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Identifying Long Term Patterns and Drivers of Vegetation Structure in an African Savanna using Stable Carbon and Nitrogen Isotopes



Image: Grassland and Scarp Forest at Rubbish Dump site near Phindisweni Gate, Hluhluwe-iMfolozi Park

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Abstract:

Savanna systems are complex and dynamic in space and time. Climate, fire, herbivory and nutrients have been identified as structuring agents of savanna form and function, but their interactions and feedbacks with one another and vegetation are poorly resolved. Increasing the spatial and temporal scope of studies will help to improve this situation, as demonstrated in recent studies in the spatial dimension in particular. This study aims to investigate vegetation and Nitrogen cycling changes over time in a diverse patch mosaic landscape in Hluhluwe-iMfolozi Park to identify drivers of vegetation structure and their dynamism over time.

Sediment from a 150cm core (taken using a Russian corer) was analyzed for stable ^{13}C and ^{15}N isotope abundances, and C: N ratio of soil organic matter. The base of the core was dated at 2380 ± 40 cal. Yr. BP. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and C: N of soil organic matter was found to be variable over time. $\delta^{13}\text{C}$ followed a pattern of stable periods of distinct abundance separated by abrupt changes; $\delta^{15}\text{N}$ and C: N underwent changes over the same periods as $\delta^{13}\text{C}$.

Vegetation follows a pattern of phase and transition as predicted by resilience theory. An aquatic vegetation phase persists around 2000 cal. Yr. BP to about 500 cal. Yr. BP, coinciding with a warm, wet period (including the Medieval Warm Period) with an open Nitrogen cycle. A C_4 grassland phase follows after a transition to cool, dry conditions coinciding with the Little Ice Age, and decreasing openness of the N cycle. Recent increasing C_3 vegetation and N-openness were attributed to atmospheric CO_2 increase and Nitrogen deposition respectively. ^{TOC data} Climate is concluded to be the major driver of vegetation at this site, and a combination of climate and vegetation are responsible for changes in Nitrogen availability. Findings are discussed in relation to landscape management. Multi-proxy evidence in future studies would be useful in validating the findings of this study.

Key words: Savanna; isotopes; vegetation change; Nitrogen; climate; Little Ice Age; Hluhluwe-iMfolozi; resilience theory.

Introduction:

Dynamism and complexity characterize savanna system structure and their interactions with biotic and abiotic modifiers (Walker 1987, Backeus 1992). The savanna biome is the most extensive of all across the globe, dominating the tropics in particular (Marchant 2010); in Africa and Australia, more than half the land surface comprises savanna, while 45% of South America and 10% of India and South-East Asia exist as savanna (Werner 1991). This extent reflects the variability contained in the definition of savannas, which is broadly a continuous grass layer punctuated by discontinuous tree cover under seasonal rainfall (Scholes and Archer 1997). Several sub-classifications of savannas have been derived from this definition, based on tree cover extent and arrangement, for example 'savanna woodland' and 'savanna grassland' (Scholes and Archer 1997). At the landscape scale, these can occur in a patch mosaic pattern, and exhibit variability in their relative proportions spatially and temporally (Wiegand *et al.* 2006, Gillson 2004). Aside from their extent, these environments are important from an economic and social point of view, as they support significant proportions of the world's livestock and wildlife rangelands and crops (Scholes and Archer 1997), and have hosted increasing human populations for millennia (Scholes and Archer 1997, Marchant 2010). The inherent relationship between savanna structure and function (Breshears and Barnes 1999) and the sensitivity of savanna structure to changes in climate and anthropogenic activity make the quest to resolve the mechanisms of tree-grass co-existence and determinants of tree density an urgent one (Sankaran *et al.* 2005, Sankaran *et al.* 2008).

The large body of research undertaken in this quest has provided little resolution in the understanding of the complexities, interactions and feedbacks of drivers of savanna structure (Scholes and Archer 1997, Sankaran *et al.* 2004, Bond 2008). Site specific studies have isolated resource-competition based mechanisms, where, for example, water or nutrients are limiting, and disturbance-based mechanisms, where disturbances such as fire and herbivory enforce demographic bottlenecks on tree populations (Sankaran *et al.* 2004, Bond, 2008). Site specificity of studies has led to contradictory findings and has greatly complicated this issue, as different patches are limited in woody cover by different vegetation determinants (Wiegand *et al.* 2005, Sankaran *et al.* 2004). Alternative hypotheses have also been generated when in fact they are not mutually exclusive drivers, but rather form part of processes that occur on different scales, nested in a hierarchical fashion (Gillson 2004). Recently, attempts have been made to overcome this problem by considering large scale environmental gradients, and the variation of drivers of savanna structure along these gradients. For example, Sankaran *et al.* 2005, 2008 found that fire, herbivory, and nutrients varied in their influence in maintaining tree density below a rainfall-predicted maximum. Other studies focus on the variation of a single driver along an environmental gradient to investigate the spatial variation in its structuring influence. For example, Aranibar *et al.*

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(2008) investigated Nitrogen cycling along land use intensity and aridity gradients in southern Africa. The conclusions of this study were that the influence of land use intensity and mean annual precipitation (MAP) were not uniform on Nitrogen mineralization and nitrification rates; furthermore, the most arid site yielded a reversal in the trend of increasing $\delta^{15}\text{N}$ signal with increasing land use intensity. Wang *et al.* (2010) conducted a similar study, investigating foliar and soil $\delta^{15}\text{N}$ during wet and dry seasons, along the Kalahari Transect, concluding that trees and grasses potentially specialize in acquisition of different resources (water and nitrogen respectively) which vary along the MAP gradient. This recent expansion in spatial perspective to isolate structuring agents has not been witnessed to the same extent in the temporal dimension, yet palaeoecological studies are useful in identifying the multitude of heterogeneity drivers operating in different patches over time, isolating ecosystem thresholds, understanding ecosystem resilience, and revealing the influence of humans on landscapes, all of which greatly inform management practice (Gillson 2009, Gillson and Ekblom 2009b).

Long term vegetation studies in African savannas have tended towards reconstructions of past landscapes, and evaluations of the role of large scale influences such as climate in these changes. For example, fossil soils (Kastanozems) were used by Eitel *et al.* (2002) to infer a more open savanna predecessor to the present scrub vegetation in northern Namibia, implying Holocene climatic change. Phytoliths permitted the identification of short grass open savanna and tall grass wooded savanna phases, and tree/shrub density in Senegal (Alexandre *et al.* 1997); from this record, late Holocene moisture was inferred. Fossil pollen analysis has provided high taxonomical resolution reconstructions in East Africa, permitting speculation on early human influence on vegetation (Lamb *et al.* 2003) and Kruger National Park, South Africa, where woody cover was the focus (Gillson and Duffin 2007). Recent work is improving insight into savanna disturbance agents such as fire and herbivory; for example, Duffin *et al.* (2008) validated the sensitivity and scope of the charcoal record as a proxy for fire characteristics, and Ekblom and Gillson (2010) generated past cattle and wildlife abundance from coprophilous fungal spores, concluding that herbivory likely only contributes to landscape patchiness and is not a primary engineer of vegetation at a local scale. Stable isotope analysis is another technique used to interrogate drivers of vegetation change in savannas, and forms the focus of this study into the role of Nitrogen in temporal vegetation dynamics in Hluhluwe-iMfolozi Park (HiP), South Africa.

Naturally occurring processes and ecosystem transformations discriminate against heavy isotopes of elements such as Carbon, Oxygen, and Nitrogen (to name a few); thus the natural abundance (ratio of heavy to normal isotope compared to a standard-denoted δ) of these heavy isotopes represent signatures of conditions under which they were deposited, or act as indicators of ecosystem transformations (Griffiths 1998). Different photosynthetic pathways (C_3 , C_4 , and CAM) discriminate

against the heavy stable Carbon isotope ^{13}C to varying extents (Boutton *et al.* 1998), and consequently have different $\delta^{13}\text{C}$ signatures (ranges -32 to -22‰, -17 to -9‰, and -30 to -10‰ respectively) (Boutton *et al.* 1998). Via carbon cycling processes, the $\delta^{13}\text{C}$ of soil carbon captures the contribution to net primary productivity of the different photosynthetic pathways (Andreux *et al.* 1990, Boutton 1996). Thus, the soil profile lends itself to chronologies of the productivity contribution of different plant communities by assessment of soil organic matter $\delta^{13}\text{C}$ at different depths (Boutton *et al.* 1998). The Nitrogen cycle has several transformation processes (nitrification, denitrification, volatilization, mineralization, and immobilization) during which differential discrimination takes place, complicating the interpretation of stable isotope (^{15}N) abundance (Hopkins *et al.* 1998). Soil $\delta^{15}\text{N}$ is an indicator of the extent to which Nitrogen is retained within a cycle relative to the amount lost, or the 'openness' of the Nitrogen cycle (Handley *et al.* 1999). Again, long term changes can be investigated by analyzing $\delta^{15}\text{N}$ of soil organic matter at different depths. Isotopes aside, the ratio of Carbon to Nitrogen in lacustrine organic matter is a good indicator of whether the sediment was derived *in situ* or originates from the surrounding matrix (Talbot and Laerdal 2000); aquatic and terrestrial vegetation, and vegetation with different photosynthetic pathways have different typical C:N ranges (Meyers 1994). For example, Lamb *et al.* (2004) examined $\delta^{13}\text{C}$ and C:N of lacustrine organic matter from Lake Tilo in Ethiopia to track vegetation changes in the surrounding savanna over the last 8840 ^{14}C -years; once again, an aim of the study was to infer climatic change too. Throughout the core, C: N was used to gauge the relative input to organic matter of aquatic and higher plants, and compared with $\delta^{13}\text{C}_{\text{organic}}$ to assess the relative contribution trees (C_3), grasses (C_4), and aquatic plants (C_3). This study benefitted from insights obtained by other studies using several different proxies, including pollen, diatoms, and stable oxygen isotopes. $\delta^{13}\text{C}$ analysis of a core taken from a small lake in Kruger National Park, South Africa allowed Gillson and Ekblom (2009a) to assess the changing dominance of C_4 grasses and C_3 trees in the savanna landscape. Vegetation dynamics were interpreted in light of openness of the Nitrogen cycle and terrestrial Nitrogen availability inferred from $\delta^{15}\text{N}$ of core sediment. It was concluded that vegetation change fitted a phase and transition pattern explained by resilience theory; nitrogen and herbivory limited grasslands switched to more wooded environments when Nitrogen availability and openness breached a threshold (1.8‰), after which the ecosystem is maintained by fire and herbivory imposing a demographic bottleneck at the tree recruitment phase. This study benefitted from charcoal analysis to assess the role of fire in response to vegetation change, and its role in the Nitrogen cycle; pollen analysis permitted a high resolution perspective to identify vegetation changes and transitions.

Resilience theory is based upon ecological concepts of persistence of stable states, thresholds, and resilience (Groffman *et al.* 2006, Gillson and Ekblom 2009a). Stable vegetation phases persist according to their ability to withstand variability in their structuring forces (resilience), until variability breaches a critical point (threshold) upon which a rapid ecosystem reorganization (transition) takes place (Gillson and Ekblom 2009a). Thresholds may be breached by dramatic, rapid disturbances, or by gradual cumulative effects that exceed the self-regulatory feedback mechanisms maintaining stability (Gillson 2009a). Temporal changes in savannas fit this model well, although transitions between woodland and grassland phases have different drivers; both changes and drivers vary over spatial scale (Gillson and Ekblom 2009a). This study aims to create a chronology of local scale vegetation change in Hluhluwe-iMfolozi Park. Using stable isotopes, the interplay between vegetation change and Nitrogen turnover and availability will be assessed to gauge the role of nutrients over time as a limitation to tree density. It is hoped that this study will provide a useful perspective on recent global phenomena such as woody encroachment, as observed in HiP and surrounds by Wigley *et al.* (2009, 2010), and Nitrogen deposition, and be of use in informing Park management policy.

It is predicted that the $\delta^{13}\text{C}$ signal will show changes in vegetation community over time, and that this change will follow a pattern defined by resilience theory. It is anticipated that the shift from C_4 grass-dominated vegetation to a C_3 -dominated system exposed by West *et al.* (2000) will be reflected in the $\delta^{13}\text{C}$ profile. Periods of C_4 prominence are expected to coincide with episodes of warmer, drier climatic conditions, reflected in the $\delta^{15}\text{N}$ signal as periods of lower Nitrogen turnover and availability, while conditions of higher Nitrogen turnover are expected to coincide with stronger C_3 vegetation influence. The hypothesis being tested in this study is the premise that the study site savanna landscape is dynamic in space and time, and that Nitrogen availability and turnover varies in its influence in structuring different savanna communities.

Study Site:

Coring took place at a wetland at Phindisweni, near Memorial Gate of Hluhluwe-iMfolozi Park, kwa-Zulu Natal (KZN) Province of South Africa. The site is situated in a hilly landscape between the coastal plain to the East, and Great Escarpment to the West. Altitude in the Park ranges from 40m-75m above sea level (Archibald *et al.* 2005); precipitation increases with rainfall in general (Balfour and Howison 2002) and ranges from around 600mm to around 1000mm per annum (Archibald *et al.* 2005). Doleritic intrusions occur extensively within the sandstone and shale formations from which soils in the area are formed (King 1970, Graham 1992).

The Hluhluwe landscape comprises of 6 vegetation types according to Mucina *et al.* (2005). Northern Zululand Sourveld and Zululand Lowveld savanna are the dominant form, with Zululand Coastal Thornveld Savanna evident in some of the eastern locations. Scarp Forest occupy high rainfall slopes, while Lowveld Riverine Forest is found on the river flats. Subtropical Freshwater Wetland vegetation occurs on wetlands such as the coring site at Phindisweni. Within the savanna landscapes, heterogeneity in tree and grass density and composition result in a patch mosaic of grazing lawns, grasslands, and thicket (Gillson 2009, unpublished).

Materials and Methods:

Core and Chronology:

Coring was conducted using a Russian Corer. The core was radio carbon dated at 2380 ± 40 calibrated years before present (cal. Yr. BP) at 148cm, and at 590 ± 30 cal. Yr. BP at 100cm, confirming the chronological sequence of deposition. OxCal Version 3.10 was used to calibrate the radio carbon dates.

Stable Isotope Analysis

Sample preparation: $\delta^{13}C$

Twelve depths (2cm, 14cm, 28cm, 40cm, 54cm, 66cm, 80cm, 92cm, 106cm, 118cm, 134cm, and 150cm) from throughout the core were selected to test whether removal of carbonates by acid washing of samples was necessary. A portion of these samples was transferred to a test tube using a metal spatula, and a small amount of 1M HCl was added. The mixture was whirlimixed, topped up with 30cm³ 1M HCl, and left overnight in an inactive oven to avoid contamination by 'Carbon rain'. Roots floating on the surface were discarded, and the test tube decanted. Flushing with miliquot water, whirlimixing and decanting using miliquot water followed, and this step repeated. Decanted sub-samples were left to dry in an oven at 40°C overnight or until dry. Once dry, sub-samples were ground to heterogeneity using a marble pestle and mortar, and all roots removed using metal forceps. The root-free fine particles were then transferred to an eppendorf tube, and stored at 1°C.

A portion of non-acid washed samples was transferred to test tubes with a metal spatula, and the test tubes placed directly into an oven at 40°C overnight or until dry. Once dry, sub-samples were ground, roots removed, and stored in the same manner as the acid washed samples.

After confirmation that acid washing was not necessary by comparison of $\delta^{13}\text{C}$ of acid washed and non-acid washed samples (carbonates did not significantly affect $\delta^{13}\text{C}$), the remaining depth samples were prepared as above, without the addition of 1M HCl and flushing with miliquot water.

Sample preparation: Total Nitrogen and $\delta^{15}\text{N}$

The same procedure as preparation for the unwashed $\delta^{13}\text{C}$ samples was followed in preparing sub-samples for $\delta^{15}\text{N}$ analysis.

In preparation of samples for both analyses, instruments (pestle, mortar, spatula, forceps, and test tubes) were flushed five times with de-ionised water after contact with each different sub-sample.

Mass Spectrometry

From the eppendorf tubes, a portion of the prepped sub-sample of each depth sample was weighed into tin caps following recommended weights derived from the acid washing test, and previous Hluhluwe soil samples. Tin caps were squashed to enclose contents, and were combusted in a Flash EA 1112 series elemental analyzer (Thermo Finnigan, Milan, Italy). The gases were passed to a Delta Plus XP IRMS (isotope ratio mass spectrometer) (Thermo electron, Bremen, Germany), via a Conflo III gas control unit (Thermo Finnigan, Milan, Italy).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values obtained were corrected using sucrose (from Australian National University), Merck gel (produced by Merck), and lentils. These standards have been calibrated against International Atomic Energy Agency standards. Carbon values obtained are relative to Pee-Dee Belemnite, and Nitrogen values relative to atmospheric N_2 . Carbon and Nitrogen stable isotopes expressed in δ notation as ‰ according to the following equation:

$$\text{Isotope abundance (‰)} = \frac{(R_{\text{sample}})}{(R_{\text{standard}} - 1)} \times 1000$$

Analysis:

STATISTICA Version 9.0 (StatSoft 2009) was used to perform Multiple Regression analysis, and Microsoft Excel (2007) to plot graphs.

- Where were the soils collected & why?
- along a transect?
- different vegetation types
- How was the hypothesis(es) achieved?

Results:

$\delta^{13}\text{C}$:

The sequence shows considerable change in the sediment $\delta^{13}\text{C}$ signal over time, with the mean of the entire sequence -19.58‰ , and standard deviation 2.83; values range from -23.97‰ to -14.07‰ (Figure 1). There are three relatively stable phases separated by four transition episodes (Figure 6). The deepest section of the core (150cm-136cm) reflects a transition from -19.96‰ (150cm) (hereafter 'transition 1') to a very stable phase of low $\delta^{13}\text{C}$ (from 136cm-94cm) (mean -22.18‰) (hereafter 'phase 1'). This phase contains the lowest $\delta^{13}\text{C}$ value of the entire sequence (-23.97‰ at 136cm). A slight trend of ^{13}C enrichment is evident across this phase to -20.82‰ at 94cm, with very little variation apparent (standard deviation 0.90). At 94cm-90cm, a sharp transition occurs (hereafter 'transition 2'), and $\delta^{13}\text{C}$ increases considerably to -15.35‰ at 90cm to a period of greater ^{13}C enrichment (hereafter 'phase 2'). This period of high $\delta^{13}\text{C}$ (mean -16.22‰) contains the sequence's highest level (14.07‰ at 54cm) and exhibits more variability than the previous stable phase (standard deviation 1.13). An equally dramatic transition concludes this phase at 40cm (hereafter 'transition 3'), during which $\delta^{13}\text{C}$ decreases sharply from -16.05‰ to -21.97‰ at 36cm. The most recent phase of stability is a relatively short, stable period (standard deviation 0.86) from 36cm-18cm with a mean value of -20.16‰ (hereafter 'phase 3'); a slight increasing $\delta^{13}\text{C}$ trend is apparent throughout to -18.70‰ at 18cm. The period of time represented by depth interval 2cm-18cm is the final transition period (hereafter 'transition 4'), during which the sediment $\delta^{13}\text{C}$ signal decreases to a point comparable with the lowest in the sequence (-23.89‰ at 4cm).

The Carbon to Nitrogen ratio (C: N) remains the lowest in the core from 136cm-94cm (mean 11.82, standard deviation 0.24) (Figure 1). An increase occurs from 94cm-78cm (11.87 to 22.24) to attain the period of highest C: N in the sequence. The depth interval from 90cm-46cm has a high C: N ratio of the sediment (mean 18.26, standard deviation 2.74) and several points in this variable section of the C: N plot reach a ratio of >20 . A rapid decline occurs from 46cm-38cm from 18.01 to 13.52, followed by a short period of fluctuation around a lower mean. Over depths 36cm-18cm, C: N has a mean of 16.28 (standard deviation 1.82), higher than depth interval 136cm-94cm where the C: N has a mean of only 11.82. A slight trend of increase in C: N over time is evident over this phase, culminating in a ratio of 20.17 at 18cm. Although C: N declines to 14.29 at 6cm, the most recent substrates for which C: N is available (4cm-6cm) show a steep increase in C: N to 17.50.

Handwritten notes:
C: N plot
The C: N ratio
is the highest
at 54cm
and lowest
at 136cm

Over the whole core, $\delta^{13}\text{C}$ and C: N show a positive relationship ($m= 0.60$, $R^2= 0.510$). $\delta^{13}\text{C}$ transition 1 and phase 1 correspond tightly to the deepest C: N signal of the sediment, both undergoing a decline from 148cm-136cm (Figure 3), then a period of stability from 136cm-94cm. The slight increasing trend observed in $\delta^{13}\text{C}$ over this episode is not reflected in the C: N trend ($R^2= 0.09$). $\delta^{13}\text{C}$ transition 2 is matched in the C: N sediment by a sharp increase (Figure 4); whereas the stable Carbon isotope abundance levels off after 90cm, the C: N continues to increase until 78cm before stabilizing. Although the $\delta^{13}\text{C}$ signal declines (via transition 3 and 4, and phase 3) following this period to eventually reach the level of that found in phase 1 and is matched in pattern by C: N, the C: N ratio remains consistently higher than its $\delta^{13}\text{C}$ phase 1 state (minimum over this interval is 13.52 at 38cm. Transitions 3 and 4 are reflected in the C: N ratio as abrupt events, although transition 3 correlates stronger ($R^2= 0.475$ -see Figure 5- compared to 0.300) with $\delta^{13}\text{C}$.

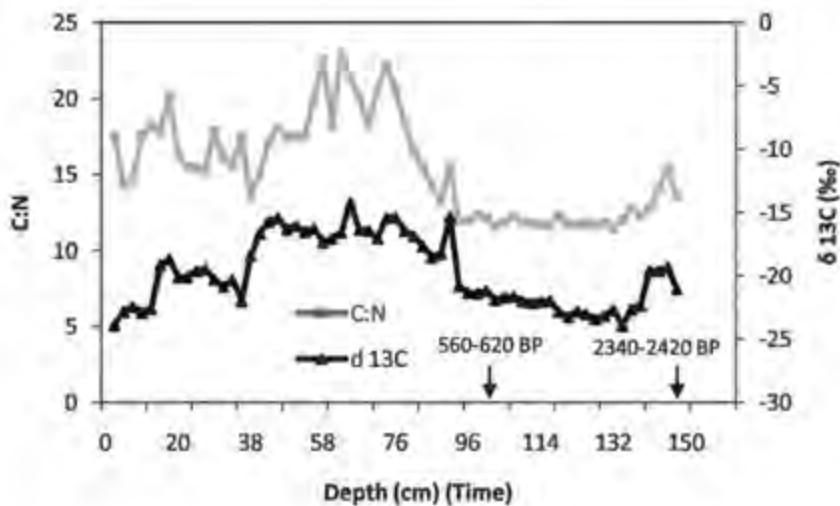


Figure 1: Sediment Carbon:Nitrogen ratio and $\delta^{13}\text{C}$ over time from Phindisweni core, Hluhluwe-iMfolozi Park.

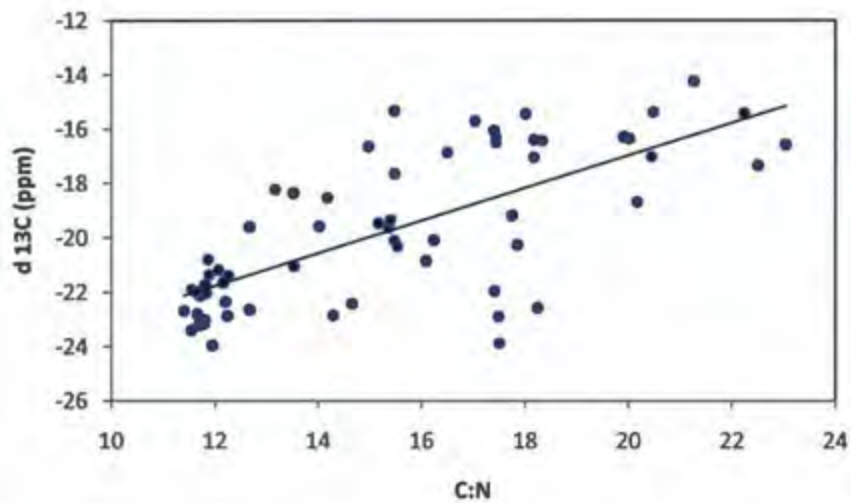
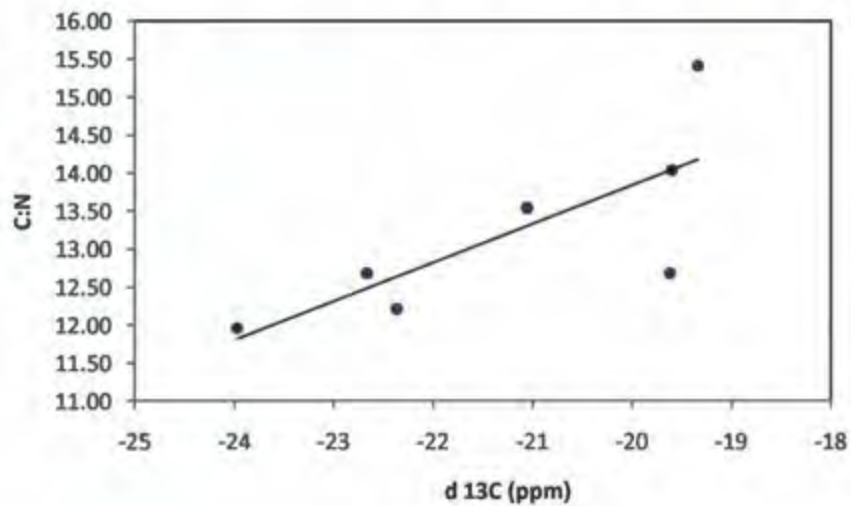


Figure 2: Sediment $\delta^{13}\text{C}$ against C: N over entire core taken at Phindisweni, Hluhluwe-iMfolozi Park. $R^2 = 0.510$, $m = 0.60$. Sediment dated near base (148cm) at 2340-2420cal. Yr. BP.



Why are the axes swapped?

Figure 3: Stable Carbon isotope abundance against C: N ratio of sediment over transition 1 (148cm-136cm) from core taken at Phindisweni, Hluhluwe-iMfolozi. $R^2 = 0.58$, $m = 0.051$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

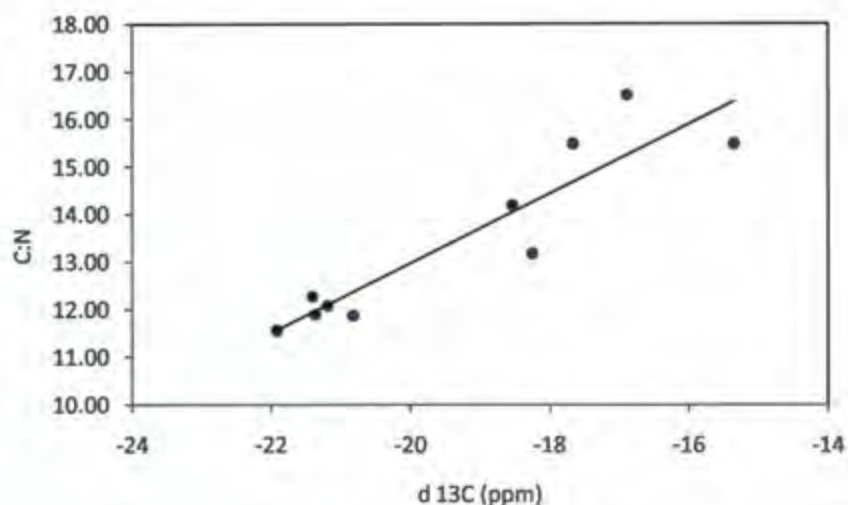


Figure 4: Stable Carbon isotope abundance against C: N ratio of sediment over depth interval 102cm-82cm, including transition 2 (96cm-90cm) from core taken at Phindisweni, Hluhluwe-iMfolozi. $R^2=0.848$, $m=0.622$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

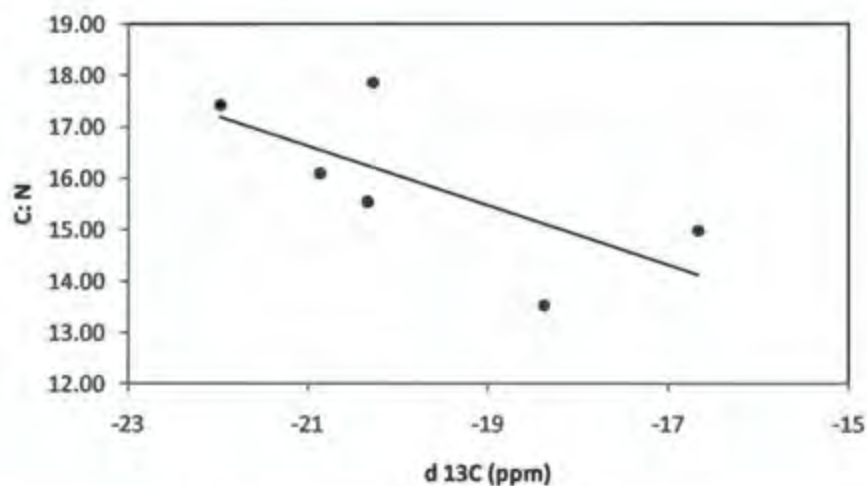


Figure 5: Stable Carbon isotope abundance against C: N ratio of sediment over depth interval 42cm-30cm, including transition 3 (42cm-36cm), from core taken at Phindisweni, Hluhluwe-iMfolozi. $R^2=0.475$, $m=-0.556$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

$\delta^{15}\text{N}$:

The $\delta^{15}\text{N}$ signal of the sequence, like the $\delta^{13}\text{C}$ signal, displays variation with depth (time) (Figure 6). The core mean is 3.72‰ and standard deviation is 2.04; values range from -0.08‰ to 6.80‰. Only two stable phases can be identified, delineated by three dramatic transition episodes. The earliest (deepest) transition occurs at the base of the core, where $\delta^{15}\text{N}$ decreases steeply from 4.18‰ to 0.55‰ between 150cm-142cm, before rapidly increasing to 6.34‰ at 134cm. This increase takes place from one of the sequence's lowest values to one of the highest. From 88cm-134cm, a stable phase occurs at the high end (mean 5.91‰ standard deviation 0.35) of the $\delta^{15}\text{N}$ gradient including the highest value throughout the core (6.80‰ at 92cm). A protracted decline follows from 36cm-88cm, over which $\delta^{15}\text{N}$ declines from 6.60‰ at 88cm to the lowest point in the sequence (-0.08‰ at 36cm) in an erratic, fluctuating manner. Following this decline, a short period of stability (16cm-36cm) fluctuates around a mean of 1.05‰ (standard deviation 0.49). The depth interval from 2cm-18cm resembles a transition period of sharply increasing $\delta^{15}\text{N}$ from 0.98‰ to 3.65‰ at 2cm.

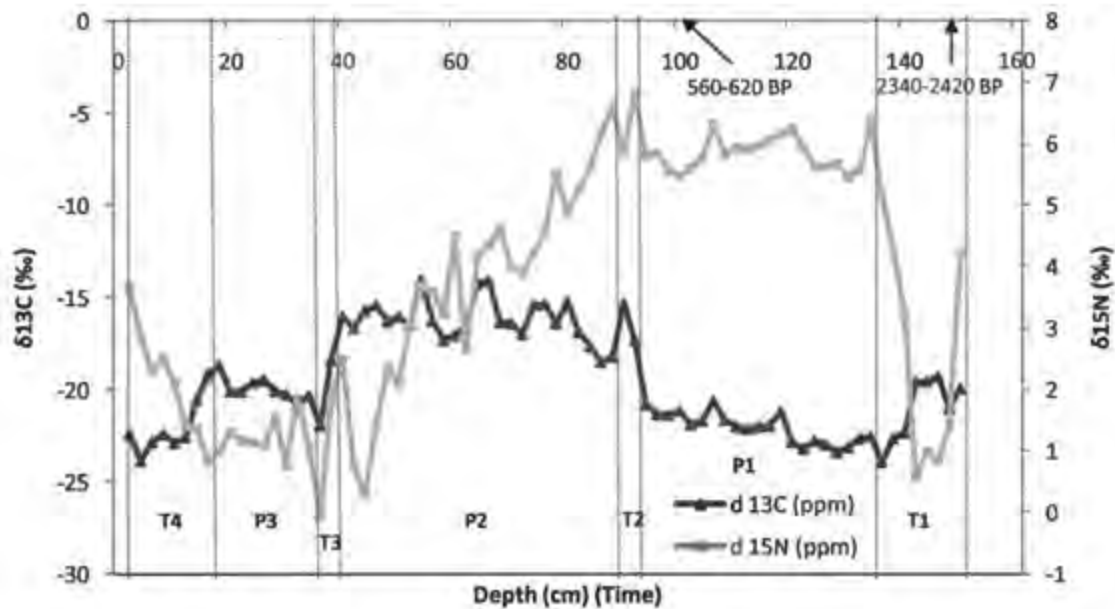


Figure 6: Sediment stable Carbon and Nitrogen isotope abundances from core taken at Phindisweni, Hluhluwe-iMfolozi Park. Sediment at 148cm Carbon dated to 2340-2420cal. Yr. BP. T1= transition 1, P1= phase 1, T2= transition 2, P2= phase 2, T3= transition 3, P3= phase 3, T4= transition 4 (see text for details).

The relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is not uniform in its magnitude or direction over the 2380 ± 40 years represented by the core (see Table 1). Overall, $\delta^{13}\text{C}$ decreases with increasing $\delta^{15}\text{N}$, although this relationship is weak. Increasing Nitrogen turnover rate accounts for 61.7% of the variability of declining $\delta^{13}\text{C}$ in transition 1 (Figure 7), while the next transition (transition 2) shows a very weak correlation between the two. Phase 2 unsurprisingly shows almost no relationship between Carbon and Nitrogen stable isotope ratios, as Carbon remains relatively constant while Nitrogen undergoes a pronounced decline from 6.60‰ to -0.08‰. Notably, phases 1 and 3 have low $\delta^{13}\text{C}$ values ($\sim -22\text{‰}$ and $\sim -20\text{‰}$ respectively) and are associated with very different $\delta^{15}\text{N}$ values (-6‰ and -1‰ respectively); the fact that low $\delta^{13}\text{C}$ phases are associated with stable $\delta^{15}\text{N}$ phases, while the higher $\delta^{13}\text{C}$ phase occurs against a period of change (decline) in $\delta^{15}\text{N}$ is also notable. None of the stable ^{13}C abundance phases correlate strongly with $\delta^{15}\text{N}$. In addition to transition 1, transitions 3 and 4 exhibit strong correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Figures 8 and 9 respectively). The changes at transitions 1 and 4 (the only relationships significant at the 5% level- see Table 1) are strikingly similar in several ways; in both scenarios, $\delta^{15}\text{N}$ declines to a similar level ($\sim -0.1\text{‰}$) at which $\delta^{13}\text{C}$ in both depths ceases to decline at a similar level ($\sim -20\text{‰}$). Subsequent increases in $\delta^{15}\text{N}$ are matched by further declines in $\delta^{13}\text{C}$ to $\sim -23\text{‰}$, although in transition 1 this level is attained at higher $\delta^{15}\text{N}$ values.

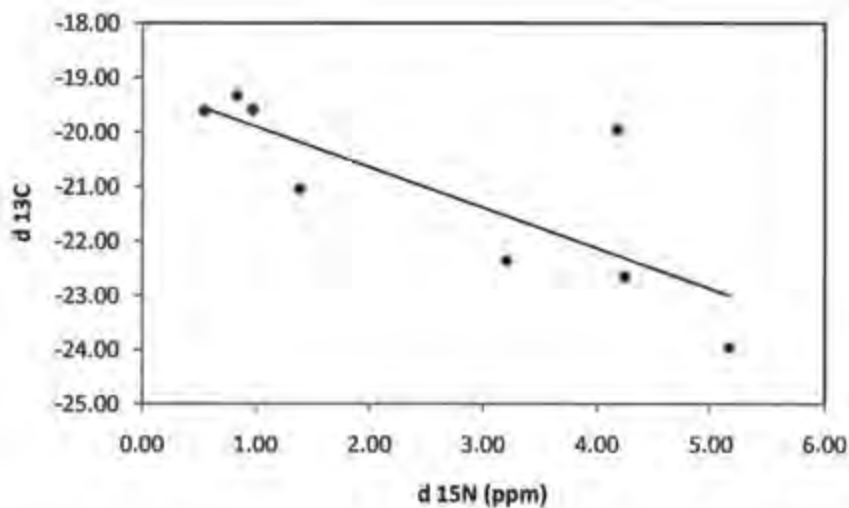


Figure 7: $\delta^{13}\text{C}$ against $\delta^{15}\text{N}$ over transition 1 (150cm-136cm) in core from Phindisweni. $R^2 = 0.617$, ($p < 0.05$) $m = -0.74$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

Transition 3 occurs as $\delta^{13}\text{C}$ declines from 16.05‰ to -21.97‰, over which $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ correlate quite strongly; although $\delta^{15}\text{N}$ is fluctuating considerably during this transition, the amount by which it changes as vegetation 'switches' is very little (0.28‰ to -0.08‰) (Figure 7).

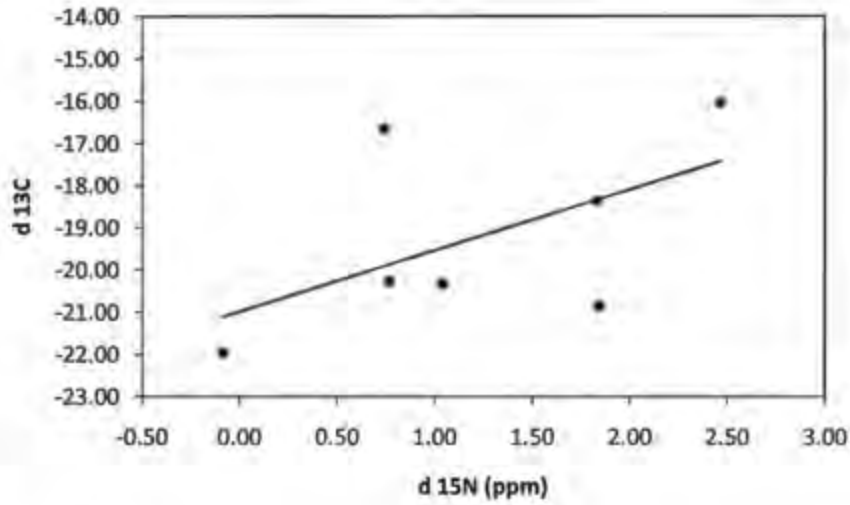


Figure 8: $\delta^{13}\text{C}$ against $\delta^{15}\text{N}$ over depth interval 42cm-30cm, including transition 3 (40cm-36cm) in core taken at Phindisweni, Hluhluwe-iMfolozi Park. $R^2 = 0.314$, $m = 1.45$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

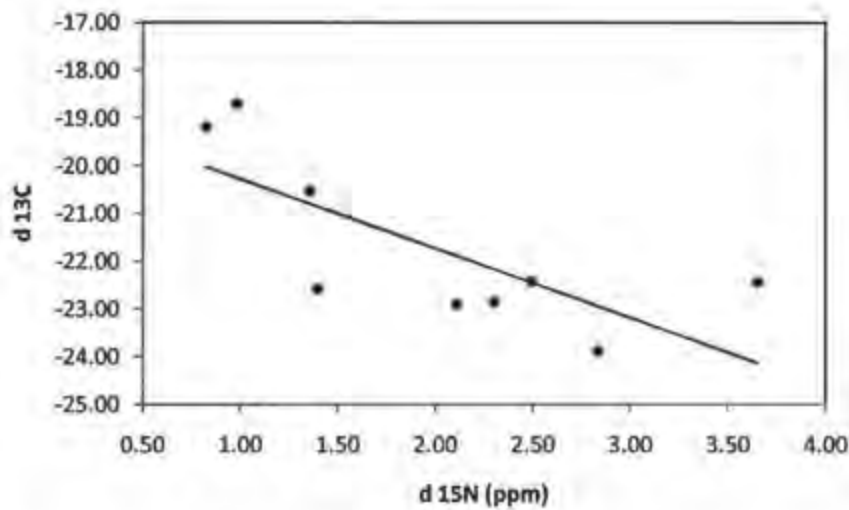


Figure 9: $\delta^{13}\text{C}$ against $\delta^{15}\text{N}$ over transition 4 in core taken at Phindisweni, Hluhluwe-iMfolozi Park. $R^2=0.564$, ($p < 0.05$) $m = -1.46$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

Table 1: Summary of regression analysis of sediment $\delta^{13}\text{C}$ vs. $\delta^{15}\text{N}$ for depth intervals of 'Phindisweni A' core. See Figure 1 and text for descriptions of phases and transitions. * denotes significance at the 5% level. Base of core (148cm) dated at 2340-2420cal. Yr. BP.

Phase/Transition	Depth (cm)	R^2	P	F	df	Gradient
Entire Core	150-2	0.027	.0162*	1.99	1, 73	-0.23
Transition 1	150-136	0.617	0.021*	9.66	1, 6	-0.74
Phase 1	136-94	0.132	0.096	3.05	1, 20	1.09
Transition 2	102-82	0.087	0.377	0.86	1, 9	1.40
Phase 2	90-40	0.067	0.201	1.73	1, 24	-0.18
Transition 3	42-30	0.314	0.191	2.28	1, 5	1.45
Phase 3	36-18	0.153	0.264	1.44	1, 8	0.67
Transition 4	18-2	0.564	0.020*	9.04	1, 7	-1.46

Over the entire sequence C: N and $\delta^{15}\text{N}$ have a weakly negative relationship ($m = -0.264$, $R^2 = 0.179$). Both undergo changes at the same points in the deep section of the core, although the relationship is negative ($R^2 = 0.699$, $m = -1.69$) (Figure 9). Whereas C: N undergoes a short increase before decreasing from 148cm-136cm, $\delta^{15}\text{N}$ declines and then increases (disproportionally to the C: N decline) over this period. The lowest C: N values over 136cm-94cm are matched by the highest $\delta^{15}\text{N}$ values. Both signals commence transitions at 90cm although they differ in extent; C: N increases to its highest levels from

78cm-46cm, while $\delta^{15}\text{N}$ declines to its lowest level at 36cm (Figure 10). More recently, $\delta^{15}\text{N}$ increases steadily to the surface while C: N increases, decreases, and increases again over the same interval.

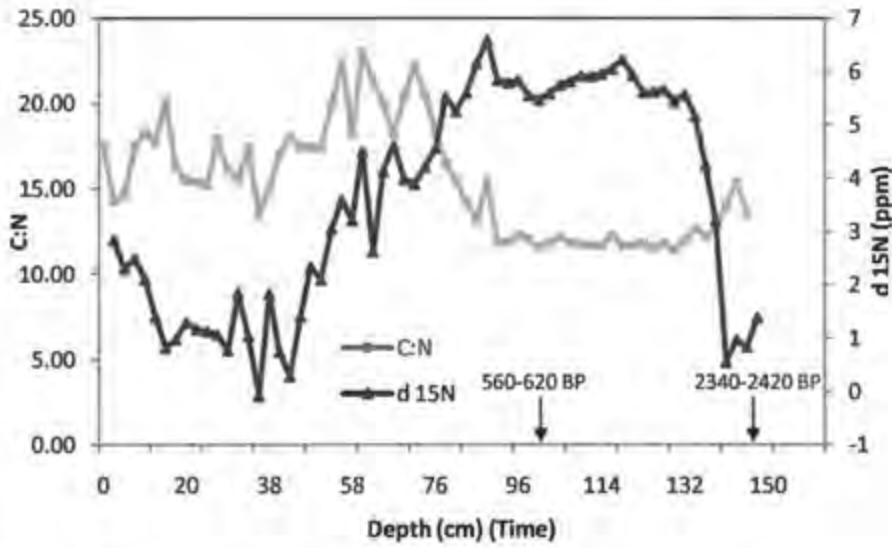


Figure 10: Sediment Carbon to Nitrogen ratio over time from core taken at Phindisweni, Hluhluwe-iMfolozi Park. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

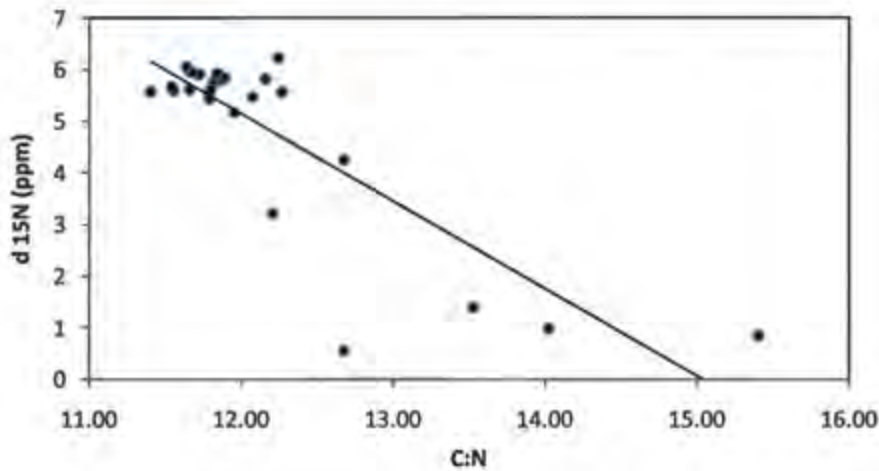


Figure 11: $\delta^{15}\text{N}$ against C: N over depth interval 148cm-94cm from core taken at Phindisweni, Hluhluwe-iMfolozi Park. $R^2 = 0.699$, $m = -1.69$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

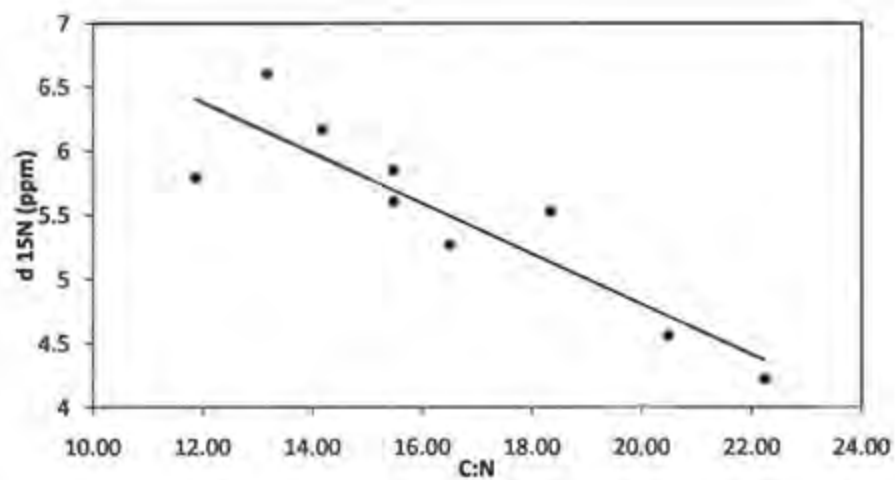


Figure 12: Sediment $\delta^{15}\text{N}$ against C: N over depth interval 94cm-74cm from core taken at Phindisweni, Hluhluwe-iMfolozi Park. $R^2 = 0.797$, $m = -0.197$. Sediment at 148cm Carbon dated at 2340-2420cal. Yr. BP.

Discussion:

Changes in Terrestrial and Aquatic Vegetation:

The vegetation, indicated by the $\delta^{13}\text{C}$ signal, at Phindisweni is variable over time, and follows a pattern of phase and transition. The base of the core captures a transition (transition 1) to a lengthy, uniform phase (phase 1) of C_3 (aquatic or tree) dominated vegetation, which occurred around 2340-2420cal. Yr. BP. Wetland core sediment C: N values typically range from 10-20, indicative of a mixture of algae and higher plant signal in the landscape (Meyers 1994). Thus phase 1 is likely an aquatic period at the site, as the low, stable C: N over this interval is an indication of strong algal influence (Lamb *et al.* 2004). Transition 1 is matched by a corresponding decrease in C: N (Figure 3), implying an increase in aquatic influence over this time. The evidence points to the base of the core capturing the onset of wet conditions which persisted for about 1400 years (transition 2 occurs just after the sediment dated at 560-620cal. Yr. BP) in a stable fashion. A pronounced warm period from 2200-2000cal. Yr. BP is evident in the temperature reconstruction of South Africa, followed by an interval of about 800 years where temperatures were at least as warm as the 1961-1991 mean, before the Medieval Warm Period occurred from *circa* 1100cal. Yr. BP to 500cal. Yr. BP (Holmgren *et al.* 1999, Holmgren *et al.* 2001). Transition 1 is closely associated temporally with the warm 'spike' while phase 1 corresponds to the interval of stable temperatures following this spike, and includes the Medieval Warm Period too. Based upon this insight, it is suggested that these warm temperatures were accompanied by higher rainfall conditions such that the Phindisweni wetland increased in extent, and supported a high proportion of aquatic vegetation. Because transition 1 represents the bottom of the wetland sediments, it is possible that this climatic change is responsible for the initiation of wetland sediment accumulation. An analysis of the diatom record of the core would provide a good test of this supposition, as would a pollen analysis to confirm the vegetation transition.

Transition 2 takes place around 500y BC, and represents a sharp vegetation change to a C_4 dominated landscape. This period of C_4 prominence persists as a fairly stable phase (phase 2) until a highly speculative date of 300cal. Yr. BP. Transition 2 is closely matched by the C: N ratio of the sediment (Figure 4), which shows a corresponding increase. $\delta^{13}\text{C}$ phase 2 coincides with the period of highest C: N ratios in the sediment from 94cm-74cm; this signal for this interval is predominantly terrestrial, as C: N ratios >20 signify higher plant dominance (Meyers 1994), and the mean for phase 2 is 18.26, with several points above 20. A terrestrial C_4 signal is most likely the signature of C_4 grasses; phase 2 therefore can be classified as grassland phase- confirmation of which should be visible in the pollen record. An aquatic element is probably retained as C_4 grasses generally have a C: N ratio between 20

and 50 (Muller *et al.* 1983). The timing of transition 2 coincides with the onset of the Little Ice Age, which arrived abruptly at 700- 500cal. Yr. BP and persisted until roughly 200cal. Yr. BP, heralding temperatures of up to 1°C lower than the 1961-1991 mean (Holmgren *et al.* 2001, Tyson *et al.* 2000). Furthermore, Norstrom *et al.* (2005)'s high resolution rainfall record from Limpopo tree isotope and wood anatomy analysis conclude the occurrence of a dry spell at 500cal. Yr. BP. It appears that the arrival of colder, drier conditions around 500cal. Yr. BP caused the extent of Phindisweni wetland to decrease, permitting an increase in the terrestrial signal. The decrease in rainfall probably caused precipitation to decrease below the 'unstable' threshold of ~650mm/ year, and lack of moisture limited tree recruitment strongly (Sankaran *et al.* 2005, Sankaran *et al.* 2008), resulting in the observed grassland phase. Phytolith and diatom analyses would be able to confirm increasing grassiness and aridity respectively.

This grassland phase comes to an abrupt end at (again highly speculatively) 300cal. Yr. BP, switching via transition 3 to a phase (phase 3) characterized by a stronger C₃ influence than the grassland phase, indicating more trees or aquatic plants. Phase 3 indicates a higher terrestrial signal than phase 1, as, although it declines (Figure 5), the C: N ratio is considerably higher (mean 16.59 compared to 11.82); this signal strengthens during the course of this depth interval. Therefore, the increased C₃ influence can largely be attributed to an increase in woody cover, although an aquatic element is likely retained as well. An increase in moisture could account for higher tree recruitment (Sankaran *et al.* 2005, Sankaran *et al.* 2008) as well as the increase in influence of aquatic vegetation. The temperature record shows that a brief spike in temperatures occurred at around 200cal. Yr. BP (Holmgren *et al.* 2001), accompanied by a small increase in rainfall around this time (Norstrom *et al.* 2005). It is possible that this short interval of warmer, wetter conditions permitted increased tree recruitment, accounting for the increase in C₃ vegetation signal, given the uncertainty in the dating of the core. In any case, the rainfall that conditions were not wet enough for an increase in wetland extent, as this increase occurs relative to a fairly low level of precipitation in the ~600y record (Norstrom *et al.* 2005). This concurs with the findings of West *et al.* (2000) and Wigley *et al.* (2009) both of whom found grasslands preceding wooded landscapes in Hluhluwe.

C₃ vegetation influence increases further to the present day over transition 4. Although the C: N declines before increasing over the most recent depths (6cm-4cm), the ratio largely indicates terrestrial input throughout this transition. A terrestrial increase in C₃ vegetation points to woody cover increasing further over transition 4; the modern trend of increasing woody density confirms this inference. It is likely, therefore, that hydrology has remained largely stable over this period, and another factor is causing higher tree recruitment, and the variability in the C: N ratio over transition 4. Within

Hluhluwe-iMfolozi Park, several explanations have arisen to explain the phenomenon of rapidly increasing woody cover. Skowno *et al.* (1999) and Walters *et al.* (2004) proposed that insufficient fire intensity was responsible for allowing enhanced germination and seedling establishment of successful woody encroaching species. Bond *et al.* (2001) identified spatial changes in disturbance (herbivory and fire) regimes as the cause of *Acacia karroo* and *Acacia nilotica* (both encroaching species) turnover. Increasing global atmospheric CO₂ have been proposed to enhance sapling recruitment (by decreasing the amount of time required to escape a set fire return interval) by Bond *et al.* (2003b), and recovery from fire and herbivory (Kgope *et al.* 2010). The magnitude and spatial extent of woody increase across a variety of land uses underlines the global nature of this phenomenon, and makes CO₂ fertilization the primary candidate as the driver (Wigley *et al.* 2009, 2010).

Lamb *et al.* (2004) found a similar range of C: N ratio of sediment from the Phindisweni core in a core taken at Lake Tilo, Ethiopia spanning the last 8840 years. The late Holocene sediments (last 2500 years) had values ranging from ~10 to ~25, with higher C: N ratios interpreted as greater terrestrial plant influence. The strong positive relationship between C: N and $\delta^{13}C$ of sediment from the Phindisweni core (Figure 2) permits the same inference. In contrast to the Phindisweni core, the Lake Tilo sediment exhibited a declining C: N ratio over the late Holocene to the surface, from which it was suggested that organic matter may have undergone diagenesis; thus it is unlikely that diagenesis has occurred in the Phindisweni core, although an analysis of the diatom record could help to confirm this. In general, Gillson and Ekblom (2009) observed higher C: N values from their cores in Kruger National Park, reporting the majority of values as at least 20. In their study, observed vegetation dynamics were tentatively linked to the regional climate fluctuations recorded by the Makapansgat Valley Cold Air Cave speleothem sequence, as interpreted by Holmgren *et al.* (1999) and Holmgren *et al.* (2001). More specifically, the general increase in regional temperature and rainfall around 800-1000cal. Yr. BP is associated with vegetation shifts observed at one of the sites (Malahlapanga).

Changes in Nitrogen availability and relationship with vegetation change:

Vegetation change correlates significantly with $\delta^{15}N$ change, which shows a sharp increase in openness of the N cycle (i.e. greater supply relative to demand, and hence higher N availability) preceded by a decreasing trend over transition i. Both vegetation and openness of the N cycle change at the same time over this transition, suggesting that Nitrogen is not the driver of the switch to the aquatic C₃-vegetated phase i. Instead, another factor that exerts an influence over Nitrogen availability is proposed to have changed over this time, such as fire or climate.

Following this transition, Nitrogen supply is very high and stable, corresponding tightly with aquatic C_3 stable vegetation phase 1. Limited soil aeration results in anaerobic conditions, which favour the microbial-mediated denitrification process (Aranibar *et al.* 2004); fractionation during this process causes an enrichment of ^{15}N in the sediment (Hopkins *et al.* 1998). This suggests that the $\delta^{15}N$ transition to a high cycling rate is in response to the increased water content of the soil at Phindisweni due to the warm, wet conditions experienced at this time and persisting for about 1400y. Figure 11 confirms this, as the increasingly aquatic signal is significantly correlated with an increase in N openness. Climate, by influencing hydrology, is therefore the likely driver of vegetation and Nitrogen cycling change at transition 1, and stability over phase 1.

Vegetation transition 2, from aquatic C_3 vegetation to terrestrial C_4 vegetation, does not correlate strongly with Nitrogen availability; this is unsurprising as the steep increase in $\delta^{13}C$ signal is not reflected in the $\delta^{15}N$ signal which merely fluctuates more about a similar level. Shortly following this vegetation transition, the Nitrogen cycling rate decreases throughout the C_4 grassland phase to the sequence's lowest levels at the end of the grassland phase. The fact that $\delta^{15}N$ responds after the vegetation change suggests that Nitrogen is not the driver of vegetation change over this transition. The decline in $\delta^{15}N$ over phase 2 also suggests that the C_4 grassland phase is not Nitrogen limited as vegetation remains stable in the face of lower N availability. In line with the work of Aranibar *et al.* (2004), Aranibar *et al.* (2008), and Wang *et al.* (2010), the decline in $\delta^{15}N$ over the inferred more arid C_4 grassland phase is surprising, suggestive of an agent other than aridity influencing the $\delta^{15}N$ signal. In light of this, the Nitrogen openness signal may reflect the observed change of vegetation, which in turn was initiated by the onset of the LIA. Figure 12 confirms this climatic influence on Nitrogen, as the increase in terrestrial signal is significantly matched by a decline in N openness. Frequent fire characterizes grassland ecosystems (Fynn *et al.* 2002, Bond 2010), and it is likely that the C_4 phase (phase 2) in the Phindisweni core is a frequently burned system as the C: N ratio widens to values even higher than those reported by Fynn *et al.* (2003) (circa 18 compared with -12 to -14) in a Natal grassland responding to frequent fire. Although Coetsee *et al.* (2010) concluded that fire has no direct effect on soil Nitrogen pools, Dijkstra *et al.* (2006) showed that frequent fire reduces N availability through enhanced volatilization, and importantly that vegetation shifts induced by the onset of fire can strongly reduce N openness through fire-adapted plant traits. Fynn *et al.* (2003) confirmed that, in a KZN grassland, burning decreased total soil N and potentially mineralizable N; the net effect of this would be a decrease in N openness (Nadelhoffer and Fry 1988). Declines in soil Nitrogen supply would be reflected as similar declines in the openness of the sediment N cycle (Hopkins *et al.* 1998). Examination of the charcoal record would be fascinating to see if in fact an increase in fire frequency

accompanies transition 2, given that fuel loads would be low under the arid conditions proposed to be prevailing at this time.

At the same time that vegetation experiences an increase in C_3 woody elements over transition 3, Nitrogen availability 'bottoms out' and stabilizes during vegetation phase 3. Over this transition, the relationship between vegetation and N openness is quite strong (Figure 7). Although $\delta^{15}N$ is fluctuating considerably over this period, only a slight decrease in N openness is evident across the vegetation switch. It is unlikely that this small decline represents a breaching of a Nitrogen availability threshold, as the vegetation switch is to a more wooded environment, and woody vegetation has higher Nitrogen requirements than non-woody vegetation (Nadelhoffer and Fry 1988, Wang *et al.* 2010). Further, if a threshold was being breached, it would be expected that the vegetation change would follow the change in N supply (availability), instead of the Nitrogen signal stabilizing after the vegetation switch. Thus it appears that once again, the openness of the N cycle is responding to the change in vegetation brought about by climate change *circa* 200cal. Yr. BP. Over phase 3, Nitrogen stabilizes at a slightly more open state than during transition 3, as would be expected under increased woody cover after Dijkstra *et al.* (2006) concluded that woody cover increases Nitrogen supply through enhanced mineralization and litter input following savanna fires.

The ongoing increase in Nitrogen openness taking place to the present day coincides with increase in woody cover over transition 4. If a difference in timing between the initiations of these changes does exist, it is a slight one, and vegetation is the first to change. Therefore, the increased N openness could be a continuation of the response seen in phase 3 where increased woody cover resulted in an increase in N openness. The drastic increase observed relative to the increase in C_3 influence (although strong) suggests that another factor could be contributing to N supply rate relative to demand. Deposition of atmospheric Nitrogen has increased dramatically during the late Holocene in response to human activity (Smil 1990). In their global perspective of the effects of additional Nitrogen fertilization on terrestrial ecosystems, Matson *et al.* (2002) conclude that increased mineralization and leaching of N generally occur, as does increased plant growth, when Nitrogen is a limitation (as it is in savannas-Sankaran *et al.* 2008). Therefore, the increased N openness in the upper section of the core could reflect the response of the Nitrogen cycle to increased N-deposition, especially considering the intensive human activities in the surrounding area which include intensive sugar cane plantations, and heavy industry in Richards Bay town. This effect could also contribute to the increase in woody vegetation observed over transition 4, given the strong correlation between $\delta^{13}C$ and $\delta^{15}N$ over this transition (Figure 8). However, this strong correlation could also arise from the effect of Nitrogen fixation by trees. Cramer *et al.* (2007) used $\delta^{15}N$ of plant material of several *Acacia* species (including major encroaching species *Acacia karroo*) in

Hluhluwe-iMfolozi Park to demonstrate their nitrogen fixing capacity, and its stimulation by grass competition. An increase in biologically fixed Nitrogen, due to increased N-fixer density, would enhance the supply of Nitrogen to the biosphere (Hopkins *et al.* 1998), reflected as an increase in Nitrogen openness as observed in transition 4.

Conclusions:

Vegetation at Phindisweni, HiP, is variable temporally, undergoing shifts in dominance of vegetation types over the last 2380 ± 40 cal. Yr represented by the core. These vegetation changes follow a phase and transition pattern, with relatively stable phases punctuated by rapid transitions as predicted by resilience theory. Stable aquatic vegetation persisted at Phindisweni from the base of the core until *circa* 500 cal. Yr. BP, after which terrestrial C_4 grassland became dominant until 200-300 cal. Yr. BP. Concurrent with other studies in the park, a more wooded phase succeeds the grassland phase; a further transition to even greater tree density is underway currently. Vegetation changes correspond well with climatic events/changes taking place over this interval. The aquatic, C_3 phase occurs during a warm, wet period that included the Medieval Warm Period; the abrupt onset of the cool, dry conditions of the Little Ice Age corresponds with the arrival of the C_4 grassland phase. A brief period of warm, wet conditions following the LIA is speculated to associate with the termination of the grassland phase, and an increase in woody cover. There is no evidence to suggest that the openness of the Nitrogen cycle is a driver of vegetation change in the vegetation history captured by the Phindisweni core, implying that nutrients are not limiting in this landscape. Instead, climate exerts the most influence over vegetation which in turn appears to be the primary influence over Nitrogen availability; hydrology-implicating climate (during the aquatic phase)- and fire (during the grassland phase) are also identified as modifiers of the openness of the N cycle. This is surprising in that it contradicts the original hypothesis that Nitrogen is (at some stages at least) the primary determinant of vegetation, although it confirms the observation made by Archibald *et al.* (2005) that soils and vegetation are not closely associated in HiP.

The most recent increase in woody cover and openness of the N cycle are probably manifestations of human derived phenomena. Anthropogenic increases in atmospheric CO_2 could be responsible for increased tree establishment, recruitment, and recovery from herbivory to cause increasing tree density. Nitrogen pollution, from agriculture and industry, and deposition are suggested to be causing the increase in Nitrogen cycling, compounded by the increase in Nitrogen-fixing tree density.

Vegetation phase 1 serves as a basic analogue of possible response of vegetation to the anticipated warming and increased moisture in the future due to climate change. From a management perspective, transition 1 is of great interest, and similarities between transitions 1 and 4 are noteworthy. The trend

in transition 4 suggests that a reversion to the aquatic state of phase 1 is possible in the future, although most recently the C: N ratio depicts increasing terrestrial influence. An examination of the pollen record is of great importance to investigate what vegetation community could be expected if this reversion is realized. Since climate appears to be the primary driver of transition 1, this sequence could be used to identify climatic thresholds that parameterize the different vegetation phases. Such thresholds, and other vegetation features illuminated by this study, are valuable to informing 'thresholds of potential concern', approaching of which initiates active management under an adaptive management policy (Gillson and Duffin 2007).

Future research could improve the evidence on which these conclusions are drawn. Multi-proxy analysis of Phindisweni sediment, namely pollen, diatoms, phytoliths, and charcoal, and archaeological evidence, would greatly improve the resolution of vegetation change detection, and help to identify other possible drivers, such as fire and human activity, causing these changes. A more comprehensive dating of the core would enable a closer examination of the co-occurrence of vegetation and climatic changes, and consequently the role of climate in driving vegetation changes.

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