUSE EXPERIMENT

Five lawn grass and 11 bunch grass species collected as ramets in HIP were grown in replicates (3–5 specimens per species) for 73 days in a greenhouse experiment at the University of Groningen (see Anderson et al. 2013 for detailed information). At the end of the experiment leaves were harvested, dried at 70 °C for 48 h and ground using similar methods as for the field samples. Foliar total C, N and P concentrations were determined as described above for the field samples.

DATA ANALYSIS

Maximum vegetation cover of the four vegetation structural types were modelled using quantile regression within the “quantreg” package in R (Koenker 2015). We used 100th and 99th conditional quantiles to estimate the upper boundary of percentage cover for each vegetation structural type. We chose for these high quantiles because we had a very high number of data points for this analysis that were largely dominated by low cover estimates, resulting in strong right-skewed frequency distributions. Lower quantiles therefore did not accurately describe the observed patterns on maximum cover, which was the main objective of our analysis. Models contained quadratic terms of annual rainfall, which best reflected the hump shaped patterns of maximum cover across the rainfall gradient.

We calculated the resorption efficiencies of N (NRE) and P (PRE) as

\[ RE = \frac{[\text{nutrient}]_{\text{green}} - [\text{nutrient}]_{\text{removed}}}{[\text{nutrient}]_{\text{green}}} \times 100\% \]

Senesced leaf nutrient concentrations were taken as a measure of resorption efficiencies both for N (NRP) and P (PRP).

For each of the five response variables (N:P ratio, NRE, PRE, NRP and PRP) we constructed two models to test the effect of our three main explanatory variables (vegetation structural type, annual rainfall and soil extractable P) using the “nlme” package in R (Pinheiro et al. 2014). First, a linear mixed effect model (LMM) was fitted with vegetation structural type, amount of rainfall and their interaction as explanatory variables. Subsequently, another LMM was fitted with vegetation structural type, soil extractable P and their interaction as explanatory variables. We subdivided this into two models instead of constructing one model with all three explanatory variables since annual rainfall and soil extractable P were correlated in a complex way (quadratic relationship, see Fig. 4a), complicating interpretation of model results. Mixed effect models were used to deal with spatial pseudo-replication as a result from our nested study design. Therefore, “site” was included as a random factor in all models. Foliar concentrations of senesced leaves (both N and P) were log transformed to meet assumption of normality. For NRE and PRE, variances between vegetation structural types were statistically different and we therefore modelled variances following Zuur et al. (2009). Satterthwaite approximation was used to estimate effective degrees of freedom, commonly used for mixed models (Satterthwaite 1946). We used backward model selection to find the minimum adequate models for each response variable based on AIC values. When the minimum adequate model showed a significant interaction term, we split up the analysis per vegetation structural type to see which vegetation structural types differed in coefficient. Post hoc Tukey’s tests (Tukey 1949) were used to investigate which vegetation structural types differed from each other.

Differences in soil extractable P between vegetation structural types and gradient of annual rainfall were investigated using ANOVA’s with both variables and interaction term included in the full model. Post hoc Tukey’s tests were used to investigate which vegetation structural types and sites differed from each other. ANOVA’s were used to investigate potential differences in foliar N:P ratios between field samples and our greenhouse experiment, with the full model containing location (field or greenhouse) and vegetation structural types (lawn or bunch grassland) and their interaction as explanatory variables. Post hoc Tukey’s tests were used to investigate which grass types and treatments differed from each other. All statistics were performed using R 3.1.1 (R Core Team 2015).

Results

MAXIMUM COVER OF STRUCTURAL TYPES

Our park-wide analysis of maximally possible cover showed distinct peaks for the different vegetation structural types in response to rainfall (Fig. 2, $P < 0.001$ for all 99% quantiles). The quantile regressions showed that bunch grasses were able to dominate over the whole rainfall gradient (493–898 mm yr$^{-1}$). However, the other vegetation structural types showed clear peaks, with maximum lawn grass cover peaking at 595 mm yr$^{-1}$, fine-leaved woody species at 706 mm yr$^{-1}$ and broad-leaved woody species at 769 mm yr$^{-1}$ (based on 100th quantile). Similar patterns were found using 99th or 100th quantiles and therefore observed patterns were qualitatively the same. However, optimal maximum cover of lawn grasses, fine-leaved and broad-leaved woody species shifted to higher rainfall using 99th quantiles (Fig. 2). Furthermore, all four vegetation structural types occur over most of the studied rainfall gradient and we observed large variation in their percentage cover.

N:P RATIOS AND NUTRIENT RESORPTION

We found over sixfold differences in foliar N:P ratios ranging from 4.4 to 27.5. Green leaf N:P ratios differed strongly between all vegetation structural types, but was not affected by rainfall (Table 1, Figs 3 and 5). The lowest N:P ratios were observed for lawn grasses (N:P < 10) while fine-leaved woody species (N:P > 16) had the highest N:P ratios (Fig. 5). Furthermore, we found no significant effect of rainfall on leaf N:P ratios for the vegetation structural types separate models (Table 2). Visual data inspection suggests lower N:P ratios around 650 mm yr$^{-1}$ annual rainfall (Fig. 3), but including a quadratic term for rainfall in the model did not prove significant ($P = 0.09$). This tendency for reduction in N:P ratios is likely caused by higher levels of soil extractable P that peaked at this intermediate amount of annual rainfall of 650 mm yr$^{-1}$ ($F_{1,55} = 22.4, P < 0.001$; Fig. 4a), making plants relatively more N limited under these conditions. Soil extractable P did not differ between vegetation structural types ($F_{2,53} = 2.17$, $P = 0.12$). Furthermore, foliar N:P ratio significantly decreased with soil extractable P (Table 1, Fig. 4b), which was also
was not affected by rainfall or soil P (Table 1, Fig. 5). Grasses resorbed the highest percentage of P (PRE >60%), while PRE of woody species (PRE <45%) was much lower. Overall, mean PRE was higher than NRE for all vegetation structural types.

Senesced leaf N concentrations ranged from 0.61% to 2.43% and were significantly different between vegetation structural types (Table 1, Fig. 5). Bunch grasses showed lowest N concentrations (N < 1%) and highest N concentrations in senesced leaves were observed for fine-leaved woody species (Fig. 5). Furthermore, no significant effects on NRP were found for rainfall. However, our models did show a significant interaction term between vegetation structural type and soil extractable P (Table 1). Closer examination of this interaction reveals that NRP for broad-leaved woody species is significantly affected by soil P (Table 2), while for the other three vegetation structural types this is not the case.

P resorption proficiency (range: 0.02-0.16%) was not affected by rainfall, but did significantly differ between vegetation structural types and sites (Table 1). Senesced leaf P concentrations were lowest for bunch grasses (Fig. 5), while the other vegetation structural types did not significantly differ. We found a significant interaction between soil P and vegetation structural types as was observed for NRP (Table 1). Nevertheless, separate linear mixed effect models per vegetation structural type revealed no significant effects of soil extractable phosphorus on either vegetation structural type.

**GREENHOUSE EXPERIMENT**

In order to see if the differences in foliar stochiometric ratios between vegetation structural types reflected inherent differences between plant species or ecosystem feedbacks we compared the field values of foliar N:P ratios to values from a greenhouse experiment using lawn and bunch grasses. Foliar N:P ratios from grasses in our greenhouse experiment were higher than from our field samples (F1,104 = 88.8, P < 0.001; Fig. 6). Furthermore, N:P ratios of lawn grasses (N: P = 14.6 ± 2.35) were lower than those of bunch grasses (N: P = 16.8 ± 3.67) (F1,104 = 10.5, P < 0.01), mimicking their differences in the field. This is further supported by the absence of a significant interaction term (F1,103 = 0.20, P = 0.65) between location (greenhouse or field) and grass

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**Fig. 2.** Cover of lawn grasslands (a), bunch grasslands (b), fine-leaved woodlands (c) and broad-leaved woodlands in Hluhluwe-iMfolozi Park over the rainfall gradient. Lines represent 100% (black line) and 99% (grey line) quantile regressions using a second order polynomial equation. Estimated maximum cover peaks at 595 mm year⁻¹ for lawn grassland, at 769 mm year⁻¹ for fine-leaved woody vegetation, and at 769 mm year⁻¹ for broad-leaved woody vegetation, while bunch grasslands achieved very high potential abundances at all positions along the rainfall gradient (493–898 mm year⁻¹). The spread in the data shows the strong potential for variation in local abundance of the vegetation structural types irrespective of rainfall.

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**Table 1.** Overall linear mixed effect model results for the effect of vegetation structural type, annual amount of rainfall and soil extractable P and their interaction as explanatory variables for foliar N:P ratios, N and P resorption efficiencies (NRE and PRE) and proficiency (Nsenesced and Psenesced).

<table>
<thead>
<tr>
<th>Vegetation type</th>
<th>N:Pgreen</th>
<th>NRE</th>
<th>PRE</th>
<th>Log Nsenesced</th>
<th>Log Psenesced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall</td>
<td>0.69</td>
<td>&lt;0.001</td>
<td>18.6</td>
<td>&lt;0.001</td>
<td>13.2</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.02</td>
<td>0.02</td>
<td>0.89</td>
<td>0.91</td>
<td>0.38</td>
</tr>
<tr>
<td>Soil P</td>
<td>78.8</td>
<td>&lt;0.001</td>
<td>18.6</td>
<td>&lt;0.001</td>
<td>13.2</td>
</tr>
<tr>
<td>Interaction</td>
<td>0.14</td>
<td>0.03</td>
<td>0.96</td>
<td>0.84</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Bold values represent significant effects at P < 0.05.
improve visual representation.

vent error bars (standard deviations within sites) to overlap and within the same site have been shifted slightly along the x-axis to pre

leaved woody species (FW

Bold values represent signi

strongly suggests that plant community composition, and structural types situated several kilometres away. This

structural types occurring several meters from each other

range of possible vegetation structures. N:P ratios differed

suggesting an important constraining effect of rainfall on the most vegetation structural types along the rainfall gradient,

structural type on nutrient ratios. We found clear peaks for

and determine the relative effects of rainfall and vegetation availability across a rainfall gradient in African savannas

Our main objective was to investigate the transition in nutrient availability across a rainfall gradient in African savannas

Discussion

Our main objective was to investigate the transition in nutrient availability across a rainfall gradient in African savannas and determine the relative effects of rainfall and vegetation structural type on nutrient ratios. We found clear peaks for most vegetation structural types along the rainfall gradient, suggesting an important constraining effect of rainfall on the range of possible vegetation structures. N:P ratios differed strongly between vegetation structural types, whereas annual rainfall did not affect N:P ratios. Thus, distinct vegetation structural types occurring several meters from each other show larger differences in N:P ratios than similar vegetation structural types situated several kilometres away. This strongly suggests that plant community composition, and associated biotic feedbacks (through fire, herbivore, decomposers, etc.), determines the availability of nutrients in our study system, overruling effects of regional rainfall gradient. The results thus suggest that Fig. 1(b) better captures the main patterns of nutrient availability in this system than Fig. 1(a). Last, as N:P ratios of both lawn and bunch grass species converged to ca. N:P = 15 when grown under sufficient nutrient supply (as expected from ecological stoichiometry theory), the observed decreased N:P ratios for grasses in the field likely not reflect inherent traits, but instead reflect differences in local nutrient availability. Overall this suggests that plant nutrient availability is directly linked to plant community composition and associated biotic feedbacks (through herbivores, fire, decomposers) instead of being strongly tied to annual rainfall.

STOICHIOMETRIC DIFFERENCES BETWEEN STRUCTURAL TYPES

In general, N:P ratios of lawn grasses were lowest (mean N: P = 8.2), followed by bunch grasses (mean N:P = 11.0), while

Table 2. Linear mixed effect model results for resorption efficiency (N_{senesced} and P_{senesced}) for the different vegetation structural types as a function of soil extractable P that showed a significant interaction term with vegetation structural type (see Table 1)

<table>
<thead>
<tr>
<th>Soil P</th>
<th>F_{1,3}</th>
<th>P</th>
<th>F_{1,5}</th>
<th>P</th>
<th>F_{1,3}</th>
<th>P</th>
<th>F_{1,5}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N_{senesced}</td>
<td>3.57</td>
<td>0.15</td>
<td>1.95</td>
<td>0.22</td>
<td>6.72</td>
<td>0.08</td>
<td>8.23</td>
<td>0.03</td>
</tr>
<tr>
<td>P_{senesced}</td>
<td>1.04</td>
<td>0.38</td>
<td>4.46</td>
<td>0.08</td>
<td>0.21</td>
<td>0.67</td>
<td>1.56</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Bold values represent significant effects at $P < 0.05$.

Fig. 3. Foliar green leaf N:P ratios across a gradient of annual rainfall for lawn grasses (LG●), bunch grass (BG●), fine-leaved woody species (FW▲, · · ·) and broad-leaved woody species (BW▲, – – –). Data points of different vegetation structural types within the same site have been shifted slightly along the x-axis to prevent error bars (standard deviations within sites) to overlap and improve visual representation.

Fig. 4. (a) The response of soil extractable P across sites spanning a rainfall gradient when sampled in lawn grass (●), bunch grass (●) and patches dominated by woody species (▲). Regression line represents all vegetation structural types including a quadratic term of annual rainfall (LM: $R^2 = 0.27$, $F_{2,63} = 11.7$, $P < 0.001$). (b) Foliar N:P ratios across a gradient of soil extractable phosphorus for lawn grasses (LG●), bunch grass (BG●), fine-leaved woody species (FW▲) and broad-leaved woody species (BW▲). Error bars represent standard deviations within sites. N:P ratios differed significantly between vegetation structural types (LMM: $F_{3,73} = 78.8$, $P < 0.001$) and decreased with soil extractable P ($F_{1,6} = 16.2$, $P < 0.001$). The overall model explained ca. 75% of the variation (Cond. $R^2 = 0.76$, Marg. $R^2 = 0.75$).
woody species showed higher ratios with fine-leaved woody species (mean N:P = 21.0) having higher N:P ratios than broad-leaved woody species (mean N:P = 15.6). These results correspond well with recent studies from South (Ratnam et al. 2008) and East Africa (Cech et al. 2008) and suggest relatively low N availability for grasses, while woody species experience relatively low P availability. Whether these results can be interpreted as strict N or P-limitation is currently still debated since there is no agreement about the actual threshold for savanna ecosystems (Koerselman & Meuleman 1996; Gusewell 2004; Cech et al. 2008; Craine, Morrow & Stock 2008). Independent experimental assessments of the type of nutrient limitation that are insensitive to P immobilization, as done by Olff & Pegtel (1994) for temperate grasslands, are now needed. Nevertheless, the use of N:P ratios as an index of relative N and P availability (as we apply in this study) is generally accepted (Sardans, Rivas-Ubach & Penuelas 2012). Furthermore, our greenhouse study showed that the differences in N:P ratios found in the field cannot be attributed to interspecific differences in optimal N:P stoichiometry, as has been suggested (Craine, Morrow & Stock 2008), where N:P ratios of grasses under sufficient nutrient supply converged to c. 15. Overall our results suggest strong feedback mechanisms on nutrient cycling linked to each vegetation structural type that result in the converged N:P ratios found.

**Feedbacks on nutrient cycling**

In African savannas, most grass biomass is consumed by large herbivores or fire. Large herbivores can increase nutrient availability by speeding up nutrient cycling through dung and urine excretion (McNaughton 1984; McNaughton, Banyikwa & McNaughton 1997). Nevertheless, this positive effect on
nutrient cycling is debated as a significant proportion of N may be lost through NH₃ volatilization and leaching outside the reach of plant roots (Ruess & McNaughton 1988; Frank & Zhang 1997; Augustine 2003). The low N:P ratios found for lawn grasses, of which most production is consumed by large herbivores (Bell 1971; McNaughton 1984), suggest relative low N availability and is in agreement with a recent study of Cech et al. (2008). These authors related the low N:P ratios of grazing lawns to the large amounts of excreta (especially P) received from herbivores. However, we did not find significant differences in soil extractable P between lawn and bunch grasslands. An explanation for this lack of increased soil P in grazing lawns might be attributed to the high numbers of white rhinos in our study area that are known to defecate in middens, resulting in a constant efflux of N and higher N mineralization rates under than away from tree canopies (Holdo, Mack & Arnold 2012; Holdo & Mack 2014). Woody species with high canopies were found to significantly increase soil-P levels, in contrast with low canopy species that experience reduced soil P concentrations (Blaser et al. 2013). A possible explanation for this discrepancy can be found in the effect of canopy height. Woody species with high canopies were found to significantly increase soil-P levels, in contrast with low canopy species that experience reduced soil P concentrations (Blaser et al. 2013).

Trees may act as pumps which bring nutrients from deep soil layers (Campbell et al. 1988) and nutrient enrichment of soils beneath trees can result from animal activity (e.g. defecation) (Dean, Milton & Jeltsch 1999). Both processes are expected to increase with tree size or canopy height. Also, fire did not affect N:P ratios of woody species after 58 years burning experience, where grass N:P and soil N both significantly decreased with recurring fire (Pellegrini et al. 2015). Altogether, this suggests that woody species have much more control on their nutrient budgets and are less affected by herbivores and fire compared to grasses.

Interestingly, whereas we found clear differences between vegetation structural types, N:P ratios did not vary with rainfall. In their meta-analysis, Dohn et al. (2013) did find consistent nutrient enrichment below tree canopies, but this effect was found across a large gradient of rainfall. This apparent overruling effect of vegetation structural type on nutrient availability has large consequences for ecosystem nutrient cycling when the distribution of the vegetation structural types changes within the ecosystem. Indeed, woody encroachment generally increases nutrient availabilities (Blaser et al. 2014), reduces N and P fluxes, and increases N-fixation rates, in a way that indicates a shift towards more P-limiting conditions (Sitters, Edwards & Venterink 2013; Sitters et al. 2015). Furthermore, the occurrence of woody encroachment generally increases with rainfall (Eldridge et al. 2011), as would be expected from the maximum cover distributions of the vegetation structural types in our study. Therefore, although the direct effect of rainfall on nutrient availability seems negligible, a clear indirect effect is present.

Fig. 7. Schematic overview of the relationship between regional environmental conditions (e.g., rainfall, or parent material P availability) and local conditions (as soil moisture, soil P availability). Solid thick line represents the expected local conditions when they correlate directly with regional environmental conditions (external driven). Dashed lines represent the maximum and minimum values for local conditions with corresponding regional environmental conditions. The suggested impact of within-system feedback mechanisms to change local conditions is highest at intermediate ranges along the environmental gradient (arrows). The x-axis represents a regional rainfall gradient in our study, with nutrient availability as possible local condition on the y-axis. The grey area represents savanna ecosystems with adjoining tropical drylands (dry end) and tropical forests (wet end).
els (DGVMs; but see Pachzelt et al. 2013), although the fundamental importance of large herbivores for savanna ecosystem functioning is widely recognized. This may explain why DGVM’s are generally poor predictors of savanna ecosystem structure and functioning (Bonan et al. 2003; Hely et al. 2006; Hickler et al. 2006; Sato, Itoh & Kohyama 2007), although improvements are made (Scheiter & Higgins 2009).

We suggest that our results reflect a more general principle, where ecosystems at intermediate levels of large-scale environmental gradients are relatively strongly subject to within-ecosystem feedbacks, whereas ecosystems at both ends of the gradient are more externally driven by these gradients (Fig. 7). For example, at low rainfall (tropical drylands, low foliar N:P ratios) and high rainfall (tropical forests, high foliar N:P ratios), the local availability of nutrients is relatively strongly determined by the regionally imposed conditions, and are thus predictable from these large-scale environmental gradients. However, at intermediate rainfall (savanna ecosystems) strong within-system feedback mechanisms operate and patches with large differences in N and P availability can be found several meters distance from each other (this study). This results in strong internal driven ecosystems, where nutrient dynamics are generally unpredictable from the regional conditions.

Conclusion

Overall, our study shows that stoichiometric ratios and nutrient resorption in African savannas are strongly different between vegetation structural types on a rather short spatial scale, irrespective of the amount of annual rainfall. Therefore, we conclude that regional variation in rainfall does not directly affect nutrient availability in African savannas, but indirectly instead, by affecting abundance distributions of the vegetation structural types. Generalizing, this suggests that nutrient dynamics at intermediate ranges of rainfall are more determined by local ecosystem organization instead of being imposed by regional conditions. Therefore, changes in local dominance from one vegetation structural type to another, especially from grasses to woody species (i.e. bush encroachment) or vice versa, might suddenly cause a large change in the relative availability of nutrients. These local interactions and within-system feedback mechanisms are essential to incorporate into regional and global modelling approaches to successfully predict future changes in nutrient dynamics.

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Data accessibility

Data deposited in the Dryad repository:

body mass and digestive strategy. Diversity and Distributions, 15, 513–522.


Local interactions determine nutrient availability


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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the seven sites.

Fig. S1. Foliar N:P ratios per grass species for both the greenhouse experiment (upper panel) and the field samples (lower panel).