Long-term effects of fire on nitrogen cycling in a broad-leaf savanna, Kruger National Park, South Africa

Corli Coetsee

Thesis for the Degree of DOCTOR OF PHILOSOPHY
at the University of Cape Town
2007

Front page: A view of the fire exclusion plot at the Shabeni Experimental Plots, from Shabeni hill.

"I told the General [Jan Smuts] I couldn't give him the ten commandments [of burning] because of the complexity of the phenomenon. On one aspect of your farm you should burn at a certain season, on another you should not burn at that particular time! You should burn thus on your grassland farm at Irene, but don't do this on your bushveld farm 50 miles away because the local ecosystems are so different. The General was disappointed..."

John Phillips at the Fire in Africa, Tall Timbers Fire Ecology Conference, Tallahassee, 1971.

To my parents,
who introduced me to the natural world in the first place

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Master of Science (Conservation Biology)

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Department of Botany
UNIVERSITY OF CAPE TOWN
July 2007

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ABSTRACT

Fire with herbivory, climate, and soil properties including nutrients are said to be important in regulating the structure and function of savanna ecosystems. Frequent fire is often held responsible for a decrease in nitrogen pools and availability and the maintenance of low fertility conditions. However, previous research in the Kruger National Park (KNP) and elsewhere found conflicting results for the effects of fire on nitrogen pools and transformation rates. The main aim of this study was to gain a better understanding of the long-term effects of fire on nitrogen cycling in the KNP.

The KNP provided an ideal opportunity for this study because of the initiation of a fire experiment in 1954. The Experimental Burn Plot (EBP) experiment was initiated in four representative landscapes of the KNP to determine the effects of fire on vegetation structure. I tested the effect of burning on nitrogen cycling and productivity in four fire treatments situated in Pretoriuskop Sour Bushveld (broad-leaf savanna). The fire treatments included a late winter, annual burn (August), late winter and summer triennial burns (August and February) and a fire exclusion treatment. Total soil nitrogen, available nitrogen, woody biomass and herbaceous production were measured. I hypothesized that vegetation adapted to low N conditions with low N foliage would be expected to dominate in frequently burnt areas if fire was to decrease N pools and fluxes. To test this, I measured grass species composition and accompanying traits across the fire gradient. I also used $\delta^{15}N$ of soil and foliage as a proxy of nitrogen cycling and measured $\delta^{15}N$ in wood to obtain a longer term perspective of fire treatment on nitrogen cycling.

The results show that fire did not affect total soil nitrogen and carbon regardless of soil depth. The high intensity triennial burn decreased the cumulative amount of available N more than the annual burn. As herbaceous production compensate for decreased woody production, frequently burnt savanna managed to stay as productive as fire excluded areas. Less frequent burning, especially in the dry season, was more detrimental to productivity than annual burning. However, the grass composition, daily rates of nitrogen mineralization and ecosystem $\delta^{15}N$ values were not affected by fire treatment. Longer term signals of nitrogen cycling ($\delta^{15}N$ values in wood) indicate that the annual burns have been moving persistently towards a more open nitrogen cycle, whereas the fire exclusion plots have had more variable $\delta^{15}N$ values through time. The effect of fire on the nitrogen cycle in this study is ameliorated by an interaction between low losses of nitrogen and limited species change-over with frequent fire. The persistence of trees, even in the annual burns and the presence of herbivores may contribute to relatively low fire intensities and the conservation of nitrogen. Furthermore, the exclusion of fire has not lead to closed canopy forest. Soil organic matter $\delta^{13}C$ values showed that this area has been in a savanna state for a considerable length of time and were not forest previously.

The two treatments with the highest cumulative N mineralization rates also had the highest forb biomass and I suggest that it is necessary to quantify the importance of N_2 fixation on the annual burns. It is also imperative that the high N deposition rates reported for the KNP be verified as these

extremely high rates of deposition will have serious implications for vegetation adapted to nutrient-poor soils.

Frequent fire in prairie and Oak savanna leads to shifts in species composition (whether grass or woody species). At my study site, frequent fire (or even the exclusion of fire) does not lead to significant shifts in species composition. These results suggest that changes in the structure of vegetation (*i.e.*, tree:grass ratio) with frequent fire may be more important in controlling N cycling than the direct effects of fire on N (*e.g.*, volatilization). Research on the effects of fire on nitrogen cycling is usually biased towards temperate systems. Furthermore, much of the fire and nitrogen research in the past has been based on single fire events and not on long-term experiments. This work has shown that fire effects on nitrogen in savannas are different from those seen in prairie and temperate savannas.

ACKNOWLEDGEMENTS

This project would not have been possible without the help and encouragement of many. I want to specially thank Prof. Nikolaas van der Merwe, without whose encouragement I would never have embarked on my present path. Thank you to my supervisors, Ed February and William Bond, who gave me the chance to do a PhD and provided the opportunity to work in the Kruger National Park. I want to thank Ed for his endless patience, all the reading of drafts and his unfailing support and encouragement. I want to thank William for keeping his door open, for inciting us to think out of the box (and arrows), and for keeping those grand ideas flowing. I also want to thank him for imparting some of his boundless enthusiasm for ecology to his students. Thank you to The Andrew Mellon Foundation and NRF for sponsorship and funding.

I had an amazing time doing fieldwork in the Kruger National Park. Thank you to all the staff in Scientific Services, Skukuza (SanParks) and Louise Swemmer. Thank you to Navashni Govender, not only for data, but also for friendship. Thanks to Rina Grant for reading some of my chapters and her kind words of encouragement, and to Nick Zambatis for weather data. Thank you to everyone I spend time with in the N'washitshaka Research Camp. Thanks to the many game guards who protected me in the field; especially to Velli Ndlovu, Moses Mathe, and Stephen Khoza. A special thanks to my field assistant, Annoit Mashele for many laughs in the field and all his hard work.

Thank you to all those people everywhere that responded when I emailed for advice. Ted Doane, whoever you are, I really appreciated you answering my calls for help! Thank you to Joe Craine for answering endless questions and all his help and support. Thank you to Willie Stock for helpful comments at the beginning of the project.

Thank you to all in the Department of Botany who made working there a pleasure. Thanks to Sandy Smuts for tackling bureaucracy on my behalf. Thank you to Gonzalo Aquilar and all the other people that supplied me with administrative and technical support. Thank you to Ian Newton for his hard work in the Archaeometry Lab. Thank you to the students in the Ecology lab and elsewhere for lightness, support and laughter (including Jasper, Fiona, Vhalinapho, Tim, Carla, Leonard, Alex, and Matt). Thank you especially to Pippin Anderson for support and friendship. Thank you to my parents and sister, nobody can ask for better! Thank you for always helping out, listening, encouraging, and for being there when I need you! Lastly, I would like to thank Ben for help in the field, for being encouraging, accommodating and always supportive and for making the dark days bright.

CHAPTER 1

EFFECTS OF FIRE ON PRODUCTIVITY AND NITROGEN CYCLING IN A BROAD-LEAF SAVANNA, KRUGER NATIONAL PARK, SOUTH AFRICA

INTRODUCTION

The aim of this study in part is to gain a better understanding of the long-term effects of fire on nitrogen cycling in the Kruger National Park (KNP). The research focuses on how fire and vegetation interacts in controlling nitrogen (N) cycling and productivity in a nutrient-poor, broad-leaf savanna. The KNP provides a unique opportunity to study the long-term effects of fire as a fire experiment was initiated in the 1950's. The experimental burn plots (referred to hereafter as the EBP's) consist of 12 fire treatments of varying frequency and season, in four string or repeats located in four different vegetation types (see Chapter 2 for background on experiment). The Kruger National Park falls within the savanna biome (Fig. 1.1). Savanna is broadly defined as an ecosystem formed by a continuous layer of graminoids and a discontinous layer of trees and/or shrubs of variable extent (Solbrig *et al.* 1996). Furthermore, tropical savanna is characterized by rainfall variability and the dry season is between three to nine months. Fire, herbivory, climate, and soil properties are considered as determinants of savanna structure (Sankaran *et al.* 2004). Fire is a prevalent ecological process in savanna and does not only influence structure, but also plant composition and nutrient cycling (Scholes and Walker 1993, Bond 1997, Scholes and Archer 1997). Frequent fire alters N availability and plant response in many different ecosystems (Knapp and Seastedt 1986, Ojima 1987, Seastedt *et al.* 1991).

Fire effects can be divided into short-term effects and cumulative long-term effects. Short-term effects include a stimulatory effect on plant production and enhanced nutrient concentrations post-fire. Higher plant production occurs as a result of an increase in N availability, the release of readily available nitrogen and phosphorus (P), the removal of accumulated detritus, and enhanced N fixation (Reich *et al.* 1990). Previous studies have suggested that enhanced net N mineralization rates and ash deposition after fire can contribute to higher plant-available nutrients (Raison 1979, Knapp and Seastedt 1986, Blair *et al.* 1998).

Research in tallgrass prairie has suggested that frequent fires will result in long-term reductions in the N capital of ecosystems (Ojima *et al.* 1994). Greater productivity under an annual burning regime, however, can be maintained despite a reduction in soil organic matter and large losses of N associated with every fire (Ojima *et al.* 1990, Blair *et al.* 1998). Some explanations for this phenomenon include increased nitrogen use efficiency (NUE) (Ojima *et al.* 1994), increased N fixation as a result of regular burning (Eisele *et al.* 1990), and the decrease of potential N losses via leaching and denitrification (Blair *et al.* 1998).

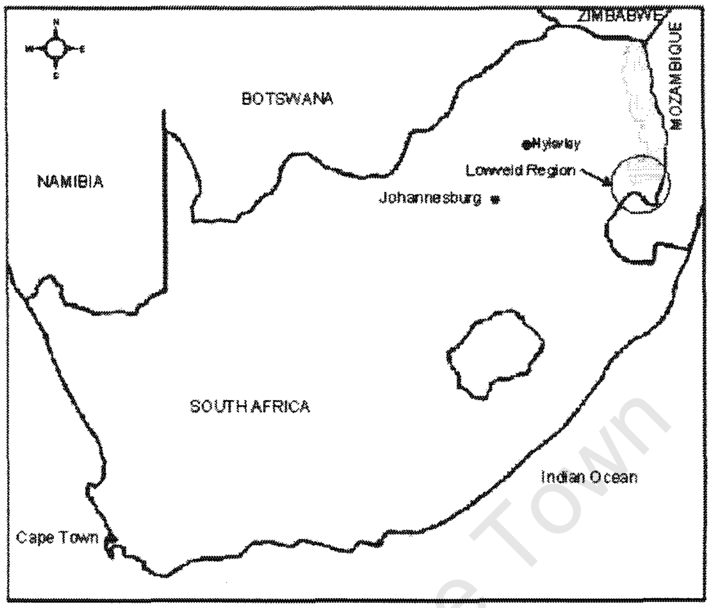

Figure 1.1. A map of South Africa with the locations of the Kruger National Park, Nylsvley, and the Lowveld.

The interactions between fire and vegetation in turn, have important implications for the animals that utilize the system by affecting their distribution, and movement as well as the quality and quantity of graze. Nitrogen and phosphorus are required in large quantities by animals and limitations of these nutrients manifest in depressed reproduction (Owen-Smith and Novellie 1982, Grant *et al.* 2000). Fire also impacts on the competition between grazing tolerant and intolerant grass species indirectly by influencing where, when and for how long animals utilize specific patches in the landscape (Archibald *et al.* 2005).

THE KNP NITROGEN CYCLE IN CONTEXT AND GENERAL EFFECTS OF FIRE ON THE NITROGEN CYCLE

There is a well developed literature on the short-term effects of fire on savanna ecosystems (Reviewed by Raison 1979). The immediate effects of fire include the removal of detritus and the loss of some nutrients such as N. The removal of detritus alters the energy environment and microclimate of the soil; this in turn results in greater solar inputs and warmer soil temperatures (Blair *et al.* 1998). N is frequently the most limiting nutrient to terrestrial plants and the effects of fire on the rate of N cycling have important consequences for plants. Nitrogen cycling in ecosystems can be divided into two cycles, an external and an internal N cycle. The internal cycle focuses on the processes that takes place within the top soil layers and between soil and plants (also known as the ecosystem) and includes plant uptake, nutrient resorption and N mineralization, nitrification, and immobilization. The external cycle includes processes such as: dry and wet nitrogen deposition; dinitrogen fixation; N leaching; denitrification and ammonia volatilization. The long-term effects of fire on these processes are important for a better understanding of productivity and nitrogen cycling in savanna systems.

External nitrogen cycle

Dry and Wet nitrogen deposition

Nitrogen is received from the atmosphere in three ways: dissolved in mist or fog, as wet deposition or dry deposition (atmospheric particles and gases) and together these make up total deposition of N (Lovett 1994). Total N deposition in the Kruger National Park (KNP) is estimated to be 21.6 kg N ha^{-1} yr^{-1} (Scholes *et al.* 2003). This number has been attributed to the fact that the Park is downwind from an area with major coal refineries. Dry deposition is estimated at 15 kg N ha^{-1} yr^{-1} and wet deposition has been variably measured as 7 kg N ha^{-1} yr^{-1} (Scholes *et al.* 2003) and 2.8 kg N ha^{-1} yr^{-1} (Mephepya *et al.* 2006). These estimates are higher that that found elsewhere as N deposition in rain is 4.3 kg N ha^{-1} yr^{-1} in Kenyan savanna (Augustine 2003) and N in bulk precipitation for North American tallgrass prairie varies between 10-20 kg N ha^{-1} yr^{-1} (Blair *et al.* 1998). Also, a study of West African and South African savannas found total N deposition to vary between 8-9 kg N ha^{-1} yr^{-1} for dry savannas and an upper range of 15-19 kg N ha^{-1} yr^{-1} for wetter savannas and forests (Galy-Lacaux *et al.* 2003). Total nitrogen deposition is much lower at Nylsvley (for location see Fig. 1.1, p.2), 100 km west of the KNP (2.5 kg N ha^{-1} yr^{-1}), apparently because it is situated upwind from the worst pollution sources in the region (Scholes and Walker 1993). It is evident in this section that the Kruger National Park has very high deposition rates compared to other savannas. This may potentiall have important implications for the systems as increased deposition may lead to eutrophication which in turn may lead to changes in species composition and ecosystem function (Vitousek *et al.* 1997, Bobbink *et al.* 1998).

Annual fire in some tropical Australian Eucalypt savannas has little effect on soil nitrogen because the relatively small losses of N are compensated for by annual N inputs in rainfall and non-symbiotic fixation in the surface soils (Holt and Coventry 1990). For Australian savanna woodland at Kapalga, annual burning leads to a net loss of 15-20 kg N ha^{-1} yr^{-1} (Cook 2001). Impacts of fire in West African savannas depend on the season of the burn (Abbadie 2006). During the dry season, close to 100% of biomass burns with large associated losses in N, while burning in the wet season leads to about 12% of biomass burned. Overall, loss of N though volatilization during fires is balanced by N inputs from deposition and dinitrogen fixation (Abbadie 2006).

Dinitrogen fixation

Dinitrogen fixation is the process by which atmospheric N_2 is converted to ammonium (NH_4^+) and constitutes a major input of N to the ecosystem. Dinitrogen fixation can be performed by bacteria existing in symbiosis with plants and associated with plant roots or by free-living bacteria such as cyanobacteria. Fixation can occur in the rhizosphere of some tropical grasses (Wullstein 1979, Miranda *et al.* 1990). Nitrogen fixed by legumes at Nylsvley has been estimated to be between 29 and 86 kg N ha^{-1} yr^{-1} (depending on the amount of acytelene reduced for every molecule dinitrogen fixed, Grobbelaar and Rösch 1981). Using natural abundance ^{15}N, it has been shown that the woody Mimosoideae in KNP are able to fix atmospheric N_2 (Scholes *et al.* 2003). Kruger estimates for fine-leaf savanna areas average around 21.0 kg N ha^{-1} yr^{-1} and for broad-leaf savanna, 4.8 kg N ha^{-1} yr^{-1}. Fixation varies in other savannas. Values of 2.2 kg N ha^{-1} yr^{-1} (Norman and Wetselaar 1960) to 12 kg N ha^{-1} yr^{-1} (Langkamp *et al.* 1979) have been reported for the Northern Territory of Australia. Symbiotic N_2 fixation is an important source of N in some Eucalypt communities and range from < 1 to 32 kg N ha^{-1} yr^{-1} but is on average about 5 kg N ha^{-1} yr^{-1} (Keith 1997). Dinitrogen fixation in West African savannas equals 12 kg N ha^{-1} yr^{-1}; 2 kg N ha^{-1} yr^{-1} from cyanobacteria, 1 kg N ha^{-1} yr^{-1} from herbaceous legumes and 9 kg N ha^{-1} yr^{-1} from grass rhizosphere microörganisms (Abbadie 2006).

Estimates for North American tallgrass prairie have shown that fixation by crustal soil cyanobacteria could be significant (~10 kg N ha^{-1} yr^{-1}) if light and soil moisture remained available for a long enough period in spring (Blair *et al.* 1998). A limited amount of work has been done on fixing by crustal soil cyanobacteria in African savannas. One estimate from a Nigerian savanna is 3.3 to 9.2 kg N ha^{-1} yr^{-1} for a surface crust cover of 30% and fixation taking place 70% of the 180 day growing season (Isichei 1980). Soil N fixation is estimated to range from 4.2 kg N ha^{-1} yr^{-1} in unburned prairie to 5.2 kg N ha^{-1} yr^{-1} in annually burned prairie (Ojima *et al.* 1990).

The effects of fire on nitrogen fixing are variable. Enhanced phosphorus concentrations in the post-burn ash may facilitate N_2 fixation, but it is also possible that the enhanced availability of N following fires might decrease N_2 fixation (Menaut *et al.* 1993).

Nitrogen leaching

As nitrate (NO_3^-) is negatively charged it is repelled by similarly negatively charged clay particles and as a result susceptible to loss by leaching (*e.g.* downwards movement of NO_3^- through the soil profile). Leaching losses of nitrogen have been estimated at 1.3 kg N ha^{-1} yr^{-1} for broad-leaf savanna in the KNP (Woghiren 2002). Although leaching losses have been found to decrease with regular fire in prairie ecosystems (Ojima *et al.* 1990), no fire influence on leaching was found for broad-leaf and fine-leaf African savanna (Meredith 1987). Inorganic N leaching in soil for West African savannas is quite small (0.2 kg N ha^{-1} yr^{-1}) but most leaching is in the form of organic N (5 kg N ha^{-1} yr^{-1}) (Abbadie 2006). Fire-adapted systems are highly conservative of nutrients and large fluxes of nutrients after fire are immobilized very rapidly resulting in little leaching of N (Kellman *et al.* 1985, Kauffman *et al.* 1994).

Nitrogen emissions

Emission rates of NO_x (NO and NO_2) from the soil vary with soil nitrogen, soil moisture content, and soil temperature and a pulse is produced with the first rains. In African savanna, most of the NO_x is NO and the fluxes are very variable (0.05-34 ng N m^{-2} s^{-1}) (Parsons *et al.* 1996, Scholes *et al.* 1997, Otter *et al.* 1999, Scholes *et al.* 2003). For the KNP, the highest background NO emission rates (20 ng N-NO m^{-2} s^{-1}) were measured on plots where fire has been excluded for more than 50 years (Parsons *et al.* 1996). For the flux tower site at Skukuza, the annual mean emission of nitrogen through burning is 0.3 g N m^{-2} yr^{-1} (3 kg N ha^{-1} yr^{-1}) (Scholes *et al.* 2003). Nitrogen emissions for the rest of the park depend on the frequency of fire and fuel loads.

Denitrification

Another major pathway for N loss from the ecosystem is through denitrification. Denitrification is the process where nitrate (NO_3^-) is converted to oxides of nitrogen (NO, NO_2, etc.) or gaseous N_2. This flux is not large in African savannas such as at Nylsvley where it is assumed to be about 5% of the nitrification flux (Scholes and Walker 1993). Potential denitrification was negligible in broad-leaf Kruger National Park savannas except in wetter areas such as wetlands and riparian zones (Fisher 2006). Also, for West African savannas, the denitrifying activity in soils is very low at all depths (Abbadie 2006).

Volatilization – microbially mediated

Volatilization is the process by which ammonium is converted to ammonia and lost to the atmosphere. Volatilization includes direct volatilization of NH_4^+ to NH_3 in the soil solution, volatilization from nitrogenous compounds in animal urine (Scholes and Walker 1993), and volatilization from plant surfaces (Blair *et al.* 1998).

Volatilization – during burning

The proportion of nitrogen volatilized during fire is a function of the amount of N accumulated in above-ground biomass, as well as the fire temperature. Combustion is almost complete above 600 °C (Raison 1979). Pyrodenitrification or volatilization during the burning of litter and dead grass is the largest denitrification flux in Nylsvley savannas (Scholes and Walker 1993). Volatilization in Kruger has been estimated at 5.5 kg N ha^{-1} yr^{-1} for fine-leaf savanna and 5 kg N ha^{-1} yr^{-1} for broad-leaf savanna (Scholes *et al.* 2003). Annually burned prairie has greater N loss due to fire over the long term than infrequently burned tallgrass prairie (Ojima *et al.* 1990). Annual losses of N due to fire in tallgrass prairie may exceed precipitation inputs of new N in frequently burned areas in some years (Blair *et al.* 1998). Volatilization losses at Nylsvley are estimated at about 2 kg N ha^{-1} yr^{-1} (0.2 g N m^{-2} yr^{-1}) (Scholes and Walker 1993). In campo limpo (Brazilian grassland), nitrogen loss associated with fire is ~ 20 kg N ha^{-1} yr^{-1}, but only ~ 5 kg N ha^{-1} yr^{-1} for cerrado *sensu stricto* (Brazilian savanna) (Kauffman *et al.* 1994).

Internal nitrogen cycling

Plant uptake

Plant uptake of N for Nylsvley was estimated to be 40.0 kg N ha^{-1} yr^{-1} by summing the increments of nitrogen in biomass, and 58.0 kg N ha^{-1} yr^{-1} by measuring the nitrogen in litterfall (Scholes and Walker 1993). The difference has been attributed to the nitrogen accumulating in woody biomass. Plant uptake (calculated by summing the increments of N in biomass) for Kruger is estimated at 51.0 kg N ha^{-1} yr^{-1} for fine-leaf savanna and 36.5 kg N ha^{-1} yr^{-1} for broad-leaf savanna (Woghiren 2002).

Litterfall (N resorption)

Binkley *et al.* (2004) have shown that there is no general tendency for species with low leaf turnover rates, greater leaf tissue N concentrations, or those that grow in nutrient poor habitats, to resorb a greater fraction of their leaf N. Tissue chemistry for oak leaves at Cedar Creek (North America), however, varied in relation to available soil N (Reich *et al.* 2001). Oak leaves had greater N resorption and greater litterfall C:N ratios in stands with low N mineralization rates. Reich *et al.* (2001) suggest that frequent fires not only depress the N cycle, but that feedbacks from the dominant grasses reinforce this depression.

N mineralization and N immobilization

Nitrogen in the soil is predominantly in an organic form. Mineralization is the process whereby organic N is converted to inorganic nitrogen, ammonium (NH_4^+) that can be taken up by microbes (immobilization) or by plants (assimilated). Ammonium can also be converted to NO_3^- by chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*) in a process called nitrification. Environmental factors (soil moisture, temperature, aeration and pH), as well as litter chemistry, determine the C:N ratio of organic matter and the decomposition rates of litter. Mineralization usually happens when the C:N ratio of the litter-microbial biomass falls below 12, and NH_4^+ is released from the system (Scholes and Walker 1993). Immobilization starts at a certain C:N ratio (usually greater than 16), ammonium is likely to be taken up immediately by microbes, and this in turn may reduce the plant available nitrogen in the soil.

Unburned watersheds in tallgrass prairie have higher concentrations of extractable N than comparable annually burned watersheds (Blair *et al.* 1998). Net N mineralization rates tend to be greater in unburned than annually burned prairie (Ojima *et al.* 1990, Blair 1997, Turner *et al.* 1997) or annually burned oak savanna (Reich *et al.* 2001). Large quantities of nutrients can be immobilized in standing detritus and surface litter that accumulates in unburned tallgrass prairie. Large amounts of N may be liberated when this litter has decomposed significantly (Blair *et al.* 1998). Mineralization of organic matter in West African savannas (Lamto) only provides 2 – 5 kg N ha^{-1} yr^{-1} (Abbadie 2006). The rest of the annual N requirement for grass primary productivity has been hypothesized to originate from root litter mineralization (Abbadie *et al.* 1992, Abbadie 2006). The supply of ash after burning induces immobilization of nitrogen and in turn, increases yearly mineralization of carbon in West African savanna (Abbadie 2006).

In the KNP, nitrogen mineralization occurs in early summer, when temperatures start to rise after winter (Scholes *et al.* 2003.). This pattern is similar to that of tallgrass prairie where extractable N concentrations are greatest in spring (Ojima *et al.* 1990). Apart from the spring mineralization flux in the KNP, there is a flux of mineralization immediately after the first spring rain (Scholes *et al.* 2003). Annual nitrogen mineralization is estimated at 58 kg N ha^{-1} yr^{-1} for broad-leaf and 78 kg N ha^{-1} yr^{-1} for fine-leaf savanna (Woghiren 2002). Immobilization has been shown to happen at all times of the year in Kruger, with a peak in autumn, except for early spring when mineralization dominates (Woghiren 2002).

PREVIOUS MODELS OF THE EFFECTS OF FIRE ON PRODUCTIVITY AND NUTRIENT CYCLING

Fire in Prairie

In this review I include previous research from prairie, and although not completely applicable to savannas, prairie is included here because savannas have a huge herbaceous component. Also much of the research on the long-term effects of fire on nitrogen cycling has taken place in prairie (North-America) rather than in savannas. Most of the long-term effects of fire on prairie productivity and nutrient cycling are based on the CENTURY simulation model. This model (Ojima *et al.* 1990, Ojima *et al.* 1994) describes how short-term effects of fire translate into the long-term behaviour of tallgrass prairie systems. The CENTURY model is an adapted version of the CENTURY soil organic matter (SOM) model (Risser and Parton 1982, Parton *et al.* 1988) and was used to simulate short-term (1-5yr) and long-term (5-20yr) impacts of fire on plant production and nutrient cycling (Ojima *et al.* 1990, Ojima *et al.* 1994). The plant production submodel simulates the dynamics of C, N and P in the live and dead above-ground plant material and roots. Plant production is controlled by precipitation and available plant nutrients. Microbes in the SOM submodel decompose plant residues and the resulting microbial products become the substrates for SOM formation. SOM was divided into three fractions based on turnover time.

The CENTURY model was modified by Ojima *et al.* (1990) to include the direct effects of burning on the ecosystem and changes included:

1. Calculating biological N_2 fixation rates as a function of the ratio of mineral N to labile P.

2. Developing a plant production model where production is a function of monthly precipitation, soil temperature, and the standing dead biomass.
3. Allowing the C:N ratio of the active SOM to float as a function of the mineral N level.
4. Increasing the nitrogen use efficiency (NUE) for burned plants.
5. Incorporating the combustion losses of above-ground C, N, and P into the model.

All data on fire effects were synthesized from the Konza and Aldous tallgrass prairie plots, Kansas (Ojima *et al.* 1990, Ojima *et al.* 1994). Data included fire effects on plant production, soil organic matter, plant combustion losses, soil nutrients and mineralization, microbial biomass, and nitrogen losses and inputs. These short-term effects were then incorporated into the CENTURY model to simulate long-term effects (Fig. 1.2).

Although both below-ground and above-ground biomass is enhanced by burning, below-ground biomass increases more (Ojima *et al.* 1990). Soil temperatures increase after burning as it removes above-ground biomass and this increase SOM N mineralization rates. The removal of plant material also decreases above-ground N immobilization, while below-ground N immobilization is increased as a result of higher root production and lower N content of burned roots. The combined long-term effects are sustained increases in plant production, which are maintained by increased NUE despite huge carbon and N combustion losses, a decrease in leaching of soil NO_3^-, decreases in N_2 and N_2O gaseous losses, and increases in N inputs due to N fixation.

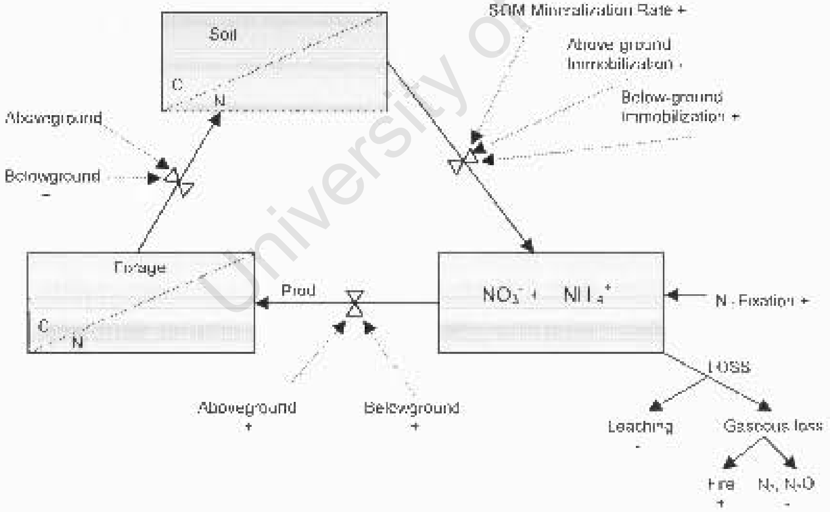

Figure 1.2. A conceptual flow diagram of the effect of annual burning on a tallgrass prairie that summarizes the cumulative long-term effects of fire, as predicted by the CENTURY model (Ojima *et al.* 1990).

Fire in savanna

In savannas an added level of complication is the difficulty in separating ecosystem processes resulting from vegetation type and structure from those due to different fire regimes. A conceptual model has been used to illustrate the effects of fire on processes controlling fluxes and pools of carbon and nitrogen in North American savanna communities (Fig. 1.3) (Reich *et al.* 2001). Fire frequencies range from annual burning to complete fire protection in oak savannas of the Cedar Creek Natural History area in east central Minnesota. Fire regime is in part responsible for the appearance of either woodlands or grasslands.

According to this conceptual model, two basic and related ideas can explain the way in which fire and vegetation controls ecosystem functioning (Reich *et al.* 2001). Net primary production (NPP) is a function of the amount of intercepted radiation and the efficiency with which the radiation is converted to biomass. Light efficiency is defined as the productivity per unit light intercepted. Secondly, NPP is related to the amount of N allocated to a canopy and the efficiency with which this N is utilised to capture carbon. Nitrogen use efficiency is defined as productivity (biomass produced) per unit canopy N.

The higher productivity in tree-dominated communities at Cedar Creek is explained by the higher leaf area index (LAI), total canopy N and a greater annual N supply (Fig. 1.3). The lower NPP in grass-dominated communities, in turn, is the result of low turnover rates of root N that lead to low amounts of litter N and therefore low mineralization rates. According to Reich *et al.* (2001) this leads to a low availability of N to build the next canopy and a small canopy with low total N leads to low C gain.

The greater LAI of the woody communities can be supported by greater annual N uptake. The higher annual N requirements are supported indirectly by a large annual litter N input of organic material of high quality (below- and above-ground) and more directly by greater annual net N mineralization. Plots with similar fire frequencies in Cedar Creek, have increased rates of N mineralization with increasing tree dominance. Although it is difficult to separate fire and vegetation effects, volatilization of nitrogen is purely a fire effect. Volatilization losses of N through fire have been estimated as 10-40 kg N ha^{-1} yr^{-1} for annual burning (Reich *et al.* 2001). Considering that N deposition at Cedar Creek is only 5-10 kg N ha^{-1} yr^{-1}, frequent fires can lead to a net loss of N capital if losses are not balanced by other N inputs (Reich *et al.* 2001).

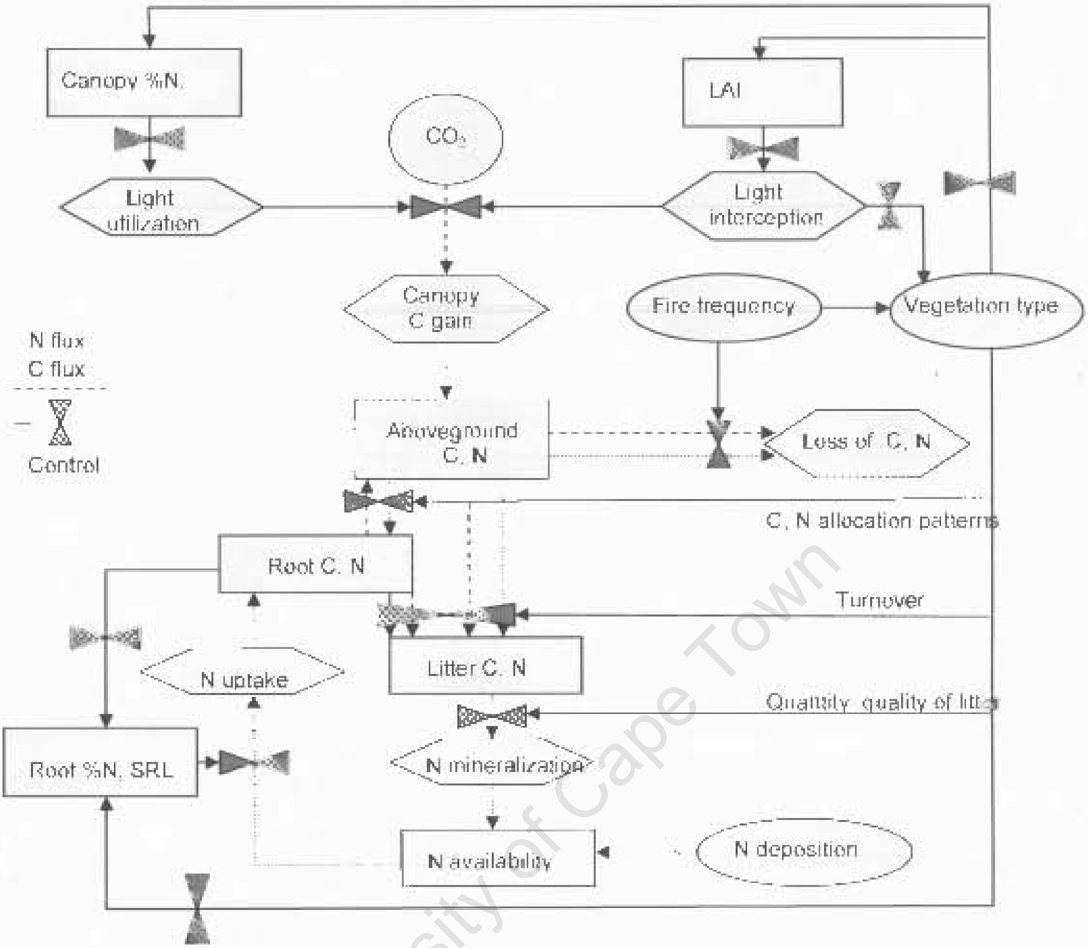

Figure 1.3. A conceptual diagram of the influence of fire frequency and vegetation type on processes controlling fluxes and pools of carbon (C) and nitrogen (N) in savanna communities at Ceder Creek, Minnesota, USA. Pools are represented by boxes, N fluxes by light dotted lines and C fluxes by dark dotted lines, controls by thin lines with valves, processes by hexagonal outlines and external of state factors by oval outlines (Reich *et al.* 2001). Productivity is greater in tree-dominated communities (low fire frequency) because of greater LAI and total canopy N (greater interception of light and greater biochemical capacity to use light), and greater annual N supply. Grass-dominated communities have low NPP because of low root turnover rates of root N leading to low amounts of litter N and in turn, to low soil N mineralization rates.

PAST RESEARCH ON FIRE, PRODUCTIVITY AND NITROGEN CYCLING IN THE KRUGER NATIONAL PARK AND AIMS OF THIS STUDY

Most of the fire research in the Kruger National Park has focused on fire management and how fire regimes influence woody community structure and the interactions between grass and trees (Trollope *et al.* 1998, Van Wilgen *et al.* 1998, Enslin *et al.* 2000, Higgins *et al.* 2000, Shackleton and Scholes 2000, O'Regan 2005, Higgins *et al.* 2007). Little research has investigated the effects of fire on soil dynamics and nitrogen cycling (Jones *et al.* 1990, Shackleton and Scholes 2000, Aranibar *et al.*

2003, Feig 2004). In fact, this is mirrored in a general dearth of system ecology studies for southern Africa (except for the work done at Nylsvley). There has been very little work on the effects of fire on the inorganic N pool and results are inconclusive (Jones *et al.* 1990, Aranibar *et al.* 2003, Feig 2004). No significant differences in net N mineralization rates with fire treatment were found for both broad-leaf and fine-leaf KNP savannas (Feig 2004). Although the results could not be tested statistically, increasing fire frequency decreased nitrogen mineralization on the N'wanetsi plots (part of the Knobthorn/Marula EBP's in fine-leaf savanna, see map Fig. 2.3, p. 19) while microbial biomass increased (Jones *et al.* 1990). The pattern does not hold for gross mineralization rates. Aranibar *et al.* (2003) found higher gross mineralization rates in annually burned plots across several EBP's. In their study, Aranibar *et al.* (2003) used the natural abundance of ^{15}N as a proxy for nitrogen cycling and found a lack of relationship between fire and $\delta^{15}N$ values of soil and woody vegetation.

The effects of fire on nitrogen fixation are also inconclusive. Enhanced phosphorus in the post-burn ash may facilitate N_2 fixation, but it is also possible that the emhanced availability of N following fires might decrease N_2 fixing. At Satara and Skukuza in the KNP, frequent fire decreased the proportions of woody N_2 fixing species (Aranibar *et al.* 2003).

Thus the strong fire effects on nitrogen cycling which exist in other systems, such as prairie and oak savanna, seem to be lacking in KNP savannas. This lack of fire effect might be the result of N inputs such as atmospheric deposition, N deposition by grazers, and N_2 fixation associated with forbs, grasses and free-living soil microorganisms (Blair 1997, Aranibar *et al.* 2003).

In this thesis, I examine the long-term effects of fire frequency and season on productivity and nitrogen cycling. The nitrogen cycle I define as both the amount of nitrogen stored in various components (pools, *e.g.*, vegetation, soil) of the system and the rate of nitrogen transfer among these components (fluxes). Specific questions that I want to address with this thesis include:

1. What are the effects of 50 years of fire exclusion (and 50 years of annual burning) on the N cycle? What are the effects of burning in different seasons (and at different intensities) on the N cycle?
2. What are the effects of 50 years of annual burning on plant functional traits in grasses? Would shifts in plant functional traits accompany fire-related changes in nitrogen cycling?
3. If N is retained in the system despite frequent fire, what are the possible causes?

The thesis is basically divided into two main themes: one (Chapter 3 to 6) that deals with the effects of fire on nitrogen cycling and another (Chapter 7 and 8) that investigate more deeply the findings of the first section and possible causes of the findings. Above- and below-ground pools (stocks), and cycling rates of nitrogen were determined (first three data chapters, Chapter 3, 4 and 5, Fig.1.4). In Chapter 6 I measured traits related to tissue longevity and nitrogen use efficiency in order to establish the relationships between grass species composition, nitrogen use efficiency, and fire frequency. Trait trade-offs occur with differing levels of disturbance and plant traits can be used as predictors of species response to environmental factors because they represent specific functional adaptations to environmental conditions. Trait trade-offs have been found across the fire gradient elsewhere and the traits have been correlated with responses to changes in nitrogen availability with varying fire frequencies (Fynn *et al.* 2005). A 'trade-off spectrum' exists among functional traits with, at one end of the spectrum, species with fast-tissue turnover and high potential for resource capture and, at the other, species with slower tissue turnover and traits that emhance resource conservation (Diaz and Cabido 1997, Diaz *et al.* 1999, Diaz *et al.* 2004). Tissue longevity, in turn, has been linked to nitrogen use efficiency.

In Chapter 7, I determined the $\delta^{15}N$ of various ecosystem fragments (*e.g.*, soil organic matter, inorganic nitrogen, and tree and grass foliage) and used it as a proxy of how fire may affect nitrogen cycling. Changes in N cycling through time were determined through an examination of the natural abundance of ^{15}N in tree-rings (Chapter 8). I expected that if fire had an effect on foliar $\delta^{15}N$, it would be most pronounced in the grass layer as their roots are in the zone most likely influenced by fire. This chapter investigates how nitrogen cycling 'openness' changed since the advent of the EBP experiment and over time by using $\delta^{15}N$ values as a proxy. Chapter 9 is a synthesis of the data chapters and includes future perspectives.

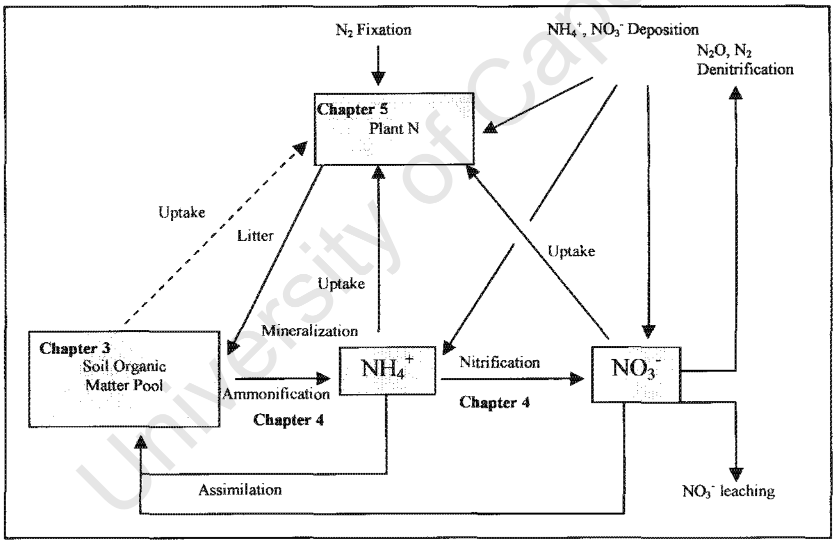

Figure 1.4. Nitrogen transformations, processes and pools and which Chapter deals with each (where relevant). Modified from Nadelhoffer and Fry (1994).

CHAPTER SUMMARY

This introductory chapter presents the broad questions that I wanted to answer with this study. The first section sets the work within the context of savanna dynamics and shortly introduces the short- and long-term effects of fire on nitrogen cycling. Thereafter I discuss the effect of fire on the external and internal nitrogen cycle. I review two previous models on the effect of fire on nitrogen dynamics in prairie and Oak savanna. Lastly, a section considers past research on the effect of fire on nitrogen cycling in the Kruger National Park and I discuss shortly the scope of each Chapter.

I used the two models that represent the most extensive fire research done to date on the long-term effects on nitrogen dynamics. These models contribute to the perception that regular fire leads to the loss of nitrogen which in turn decreases ecosystem nitrogen fluxes and pools in the long-term. Research done in Africa and South America on the other hand, indicates that nitrogen lost by fire is replaced in some way (*e.g.*, through N deposition or fixation). Previous research done in the Kruger National Park also suggests that nitrogen loss through fire is not as substantial as previously believed or that N lost is compensated by N inputs. This work has largely been prompted by this divergence in the findings on the effects of fire on nitrogen cycling.

CHAPTER 2

BACKGROUND TO THE KRUGER NATIONAL PARK'S MANAGEMENT FRAMEWORK, THE EXPERIMENTAL BURN PLOTS AND A DESCRIPTION OF THE STUDY AREA

INTRODUCTION

The main aim of this study is to examine the long-term effects of specific fire regimes on the productivity and nutrient cycling of a broad-leaf nutrient-poor savanna. In order to achieve this goal, measurements were made on the long-term Experimental Burn Plots (EBP) in the Kruger National Park (KNP). The Sourveld EBP Plots are situated in the Lowveld Sour Bushveld in the south-west section of the KNP. These plots are part of a long-term fire experiment that has been initiated more than 50 years ago and provide a unique opportunity to study the long-term effects of specific fire regimes. This chapter includes a brief history of fire management in the KNP and an introduction to the EBP's. The chapter also gives a background to the biophysical characteristics of the Sourveld Plots.

THIS STUDY AND THE BROADER KNP MANAGEMENT FRAMEWORK

The 1986 Masterplan for the Kruger National Park (Joubert 1986, with addenda 1993) provided a cohesive plan for the management of the Park. This plan stipulated that the document and its policies should be formally reviewed every five years with changing circumstances and new insights (Braack *et al.* 1997). The review process culminated in the creation of Volume VII; an objectives hierarchy for the management of the KNP (Braack *et al.* 1997). The objectives hierarchy was reviewed post-1993 and the current objectives are summed in Supporting document 1; Kruger Park Management Plan October (2006).

The mission statement of the KNP is "*to maintain biodiversity in all its natural facets and fluxes and to provide human benefits in keeping the mission of South African National Parks in a manner which detracts as little as possible from the wilderness qualities of the Kruger National Park*". Biodiversity in this context should be interpreted as described by Noss (1990) and incorporates compositional, structural and functional attributes at four levels of organization (genes, species, communities and landscape). The mission statement can be fragmented into various "Objectives" and these are aimed at supporting and ensuring achievement of the Mission. Objectives are merely statements of intent and are not necessarily achievable. The objectives are used to support the achievement of the Mission by setting goals which are achievable, testable and auditable targets (Braack *et al.* 1997).

To maintain biodiversity effectively Thresholds of Potential Concerns (TPC's) were developed to track changes that occur and intervene when these changes are due to unacceptable human influence

(Braack *et al.* 1997). Thresholds of potential concern (TPC's) are upper and lower levels of acceptable change along a continuum of change for a selected environmental indicator (Biggs and Rogers 2003). When the upper or lower level of acceptable change is reached or modeling predicts it will be reached, an assessment of the causes of the extent of change are made and result in either a management action or a re-calibration of the threshold to a more realistic or meaningful level (Braack *et al.* 1997, Van Wilgen *et al.* 1998, Biggs and Rogers 2003). TPC's form the basis of an inductive approach to adaptive management because they are invariably hypotheses of limits of acceptable change in ecosystem structure, function, and composition (Biggs and Rogers 2003).

Research plays an important part in the setting of goals, the measuring of goal attainment and the monitoring of biodiversity change. No research can exist in isolation and it makes sense that research conducted in the KNP should fit into the well-defined objectives framework. The biodiversity objective under the Mission Statement includes various subgroup objectives of which the Ecosystem Objective is one. The research objective aims to "promote through an integrated approach an understanding of the influences of factors (*e.g.*, fire, herbivory, disease, predation, pollination, nutrient cycling) affecting ecosystem diversity and dynamics". Two sub-objectives are relevant to this work, namely fire and nutrient cycling. The exact objectives that this study falls under are illustrated in Fig. 2.1 for fire research and Fig.2.2 and these figures are simplified adaptations of the objective hierarchy from Supporting document 1, Kruger Park Management Plan October 2006.

THE FIRE MANAGEMENT HISTORY AND EXPERIMENTAL BURN PLOTS (EBP'S) OF THE KRUGER NATIONAL PARK

Fire management in Kruger has gone through many different stages as scientific knowledge improves and management focus changed (O'Regan 2005). Fire management can be divided into six phases since the establishment of the Kruger National Park (Van Wilgen *et al.* 1998, Van Wilgen *et al.* 2000, Van Wilgen *et al.* 2004). From 1926 to 1947, limited burning was very occasionally allowed to provide green grazing for wildlife. From 1948 to 1956, firebreaks were implemented to aid protection against fire. Limited prescribed burning took place but no area was burned more than once every five years. The exclusion of fires led to very high fire intensities as a result of which the EBP experiment was initiated in the early 1950's to investigate the effects of fire on vegetation structure.

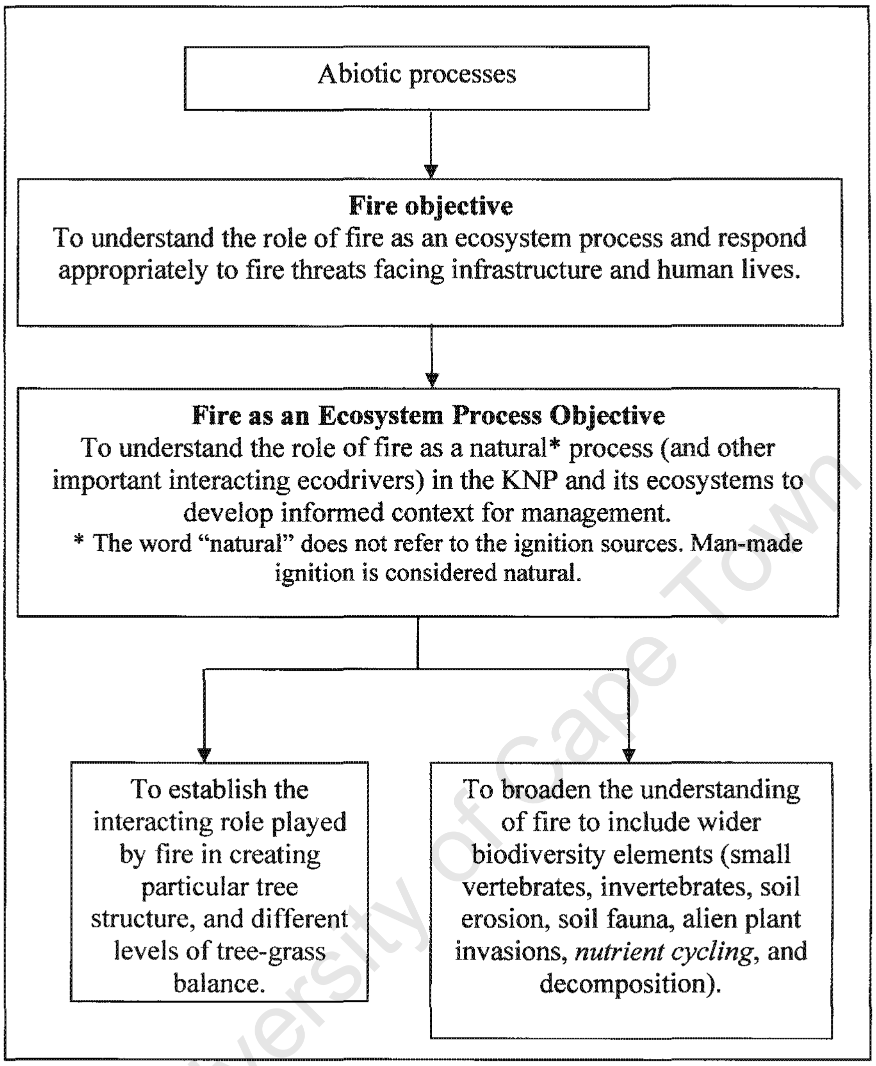

Figure 2.1. Fire research objectives and sub-objectives pertinent to this study, (Supporting document 1. Kruger Park Management Plan October 2006).

From 1957 to 1980, prescribed burns took place in early spring after the first rains at intervals of three years. From 1981 to 1991, the fixed prescribed burning was amended to time fire according to fuel load, post-fire age and mean annual rainfall. Fire season was also varied between late winter, mid-summer and autumn. From 1992 to 2001, "natural" fires (lightning-ignited) were allowed to burn freely and fires of human origin were suppressed. At the present time, point-ignitions are used to start fires and the present fire policy includes a combination of patch burns, fire suppression in areas where fire is not considered necessary and tolerance of all lightning fires. Fires are ignited in suitable areas after grass-sward composition and grass fuel loads are measured.

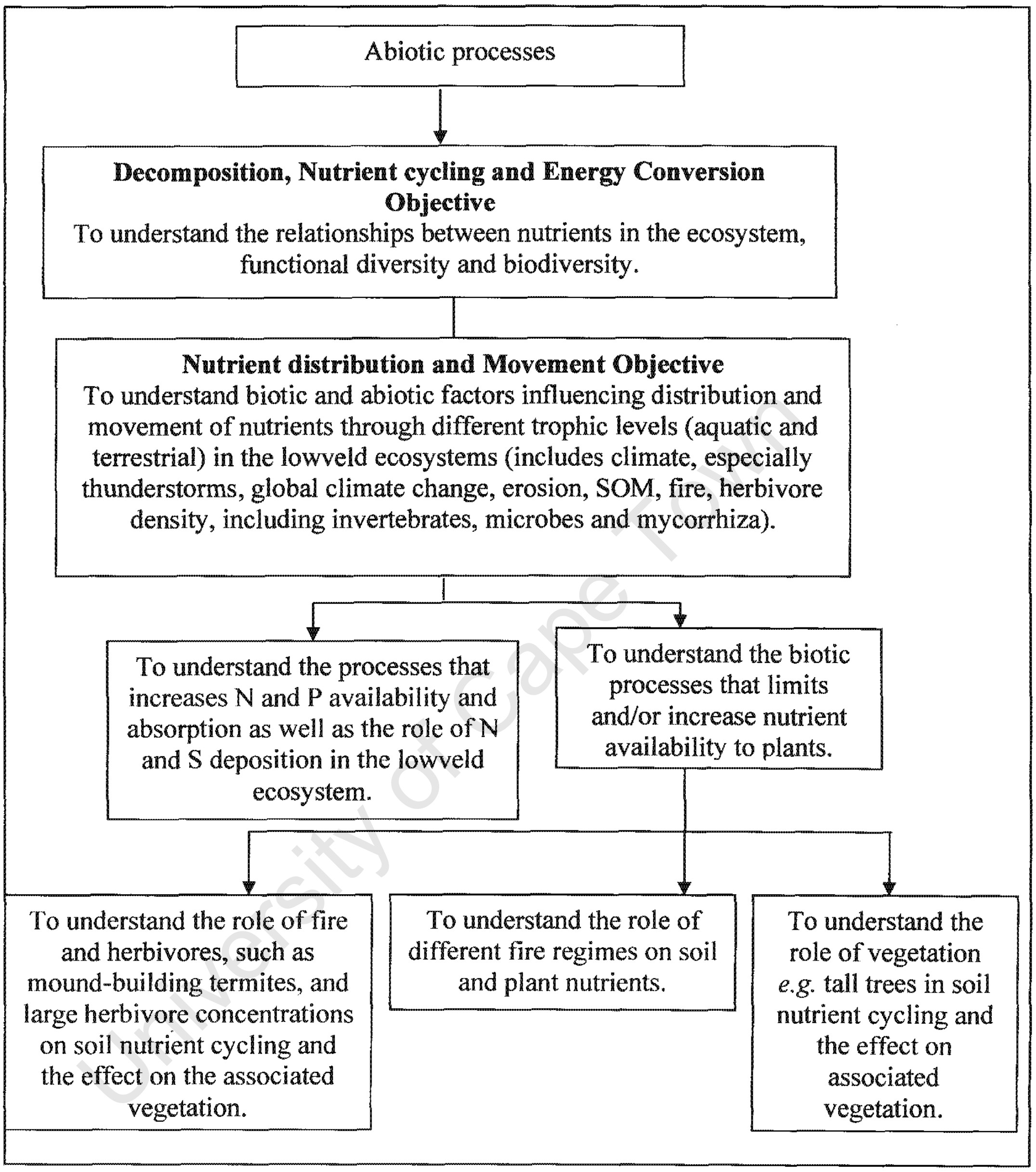

Figure 2.2. Nutrient research objectives pertinent to this study (Supporting document 1. Kruger Park Management Plan October 2006).

Point-ignited fires are allowed to burn until a monthly target is reached and the target area increases (≤ 50%) with increasing grass biomass at the start of the fire season. This policy is adaptive management driven and promotes heterogeneity of effects on the biota, thereby promoting and maintaining biodiversity (Van Wilgen *et al.* 2003).

More recent fire management policies (early 1990's to 2002) have come about under the influence of non-equilibrium theories of savanna dynamics (Van Wilgen *et al.* 2003). Non-equilibrium models emphasize that the changes in tree abundance are caused by the interactions between organisms (biotic instability), disturbance and environmental stochasticity, which are superimposed on the environmental template created by climate and edaphic factors (Coughenour and Ellis 1993, Gillson 2004). Savannas are seen as independent ecosystem entities where disturbance, such as fire, mediates the coexistence of the tree-grass components. Fire management policies affect spatial heterogeneity of the fires, seasonal distribution and intensity (Van Wilgen *et al.* 2004).

The EBP experiment in the Kruger National Park is one of few such long-term experiments worldwide (Aldous 1934, Tainton and Mentis 1983, Ojima *et al.* 1994, Peterson and Reich 2001, Russell-Smith *et al.* 2003). The EBP's were established on four of the six major vegetation types in the KNP during 1954. The fire research program was initiated to address problems that arose from the suppression of fire in the Park pre-1954 (Trollope *et al.* 1998). These problems included serious deterioration in the nutritional value of the grazing, and a significant increase in the density and volume of the woody vegetation (Van der Schijff 1958, Trollope *et al.* 1998). The vegetation types in which the experiment was laid out include Mopani shrubveld between Shingwedzi and Letaba, *Sclerocarya/Acacia nigrescens* savanna at Satara, *Combretum* woodland at Naphe, and *Terminalia* woodland at Pretoriuskop (see Fig. 2.3).

The set of treatments together (usually 12) is referred to as a 'string'. The treatments in every string include high intensity, biennial and triennial August and October burns, intermediate intensity, biennial and triennial April burns, as well as an annual August burn, and low intensity, biennial and triennial December and February burns (Biggs *et al.* 2003). Each of the four regions has four strings (or repeats); the Mopani region (Dzombo, Nshawo, Mooiplaas and Tsende), the Satara region or Knobthorn/Marula plots (Satara, N'wanetsi, Marheya and Lindanda), the Naphe region or Combretum plots (N'waswitshaka, Skukuza, Napi and Biyamiti) and the Pretoriuskop region or Sourveld plots (Shabeni, Numbi, Kambeni, and Fayi). This study took place in the Pretoriuskop region at the Sourveld plots (see Fig. 2.3).

Figure 2.3. A map of the Kruger National Park showing the location of the four Experimental Burn Plot sites. Landscapes are according to Gertenbach (1983).

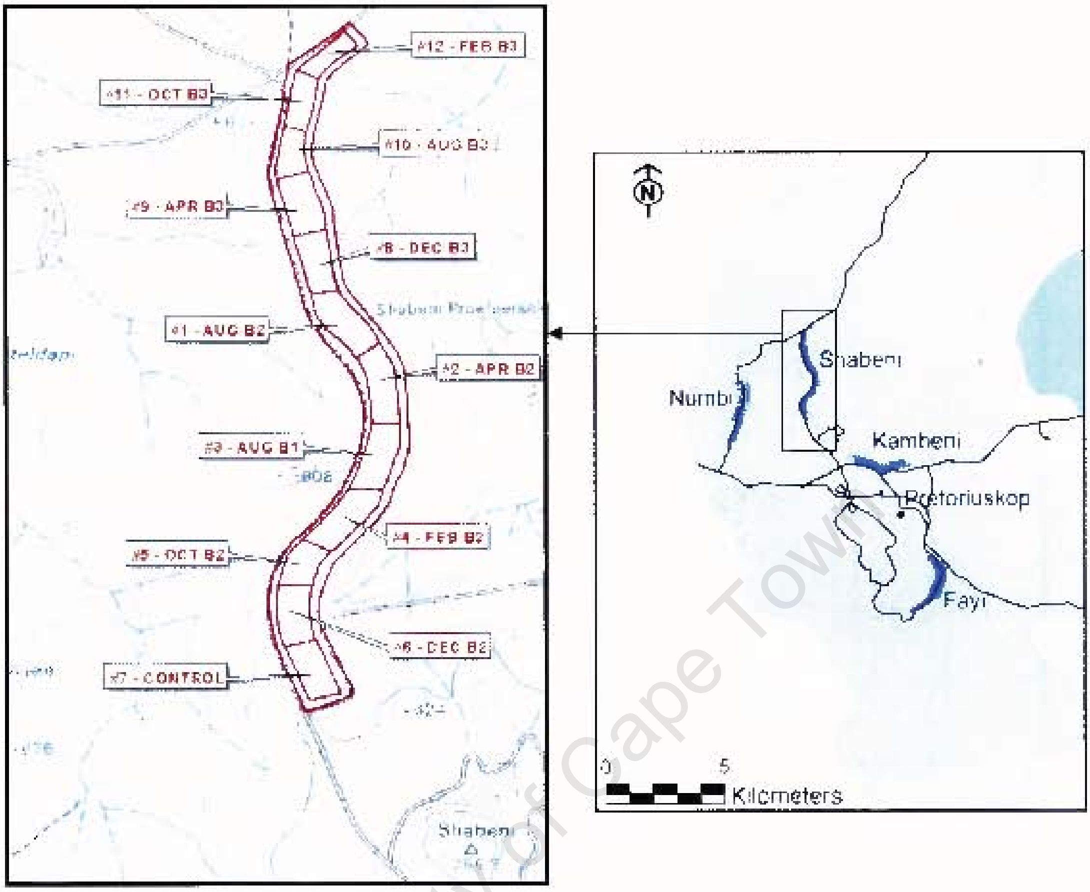

Figure 2.4. The south-western section of the Kruger National Park with the Sourveld Experimental Burn Plots with more detail for Shabeni. The four treatments in the Shabeni string at which permanent sampling points were implemented were Aug 1 (annual burn), Aug 3 (triennial August burn), Feb 3 (triennial February burn) and FE (fire exclusion).

EBP'S AND TREATMENTS RELEVANT TO THIS STUDY

The study focused on the Sourveld EBP's which are situated in the Pretoriuskop area. Intensive measurements were made at the Shabeni string, but the other strings (*i.e.*, Numbi, Kambeni, and Fayi) were used in some instances as replicates. Four treatments were selected for this study, the fire exclusion or no burn (FE) (Fig. 2.5), annual burn in late winter (Aug 1) (Fig. 2.6), triennual burn in late winter (Aug 3), and triennial burn in late summer (Feb 3) (see Fig. 2.4 for Shabeni string). Each plot is approximately 370 m x 180 m and covers ~ 7 ha. To establish the long-term effects of fire on productivity and nitrogen cycling, monthly measurements were implemented at Shabeni. These measurements included grass clipping for herbaceous biomass estimates and the measurement of *in situ* net N mineralization. Ten permanent sampling points were set up in every treatment. The sampling points were situated on two transects parallel to each other across the plots in a north-south direction. Each sampling point was 30 m apart, marked with a Y-standard and fitted with a number.

Two 1 m^3 cages were placed in the vicinity of a Y-standard (for a total of 16 per treatment): one cage to measure herbaceous biomass accumulation and another fitted with a shade-cloth bottom for leaf litter. The cages were constructed from 100 mm x 100 mm square grids, 6 mm diameter steel concrete reinforcing. Other measurements taken at the permanent sampling points were fine root biomass, fine root growth rates, and resin-bag inorganic N accumulation.

Table 1.1. The permanent sampling points at the Shabeni string. Points are either under a canopy (C) of a large tree (large trees are taller than 3 m with spreading crown), subcanopy (SC) which is in the vicinity of a large tree but not under the crown or amongst smaller trees with variable influence of leaf litter or shade, otherwise in the open (O).

Treatment	1	2	3	4	5	6	7	8	9	10
FE	C	C	SC	SC	C	C	SC	O	SC	SC
Aug 1	SC	SC	SC	SC	C	O	O	SC	C	C
Aug 3	O	SC	O	SC	C	SC	O	SC	SC	O
Feb 3	C	SC	O	O	SC	SC	SC	O	O	C

Figure 2.5. The fire exclusion plot at Shabeni.

Figure 2.6. The August annual burn at Shabeni.

BIOPHYSICAL CHARACTERISTICS

Geographical location

The Kruger National Park (KNP) is located in the northeast of South Africa and spans two provinces, the Northern (Limpopo) and Mpumalanga provinces (22°25′ to 25°32′ S and 30°50′ to 32°2′ E). The park covers an area of about 2 million hectares, but forms part of a much bigger conservation area, which include private game reserves, and transfrontier areas extending into Mozambique and Zimbabwe.

Climate

The Sourveld Plots fall into the lowveld bushveld zone with a rainfall of 500-700 mm yr^{-1} (mean annual precipitation is 677 mm for this vegetation type, Mucina and Rutherford 2006) and potential evaporation of 6 mm day^{-1} (Venter *et al.* 2003), but the long-term average for Pretoriuskop is 743.6 mm (Gertenbach 1983). The rain occurs mainly in summer (October to April), with some out of season showers (Fig. 2.7). Most of the rainfall is delivered in powerful convective thunderstorms which can lead to high erosion rates on unprotected soil. Temperatures are generally warm and winters are very moderate (temperatures seldom below 10°C and frost mostly absent). Summer temperatures, however, can be very high with high humidity. Winter, on the other hand, has low humidity. Humidity is an important factor in controlling the rate of spread and intensity of fires (Venter *et al.* 2003).

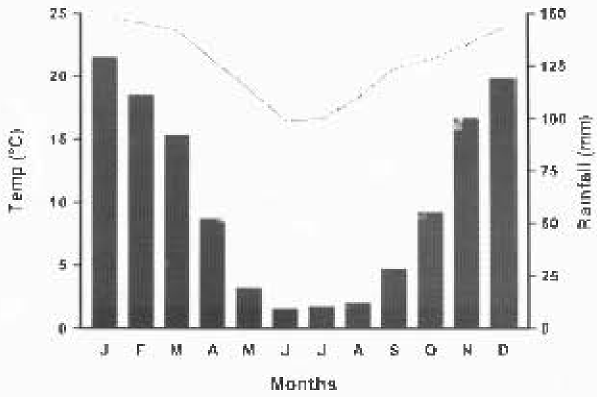

Figure 2.7. Rainfall (mm) and temperature data (°C) for Pretoriuskop (data from Scientific Services, Skukuza).

Geomorphology and soils

The Sourveld Plots lie within the 'moderately undulating plains' Landform Type, which has a small percentage of areas of slopes more than 15° (~2%) and around 20% of slopes less than 2° (Venter *et al.* 2003). The KNP is roughly divided into north-south bands of different geology with underlying granitic rock in the west and basaltic rock in the east (Venter *et al.* 2003). Granitic rocks of the Basement complex underlie the Sourveld Plots and the landscape has very distinctive catenal sequences of soils from crests to valley bottom. At the crest, reddish or yellowish brown sand; midslope, gray hydromorpic sand and clay (known as seeplines; seasonally waterlogged bands of soil on the contour); footslopes, grayish brown sodic duplex soils (better known as sodic sites referring to the presence of sodium), and mixed alluvial soil; valley bottoms, complex association of deep brown sand and calcareous clay as well as rocky outcrops (Venter *et al.* 2003). The experimental burn plots are located on midslope to crest and sodic sites are excluded.

The soil underlying the Sourveld Plots is formed from basement granites (mostly on crests or midslope). The plots feature deep red sandy loam soils with minor areas of duplex soils, shallow soils, or clay soil associated with intrusions of basic lavas (Unpublished Report). The soil is usually at least 80 cm deep (in a few cases deeper than the height of the auger, ~1.2 m), and contains between 18 and 20% clay on the crests and hillslopes (Fischer 2006). As a result of the low clay-forming potential and the coarse texture of the Quartz and K-feldspar minerals in the granites, these soils are highly leached as soluble products wash out (Venter *et al.* 2003).

Footslopes are nutrient-rich as fine materials are leached from sandy hilltops and crests. The soil of the area has been classified (Venter 1990), and includes the Hutton (Orthic A over Red Apedal B), Bainsvlei (Orthic A over Red Apedal B over Soft Plinthic B), Clovelly (Orthic A over Yellow-brown Apedal B), Cartref (Orthic A over E horizon over Lithocutanic B) and Avalon Forms (Orthic A over Yellow-brown Apedal B over Soft plintic B) (Forms according to Soil Classification Working Group

1991). Soils of the Shabeni string are mostly sandy loam and sandy clay loam and, on the lower slopes, Kroonstad and Longlands form. In terms of the World Reference Base Classification system soils at the Sourveld Plots include Rhodic Lixisols, Paraplinthic Cambisols, Dystric Regosols, and Paraplinthic Lixisols (FAO 1998). Granite soils from the Sourveld EBP's contain an average of 0.87 % carbon and 0.05 % nitrogen with an average of 200 mg kg^{-1} total phosphorus (Feig 2004).

Vegetation

The soils of the KNP are very closely associated with distinct vegetation assemblages. Granitic parent material gives rise to nutrient-poor, sandy soils (Scholes *et al.* 2003). Savannas on the nutrient-poor soils tend to be dominated by deciduous, broad-leaf trees in the families Combretaceae (especially *Combretum* and *Terminalia* spp.) and Ceasalpinaceae. These species are undefended (low nitrogen and therefore not very palatable) or chemically protected from mammal herbivory (no thorns). The grasses are often unpalatable, wiry, and sparse or tall and thatchy, although the more palatable *P. maximum* grows in association with trees.

The vegetation around Pretoriuskop has been classified as Lowveld Sour Bushveld by Gertenbach (1983). This landscape type correlates with the KNP 1 Lowveld Sour Bushveld of Acocks (1988) or the SVl 10 Pretoriuskop Sour Bushveld (Mucina and Rutherford 2006). The vegetation of the Shabeni EBP varies with fire treatment, but is mostly open tree savanna with relatively few shrubs, and a well-developed herbaceous layer. The woody layer is dominated by a tree *Terminalia sericea* Burch. Ex DC., and the shrub *Dichrostachys cinerea* Wight & Arn. Other species of importance include *Sclerocarya birrea* (A. Rich.) *Hochst*, *Strychnos madagascariensis* Poir., *Combretum collinum Fresen.* subsp. *suluense* (Engl. & Diels) Okafor, *C. zeyheri* Sond., *C. apiculatum* Sond., *C. molle* R. Br. Ex G. Don, *Ximenia caffra* Sond.. The Pretoriuskop EBPs are located in two distinct vegetation types, namely the open and the closed *Terminalia sericea* \ *Combretum* woodlands of the Pretoriuskop region (O'Regan 2005). The Numbi and Shabeni replicates are in the open *Terminalia sericea* \ *Combretum* woodlands, and the Kambeni and Fayi replicates are in the closed *Terminalia sericea* \ *Combretum* woodlands. See Table 2.2 for a summation of woody vegetation density and the most common southern EBP tree species.

The herbaceous layer is tall and dense including both unpalatable species of grass such as *Hyperthelia dissoluta* (Nees ex Steud.) Clayton, *Elionurus argenteus* (Spreng.) Kunth, *Hyparrhenia hirta* (L.) Stapf, *H. filipendula* (Hochst.) Stapf var *pilosa* (Hochst.) Stapf, *Schizachyrium sanguineum* (Retz.) Alst., *Pogonarthria squarrosa* (Roem. & Schult.) Pilg., and more palatable grass species such as *Digitaria eriantha* Steud., *Panicum maximum* Jacq., *Heteropogon contortus* (L.) Roem. & Schult., *Eragrostis superba* Peyr., *Urochloa mosambicensis (Hack.) Dandy*, *Setaria sphacelata (Schumach.) Moss* var *sphacelata*, *S. Sphacelata* var *torta* (Stapf) Clayton, *Diheteropogon amplectens* (Nees)

Clayton, and *Themeda triandra* Forssk. (Gertenbach 1983). See Table 2.3 for a list of the grass species on the southern EBP's.

Scientific nomenclature

Scientific nomenclature follows Gibbs Russel *et al.* (1990) and Retief and Herman (1997).

CHAPTER SUMMARY

This chapter places the study within the broader Kruger National Park management framework and addresses the two sub-objectives of the Management Plan within which the study's objectives and aims fall. The history and the lay-out of the experimental burn plot experiment are discussed, as well as the biophysical characteristics of the Sourveld Plots. Most of the study focused on the Shabeni string of the Sourveld Experimental Burn Plots (Pretoriuskop), but the other strings (*i.e.*, Numbi, Kambeni and Fayi) were used as replicates where applicable.

APPENDIX

Table 2.2. Plant density and dominant woody species for the different fire frequencies across all four southern EBP strings, compiled using O'Regan (2005).

Fire frequency	Height class	Plants/ha	Dominant woody species
Annual	< 1 m	> 19000	*Dichrostachys cinerea* *Catunaregam spinosa* *Terminalia sericea* *Strychnos madagascariensis*
	1 – 3 m	> 4500	*Dichrostachys cinerea* *Terminalia sericea*
	3 – 5 m	< 310	*Dichrostachys cinerea* *Terminalia sericea*
	> 5 m	< 230	*Terminalia sericea* *Strychnos madagascariensis* *Dichrostachys cinerea* *Sclerocarya birrea*
Triennial	< 1 m	< 14500	*Dichrostachys cinerea* *Terminalia sericea* *Maytenus senegalensis* *Catunaregam spinosa*
	1 – 3 m	> 7500	*Dichrostachys cinerea* *Terminalia sericea* *Maytenus senegalensis* *Diospyros lycioides*
	3 – 5 m	> 600	*Dichrostachys cinerea* *Terminalia sericea* *Acacia gerrardii*
	> 5 m	> 250	*Dichrostachys cinerea* *Peltophorum africanum* *Acacia gerrardii*
Fire exclusion	< 1 m	> 20000	*Euclea natalensis* *Ochna natalitia* *Dichrostachys cinerea* *Maytenus senegalensis*
	1 – 3 m	>12000	*Dichrostachys cinerea* *Terminalia sericea* *Ochna natalitia*
	3 – 5 m	> 2000	*Terminalia sericea* *Dichrostachys cinerea* *Peltophorum africanum*
	> 5 m	> 400	*Terminalia sericea* *Sclerocarya birrea*

Table 2.3. Grass species lists and classifications for four southern EBP strings, data from Scientific services, KNP.

Code	Species	Palatability	Physiology
Ariads	*Aristida adscensionis*	Unpalatable	C4, NADP-me
Ariconcon	*Aristida congesta* var *congesta*	Unpalatable	C4, NADP-me
Ariconbar	*Aristida congesta* var *barbicollis*	Unpalatable	C4, NADP-me
Botbla	*Bothriochloa bladhii*	Unpalatable	C4, NADP-me
Botins	*Bothriochloa insculpta*	Unpalatable	C4, NADP-me
Brabri	*Brachiaria brizantha*	Palatable	C4, PCK
Branig	*Brachiaria nigropedata*	Palatable	C4, PCK
Cymexc	*Cymbopogon excavatus*	Unpalatable	C4, NADP-me
Cymval	*Cymbopogon validus*	Unpalatable	C4, NADP-me
Cyndac	*Cynodon dactylon*	Intermediate	C4, NAD-me
Digarg	*Digitaria argyrograpta*	Palatable	C4, NADP-me
Digeri	*Digitaria eriantha*	Palatable	C4, NADP-me
Diglon	*Digitaria longiflora*	Intermediate	C4, NADP-me
Dihamp	*Diheteropogon amplectens*	Palatable	C4, NADP-me
Eragum	*Eragrostis gummiflua*	Unpalatable	C4, NAD-me
Erahet	*Eragrostis heteromera*	Intermediate	C4, NAD-me
Eraina	*Eragrostis*	Intermediate	C4, NAD-me
Eraleh	*Eragrostis lehmanniana* var. *lehmanniana*	Intermediate	C4, NAD-me
Erarig	*Eragrostis rigidior*	Unpalatable	C4, NAD-me
Erasup	*Eragrostis superba*	Intermediate	C4, NAD-me
Erista	*Eriochloa stapfiana*	Intermediate	C4, NAD-me
Hetcon	*Heteropogon contortus*	Intermediate	C4, NADP-me
Hypdis	*Hyparrhenia dissoluta*	Unpalatable	C4, NADP-me
Hypfil	*Hyparrhenia filipendula* var. *pilosa*	Intermediate	C4, NADP-me
Loufla	*Loudetia flavida*	Unpalatable	C4, NADP-me
Lousim	*Loudetia simplex*	Unpalatable	C4, NADP-me
Melrep	*Melinis repens*	Unpalatable	C4, PCK
Miccaf	*Microchloa caffra*	Unpalatable	C4, PCK
Pandeu	*Panicum deustum*	Palatable	C4, PCK
Panmax	*Panicum maximum*	Palatable	C4, PCK
Passcr	*Paspalum scrobiculatum*	Intermediate	C4, NADP-me
Perpat	*Perotis patens*	Unpalatable	C4, NAD-me
Pogsqu	*Pogonarthria squarrosa*	Unpalatable	C4, NAD-me
Schsan	*Schizachyrium sanguineum*	Unpalatable	C4, NADP-me
Setsph	*Setaria sphacelata* var. *sphacelatata*	Palatable	C4, NADP-me
Spoafr	*Sporobolus africanus*	Intermediate	C4, PCK
Spoioc	*Sporobolus ioclados*	Palatable	C4, NAD-me
Thetri	*Themeda triandra*	Palatable	C4, NADP-me
Traber	*Tragus bertorianus*	Unpalatable	C4, NAD-me
Traspi	*Trachypogon spicatus*	Intermediate	C4, NADP-me
Trigra	*Trichoneura grandiglumis*	Unpalatable	C4, NAD-me
Trimon	*Tricholaena monachne*	Intermediate	C4, PCK
Uromos	*Urochloa mosambicensis*	Intermediate	C4, PCK
Urooli	*Urochloa oligotricha*	Intermediate	C4, PCK
Uropan	*Urochloa panicoides*	Unpalatable	C4, PCK

CHAPTER 3

FIRE AND VEGETATION EFFECTS ON TOTAL SOIL NUTRIENTS (NITROGEN, CARBON AND PHOSPHORUS) IN A BROAD-LEAF SAVANNA, KRUGER NATIONAL PARK.

INTRODUCTION

Savannas are shaped by agents of disturbance, and the biota of savannas has evolved in the presence of fire (Menaut *et al.* 1993). Fire frequency, intensity and season affect both environment and biota directly and indirectly. Fires alter energy, nutrient and water fluxes between soil, plants and the atmosphere by reducing plant biomass and litter, and these changes may influence the long-term nutrient status and productivity of the system (Frost and Robertson 1987). Fires also kill organisms, damage living tissue, modify growth and reproductive rates, change resource availability and use and alter competitive and other interactions between organisms (Frost and Robertson 1987). Resources such as nutrients and nitrogen in particular, in turn, affects ecosystem characteristics including rates of primary productivity, organic matter decomposition, plant water use efficiency, diet selection of consumer organisms and plant species composition (Risser and Parton 1982) . The aim of this chapter is to examine the effect of specific fire regime on total soil carbon (C), nitrogen (N) and phosphorus (P).

Nitrogen and phosphorus are found in organic form (*i.e.*, part of carbon-based molecules) for the major biological portion of their cycles and need to be liberated from the carbon bond before becoming available for plant uptake (*i.e.*, inorganic or elemental form) (Scholes and Walker 1993). Soil carbon and nitrogen are tightly linked and their proportion relative to each other control the mineralization of N. Frequent fire decreases (Bird *et al.* 1999), increases (Trapnell *et al.* 1976, Ojima *et al.* 1990) or has no effect on total soil C (Blair *et al.* 1998, Wan *et al.* 2001). The effect of fire on soil organic C is variable because the combustion of carbon varies with fire intensity and frequency and C inputs with vegetation structure and season of burn (Bird *et al.* 2000). Decomposition of leaf and root litter makes a contribution to soil organic matter and replenishes C lost through burning (Fynn *et al.* 2003). Higher soil carbon with regular burning occurs in some instances where charcoal accumulates after fire (Frost and Robertson 1987).

The amount of nutrients lost with fire and the accompanying effect on soil nutrients is strongly associated with vegetation structure (Kauffman *et al.* 1994, DeCastro and Kauffman 1998, Reich *et al.* 2001). The tree:grass ratio governs fuel load, vegetation structure and vegetation nutrient status, and the associated effects on N loss. The presence of trees also alters soil chemistry; higher soil nutrients are often associated with tree canopies (Kellman 1979, Callaway *et al.* 1991, Belsky 1994, Scholes and Archer 1997). Fire seldom decreases total soil N (Binkley *et al.* 1992, Kauffman *et al.* 1994, Blair *et al.* 1998, Wan *et al.* 2001, Aranibar *et al.* 2003). However, there have been some cases where a decrease has been reported (Menaut *et al.* 1993, Aranibar *et al.* 2003). Nitrogen loss during fire occurs through volatilization and wind-born transport of particulate matter (Bustamante *et al.* 2006). It has been suggested that lost N is replaced through N fixation and N deposition (Medina 1982, Frost and Robertson 1987, Aranibar *et al.* 2003).

In a meta-analysis done by Wan *et al.* (2001) which included studies done in chaparral, prairie, forests, and tropical savannas (*i.e.*, India, South America and Australia) fire had no significant influence on total soil N amount or concentration. For tropical savannas from West Africa, South America and India, fire had either no effect on total soil carbon and nitrogen or the effects depended on the season of burn (Menaut *et al.* 1993, Kauffman *et al.* 1994). Early burn and no burn plots had comparable soil nitrogen status in West African savannas, but late burning (at the onset of the wet season) led to the impoverishment of the soil.

Soil phosphorus (P) concentrations have implications for the nitrogen cycle and might also limit plant productivity (Blair *et al.* 1998). Phosphorus volatilizes at high temperatures and burning converts phosphorus to an inorganic form that is returned to the soil surface in ash and is readily usable by plants (Eisele 1990, Menaut *et al.* 1993). Frequent fire often increases concentrations of P and in the long term, P tends to be higher in burned plots (Menaut *et al.* 1993, Ojima *et al.* 1990). The high volatilization temperatures and the relative insolubility of P which makes it less vulnerable to leaching (Frost and Robertson 1987), both contribute to the insensitivity of P to fire effects. Inputs of inorganic P may stimulate N fixation which contributes to the sustained productivity of the system and might also replenish N lost through burning (Seastedt and Ramundo 1990, Menaut *et al.* 1993, Kauffman *et al.* 1994).

Fire effects on total soil nutrients are highly variable in South African systems. For South African grasslands at Ukulinga in KwaZulu/Natal, frequent fire decreased organic C only in the surface 2 cm of the soil while total N decreased in the surface 6 cm (Fynn *et al.* 2003). In the Kruger National Park, fire research has been conducted at the Experimental Burn Plots (EBP's) which consists of 12 treatments at four strings at four locations (see Fig. 2.3, p. 19). Long-term fire exclusion increased organic carbon and nitrogen at the N'wanetsi string of the Knobthorn/Marula EBP plots in semi-arid savanna. This could not, however, be tested statistically (Jones *et al.* 1990). For other strings in semi-arid savanna (Satara, Marheya and N'wanetsi), fire exclusion increased organic carbon but had no effect on total soil nitrogen (Shackleton and Scholes 2000). At N'waswitshaka, Skukuza, and Satara, frequent burning increased soil C:N ratios. Total soil N was not affected, except in the Skukuza string in the Naphe region where fire exclusion increased total soil N (Aranibar *et al.* 2003).

To investigate the role of fire in 'shaping' nutrient budgets, net changes in total soil C, N and plant available P, were measured in a broad survey of different fire treatments at the Shabeni string. The effects of fire on inorganic N, which represents less than 2 % of total soil N, are addressed in Chapter 4. Total C, N and plant available P was sampled to a depth of 10 cm, which is where the majority of changes are likely to occur (Raison 1979). Secondly, in three treatments (Aug 1, Aug 3 and FE), I examined the effect of fire on different depths to test whether there where changes in the surface layers and below 10 cm. Finally, fire exclusion increases tree cover and as soil nutrients can be higher under tree canopies, I measured soil C and N under large canopies and in the open.

METHODS

Study area

Most of the measurements took place at the annual burn (Aug 1), triennial August (Aug 3), triennial February (Feb 3), and fire exclusion (FE) treatments of the Shabeni string of the sourveld EBP's. The area in which this string is situated as well as an overview of the EBP experiment is described in Chapter 2.

Data collection and analysis

Base-line survey

To examine the effects of fire on total N and other nutrients, a base-line soil survey was done in the Shabeni string during summer (January 2004). Permanent sampling points were established in each of the four treatment plots at the Shabeni string (see Table 1.1, p.21). Points were 30 m apart situated on two transects in each plot, five points per transect. Transects ran parallel and were approximately 100m apart. At every permanent sampling point, within 1 m^2, 4 cm diameter by 15 cm long soil cores were extracted. The soils were air-dried for a week and ground to a powder using a hammer mill. The ten samples per treatment were analysed for total soil N, organic carbon (C), and plant available phosphorus (P) at the analytical laboratories of the Agricultural Research Council in Pretoria. Total carbon and nitrogen were determined with CN analyser using total (Dumas) combustion and the plant available P was analysed using Bray I (Soil Analysis Work Committee 1990). Soil phosphorus was extracted by a solution consisting of HCl and HN_4F (Bray I). The amount of P is determined by measuring the intensity of blue color developed in the filtrate when treated with ammonium molybdate-hydrochloric acid solution.

Sampling with depth

Soils were sampled at seven depths in spring 2003 (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60+ cm) to measure total soil nitrogen and total soil carbon through the soil profile. Three treatments (not the Feb 3) were sampled at Shabeni. Sampling points were randomly chosen and for each of the four sampling points per treatment, one sample was taken under the canopy of an adult *Sclerocarya birrea* tree and another away from the canopy, amongst grass. Soils were sifted to remove organic material and roots, airdried and ground.

Statistical analyses were performed using the software package JMP 5.0.1 (SAS Institute, Cary, NC, USA). Dependent variables were tested for normality. Parameters were transformed where necessary. Different factor ANOVAS were used for the different datasets. A one-way, one factor ANOVA was used to test the effect of fire treatment on total soil nutrients. A two-factor model was used to test the effect of canopy and depth on total soil nitrogen.

RESULTS

Base-line survey

Table 3.1. Baseline analysis of soil nutrients (carbon, nitrogen and phosphorus) for four fire treatments in the Shabeni string. No significant differences occurred between treatments ($P > 0.05$). Means ± standard deviations.

	[C] (%)	[N] (%)	C:N	P (mg kg^{-1})
r^2	0.10	0.20	0.01	0.10
P	0.29	0.05	0.94	0.30
Aug 1	1.10 ± 0.08	0.08 ± 0.005	14.0 ± 0.5	3.45 ± 0.45
Aug 3	1.25 ± 0.08	0.09 ± 0.005	13.9 ± 0.5	2.83 ± 0.45
Feb 3	1.11 ± 0.08	0.08 ± 0.006	14.3 ± 0.5	3.41 ± 0.45
FE	1.30 ± 0.09	0.10 ± 0.006	13.9 ± 0.5	5.03 ± 0.47

The base-line study examined the effect of fire on total soil nutrients (Table 3.1). Fire had no significant effect soil C and P; however, excluding fire increased total N ($P = 0.05$). Although excluding fire increased total soil N, this was limited to the upper 2 cm of the soil.

To compute the total soil N, it was necessary to measure total soil N with depth and tree cover. There was no significant relationship between fire treatment and soil N (Fig. 3.1 for total soil N). Nitrogen content decreased significantly with depth (see Table 3.2, Fig. 3.1) and the presence of canopies increased total soil N to a depth of 5 cm (Fig. 3.2).

Sampling at depth

Table 3.2. A Two-factor model showing F-ratios and significance levels (***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$) on the effects canopy (under adult *Sclerocarya birrea* and in the open), and depth (D1: 0-2 cm, D2: 2-5 cm, D3: 5-10 cm, D4: 10-20 cm, D5: 20-40 cm, D6: 40-60 cm, D7: 60+ cm) on nitrogen and carbon concentrations.

Factor	N (%)	C (%)
Whole model r^2	0.73***	0.73***
Canopy	26.59***	23.16***
Depth class	53.77***	53.58***
C * DC	2.04	1.89

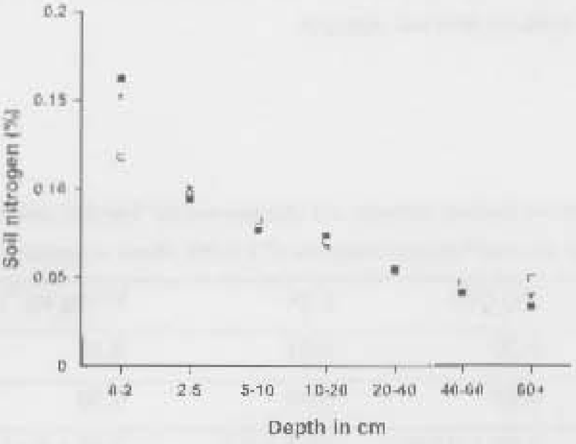

Figure 3.1. Total soil N (%) at different depths for three fire treatments; (□ annual burn, ■ triennial August burn, ★ fire exclusion).

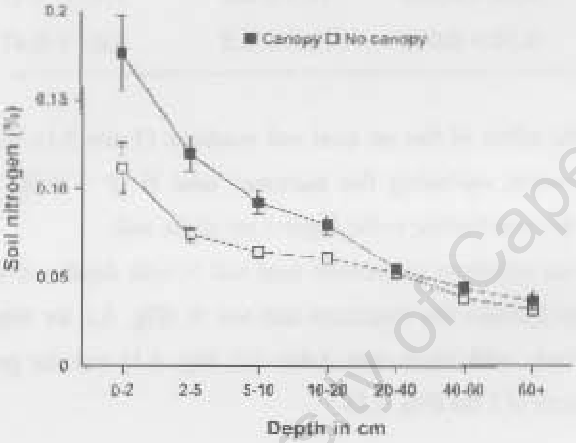

Figure 3.2. Total soil N (%) for different depths, under and away from the canopy of adult *Sclerocarya birrea*.

Soil N concentrations were computed separately for each treatment, averaged for each depth, under and away from the canopy (the values are summed in Table 3.3).

Table 3.3. Total soil N (kg N ha^{-1}) for each treatment, under and away from tree canopies.

	Aug 1	Aug 3	FE
Canopy	5729.7	5661.5	5272.1
No canopy	3730.6	4153.8	4621.7

The annual burn has around 25% tree cover, and the fire exclusion – 80%. When the amount of total soil N in each plot is multiplied by that of canopy cover, the fire exclusion plot has about 900 kg ha^{-1} more N than the annual burn. This is only a rough estimate and the difference could not be tested statistically. However, on a plot level, the annual burns may have less total N in soil than the fire exclusion treatment.

DISCUSSION

After more than 50 years, fire treatment had no effect on the ratio between soil carbon and nitrogen nor were soil C and N concentrations affected through the profile. The lack of response of total soil C and N to fire treatment is not entirely unexpected as a number of studies have shown that frequent fire does not influence total soil N and C (Trapnell *et al.* 1976, Blair 1997, Turner *et al.* 1997). Low fire intensities may increase soil carbon as a result of the addition of recalcitrant partly-combusted material (charcoal), and higher plant productivity (Raison 1979). Offsetting the reduced above-ground input, total soil C increases as a result of increased root growth and turnover in more frequently burned situations (Reich *et al.* 2001).

Organic C was decreased in the upper 2 cm by autumn and winter burning in southern tall grassland at Ukulinga, KwaZulu/Natal (Fynn *et al.* 2003). Soil organic C (SOC) in the Kruger National Park was variously affected by frequent burning; fire had no effect at Skukuza and N'waswitshaka, while decreasing total C at Marheya and N'wanetsi (Jones *et al.* 1990, Aranibar *et al.* 2003, Mills 2003). Skukuza and N'waswitshaka are on sandy, highly-leached, granite soils and Marheya and N'wanetsi are on clayey, basaltic soils. This suggests that it is either the soil texture or low fertility of the sandy soils that confers some resistance to the loss of C through fire. Shabeni, when compared to other ecosystems globally, has relatively nutrient-poor soil, but is comparable in soil fertility to other African savannas (see Table 3.4). Aranibar *et al.* (2003) found that fire had no effect on soil C at the Satara string, while Shackleton and Scholes (2000) found that fire decreased soil C at the same string. They conducted their research during different rainfall regimes and at different depths (Shackleton and Scholes 2000; relative dry period at 2 – 8 cm depth vs. Aranibar *et al.* 2003; very high rainfall, < 5 cm, this study; high rainfall, 10 cm), which may explain the discrepancy in findings.

In a meta-analysis done by Wan *et al.* (2001) on 78 studies including forests, grassland, and shrubland, the response of soil N amount was not significantly affected by fire. Alternative summer/winter burning increased total soil N in a temperate mixed-grass mesquite savanna (Dai *et al.* 2006). In the KNP, burning decreased total soil nitrogen with higher concentrations in the fire exclusion plots of Skukuza, Numbi, Satara and Mopani (Aranibar *et al.* 2003, Mills 2003). Fire had no effect on soil nitrogen at the Satara, Meraya, Nwanetsi, and Nwashitshaka strings (Shackleton and Scholes 2000, Aranibar *et al.* 2003). As was seen in this study, fire decreased total soil N at Satara only at the very surface layers of soil (2 cm, Mills 2003), while fire had no effect in deeper soil (5 cm, Aranibar *et al.* 2003).

Fire often does not have an effect on total N because fire-induced changes are so small compared to total soil N. A model from Nylsvley estimated loss of N through fire as around 0.2 g N m^{-2} y^{-1} and this was only about 6% of the nitrogen returned annually to the soil by litterfall and root turnover (Scholes and Walker 1993). Volatilization of N by fire was estimated as 0.5 g N m^{-2} y^{-1} for broad-leaf Kruger savanna, and may be balanced by wet and dry deposition (Scholes *et al.* 2003). N volatilization losses caused by regular burning are often balanced by N inputs in non-associative N fixation, or N deposition in rain (Jorgensen and Wells 1971, Medina 1982, Holt and Coventry 1990, Menaut *et al.* 1993).

Soil depth is important in the detection of fire effects, but fire did not decrease total soil C or N at the surface or at deeper soil depth in this study. It has been suggested that major changes in soil nutrient pools as a result of burning will take place in the upper 0 – 2 cm of mineral soil (DeBano and Conrad 1978, Kutiel and Naveh 1987, Mills and Fey 2003) and differences in total C and N are sometimes only detected in the upper 2 cm (Fynn *et al.* 2003, Ojima *et al.* 1994). Soil C and N volatilize only above 200 °C (Pyne *et al.* 1996) and increases in soil temperatures are usually less than 50 – 80 °C in the surface 3 – 4 cm of soil (Raison 1979). Soil temperatures, in many studies (see Review by Raison 1979) were directly associated with fuel load and the structure of the vegetation; heaped or thick vegetation (such as in slash-and-burn clearing) caused the highest soil temperatures. Volatilization losses are probably less than expected in the less intensely burned annual plot and higher in the triennial burns (when comparing the same season) where fuel load builds up over three years. The triennial burns, on the other hand, have enough time since previous fire for N inputs to balance higher N losses.

The exclusion of fire lead to higher canopy cover and soil C and N was higher under canopies in this study. The presence of tree canopies often enhances pools of soil nutrients (and nitrogen) and their fluxes (Georgiadis 1989, Frost and Edinger 1991, Isichei and Muoghalu 1992, Mordelet *et al.* 1993, Belsky 1994). The enrichment of soil nutrients under the canopy can be attributed to different mechanisms and include a) the tree may draw nutrients from deep soil layers and from areas beyond the canopy and deposit it under the canopy in the form of litter or canopy leaching (Kellman 1979, Belsky 1994, Scholes and Hall 1996), b) the tree may act as an atmospheric dust trap (Escudero *et al.* 1985, Bernhard-Reversat 1988), and c) mammals and roosting birds may be attracted by shade and refuge and add nutrients in droppings (Georgiadis 1989, Belsky 1994). The tree cover is much higher in the fire exclusion plot (see Chapter 5). Despite this, the annual burn plot had only two sampling points in the open and the rest were also shaded (see Table 1.1). At the plot scale, the higher tree cover may lead to higher total soil C and N in the fire exclusion plot.

Frequent fire did not affect plant available phosphorus in this study. The fractions of P which are available for uptake by plants are more complicated than for nitrogen. Phosphorus can be divided into three fractions; the occluded phosphorus which is not available for uptake, moderately available P which includes organic P and readily available P which is measured by resin extraction (Scholes and

Walker 1993). Volatilization of P only occurs at very high temperatures (> 600 °C, DeBano *et al.* 1998). As a result of the low volatilization losses of P, P is deposited in ash with every fire (Ojima *et al.* 1990). Fire had no effect on organic soil P at Satara in KNP (Shackleton and Scholes 2000). Fire increased P in burned plots regardless of the date of burning in West African savannas (Menaut *et al.* 1993) and in Zambian miombo woodland (Strømgaard 1992). The high soil temperatures produced during slash-and-burn clearing, transforms non-plant available organic and occluded soil P into plant-available mineral forms (Giardina *et al.* 2000). Although around 80% of the residual above-ground P was located in the ash component after a slash fire in dry Brazilian forest, around 60% of this P was lost subsequently as a result of wind erosion (Kauffman *et al.* 1993).

Except for the surface 2 cm of soil, frequent fire did not alter total soil nutrient concentrations in this study. The long evolutionary history of these savannas with fire may have conferred mechanisms which increases resistance to fire-driven change. These results reinforce the concept that regular fires do decrease nutrient stocks in the top layers of the soil. This also does not support the idea that the exclusion of fire leads to quicker tree recruitment and growth through the positive effects on soil nitrogen.

Table 3.4. Total soil nutrients C, N (%) and P ($\mu g\ g^{-1}$) for southern African and other savannas, grassland and temperate ecosystems.

	Total soil C	Total soil N	Soil P	C:N ratios
Broad-leaf savanna, Kruger National Park				
Shabeni, this study	1.10-1.53	0.08-0.11	2.83-5.03	14
African savanna, Nigeria (Moore 1960, Egunjobi 1971, Oguntala 1980)				
Early burn		0.07-0.18	4.2-6.1	
Late burn		0.04-0.13	2.7-6.5	
Unburned		0.05-0.19	2.6-3.8	
African savanna, Nigeria (Isichei and Muoghalu 1992)				
Guinea savanna	1.28-2.44	0.05-0.09	2.51-6.73	15
African savanna, Côte d'Ivoire (Abbadie and Lata 2006)				
Lamto savanna	0.8-1.5	0.04-0.08		
***Trachypogon* savanna, Venezuela** (Medina 1982)				
	1.23	0.07	2.30	
Grasslands				
Tarangire National Park, Masai Mara (Van de Vijver 1999)				
Grasslands	3.04-10.61	0.06-0.16	2.00-11.00	
Serengeti-Mara (Ruess and Seagle 1994)				
17 sites, mixed	0.93-4.23	0.05-0.32	2.07-126.98	10-20
Serengeti-Mara (Ruess and McNaughton 1987)				
5 grasslands	1.70-4.52	0.14-0.39	12.6-130.0	12-16
Prairie				
Yellowstone National Park (Augustine and Frank 2001)				
Grazed		0.10-0.25		
Ungrazed		0.09-0.18		
Wind Cave National Park prairie, South Dakota (Holland and Detling 1990)				
Mixed-grass prairie		0.17-0.25		
Riparian meadows, Yellowstone National Park (Sirotnak and Huntly 2000)				
		0.29-0.70		13-18
Tropical forest, Amazon basin (Neill *et al.* 1999)				
	1.68	0.13		13
Fynbos, Cape floristic region (Stock *et al.* 1995)				
Fynbos		0.001		35
Strandveld		0.003		21

CHAPTER 4

THE EFFECTS OF FIRE ON N AVAILABILITY IN A BROAD-LEAF SAVANNA, KRUGER NATIONAL PARK.

INTRODUCTION

Fire and herbivory with water, light and nutrient availability are the five most important factors that constrain savanna structure, functioning and dynamics (Bond *et al.* 2003, Sankaran *et al.* 2004, Sankaran *et al.* 2005, Abbadie *et al.* 2006). Nutrient availability and soil fertility influences species composition, morphology, forage chemistry and the degree and type of herbivory (Scholes 1990) and constrains plant productivity in many systems (Knapp *et al.* 1998, Reich *et al.* 2001). Fire initiates changes in ecosystems that alter nitrogen (N) cycling and soil processes (Knapp *et al.* 1998). The immediate effects of fire are the removal of accumulated litter and above-ground biomass as well as the loss of nutrients such as nitrogen as particulates and through volatilization (Blair *et al.* 1998). Longer term indirect effects of fire may include decreases in the availability of N by decreasing litter quality (Knapp *et al.* 1998). Fire therefore has both direct effects on savanna structure and function, and indirect effects through nitrogen cycling and availability. The aim of this chapter is to examine the effects of fire on nitrogen availability in a broad-leaf savanna in the Kruger National Park.

Fire has been described as "a herbivore that does not require protein for growth" (Bell 1982, as in Wedin 1995). While herbivores accelerate the turnover of N (Holland and Detling 1990, Frank and Evans 1997, Frank 1998, Frank *et al.* 2000, Johnson and Matchett 2001), it has been suggested that fire reinforces N limitation over the long term by volatilizing N in soil and plant litter (Raison 1979, Ojima *et al.* 1994, Wedin 1995). Apart from the physical effects of fire on soil, such as increasing soil temperatures, fire also creates new microclimates through the removal of litter and standing crop and by the addition of ash in grassland. Fire does not always reduce available N and in the short term the elevated soil temperatures and addition of ash after burning may lead to increased N mineralization rates (Raison 1979, Singh 1994, Knapp *et al.* 1998, Jensen *et al.* 2001). In savannas, changes in nutrient availability remain very small with regular burning as savanna systems are more orientated towards mineralization than humification (Menaut *et al.* 1993). As a result the N susceptible to loss through burning is negligible (Kauffman *et al.* 1994). The effects of fire on savanna may depend more on the structural vegetation changes brought about by fire than by fire itself (Menaut *et al.* 1993).

A large amount of research has been done on **short-term, post-fire** inorganic nitrogen (N) concentrations, and most of this work focuses on the effects of fire in grasslands and forests (Briggs and Knapp 1995, Blair 1997, Turner *et al.* 1997, Wan *et al.* 2001). Although some research has considered the **long-term** effects of fire on net N mineralization rates and cumulative N mineralization in savannas (Reich *et al.* 2001, Aranibar *et al.* 2003), the majority of studies on N mineralization have focussed on prairie and other grass-dominated systems (Risser and Parton 1982, Ojima *et al.* 1990, Seastedt and Ramundo 1990, Ojima *et al.* 1994, Blair 1997, Turner *et al.* 1997, Johnson and Matchett 2001, Fynn *et al.* 2003).

With frequent burning, net N mineralization may decrease and hence the rates at which N becomes available for plant uptake (Ojima *et al.* 1990, Seastedt and Ramundo 1990, Johnson and Matchett 2001, Reich *et al.* 2001, Bustamante *et al.* 2006). This is not always the case as frequent periodic burning enhances N availability in some systems such as ponderosa pine stands (DeBano *et al.* 1998). In South Africa, Jones *et al.* (1990) and Fynn *et al.* (2003) found that frequent burning resulted in a decrease in N mineralization. In contradiction to these studies, Aranibar *et al.* (2003) found higher gross N mineralization with frequent burning for both nutrient-rich (fine-leaf) and nutrient-poor (broad-leaf) savanna. Working at the same site in the nutrient-poor broad-leaf savanna at Pretoriuskop, Feig (2004) found fire frequency to have no influence on net N mineralization. These inconsistent results may be attributed to the use of different methods to determine nitrogen availability (W. Stock pers comm.). Furthermore, measurements for the different studies were taken in different seasons. For instance, Aranibar *et al.* (2003) collected soil once during the wet season in 2000 and used NO_3^--^{15}N and NH_4^+-^{15}N pool dilution methods to calculate gross N mineralization and nitrification in the laboratory. Jones *et al.* (1990) collected soil once during the late 1988 wet season and incubated the soils anaerobically in the laboratory to calculate potential nitrogen mineralization.

Here I compare the effect of fire on nitrogen availability using three different methods, *in situ* cores, laboratory incubations and resin bags. Net nitrogen mineralization was also measured for a full growing season to account for interannual variation. The primary objective, however, was to ascertain whether frequent fire decreased nitrogen availability in a mesic South African savanna.

METHODS

Study area

The measurements were made on four treatments of the Shabeni string of the sourveld EBP's at Pretoriuskop (see Chapter 2 for detail). These treatments include the August annual burn (Aug 1), the August triennial burn (Aug 3), the February triennial burn (Feb 3), and the fire exclusion treatment (FE) of the Shabeni string of the sourveld experimental burns (EBP's) at Pretoriuskop. The Shabeni string is the only string where the fire exclusion plot has not burned accidentally since the advent of the EBP experiment. These treatments were chosen to include an annual burn (of which the August annual is the only annual burn) and both a high and low intensity triennial burn. The only exceptions where these treatments were not used were for the inorganic N sampling with depth (only the annual, August triennial and fire exclusion), and the measurements of potential N mineralization, where soil was collected from treatments in all four southern EBP strings. Figure 4.1 and 4.2 contain long-term average rainfall data for the Pretoriuskop area, as well as shorter-term rainfall data for Shabeni (data from Scientific Services, Skukuza).

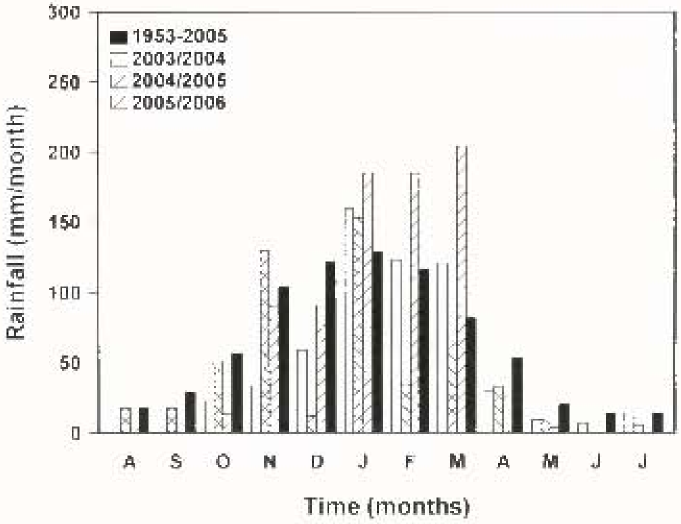

Figure 4.1. The long-term monthly rainfall data for Pretoriuskop, as well as the rainfall for Shabeni for the period that the fieldwork took place, January 2003- May 2006.

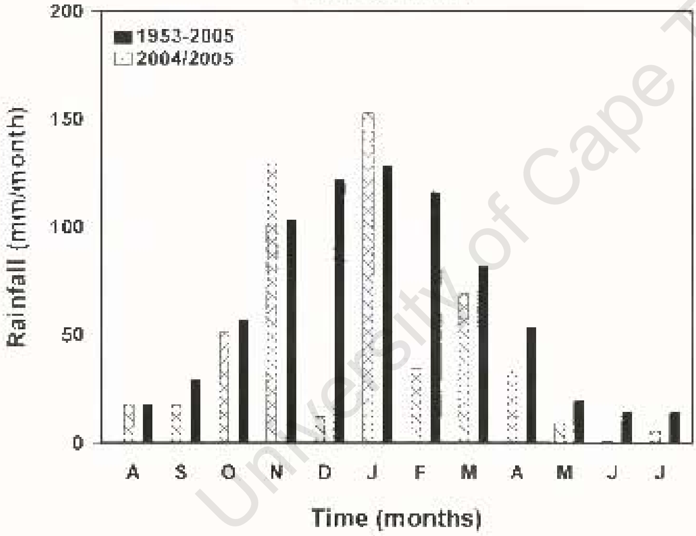

Figure 4.2. The long-term average monthly rainfall vs. the rainfall for the time that the *in situ* soil incubations took place to measure net N mineralization (August 2004 to June 2005).

Data collection and analysis

To examine the effect of fire on nitrogen availability, seasonal inorganic nitrogen concentrations and net N mineralization rates were measured under four different fire regimes. In three treatments (Aug 1, Aug 3 and FE), I examined the effect of burning on the inorganic N concentrations at seven different depths to test whether there where changes between the surface and deeper soil layers. Fire exclusion often increases tree cover and it has been shown that N availability is higher under tree canopies (Kellman 1979, Callaway *et al.* 1991, Belsky 1994, Scholes and Archer 1997). To account for the canopy effect on N availability, I measured inorganic N concentrations under large canopies and in the open.

Net N mineralization rates were measured *in situ* with cores (Knoepp and Swank 1998, as adapted from Adams and Attiwill 1986) and resin bags (Binkley and Matson 1983). Potential N mineralization was measured by performing laboratory incubations (Wedin and Pastor 1993). Potential N mineralization rates are often higher than *in situ* soil incubations as incubation conditions are kept at an optimum for microbial activity. Net N mineralization was measured *in situ* for a full growing season. The *in situ* cores were covered with plastic to prevent leaching of nitrate. It is questionable whether soil moisture equilibrium occurs between soil in and out of the core. To account for micro-climate fluctuations, ion exchange resin bags were used which are sensitive to in situ temperature and moisture regimes (Binkley 1984, Binkley *et al.* 1986). Resin bags, however, do not provide flux estimates and cannot be used to examine N mineralization and immobilization transformations independently (Zou *et al.* 1992).

Inorganic nitrogen

Soils were sampled at seven depths (0-2 cm, 2-5 cm, 5-10 cm, 10-20 cm, 20-40 cm, 40-60 cm, 60+ cm) for three treatments of the Shabeni string (excluding the February triennial burn) in spring 2003 to determine the concentrations of inorganic nitrogen. Sampling points were randomly chosen and for each of the four sampling points per treatment, one sample was taken under the canopy of an adult *Sclerocarya birrea* tree and another away from the canopy, amongst grass. Soils were collected by augering down to the specified depth. All collected soil was kept cool by storing in a refrigerator at 4°C until analysed within 48 hours. Soil nitrogen was extracted from 10 g of sieved field-moist soil, shaken for one hour in 45 ml of 1mol/L KCl solution. The soil/KCl mixture was then centrifuged for 10 min at 5000 rpm. The supernatant was kept frozen before analysis for NO_3^--N and NH_4^+-N concentrations at the Department of Oceanography, University of Cape Town.

Nitrogen mineralization

In situ net N mineralization rates – in situ core method

Mineralization rates were determined monthly for the 2004/2005 growing season at each of the ten permanent sampling points in each treatment (Chapter 2, pg. x). Net rates of N transformations were measured using an in situ core method as used by Knoepp and Swank (1998), modified from Adams and Attiwill (1986). Two 25 cm long, 4 cm inside diameter, steel cores were driven 10 cm into the soil within 30 cm of each other. One core was removed immediately for the time zero determination of soil NO_3^--N and NH_4^+-N concentrations. The paired core was left and retrieved after incubating in the field for 28 days.

One subsample (~20g) was placed in a 105° C oven for more than 12 hours to obtain oven-dry weight. One ten gram subsample was shaken with 45 ml. of 1 M KCl for 1 h to extract NO_3^--N and NH_4^+-N. The soil/KCl mixture was then centrifuged for 10 min at 5000 rpm and the supernatant analysed for NO_3^--N and NH_4^+-N at the Institute of Soil, Climate and Water of the Agricultural Research Council (ARC), Pretoria.

Daily net N mineralization rates for specific incubation periods were calculated as:

$(N_F - N_I)$/(incubation time in days),

where N_F is the final concentration of total extractable N (NH_4^+-N + NO_3^--N) in post-incubation cores and N_I is the initial concentration of total extractable N in the adjacent paired core taken at the start of the incubation period.

Net nitrification rates were calculated as:

$(NO_3^-\text{-}N_F - NO_3^-\text{-}N_I)/D$,

where NO_3^--N_F is the final concentration of NO_3^--N in the post-incubation core, NO_3^--N_I is the initial concentration of total extractable NO_3^--N in the adjacent core at the start of the incubation period, expressed as $\mu g\ NO_3^-\text{-}N\ g^{-1}\ d^{-1}$, and D is incubation time in days. Annual rates of mineralization and nitrification of a treatment were estimated as the sum of the values from the different incubation periods.

To calculate gravimetric soil water content, 20 g of soil was weighed and dried at 65 °C until constant weight. The following formula was used to calculate gravimetric soil water content:

θ_g = [(g moist soil) – (g dry soil)]/(g dry soil),

where θ_g is gravimetric soil water content as g H_2O/g dry soil (Jarrell *et al.* 1999).

Gravimetric soil water content was then used with the concentration of the ion in extract, mass of soil and volume of extract to calculate mass N/ mass soil (Robertson *et al.* 1999).

μg element/g soil = (C x V)/W,

where C is the concentration of ion in extract, V is the volume of extract plus water in the soil sample, and W is the dry mass of the soil less water content.

Potential nitrogen mineralization – Laboratory incubations

The N mineralization potential of soil can be assessed independently of short-term environmental conditions such as temperature and moisture through laboratory incubations (Wedin and Pastor 1993). Five samples from each of the four treatments were taken for all southern EBP strings. Soils were air-dried and processed in the Nitrogen Laboratory in the Department of Botany, UCT. The moisture content of soils was adjusted to 60% water-filled pore space (WFP). Two samples of 10 g for each sampling point were weighed before one set was immediately analysed for inorganic N (NO_3^- and NH_4^+). The paired samples were covered with para-film (permeable to O_2 and CO_2 but not H_2O) and kept in a humidified incubator at 25 °C for 16 days before analysing for inorganic N concentrations.

Resin bag method

Ion-exchange resins have the potential to mimic nutrient uptake by plant roots (Lajtha *et al.* 1999). Ten resin bags per treatment were placed in close proximity to the sampling points used for the *in situ* core method (method adapted from Binkley *et al.* 1986). Every bag contained approximately 8 g of mixed bed resin (mixed anion and cation exchange Dowex MR3 resin in bags made from printers' screen). Bags were buried horizontally at 5 cm depth and left in the field for a full year (August 2004 to August 2005). Resin bags were then collected, larger roots and stones were removed by hand and the bags were rinsed with deionised water to remove all smaller roots and soil. Thereafter the resin bags were placed in jars and extracted overnight with 100 ml of 2 M KCl and analysed for inorganic nitrogen concentrations (NH_4^+-N + NO_3^- -N). The rate of inorganic nitrogen accumulation was reposted as mmol per resin bag surface (*i.e.*, 40 cm^2). The accumulation of ions in the resin bags depends strongly on nitrogen mineralization rate as NO_3^- moves into the bags more readily than NH_4^+ (Binkley 1984).

Statistical analysis

Statistical analyses were performed using the software package JMP 5.0.1 (SAS Institute, Cary, NC, USA). Dependent variables were tested for normality and parameters were transformed where necessary (*i.e.*, NO_3^--N was log transformed). Different factor ANOVAS were used for the different datasets. A three factor model was used to test which parameter (*i.e.*, fire treatment, soil depth, and canopy) explained the most variation in soil inorganic concentrations differed between fire treatments. Significance of main treatment effects of treatment and month on various indices of nitrogen availability was determined using a two-factor ANOVA. Significant differences ($P < 0.05$) among months and treatments were determined using a Tukey HSD test.

RESULTS

Concentration of inorganic N with depth

When analysed with a three factor model (fire treatment, canopy/open and depth), there were no significant differences in soil moisture and NH_4^+ between the different fire treatments when sampled in spring before the rains (Table 4.1, $P > 0.05$). NO_3^- concentrations, however, were lower in the annual burn than the fire exclusion treatment. The presence of tree canopies increased NO_3^- but not NH_4^+ concentrations with the higher NO_3^- under canopies persisting to a depth of 5 cm. Soil moisture was greater under canopies than away from canopies. The concentrations of both NH_4^+ and NO_3^- varied with soil depth, with the highest concentrations in the shallowest soil layer, while soil moisture increased with depth (Fig. 4.3).

Table 4.1. A Three-factor model showing F-ratios and significance levels (***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$) for factors: fire treatment (T), canopy (C; under adult *Sclerocarya birrea* and away from trees), and depth class (DC; D1: 0-2 cm, D2: 2-5 cm, D3: 5-10 cm, D4: 10-20 cm, D5: 20-40 cm, and D6: 40-60 cm) on soil moisture and inorganic nitrogen concentrations.

Factor	Soil Moisture	NO_3^-	NH_4^+
Whole model r^2	0.66***	0.68***	0.33*
Treatment (T)	1.20	5.23**	0.29
Canopy (C)	18.81**	29.78***	2.60
T x C	2.36	4.21*	1.75
Depth class (DC)	26.62***	24.95**	4.04**
T x DC	0.67	0.89	1.42
C x DC	1.65	2.38*	1.23

Analysis was repeated using a *t*-test to establish if the effect that canopies had on soil moisture and inorganic N concentrations were similar across fire treatments (Fig. 4.4). Soil moisture was higher under canopies than in the open in the fire exclusion treatment ($t_{36} = -2.12$, $P = 0.04$). Tree canopies increased NO_3^- concentrations in the fire exclusion treatment ($t_{36} = -2.89$, $P = 0.007$). NH_4^+ under canopies was not affected by fire exclusion and was the dominant form of inorganic N across treatments.

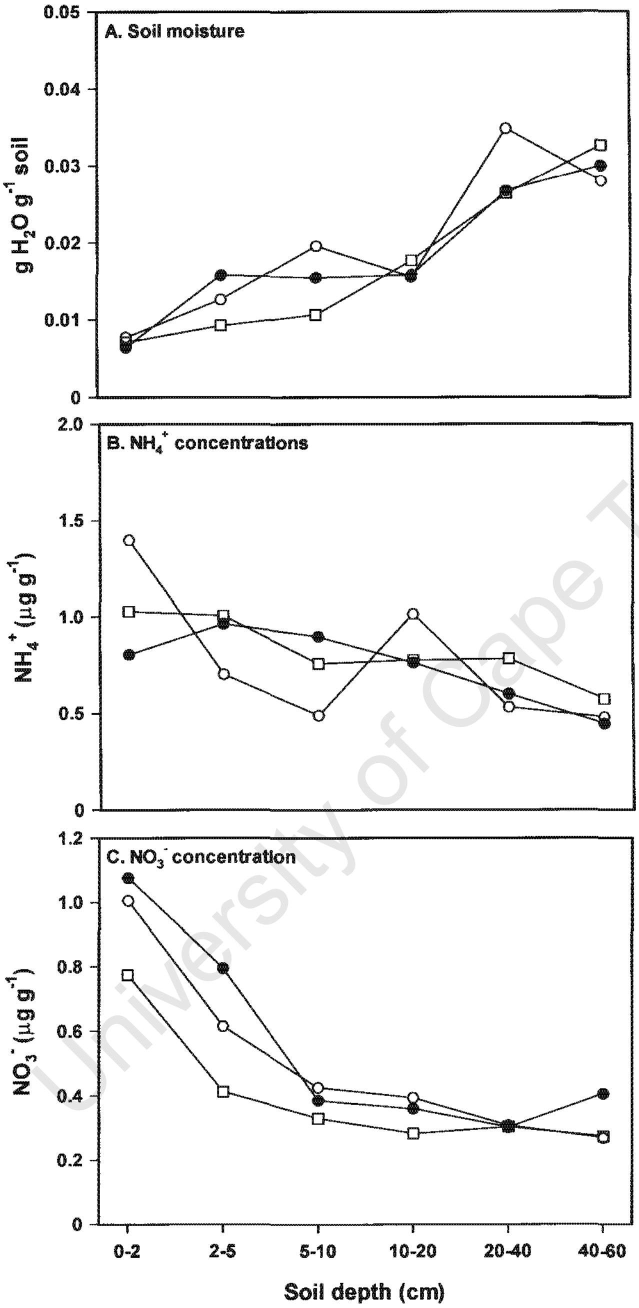

Figure 4.3. Soil moisture and inorganic N concentrations sampled in spring, with six depths for three different treatments, annual August (□), triennial August (○), and Fire exclusion (•) at Shabeni.

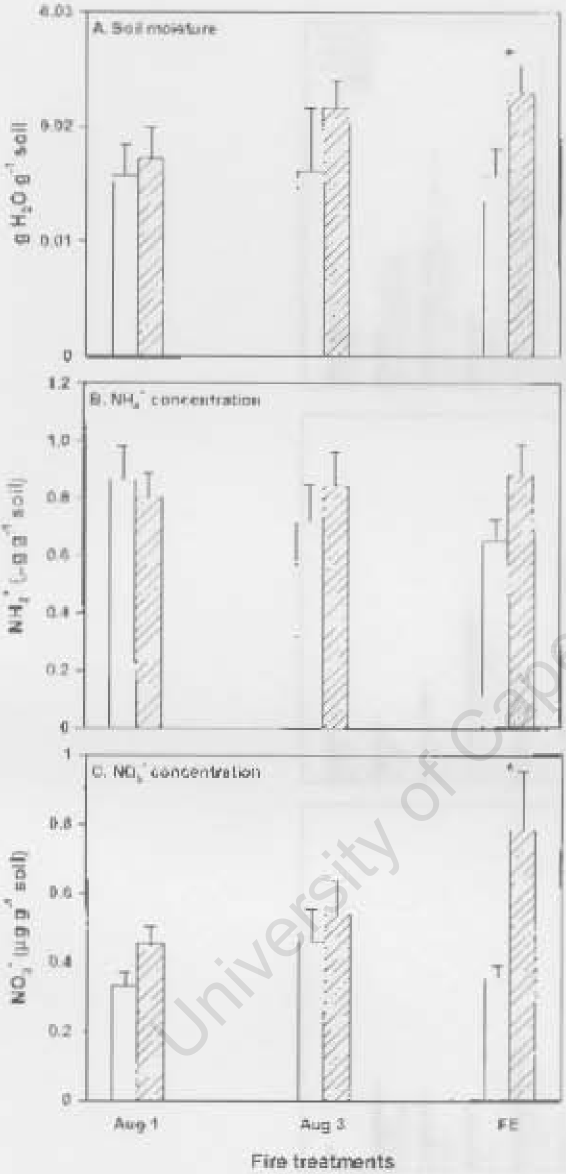

Figure 4.4. Soil moisture and inorganic N concentrations for Shabeni for each fire treatment (across the soil profile), under and away from trees; * $P < 0.05$ for differences between canopy (shaded bar) and open (unshaded bar).

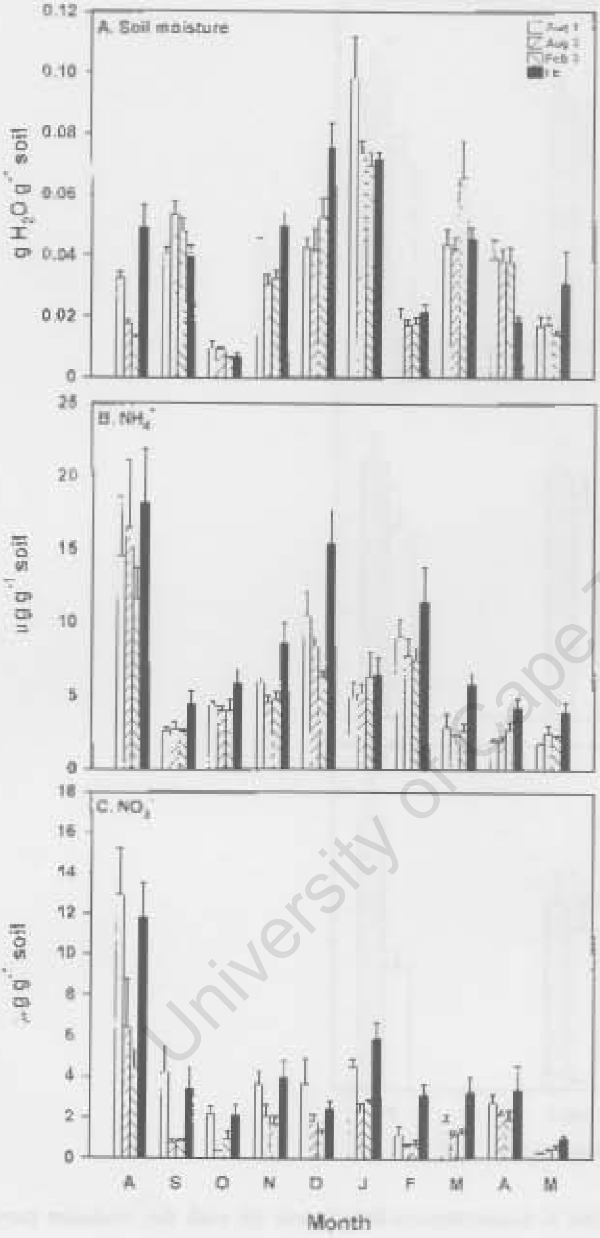

Figure 4.5. Monthly soil moisture (g H_2O g^{-1} soil, August to May) and inorganic nitrogen concentrations (µg g^{-1}) for each of the four treatments measured at Shabeni. Error bars are standard errors.

Temporal (monthly) rates of net N mineralization and nitrification

Inorganic N concentrations and soil moisture

Throughout the growing season (September 2004 to June 2005), soil moisture did not vary among treatments in the surface 15 cm of soil, but did vary temporally (Fig 4.5A). On average, soil moisture was greatest in January in the annual burn (0.17 g H_2O g^{-1} soil) and least in October in the annual burn (0.002 g H_2O g^{-1} soil).

Inorganic nitrogen concentrations were the highest at the end of the dry season. Averaged across all sampling dates, mean concentrations of NH_4^+ in the surface soil were greater in the fire exclusion (FE) treatment when compared to the burned treatments (Table 4.2, $P < 0.0001$). NH_4^+ was greater in the fire exclusion treatments in November/December when compared to the February triennial burn (Fig. 4.5B, $P < 0.05$). NO_3^- concentrations, averaged across sampling dates, were greater in the Aug 1 and FE treatments than the triennially burned treatments (Table 4.2, $P < 0.0001$). NO_3^- concentrations were greater in the Fire exclusion and Aug 1 in August and September when compared to the triennial burns (Fig 4.5C, $P < 0.05$).

Table 4.2. Mean daily inorganic N concentrations compared among fire treatments. Values in brackets are standard errors. Different lower case letters denote significant differences at $P < 0.05$

	Aug 1	Aug 3	Feb 3	FE
NH_4^-	5.83^b (0.49)	5.53^b (0.48)	5.04^b (0.48)	8.42^a (0.77)
NO_3^-	3.71^a (0.49)	1.81^b (0.25)	1.62^b (0.26)	4.03^a (0.26)

Net nitrogen mineralization

The inorganic nitrogen values obtained for February were left out during the analysis as the integrity of the samples at the end of incubation was questioned. Except for the lower August/September rates (-0.46 ± 0.05 μg g^{-1} soil day^{-1}, $P < 0.0001$) and the higher March/April rates (0.33 + 0.05 μg g^{-1} soil day^{-1}, $P < 0.0001$), there were no statistically significant differences between monthly fluxes (Fig. 4.6). Fire treatment only affected fluxes in two months; in August/September, the Feb 3 treatment has significantly ($P < 0.0001$) higher net N mineralization rates than the other treatments. Compared with the triennial burn, the annual burn had higher N mineralization rates in February/March ($P < 0.0001$).

Table 4.3. Mean daily rates of N cycling (μg g^{-1} day^{-1}) measured *in situ* compared among fire treatments. Values in brackets are standard errors. Different lower case letters denote significant differences at $P < 0.05$.

	Aug 1	Aug 3	Feb 3	FE
N mineralization	0.16 (0.05)	0.08 (0.02)	0.14 (0.03)	0.11 (0.04)
Nitrification	0.09^{ab} (0.03)	0.06^{b} (0.02)	0.15^{a} (0.02)	0.13^{ab} (0.03)

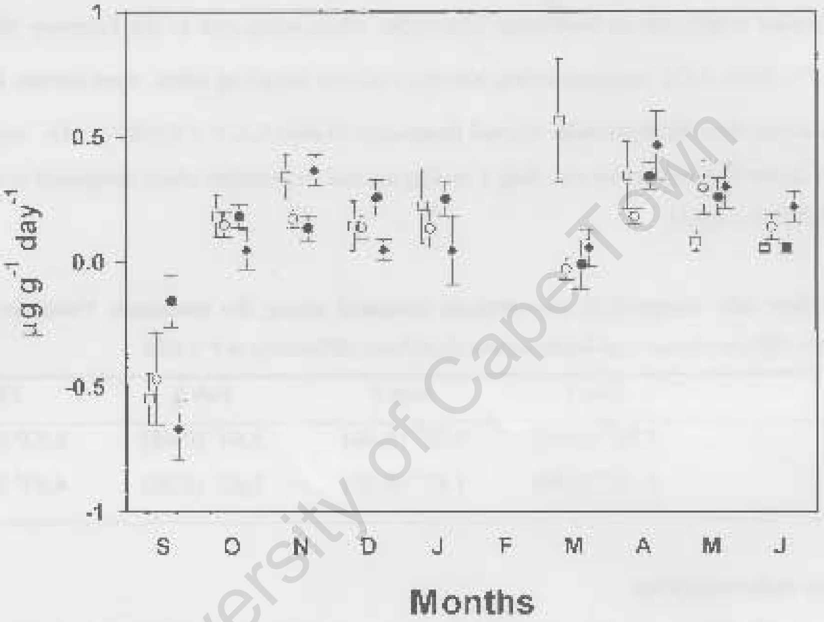

Figure 4.6. Monthly variation in the rates of net N mineralization (μg g^{-1} soil day^{-1}) in soil from each of the four treatments; August annual (□ Aug 1), August triennial (○ Aug 3), February triennial (● Feb 3) and Fire exclusion (♦ FE). Error bars are standard errors.

Field and laboratory incubations may differ in their mineralization potential as the conditions under which the incubation progresses are different. For instance, there are no new inputs to replenish the substrate of laboratory soil incubation, but mineralization proceeds under uniform conditions. On the other hand, in the field, mineralization does not proceed under uniform conditions (Handley and Scrimgeour 1995). Both the resin bag method (Fig. 4.7) and the laboratory incubations for potential N mineralization (Fig. 4.8), however, supported the *in situ* incubations and available N did not vary with fire treatment. As NO_3^- is more mobile in the soil than NH_4^+, NH_4^+ will be under-represented in the resin.

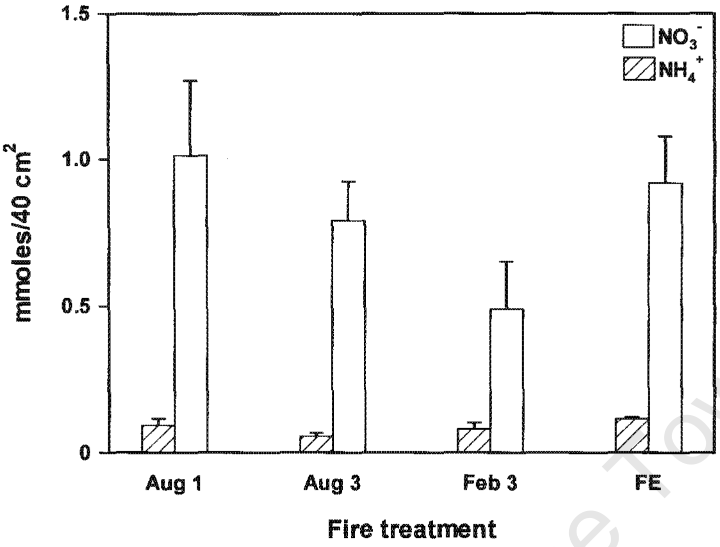

Figure 4.7. Resin absorbed inorganic nitrogen concentrations (mmoles per resin bag surface - 40 cm^2) for the different fire treatments.

Table 4.4. A Two-factor model showing F-ratios and significance levels (***P < 0.001, **P < 0.01 and *P < 0.05) on the effects of treatment and month on nitrogen concentrations, and N transformations.

	Whole model r^2	Treatment (T)	Month (M)	T x M
NH_4^+	0.52***	618.63***	6158.87***	0.76
NO_3^-	0.54***	17.83***	33.90***	2.32**
Mineralization	0.46***	1.40	28.84***	2.55***
Nitrification	0.52***	4.78**	35.76***	1.52

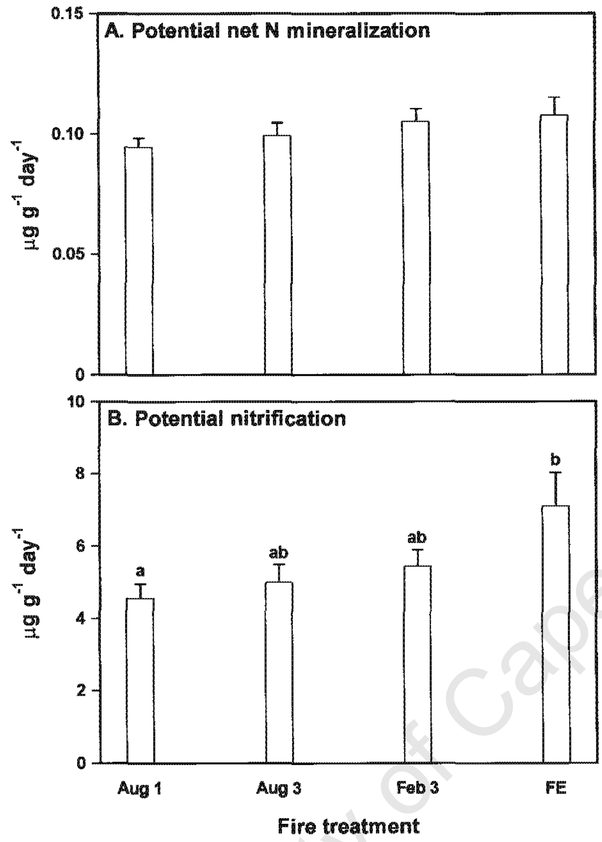

Figure 4.8. Potential net N mineralization and nitrification from laboratory incubations. Different lower case letters denote significant differences at $P < 0.05$.

Nitrification differed significantly between months (Fig. 4.3, $P < 0.0001$) and was the highest in January (0.33 ± 0.03 µg g^{-1} soil day^{-1}) and the lowest in September (-0.17 ± 0.03 µg g^{-1} soil day^{-1}). Nitrification (*in situ*), however, did differ between treatments and was in general (across the growing season) higher in the Feb 3 when compared with the Aug 3 treatment (Fig. 4.9, $P = 0.003$). For the laboratory incubations, nitrification was higher in the fire exclusion treatment than the annual burn treatment (Fig. 4.8).

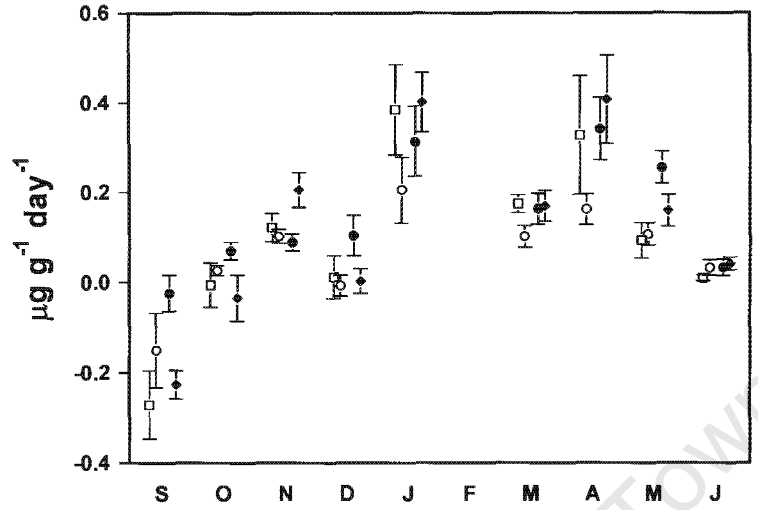

Figure 4.9. Monthly variation in the rates of net nitrification (µg g^{-1} soil day^{-1}) in soil from each of the four treatments; August annual (□ Aug 1), August triennial (○ Aug 3), February triennial (● Feb 3) and Fire exclusion (♦ FE). Error bars are standard errors.

Cumulated over one year, the annually burned treatment mineralized the most N and the August 3 treatment had the least amount of N mineralized during the growing season (~ 9 months, Table 4.5, Fig. 4.10). Plant available N is traditionally seen as the sum of rates of N mineralization and this involves the summation of the amounts of N mineralized (positive rates) and immobilized (negative rates) during all incubations. The Aug 1 had the highest cumulative (long-term) mineralization rates, followed by the Feb 3 treatment with the Fire exclusion and Aug 3 treatments having lower long-term rates.

Table 4.5. Cumulative annual rates of N transformation processes (µg. g^{-1} soil) among the different fire treatments.

	Aug 1	Aug 3	Feb 3	FE
N mineralization	49.0	26.8	46.9	27.6
Nitrification	25.2	17.5	40.5	33.9

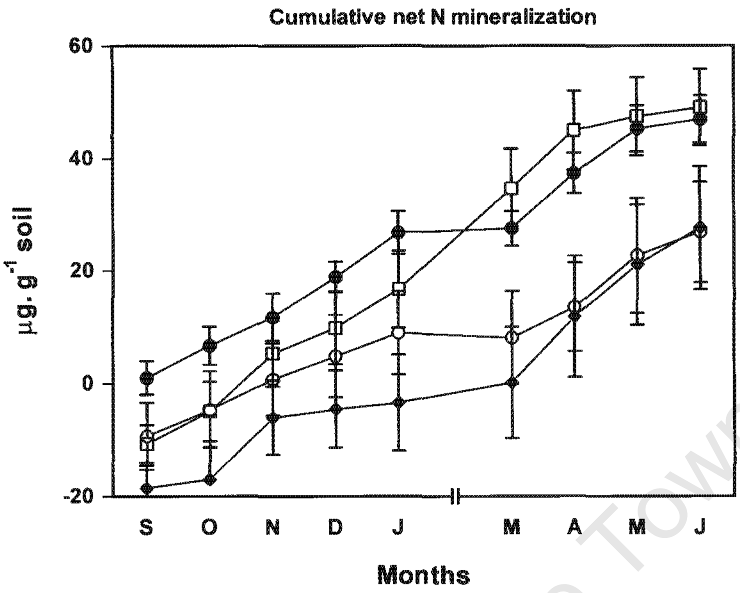

Figure 4.10. Cumulative net N mineralization rates (µg. g^{-1} soil) for the growing season 2004/2005 for four different fire treatments; Annual August burn (□), Triennial August burn (○), Triennial February burn (•), and Fire exclusion (♦).

DISCUSSION

It has been suggested that the different results for the EBP's nitrogen availability in the KNP are related to the use of different methods for determining N mineralization rates (W. Stock pers comm). I used different methods to measure N availability in an effort to allay fears that results will be method-dependent. Regardless of the method used, fire did not decrease plant-available nitrogen in this study (Table 4.6 for comparison with other systems) and in fact, the cumulative rates were positively influenced by annual burning.

A number of studies have shown that frequently burned prairies have lower net N mineralization rates than unburned plots (Ojima *et al.* 1990, Ojima *et al.* 1994, Blair 1997). Blair *et al.* (1998) however, suggested that burned and unburned prairie have similar net N mineralization rates. In North American Oak savanna, Reich *et al.* (2001) showed that net N mineralization decreased with increased fire frequency. In nutrient-poor West African savannas, however, fire frequency had no effect on nitrification and mineralization (Abbadie *et al.* 1992, Menaut *et al.* 1993). In India on the Vindhyan plateau, Singh (1994) found that nitrogen mineralization rates were similar in protected and frequently burned savannas. Although much **short-term, post-fire** research on N has been conducted in Australian and South American savannas (Raison 1979, Batmanian and Haridasan 1985, Cook 2003, Nardoto *et al.* 2006), studies on the **long-term** effects of fire on nitrogen availability and N mineralization rates are scarce. Mistry (1998) in his review of fire in *cerrado*, focussed on the differences in nitrogen loss when different *cerrado* types were burned but failed to mention the effects of burning on N mineralization and availability. He did however suggest that the amount of nutrients lost to *cerrado* with burning represents a minor portion of the total ecosystem N and is likely to be replaced by natural N inputs.

Although frequency of fire had no effect in southern African tall grasslands, potential mineralizable N was greatly reduced by burning (Fynn *et al.* 2003). It has been suggested that nutrient availability increases after burning in South African savannas (Nylsvley) and that N mineralization may increase over the longer term as a result of higher soil temperatures due to reduced cover (Scholes and Walker 1993). At N'wanetsi on the Satara EBP's in the KNP, the fire exclusion plot tended to have higher potential net N mineralization rates than the burned plots (Jones *et al.* 1990). Feig (2004) on the other hand found no significantly different N mineralization rates between the different fire treatments on granite soils. Annually burned sites, however, had positive average rates while fire exclusion plots had negative rates. Aranibar *et al.* (2003) found increased gross N mineralization with increased fire frequency at the Skukuza plots (Combretum EBP's).

Why would annual burning have a positive effect on N mineralization in savanna but not in grassland and prairie? An explaination lies in the differences between the systems. First, my study was done with grazers present and the annual burns in particular are heavily utilised by grazers. Grazers may ameliorate the effects of fire on the nitrogen cycle. Grazers conserve about 50% of nitrogen that would otherwise be lost through fire (Hobbs *et al.* 1991). In the absence of grazers, prairie net N mineralization rates start to decline with frequent burning. In the presence of grazers however, fire driven nitrogen losses are compensated by annual inputs from dry and wet deposition as well as microbial fixation (Ojima *et al.* 1994). Removal of biomass by herbivores decreases fire intensity and also move N from above-ground to below-ground pools via urinary and fecal excretion where it is less susceptible to volatilization during burning (Hobbs *et al.* 1991). Grazers also increase N directly through dung and urine when grazing on the regrowth after fire.

Secondly, the inherent characteristics of savanna structure confer resistance to nitrogen loss. Kauffman *et al.* (1994) have shown that the quantity of above-ground N that is susceptible to loss by fire in Brazilian *cerrado* is minute (~2.5%) when compared to soil N pools. When grass dominated savanna burned, more than 90% of the N pool was lost, while less than 56% was lost in tree dominated communities (Kauffman *et al.* 1994). As a result, in a prairie post-fire environment, high nutrient loss occurs through erosion following rainfall as there is no standing dead matter or litter left to alleviate erosion (Seastedt and Ramundo 1990). For savanna, losses of N through fire are often compensated for by N deposition and N fixation (Medina 1982, Menaut *et al.* 1993, Jensen *et al.* 2001).

In their review chapter of nutrient dynamics in tropical savannas, Menaut *et al.* (1993) discussed the discrepancies which exist in the evaluation of the effects of fire on nitrogen. The diversity in conclusions was attributed to an inherent negative bias against fire and the complexity of flammable ecosystems which behaves dissimilarly. Even though fire is perceived as having negative effects on nitrogen availability, regular fire seldom decreases productivity in prairie or savanna (Isichei 1980, Medina 1982, Ojima *et al.* 1990, Menaut *et al.* 1993, Ojima *et al.* 1994, Jensen *et al.* 2001, Abbadie *et al.* 2006).

Table 4.6. Mean annual rates of N mineralization (µg g^{-1} yr^{-1}, kg ha^{-1} yr^{-1}), and NH_4^+ and NO_3^- concentrations (µg g^{-1}) for southern African savanna and other ecosystems, including savannas elsewhere.

	NH_4^+	NO_3^-	N mineralization (µg g^{-1} day^{-1})	Cumulative Nmin (kg ha^{-1} yr^{-1})
Broad-leaf African savanna				
Shabeni, this study				
Aug 1	5.90	3.67	0.16	55.9 (0-10 cm)
Aug 3	5.55	1.73	0.06	30.6
Feb 3	5.38	1.61	0.14	53.5
FE	8.64	3.79	0.11	31.5
Other broad-leaf savanna in KNP (Woghiren 2002)				
			-0.15-0.2	58
Broad-leaf savanna, Nylsvley (Scholes and Walker 1993)				
			0.2-0.4	
Other African ecosystems				
Serengeti-Mara (Ruess and Seagle 1994)				
17 sites (ave.)	4.55	2.45	0.22	
Grassland Serengeti-Mara (Ruess and McNaughton 1987)				
5 grassland sites	4.20-11.18	0.92-5.31	0.05-3.17	
Other savanna				
Oak savanna, Cedar Creek (Ritchie *et al.* 1998)				
Grazed/Ungrazed	0.75-1.0	0.1-0.2		
Oak savanna, Cedar Creek (Reich *et al.* 2001)				
Unburned				80-160
Annually burned				< 40
Prairie				
Riparian meadows, Yellowstone National Park (Sirotnak and Huntly 2000)				
With voles			-4.7-7.0	
No voles			0.4-0.7	
Yellowstone National Park (Augustine and Frank 2001)				
Grazed	2.73-8.92		0.54-0.63*	
Ungrazed	2.98-5.03		0.35-0.65*	
Wind Cave South Dakota prairie dog colonies (Holland and Detling 1990)				
Uncolonized		0.3-7.0	0.18-0.41	
Colonized		2.6-9.4	0.07-0.29	
Yellowstone National Park (Tracy and Frank 1998)				
Grazed grassland			0.28	
Ungrazed grassland			0.16	
Boreal Forest, Isle Royale (Pastor *et al.* 1993)				
Control site, browsed			1.4-1.9	
Exclosure			1.5-3.2	
Tropical forests (Vitousek and Matson 1988)				
Costa Rica/La Selva	4.6-8.5	6.5-11.7	2.6-3.9	
Panama	5.3-6.0	4.0-7.5	10.2-15.2	
Brazil	2.5-4.2	0.5-1.4	0.9-4.0	

*Potential N mineralization

CHAPTER 5

THE EFFECTS OF FIRE ON NITROGEN UPTAKE AND THE PRODUCTION OF BIOMASS IN A BROAD-LEAF SAVANNA, KRUGER NATIONAL PARK

INTRODUCTION

Fire with herbivory, water, light and nutrient availability are the most important factors that constrain savanna structure, functioning and dynamics (Bond *et al.* 2003, Sankaran *et al.* 2004, Sankaran *et al.* 2005, Abbadie *et al.* 2006). Fire influences the balance between trees and grasses (Scholes and Walker 1993, Higgins *et al.* 2000) which in turn, controls productivity (Belsky 1994, Reich *et al.* 2001). The effect of fire on nutrient cycling is however unclear. It has been suggested that the loss of nitrogen is high in frequently burned systems as nitrogen volatilizes at relatively low temperatures (Seastedt and Ramundo 1990). In Oak savanna, soil nitrogen (N) mineralization rates decrease with frequent fire. As a result low N availability contributes to a smaller canopy size (*i.e.*, low total leaf area) and total canopy N (Reich *et al.* 2001). Reich *et al.* (2001) found that areas with higher tree dominance (lower fire frequency) may have higher productivity and higher rates of nutrient cycling which support foliage of high quality (*i.e.*, high nitrogen concentrations). Foliage quality, in turn, has broader ecosystem implications. For instance, Grant and Scholes (2006) showed that for the Kruger National Park (KNP), more animals are attracted to areas with higher quality forage (grass). At a finer scale, patches preferentially utilised by animals in the KNP, tended to be higher in quality as exhibited by higher concentrations of foliar N, phosphorus, and sodium.

Vegetation structure has important consequences for the amounts of N lost during fires in Brazilian *cerrado* (savanna); less N is lost when *cerrado* burns compared to both grassland and forest (Kauffman *et al.* 1994, DeCastro and Kauffman 1998). Frequent burning in Oak savanna leads to low rates of N cycling, leaf area index (LAI) and productivity (Reich *et al.* 2001). Less frequently burned, tree dominated Oak savanna has high LAI and leaf percentage N which results in high annual N requirements. Reich *et al.* (2001) found that the high N requirements are supported by a large annual N input in organic matter (litter) of high tissue quality (lower C:N ratio) and also by the greater annual net N mineralization.

In prairie systems, frequent fire may cause a reduction in soil organic matter, microbial biomass, nitrogen availability and increased nitrogen immobilization (Ojima *et al.* 1990, Ojima *et al.* 1994). Production, however, is consistently higher in annually burned prairie when compared to unburned prairie as a result of increased nitrogen use efficiency (NUE) of the dominant species (Ojima *et al.* 1994). The high potential for N immobilization in the litter and root detritus of the dominant C_4 grasses translates into decreased N turnover and reduced N availability (Wedin 1995). The high allocation to root production, as well as high C:N foliar and lignin:N ratios contributes to a rapid accumulation of fuel load which in turn increases fire frequency (Blair *et al.* 1998). Ecosystem processes may therefore be severely altered if the dominant vegetation or vegetation structure changes with altered fire regime (Chapin *et al.* 1997, Knops *et al.* 2002, Chapman *et al.* 2005).

Frequent fire in southern African savannas (Nylsvley) decreases woody biomass and increases herbaceous production over the long-term (Scholes and Walker 1993). Only a small proportion of primary production is consumed by fire at Nylsvley leading to low loss of humified organic matter and low pyrogenic losses of N (Scholes and Walker 1993). A small amount of work has been done on the effects of fire on nitrogen cycling in the Kruger National Park with the focus on below-ground processes (Jones et *al.* 1990, Shackleton and Scholes 2000, Aranibar *et al.* 2003, Feig 2004, Mills and Fey 2004).

Except for Oak savanna, annually burned systems seem to be very productive. This persistent high productivity with frequent burning is sometimes linked to increased nitrogen use efficiency of the dominant vegetation. The effect on nitrogen cycling is less clear and may depend on both fire frequency and season of burn. Some ecosystems rapidly replace lost N through natural N inputs, while others have a reduction in available N with frequent fire. This can be a consequence of both large N losses with fire as well as a change in litter chemistry. As considerable dichotomy exists in the literature regarding the effects of fire on the quality and quantity of vegetation, the primary objective of this chapter is to gain a better understanding of the role of fire on nitrogen cycling and primary production in a mesic savanna. I examined the effects of fire frequency on both the quality and quantity of fine root biomass, above-ground biomass and litter. I do this to determine the relationship between fire frequency, vegetation structure and nitrogen pools. Although the focus of this chapter is above-ground, fine root biomass was included as it interacts with above-ground litter quantity and quality to determine decomposition rates and, ultimately, nitrogen availability.

METHODS

Study area

Above-ground biomass and productivity were determined for the four relevant treatments on the Shabeni string of the experimental burn plots (EBP's). The Shabeni string was chosen for this study as it is the only string in the southern (EBP's) where the fire exclusion plot has not burned since the advent of the experiment in 1954. Despite the fact that the Fayi fire exclusion plot was burned in 1991 and 2002, the Numbi fire exclusion in 2002 and Khambeni in 2004, these strings were used as replicates. All four strings (Shabeni, Fayi, Numbi, and Khambeni) were sampled for foliar carbon and nitrogen on the annual August burn (Aug 1), triennial August burn (Aug 3), triennial February burn (Feb 3) and Fire exclusion (FE) plots. The areas in which the strings are situated as well as background to the EBP experiment are described in Chapter 2 (page 24 to 29).

Vegetation

The timing of fires has important implications for the recovery of post-burn vegetation (O'Regan 2005). The February (wet season) treatments are less intensely burned on average than the August treatments (1049 ± 204 kJ m^{-1} s^{-1} vs. 2417 ± 465 kJ m^{-1} s^{-1} and 3653 ± 200 kJ m^{-1} s^{-1} for August annual and triennial respectively, Govender *et al.* 2006). These treatments tend to burn slowly with tall grass stalks, such as *Hyparrhenia dissoluta*, remaining un-burned. *Panicum maximum* growing under trees is still green in February protecting the trees and under-canopy seedlings from fire. Regeneration and recovery of the vegetation is relatively quick if rains continue into autumn (O'Regan 2005). However, if the rains end early in the wet season, the plots are grazed, browsed, and trampled heavily throughout the winter and only recover in the following wet season.

The August (end of winter) burns tend to be more intense as the grass and herbaceous layer is very dry. *Panicum maximum* is dry in August, as a result of which the trunks and lower branches of large trees are often badly scorched. Fire intensities in the annual burns are lower than the August triennial burns as there is less fuel over the long term (Govender *et al.* 2006). Top kill of shrubs in the August burns is very high but many trees are less susceptible to fire damage because it is a time of dormancy and low resource utilization (O'Regan 2005). Post-fire recovery of the vegetation after August fires in the Pretoriuskop region is slow because the first rains usually occur in October or November.

Measurements

Biomass and productivity

Leaf biomass

Leaf biomass was obtained for each treatment by using two different methods; leaf area index (LAI) and by collecting leaf litter. Fifty LAI measurements were taken with a Li-Cor LAI-2000 Plant Canopy Analyser (LI-COR, Lincoln, NE, USA) in each of the study plots. Measurements were made on five parallel transects 50 m apart. The fifth transect was centered on the middle of the plot. Leaf biomass was estimated by converting tree LAI to biomass based on specific leaf area (SLA) which is the amount of leaf area per unit leaf mass by using the following equation from Chapin *et al.* (2002):

$$\textit{Biomass} = \mathrm{SLA/LAI}.$$

Leaf area was determined using the computer program SIGMASCAN (Systat Software Inc., San Jose, CA, USA) from digital photos taken of the leaves of *Terminalia sericea*, *Sclerocarya birrea* and *Euclea divinorum* (100 leaves per species). After measuring area in SIGMASCAN, leaves were dried at 60 °C for two days and then weighed. SLA was then determined from the equation:

$$\mathrm{SLA} = \text{leaf area/dry weight}.$$

In the winter of 2004, an attempt was made to collect litter in plastic bins. Unfortunately, animals such as rhino and hyena kept disturbing the bins. The sampling methodology was modified for the second winter (2005) when 1x1 m cages were placed in a transect parallel to permanent sampling points (permanent sampling points explained in Table 1.1, p.21). Litter was collected monthly (starting in August, continuing through winter) in these cages of which the bottom and lower parts of the sides (50 cm high) were covered with shade cloth. Seasonal leaf biomass was assumed to equal litter biomass (when collected monthly) as most trees in this study area were deciduous.

Woody biomass

Annual above-ground net primary production (ANPP) was assumed to be the sum of herbaceous biomass, combined with an estimate of annual new wood production (Reich *et al.* 2001). Allometric relationships can be used successfully to estimate woody biomass and above-ground production in forest (Chambers *et al.* 2001, Baker *et al.* 2004, Chave *et al.* 2004) and savannas (Rutherford 1976, Guy 1981, Goodman 1990, Shackleton 1997). The most important source of error in predicting biomass is related to the choice of allometric model (Chave *et al.* 2004). I chose to use two different approaches; the savanna specific formulas of Rutherford (1976), Shackleton (1997) and Goodman (1990) and the method of Guy (1981).

The savanna specific formulas are:

$\mathrm{Ln}(10000(M_1)) = 0.94 \ln (D^2) - 4.28$ (Goodman 1990),
$\mathrm{Log10}(M_2) = 2.63 (\log C) - 2.58$ (Shackleton 1997),
$\mathrm{Log}M_3 = 2.52 (\log C) - 2.46$ (Shackleton 1997),
$\mathrm{Log10}(M_4) = 2.59 (\log C) - 2.61$ (Shackleton 1997),
$\mathrm{Ln}(10000(M_5)) = 0.91 \ln (D^2) - 3.61$ (Goodman 1990),

where M_1 is the total woody mass of general broad-leaf species, M_2 is the total woody biomass of *Acacia* species, M_3 is the total woody biomass of *Dichrostachys cinerea*, M_4 is the total woody biomass of *Terminalia sericea*, and M_5 is the total woody biomass of *Euclea* species, D is diameter in cm, and C is circumference in cm.

Guy (1981) developed his allometric relationships for trees and shrubs to measure elephant damage and the effects of fire in different kinds of Miombo woodland in Zimbabwe. Trees were defined as plants with a stem diameter of 6 cm or more and a height of 3 m or more. Biomass relationships were obtained during Guy's (1981) study and it was found that the total biomass from a tree could be predicted from:

$$\text{Mass} = 0.0549 \times (\text{diameter})^{2.5101}$$

Shrubs were defined as woody plants with a stem diameter of more than 1 cm and less than 3 m tall. Multi-stemmed plants with stem diameters of less than 6 cm were treated as single shrubs. Shrub biomass was calculated from the equation:

$$\text{Mass} = 1.2102 \times (\text{canopy volume})^{0.9138},$$

where canopy volume = height x width (m).

Woody biomass was determined for one season as a woody survey was done during 2000 (data from W.J. Bond, unpublished). During this survey, in three 250 x 1 m transects, all trees and shrubs were measured and the following noted: species, canopy height, canopy width, number of stems and diameter of all stems. The August triennial burn was not sampled during the Bond survey and alternative methods had to be employed to infer biomass for this treatment. Biomass therefore, could only be inferred from the basal area as computed from 'Angle count sampling' (Brack 1997). Basal area may be seen as a summary of the number and size of trees in a stand. Whether the tree is included in an angle count depends on the basal area of the tree and its proximity to the sampling point. An optical wedge is used to count trees of a specified diameter which will add a known amount of basal area (Brack 1997).

The only other recent woody data for the EBP plots originated between 1996 and 1999. These surveys included the size-class, height and species of each woody individual in two belt transects (105-500 x 2 m in size). Analyses of these surveys are reported in O'Regan (2004) and Higgins *et al.* (2007). Wood production was assumed to be 3% of total woody biomass (Shackleton 1997).

Herbaceous biomass

Herbaceous production includes the growth from grasses and forbs. Grass production was measured by clipping eight x 1 m^2 plots in each treatment, clipping commenced at the beginning of summer 2003 (December) to May 2004. Cages (1 m^3) were constructed to keep out the bigger herbivores (small buck to elephant) and placed over the clipped areas. Eight cages were distributed for every treatment in a transect running parallel to the road (every 50 m). Subsamples were then clipped once a month through the summer to establish production for the growing season. An adjoining 1x1 m plot was clipped outside every cage during 2004 in an attempt to determine herbaceous production lost to herbivory (insect herbivory was assumed to be equal across all treatments). All clipped material was dried (70°C, 48h) and weighed. Some of the cages were repeatedly disturbed by large mammals (elephant and rhino) as a result of which the data from these were left out of the analysis. For the second season, biomass was only clipped three times during the growing season because of the possibility that frequent clipping will encourage growth.

Foliar nitrogen and carbon status

Carbon to nitrogen ratios were used to develop an understanding of the effects of fire on foliar and litter chemistry. Foliage of three grasses (*Setaria sphacelata*, *Panicum maximum* and *Heteropogon contortus*) and two tree species (*Sclerocarya birrea* and *Terminalia sericea*) were collected during mid-summer (February 2004). Sampling was done in the four fire treatments across all southern EBP strings and analysed for concentrations of N and carbon (Table 5.1). Three individuals from each grass species were pooled at each sampling point to create one sample with three samples per plot. Four individual trees were used to create one sample with the amount of leaves taken dependent on the size of the leaves (three samples per plot). Only young green material was used for both the grass and tree sample. Nitrogen was analysed using a Leco FP528 nitrogen analyser (St. Joseph, Michigan, USA). This method quantitatively determines the amount of nitrogen (%) in all forms (i.e, ammonium, nitrate, protein and heterocyclic nitrogen) in botanical materials using an induction furnace and a thermal conductivity detector (Horneck and Miller 1998). The Leco FP528 nitrogen analyser implements the Dumas method where the sample is combusted at 850°C in an oxygen-rich atmosphere. A portion of the combustion gases is scrubbed of water and carbon dioxide (CO_2) and passed through a hot copper column. Resulting nitrogen gas is then measured by thermal conductivity in a helium carrier. The carbon was measured in a Carlo Erba NCS 2500 Elemental Analyser (Carlo Erba Instruments, Milan, Italy) while the samples were analysed for $\delta^{13}C$ (data in Chapter 8).

Table 5.1. Sample design for tree and grass foliar chemistry. Fire treatments sampled in each string included the August annual, August triennial, and February triennial burns, as well as the Fire exclusion.

	Tree samples per plot	Grass samples per plot
Shabeni	2 species x 3	3 species x 3
Numbi	2 species x 3	3 species x 3
Kambeni	2 species x 3	3 species x 3
Fayi	2 species x 3	3 species x 3
Total	96	144

Root biomass and N content

A point in time measurement of root biomass was made in January 2005. Three holes were augured to a depth of 25 cm at each of the ten permanent sampling points using a 4 cm diameter auger (see Table 1.1, p. 21). The three samples per point (adding up to 30 per burn treatment) were weighed and sieved separately to retrieve roots. The three samples were then pooled to ensure a large enough sample for analysis. Roots were analysed for concentrations of N and C, as well as $\delta^{13}C$ values. Photosynthetic isotope fractionation provides an opportunity to distinguish between C_3 and C_4 plants (Vogel 1978, Cerling *et al.* 1997). C_3 and C_4 types have inherent biochemical and anatomical differences and as a result discriminate against CO_2 to different degrees during photosynthesis (Farquhar *et al.* 1989). Grasses in the Kruger National Park have a C_4 photosynthetic pathway (mean values of -12.5 ‰) while most woody plants are C_3 (-26.5 ‰, Vogel 1978).

$\delta^{13}C$ values can be used to calculate the percentage of fine roots in the sample by using the following equation (adapted from Still *et al.* 2003):

$$\%C_{grass} = (\delta^{13}C_{tree} - \delta^{13}C_{measured})/(\delta^{13}C_{tree} - \delta^{13}C_{grass}) \times 100,$$

where $\%C_{grass}$ is the percent C_4 contribution, $\delta^{13}C_{tree}$ is the carbon isotopic composition of C_3 vegetation, $\delta^{13}C_{grass}$ is the carbon isotopic composition of C_4 vegetation, and $\delta^{13}C_{measured}$ is the isotopic composition of the measured sample.

Plant samples were combusted in a Carlo-Erba system (Carlo Erba NCS 2500 Elemental Analyser, Carlo Erba Instruments, Milan, Italy), analysed on a GC-IRMS (Finnigan MAT 252 IRMS, Finnigan, Bremen, Germany), and results are reported relative to the internationally accepted carbonate isotope standard PDB (Chicago Pee Dee Belemnite). Carbon $^{13}C/^{12}C$ ratios are calculated relative to this standard from the equation:

$$\delta^{13}C = ((R_{sample}/R_{Std})-1) \times 1000,$$

where R_{sample} and R_{Std} are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. Replicate samples were reproducible to 0.25 ‰.

Carbon concentrations were measured with the same system as ^{13}C during the isotopic analysis. Nitrogen concentration was analysed using a Leco FP528 nitrogen analyser (St. Joseph, Michigan, USA) (Horneck and Miller 1998).

Statistical analysis

Statistical analyses were performed using the software package JMP 5.0.1 (SAS Institute, Cary, NC, USA). Dependent variables were tested for normality. Parameters were transformed where necessary. Different factor ANOVAS were used for the different datasets. A one-way, one factor ANOVA was used to test the effect of fire treatment on root, and leaf litter biomass and nutrient concentrations. A three-factor model was used to test the effect of string, species and treatment on foliar nutrient concentrations. Significant differences ($P<0.05$) among means were determined when necessary using a Tukey HSD test.

RESULTS

Above-ground biomass and productivity

Tree leaf litter can be used as an estimate of leaf production as woody plants in broad-leaf savanna are more than 95% deciduous (Scholes and Walker 1993). Leaf biomass as calculated from litter was higher than that calculated from the LAI measurements, especially in the August annual and February triennial burns (Table 5.2). As expected, the fire exclusion treatment produced the most leaves. The August triennial burn produced less leaf biomass than the August annual and February triennial burns.

Table 5.2. Leaf biomass (kg ha^{-1}) for the four fire treatments in the Shabeni string. Treatments include the August annual burn (Aug 1), August triennial burn (Aug 3), February triennial burn (Feb 3), and Fire exclusion treatment (FE).

Biomass	Aug 1	Aug 3	Feb 3	FE
[1]Leaf	248	221	354	538
Litter	358	203	492	596

[1] Leaf biomass as computed from LAI (see methods section).

The two methods used gave comparable results for woody biomass. The February triennial burn had intermediate values between the August annual burn and the Fire exclusion plot (see Table 5.3). Wood N concentrations varied between 0.20 to 0.27% (annually burned vs. fire exclusion, from Chapter 7) for *Sclerocarya birrea*. This value was used to calculate the proportion N contained in wood for each treatment. The long-term average wood biomass increment is around 6% per annum in Nylsvley leading to wood production of around 90 g DM m^{-2} yr^{-1} (Scholes and Walker 1993). Shackleton (1997), on the other hand, found wood production in Kruger to average around 3% of wood standing crop which translates to around 60 g DM m^{-2} yr^{-1} on average. I used Shackleton's figure for wood production as the study took place in the Kruger National Park. If wood production is assumed to be 3% of standing wood crop, then wood production in the Annual burn is about half that of the Fire exclusion with the February triennial burn intermediate (531 kg ha^{-1} vs. 1062 kg ha^{-1} vs. 791 kg ha^{-1}).

Table 5.3. Above-ground woody biomass (kg ha^{-1}) and annual production of wood for the four fire treatments in the Shabeni string.

Biomass		Aug 1	Aug 3	Feb 3	FE
‡Woody	Wood	16785	na	23730	38001
	Total	17110	12319*	26602	35403
†Wood production		531	370*	791	1062

‡Total wood biomass (not including leaf) computed from the 2000 data using the formula's of Rutherford (1976), Shackleton (1997) and Goodman (1990) for specific savanna trees as well as combined formulas for *Acacia* spp., *Euclea* spp. and other broad-leaf trees. Total woody biomass (leaf and wood) computed from 2000 data using the method of Guy (1981).

*No woody data exists for the August triennial plot; Aug 1 x 0.72 Aug 3 basal area (BA) is 72% of the Aug 1 BA, as computed from relascope readings.

†Wood production was assumed to be 3% of total woody biomass (Shackleton 1997).

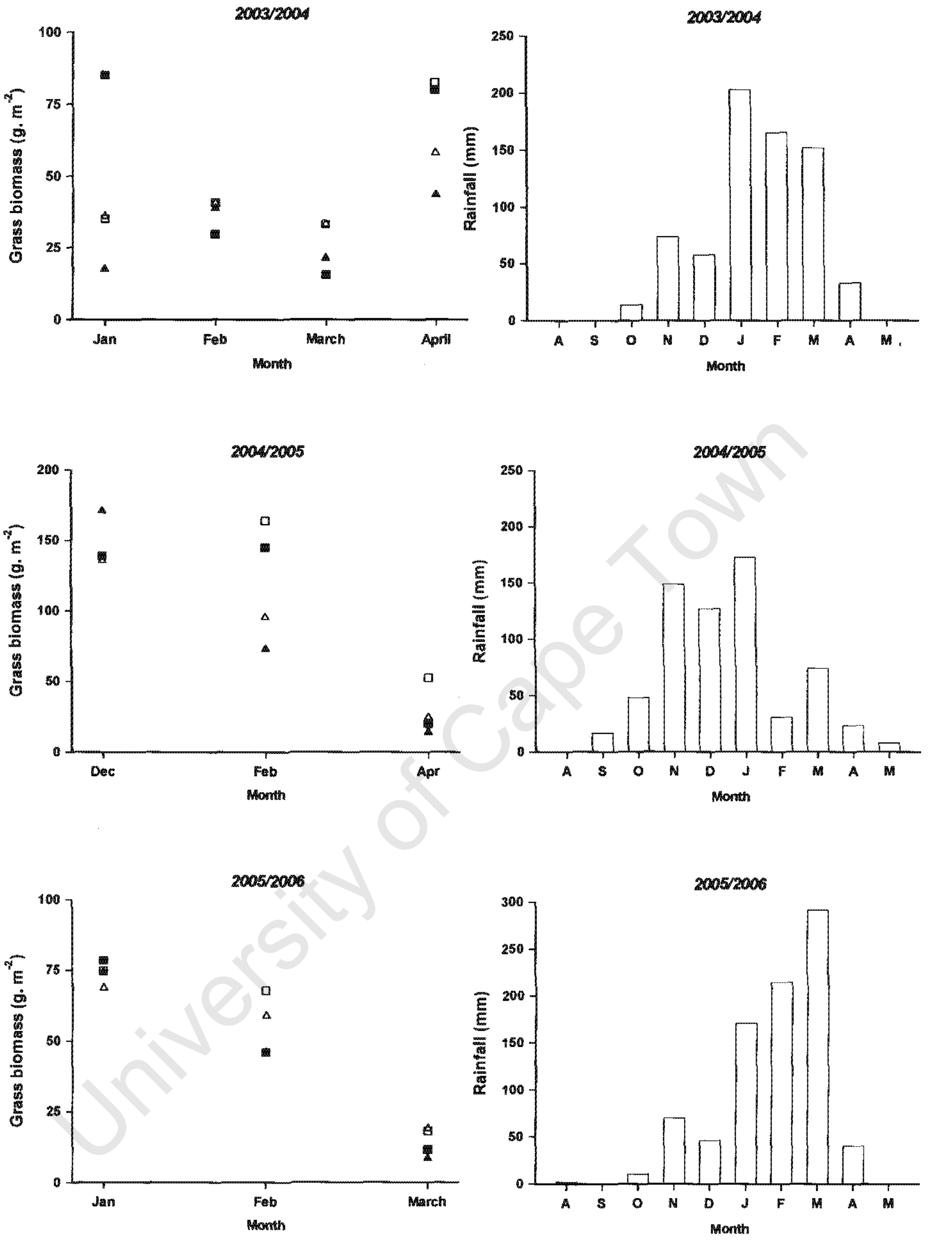

Figure 5.1. Grass biomass for three seasons starting in 2003. Annual burn (□), Fire exclusion (■), Triennial August burn (Δ), and the Triennial February burn (▲).

Foliar nitrogen and carbon status

Averaged across all four treatment locations and for the five species measured, treatment had a significant effect on foliar N concentrations [N] ($P < 0.05$, Table 5.5). Across species, annual burning decreased [N] by 1.05 mg g^{-1} compared to fire exclusion plots, with triennial burn treatments intermediate in [N]. However, looking at species individually, frequent fire only decreased [N] in *P. maximum*. There were no differences in foliar C concentrations among treatments. As burning decreased [N] and had no effect on [C], annual burning increased mass-based foliar C:N ratios, while the Fire exclusion plots had lower C:N ratios (37.7 ± 0.7 vs. 34.8 ± 0.7) (Table 5.6). Fire, however, had no effect on foliar nutrient concentrations or on C:N ratios ($P > 0.05$) at Shabeni.

Table 5.5. Analysis of the effect of string (S), fire treatment (T), species (Sp) and the interactions on [C], [N], and C:N ratios; F-values, Sum of Squares (in parentheses) and degrees of freedom (df) are given at a level of significance ($*P < 0.05$, $**P < 0.01$ and $***P < 0.001$).

		[N]	[C]	C:N
	r^2	0.77***	0.55***	0.78***
	d	F (SS)	F (SS)	F (SS)
S	3	3.07* (0.36)	4.77** (115.37)	0.32 (28.73)
T	3	3.74* (0.45)	0.16 (3.85)	4.02** (363.25)
S * T	9	2.28* (0.81)	1.34 (97.32)	2.11* (573.37)
Sp	4	130.07*** (20.67)	31.74*** (1022.65)	145.02*** (17478.39)
S * Sp	12	3.41*** (1.63)	1.04 (101.17)	3.13** (1133.66)
T * Sp	12	2.81* (1.34)	1.88* (181.98)	2.38** (860.87)

Species had different nitrogen and carbon concentrations, as well as C:N ratios ($P < 0.001$, Table 5.5). In general, *P. maximum* had the highest nitrogen concentrations and the lowest carbon concentrations (Table 5.6). The two trees had the highest [C] and *H. contortus* had the lowest N concentrations. Differences among species in C and nutrient concentrations depended on the treatment (Table 5.6). The grasses, *H. contortus* and *Setaria sphacelata* had the highest C:N ratios, *P. maximum* the lowest C:N, with the two tree species intermediate. The ranking of C:N ratios among species were constant across sites and treatments. The only anomaly was *T. sericea* in the annually burned treatments, which had lower C:N ratios than *P. maximum*.

Table 5.6. Means (with standard errors) for [C] (%), [N] (mg g^{-1}), and C:N ratio with string, fire treatment and species from Tukey's HSD; August-triennial (A3), February-triennial (F3), Fire exclusion (FE), and August-annual (A1). Means sharing the same lowercase letter does not differ significantly ($P < 0.05$).

	String		Fire treatment		Species	
[C]	Fayi	44.82 (0.64)^{a}	FE	44.15 (0.38)^{a}	*Sclerocarya birrea*	46.5 (0.4)^{a}
	Numbi	42.96 (0.40)^{a}	A3	44.30 (0.37)^{a}	*Terminalia sericea*	46.5 (0.4)^{a}
	Shabeni	44.49 (0.40)^{ab}	F3	43.86 (0.37)^{a}	*Heteropogon contortus*	42.8 (0.4)^{b}
	Khambeni	43.79 (0.39)^{b}	A1	43.83 (0.37)^{a}	*Setaria sphacelata*	42.3 (0.4)^{b}
					Panicum maximum	41.8 (0.4)^{b}
[N]	Fayi	13.79 (0.58)^{a}	FE	13.71 (0.23)^{a}	*Panicum maximum*	16.4 (0.3)^{a}
	Khambeni	13.22 (0.56)^{ab}	A3	13.50 (0.23)^{a}	*Sclerocarya birrea*	15.3 (0.3)^{ab}
	Numbi	13.18 (0.48)^{ab}	F3	12.81 (0.23)^{a}	*Terminalia sericea*	14.9 (0.3)^{b}
	Shabeni	12.64 (0.53)^{b}	A1	12.78 (0.23)^{a}	*Setaria sphacelata*	10.4 (0.3)^{c}
					Heteropogon contortus	8.8 (0.3)^{d}
C:N	Numbi	36.08 (1.34)^{a}	A1	37.69 (0.72)^{a}	*Heteropogon contortus*	50.5 (0.8)^{a}
	Shabeni	36.08 (1.00)^{a}	F3	36.90 (0.72)^{ab}	*Setaria sphacelata*	41.1 (0.8)^{b}
	Fayi	40.02 (4.46)^{a}	A3	34.89 (0.72)^{b}	*Terminalia sericea*	31.8 (0.8)^{c}
	Khambeni	35.86 (1.57)^{a}	FE	34.81 (0.72)^{b}	*Sclerocarya birrea*	30.6 (0.8)^{c}
					Panicum maximum	26.3 (0.8)^{d}

The august triennial had slightly lower litter nitrogen concentrations than the other fire treatments (Table 5.7). Incidentally, the august triennial trees also had the highest N resorption in tree (*T. sericea*) leaves at the end of the growing season.

Table 5.7. Indices of woody litter nitrogen concentrations for the four fire treatments. Means sharing the same lowercase letter does not differ significantly ($P < 0.05$).

	Treatment			
	Aug 1	Aug 3	Feb 3	FE
N_{area} (mg cm^{-2})	0.22^{a} (0.01)	0.22^{a} (0.01)	na	0.15^{b} (0.01)
$N_{resorption}$ (%)	0.55	0.60	0.54	0.51
Litter N (%)	0.65	0.55	0.68	0.71
Total litter N (kg ha^{-1})	2.32	1.12	3.34	4.23

Fine root biomass and nutrient status

Excluding fire increased the nitrogen concentrations of fine roots when compared with the burn treatments ($r^2 = 0.50$, $P < 0.0001$). Roots from the fire exclusion plots also had lower C:N ratios than those from the burned plots ($r^2 = 0.41$, $P = 0.0003$). Although Aug 1 and Feb 3 burns had lower root biomass, when compared with the other treatments, they were not significantly different (see Table 5.8). Differences in wood biomass among burn treatments are reflected in the proportion of fine root attributable to trees and forbs. Of the fine root material in the fire exclusion plot, 42% can be attributed to trees (and forbs). In the August triennial burn however, only 9% of the root material are tree (and forb) roots.

Table 5.8. Mean values of root nutrient status and biomass. Values in brackets are standard errors. Means sharing the same lowercase letter do not differ significantly ($P < 0.05$). N_{fr} = fine root N.

	Aug 1	Aug 3	Feb 3	FE
[N] (%)	0.59^b (0.06)	0.53^b (0.01)	0.65^{ab} (0.03)	0.85^a (0.07)
C:N ratio	76.3^{ab} (5.7)	88.9^a (3.4)	66.6^{bc} (5.9)	50.1^c (3.3)
% C_3	16.8	9.4	23.5	41.6
kg fine root ha^{-1}	143^a (16)	200^a (20)	147^a (16)	202^a (25)
kg N_{fr} ha^{-1}	0.84	1.06	0.96	1.72

Total N stocks in vegetation

In general, nitrogen stocks are the highest in the fire exclusion plot and lowest in the August triennial burn. Most of the nitrogen however, is contained in woody biomass, biasing nitrogen stocks in the FE treatment. The burned treatments may be more productive with higher nitrogen pools when only considering the fraction of biomass that turns over annually (*e.g.*, herbaceous biomass, leaves, new wood, etc.). However, as a large proportion of total vegetation biomass is in the grass, the nitrogen pools on the different treatments are ultimately correlated with rain. Grass production was stimulated in the annual burns when the rain commenced early in the growing season but higher in the fire exclusion in a year when rains only started later in the season (Fig 5.1).

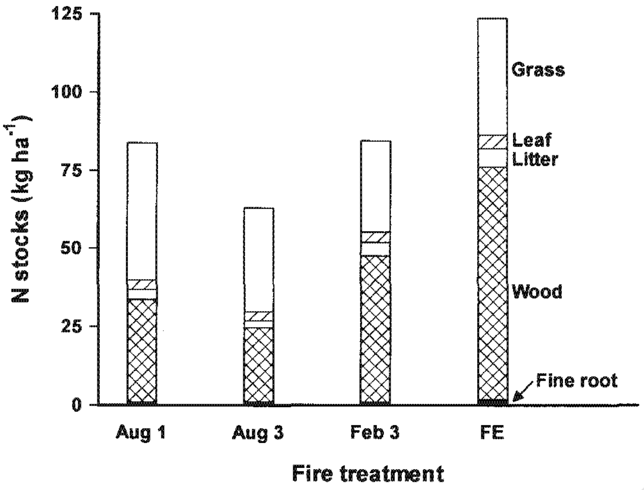

Figure 5.2. Total nitrogen (kg ha^{-1}) in the above-ground and fine root biomass for four fire treatments in the Shabeni string. Nitrogen concentrations for the various plant fractions were sampled at different times of the year, mostly during mid-summer (January to February 2005). Values were determined from the standing biomass as estimated in Table 5.2, 5.3, 5.4 and nitrogen concentrations in Table 5.6, 5.7 and 5.8. Grass biomass is the mean of biomass for the three study years.

Total productivity

Productivity was higher in the annual burn and fire exclusion plot than the triennial burns. Whereas excluding fire increased woody production (wood and leaves), this increase was compensated for by herbaceous production on the annual burns. The increase in woody production when changing from burning triennially in February to excluding fire (~500 kg ha^{-1} yr^{-1}) was the same between the two triennial burns. In other words, woody productivity was approximately 500 kg ha^{-1} yr^{-1} higher in the February triennial burn than the August triennial burn. Generally, although woody biomass contributed substantially to total above-ground biomass, herbaceous production was much higher than woody production.

higher numbers of juvenile trees than unburned areas. This would result in higher productivity in the burned areas because of faster growth rates in juveniles. As expected, fire exclusion was found to increase woody biomass production. However, the season of burn had a relatively large impact on woody productivity with August burns being much more severe than February burns (Fig 5.3). This supports findings from other savannas where it has been shown that the ratio of grass to woody plants is highest with late-dry season fires (*i.e.*, August) and lowest with late-wet season fires (*i.e.*, February, Frost and Roberston 1987).

Fire exclusion in Oak savanna resulted in a switch to forest and increased N cycling (Reich *et al.* 2001). Although the largest amount of above-ground N (largest N pool) was found in the fire exclusion treatment in this study (Fig 5.2), most of the N was stored in wood. This N was therefore not available for recycling back to the soil in the short-term. Savanna tree litter generally decomposes at a slower rate than grass litter (Scholes and Walker 1993). Combined with this, grasses that are abundant in the annually burned plots such as *H. contortus* and *S. sphacelata* have relatively high N concentrations (9-10 mg g^{-1}, see Table 5.9 for a comparison between systems). In this study, frequent fire was found to shift savanna to a more open state with high grass production. However, these grass-dominated areas did not represent the lowest N pools (Fig. 5.2). For nitrogen fluxes, the results in Chapter 4 show that mineralization was not decreased on the annual burns. This suggests that the feedback mechanism of frequent burning on low productivity found in Oak savanna did not apply to this system. Comparing these results with an Oak savanna is essentially comparing grassland with forest. Although both annual burns and forest are classified as savanna, the differences in N cycling are not directly comparable as forest often has higher biomass and N stocks than grasslands.

Fire is often perceived to have a negative effect on plant production through its effects on nitrogen availability. However, frequent fire seldom decreases productivity in prairie or savanna (Isichei 1980, Medina 1982, Ojima *et al.* 1990, Menaut *et al.* 1993, Ojima *et al.* 1994, Jensen *et al.* 2001, Abbadie *et al.* 2006). The negative perception of fire and its effect on N availability originated from studies done in North American prairie (Ojima *et al.* 1990, Ojima *et al.* 1994) and forest (Gillon and Rapp 1989, Baird *et al.* 1999). Although nitrogen is lost in savanna fires, these losses are rapidly replaced by natural inputs (Holt and Coventry 1990, Kauffman *et al.* 1994, Franklin *et al.* 1997, Mistry 1998, Jensen *et al.* 2001, Bustamante *et al.* 2006).

Accurate measurements of primary productivity were not within the scope of this study. Here, estimates of primary productivity, however, showed that annual burning did not decrease above-ground productivity. The effects of fire treatment on productivity were variable and depended to a large extent on the timing and amount of rainfall. The high intensity triennial August burns have a greater effect on productivity and nitrogen stocks than the annual burns. Intensity rather than frequency is therefore important when using fire as a management tool (*i.e.*, controlling woody encroachment) in these savannas. However, the exact mechanism responsible for the sustained high productivity and the lack of a strong effect on nitrogen cycling in the annually burned treatment remains unclear.

Table 5.9. Concentrations (% and mg g^{-1}) of mineral elements in foliage and litter from southern African savannas and elsewhere.

THIS STUDY	C (%)	N (mg g^{-1})	P (mg g^{-1})
Grasses			
Panicum maximum	41.76	16.40	1.68
Setaria sphacelata	42.29	10.45	1.10
Heteropogon contortus	42.78	8.78	0.84
Woody plants			
Sclerocarya birrea	46.51	15.32	0.91
Terminalia sericea	46.50	14.86	1.08
Litter			
Tree leaf litter		5.50-7.10	
Fine roots		5.30-8.50	
OTHER SOUTHERN AFRICAN STUDIES			
Nylsvley broad-leaf savanna (Scholes and Walker 1993)			
Grasses			
Eragrostis pallens	45.2	5.16	0.63
Digitaria eriantha	41.6	8.23	0.99
Woody plants			
Burkea Africana	45.8	12.10	0.68
Terminalia sericea	45.9	8.41	0.59
Litter			
Tree leaf litter		6.40	0.63
Fine roots	42.0	10.84	0.49
AFRICAN STUDIES			
Olokemeji Forest Reserve, Nigeria (Oguntala 1980)			
Late burn ground flora		7.00	
Early burn		7.50	
No burn		9.80	
Leaf litter (late burn to no burn)		6.70-8.60	
OTHER NON-AFRICAN STUDIES			
Oak savanna (Ritchie *et al.* 1998)			
Plant tissue		~14.00-16.00	
Litter		~6.00-8.00	
Roots		~6.00-12.00	
Oak savanna (Reich *et al.* 2001)			
Oak leaf N		22.4	
Litter		9-14	
Konza prairie (Knapp 1985)			
Andropogon gerardii, burned		2.5	
Andropogon gerardii, unburned		1.77	
Rocky Mountain National Park (Menezes *et al.* 2001)			
Ungrazed Salix spp.	57.2	12.5	
Grazed Salix spp.	56.2	14.9	
Moist tropical forests (Vitousek and Sanford 1986)			
Montane sites		9.9-17.8	
Moderately fertile soils		20.8-25.4	

CHAPTER 6
FUNCTIONAL TRAITS AND GRASS SPECIES COMPOSITION ACROSS THE FIRE GRADIENT

INTRODUCTION

Fire regimes alter N availability in many systems (Knapp and Seastedt 1986, Ojima 1987, Seastedt *et al.* 1991, Reich *et al.* 2001). Burning also changes the post-fire environment through removal of biomass and litter, leading to increased soil surface temperatures, radiation, and decreased soil moisture (Ojima *et al.* 1994). Without fire plants have to adapt to conditions of low light availability. With frequent fires, however, plants may have to adapt to low soil nitrogen, mineralization and availability (Blair 1997). A controlled fire experiment (EBP) was initiated in the early 1950's in the Kruger National Park (KNP) in an attempt to clear some of the controversy that existed at the time regarding the most appropriate burn practices (Van Wilgen *et al.* 1998, Trollope *et al.* 1998, Van Wilgen *et al.* 2000, Van Wilgen *et al.* 2004). The experiment was laid out in four vegetation types and treatments of varying frequency and season have been conducted since 1954. The set of treatments together (usually 12) is referred to as a 'string' and there are four strings in every vegetation type. Fire treatments in time have become dominated by a suite of plant species adapted to the specific environmental conditions in every treatment.

Changes in plant species composition can impact ecosystem nitrogen cycling (Ojima *et al.* 1990, Wedin 1995, Knops *et al.* 2002, Chapman *et al.* 2005).When prairie is frequently burned soil nitrogen decreases and the grasses that dominate are high in lignin and have low tissue N concentrations. The result is low forage quality, slow litter turnover, high rates of N immobilization in decomposing litter, and reduced soil net N mineralization and availability. Feedbacks on nitrogen use occur through the influence of litter properties on soil N cycling where the dominant grasses maintain high productivity through increased nitrogen-use efficiency in frequently burned areas (Ojima *et al.* 1994, Wedin 1995). Shifts in grass species composition with different fire frequencies can be explained by Tilman's (1982) R* concept. Here, the competitive dominant may be the species that can reduce the concentration of the limiting resource to the lowest level and in this way out-compete species that are less efficient in the use of the resource.

Previous vegetation change studies done on the EBP's in the Kruger National Park concentrated on the woody component (Trollope *et al.* 1998, Enslin *et al.* 2000, Shackleton and Scholes 2000). The EBP's provide a unique opportunity to study the long-term consequences of fire with the resultant establishment of a suite of species adapted to the set of conditions generated. Plant traits can be used as predictors of the response of species to environmental factors. Not only do plant traits reflect specific functional adaptations to variation in the physical environment, but they also reflect trade-offs among different functions in a plant (Westoby 1998, Craine *et al.* 2002, Fynn *et al.* 2005, Fynn and

O'Connor 2005, Lavorel *et al.* 2005). From syntheses of empirical and theoretical studies, at least four axes of plant specialisation have been proposed (Westoby *et al.* 2002, Lavorel *et al.* 2005). This includes a specific leaf area – leaf life span trade-off axis that is associated with turnover time of plants, including turnover through herbivory, nutrient residence times and the rate of response to favourable growth conditions (Diaz *et al.* 2004, Lavorel *et al.* 2005). Disturbance, such as fire, may lead to the dominance of certain species, depending on these trade-offs.

There has been some research on the effects of fire on grass composition and accompanying traits, but the underlying plant strategies (*e.g.*, stress tolerant vs. good competitor) are not clear (Belsky 1992, Fynn *et al.* 2004, Fynn *et al.* 2005). In this chapter I examine the effects of fire on grass species composition to establish whether grasses arrayed according to those traits associated with increased nitrogen-use efficiency. A suite of 6 traits were measured in the 15 dominant species. To test whether soil characteristics could explain species abundance across the fire gradient, I measured several soil nutrients as well as pH across treatments.

METHODS

Study area

Data from all four southern EBP strings (*i.e.*, Shabeni, Numbi, Kambeni and Fayi) were used (See Fig. 2.4, p. 20). Each string includes 12 treatments that vary both in fire frequency and timing: August annual burn, biennial and triennial burns in late winter (August), spring (October), early-summer (December), mid-summer (February) and autumn (April), as well as a fire exclusion treatment. A comprehensive description of the study area, as well as background on the Experimental Burn Plot Experiment is described in Chapter 2. To standardize fire frequency and intensity, fire treatments were grouped into average burn in ten years (data from Scientific Services, Skukuza) and also into low, medium and high intensity burns (Govender *et al.* 2006).

Archaic granite of the Swaziland system underlies this area and the plots are part of an undulating landscape (Gertenbach 1983). Soils in the area belong to the Clovelly and Hutton Forms and are derived from the underlying granite and, as a result, relatively nutrient poor (Van Wyk 1971, Forms according to the South African Soil Classification Working Group 1991). Precipitation is generally restricted to the summer months (October to April) and ranges between 600 and 1000 mm with an average of 743.6 mm for Pretoriuskop (Gertenbach 1983). Mean annual temperature for the sites is 22°C with winter temperatures seldom below 10°C.

Grass traits

A total of 6 traits (*i.e.*, Lignin/N, leaf tissue density, root tissue density, tensile strength, nutrient concentrations, and root diameter) were measured on each of three individuals of 14 dominant grasses as well as *Panicum deustum* in the southern EBP's (see Table 6.1). *P. deustum* was chosen as it is one of few grasses that grow well in shady places. As there are so few fire exclusion plots, I wanted to include an extra grass that would represent the conditions of the fire exclusion plots. All grasses were collected from an area just south of the Shabeni string. Grass traits were chosen to distinguish those grasses that will dominate at high N situations (high N species) from those who would do better in low N situations (low N species) (Tilman 1982). Low N species maintains greater biomass than high N species by producing more tissues with lower N (increased nitrogen use efficiency and higher tissue C:N) and by greater longevity (Craine *et al.* 2002). I used tensile strength and tissue density as a proxy for longevity as tougher, dense leaves should live longer. If fire should decrease the availability of N, then those species that have a low nutrient strategy (*e.g.*, low rates of physiological activity, tough, dense, long-lived tissues that have low nutrient concentrations above- and below-ground, Craine *et al.* 2002), should have an advantage.

Tensile strength

To determine leaf tensile strength I used the method of Balsamo *et al.* (2006). Leaves were secured vertically between two clamps approximately 5 cm apart. A bucket was then hung from the lower clamp with a Pesola balance between leaf and bucket. The bucket was progressively loaded with small weights until the leaf snapped. Tensile strength was computed as follows:

$$T = (M * N)/(LT * LW),$$

where M is the mass at which the leaf broke, N is the Universal gravitational constant, 6.672×10^{-11} N m^2 kg^{-1}, LT equals leaf thickness (mm), and LW is the leaf width (mm). Leaf tissue density was calculated as the ratio of subsample mass and total area of the subsample.

Leaf nutrients and lignin

Leaf samples were analysed for the following nutrient concentrations: N, P, K, Ca, Mg, Na, Mn, Cu, and Zn. Nitrogen was determined with a LECO FP528 N-analyser (St. Joseph, Michigan, USA) and the rest of the elements were determined with an ICP-OES analyser (Optima 3000XL, PerkinElmir, Waltham, Massachusetts, US) according to the method of Kalra (1998). Leaf samples were also analysed for Acid Detergent Lignin using the Method for determining Acid Detergent Lignin in beakers (Hattas *et al.* 2005). This process involves a series of extractions to determine the fiber content of a plant sample. Extractions are done in order of Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and then lastly, Acid Determined Lignin (ADL).

Root density

Root samples for the 15 species were obtained by removing a 20 cm x 20 cm square volume of soil around each root system. Excess soil was removed in the field. Roots were then washed until free of soil. A representative subsample was scanned on a Hewlett Packard 6100 scanner at 600 dpi. As some root systems had very few fine roots (< 1 mm), a larger sample was taken which incorporated all diameter roots (< 3 mm). Average diameter, total root length and root volume of the subsample were determined with WinRhizo (Régent Instruments, Quebec, Canada). Subsamples were then dried and weighed and root tissue density was calculated as the ratio of subsample mass and total root volume.

Vegetation sampling

Grass (herbaceous) composition was sampled in 2001 using a step-point technique (data from Scientific Services, Skukuza). For each plot, points placed ~2 m apart were sampled on two diagonal transects, adding up to 200 points per plot. The distance of the nearest rooted species and each point was recorded, as well as tuft diameter. The frequency for each species was calculated as the number of points where that species was encountered out of the total number of points sampled. Basal cover was taken as the mean point to tuft distance of a species per plot. Traits were measured for fifteen grass species.

Table 6.1. Characteristics of the 15 grasses used in this study. Growth forms include decumbent stoloniferous (DS); culmless bunch vegetative shoots with below-ground position of meristems (CLV); and culmed bunch vegetative shoots with axillary buds above-ground level (CV). Perennial (P), annual (A), biennial (B).

Grass species‡	Abbr.	Life history‡	Palatability‡	Physiology†	Growth Form*
Cynodon dactylon	Cyndac	P	Intermediate	NAD-me	DS
Digitaria eriantha	Digeri	P	Palatable	NAD-me	DS
Diheteropogon amplectens	Dihamp	P	Palatable	NADP-me	CV
Eragrostis rigidior	Erarig	P	Palatable	NAD-me	
Heteropogon contortus	Hetcon	P	Intermediate	NADP-me	CV
Hyperthelia dissoluta	Hypdis	P	Unpalatable	NADP-me	
Hyparrhenia filipendula var. *pilosa*	Hypfil	P	Intermediate	NADP-me	
Loudetia flavida	Loufla	P	Unpalatable	NADP-me	
Melinis repens	Melrep	A/B	Unpalatable	PCK	CV
Panicum deustum	Pandeu	P	Palatable	PCK	CLV
Panicum maximum	Panmax	P	Palatable	PCK	CLV
Pogonarthria squarrosa	Pogsqu	B	Unpalatable	NAD-me	
Schizachyrium sanguineum	Schsan	P	Unpalatable	NADP-me	
Setaria sphacelata var. *sphacelata*	Setsph	P	Palatable	NADP-me	CLV
Urochloa mosambicensis	Uromos	A/B	Intermediate	PCK	DS

‡Van Oudtshoorn (1991), intermediate palatable species are usually palatable early in the growing season, but lose palatability as the growing season progress, †Gibbs Russell *et al.* (1990), *R.W.S. Fynn Pers. Comm.

Soil sampling

A composite sample (*i.e.*, three 10 cm deep soil cores) of five randomly distributed points was taken for every plot. The soils were air-dried and assayed for total carbon, nitrogen, and phosphorus, extractable calcium, magnesium, potassium, manganese, zinc, sodium and pH by the institute for Soil, Climate and Water in Pretoria, using standard procedures.

Data analyses

To understand the influence of fire on herbaceous species composition, the relative species abundance for every treatment was compared through non-parametric multivariate analysis using PRIMER (Clarke 1993). Total abundances of each species sampled were square-root transformed and a Bray Curtis similarity matrix computed (Clarke 1993). Statistical significance of differences between the treatments was tested by one-way ANOSIM developed by Clarke (1993). The ANOSIM computes a test statistic reflecting the observed differences among replicates between sites (treatments), contrasted with differences among replicates within sites (treatments). For a full explanation of the ANOSIM procedure see Pandolfi and Minchin (1995). To understand the differences in species composition between treatments, I calculated similarity percentages (SIMPER; see Clarke and Warwick 1994). The SIMPER results indicate specifically which species are responsible for the results obtained from the ANOSIM tests by comparing the average abundance of species between treatments. The average dissimilarity between pairs of strings within treatment was computed. The average is then broken down into separate contributions from every species, resulting in an average term. Species that have large values of the average term and large values of the ratio of the average term to its standard deviation are important in characterizing the differences between treatments.

I used the program PC-ORD 4 (McCune and Mefford 1995) and performed a non-metric multidimensional scaling (NMDS, Kruskal 1964, Mather 1976) technique to discriminate between the most important axes of variation in the dominant grass species' abundances. Untransformed abundance values were standardized by the maximum attained by each species which equalizes the potential contributions of species to the overall dissimilarity in composition. NMDS is an effective method for multivariate data reduction and analysis of ecological community data sets and is considered to be one of the most powerful ordination techniques available (McCune and Grace 2002). NMDS is well suited to data that are non-normal or are on arbitrary or discontinuous scales (McCune and Grace 2002).

NMDS uses an iterative search for ranking and placement of n entities (samples) in k dimensions (ordination axes) that minimizes the stress of the k-dimensional configuration. The "stress" value is a measure of the departure from monotonicity in the relationship between the dissimilarity (distance) in the original p-dimensional space and in the reduced k-dimensional space (Clarke 1993). The lower the stress value, the better the representation of the plots in the multidimensional space. The primary objective is to find a representation of data in the fewest dimensions so that the distances in the ordination space reflect the similarities between plots as closely as possible. An initial run was made using a 6 dimensional space, Sørensen distance measure (also known as Bray-Curtis) and 100 iterations.

To find the lowest number of axes at which the reduction in stress gained by adding another axis was small, I examined a plot of stress versus iteration. A final run of 100 iterations was made using three axes with a randomly selected starting configuration. The stress obtained from the data was compared to the stress from 50 runs of randomized versions of data. Stress values less than 0.2 (20%) generally produce interpretable results (McCune and Grace 2002). The results of the NMDS technique were used to correlate axes with principal traits axes and soil characteristics. Soil variables were related to the strongest gradients (axes) in species composition using overlays and correlation coefficients.

To determine the relationship among functional traits across species, a principal component analysis (PCA) was performed on 6 functional traits, using a correlational matrix structure. PCA was performed using the statistical program JMP 5.0.1.2 (SAS Institute).The first two axes of the PCA were the most biologically meaningful and reported here. The trait axes were correlated with the NMDS axes using JMP 5.0.1.2 (SAS Institute).

RESULTS

Species composition

Annual burn treatments burned an average of nine times in ten years, and one fire exclusion plot received an accidental burn during the sampling period (Fayi accidentally burned during 1991). The 2001 herbaceous survey (data from Scientific Services, Skukuza) found 45 grass species at the Sourveld EBP's (see Table 6.8 for species list and nomenclature). The ANOSIM analysis in PRIMER showed few differences in species abundances between the different fire frequencies (Table 6.2). The only significant difference was between annual burns and the fire exclusion plots. For fire intensity, the only significant difference was between the high intensity burns and fire exclusion treatments. There were no significant differences between the species composition of the strings. It is important to note that *P*-values, in this case, are not a good indicator of significance as they are dependent on the number of replicates (McCune and Mefford 1999).

The SIMPER dissimilarity analysis calculates which species are most responsible for the differences between treatments as shown by the ANOSIM results. The four species (or groups) most responsible for the difference between the annual burned and fire exclusion were *Aristida congesta* subsp. *congesta*, *Pogonarthria squarrosa*, *Urochloa mosambicensis*, and the sedges (Table 6.3). All of these were present in the annual burns but not in the fire exclusion plots. The fire exclusion and high burn intensity treatments were distinguished by *Pogonarthria squarrosa*, *Perotis patens*, *Hyperthelia dissoluta*, *Setaria sphacelata*, and *Heteropogon contortus* (Table 6.3). SIMPER ranks species on their average contribution to the overall dissimilarity between treatments, not on the gross differences in average abundance. A species with a high ranking (based on abundance) but low mean-to-standard deviation ratio will not be a good discriminating species (Clarke 1993). According to the mean-to-standard deviation ratios, the differences in species abundances between the no burn and high intensity burns are not pronounced.

Table 6.2. Analysis of similarity (ANOSIM) results for differences in grass composition between fire treatments for the Sourveld EBP's (Pretoriuskop). Treatments are divided according to intensity and frequency (average burns in ten years). *R* is the ANOSIM statistic, based on the rank of the Bray-Curtis values. The strings are Fayi (F), Kambeni (K), Numbi (N), and Shabeni (S).

	R-stat		*R*-stat		*R*-stat
Frequency		Intensity		String	
Overall	0.23	Overall	0.23	Overall	0.25
4,9	0.28	High, and No	0.64*	Fayi and Kambeni	0.30
4,6	0.37	High and Low	0.15	Fayi and Numbi	0.29
4,3	0.00	Med and High	0.21	Fayi and Shabeni	0.23
4,1	0.36	Med and No	0.43	Kambeni and Numbi	0.15
3,9	0.29	Med and Low	0.12	Kambeni and Shabeni	0.21
3,6	0.03	No and Low	0.35	Numbi and Shabeni	0.30
3,1	0.42				
9,6	0.29				
9,1	0.68*				
6,1	0.41				

**R* approaches 1 if all replicates within sites (treatments) are more similar to each other than any replicate from different sites (treatments).

Table 6.3. Contributions of species that distinguish between fire treatments (SIMPER procedure from PRIMER software package, Clarke and Warwick 1994). The dissimilarity's are shown for those treatments significantly different in the ANOSIM analysis. The species shown have the largest discrepancies in average abundance between treatments. Abundance data are square-root transformed.

	Avg abundance*		Avg term†	Ratio‡
Frequency	Nine (annual)	No burn		
Aristida congesta	0.78	0.00	1.18	5.94
Pogonarthria squarrosa	3.05	0.35	4.08	3.99
Urochloa mosambicensis	1.13	0.00	1.72	2.17
Sedge	1.75	0.00	2.63	1.95
Intensity	High	No burn		
Pogonarthria squarrosa	1.44	0.35	1.68	1.70
Perotis patens	1.31	0.53	1.41	1.59
Hyperthelia dissoluta	3.92	1.76	3.85	1.54
Setaria sphacelata	3.86	5.45	4.31	1.52
Heteropogon contortus	3.04	2.18	2.20	1.50

* The mean abundance for each species in each fire treatment across strings. † The average contribution for a particular species to the overall similarity (among all species) within in every fire treatment. ‡ Ratio of average term to its standard deviation. Species with higher ratio values relative to other species better discriminate between the treatments.

Certain species array across the fire gradient; being more dominant in frequently burned plots than fire exclusion plots. In general (across all strings), *P. maximum* was abundant in the fire exclusion plots and *H. contortus* and *P. squarrosa* more abundant in the annual burns. When considering strings separately the pattern did not always hold. *P. maximum* was more abundant in the intermediate frequency plots than in the fire exclusion plots at Fayi and Kambeni (Fig 6.1). The abundance of certain species such as *S. sphacelata* varied primarily with related string. *S. sphacelata* was frequently found in Fayi and Shabeni, regardless of frequency of burning.

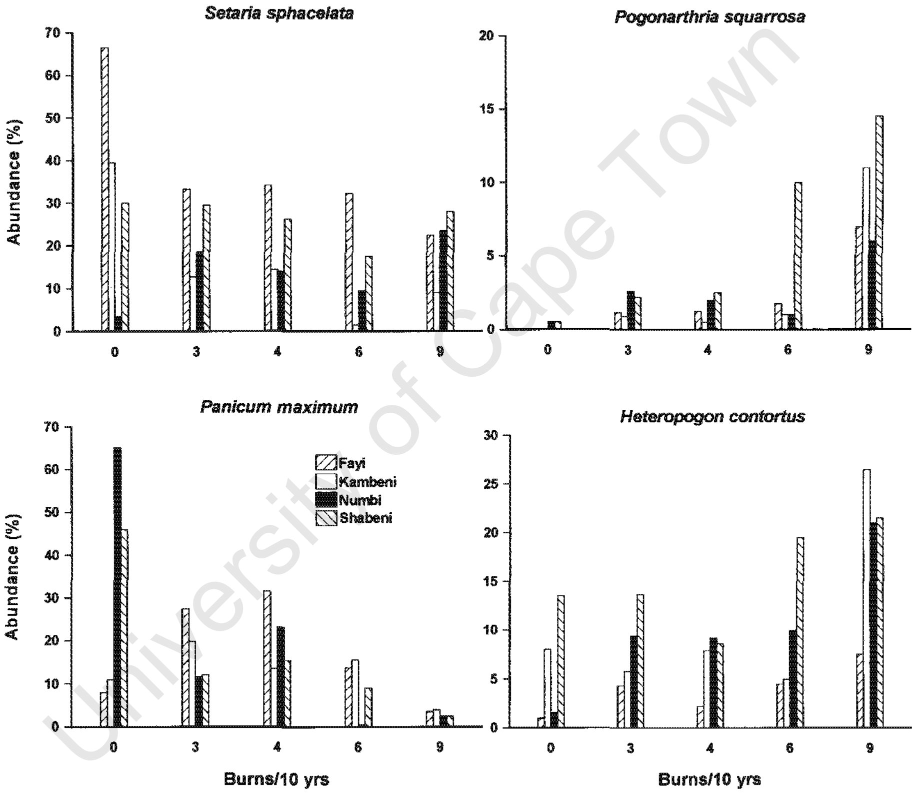

Figure 6.1. Relative abundances of four grass species across the fire frequency gradient.

Whereas the PRIMER analyses were used to examine differences in the fire treatments and all the species were included, the NMDS ordination was only performed with the 15 grass species used in the traits analysis. For the NMDS ordination, the greatest reduction in 'stress' (McCune and Mefford 1999) was achieved with a three dimensional solution (see Fig 6.2 and Fig 6.3 for 2-dimensional representation of axes). The final NMDS solution had a stress value of 0.09 and final instability equaled $<10^{-4}$. The proportions of variance (coefficients of determination R^2 for the correlations

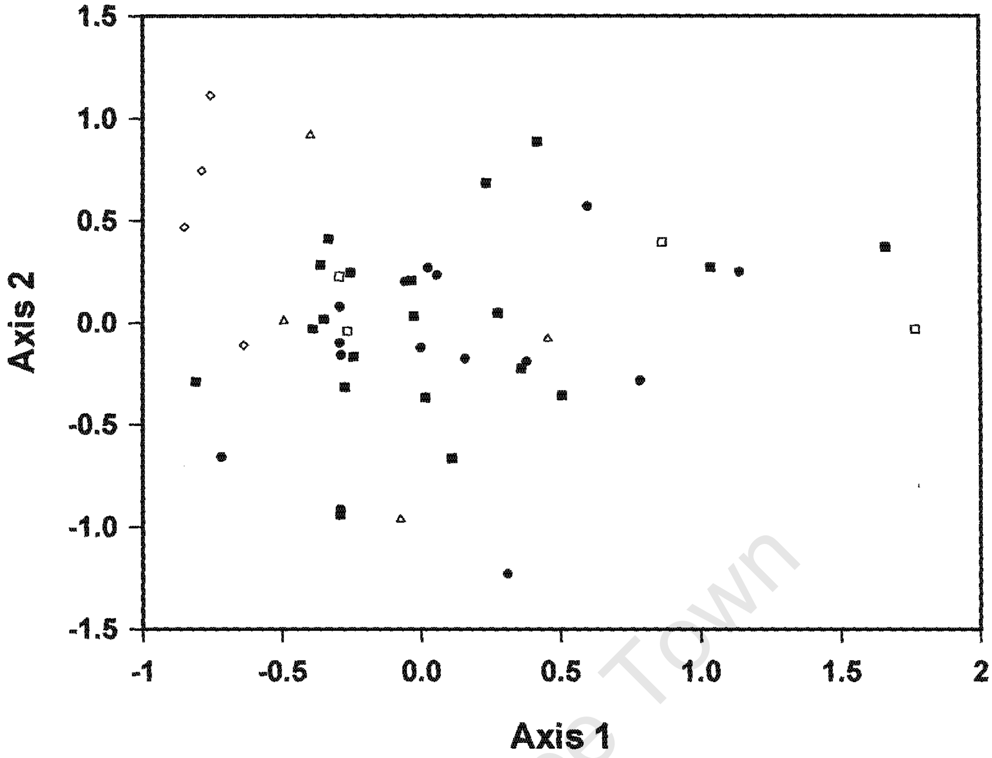

Figure 6.2. Ordination of the different frequency burn plots in the strings of the Sourveld EBP's (Axis 1 vs. Axis 2). The plots have been labeled according to the mean amount of years the plot burned every ten years since the advent of the EBP experiment; nine burns (◊), three burns (■), four burns (•), six burns (Δ), and fire exclusion plots (□), Fayi burned once since the establishment of the burn plots.

between ordination distances and Sørensen distance in the original multi-dimensional space) represented by the three axes were 0.45, 0.26 and 0.23 respectively (cumulatively $R^2 = 0.94$).

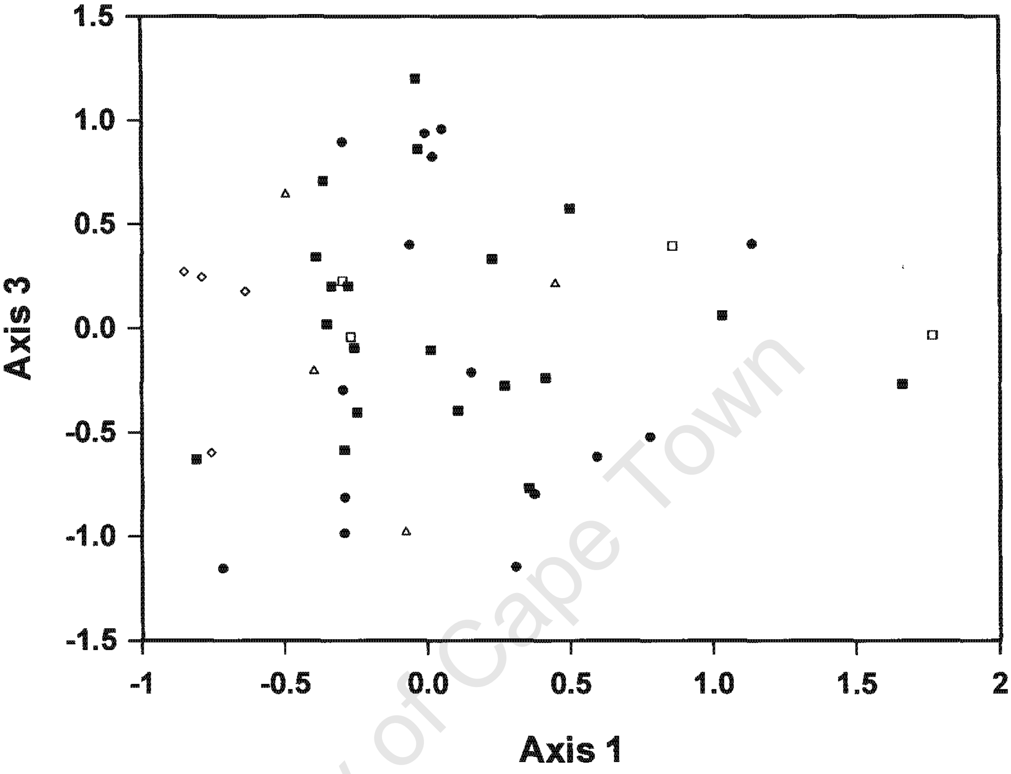

Figure 6.3. Ordination of the different frequency burn plots in the strings of the Sourveld EBP's (Axis 1 vs. Axis 3). The plots have been labeled according to the mean amount of years the plot burned every ten years since the advent of the EBP experiment; nine burns (◊), three burns (■), four burns (•), six burns (Δ), and fire exclusion plots (□),Fayi burned once since the establishment of the burn plots.

Axis 1 was correlated with fire frequency (r^2 = 0.20, Table 6.4). *H. contortus*, *P. squarrosa* and *Schizachyrium sanguineum* were more likely to be found in an annual burn, while *P. deustum* and *P. maximum* were more likely to be found in the fire exclusion plots (Table 6.5). Axis 2 separated plots more likely to be dominated by the tall unpalatable grasses *Hyperthelia dissoluta*, *Hyparrhenia filipendula*, and *Schizachyrium sanguineum* (see Table 6.5) from those dominated by *Digitaria eriantha, Eragrostis rigidior, P. squarrosa,* and *Urochloa mosambicensis* and was weakly correlated with the intensity of burns (r^2 = 0.17). Axis 3 separated those plots that were more likely to be dominated by *Setaria sphacelata* from those more likely dominated by the tall thatch grasses, *H. filipendula*, and *H. dissoluta*. None of the axes correlated well with soil characteristics (Table 6.4). Axis 1 was weakly correlated with nitrogen (r^2 = 0.15) and Axis 3 with phosphorus (r^2 = 0.16). It appears as if *P. deustum* and *P. maximum* were more likely to occur in plots with higher soil N content. Plots did not separate well according to the strings in which they were situated and none of the NMDS axes were correlated with strings (Table 6.4, Fig. 6.4).

Table 6.4. Correlations (r^2) of NMDS species abundance axes with site, fire treatment and soil characteristics.

		Axis 1	Axis 2	Axis 3
Site	String	0.04	0.01	0.05
Fire treatment	Month	0.02	0.13	0.08
	Intensity	0.03	0.17	0.06
	Frequency	0.20	0.08	0.01
Soil characteristic	P	0.03	0.05	0.16
	K	0.03	0.01	0.07
	Calcium	0.09	0.03	0.00
	Mg	0.06	0.03	0.03
	Na	0.07	0.05	0.02
	pH	0.01	0.01	0.00
	N	0.15	0.10	0.01
	Carbon	0.08	0.14	0.04

Table 6.5. Results of the non-metric multidimensional scaling (NMDS) ordination; correlations between ordination axes and species abundance; Pearson's *r*. The proportions of variance represented by the three axes were 0.45, 0.26 and 0.23 respectively (cumulatively $r^2 = 0.94$).

Grass species	Axis 1	Axis 3	Axis 2
	r	*r*	*r*
Cynodon dactylon	0.15	-0.08	-0.24
Digitaria eriantha	0.07	**0.60**	-0.06
Diheteropogon amplectens	-0.17	-0.17	0.08
Eragrostis rigidior	-0.28	**0.47**	-0.22
Heteropogon contortus	**-0.54**	**0.45**	-0.31
Hyperthelia dissoluta	-0.30	**-0.70**	**-0.76**
Hyparrhenia filipendula	-0.21	**-0.72**	**-0.59**
Loudetia flavida	-0.15	-0.21	-0.01
Melenis repens	0.12	0.19	-0.13
Panicum deustum	0.39	-0.36	-0.04
Panicum maximum	**0.86**	0.05	-0.22
Pogonarthria squarrosa	-0.41	0.44	-0.56
Schizachyrium sanguineum	-0.42	-0.41	-0.20
Setaria sphacelata	-0.32	0.43	**0.80**
Urochloa mosambicensis	-0.09	0.34	-0.27

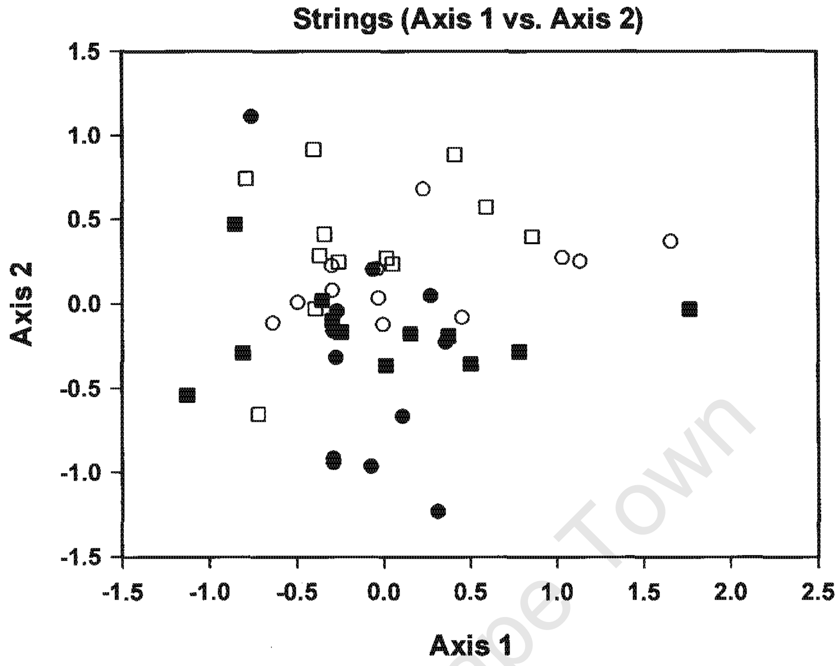

Figure 6.4. Non-metric multi-dimensional scaling (NMDS) ordination of strings in the Sourveld EBP's. Strings include Shabeni (□), Fayi (○), Numbi (■), and Kambeni (•). Although the NMDS was calculated in three dimensions, this graph shows the first and second dimension.

Traits

The principal components analysis of twelve plant functional traits (Table 6.6; Fig. 6.5) revealed two axes that together accounted for 53% of the explainable variation in traits. The first axis separated robust or tough from not so tough species. The less robust species, which scored high on Axis 1, had leaves with low tissue density, low tensile strength, and were thick and wide. The leaves of these species also had high concentrations of N, K Mg and Ca concentrations, but did not differ in P concentrations. The less robust species had roots with high tissue density. Axis 1 explained 36% of the explainable variation in functional traits. Axis 2, which explained half as much variation as Axis 1 (17%), primarily separated the short stoloniferous *Cynodon dactylon* from *Loudetia flavida*. In contrast with *L. flavida*, *C. dactylon* was characterized by high leaf tissue density, narrow leaves with high tensile strength, high leaf N, P and K concentrations, and low leaf lignin. L. flavida had low tissue density and leaf N, P and K concentrations, but high leaf lignin.

Table 6.6. Loadings for the principal component analysis (PCA) for functional traits. Axis 1 explained 36% of the explainable variation in traits, 5 times more than expected by chance. Axis 2 explained 17% of the explainable variation, 2.4 times more than expected by chance.

	Axis 1	Axis 2
Leaf tissue density	-0.39	0.31
Leaf thickness (mm)	0.40	0.11
Leaf width (mm)	0.30	-0.35
Tensile strength	-0.26	0.40
[Lignin]	0.05	-0.50
[N]	0.17	0.32
[P]	0.06	0.25
[K]	0.38	0.29
[Ca]	0.39	0.21
[Mg]	0.37	0.19
Root tissue density (g.cm^{-3})	0.24	-0.11
Ave root diameter	0.03	0.15

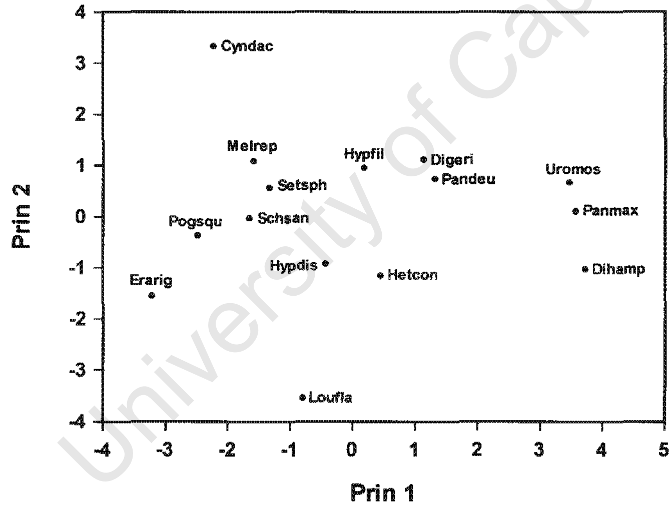

Figure 6.5. Principal component analysis (PCA) on functional traits' principal components; Prin 1 vs. Prin 2.

Table 6.7. Correlations (r^2) of NMDS species abundance axes and principal components from the PCA of traits.

		Axis 1	Axis 2	Axis 3
PCA principal components	Traits Axis 1 (Prin 1)	0.12	0.004	0.007
	Traits Axis 2 (Prin 2)	0.18	0.002	0.05

The differences in species that accounted for the separation of plots along axes were not due to differences in measured functional traits. The strongest correlation between traits was with Axis 1 (see Table 6.7, $r^2 = 0.18$, not significant).

DISCUSSION

Fire regimes alter N availability in many systems (Knapp and Seastedt 1986, Ojima 1987, Seastedt *et al.* 1991, Reich *et al.* 2001). Frequent fires may lead to low soil nitrogen, mineralization and availability (Ojima *et al.* 1994, Reich *et al.* 2001) with a suite of species adapted to these low N conditions becoming dominant in frequently burned sites (Blair 1997). In frequently burned prairie, C_4 grasses that are very nitrogen use efficient become dominant (Ojima *et al.* 1990, Ojima *et al.* 1994). Using the long-term experimental plots in the Kruger National Park, I investigated whether species arrayed on the fire gradient according to traits relating to nitrogen use efficiency. A secondary aim was to establish if fire had an effect on species composition and to identify which species were affected by specific fire treatments.

Westoby *et al.* (2002) proposed that at least four axes of plant specialisation can be considered. The first and best understood axis is represented by the specific leaf area-leaf life span trade-off and is associated with the turnover time of plant parts, nutrient residence times and rate of response to favourable conditions (Lavorel *et al.* 2005). Similarly, a 'trade-off spectrum' in functional traits exists that separates species with fast-tissue turnover and high potential for resource capture from those species with slower tissue turnover and traits that enhance resource conservation (Reich *et al.* 1997, Diaz *et al.* 1999, Reich *et al.* 1999, Aerts and Chapin 2000, Diaz *et al.* 2004). To test whether traits separated species on the universal first axis of specialisation, I performed a principal component analysis of the traits measured in the dominant grass species.

Slow-growing species generally tend to have tough (high tensile strength), dense and long-lived leaves with low foliar nutrient concentrations and are relatively unpalatable to herbivores (Grime *et al.* 1997, Aerts and Chapin 2000, Craine *et al.* 2002). Broadly, PCA Axis 1 separated fast-growing palatable grasses from slow-growing unpalatable species. Among the grass species at KNP, less palatable *P. squarrosa* and *M. repens* are separated from the more palatable *P. maximum* and *U. mosambicensis* on Axis 1. The lack of high phosphorus concentrations for fast-growing species is the only exception in the group of associated traits with greater foliar N:P for fast growing grass species at KNP.

I performed a NMDS ordination procedure to discern main gradients in the dominant grass species composition and to test whether the traits axes correlated with fire gradients. Although the first NMS axis was weakly correlated with fire frequency and the second with fire intensity, the PCA (*i.e.*, traits) axes were not related to any of the NMDS axes. Neither did fire treatment have any strong effects on soil characteristics. Fynn *et al.* (2003) found that winter burning in grassland resulted in lower soil nitrogen than spring burning and that the competitive balance between species was related to the effect of fire on soil resources such as nitrogen. In this study however, frequent or intense fires did not increase the dominance of grass species with higher nitrogen use efficiency.

Axis 1 of the NMDS separated *H. contortus* from *P. maximum* which agrees with the annual burn vs. fire exclusion treatment difference shown by the ANOSIM procedure. *P. maximum* thrives in some of the fire exclusion plots and tolerates shading well. *P. maximum* has culmless vegetative shoots with below-ground meristems which may not require light to initiate tillering. This species is therefore well suited to the shaded conditions of the fire exclusion plots (R.W.S. Fynn pers. comm.). In comparison, *H. contortus* often increases with frequent fire (Uys *et al.* 2004, Fynn *et al.* 2005). *H. contortus* has culmed vegetative shoots with axilliary buds above ground level, which may require light to initiate tillering. It is a fast growing species with copious seed production and its success in high fire environments have been attributed to germination of seed being favoured by high soil surface temperatures post-fire (Stocker and Mott 1981). *Andropogon gerardii*, a grass that dominates in frequently burned prairie, has certain physiological adaptations that enable it to adapt to the lower moisture, high light conditions of frequently burned areas (Knapp 1985). For example, it has the ability to maintain high rates of carbon gain over a greater range of leaf temperatures and at lower water potential than a less dominant *Panicum* species (Knapp 1985). *H. contortus* and *P. maximum* appear to employ a 'tolerance' strategy (MacDougall and Turkington 2004). The mechanism that confer tolerance is different though, *H. contortus* and *P. maximum* have trade-offs between the ability to grow rapidly (and tolerate disturbance well) and the ability to recruit within resource-limited (tolerate low light) understoreys that develop as plant communities approach equilibrium.

Fire intensity did explain some of the variation in the second NMDS axis. It is difficult to separate the fire intensity, fire frequency and month of burn as both fuel load (amount of years that fuel load can accumulate) and moisture content of the fuel (month of burn) plays a role in determining fire intensity (Govender *et al.* 2006). An example of this is the annual burns that take place in August and burn at intermediate intensities as fuel load has only one year to accumulate. The August and October biennial and triennial burns usually burn at higher intensities as the fuel has low moisture content and two or three years of biomass accumulation. Plots with high abundance of tall thatch grasses (*i.e.*, *Hyperthelia dissoluta*, *Hyparrhenia filipendula*, and *Schizachyrium sanguineum*) did separate from the rest of the grasses on the second axis of the ordination. From the results, these grasses appear to be adapted to high intensity, intermediate frequency burns. I speculate that these grasses grow slowly and are dormant early in the season when the high intensity burns take place. Further, as the high intensity burns happen only every second or third year, they subsequently have enough time to mature and seed between fires. Early season fire in prairie leads to matrix (late successional, slow-growing) grasses, whilst killing shoots of early-growing grasses (Engle *et al.* 2000).

To determine which species were responsible for a fire treatment effect in the NMDS ordination, species composition was further investigated with the use of PRIMER. The results of this analysis show that the fire exclusion plots were the most different from either the most frequently burned plots or those burned at the highest intensities. Excluding the fire exclusion plots, frequency or intensity of fire had little effect on grass species abundance. Of the three grasses responsible for the difference between the annually burned and fire exclusion plots, *P. squarrosa* and *A. congesta* frequently occur in disturbed places and are avoided by grazers (Van Oudtshoorn *et al.* 1991). Annual burns are subjected to high grazing pressure (Mills 2003), as a result of which unpalatable grasses should be advantaged. *U. mosambicensis*, a grass that does not occur on the fire exclusion plots, also frequents disturbed areas, and tolerates intensive grazing (Van Oudtshoorn *et al.* 1991). *U. mosambicensis* is decumbent stoloniferous. This is a growth form that confers high resistance to disturbance through fast vegetative regeneration (Sarmiento 1992).

As opposed to results obtained for Prairie grasses (Ojima *et al.* 1994, Wedin 1995), my analyses show that grass species composition at Pretoriuskop in the KNP is not strongly influenced by fire frequency. Grass species did not array on the fire gradient according to those traits that correlated with high nitrogen use efficiency. Grass species composition was thus not driven in a 'bottom-up' way by the effect of fire on nitrogen availability. Rather, the differences in species composition for plots on the extremes of the fire gradient (*e.g.*, annual burns vs. fire exclusion) were primarily related to traits that confer tolerance to high disturbance (frequent or intense fire) or tolerance of low light conditions. The results indicate that the control on grass species composition is primarily 'top-down', with species sorted across treatments depending on plant traits that reflect responses to the variation in the physical environment brought about by fire.

APPENDIX

Table 6.8. Grasses occurring at the Sourveld Experimental Burn Plots, palatability (Pal) includes palatable (P), unpalatable (U) and palatable early in the growing season (I).

Abbr.	Species	Nomenclature	Pal	Physiology
Ariads	*Aristida adscensionis*	(Nees) Dur. & Schinz	U	C4, NADP-me
Ariconcon	*Aristida congesta* var. *congesta*	Roem & Schult	U	C4, NADP-me
Ariconbar	*Aristida congesta* var. *barbicollis*	(Trin. & Rupr.) De Winter	U	C4, NADP-me
Botbla	*Bothriochloa bladhii*	(Retz.) S.T. Blake	U	C4, NADP-me
Botins	*Bothriochloa insculpta*	(A.Rich.) A. Camus	U	C4, NADP-me
Brabri	*Brachiaria brizantha*	(A. Rich.) Stapf	P	C4, PCK
Branig	*Brachiaria nigropedata*	(Fical. & Hiern) Stapf	P	C4, PCK
Cymexc	*Cymbopogon excavatus*	(Hochst.) Stapf ex Burtt Davy	U	C4, NADP-me
Cymval	*Cymbopogon validus*	Stapf ex Burtt Davy	U	C4, NADP-me
Cyndac	*Cynodon dactylon*	(L.) Pers.	I	C4, NAD-me
Digarg	*Digitaria argyrograpta*	(Nees) Stapf	P	C4, NADP-me
Digeri	*Digitaria eriantha*	Steud.	P	C4, NADP-me
Diglon	*Digitaria longiflora*	(Retz.) Pers.	I	C4, NADP-me
Dihamp	*Diheteropogon amplectens*	(Nees) Clayton	P	C4, NADP-me
Eragum	*Eragrostis gummiflua*	Nees	U	C4, NAD-me
Erahet	*Eragrostis heteromera*	Stapf	I	C4, NAD-me
Eraina	*Eragrostis inamoena*	K. Schum.	I	C4, NAD-me
Eraleh	*Eragrostis lehmanniana* var. *lehmanniana*	Nees	I	C4, NAD-me
Erarig	*Eragrostis rigidior*	Pilg.	U	C4, NAD-me
Erasup	*Eragrostis superba*	Peyr.	I	C4, NAD-me
Erista	*Eriochloa stapfiana*	Clayton	I	C4, NAD-me
Hetcon	*Heteropogon contortus*	(L.) Roem. & Schult.	I	C4, NADP-me
Hypdis	*Hyperthelia dissoluta*	(Nees x Steud.) Clayton	U	C4, NADP-me
Hypfil	*Hyparrhenia filipendula* var. *pilosa*	(Hochst.) Stapf	I	C4, NADP-me
Loufla	*Loudetia flavida*	(Stapf) C.E. Hubb.	U	C4, NADP-me
Lousim	*Loudetia simplex*	(Nees) C.E. Hubb.	U	C4, NADP-me
Melrep	*Melinis repens* subsp. *repens*	(Willd.) Ziska	U	C4, PCK
Miccaf	*Microchloa caffra*	Nees	U	C4, PCK
Pandeu	*Panicum deustum*	Thund.	P	C4, PCK
Panmax	*Panicum maximum*	Jacq.	P	C4, PCK
Passcr	*Paspalum scrobiculatum*	L.	I	C4, NADP-me
Perpat	*Perotis patens*	Gand.	U	C4, NAD-me
Pogsqu	*Pogonarthria squarrosa*	(Roem. & Schult.) Pilg.	U	C4, NAD-me
Schsan	*Schizachyrium sanguineum*	(Retz.) Alst.	U	C4, NADP-me
Setsph	*Setaria sphacelata* var. *sphacelata*	(Schumach.) Moss	P	C4, NADP-me
Spoafr	*Sporobolus africanus*	(Poir.) Robyns & Tournay	I	C4, PCK
Spoioc	*Sporobolus ioclados*	(Trin.) Nees	P	C4, NAD-me
Thetri	*Themeda triandra*	Forssk.	P	C4, NADP-me
Traber	*Tragus bertorianus*	Schult.	U	C4, NAD-me
Traspi	*Trachypogon spicatus*	(L.f.) Kuntze	I	C4, NADP-me
Trigra	*Trichoneura grandiglumis*	(Nees) Ekman	U	C4, NAD-me
Trimon	*Tricholaena monachne*	(Trin.) Stapf & C.E. Hubb.	I	C4, PCK
Uromos	*Urochloa mosambicensis*	(Hack.) Dandy	I	C4, PCK
Urooli	*Urochloa oligotricha*	(Fig. & De Not.) Henr.	I	C4, PCK
Uropan	*Urochloa panicoides*	Beauv.	U	C4, PCK

Table 6.9. Traits information for the grasses used in the traits analysis. N and P (mg kg^{-1}) and K, Ca and Mg (me $100g^{-1}$). Root and leaf tissue density (g cm^{-3}).

Species	Lignin/ N	Leaf tissue density	Root tissue density	Tensile strength	N	P	K	Ca	Mg	Root diameter (mm)	Leaf thickness (mm)	Leaf width (mm)
Cyndac	2.64	0.06	0.03	14.8	1.85	0.10	1.27	0.27	0.14	0.44	2.25	0.14
Digeri	4.90	0.10	0.19	1.5	1.33	0.23	1.94	0.24	0.14	0.36	5.26	0.35
Dihamp	6.30	0.17	0.13	2.8	1.15	0.09	1.74	0.43	0.25	0.44	8.18	0.71
Erarig	11.79	0.10	0.07	5.2	0.69	0.12	0.87	0.16	0.06	0.53	4.75	0.19
Hetcon	6.68	0.06	0.32	2.7	1.16	0.10	1.49	0.28	0.14	0.47	5.38	0.32
Hypdis	5.98	0.05	0.39	3.0	1.12	0.43	1.33	0.21	0.17	0.46	4.79	0.24
Hypfil	5.37	0.05	0.27	3.8	1.18	0.11	1.34	0.28	0.17	0.36	6.81	0.21
Loufla	7.99	0.13	0.20	2.3	1.18	0.08	0.73	0.22	0.10	0.45	3.55	0.60
Melrep	7.31	0.06	0.12	11.7	0.96	0.22	1.44	0.30	0.14	0.53	3.54	0.26
Pandeu	5.51	0.02	0.31	9.6	1.39	0.16	1.68	0.29	0.24	0.55	6.77	0.21
Panmax	4.92	0.13	0.23	1.0	1.64	0.15	2.49	0.31	0.20	0.38	8.10	0.62
Pogsqu	8.27	0.06	0.10	5.7	0.85	0.11	1.36	0.19	0.11	0.35	4.77	0.18
Schsan	6.18	0.10	0.11	4.5	1.08	0.11	1.31	0.22	0.14	0.53	2.97	0.30
Setsph	4.68	0.08	0.22	4.0	1.52	0.10	1.66	0.21	0.11	0.50	3.92	0.24
Uromos	4.87	0.11	0.26	1.9	1.50	0.14	2.79	0.42	0.20	0.42	13.93	0.31

CHAPTER 7

^{15}N NATURAL ABUNDANCE IN VEGETATION AND SOIL IN A MESIC SAVANNA, KRUGER NATIONAL PARK

INTRODUCTION

Variations in the natural abundance of ^{15}N in soil and plant material may be used to determine environmental and disturbance effects on nitrogen (N) cycling (Garten and Van Miegroet 1993, Frank and Evans 1997, Handley *et al.* 1999, Frank *et al.* 2000, Cook 2001). Soil δ^{15}N is a function of a number of external factors including the rate and isotopic composition of N inputs, the rate of soil N transformations, and the isotopic composition of N lost from the soil (Nadelhoffer and Fry 1994). Processes leading to loss of N, *e.g.* ammonia volatilization, nitrification followed by leaching, and denitrification, discriminate against the heavier ^{15}N and increase ecosystem and foliar δ^{15}N values (Högberg and Johannisson 1993, Shearer and Kohl 1986). Fire increases N loss through volatilization, but may also enhance denitrification and leaching losses (Mariotti *et al.* 1981, Blackmer and Bremmer 1977), as well as N mineralization (Shearer and Kohl 1986).

Fire is thought to play a major role in influencing total ecosystem N and available N in mesic savannas with conflicting results from a number of studies (Holt and Coventry 1990, Kauffman *et al.* 1994, Mistry 1998, Reich *et al.* 2001, Schmidt and Stewart 2003, Bustamante *et al.* 2006). It has been suggested that fires decrease the proportional flux of ecosystem N into organic matter storage pools resulting in an open nitrogen cycle (higer transformation rates of N) in which nitrogen is relatively ^{15}N enriched (Handley *et al.* 1999). Conversely, the repeated pyrogenic losses of nitrogen in burned mesic Australian savanna creates a relatively 'closed' nitrogen cycle in which nitrogen is relatively ^{15}N depleted (Cook 2001). At another Australian savanna close by, annual burning resulted in a trend of enriched soil and foliar values compared to less frequently burned savanna (Schmidt and Stewart 2003). These conflicting results show that the role of fire in nitrogen cycling in savannas is still poorly understood.

In this chapter I used ^{15}N natural abundances to examine nitrogen cycling processes. The Shabeni string of the experimental burn plots (EBP's) provides a unique opportunity to examine the long-term effects of different fire treatments on ecosystem δ^{15}N. In order to test whether frequent fire decreased the 'openness' of the nitrogen cycle, I determined plant and soil δ^{15}N of different fire treatments. Openness (vs. closed) is defined in relation to the importance of internal cycling of N versus inputs and outputs to plant nutrition (Austin and Vitousek 1998). 'Open' systems usually have large losses of N and the processes associated with N loss discriminate against ^{15}N such that ecosystems with large losses of N become enriched in ^{15}N (Högberg and Johannisson 1993, Eshetu and Högberg 1997). Therefore stable isotope analysis provides an opportunity to determine the role of fire on nitrogen cycling in savanna systems.

METHODS

Study area

The study site is situated in the southern part of the Kruger National Park (see Fig. 2.3, p. 19). Vegetation was collected from four fire treatments in all four strings of the Sourveld Experimental Burn Plots (*Terminalia* woodland strings). Roots were sampled from four treatments of the Shabeni string, the annual burn (Aug 1), triennial August burn (Aug 3), triennial February burn (Feb 3), and Fire exclusion (FE). Soils were sampled at different depths at only three treatments (Aug 1, Aug 3 and FE) of the Shabeni string. Sample date and which strings were sampled are summed in Table 7.1 The area in which the strings are situated as well as background on the EBP experiment are described in Chapter 2.

Table 7.1 Summation of the sample dates and strings.

Class	Time	Treatments/String	N
Soils	Spring 2003	Aug 1/Aug 3/FE at Shabeni	168
Vegetation	Summer 2004	Aug 1/Aug 3/Feb 3/FE at all 4 strings	160
Roots	Summer 2005	Aug 1/Aug 3/FE at Shabeni	40

Data collection and analysis

Natural abundance of ^{15}N

It is possible to identify the origin of nitrogen assimilated by the plant through a comparison of the abundance of ^{15}N in the plant and those of possible sources (Abbadie *et al.* 1992, Garten 1993, Abbadie and Lata 2006). To achieve this objective, δ^{15}N values were determined for tree and grass foliage, fine roots, organic soil N, soil NH_4^+-N, NO_3^--N, and wet deposition NH_4^+-N, NO_3^--N. All samples were analysed for %N, and δ^{15}N. Samples were weighed according to rough prior estimates of %N, sealed in a tin capsule and combusted at 1600 °C. The resultant gases were cleaned on-line in a Carlo Erba NCS 2500 Elemental Analyser (Carlo Erba Instruments, Milan, Italy), before being introduced to a Finnigan MAT 252 ratio mass spectrometer (Finnigan, Bremen, Germany) in the Archaeometry Laboratory at the University of Cape Town. The results are expressed in δ notation as:

$$\delta_{Sample}\ (‰) = [(R_{Sample})/(R_{Standard})-1]\ x1000$$

where δ_{Sample} represents δ^{15}N, and R is the molar ratio of the heavier to the lighter isotope for the standard or sample. Nitrogen isotope results were related to atmospheric N_2 by measuring various secondary standards whose value relative to atmospheric N_2 is known. The reproducibility of the results for homogenous material is better than 0.25‰.

Soil at different depths

Soils were sampled at different depths in spring (September 2003) to analyse for total soil nitrogen and natural abundance of ^{15}N with depth in each fire treatment. Soil depths were divided into seven classes: 0-2 cm (D1), 2-5 cm (D2), 5-10 cm (D3), 10-20 cm (D4), 20-40 cm (D5), 40-60 cm (D6), and > 60 cm (D7). Three treatments were sampled at Shabeni and included the August annual (Aug 1), August triennial (Aug 3) and the Fire exclusion treatment (FE). Sampling points were randomly chosen and for each of the four sampling points per treatment, one sample was taken under the canopy of an adult *Sclerocarya birrea* tree and another away from the canopy, amongst grass. Soils were sifted to remove organic material and roots, airdried and ground.

Root biomass and N content

A point in time measurement of root biomass was made in January 2005. Three holes were augured to a depth of 25 cm at each of 10 points (in the vicinity of each of the ten permanent sampling points, see Chapter 2). The three samples per point (adding up to 30 per burn treatment) were weighed and sieved separately to retrieve roots. These samples were then pooled to ensure a large enough sample for analysis. Roots were analysed for concentrations of N and $\delta^{15}N$ values.

Foliar $\delta^{15}N$ values

Foliage of three grasses (*Setaria sphacelata*, *Panicum maximum* and *Heteropogon contortus*) and two tree species (*Sclerocarya birrea* and *Terminalia sericea*) was collected during mid-summer (February) 2004. Foliage from at least four different individuals was pooled to create one sample. This was repeated three times per species for every treatment. Foliage was left for several days in a drying room and then ground using a hammer mill. Samples were then analysed for $\delta^{15}N$.

Soil and rainwater nitrate and ammonium $\delta^{15}N$ values

Soil samples were collected by hammering a 4 cm diameter steel core 10 cm into the soil. Sampling took place early in the season (November 2006) and three soil cores were combined into one sample. Three samples each were taken from established experimental plots, three with only grass and three with only one small *Terminalia* tree (no grass). One 10 g subsample was shaken with 45 mL of 1 M KCl for 1 h to extract NO_3^--N and NH_4^+-N. The soil/KCl mixture was then centrifuged for 10 min at 5000 rpm and the supernatant frozen.

Rainwater was collected during three different rainfall events between November 2006 and January 2007. The rainwater was collected in a polyethylene funnel connected to a container rinsed with a weak hydrochloric acid (HCl) solution beforehand to remove any contaminants (L.O. Hedin pers. comm.). The funnel and container was only placed outside during the rainfall event and removed when the rain stopped in order to minimize polluting rainwater with dust. Both KCl extracts and rainwater were analysed at the Water Sciences Laboratory at the University of Nebraska (Lincoln, NE, USA). All samples were acidified with sulphuric acid (H_2SO_4) before transport as recommended by the laboratory. Samples were analysed for nitrate concentrations by using ion chromatography. Nitrate was then converted to $(NH_4)SO_4$ by the steam distillation method of Bremner and Keeney (1965) with a modification by Gormly and Spalding (1979). Subsequently, $(NH_4)SO_4$ was oxidised to N2 in a vacuum preparation system. $\delta^{15}N$ was measured by the procedure of Bremner and Edwards (1965) and Miyaka and Wada (1967) by analysing purified nitrogen samples against atmospheric nitrogen.

Determination of input nitrogen source

Nadelhoffer and Fry (1988) have shown that in well-drained, non-ploughed ecosystems, isotopic discrimination against ^{15}N during decomposition is primarily responsible for the observed patterns of enriched $\delta^{15}N$ with depth and not the varying preservation of litter components with different ^{15}N abundances. The decrease in nitrogen content and corresponding increase in $\delta^{15}N$ of soil nitrogen during decomposition follows Raleigh distillation kinetics (Nadelhoffer and Fry 1988, Evans and Ehleringer 1993). Raleigh distillation describes the process in which the isotopic composition of the product varies as a result of the extent of the reaction. The following equation describes the correlation (Mariotti *et al.* 1981) as:

$$\delta^{15}N_{s,t} = \delta^{15}N_{s,0} + \ln (C_t/C_0)$$

where $\delta^{15}Ns,t$ is the isotopic composition of the substrate at time t, $\delta^{15}N_{s,0}$ is the isotopic composition of the substrate at the start of the reaction, ε the isotopic enrichment factor associated with the reaction, and C_t/C_0 the fraction of substrate remaining at time t. The isotopic value and concentration of the original substrate are seldom known and the above equation can be rewritten as

$$\delta^{15}N_{s,t} = k + \varepsilon \ln ([N])$$

where $\delta^{15}N_{s,0}$ are replaced by a constant k and C_t/C_0 by soil nitrogen concentration ([N]) (Mariotti *et al.* 1988, Evans and Ehleringer 1993).

Statistical analysis

Statistical analyses were performed using the software package JMP 5.0.1 (SAS Institute, Cary, NC, USA). Dependent variables were tested for normality, and parameters were transformed where necessary. A three-factor model was used to test the effect of site, canopy and depth on soil $\delta^{15}N$ values. SMART 2 (Warton *et al.* 2006) was used to test for differences in slope and elevation of regression correlations between $\delta^{15}N$ and ln([N]) between the different fire treatments.

RESULTS

δ^{15}N with depth

Soils were enriched in ^{15}N with depth at my study site on the Shabeni string, with depth explaining most of the observed variation (Table 7.2). Tukey's test for multiple comparisons of means indicated that the surface 2 cm was significantly different ($P < 0.0001$) from 2-5 cm (+1.8 ‰), 2-5 cm (+2.81 ‰) from 5-20 cm (+3.51, +4.16‰), and 5-20 cm from 20-60+ cm (+5.10, +5.28, +5.60 ‰). Soil nitrogen content decreased from 1.31 ± 0.06 mg N.g^{-1} soil at the surface to 0.41 ± 0.06 mg N.g^{-1} soil at 60 cm (Fig. 7.1). Fire treatment had an effect on the soil δ^{15}N (Table 7.2, $F = 9.22$, $P = 0.04$). Across all depths, less frequent burning and fire exclusion enriched soils in ^{15}N when compared to annual burns (4.27 ± 0.12 and 4.00 ± 0.12 vs. 3.32 ± 0.14 ‰, $P = 0.002$). Fire, however, had no effect on soil δ^{15}N at the individual depths (Table 7.2, $F = 0.19$, $P = 0.82$).

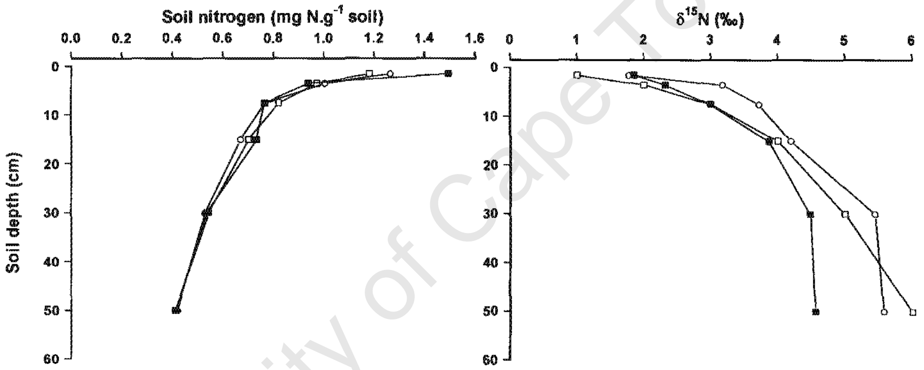

Figure 7.1. Nitrogen content (mg N.g^{-1} soil) and isotopic composition of soils (δ^{15}N in ‰) collected from the Sourveld Experimental Burn Plots (EBP's). Soils were collected from three different fire treatments; annual August burn (■), triennial August burn (□), and the Fire Exclusion treatment (○). Overall, the Fire Exclusion treatment and triennial burns were enriched in ^{15}N over the annual burns ($P < 0.0001$), but no differences were found at individual depths.

Table 7.2. A Three-factor model showing F-ratios (Sum of Squares in parentheses) and significance levels (***P < 0.001, **P < 0.01 and *P < 0.05) on the effects of fire treatment (Aug 1, Aug 3 and FE), canopy (C, under trees and away from trees), and depth (DC, D1: 0-2 cm, D2: 2-5 cm, D3: 5-10 cm, D4: 10-20 cm, D5: 20-40 cm, D6: 40-60 cm, D7: 60+ cm) on soil (organic matter) $\delta^{15}N$ values.

Factor	$\delta^{15}N$
Whole model r^2	0.75***
	F(SS)
Treatment	6.80** (9.22)
Canopy	1.52 (1.03)
Treatment * Canopy	0.09 (0.12)
Depth class	51.97*** (211.52)
Treatment * Depth Class	0.73 (5.93)
Canopy * Depth Class	0.75 (3.07)

Neither the slopes ($P = 0.053$) nor the intercepts ($P = 0.056$) for the relationships between $\delta^{15}N$ and ln([N]) were different for the different fire treatments. Although significant (Fig. 7.2), the relationship between $\delta^{15}N$ and ln([N]) was less pronounced in the annual burn treatment.

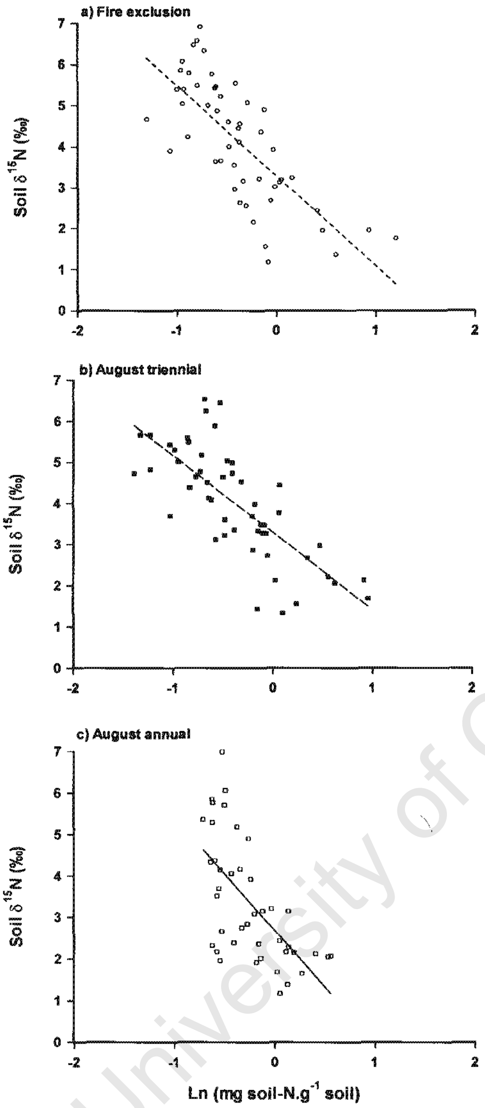

Figure 7.2. The relationship between nitrogen isotope ratio ($\delta^{15}N$) and ln(nitrogen content) for soils from the three fire treatments, a) Fire exclusion plot ($y = -2.55x + 3.15$, $r = -0.74$, $P < 0.0001$), b) August triennial burn ($y = -2.19x + 3.24$, $r = -0.76$, $P < 0.0001$), and c) August annual burn ($y = -2.02x + 2.74$, $r = -0.36$, $P < 0.0001$).

Root $\delta^{15}N$ values

Whereas the exclusion of fire increased root nitrogen concentrations when compared to the August burns, fire had no effect on root $\delta^{15}N$ (Table 7.3).

Table 7.3. Mean values of root nitrogen concentration and $\delta^{15}N$ values. Values in brackets are standard errors. Means sharing the same lowercase letter did not differ significantly ($P < 0.05$).

	Aug 1	Aug 3	Feb 3	FE
$\delta^{15}N$ (‰)	1.88^{a} (0.34)	1.13^{a} (0.38)	1.03^{a} (0.14)	1.07^{a} (1.80)
[N] (%)	0.59^{b} (0.06)	0.53^{b} (0.01)	0.65^{ab} (0.03)	0.85^{a} (0.07)

Foliar $\delta^{15}N$ values

Foliar measurements were made across all four strings of the Sourveld EBP's. Burning (as compared to fire exclusion), with the effect averaged across strings, increased foliar $\delta^{15}N$ values significantly (Table 7.4, $F = 8.42$, $P = 0.002$). The February triennial burns were enriched by 0.51‰ when compared to the fire exclusion treatment (0.66‰ ± 0.10 vs. 0.15‰ ± 0.10, respectively), with August triennial and the annual burn treatments intermediate (Table 7.5).

Table 7.4. Analysis of the effect of string, fire treatment, species and the interactions on $\delta^{15}N$ values; F-values, Sum of Squares (in parentheses) and degrees of freedom (df) are given at a level of significance (*P < 0.05, **P < 0.01 and ***P < 0.001).

		$\delta^{15}N$
	r^2	0.57***
	df	*F* (SS)
String	3	5.74** (9.35)
Treatment	3	5.21** (8.42)
String * Treatment	9	4.41*** (21.24)
Species	4	33.81*** (72.52)
String * Species	12	2.83** (18.51)
Treatment * Species	12	0.99 (6.37)

Table 7.5. Means with standard errors for $\delta^{15}N$ values (‰) with string, fire treatment and species from Tukey's HSD; Fayi (F), Khambeni (K), Numbi (N) and Shabeni (S), August-triennial (Aug3), February-triennial (Feb 3), Fire exclusion (FE), and August-annual (Aug 1), *Panicum maximum* (Panmax), *Setaria sphacelata* (Setsph), *Heteropogon contortus* (Hetcon), *Sclerocarrya birrea* (Sclbir), and *Terminalia sericea* (Terser). Means sharing the same lowercase letter does not differ significantly ($P < 0.05$).

String	$\delta^{15}N$	Fire treatment	$\delta^{15}N$	Species	$\delta^{15}N$
K	0.72 ± 0.10^{a}	Feb 3	0.66 ± 0.10^{a}	Sclbir	1.17 ± 0.10^{a}
F	0.61 ± 0.09^{ab}	Aug 3	0.54 ± 0.09^{a}	Terser	1.12 ± 0.11^{a}
S	0.34 ± 0.09^{bc}	Aug 1	0.53 ± 0.09^{a}	Panmax	0.22 ± 0.11^{b}
N	0.22 ± 0.10^{c}	FE	0.15 ± 0.10^{b}	Setsph	-0.07 ± 0.11^{b}
				Hetcon	-0.07 ± 0.11^{b}

Table 7.6. Means with standard errors for $\delta^{15}N$ values (‰) for the different species in each fire treatment and string (tree data are pooled), grasses are *Panicum maximum* (Panmax), *Setaria sphacelata* (Setsph), *Heteropogon contortus* (Hetcon), August-triennial (Aug3), February-triennial (Feb 3), Fire exclusion (FE), and August-annual (Aug 1).

	Tree	Panmax	Setsph	Hetcon
Treatment				
Aug 1	1.31 ± 0.10	0.21 ± 0.15	0.15 ± 0.33	-0.39 ± 0.11
Aug 3	1.19 ± 0.17	0.06 ± 0.28	0.18 ± 0.29	0.05 ± 0.23
Feb 3	1.28 ± 0.21	0.48 ± 0.18	-0.09 ± 0.24	0.34 ± 0.42
FE	0.77 ± 0.17	0.04 ± 0.24	-0.51 ± 0.15	-0.27 ± 0.17
String				
Fayi	1.31 ± 0.12	0.63 ± 0.21	-0.01 ± 0.21	-0.22 ± 0.16
Khambeni	1.08 ± 0.17	0.75 ± 0.22	0.15 ± 0.28	0.50 ± 0.42
Numbi	1.07 ± 0.22	-0.08 ± 0.15	-0.57 ± 0.09	-0.37 ± 0.14
Shabeni	1.09 ± 0.15	-0.43 ± 0.12	0.14 ± 0.38	-0.22 ± 0.13

Strings had significant differences in $\delta^{15}N$ values (Table 7.4, $F = 9.35$, $P = 0.001$). In general, Khambeni had significantly enriched foliar $\delta^{15}N$ when compared with Numbi and Shabeni (Table 7.5). The range of differences in $\delta^{15}N$, as well as the effect of fire on $\delta^{15}N$, however, depended on the string. The Shabeni fire exclusion plot, for instance, had the lowest foliar $\delta^{15}N$ values of all plots whereas the annual burn treatment had enriched foliage (-0.27‰ ± 0.25 vs. 0.72‰ ± 0.25, $P = 0.04$). However, this difference could be attributed to one species, *T. sericea*, whereas all other species were not affected by burning. For Fayi on the other hand, the fire exclusion and annual treatments had very similar $\delta^{15}N$ values. Variability between strings had a greater effect on grass $\delta^{15}N$ values than fire (Table 7.5).

Trees, on the other hand, were more strongly affected by fire with the least enriched values in the fire exclusion plots (Table 7.6).

Fine root $\delta^{15}N$ in this study (trees and grasses mixed) incorporates both NO_3^--$\delta^{15}N$ and NH_4^+-$\delta^{15}N$ signatures (Fig. 7.3). The more enriched $\delta^{15}N$ values of the trees indicate that trees utilize slightly more NH_4^+ than grasses or access N from deeper ^{15}N enriched soil layers. The positive soil $\delta^{15}N$ indicates that precipitation N is not a primary source of N to this system.

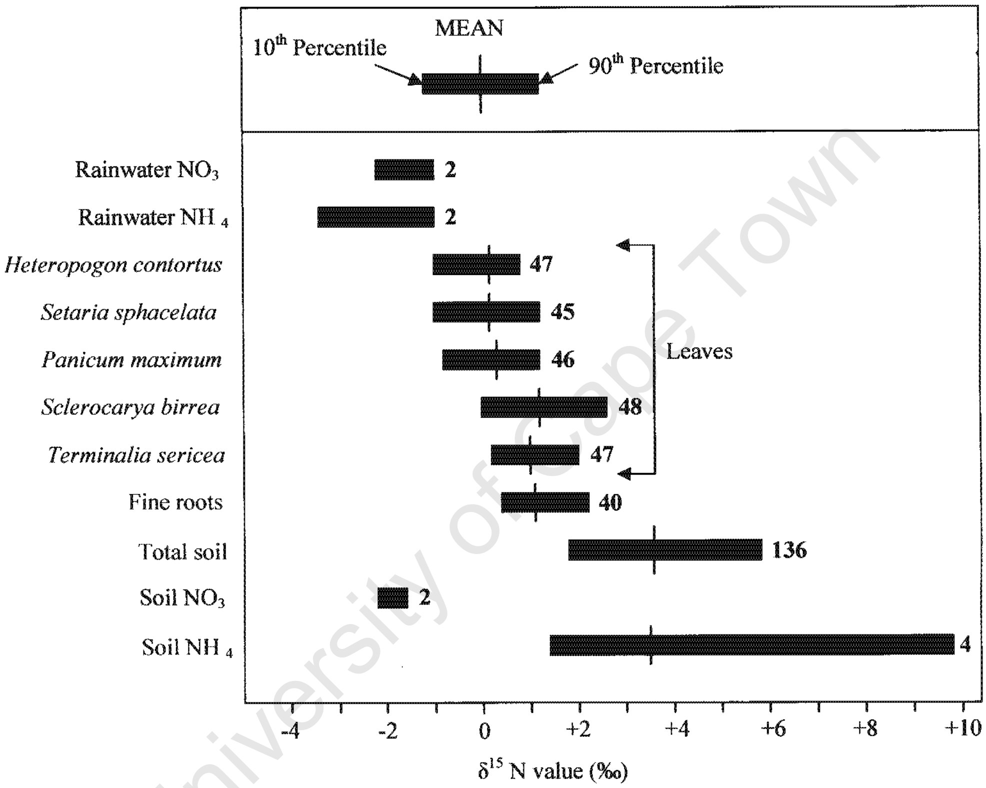

Figure 7.3. Measurements of ^{15}N abundance in rainwater, inorganic nitrogen, leaves, fine roots, total soil nitrogen, and soil inorganic nitrogen. Sample sizes (N) indicated in bold print. Mean values with the 10th and 90th percentile shown for $N \geq 4$. Total soil nitrogen includes values from the soil surface to a depth of 60 cm.

DISCUSSION

To examine the effects of fire nitrogen pools and transformation rates on the Shabeni string of the EBP's in the Kruger National Park, natural abundance of ^{15}N in soil and foliage were used as a proxy of the 'openness' of the N cycle. It has been argued that any factor (*e.g.*, fire) that decreases the residence time of nitrogen may 'push' ecosystems towards ^{15}N enrichment and enriched $\delta^{15}N$ values (Schulze *et al.* 1991, Handley *et al.* 1999). Alternatively, the repeated pyrogenic losses of nitrogen may create a relatively 'closed' nitrogen cycle in which nitrogen is relatively ^{15}N depleted. Cook

(2001) compared frequently burned semi-arid and mesic savannas with rainforest and mesic savannas that burned infrequently, and found that foliar $\delta^{15}N$ values were more enriched in rainforest and fire-excluded mesic savanna than frequently burned savannas. In the present study however, burning had no consistent effect on foliar $\delta^{15}N$ values and burning at Shabeni had no effect on soil $\delta^{15}N$ at individual depths. The difference between strings had the same order of magnitude effect on foliar $\delta^{15}N$ than fire. Previous work has also shown limited effects of fire on ecosystem $\delta^{15}N$ values. Burning had no effect on foliar $\delta^{15}N$ in Australian woodland (Mordelet *et al.* 1996). Fire also had no effect on foliar $\delta^{15}N$ values of a mesic Australian savanna that had been unburned for six years and a nearby site that had been burned annually for four years (Schulze *et al.* 1998). A previous study in the Kruger National Park found no significant differences in soil $\delta^{15}N$ values along fire gradients (Aranibar *et al.* 2003).

Foliar $\delta^{15}N$ has limited use to trace the source of nitrogen that the plant is using because plant $\delta^{15}N$ values are not only a product of the N source but are also influenced by the rooting depth of the plant (Högberg 1997), mycorrhizal associations (Hobbie *et al.* 2000, Handley *et al.* 1993, Handley *et al.* 1994), fractionations from internal N recycling (Evans 2001, Gebauer and Schulze 1991), and intra-plant variation (Evans 2001). Past studies, however, have identified the origin of nitrogen assimilated by the plant by comparing the abundance of ^{15}N in the plant and those of possible sources (Abbadie *et al.* 1992, Garten 1993, Abbadie and Lata 2006). Trees had more enriched $\delta^{15}N$ values in this study and Aranibar *et al.* (2003) found that most trees without indications of N_2 fixation had foliar $\delta^{15}N$ signatures of > 3.5 ‰ in the KNP. Although this indicates that trees do indeed utilize a nitrogen source with higher $\delta^{15}N$ than grasses (Fig 7.3), it is also possible that trees take up inorganic N from enriched depths.

The intricateness of the system in the present study proscribes the use of the correlation between soil nitrogen concentrations and $\delta^{15}N$ values to infer the ^{15}N signature of the input source. The result in Fig 7.2 show, however, that annual burning disrupts the correlation between soil nitrogen concentrations and $\delta^{15}N$ values. This indicates that the fractionation process that leads to enriched soil $\delta^{15}N$ values with depth is interrupted in the annual burns. One possible explanation is that the continual removal of herbaceous litter in the annual burns leads to very low litter inputs. Nadelhoffer and Fry (1988) found differences in surface soil $\delta^{15}N$ for plots where litter was removed and added.

Some uncertainty remains as to why frequent fires do not decrease N availability or lead to a 'closed' N cycle in this broad-leaf savanna. However, previous research has shown that losses of N through volatilization need not lead to altered system N values as nitrogen is replaced through N fixation or N deposition (Kauffman *et al.* 1994, Mistry *et al.* 1998, Bustamante *et al.* 2006). The results of this study indicate that the inherent variability in the landscape (and associated soil characteristics) has more of an effect on ecosystem ^{15}N than fire.

CHAPTER 8

TEMPORAL CHANGES IN ^{15}N OF WOOD AND ^{13}C OF SOILS AT THE EXPERIMENTAL BURN PLOTS AT SHABENI IN THE KRUGER NATIONAL PARK.

INTRODUCTION

The natural abundance of ^{15}N in foliage has been used for some time to infer soil nitrogen (N) cycling processes (Shearer *et al.* 1974, Garten 1993, Garten and Van Miegroet 1993, Austin and Vitousek 1998, Schmidt and Stewart 2003). Mean foliar δ^{15}N values and mean enrichment factors ($\delta^{15}N_{leaf}$ - $\delta^{15}N_{soil}$) are positively correlated with net N mineralization and net nitrification potentials in soil (Garten and Van Miegroet 1993, Emmett *et al.* 1998). In systems with high rates of N deposition or high ambient N availability, the natural abundance of ^{15}N may increase over time in the vegetation (Gebauer and Schulze 1991, Högberg 1997). This has been attributed to the accompanying increase in N transformation processes such as net nitrification. These can lead to ^{15}N enrichment of the soil through increased leaching and/or gaseous loss of the lighter N isotope (Shearer *et al.* 1974, Högberg 1990). Working in coniferous European forests Emmett *et al.* (1998) has used enriched δ^{15}N values of foliage to show increases in the rate of N cycling. Alternatively, the low ^{15}N abundance of N deposited in some pollution sources (including nitrogenous air pollutants such as NH_3) may lead to more depleted ecosystem δ^{15}N values (Köchy and Wilson 2001, Stewart *et al.* 2002, Bukata and Kyser 2007).

The use of natural abundance nitrogen isotope values in tree rings to investigate the long-term biochemical behaviour of nitrogen is a relative new concept. Using changes in ^{15}N in tree rings through time, Poulson *et al.* (1995) demonstrated changes in atmospheric deposition of nitrogen and the effects this had on forested ecosystems. A few studies since have used the δ^{15}N values of tree rings to infer temporal changes in the availability of nitrogen and land-use change (Puñuelas and Estiarte 1997, Hart and Classen 2003, Saurer *et al.* 2004, Bukata and Kyser 2005, Bukata and Kyser 2007). In this chapter I use the δ^{15}N values of tree rings in adult *Sclerocarya birrea* on the annual and fire exclusion plots of Shabeni to determine the effects of fire on the nitrogen transformation rates through time.

The long-term experimental burn plots in the Kruger National Park (KNP) offer a unique opportunity to investigate the effects of fire on the nitrogen pools and transformation rates through time. Trees on the different treatment plots have been subjected to the same fire frequency and accompanying effects on nitrogen for more than 50 years. In Chapter 2 I showed that frequent fires have had very little effect on available N in the KNP. This work supported the conclusion of Aranibar *et al.* (2003). The high nitrogen deposition values of ~22 kg N ha^{-1} ha^{-1} (Scholes *et al.* 2003) have been used as an explanation for these results. An examination of the ^{15}N natural abundance in tree rings through time should give us a better understanding of the increases in N deposition in the recent past.

Increases in available N may favour C_3 trees over C_4 grasses in savanna systems. Changes in the ratio of trees and grasses through time have been documented in a number of studies using stable carbon isotope ratios of soil organic matter (Mariotti and Peterschmitt 1994, Desjardins *et al.* 1996, Schwartz *et al.* 1996, De Freitas *et al.* 2001, Delégue *et al.* 2001, Pessenda *et al.* 2001). To examine whether tree density has been increasing over time, I compared soil organic matter $\delta^{13}C$ values with depth for the two treatments.

METHODS

Study site

The study site is on the annually burned and fire exclusion treatments of the Shabeni string in the southern Experimental Burn Plots (EBP's) in the Kruger National Park (31°10'E, 25°10'S). Shabeni is one of four replicate treatments in the southern Experimental Burn Plots (EBP's) and is located to the northeast of Shabeni Hill, approximately seven kilometers east of the Pretoriuskop tourist camp. A detailed description of the layout and background on the experiment is presented in Chapter 2.

The treatments had similar biomass and tree densities at the initiation of the EBP experiment in 1954 (O'Regan 2005). Before the initiation of the EBP's, fires were controlled and the area burned every 5 years on average (Van Wilgen *et al.* 2004). At the onset of the EBP experiment fire was excluded from one treatment and this plot has been unburned for more than 50 years, while another has been burned every year in August. The exclusion of fire in the Pretoriuskop sourveld resulted in an increase in the density of the woody vegetation and an increase in species diversity and species evenness (O'Regan 2005). However, although the fire exclusion has higher species diversity than the August triennial burns, it does not differ from the August annual burns (O'Regan 2005). Higgins *et al.* (2007) found that the density of the woody individuals was unresponsive to fire, but that the relative dominance of small individuals was very responsive. Burn plots in the Pretoriuskop region gained woody biomass even when burned annually (Higgins *et al.* 2007). Trees in the annual burn stayed at the same density for the first 15 years after the initiation of the EBP, but density tripled in the subsequent 27 years (O' Regan 2005).

Materials and methods

Wood $\delta^{15}N$

The sampling was carried out in two phases; during the summer of 2004 a single tree per treatment was sampled to determine if any differences in wood ^{15}N existed between the treatments. A follow-up sample was carried out during the summer of 2005 when five more trees per treatment were sampled.

The sampled trees were randomly selected across the treatments. All and all 12 trees were sampled from two different fire treatment plots.

Samples of *Sclerocarya birrea* were obtained using a 5 mm-diameter increment borer at breast height. Cores were taken perpendicular to the tree trunk and as close as possible to the centre of the tree or if the tree was very thick, to the full extent of the borer. The cores were oven dried at 65°C prior to analysis. Cores from the two sampling periods were treated differently. The first two cores were cut up into 30 mg sections with a steel blade and half of every section used was for analysis. The second set of five cores each was cut up into 5 mm sections to provide enough material for analysis (more than 30 mg) and every second section was used to perform analysis.

Savanna tree rings are in many cases not annual (Lilly 1977, Shackleton 1997, February *et al.* 2006). Trees may have visible annual rings in times of rapid growth, but rings are 'missing' during periods of slow growth, such as when experiencing a drought. Consequently the aging of trees by densitrometry (and the enumeration of 'growth rings') was deemed to be unreliable. Four dates from the initially sampled trees where obtained through AMS-radiocarbon dating. The samples were analysed at QUADRU (Quaternary Dating Research Unit) at the CSIR, Pretoria. Calendar dates (AD) are estimated by calibrating conventional radiocarbon ages (y BP). The atmospheric level of ^{14}C, however, has been variable from 1650 to 1950 due to the release of inactive carbon from fossil fuels and this precludes the determination of one date for samples from this time period. I used data from two surveys done on the EBP's during the 1970's (Scientific Services, Skukuza) and 2000's (Bond unpublished data) to determine how much trees have grown in the last 30 years. By comparing all the trees larger than 7 m, I determined that adult trees grew an average of 1.8 mm yr^{-1} (N = 10). J.J.T. Verweij (unpublished data) has determined that adult *S. birrea* grows about 0.5 mm yr^{-1} in the Pretoriuskop area. Taking these growth rates into consideration, the EBP experiment was initiated between 50 mm and 100 mm into the core (away from the y axis, see Fig 8.1 and 8.2).

The first set of cores were analysed at the UC Davies Stable Isotope Facility where the samples were run on a Europa 20-20, Europa Anca (PDZ Europa Ltd., Sandbach, UK). A Carbosorp CO_2 trap (NaOH on clay support) kept CO from co-eluting with N_2 and a cryotrap was used between the Carbosorp CO_2 trap and the GC column to remove final traces of CO_2 (as well as NO_x and H_2O). The second set of cores were analysed at the Archaeometry Laboratory, University of Cape Town. Samples weighing approximately 35 mg were sealed in a tin capsule and combusted at 1600 °C. The resultant gases were cleaned on-line in a Carlo Erba NCS 2500 Elemental Analyser (Carlo Erba Instruments, Milan, Italy), before being introduced to a Finnigan MAT 252 ratio mass spectrometer (Finnigan, Bremen, Germany). The results are expressed in δ notation as:

$$\delta_{Sample} (‰) = [(R_{Sample})/(R_{Standard})-1] \text{ x}1000$$

where δ_{Sample} represents $\delta^{15}N$, and R is the molar ratio of the heavier to the lighter isotope for the standard or sample. Nitrogen isotope results were related to atmospheric N_2 by measuring various secondary standards whose value relative to atmospheric N_2 is known. The reproducibility of the results for homogenous material was better than 0.25‰. The size of the sample was reduced to 15 mg after initial large sample sizes caused some of the carbon to be converted to CO. A Carbosorb CO_2 trap was placed before an $MgPCO_3$ trap to keep CO from co-eluting with N_2 and to absorb excess CO_2 and H_2O. Due to the large amount of sample needed to measure the low N concentrations in the wood, the usual measurement procedure for organics was modified by measuring a blank (empty folded tin capsule) after every sample.

Resinous species such as pine (*Pinus* spp.) require pre-treatment to remove extractable N-compounds as the isotopic composition of the bio-available nitrogen with non-structural nitrogen bearing compounds removed reflects the isotopic composition of the nitrogen incorporated in the sapwood of resinous species (Sheppard and Thompson 2000). Samples were not pre-treated to leach water labile nitrogenous compounds as pre-treatment in earlier studies had negligible effects on the weight percent nitrogen or the $\delta^{15}N$ value of the samples for nonresinous species (Bukata and Kyser 2005). Nonresinous species (which includes *Sclerocarya birrea*) also have far less non-structural nitrogen in the heartwood when compared with resinous species (Bukata and Kyser 2005). The low N concentration of wood (0.3-0.7 g N kg^{-1}) further precludes extraction of specific N compounds (Poulson *et al.* 1995).

Soil $\delta^{13}C$

Soils were sampled in spring (September 2003) to measure the concentrations of total soil carbon, and the natural abundance of ^{13}C with depth in every treatment. Soil depths were divided into seven classes: 0-2 cm (D1), 2-5 cm (D2), 5-10 cm (D3), 10-20 cm (D4), 20-40 cm (D5), 40-60 cm (D6), and > 60 cm (D7). Three treatments were sampled at Shabeni and included Aug 1, Aug 3 and FE (not the triennial February burn). Sampling points were randomly chosen and for each of the four sampling points per treatment, one sample was taken under the canopy of an adult *Sclerocarya birrea* tree and another away from the canopy, amongst grass. Soils at the surface were sampled manually with a trowel. Thereafter, soil at depth was sampled with a 4 cm-diameter auger marked at 20 cm intervals. Soils were sifted at progressively finer resolution to remove organic material and roots after which they were airdried and ground.

RESULTS

Nitrogen isotope variations

For this study, I assumed that the EBP experiment was initiated at between 50 and 100 mm. In the initial cores (Fig. 8.1) the fire exclusion plot and annual burn showed divergence in $\delta^{15}N$ patterns. $\delta^{15}N$ values ranged from 0.67 to 3.23 ‰ in the annually burned treatment tree and from -0.31 to 3.46 ‰ in the fire exclusion treatment tree (Fig. 8.1). The annual burn core shows a steady enrichment in ^{15}N

over time (~1 ‰ at 225 mm to 3 ‰ at 40 mm), with a slight decline in the last 40 mm. The $\delta^{15}N$ values from the fire exclusion plot core declines from around a 200 mm depth to 70 mm (~2 ‰ to -0.3 ‰), where after wood becomes enriched in ^{15}N. The two cores converge in the most recent wood at 2.5 ‰.

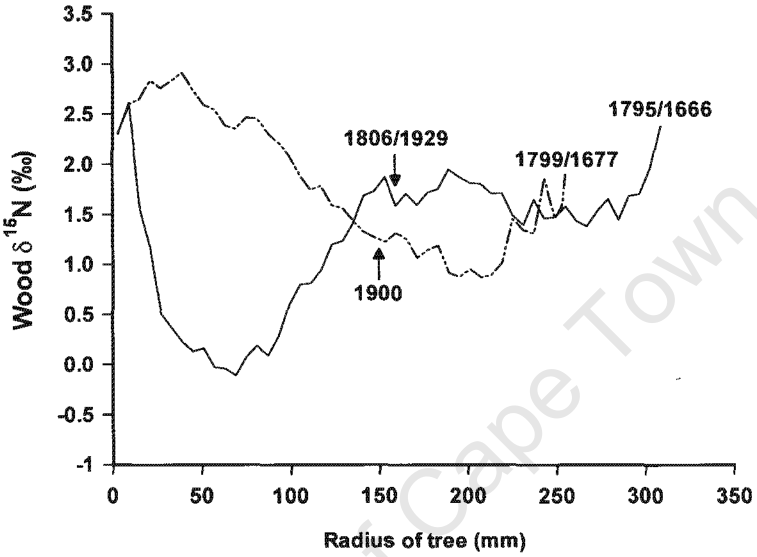

Figure 8.1. Wood $\delta^{15}N$ for the two trees sampled initially from the annually burned plot (dotted dashed line) and the fire exclusion plot (solid line) at Shabeni. Radius is marked from the bark (0) to the inner core (350).

The pattern seen in the initial core sampling is upheld to varying degrees in the second sampling period's $\delta^{15}N$ data (Fig. 8.2 A, B). Trees in the fire exclusion treatment showed very little overall change of ^{15}N over time, except around 50 mm depth were $\delta^{15}N$ become enriched (Fig. 8.2 A). The trees from the annual burn treatment show enrichment of $\delta^{15}N$ values over time from a 100 mm depth (Fig. 8.2 B).

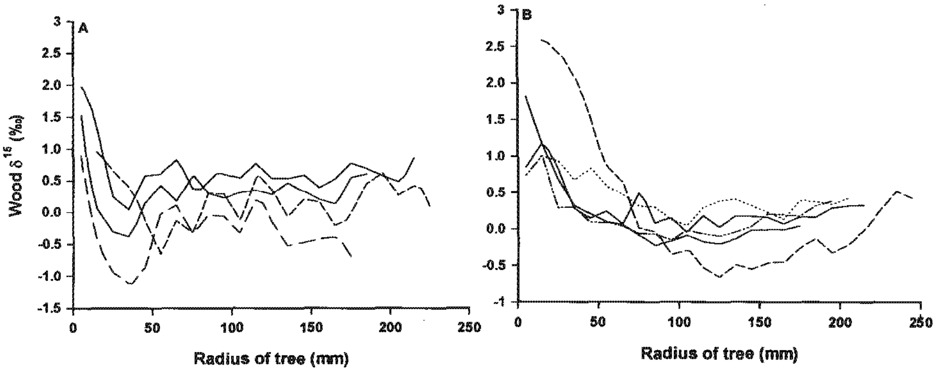

Figure 8.2. *Sclerocarya birrea* wood δ^{15}N for A) four cores from the fire exclusion plot and B) five cores from the annual burn plot. Radius is marked from the bark (0) to the core (250).

Soil δ^{13}C values

Differences between the top and bottom δ^{13}C of the soil profile ranged between 2.04 ‰ in the annual burn plot to 5.75 ‰ in the fire exclusion plot (Fig. 8.3). In both the annual and fire exclusion plots, some of the profiles under trees had a higher C3 input in the SOM. The mean δ^{13}C of the top 2 cm was -22.49 ‰ and -14.73 ‰ for the 40 – 60 cm depth. Profiles are remarkably consistent considering that profiles were taken across several 100 m^2.

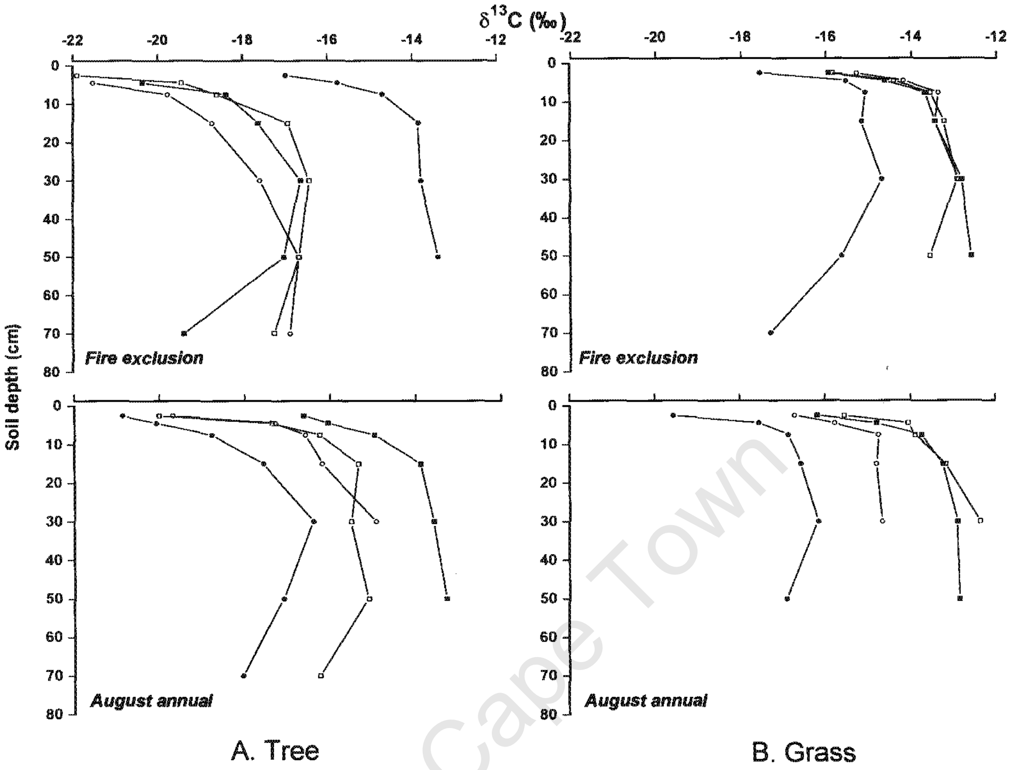

Figure 8.3. Soil organic matter $\delta^{13}C$ values with soil depth for the annually burned and fire exclusion plots at Shabeni, A) samples taken from under tree canopy, and B) in the open, under grass.

DISCUSSION

Although frequent fire may lead to decreases in available nitrogen in some ecosystems (Ojima *et al.* 1990, Reich *et al.* 2001), the results from Chapter 4 and 5 suggested that frequent fire had limited effects on nitrogen pools and available N. In order to gain an understanding of the longer term patterns of annual burning and fire exclusion on nitrogen cycling, wood $\delta^{15}N$ values were determined. Whereas the annual burn cores showed consistent enrichment in ^{15}N over time, the fire exclusion seems to have an initial depletion of ^{15}N in wood after the EBP experiment was initiated. Thereafter, $\delta^{15}N$ values become more enriched in recent times.

Fire limits the recruitment and growth of woody species with tree and shrub biomass increasing as fire frequency decreases (Scholes and Archer 1997, Higgins *et al.* 2000, Tilman *et al.* 2000, Silva *et al.* 2001). Previous research, however, suggested that woody biomass has increased in both treatments (Higgins *et al.* 2007). I used SOM $\delta^{13}C$ values to investigate whether I could detect an increase in woodiness for both treatments. The main controls of $\delta^{13}C$ on SOM are plant litter inputs, although isotope fractionation and humification can also influence $\delta^{13}C$. Although SOM becomes ^{13}C-enriched with increasing depth in the soil profile, this enrichment is usually < 4 ‰ and more likely 1-2 ‰

(Nadelhoffer and Fry 1988). Taken this into consideration, it is possible to determine if a change in vegetation has taken place (Mariotti and Peterschmitt 1994, Desjardins *et al.* 1996). $\delta^{13}C$ values in SOM indicated that the area where the present study took place has been in a savanna state for a long time. The area does not show the forest-savanna oscillations of many other savanna forest ecotones (Mariotti and Peterschmitt 1994, Martinelli *et al.* 1996, De Freitas *et al.* 2001). The more depleted $\delta^{13}C$ in the surface soil indicates that woodiness has increased in recent times for both the fire exclusion and annually burned plots. Although this can also indicate that forbs have been increasing, results from Chapter 5 shows that there are very few forbs in the fire exclusion plot. This increase in woodiness has probably been taking place for at least the last 100 years and does not explain the discrepancies in the wood $\delta^{15}N$ patterns for the two plots.

Why would excluding fire decrease $\delta^{15}N$ values? Increases in tree and shrub biomass have an effect on N cycling including mineralization and nitrification rates. Bukata and Kyser (2005) found that the natural abundance of ^{15}N in red oak (*Quercus rubra*) and white oak (*Quercus alba*) wood shifted when rates of nitrification and nitrate leaching increased after tree clearing. Högberg and Alexander (1995) found changes in foliar $\delta^{15}N$ associated with varying tree density and litter accumulation in West-Africa and assumed that density variation altered local patterns of N cycling. The loss of N or N availability from terrestrial ecosystem may be dependent on successional patterns of biomass accumulation (Vitousek and Reiners 1975). Temperate forests that recover from disturbance have high N uptake potential with concurrent short-term declines in N availability (Pardo *et al.* 1995). Other factors may decrease N availability on a longer timescale and include the recovery of N immobilization potential via carbon additions, soil matter accumulation and the production of coarse woody debris (Hooker and Compton 2003, McLauchlan *et al.* 2007). I suspect that the initial increase in tree density in the fire exclusion plot lead to high immobilization of nitrogen. Although this explains the pattern of less enriched wood $\delta^{15}N$ over time, it is still unclear why the most recent wood became enriched in ^{15}N.

The main air transport corridor over southern Africa flows in a south eastern direction placing the Kruger National Park (KNP) downwind from major coal refineries. N deposition values for the Kruger National Park have been reported to be the highest in Africa (Galy-Lacaux *et al.* 2003, Scholes *et al.* 2003). Increased deposition leads to eutrophication which in turn may lead to changes in species composition and ecosystem function (Vitousek *et al.* 1997, Bobbink *et al.* 1998). Nitrogen isotope signatures ($\delta^{15}N$) in wood have been successfully used to detect N pollution signals, increased N deposition and soil nitrogen availability (Puñuelas and Estiarte 1997, Köchy and Wilson 2001, Hart and Classen 2003, Saurer *et al.* 2004, Bukata and Kyser 2005, Bukata and Kyser 2007).

The enrichment of both fire treatments in the most recent wood indicates that there may be a process involved that influence both treatments. Although data on the N isotopic signature of the dry deposition for the Kruger National Park is lacking, previous work suggests that deposition originating from coal combustion has positive δ^{15}N values (Heaton 1986). In Europe, tree ring δ^{15}N values increased by up to 8 ‰ next to polluted highways because of ^{15}N enriched NO_x pollution (Ammann *et al.* 1999, Saurer *et al.* 2004). Wood may become enriched in ^{15}N if the N in dry deposition has a positive N isotopic signature, or if high amounts of N enter the cycle in the KNP.

To reiterate, vegetation and fire effects on nitrogen interact to display δ^{15}N trends in wood cores. Nitrogen cycling in the annual burn plot is mostly driven by the effects of fire on the availability and losses of nitrogen and secondly by increases in woody density (which occurs regardless of fire). The fire exclusion plot on the other hand, initially had large litter inputs which may have lead to slower decomposition and less available N. This in turn leads to more depleted δ^{15}N values. However, the possibility that wood δ^{15}N may be influenced by an extraneous N source such as N in dry deposition should not be ignored and may explain the enriched δ^{15}N in the most recent wood.

CHAPTER 9
SUMMARY AND SYNTHESIS

INTRODUCTION

The rate of nutrient cycling is a major factor controlling biomass productivity in many ecosystems. Nitrogen (N) is often the most limiting nutrient and its cycle is influenced by the amount of nitrogen stored in various components (pools, *e.g.*, vegetation, soil) of the system and the rate of nitrogen transfer among these components (fluxes) (Keith 1997). Few studies have investigated the effects of fire on soil dynamics and nitrogen cycling in the Kruger National Park and the results are inconclusive (Jones *et al.* 1990, Shackleton and Scholes 2000, Aranibar *et al.* 2003, Feig 2004). Research from elsewhere has also found varying effects of fire on the nitrogen cycle; fire either decreases (Oguntala 1980, Risser and Parton 1982, Cook 2001, Reich *et al.* 2001, Grogan *et al.* 2002, Fynn *et al.* 2003), increases (Covington and Sackett 1986, Dai *et al.* 2006) or has no effect (Mistry 1998, Pivello and Coutinho 1992, Aranibar *et al.* 2003, Feig 2004) on nitrogen pools and available N.

In order to better understand the long-term effects of fire frequency and season on nitrogen cycling and productivity in savannas, I measured aboveground and belowground pools (stocks), and cycling rates of nitrogen. The natural abundance of ^{15}N was used as a proxy for nitrogen availability where appropriate. Vegetation adapted to low N conditions with low N tissues would be expected to dominate in frequently burned areas if fire was found to decrease the rates that nitrogen become available. Herbaceous composition and traits related to nutrient use were measured to determine if this shift with frequent fire took place. Measurements were taken on the Shabeni string of the southern or Sourveld experimental burn plots (EBP's), but the other three strings of the southern EBP's were used as replicates where applicable. The EBP's provide a unique opportunity to study the long-term effects of fire on nitrogen cycling as plots have been subjected to the same fire treatment for more than 50 years. The effects of fire, and specifically the effects of frequent fire (annual and triennial burning), on aspects of ecosystem function are summed up in the following section.

IMPACT OF FIRE ON NITROGEN

Fire leads to losses of N through volatilization in the short-term and changes in vegetation structure and species composition in the longer-term. N is easily lost during fuel combustion as N volatilizes at relatively low temperatures (~200°C) (Raison 1979, DeBano *et al.* 1998). Grass dominates in frequently burned Oak savanna. Total productivity in these communities is decreased due to low turnover rates of root N resulting in decreased litter N and associated N mineralization rates (Reich *et al.* 2001). This results in low N availability resulting in reduced growth rates for the woody component.

In other systems frequent fire does not necessarily impact negatively on productivity, but slows down nitrogen (N) cycling. Vitousek (2004) diagrammatically described components of a plant-soil microbial positive feedback in nutrient poor forests. In these systems nutrients are utilised more efficiently than in nutrient rich forests through an increase in the residence time of nutrients in the canopy. This happens through increased leaf longevity and the resorption of nutrients from senescing tissues resulting in low nutrient levels in litter. Low quality litter takes longer to decompose, immobilizes nutrients during decomposition and the overall effect is a reduction in nutrient availability (Vitousek 2004).

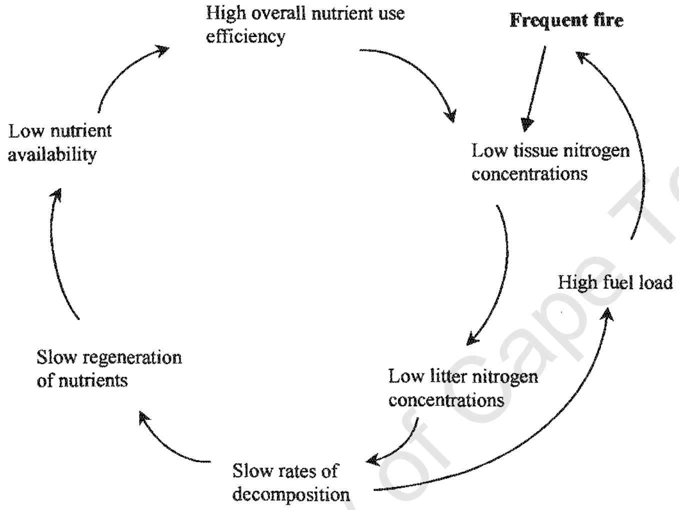

Figure 9.1. A feedback mechanism describing the effects of frequent fire in prairie. Adapted from Vitousek (2004)

I adapted Vitousek's diagram (Fig 9.1) in order to visualize what happens in frequently burned prairie. Burned prairie vegetation produces large amounts of low quality (wider C:N ratios) roots, and as a result, large amounts of below-ground litter which immobilizes large amounts of N. The subsequent decrease in N availability creates a positive feedback where plants with high nitrogen use efficiency (NUE) have an advantage. This low quality litter with slower decomposition rates further advances conditions of low nitrogen availability which in turn promotes high NUE plants. The higher below-ground production following fire, and inputs of litter with wide C:N ratios decreases N mineralization rates (Ojima *et al.* 1994). The slower decomposition rates caused by wide C:N and lignin/N ratios in these grasses together with their high productivity leads to rapid fuel accumulation and increased fire frequencies (Wedin 1995).

FIRE EFFECTS ON NITROGEN IN A MESIC SAVANNA OF THE KRUGER NATIONAL PARK

In my study, frequent fire did not depress the nitrogen cycle. By nitrogen cycle, I refer to plant available N and the N capital measured as total N pools in litter and soils. Frequent burning did not decrease total soil N or plant available N regardless of soil depth. The total amount of N mineralized in a season was increased by annual burning when compared with excluding fire. Frequent fire did not consistently decrease or increase ecosystem $\delta^{15}N$ values. In fact, the variability between the strings (replicates) was larger than between fire treatments within strings.

Not only did the tree layer persist in the annual burns, it also has a higher woody biomass than the triennial August burn. The triennial August burn was already visibly destructive to trees 20 years after the initiation of the EBP experiment (Van Wyk 1971). Frequent fire did not lead to the dominance of grasses adapted to low nitrogen conditions or grasses with low nutrient characteristics. Frequent burning did not decrease nitrogen concentrations of tree or herbaceous foliage except for *Panicum maximum* where the fire exclusion had higher concentrations than the annual burn. The only difference in inorganic N concentrations was in the fire exclusion plots were NO_3^- was higher under the canopy than in any other treatment. This may explain the higher N concentrations of *P. maximum* in the fire exclusion as it grows under tree canopies.

This work is in agreement with previous research from the KNP that did not show changes in plant available N and total soil N, or $\delta^{15}N$ with frequent burning (Feig 2004, Aranibar *et al.* 2003). What we see in Kruger is paralleled by findings in studies from other savannas where frequent fire (every 2 to 3 years) does not lead to long-term losses of ecosystem nitrogen (Holt and Coventry 1990, Mistry 1998, Medina and Silva 1990, Miranda *et al.* 2002, Abbadie 2006). With annual burning, however, some of these savanna systems may experience N depletion (Cook 2001).

The season of burn, which also affects the intensity of the burns, had as much of an effect on the N cycle (and sometimes more) as the frequency of burn. The lower intensity February burn leads to higher cumulative N availability than the high intensity August burn. The higher N availability was accompanied by higher herbaceous, woody and forb production on the February burns. The August triennial burn had the lowest total above-ground productivity. This treatment also had the lowest quality roots (i.e., widest root C:N ratios) which may have result in slower decomposition rates and lower cumulative N mineralization.

WHAT GOVERNS WHETHER FREQUENT BURNING DECREASES ECOSYSTEM N POOLS AND FLUXES?

1. The amount of N volatilized with each fire?

Within the process of volatilization ammonium is converted to ammonia and lost to the atmosphere. Significant losses of N occur at fire temperatures above 400°C (Cass *et al.* 1984) and combustion is almost complete above 600°C (Raison 1979). Losses due to volatilization in savanna have been reported to vary between 4 and 33 kg N ha^{-1} yr^{-1} (Frost and Robertson 1985).

The amount of N volatilized is system-dependent. Boerner (1982) argued that nutrient losses due to fire in nutrient-poor (oligotrophic) ecosystems are much greater than in nutrient-rich (eutrophic) ecosystems. The reason for this is that most of the nutrients are aboveground in nutrient-poor systems such as chaparral and pine forests and therefore susceptible to consumption by fire. Boerner (1982) suggested that in temperate grasslands, where most of the total biomass is belowground, fire impacts should be less. However, in some pine forests, excluding fire results in an accumulation of litter that retards organic N mineralization (Covington and Sackett 1986) and frequent fire actually increases total soil N (Heyward and Barnette 1934, Wells 1971). Frequent fire in grasslands (and prairie), on the other hand decreases N cycling (Ojina *et al.* 1990, Ojima *et al.* 1994, Fynn *et al.* 2003).

A factor that may be more important than how nutrient-rich the system is, relates to the vegetation structure of the system. For instance, more N is lost in open cerrado (grass dominated) than closed cerrado (tree dominated) as herbaceous biomass is the primary fuel source in savanna (Kauffman *et al.* 1994). Savanna fires are usually surface-fires that consume the grass layer but not trees taller than 2-4 m (Bond and Keeley 2005). Consequently, the presence of trees should decrease fire N losses as N is conserved in wood and leaves that do not burn. An increase in tree cover often results in a decrease in grass cover and in the process decreases herbaceous fuel loads (Frost and Robertson 1987). Williams *et al.* (1999) found that excluding fire for 2 to 4 years in Eucalypt savanna lead to a build-up of near maximal fuel levels. These high fuel loads caused fires of high intensities and woody stem death in a single high intensity fire was comparable to annual burning where fire intensities were much less. The loss of N will also depend on the time of year as this would determine how much moisture is in the fuel and therefore intensity of burn, as well as how vulnerable vegetation is to burning. In West Africa, burning in the dry season caused an almost 100% loss of herbaceous biomass but burning earlier (when wetter) caused a loss of around 12% (Abbadie 2006). Annual burning does not eliminate trees in the broad-leaf savanna of the Kruger National Park. High intensity fires also have higher flame heights that may consume more tree foliage. I suggest that the high intensity August triennial burns cause larger losses of N than the lower intensity February burns.

2. Loss of N is replaced by N inputs

N fixation and N deposition

Frost and Robertson (1987) suggested that if burned areas have higher rates of N-fixation than unburned areas, losses due to fire would be balanced. However, if burned and unburned areas receive the same amount of N inputs through N fixation and deposition, there is no reason to expect that losses of N from fire would be replaced. West African savannas have relatively high rates of fixation by cyanobacterial crusts. The highest densities of these crusts are found in open shrub savanna (which burns frequently) and it has been suggested that N losses may be replaced to significant extent by algal crust fixation (Isichei and Sanford 1980, Abbadie 2006). Although nitrogen is lost in savanna fires, these losses are reported to be rapidly replaced by natural inputs (Holt and Coventry 1990, Kauffman *et al.* 1994, Franklin *et al.* 1997, Mistry 1998, Jensen *et al.* 2001, Bustamante *et al.* 2006).

Increased symbiotic nitrogen fixation by woody species can be ruled out as previous work has shown that fixation does not increase with regular fire (Aranibar *et al.* 2003). In my study, the two treatments with the highest cumulative N mineralization (annual and February triennial) had three to four times as much forb biomass as the other treatments. Many of the forbs in this system are legumes (Van Wyk 1971) and most symbiotic N fixation in southern Africa is performed by leguminous forbs (Scholes 1997).

Large N deposition rates originating mainly from coal burning have been reported for the Kruger National Park (~22 kg N ha^{-1} yr^{-1}, Scholes *et al.* 2003). This number is exceptionally high as Amersfoort, which is in the middle of an anthropologically modified and industrialized area in South Africa, only receives 15 kg N ha^{-1} yr^{-1} (Galy-Lacaux *et al.* 2003). Regardless of the accuracy of this figure, a value of less than half of this would replace the N lost via volatilization.

Herbivores

Herbivores are an integral component of African savanna. In this study, herbivores congregated on the EBP plots post-burn and they may redistribute some of the nitrogen that would have been lost with burning. The dense vegetation of the fire exclusion plot is not frequented by grazing antelope as they prefer more open areas. Herbivores promote nitrogen cycling and nitrogen retention in other systems such as prairie and grasslands (Seagle *et al.* 1992, Frank *et al.* 1994, Frank *et al.* 2000).

3. Shifts in species composition (and community)?

Another possible explanation for fire effects on N cycling is that different fire regimes cause plant species composition to change and these changes drive changes in N. Many studies have indicated that plant species composition directly controls the functioning of ecosystems including decomposition and N availability (Hart *et al.* 1994, Chapin *et al.* 1997, Schmidt *et al.* 1997, Van der Krift 2001, Knops *et al.* 2002, Chapman *et al.* 2005).

Two different mechanisms may be at work in savannas, a change in grass species with different fire regimes and a change in tree:grass ratios. As I have explained in Fig. 9.1, frequent fire may lead to a change in the dominant grass species which leads to changes in N availability. This change is not always driven by altered nitrogen conditions brought about by fire, but may also be directly related to morphological traits such as height, position of tiller initiation and position of meristems (Fynn *et al.* 2005). If only some species are adapted to frequent disturbance (*i.e.*, fire), a complete species shift will take place with frequent fire. The species shift will then drive the nitrogen dynamics. An example of this is where the alien grass, *Andropogon gayanus*, invaded northern Australian ecosystems (Rossiter *et al.* 2003). Areas dominated by this grass burn at very high fire intensities, which is detrimental to the native species. In turn, *A. gayanus* decreases available nitrate levels by 70% compared with native vegetation (Rossiter *et al.* 2004).

Fire, however, can also affect savanna structure. Fire exclusion in Oak savanna leds to forest invasion (Reich *et al.* 2001). The shift to forest lead to a much higher annual N cycle, defined as either N mineralization or litterfall N. Nitrification levels are also much higher in forest than savanna in Lamto despite the fact that physical and chemical characteristics of soils are very similar (Abbadie and Lata 2006).

FUTURE PERSPECTIVES

Little work has been done in southern African savannas on how individual species (and the associated tissue chemistry) influences N cycling. For instance, are the thick stands of *Panicum maximum* responsible for increased NO_3^- concentrations under the canopies of adult *Sclerocarya birrea* in the fire exclusion plot (Chapter 4), or is this an effect of the tree canopy?

Previous work found that woody legume N_2 fixation did not increase with frequent fire in the KNP, but it is important to quantify the input from leguminous forbs in frequently burned areas. We also lack knowledge on how frequent fire will affect or encourage N_2 fixation associated with grasses and soil crusts. Although it is recognized that fire losses are replaced by N inputs in savannas, we still have a poor understanding of the mechanisms.

It is imperative to verify the high N deposition rate of 22 kg N ha^{-1} yr^{-1} (Scholes *et al.* 2003). Values of 8-14 kg N ha^{-1} yr^{-1} for temperate and sub-tropical savannas have been considered high as they rival values observed from areas undergoing N pollution and saturation (Asner *et al.* 2001). Such high deposition values may potentially have profound effects on grass and tree species adapted to the relatively nutrient-poor granite soils of the KNP.

LAST COMMENTS

This work has improved our understanding of the effects that a frequent fire regime will have on nitrogen pools and available N in an African broad-leaf savanna. The perception that fire will decrease N pools and transformation rates has for the most part been based on measurements taken during single fire events. Few studies have used long-term burn experiments to establish what the long-term outcome of a specific fire regime on N cycling would be (Ojima *et al.* 1994, Reich *et al.* 2001, Fynn *et al.* 2003). This is partly due to the scarcity of such long-term experiments and these studies are particularly lacking in savannas. Furthermore, many studies compare annual burning with fire exclusion which may equal comparing grassland with forest as savanna may be replaced by forest in the absence of fire (Keay 1951, Charter and Keay 1960, Ramsay and Rose 1963, Menaut 1977, San José and Farinas 1983, Carson and Abbiw 1990, Chidumayo 1990, Swaine *et al.* 1992). As many savannas are maintained by fire (Bond *et al.* 2003), I suggest that a situation where savanna persists while never burned unlikely. The focus therefore should shift to differences between burn treatments and away from comparing fire exclusion to annual burning.

The broad-leaf savannas of the Kruger National Park seem to have mechanisms in place that confer resistance to perturbation by fire and are remarkably resilient. Fifty years of annual fire has not led to grassland, neither has 50 years of fire exclusion led to closed canopy forest. Examples of these include the physiological adaptations, such as thick bark and resprouting, which make savanna trees very resistent to damage by fire. Furthermore, in the absence of fire, large herbivores ensure that moribund material does not accumulate. Results of SOM ^{13}C indicates that the area has been in a savanna (mixed trees and grass) state for a considerable length of time. The same applies to the effects on the nitrogen cycle; the system is in balance with the losses of N replaced by N inputs.

Future work should be directed to highlight the important distinctions in the way that African savannas function. It is imperative that we use our understanding of savanna function to build our own perspectives. We can do this by modifying existing models of ecosystem function to suit our unique situation and not by trying to explain our results by using existing models *pro forma.*

Chapter 10

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