

# Charting the Quality of Forage

**Measuring and Mapping  
the Variation of Chemical  
Components in Foliage with  
Hyperspectral Remote Sensing**



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# Charting the Quality of Forage

## Measuring and Mapping the Variation of Chemical Components in Foliage with Hyperspectral Remote Sensing

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Proefschrift ter verkrijging van de  
graad van doctor, op gezag van de rector  
magnificus van Wageningen Universiteit,  
Prof. Dr. Ir. L. Speelman, in het openbaar  
te verdedigen op vrijdag één juli 2005 des  
namiddags te half vier in het auditorium  
van het ITC, te Enschede

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# Contents

Samenvatting		xi
Summary		xii
Acknowledgements		xiii
General Introduction		1
1.1	Herbivore distribution: Sketching a background	3
1.2	A herbivore's view of the landscape	3
1.3	Hyperspectral remote sensing	4
1.4	Plant chemical composition	5
1.5	Study objectives	5
1.6	Study Area	6
1.7	Thesis outline.	7
Mopane Responses to Soil Properties and Physical Damage		9
2.1	Introduction	11
2.2	Methods	13
2.2.1	<i>Plant material and growing conditions</i>	13
2.2.2	<i>Sampling</i>	13
2.2.3	<i>Chemical analysis</i>	14
2.2.4	<i>Statistical analysis</i>	14
2.3	Results	15
2.3.1	<i>Effect of soil treatments</i>	15
2.3.2	<i>Effect of leaf removal</i>	16
2.4	Discussion	17
2.4.1	<i>Effect of soil nutrients</i>	17
2.4.2	<i>Effect of leaf removal</i>	18

	Lab-Spectroscopy of Mopane Biochemistry	21
3.1	Introduction	23
3.2	Methods	24
	3.2.1 Greenhouse setup	24
	3.2.2 Spectrometry	25
	3.2.3 Chemical analysis	26
	3.2.4 Statistical Analysis	26
3.3	Results	28
	3.3.1 Foliar chemistry & plant size	28
	3.3.2 Reflectance spectra.	29
	3.3.3 Derivative spectra.	31
3.4	Discussion	33
	 Nitrogen Detection Across Plant Species	 37
4.1	Introduction	39
4.2	Methods	40
	4.2.1 Data collection / biochemical analysis	40
	4.2.2 Data processing	42
	4.2.3 Index calculation and correlation	42
	4.2.4 Between species correlation	42
	4.2.5 Similarity of species	43
4.3	Results	43
	4.3.1 Correlation between NRI and nitrogen	43
	4.3.2 Between species correlation	46
	4.3.3 Similarity of species	46
4.4	Discussion	47
	 Detecting Foliar Nutrients and Trace Elements	 53
5.1	Introduction	55
5.2	Methods	57
	5.2.1 Study areas	57
	5.2.2 Field sampling	57
	5.2.3 Chemical analysis	57
	5.2.4 Spectral processing	57
	5.2.5 Statistics	58
5.3	Results	59
5.4	Discussion	64

Detecting Foliar Condensed Tannins in Mopane		65
6.1	Introduction	67
6.2	Methods	68
	6.2.1 Data Collection / Study Area	68
	6.2.2 Processing of Spectra	69
	6.2.3 Red Edge and 'Infrared Edge'	70
	6.2.4 Statistical Methods	70
6.3	Results	72
6.4	Discussion	76
Natural Variation in Mopane Biochemistry		79
7.1	Introduction	81
7.2	Methods	82
	7.2.1 Study area	82
	7.2.2 Data collection	83
	7.2.3 Chemical analysis	84
7.3	Data analysis.	84
	7.3.1 Data transformation	84
	7.3.2 Fire effects	84
	7.3.3 Parent material	84
	7.3.4 Relations	85
7.4	Results	85
	7.4.1 Fire effects	85
	7.4.2 Parent material	86
	7.4.3 Relations	86
	7.4.4 Reduction of variables	87
7.5	Discussion	88
Box 1. Herbivory and Plant Chemical Composition.		91

	Mapping Phenolic Compounds in Mopane Woodland	93
8.1	Introduction	95
8.2	Methods	96
	8.2.1 Study area	96
	8.2.2 Validation	97
	8.2.3 Chemical Analysis	97
	8.2.4 Image acquisition and processing	97
	8.2.5 Band selection	98
	8.2.6 Neural Network	98
	8.2.7 Optimizing neural net settings	99
	8.2.8 Masking	100
8.3	Results	100
	8.3.1 Band selection	100
	8.3.2 Optimizing neural network settings	103
	8.3.3 Polyphenol and condensed tannins	103
	8.3.4 Prediction accuracy	103
	8.3.5 Spatial patterns	105
8.4	Discussion	106
	8.4.1 Complete images	107
	8.4.2 Masked images	107
	8.4.3 Between flight-line differences	108
	8.4.4 Relevance for ecology & management	108
	Hyperspectral Remote Sensing and Vegetation Chemical Composition: Looking towards the Future	111
9.1	Introducing the synthesis	113
	9.1.1 Understanding chemical composition of foliage	114
	9.1.2 Measuring chemical composition of foliage	115
	9.1.3 Mapping chemical composition of foliage	117
9.2	Plant-herbivore systems	119
	9.2.1 Foraging strategies in the landscape	120
9.3	Conceptual model	121
	9.3.1 Foraging herbivore	122
	9.3.2 Balancing intake ratios	122
	9.3.3 Intake strategies	123
	9.3.4 One-component intake control	125
	9.3.5 Two-component intake control	125



References	131
Appendices	145
Appendix 1. Kruger National Park fire frequency map	147
Appendix 2. Kruger National Park geography maps.	149
Appendix 3. Foliar Condensed Tannin in Mopane.	151
Appendix 4. Foliar Total Polyphenol in Mopane.	153
Appendix 5. Distribution of Colophospermum mopane.	155
Résumé	157
ITC dissertation list	159
Author's Publication list	165



# Samenvatting

## ‘Kartering van Voedselkwaliteit’

Het meten en karteren van de variatie in blad-chemische samenstelling door middel van hyperspectraal remote sensing.

*D*e draagkracht van *rangelands* voor herbivoren wordt bepaald door zowel de hoeveelheid, als de kwaliteit van de aanwezige plantbiomassa. Het schatten van de beschikbare hoeveelheid biomassa is eerder bestudeerd, en hiervoor zijn betrouwbare methodes ontwikkeld. Het bepalen van de kwaliteit van bladbiomassa wordt normaliter gedaan door middel van chemische analyse van monsters in een laboratorium. Deze analyse methoden kosten veel tijd en geld. Een aantal studies heeft aangetoond dat het mogelijk is om met reflectiemetingen onder gecontroleerde omstandigheden de chemische samenstelling van vegetatie monsters te bepalen.

In dit proefschrift is een aantal methoden onderzocht die gebruikt kunnen worden om de chemische samenstelling van vegetatie te meten met behulp van *remote sensing* technieken, en worden de factoren die variatie in chemische samenstelling veroorzaken begrijpelijk gemaakt.

Uit de resultaten blijkt dat de hoeveelheid beschikbare stikstof en fosfor in de bodem de reflectiespectra van een tropische boomsoort (*Colophospermum mopane*), gekweekt in een kas, beïnvloeden. Alhoewel meerdere studies hebben aangegeven dat plantschade de chemische samenstelling van bladmateriaal kan beïnvloeden, heb ik dit in een kasexperiment niet kunnen bevestigen. Om betere lichtreflectie indices te vinden voor het bepalen van stikstof concentratie in meerdere plantensoorten, is gebleken dat voor verschillende plantensoorten verschillende indices nodig zijn voor het optimaal voorspellen van de stikstof concentratie. Voor spoorelementen in het blad vond ik hetzelfde effect. De verschillen tussen soorten blijken groot genoeg om een veralgemenisering van reflectie-modellen in de weg te staan.

Variatie in plant-chemische samenstelling en vegetatieopbouw in het noorden van Kruger National Park (Zuid Afrika) is voornamelijk afhankelijk van het voorkomen van terreinbranden, wat weer gerelateerd is aan de geologische ondergrond.

Dit proefschrift combineert ten slotte de in de vorige hoofdstukken gegenereerde kennis betreffende ecologie en remote sensing in een neuraal netwerk, waarmee de concentratie van gecondenseerde tanines en totale polyphenolen in een stuk mopane savanna in kaart werd gebracht. Met een voorspellende nauwkeurigheid van 60 – 70% blijkt dit model succesvol. Dit betekent een stap voorwaarts in technieken om de chemische samenstelling van vegetatie te karteren. De verworven kennis is goed bruikbaar bij het analyseren van herbivoor-verspreiding, en het testen van optimale fourageer-theoriën

# Summary

## ‘Charting Forage Quality’

### Measuring and Mapping the Variation of Chemical Components in Foliage with Hyperspectral Remote Sensing

Rangeland carrying capacity is determined by both the quantity, as well as the quality, of forage. The estimation of the quantity of rangeland foliage and its spatial distribution has been studied and reliable results achieved. Traditional methods to determine quality involve collection of field samples and their analysis in a laboratory using wet chemical techniques. These analysis methods are time consuming and expensive. Previous studies have demonstrated that laboratory spectroscopy can be used to determine the concentration of leaf chemical compounds.

In this study I demonstrate a number of methods to measure the chemical composition of foliage using hyperspectral remote sensing techniques, and try to predict the interspecific variation of chemical composition in vegetation.

Summary

The results show that soil nitrogen and phosphorus content affect the spectral reflectance signatures of a tropical tree species (*Colophospermum mopane*), grown in a greenhouse. Although several studies have indicated potential effects of herbivory on plant-chemical composition, this could not be confirmed. In an attempt to find better spectral indices to detect foliar nitrogen concentration across multiple species, it was shown that there are differences between species for the optimal spectral regions used to measure nitrogen concentration. For foliage trace-elements I found large differences between species thereby confounding the ability to generalize prediction-models.

Variation of mopane foliar chemistry and forest structure in the north of Kruger National Park, South Africa, was mainly dependent on fire history, which is closely related to the parent material of the soil on which the plants were located.

This thesis concludes by combining the ecological and remote sensing knowledge generated in previous chapters into a neural network model to predict the concentration of Condensed Tannins and Polyphenols in a stretch of mopane woodland in Kruger National Park from hyperspectral data. With an accuracy of approximately 60-70% this model proved to be successful. This is a step towards mapping the chemical composition of foliage, which may subsequently be used to analyze herbivore distribution patterns in space and time, and test optimal foraging theories.

# Acknowledgements

*“By three methods we may learn wisdom:  
first by reflection, which is noblest; second by imitation, which is  
easiest; and third by experience, which is the bitterest.”*

*Confucius (551-479 B.C.)*

The fundamentals for this thesis were laid over 2300 years ago when, in 347 B.C., Aristotle was born in the village *Stagirus* in Greece. With the theory of syllogisms, he made major advancements in logical thinking. The basic ideas of this theory are still embedded in logical reasoning now, more than 23 centuries later. He showed us that by linking an unknown object to a basic truth, a new conclusion can be formulated about the unknown object. And what more is science than this? We use existing knowledge to explain patterns or processes by linking this knowledge to the observed patterns and processes. We thus build on the knowledge of those before us and construct new foundations for scientists to come (‘If I have seen farther than others, it is because I was standing on the shoulders of giants.’; Isaac Newton, 1642-1727).

Moving our focus to the current day and age, this thesis builds on the knowledge and support that I received from several individuals and institutions over the past 3 ½ years. Because most people will not read the whole thesis, but are merely interested in the acknowledgements (am I in it?) I placed the acknowledgements at the beginning of this thesis. This way, my dear friends, you will not have to search too long.

First and foremost I would like to thank ITC for giving me the opportunity to develop my skills, by giving me a fully funded PhD (AIO) position to work on this thesis. Particularly the commitment to pay for a hyperspectral mission in Kruger National Park is quite an investment to make, especially since the core of this project consisted of only two PhD students.

Directly after this follow of course my promoters: at ITC, Andrew Skidmore, and at Wageningen University, Herbert Prins. Herbert & Andrew: Thank you so much for all the advice and support. I could not have written this thesis without you! You guided me through the long process of growth from my MSc graduation to now, a defending PhD candidate. Andrew & Herbert, thank you for your support, ideas, corrections, and seemingly endless discussions. Often we disagreed on the direction to take, but you were always ready to listen to yet another idea for a field experiment, paper or hypothesis.

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The combined supervision worked out very well, with one person often criticizing the changes made to papers, after suggestions from the other. You have helped me to realize that the choices you make should be your own, supported by scientific arguments. Thank you for helping me to grow as a scientist, and for opening doors, which seemed impossible to open to me.

To my parents, I can only say: Words are not enough. Ever since I made the choice to aim at a career in science you have given me the mental support I at times needed more than anything. You helped me to get across frustrations, and broke down boundaries that were impossible to cross for me. You stimulated me to get most out of life, for which I can only be grateful.

On a day-to-day basis there were many people who helped me to work ideas into methods and scientific papers. Of course, I cannot possible mention everybody, and will not attempt to do so. But a few people need mentioning.

First of all: Sip van Wieren. Whenever a problem arrived, you were there with suggestions; whether it was to arrange a greenhouse for plants to be grown, or to find that link that can turn a nice story into a scientific paper. We worked together on a number of papers, and I hope we can continue to do so.

In general, the Resource Ecology Group at Wageningen University made me feel at home, whenever I was there for a day or week to do laboratory work. Discussions on tannins (Jantineke), competition (Jasja & Nicol), and herbivore distribution and resources (Geerten & Thomas) greatly improved many afternoons and gave new insights in the direction to take. Several other people in Wageningen were always willing to discuss statistics or ecological theories with me, such as Fred, Frank, and Claudius. All the chemical analyses were performed in the laboratory at the resource ecology group. Jan and Tjakkie: Thank you so much for all those little hints and advices on how to perform the endless stream of chemical analyses. Your presence in the laboratory made the trip to Wageningen more than worth the long train rides.

Whenever I needed to be in Wageningen, I found a roof, bed, and often a dinner and breakfast with a few dedicated friends, who acted insulted if I did not stop by for dinner after my one-day visits to Wageningen. Joris, Mariska, Mark, and Christien: You deserve so much more than a simple ‘thank you’! You saved me precious hours during lab-work, and while my greenhouse experiment was running.

During the bulk of my thesis I worked in my office at ITC. There, the workload was made lighter by the talks with people like Jan de Leeuw, Wouther Siderius, Iris van Duren, Alfred Stein and Freek van de Meer. I would like to thank you for the scientific insights, literature searches, and presence, which have made life at ITC a good experience. In general, the PhD community made the workload lighter. Especially the Friday afternoon PhD drinks provided a nice opportunity to socialize with all those unknown faces, who over time became friends. Everybody was special in his or her own way, I am not going to even try and name each and every PhD student individually. I thank you all.

I could not have written this thesis without technical support, and the help I received from the geo-technical support team at ITC helped me to quickly get footing in a previously unknown field. Whenever I got stuck, there was always the support team to solve the technical or programming problems. Boudewijn, Jelger, Wan, Henk: Thank you! Harald: thank you for your insights in spectrometry.

This thesis is full of graphs and figures. Backups of my thesis were made on DVD throughout the project. For this I owe thanks to two people at ITC; the magical team ‘Job and Benno’. Even in the most stressful moments, the work gets done, quickly and professionally. They are amongst the most efficient and reliable people I have met at ITC. Every day ready with a joke to make you smile. Job and Benno: Keep it up. Ronnie thanks for reproducing this thesis in the short time left at the end of the thesis writing phase.

To get all the literature has been a challenge. My gratitude therefore goes out to the ITC library team. Particularly to Carla and Petry, who made it their ‘hobby’ to locate papers published years ago in local African journals.

The Scientific Board of the Kruger National Park gave permission to work in this magnificent park. Without co-operation from their side, this thesis would have stopped before the work had started. For this and logistics support, I thank Rina Grant, Mike Peel, and Harry Biggs. In and around the park Bruce and Theresa in Senalala provided housing for our field mission in the southern part of the park. In the Shingwedzi camp, Angela and Abri were always there for us, and solved every little problem we might have had. From providing us with 4WD vehicles when the roads were soaked with water, to quick access to game guards, even at last-minute trips.





# 1

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## General Introduction

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*“A problem well stated is a problem half solved”*

*C.F. Kettering (1876-1958)*





# Is the world greener on the other side?

## 1.1 *Herbivore distribution: Sketching a background*

*I*n a world full of resources, a herbivore continuously has to make a choice: 'Where to eat what next?', 'Move to the next shrub, or tuft of grass?', or 'Stay and locally deplete resources?'. Although these choices are most likely not made at a conscious level, they are certainly important for the herbivore's fitness. What determines these choices is unclear. Is the concentration of nutrients, such as nitrogen and phosphorus, the main factor that determines this choice (Cook et al. 1976) ? Or are fibre-content and the concentration of digestibility lowering compounds, such as condensed tannins, the driving factors behind herbivore distribution and resource selection (Bernays et al. 1989, Levin 1971) ? Or maybe the distribution of herbivores is a response to differences in predator-density (White et al. 2003) ?

## 1.2 *A herbivore's view of the landscape*

Based on feeding preference, herbivores can be split into three groups: Browsers, grazers, and between those, intermediate feeders (Gordon 2003). Grazers are commonly defined as relatively non-specific feeders, and the bulk of their diet consists of monocots and herbs. Browsers on the other hand are often very specific feeders and may select individual leaves when feeding, with a large part of their diet consisting of foliage, buds and stems of trees and shrubs (browse). These two resources -grasses versus browse- are quite distinct in their digestive properties. Grasses generally contain thicker cell walls, which are less digestible due to a higher concentration of slowly digestible plant fibres, such as cellulose, compared to browse. Digestion of these fibres is made more difficult by lignification of the cell wall and the amount of lignification in grasses varies drastically among seasons and plant age (Van Soest 1987). To digest the fibres encountered in forage, all ruminants have one or more portions of the foregut or intestine enlarged, to house microbes that ferment plant-fibres (Burk 2002, Dougherty et al. 1964). Although cell walls in browse are generally not as thick as in grasses (Demment and Soest 1985), browsers have to overcome a wide array of herbivore defences when feeding. Browse may contain a combination of mechanic (spines & thorns) and chemical (phenolics, including tannins which may reduce protein digestibility, terpenes, and toxins) defences.

Although it has been shown that the distribution of grazing herbivores is tightly linked to the concentration of nutrients in grass (grazers on the Serengeti Plains have been found to concentrate in areas where foliar nutrient concentration is high (McNaughton 1988, 1990, Seagle and McNaughton 1992) and the annual migration of blue wildebeest is related to increased phosphorus requirements during the lactation period (Voeten 1999)), several studies have shown the potential anti-herbivore effects of phenolic compounds in vegetation (McArthur and Sanson 1993, Mole 1989, Robbins et al. 1987b) and herbivores may avoid forage rich in condensed tannins (Cooper et al. 1988, Furstenburg and Hoven 1992, Matson et al. 2004, Owen-Smith 1994). As a consequence it can be expected that browser distribution may be largely explained by the amount and concentration of herbivore deterring mechanisms in trees and shrubs.

As illustrated, understanding the chemical composition of individual plants, and the variation of plant chemical composition across the landscape can help to understand herbivore distribution. This understanding requires extensive datasets on the chemical composition of vegetation, sampled at high spatial resolution. To build this dataset, a quick and objective way to measure the quality and quantity of resources is critical. To this end, the development of remote sensing, and in particular hyperspectral remote sensing, offers interesting possibilities.

### **1.3                    *Hyperspectral remote sensing***

When light encounters a molecule in its path, the light may scatter and, depending on the properties of the intercepting molecule, the energy of parts of the light spectrum is absorbed or scattered by the atomic bonds, electrons or atoms in the molecule. The wavelength at which this absorption takes place are specific for the atomic bonds, and the molecular architecture of the object, which make identification of individual chemical components possible. By measuring the amount of light that reflects from a surface it may therefore be possible to identify the composition of that surface.

This principle has been used for a long time to describe the earth surface, starting with aerial photography, and, since roughly 35 years, with satellite-based sensors, which is commonly referred to as Remote Sensing. With the development of better sensors and faster computers a new field of research is emerging: 'Hyperspectral remote sensing'. The 'hyper' in hyperspectral refers to the many light sensors with a very narrow sensitive range. The resulting data often has over 100 contiguous bands, each 10 nm or less wide, and each measurement results in a semi-continuous spectrum over the sensitive range. The high number of bands, and the narrow sensitive range, enables the detection of changes in narrow absorption features that may go undetected when using broad-band sensors. This high sensitivity makes it realistic to try to detect changes in chemical composition of vegetation using

spectrometry. When the individual sensors are combined into a sensor array it is possible to measure the reflectance spectra for individual pixels of an image, called imaging spectrometry. By then analysing the spectral features of the individual pixels we can spatially map the properties of the earth surface.

#### **1.4 Plant chemical composition**

The importance of soil properties and climatic variables, as well as external disturbances such as fire and herbivory on plant chemical composition has long been recognized and described in many studies (Adams and Rieseke 2003, Cornelissen and Fernandes 2001, Dudt and Shure 1994, Kraus et al. 2004b, Langevelde et al. 2003, Vijver et al. 1999). Nitrogen and phosphorus in the soil may directly affect the concentration of these same elements in the plant (Ferwerda et al. In Press), and, when only available in low concentration, can affect basic physiological processes such as chlorophyll functioning and cell wall maintenance (Saneoka et al. 2004, Zhao et al.). To fully appreciate the ecological usefulness of hyperspectral remote sensing it is therefore important to link the effects of external factors to the chemical properties of the vegetation and to determine whether hyperspectral remote sensing is capable of capturing the variation in vegetation quality that is the result of differences in soil, climate or other environmental factors.

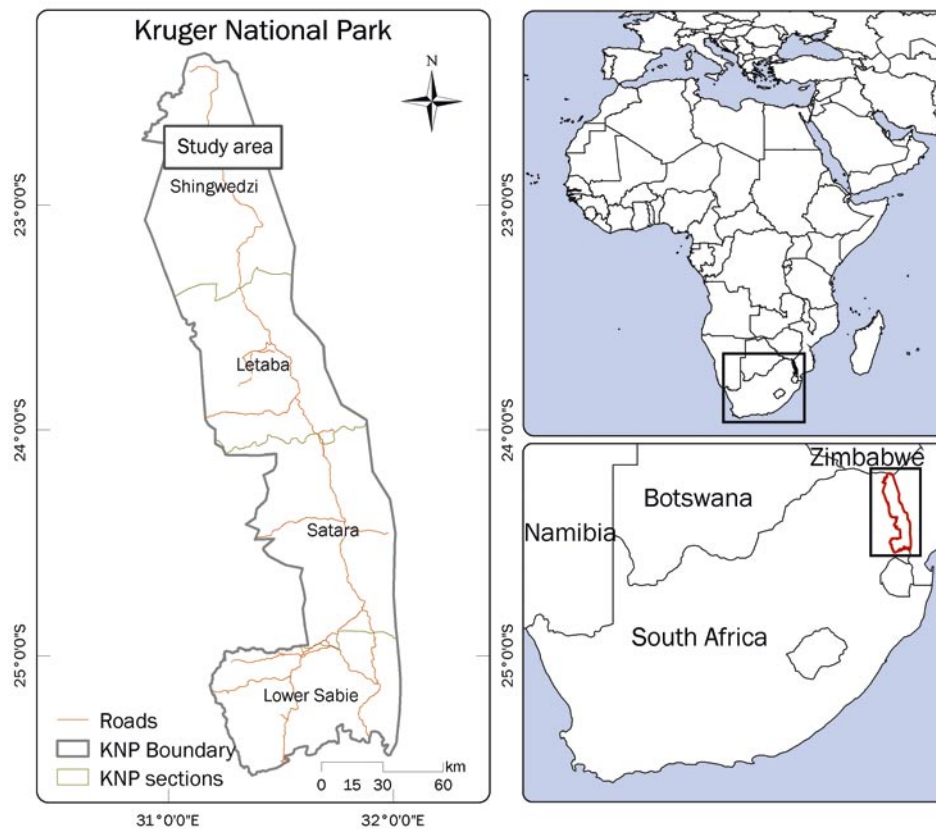
#### **1.5 Study objectives**

The main objectives of this study were (1) to test if nutrient content and deterrent content of woody vegetation can be predicted from high spectral resolution reflectance data, (2) to detect wavebands sensitive to changes in nutrient and deterrent levels in woody vegetation, and (3) to understand how the chemical composition of savanna trees is determined by environmental factors.

The thesis work was performed in parallel with a study by Mutanga (2004), who showed that it is possible to detect the variation in nitrogen concentration of grass using hyperspectral remote sensing under controlled and natural conditions. The combination of both studies gives an overview of the detection of nutrient and deterrent concentration in grasses and woody plants in a South African ecosystem.

## 1.6 Study Area

The major part of this study was conducted in the far north of Kruger National Park, in a region called 'The Northern Plains', just north of Shingwedzi Camp (Figure 1.1). It covers an area of approximately 30 by 6 kilometres from east to west, to cover the main north-south running geological formations. These are from west to east: (1) sandstone with quartzites and some Andesite, (2) shales, and (3) basalts rich in olivine (Venter 1990). The area underlain by sandstones (the 'sandveld') is mainly flat; soils are deep to moderately deep, well-drained, medium and fine sands with some admixture of coarse sand. The shale-based areas are slightly hilly and some rock outcrop may occur. Soils are of variable depth and drainage, fine loamy to clayey and may show an enrichment of clay and cations in the subsoil. The area underlain by basalt is flat with poorly drained, shallow to moderately deep, vertic clays (Venter 1990).



**Figure 1.1.** The location the study area and Kruger National Park.

The vegetation in the study area consists of patches of mopane woodland, intersected with open areas of grassland and bare sands in the west of the study area, and mopane shrubland with tall grasses in the east. Two major drainage lines, which are dominated by dense wetland vegetation with *papyrus* and tall grasses, run from north to south across the study area. Along these drainage lines salt-flats occur, which are almost completely bare of vegetation. The eastern section of the study contains an enclosure, which is used to breed roan antelopes and is separated from the rest of the park by double firebreaks and wildlife fencing. The enclosure is clearly visible on satellite images as a rectangle of about two by two kilometres: as a result of a different fire regime, and the absence of elephants, the vegetation here is denser than on the rest of the basaltic soils.

Mopane (*Colophospermum mopane*, (J. Kirk ex Benth) J. Leonard) is a tree species that covers large parts (> 550.000 km<sup>2</sup> (Timberlake 1995, Appendix 5)) of southern Africa and is used for many domestic purposes, such as firewood, construction and ropes (Madzibane and Potgieter 1999, Mashabane et al. 2001). Deterrent levels in mopane are also high, and this effectively prevents continuous browsing by many herbivores for most of the year (Styles and Skinner 1997), and only African elephants (*Loxodonta africana*) (Ben-shahar 1996, Ben-Shahar and Macdonald 2002, Smallie and O'Connor 2000), eland (*Tragelaphus oryx*) (Styles and Skinner 1997), and some insects are known to regularly use mopane as a food source. However, just before the rainy season starts, leaf flush of mopane starts, providing many browsers with protein-rich forage, when all other trees are still leafless (Styles and Skinner 1997). In context of the importance of this tree species in the region for both wildlife distribution and local uses, it was decided to investigate the relation between the reflectance properties of individual mopane leaves, the chemical composition of mopane leaves and the environmental conditions controlling this chemical composition.

## **1.7 Thesis outline.**

Each chapter of this thesis, except for the introduction and the synthesis, has been written as an individual paper, and can be read independently of the rest of the thesis. All chapters were submitted as research papers to peer-reviewed journals, and the journal to which each chapter was submitted, as well as the title of the submitted paper, are mentioned at the beginning of each chapter. To maintain a consistent style throughout the thesis, the used abbreviations, names and referencing style were standardized, and may be different from the submitted papers. However, deviations from the submitted papers were minimized, and no sections were removed, revised, or added to the chapters, except for chapter 8, where the submitted paper is a condensed version of the chapter. As a consequence, an overlap occurs in the introductions and methods of the different chapters.

This thesis can be subdivided into three sections, each of which is related to the scale at which the work was performed.

The first section describes the effects of soil properties and physical damage on plant chemical composition (Chapter 2) under controlled conditions. The resulting differences in spectral signatures were identified using a GER 3700 field spectrometer (Chapter 3). This work was carried out in a greenhouse experiment in the Netherlands, and comprises two chapters.

The second section describes methods to predict the variation in chemical composition of woody plants, using field spectrometry. These three chapters are based on work in the Netherlands, Spain and South Africa, and focus on the detection of nitrogen using normalized ratio indices (Chapter 4), the detection of a range of nutrients and trace elements in 4 different plant species (Chapter 5), and on a method to detect tannin content in mopane leaves (Chapter 6).

The last section identifies the relation between environmental factors and the variation in foliar chemical composition of Mopane (Chapter 7), and shows that it is possible to map patterns of condensed tannin and total polyphenol concentration in Mopane woodland in South Africa using HyMap imagery (Chapter 8).

This thesis ends with a summary and interpretation of the results, and a discussion of the potential and future research aims in the combined fields of chemical ecology and hyperspectral remote sensing of plant chemical composition (Chapter 9).

The literature used in the individual chapters show substantial overlap between chapters, and has therefore been combined in one common literature list, which was placed directly after the last chapter. Finally, maps of the main study area (Kruger National Park) as well as large maps of the concentration of condensed tannin and total polyphenol in mopane can be found in the appendices, at the end of the thesis.



# 2

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## Mopane Responses to Soil Properties and Physical Damage

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*“It is not the strongest of the species, nor the most  
intelligent, that survives. It is the one that is the most  
adaptable to change.*

*C. Darwin (1809-1882)*



### **Abstract**

The effect of available soil nitrogen and phosphorus and the effect of simulated browsing (leaf removal) on foliar condensed tannin concentration were tested on young *Colophospermum mopane* (J. Kirk ex Benth) J. Leonard (Mopane) plants. Although clear differences in growth occurred between different levels of soil nitrogen, no differences in foliar condensed tannin concentration were found. Changes in available soil phosphorus and physical damage did not affect the plants growth or chemical composition. The complete absence of response of condensed tannin concentration to physical damage and soil nutrients may be related to the age of the trees in this study. It is proposed that existing theories on the interaction between soil properties and carbon based defences in trees are expanded, to include the potential responses of young trees to different soil nutrient levels.

### *In press as:*

Jelle G. Ferwerda, Sipke E. Van Wieren, Andrew K. Skidmore & Herbert H.T. Prins. *Inducing condensed tannin production in Colophospermum mopane: Absence of response to soil N and P fertility and physical damage.* Plant and Soil.

## 2.1 Introduction

Even in dry, herbivore rich savannas, vegetation quantity is usually not limiting herbivore densities (The green world theory, (Hairston et al. 1960)). Therefore, plant species may reduce herbivore pressure through chemical and physical defences (Boege 2004, Karban and Myers 1989, Mole 1989 and references therein). One form of chemical defence is the production of condensed tannins. Tannins are estimated to be the fourth most abundant biochemical produced by vascular plants (Hernes and Hedges 2000) and are important in ecosystem processes (Kraus et al. 2003, Kraus et al. 2004a, Northup et al. 1995, Sjoberg et al. 2004).

The high costs associated with production of tannins are thought to impose a selective penalty on plants, but these are thought to be traded off against improved herbivore defence. Two types of tannins exist: hydrolysable and condensed tannins. Low levels of hydrolysable tannins in forage may increase nutrient intake by temporal binding of proteins (Bernays et al. 1989, Class et al. 2003, Mole 1989) whereas condensed tannins can reduce protein availability (Bernays et al. 1989, Mangan 1988, Mole 1989, Robbins et al. 1987a, Van Soest 1987) and may influence forage selection (Belovsky and Schmidt 1994, Class et al. 2003, Cooper et al. 1988, Matson et al. 2004). This makes understanding the factors that influence tannin production by plants important to understand ecosystem functioning.

Tannins are part of a larger chemical group, the polyphenols. The variation in polyphenol concentration of plants has been related to potential leaf age (Coley 1988) and environmental factors, such as soil properties (Osier and Lindroth 2001), temperature stress (Rivero et al. 2001), light intensity (Dudt and Shure 1994), and herbivory (Furstenburg and Hoven 1992, Kraus et al. 2003, 2004b, Osier and Lindroth 2001 and references therein), but these relations are not clearly understood, and different studies show different results (Styles and Skinner 1997). Although it may be generally believed that chemical defences can be induced, and result in lower herbivore pressure, this theory is strongly debated (Koricheva et al. 2004, Nykanen and Koricheva 2004).

A number of hypotheses have been proposed, which explain changes in foliar phenolic compound production in response to soil nutrient levels and herbivory. Coley et al. (1985) and Coley (1988) link differences in the concentration of condensed tannins and fibres between species to intrinsic growth rates, leaf longevity of plants, and herbivore pressure. These authors hypothesize that the concentration of condensed tannins increases with increasing leaf longevity, decreases with increasing growth-rate and increases with increasing herbivore pressure. The carbon-nutrient balance hypothesis (Bryant et al. 1983) predicts a shift from nitrogen-based to carbon-based defences in a situation where soil nutrient levels become more limited, because of the relative low cost of carbon, resulting in an increase of phenolic compound concentration. In response to herbivory, they predict that plants respond to browsing by producing shoots with well-developed chemical defences.

The recent process-based 'protein competition model of phenolic allocation' by Jones and Hartley (1999) is an extension of the carbon-nutrient balance hypothesis. This model explains relative phenolic compound production of plants through an internal chemical balance between nitrogen demand for protein production and phenolic compound production. In this model, *phenylalanine* controls the production of polyphenols and proteins, which in turn is regulated by soil nitrogen and phosphorus availability. According to the model, phenolic compound production increases with decreasing soil nitrogen and phosphorus. Jones and Hartley (1999) furthermore predict an increase in the production of phenolic compounds in response to physical damage to the plant as a result of induced *phenylalanine ammonia lyase* concentration.

The presented study is part of a broader study on the effects of herbivores on savannah woodland structure and chemical composition, with a focus on *Colophospermum mopane* (J. Kirk ex Benth) J. Leonard (mopane) woodlands, which cover an area of 550,000 km<sup>2</sup> in southern Africa (Mapaure 1994). Mopane is used for many domestic purposes such as firewood, construction, and ropes (Madzibane and Potgieter 1999, Mashabane et al. 2001). Although mopane leaves are rich in proteins, deterrent tannin levels in mopane are also high and effectively prevent continuous browsing by many herbivores for most of the year (Styles and Skinner 1997). Only African elephants (*Loxodonta africana*) (Ben-shahar 1996, Ben-Shahar and Macdonald 2002, Smallie and O'Connor 2000), eland (*Tragelaphus oryx*) (Styles and Skinner 1997), and some insects are known to regularly use mopane as a food source. Also, seasonal browsing by the greater kudu (*Tragelaphus strepsiceros*) and other, smaller ruminants (Styles and Skinner 1997) have been reported to occur. Cattle cannot survive the dry-season on mopane leaves alone because of low intake rates (Ludeman 1966 in Timberlake 1995), but when mixed with maize, urea and bone meal, mopane can be used as 'bush meal' for cattle to survive dry periods, making it an important food source to reduce livestock losses in the dry season (Timberlake 1995).

Mopane trees were grown in a greenhouse experiment on soils with different levels of available nitrogen and phosphorus, to test how foliar condensed tannin concentration in mopane is affected by soil nutrients and herbivory. To test the effects of severe browsing by herbivores on foliar condensed tannin levels, leaves were removed from the plants after 4 months of growth and left to regrow foliage for 2 months. Based on work by Bryant et al. (1983), Coley (1988), Coley et al. (1985), and Jones and Hartley (1999), the following hypotheses were formulated: Increased soil nitrogen levels result in a decrease of leaf condensed tannin concentration; An increase in soil phosphorus results in a decrease of condensed tannin concentration of leaves; Physical damage by removal of leaves induces condensed tannin production which results in an increase of condensed tannin concentration in leaves.

## 2.2 Methods

### 2.2.1 Plant material and growing conditions

Seeds of mopane were obtained through the Skukuza nursery, in Kruger National Park, and transported to the Netherlands. The one-seeded pods of mopane have a thick husk, and in order to increase the germination success, this protective outer layer was removed from all seeds. The peeled seeds were soaked for 24 hours in soil saturated with water to initiate the germination process, after which they were placed at 20° C for one week to germinate. By then approximately 90% of the seeds had germinated, and the seedlings were transferred to plastic plant containers (diameter 15 cm, height 20cm) with 2 seedlings per pot, to compensate for the potential loss of seedlings after transplanting. The seedlings were allocated one of four soil treatments described below. All treatments were based on the same initial mixture of 1 unit of black soil added to 7 units of coarse sand, to which powdered nitrogen fertiliser ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) or phosphorus fertiliser ( $\text{P}_2\text{O}_5$ ) was added. The lowest nutrient levels were based on concentrations found in East African savannas (Ludwig et al. 2004). Twelve pots were filled with untreated sand / soil mixture (Treatment N-P-; 3.25 g N m<sup>-3</sup> and 2.1 g P m<sup>-3</sup>). The three other series of twelve pots contained an additional 35.75 g N m<sup>-3</sup> (Treatment N+P-; 39.00 g N m<sup>-3</sup> and 2.1 g P m<sup>-3</sup>), an additional 17.4 g P m<sup>-3</sup> (Treatment N-P+; 3.25 g N m<sup>-3</sup> and 19.5 g P m<sup>-3</sup>), or both (Treatment N+P+; 39.00 g N m<sup>-3</sup> and 19.5 g P m<sup>-3</sup>). The levels of nutrients applied were adapted from those used in a field experiment by Ludwig et al. (2001). As anticipated, a number of plants died after transplanting, leaving only one plant per pot. Therefore it was decided to only include the most successful (tallest) plant for all pots in this study. After two months, the smaller of two plants was removed from each pot, taking care to minimize disturbance to the rest of the substrate and to the other plant. A latin-squares design randomly distributed the treatments. Plants were grown in daylight (from May to November) with the photoperiod extended to 12 hours with greenhouse lights. The automated greenhouse control system monitored air temperature and humidity throughout the experiment. Temperature was kept at 25° C during the day, and 20° C at night, air humidity was kept over 70%, and plants were watered once a day.

### 2.2.2 Sampling

During the growth, basic physical parameters, such as stem length and number of leaves, were recorded to determine the effect of soil nutrients on plant growth. After four months, the plants were large enough to allow sampling of leaves for chemical analysis.

Furstenburg and Hoven (1992) found an increase in foliar condensed tannin concentration in *Acacia nigrescense*, within 30 to 100 minutes after browsing by giraffes commenced. This elevated concentration of foliar condensed tannin remained for 40 to 66 hours. Therefore, in the current study sampling was done in phases. Except for a few small plants of which all leaves were collected, and a control group, of which no leaves were collected, all mature leaves on one side of the main-stem were collected after 4 months of growth (t=0). The leaves on the other side of the main-stem were removed after 1 day for half of the remaining plants (t=1), and for the other half after 4 days (t=4). Two months after sampling, most plants had re-sprouted, and all fully developed leaves were collected of all previously sampled plants (t=60), as well as of the control group, of which no leaves had been removed before (Control). After harvest all leaves were dried at 70° C for 48 hours, and stored for analysis.

### 2.2.3 Chemical analysis

Condensed tannin concentration was determined for all sample sets. Condensed tannin was extracted from ground leaves following Hagerman (1998, 2002) and the concentration was determined with the improved acid butanol assay (Porter et al. 1986). Purified Quebracho tannin was used to standardize the measurements (Waterman and Mole 1994). Because the reactivity of tannins to the acid butanol assay is species-specific, the obtained concentration is a relative measure of concentration, and cannot be used to calculate absolute concentrations (Waterman and Mole 1994).

Nitrogen and phosphorus concentration was determined for the samples of t=0. A mixture of sulphuric acid, selenium and salicylic acid was used for destruction of the samples (Novozamsky et al. 1983) after which nitrogen and phosphorus concentration were measured with a Skalar San-Plus auto analyser. Some of the plants were small, and not all analyses could be performed for all samples, therefore differences in the number of samples available for statistical analysis occurred between treatments.

### 2.2.4 Statistical analysis

A Shapiro-Wilk's W test for normality was performed on arcsine transformed data of the condensed tannin, nitrogen and phosphorus concentration of leaves, N:P-ratio, and condensed tannin to nitrogen ratio, as well as on the number of leaves and stem length, for the individual treatments. These factors did not deviate from normality. Therefore we used a factorial ANOVA to test for differences in leaf condensed tannin, nitrogen, phosphorus, Nitrogen to phosphorus ratios, and condensed tannin to nitrogen ratios between treatments at t=0, with soil nitrogen (low and high) and soil phosphorus (low and high) as factors. Tukeys' unequal n HSD test was used to discriminate groups for which differences occurred.

Differences in foliar condensed tannin concentration between  $t=0$ ,  $t=1$ ,  $t=4$  and  $t=60$  were determined using a one-way ANOVA. A one-sided t-test was used to test whether the foliar condensed tannin concentration of the control group is lower than the foliar condensed tannin concentration of samples from  $t=60$ .

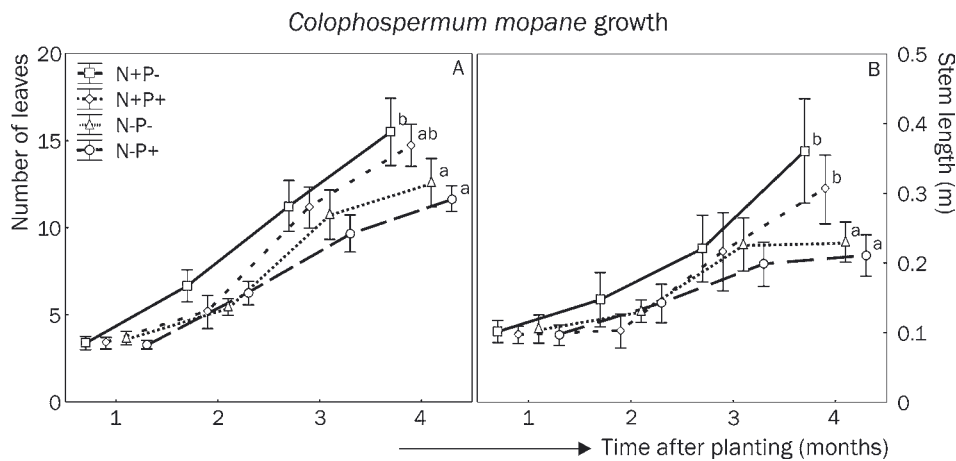
## 2.3 Results

### 2.3.1 Effect of soil treatments

The growth (measured through the number of leaves and stem length), and foliar nitrogen concentration were higher for plants growing on soils with an increased soil nitrogen than those growing on soils with low nitrogen (Table 2.1, Figure 2.1). This effect was not influenced by soil phosphorus concentration (Table 2.1, Figure 2.1). The foliar phosphorus concentration was not influenced by soil nutrients (Table 2.1). The nitrogen to phosphorus ratio in plants growing on high nitrogen soils was more than double that of the plants growing on soils with lower nitrogen concentration (Table 2.1). Foliar concentration of condensed tannin was not affected by the amount of nitrogen or the amount of phosphorus in the soil (Table 2.1).

**Table 2.1.** Average foliar condensed tannin (CT) concentration (quebracho tannin equivalents in  $\text{mg g}^{-1}$ ), nitrogen (N,  $\text{mg g}^{-1}$ ), phosphorus (P,  $\text{mg g}^{-1}$ ), N:P-ratio, and CT:N-ratio in mopane leaves after growing seedlings for 4 months ( $t = 0$ ) on four different soil nutrient levels. (N-: Low soil N, N+: High soil N, P-: Low soil P, P+: High soil P). Groups (a or b) were discriminated with a Tukeys' unequal N HSD test, after significant differences ( $p \leq 0.05$ ) were detected using a factorial ANOVA on arcsine transformed data, with factors: soil N (low and high) and soil P (low and high)

Treatment	N- P-	N- P+	N+ P-	N+ P+
n	7	9	8	11
CT	381 <sup>a</sup>	430 <sup>a</sup>	400 <sup>a</sup>	335 <sup>a</sup>
95% Conf. Int.	260-526	314-564	282-538	243-443
N	13.66 <sup>a</sup>	13.97 <sup>a</sup>	24.83 <sup>b</sup>	23.72 <sup>b</sup>
95% Conf. Int.	10.24-17.55	10.91-17.42	20.46-29.62	20.05-27.69
P	2.17 <sup>a</sup>	2.27 <sup>a</sup>	1.82 <sup>a</sup>	1.61 <sup>a</sup>
95% Conf. Int.	1.64-2.78	1.79-2.81	1.37-2.34	1.24-2.02
N:P-ratio	6.37 <sup>a</sup>	6.29 <sup>a</sup>	13.68 <sup>b</sup>	15.02 <sup>b</sup>
95% Conf. Int.	5.08-7.81	5.14-7.54	11.93-15.52	13.47-16.65
CT:N-ratio	0.385 <sup>a</sup>	0.332 <sup>a</sup>	0.163 <sup>a</sup>	0.141 <sup>a</sup>
95% Conf. Int.	0.214-0.605	0.191-0.512	0.066-0.304	0.062-0.251



**Figure 2.1.** Growth of mopane seedlings on different soil nutrient levels during the first four months after planting, measured by (A) the average number of leaves and (B) the average main stem length. Initial sampling took place four months after planting ( $t=0$ ). Averages were calculated for all plants grouped by treatment. The graphs of individual treatments are offset along the time axis for clarity. Groups (a or b) were discriminated with a Tukeys' unequal  $n$  HSD test, after significant differences ( $p \leq 0.05$ ) were detected using a factorial ANOVA, with factors: soil N (low and high) and soil P (low and high)

### 2.3.2 Effect of leaf removal

There was no difference in foliar condensed tannin concentration between plants damaged by removal of leaves, and the control group (Table 2.2), although the foliar condensed tannin concentration of leaves did increase with time (Table 2.2).



**Table 2.2.** Average foliar condensed tannin (CT) concentration (Quebracho tannin equivalents in  $\text{mg g}^{-1}$  dry weight), 95% confidence interval (95% C.L.), and number of samples of mopane leaves, harvested at different times as described in the methods section. The first samples ( $t=0$ ) were collected after 4 months of growth. Groups (a or b) were determined using a one-way ANOVA in combination with an unequal  $n$  HSD posthoc test. The difference between samples collected at  $t=60$  and the control group was tested using a Tukeys  $t$ -test, using a 95% significance threshold.

Sample	Date	CT	95% C.L.	$n$
$t = 0$	September 11 <sup>th</sup>	383 <sup>a</sup>	334-435	35
$t = 1$	September 12 <sup>th</sup>	385 <sup>a</sup>	312-467	15
$t = 4$	September 15 <sup>th</sup>	429 <sup>a</sup>	353-511	16
$t = 60$	November 10 <sup>th</sup>	589 <sup>b</sup>	528-653	35
Control	November 10 <sup>th</sup>	552 <sup>b</sup>	470-640	8

## 2.4 Discussion

### 2.4.1 Effect of soil nutrients

We did not find any differences in foliar condensed tannin concentration for mopane seedlings grown on soil with different nitrogen and phosphorus concentration (Table 2.2). These results do not agree with results found in other studies on other plant species. These predicted a decrease of phenolic compounds with an increase of soil nutrients (Coley et al. 1985, Jones and Hartley 1999). This relationship was recently confirmed by Kraus et al. (2004b), who found significant increases of foliar total phenol and condensed tannin concentrations in response to reduced soil fertility for one year old trees grown under controlled conditions. Kraus et al. (2004b) also found significant changes in the ratio between condensed tannin and nitrogen, in response to fertilization. In the present study this difference did not occur (Table 2.1). This could indicate that neither phosphorus nor nitrogen were limiting for growth in this study. However, the strong differences in growth for plants grown on different nitrogen levels (Figure 2.1) indicate that nitrogen-limitation occurs for the low nitrogen treatment. There is no significant effect of soil phosphorus concentration on the growth of mopane seedlings (Figure 2.1). This indicates that the amount of phosphorus available to the plant may not be limiting growth, even when no phosphorus is added to the original soil mixture.

Koerselman and Meuleman (1996) and Verhoeven et al. (1996) suggested using the ratio between leaf nitrogen and phosphorus as an indicator of the type of nutrient limitation, where a ratio of more than 16 indicates phosphorus limitation, and a ratio of less than 14 indicates nitrogen limitation, based on wetland studies in temperate climates. Ludwig et al. (2001) found that a N:P-ratio of 12 or higher indicates phosphorus limitation in an open savanna. This suggests that variation exists in N:P-ratio thresholds for different ecotypes. In this study we found an increase of N:P-ratios from 6.45 for plants grown on nitrogen poor soils, to 15 for plants grown on nitrogen rich soils, which was not influenced by soil phosphorus concentration (Table 2.1). Again, this suggests that the phosphorus levels in this study were probably not limiting for mopane.

#### 2.4.2 *Effect of leaf removal*

Physical damage to the plants by removal of leaves did not influence condensed tannin concentration in the remaining leaves (Table 2.2). The concentration of foliar condensed tannin in leaves which had re-grown after complete defoliation, collected at  $t=60$ , is significantly higher than the concentration in samples collected at  $t=0$ ,  $t=1$ , and  $t=4$ , with  $t$  in days after the first sampling date (Table 2.2). This difference cannot be attributed to the effect of removal of leaves: When comparing the foliar condensed tannin concentration in leaves that re-grew after plants were completely defoliated ( $t=60$ ) with those from the control group (plants which had not been defoliated) there is no difference in concentration ( $p=0.29$ ). The increase of condensed tannin over time is probably the result of plant ageing. Young plants often have a different physiology than mature plants. The young plants invest a lot of energy in the development of biomass. As described in the protein competition model (Jones and Hartley 1999), growth results in allocation of carbon for protein production, instead of polyphenol production. This would result in lower phenol concentration in young plants compared to older plants, and may dominate the effect of soil properties on the production of phenolic compounds.

Bryant et al. (1983) suggested that high tannin levels in young boreal forest trees have evolved in response to the high risk of browsing by hares. Based on this, combined with the theory that condensed tannin concentration is negatively correlated to the potential growth rate of a species (Coley 1988, Coley et al. 1985), we propose that juvenile plants may exhibit higher levels of tannins than mature plants for slow growing species, or species which invest a lot of energy in development of root systems, before developing above-ground biomass. Fast growing species can reduce biomass loss from browsing by increasing vertical growth. These plants therefore allocate carbon to growth instead of to the production of C-based defences. Therefore these species will exhibit lower levels of condensed tannin in the juvenile form than older species. When we apply this hypothesis to mopane, we notice that under ideal growing conditions, mopane plants can reach a main stem length of up to 0.5 m in four months, and even nutrient limited plants reach an average height of 0.2 m in four months (Table 2.1). Under field conditions this tree can reach up to 4 m

height within 8 years (Sharma et al. 1989). It can therefore be considered to be a fast growing species. And although an increase of foliar condensed tannin concentration in time occurs, the effect of nutrient limitation on the tannin concentration is absent, which is in line with the hypothesis presented here.



# 3

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## Lab-Spectroscopy of Mopane Biochemistry

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*“Where there is much light, the shadow is deep”*

*J.W. von Goethe (1749-1832)*



**Abstract**

Reflectance and derivative spectra of greenhouse-grown *Colophospermum mopane* (mopane) were analyzed to investigate whether the interactive effect of available soil phosphorus and nitrogen on plant-chemical composition can be detected using non-destructive techniques. Reflectance spectra were only affected by soil nitrogen content. However, derivative spectra showed significant differences as a result of differences in soil nitrogen and soil phosphorus. Several spectral bands which showed different spectral signatures between soil treatments also showed significant correlation to foliar condensed tannin, phosphorus and nitrogen content, which forms the link between soil properties and the reflectance properties of leaves. In a wider application, this study shows that it is possible to detect different levels of soil phosphorus and nitrogen through the reflectance properties of vegetation, and the potential to detect the effect of soil properties on the plant's basic physiology through the use of hyperspectral remote sensing.

*Submitted as:*

Jelle G. Ferwerda, Andrew K. Skidmore, Sipke E. van Wieren & Herbert H. T. Prins. *What's in a leaf? Soil N and P affect reflectance signatures of individual Colophospermum mopane leaves.* International Journal of Remote Sensing.

### 3.1 Introduction

The spatial distribution of herbivores has been linked to the quantity and quality of resources, and a number of studies have suggested that resource quality may be a key factor that determines the spatial distribution of species, especially on a seasonal basis: Herbivores on the Serengeti Plains have been found to concentrate in areas where mineral concentration in vegetation is high (McNaughton 1988, 1990, Seagle and McNaughton 1992) and the annual migration of blue wildebeest is related to increased phosphorus requirements during the lactation period (Voeten 1999). For browsers, it has been suggested that certain chemical components of tree leaves, such as condensed tannin and other polyphenols, deter herbivores (Bell 2001, Belovsky and Schmidtz 1994, Cooper et al. 1988, Mole 1989), and regions or even individual plants with relatively higher concentrations of condensed tannins would therefore be avoided by herbivores.

To understand the spatial distribution of herbivores it is thus important to know the spatial variation in chemical composition of vegetation. Although the chemical composition of plants can be measured, the analyses involved are usually labour-intensive and expensive. This makes detailed studies at ecosystem level impractical. To assess vegetation at the scale necessary to study ecosystem processes applicable to large herbivores, an improved method for the detection of vegetation quality is required.

One of these methods is hyperspectral remote sensing. This technique measures light reflectance in narrow wavebands of 10 nm or less, which makes it possible to detect minimal variations in the reflectance spectra, caused by differences in chemical composition and physiology of the samples. Laboratory spectroscopy has been developed, tested, and is now used on a commercial basis to analyse the chemical composition of dried, and ground samples (Rabkin 1987). The use of spectroscopy to predict chemical composition of living plants is still in an experimental phase, although several studies have recently shown the potential to detect chemical composition and structural variables in living vegetation using this technique, under a variety of circumstances (Coops et al. 2003, Curran et al. 1992, Goel et al. 2003, Mutanga et al. 2003, Zarco-Tejada et al. 2004).

These studies have mainly looked at the link between the foliar nutrient or chlorophyll concentration of grasses, such as maize (Haboudane et al. 2002, Strachan et al. 2002), savanna grasses (Mutanga et al. 2003) or wheat (Hansen and Schoerring 2003, Lilienthal et al. 2000), and light absorption. Relatively few studies have worked on woody plants (But see Coops et al. (2003), Hernes and Hedges (2004), Yoder and Pettigrew-Crosby (1995), and Zarco-Tejada et al. (2004)) and the studies that have worked on woody species have, to our knowledge, not looked at the direct effects of soil phosphorus and nitrogen on the reflectance spectra. It is however well-known that these two elements have a strong interactive effect on the physiology and growth of plants (Kim et al. 2003, Marschner 1988). Available nitrogen affects the

cell structure and physiology of leaves (Saneoka et al. 2004, Syros et al. 2004) which in turn affects reflectance signatures. Foliar nitrogen concentration is commonly considered as a proxy for leaf protein concentration, and has a strong correlation with chlorophyll concentration and functioning (Zhao et al.). The ratio between foliar nitrogen concentration and foliar phosphorus concentration has been suggested as an indicator of the type of nutrient limitation in plants (Koerselman and Meuleman 1996, Verhoeven et al. 1996).

Biomass and canopy structure change light reflectance through BRDF effects and increased scattering. Since nutrient availability affects plant production (McInnis et al. 2004, Zhao et al.), the total amount of biomass produced in a fixed period of time (e.g. the duration of a greenhouse experiment) depends on nutrient availability. Therefore, to obtain reliable reflectance measurements, the amount of biomass needs to be standardized, which was achieved by measuring the reflectance properties of a single leaf section.

Mopane (*Colophospermum mopane*) is a tree species that covers large parts (> 550.000 km<sup>2</sup>, (Mapaure 1994)) of southern Africa and is used for many domestic purposes, such as firewood, construction and ropes (Madzibane and Potgieter 1999, Mashabane et al. 2001). Leaf biochemical deterrent levels in mopane are also high, and this effectively prevents continuous browsing by many herbivores for most of the year (Styles and Skinner 1997). Only African elephant (*Loxodonta africana*) (Ben-shahar 1996, Ben-Shahar and Macdonald 2002, Smallie and O'Connor 2000), eland (*Tragelaphus oryx*) (Styles and Skinner 1997), and some insects are known to regularly use mopane as a food source.

In context of the importance of this tree species in the region for both wildlife-distribution and local uses, as well as its extensive areal extent, it was decided to investigate the relation between the reflectance properties of individual mopane leaves, and available nitrogen and phosphorus in the soil, under controlled light conditions. To control growth conditions the plants were grown in a greenhouse.

## 3.2 Methods

### 3.2.1 Greenhouse setup

Seeds of mopane were obtained from the Skukuza nursery, in Kruger National Park, and transported to the Netherlands. The one-seeded pods of mopane have a thick husk, and in order to increase the germination success, this protective outer layer was removed from all seeds. The peeled seeds were soaked for 24 hours in water-saturated soil to initiate the germination process, after which they were placed at 20° C for one week to germinate. The seedlings were then transferred to



plastic plant containers (diameter 15 cm, height 20 cm), with 2 seedlings per pot to compensate for the potential loss of seedlings after transplanting. The seedlings were allocated one of sixteen soil treatments, with 12 repetitions, all of which were based on the same initial mixture of 1 unit of black soil in 7 units of coarse sand, to which powdered nitrogen fertilizer ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ) or phosphorus fertilizer ( $\text{P}_2\text{O}_5$ ) was added. This resulted in 4 different levels of nitrogen (Table 3.1) in combination with 4 levels of soil phosphorus (Table 3.1). The lowest nutrient levels were based on concentrations found in East African savannas (Ludwig et al. 2004). The maximum levels of nutrients applied were adapted from those used in a field experiment by Ludwig et al. (2001), where 3 applications of N ( $67 \text{ kg ha}^{-1}$ ) and P ( $27 \text{ kg ha}^{-1}$ ) were applied over one full growing season. A latin squares design randomly distributed the treatments. Plants were grown under a 12 hour light / 12 hour dark cycle, providing additional light using commercial greenhouse lights. Temperature was kept at  $25^\circ \text{C}$  during the day and  $20^\circ \text{C}$  at night, air humidity was kept over 70%, and plants were watered once a day.

**Table 3.1.** Levels of available soil nitrogen and phosphorus used for the 16 treatments ( $\text{g m}^{-3}$ )

Level	$L_1$	$L_2$	$L_3$	$L_4$
Nitrogen	3.25	6.5	13	39
Phosphorus	2.1	3.25	6.5	19.5

As anticipated, a number of plants died after transplanting, leaving only one plant per pot. Therefore it was decided to only include the most successful (tallest) plant for all pots in this study. Two months after seeding, the smaller of two plants was slowly pulled from each pot, taking care to minimize disturbance to the substrate and to the other plant. After four months, the remaining plants were large enough to allow spectral measurements to be taken of the leaves. At this moment, stem length and number of leaves were recorded to determine the effect of soil nutrients on plant growth. To investigate the effect of soil phosphorus and nitrogen on leaf nutrient and deterrent status, leaves were sampled of plants grown on the four most diverse soil nutrient combinations ( $\text{N}_{L_1}\text{P}_{L_1}$ ,  $\text{N}_{L_1}\text{P}_{L_4}$ ,  $\text{N}_{L_4}\text{P}_{L_1}$ ,  $\text{N}_{L_4}\text{P}_{L_4}$ , Table 3.1), and these samples were chemically analyzed for nutrients (nitrogen and phosphorus) and deterrents (condensed tannins) and their interaction in time (Chapter 2, Ferwerda et al., *In Press*).

### 3.2.2 Spectrometry

Reflectance measurements were taken of individual mopane leaves. To standardize between readings, a fixed setup between light source, sensor and target was constructed. To reduce light scattering from the measurement setup, surfaces with high reflectance were painted black with so-called blackboard paint, which has a low light reflectance (< 5%) over the 350 nm to 2500 nm range used in this study. Leaves were attached to a small platform (5 cm \* 15 cm) and reflectance

measurements were taken using a GER 3700 spectrometer (Geophysical and Environmental Research Corporation, Buffalo, New York), equipped with a 1.5 metre long fibre-optic cable. The fibre optic cable was attached to a thin aluminium rod, pointing at the centre of the leaf at 15 degrees off-nadir. A light source (Lowel Pro-Light with 14.5V/50W/3200°K JCV halogen lamp), pointing at the centre of the leaf, was placed at 30 degrees off-nadir. To avoid problems with reflectance hotspots, the sensor and incoming light were placed at a projected angle of 90 degrees.

Of each plant, the youngest fully developed leaf was placed on the platform, and kept in place by two thin rubber bands across the leaf base and tip, taking care not to damage the leaf or plant in the process. The leaves of mopane consist of two symmetrical leaflets, which were placed, with a slight overlap, next to each other on the platform. Ten reflectance measurements were taken of the leaf and measurements were calibrated against a Spectralon Reflectance Target.

### 3.2.3 Chemical analysis

For a subset of plants the foliar nitrogen, phosphorus and condensed tannin concentration was determined, as part of another study on the effects of soil properties on the chemical composition of mopane (Chapter 2, Ferwerda et al. *In Press*): After taking reflectance measurements, the leaves on one side of the plants were collected, dried at 70° C for 48 hours, and stored for analysis.

Condensed tannin was extracted following Hagerman (1988, 2002) and the concentration was determined with the improved acid butanol assay (Porter et al. 1986). Purified quebracho tannin was used to standardize the measurements (Waterman and Mole 1994). A mixture of sulphuric acid, selenium and salicylic acid was used for destruction of the samples (Novozamsky et al. 1983) after which nitrogen and phosphorus concentration were measured with a Skalar San-Plus auto analyser.

### 3.2.4 Statistical Analysis

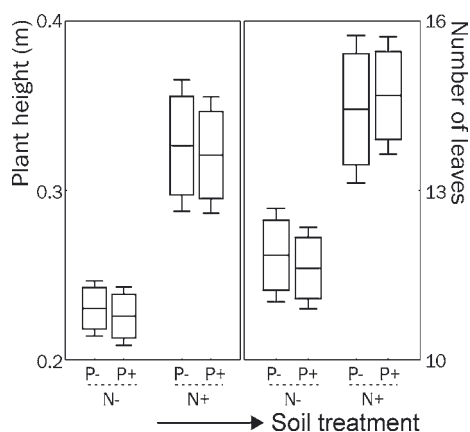
#### *Foliar chemistry & plant size.*

A Shapiro-Wilk's W test for normality was performed on arcsine transformed data of the condensed tannin, nitrogen and phosphorus concentration of leaves, and N:P-ratio, as well as on the number of leaves and stem length, for the individual treatments. These factors did not deviate from normality. Therefore we used a factorial ANOVA to test for differences in leaf condensed tannin, nitrogen, phosphorus, N:P-ratio and the number of leaves and main stem length between treatments with soil nitrogen ( $L_1$  and  $L_4$ , Table 3.1) and soil phosphorus ( $L_1$  and  $L_4$ , Table 3.1) as factors. Tukeys' unequal n HSD test was used to discriminate groups for which differences occurred.

### Spectrum Analysis.

The ten spectral readings per plant were averaged to reduce noise levels, resulting in one average spectrum per plant. From these readings derivative spectra were calculated, using an adjusted version of the seven band moving spline smoothing technique (Savitzky and Golay 1964, Tsai and Philpot 1998). The parameters of the moving polynomial were used to directly calculate the derivative at the centre waveband of the moving spline window, instead of smoothing the spectra first and then calculating the derivative spectra from the smoothed spectra.

To test whether the mean reflectance for each waveband is different between treatments, a two-way analysis of variance with interaction was carried out for each wavelength, for both reflectance and derivative spectra. To increase the statistical power of the test, the individual treatments were combined into four treatment groups. The four nitrogen treatments were reduced to two groups, one consisting of the two highest nitrogen treatments (N+), and with the two lowest nitrogen treatments (N-). The same was done for phosphorus (P+ and P-). Regions where reflectance and derivative spectra were different between treatments were compared to known absorption features found in literature.



**Figure 3.1.** Size of mopane seedlings on different soil nutrient levels measured after 4 months of growth. Averages were calculated for all plants grouped by treatment with 95% confidence interval (boxes) and 99% confidence intervals (bars). N+, N-: High respectively low soil nitrogen. P+, P-: High respectively low soil phosphorus

To further link these regions to the chemical composition of the plants in this study, the correlation between each waveband of the reflectance and derivative data and the foliar nitrogen, phosphorus and condensed tannin concentration was calculated, for those samples of which this information was available ( $n=35$ ). To determine how much variation in the derivative spectrum can be explained by the three chemical components, linear regression models were constructed. For each waveband the coefficient of determination was calculated between the concentration of nitrogen, phosphorus and condensed tannin and the derivative spectrum. For each model the chemical components which made a significant ( $p \leq 0.05$ ) contribution to the regression model were registered.

### 3.3 Results

#### 3.3.1 Foliar chemistry & plant size

The growth (measured through the number of leaves and stem length), and foliar nitrogen concentration were higher for plants growing on soils with increased soil nitrogen than those growing on soils with low nitrogen (Figure 3.1, Table 3.2). This effect was not influenced by soil phosphorus concentration (Table 3.2). The foliar phosphorus concentration was not influenced by soil nutrients (Table 3.2). The N:P-ratio in plants growing on high nitrogen soils was more than double that of the plants growing on soils with lower nitrogen concentration (Table 3.2). Foliar concentration of condensed tannin was not affected by the amount of nitrogen or the amount of phosphorus in the soil (Table 3.2, Ferwerda et al. *In Press*).

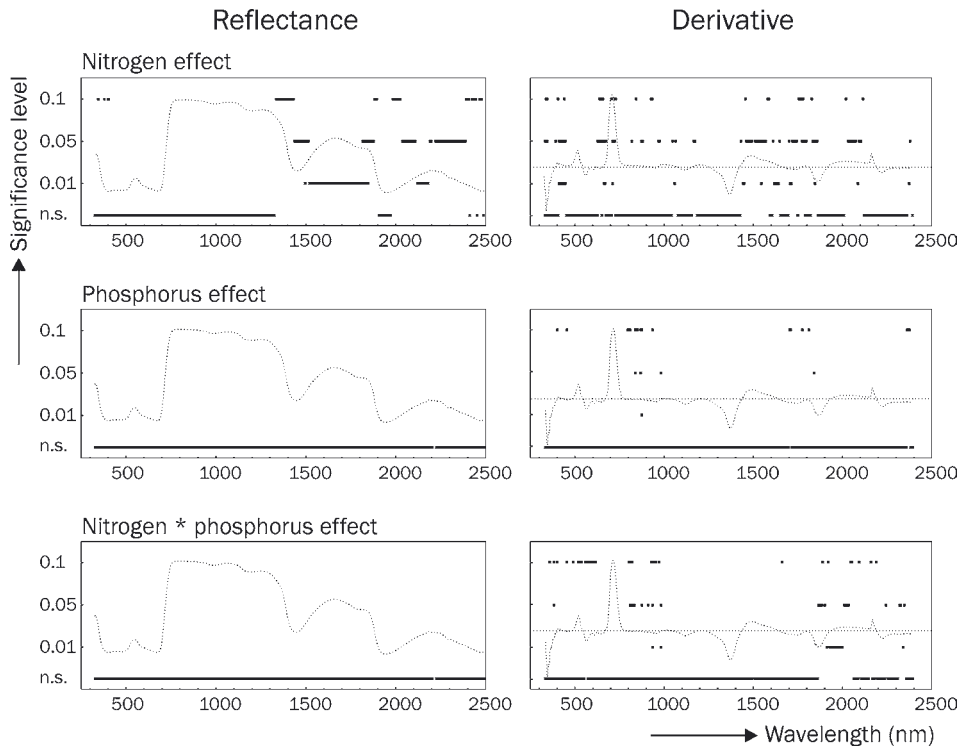
**Table 3.2.** Average foliar condensed tannin (CT) concentration (quebracho tannin equivalents in  $\text{mg g}^{-1}$ ), nitrogen (N,  $\text{mg g}^{-1}$ ), phosphorus (P,  $\text{mg g}^{-1}$ ), and N:P-ratio in mopane leaves after growing seedlings for 4 months on four different soil nutrient levels. Groups (a or b) were discriminated with a Tukeys' unequal N HSD test, after significant differences ( $p \leq 0.05$ ) were detected using a factorial ANOVA on arcsine transformed data, with factors: soil N ( $L_1$  and  $L_4$ ) and soil P ( $L_1$  and  $L_4$ ).

Treatment	$N_{L1}$ $P_{L1}$	$N_{L1}$ $P_{L4}$	$N_{L4}$ $P_{L1}$	$N_{L4}$ $P_{L4}$
n	7	9	8	11
CT	381 <sup>a</sup>	430 <sup>a</sup>	400 <sup>a</sup>	335 <sup>a</sup>
95% Conf. Int.	260-526	314-564	282-538	243-443
N	13.66 <sup>a</sup>	13.97 <sup>a</sup>	24.83 <sup>b</sup>	23.72 <sup>b</sup>
95% Conf. Int.	10.24-17.55	10.91-17.42	20.46-29.62	20.05-27.69
P	2.17 <sup>a</sup>	2.27 <sup>a</sup>	1.82 <sup>a</sup>	1.61 <sup>a</sup>
95% Conf. Int.	1.64-2.78	1.79-2.81	1.37-2.34	1.24-2.02
N:P-ratio	6.37 <sup>a</sup>	6.29 <sup>a</sup>	13.68 <sup>b</sup>	15.02 <sup>b</sup>
95% Conf. Int.	5.08-7.81	5.14-7.54	11.93-15.52	13.47-16.65

### 3.3.2 Reflectance spectra.

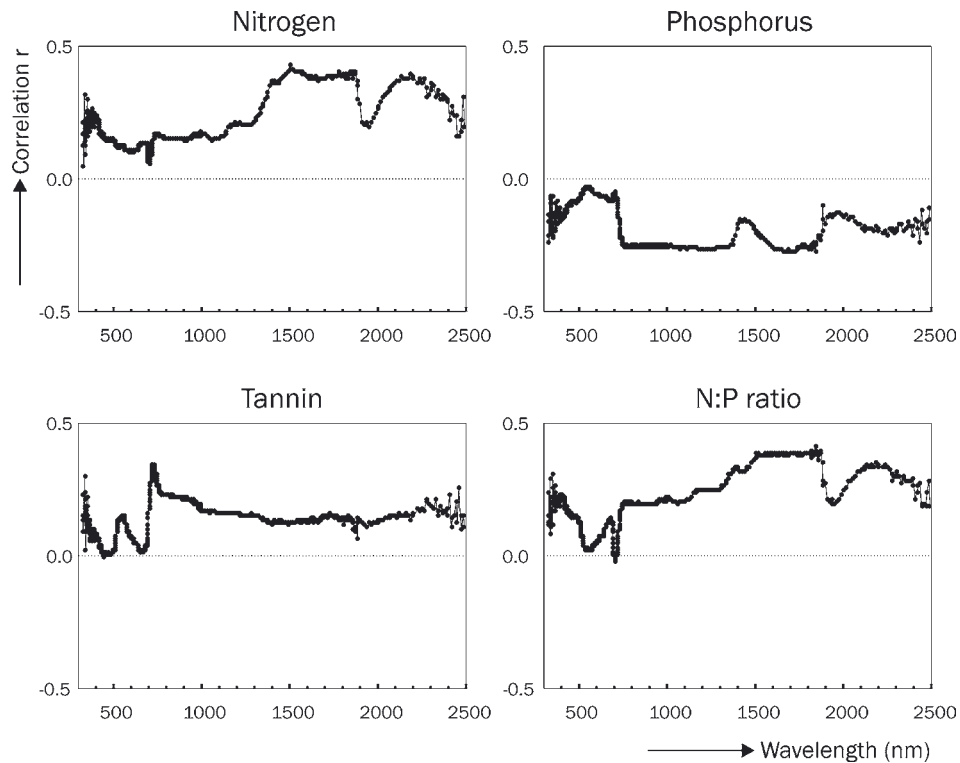
#### Differences between soil treatment

The reflectance of plants grown on nitrogen-rich and on nitrogen-poor soils differed in a number of spectral regions (see figure 3.2). These occur around 400 nm, between 396 nm and 407 nm ( $p \leq 0.1$ ), and from 1350 nm to 2480 nm ( $p \leq 0.1$ ). Confidence levels were below  $p \leq 0.05$  from 1441 nm to 1879 nm, and from 2041 nm to 2349 nm. Confidence levels below  $p \leq 0.01$  occurred for bands between 1523 nm and 1819 nm, and between 2114 nm and 2195 nm.



**Figure 3.2.** Overview of ANOVA results, testing for differences in reflectance spectra and derivative spectra between plants grown on high and on low concentrations of soil nitrogen, soil phosphorus, and the interaction between these two. The significance levels were grouped: 0.01:  $p \leq 0.01$ , 0.05:  $p \leq 0.05$  and 0.1:  $p \leq 0.1$ .

There was no effect of soil phosphorus levels on the reflectance at any waveband. The interaction effect between phosphorus and nitrogen on reflectance was not significant at any waveband either.



**Figure 3.3.** Correlation between the ratio of foliar nitrogen and phosphorus, the foliar concentration of nitrogen, phosphorus, condensed tannin and the reflectance spectra of mopane plants.

*Relation with foliar chemical composition.*

A number of bands showed an increased correlation between the three chemical components and reflectance data (see figure 3.3). For foliar nitrogen, local correlation maxima were observed at 1501 nm ( $r=0.43$ ), 1874 nm ( $r=0.40$ ) and 2185 nm ( $r=0.40$ ). For foliar phosphorus, correlation ranged between  $r=0$  and  $r=-0.25$  for all bands, without any specific maxima. Correlation between foliar condensed tannin concentration and reflectance reached a maximum of  $r=0.34$  at 726 nm, without any other clear maxima. The N:P-ratio showed regions of higher correlation similar to those observed for foliar nitrogen alone.

### 3.3.3 Derivative spectra.

#### *Differences between soil treatments.*

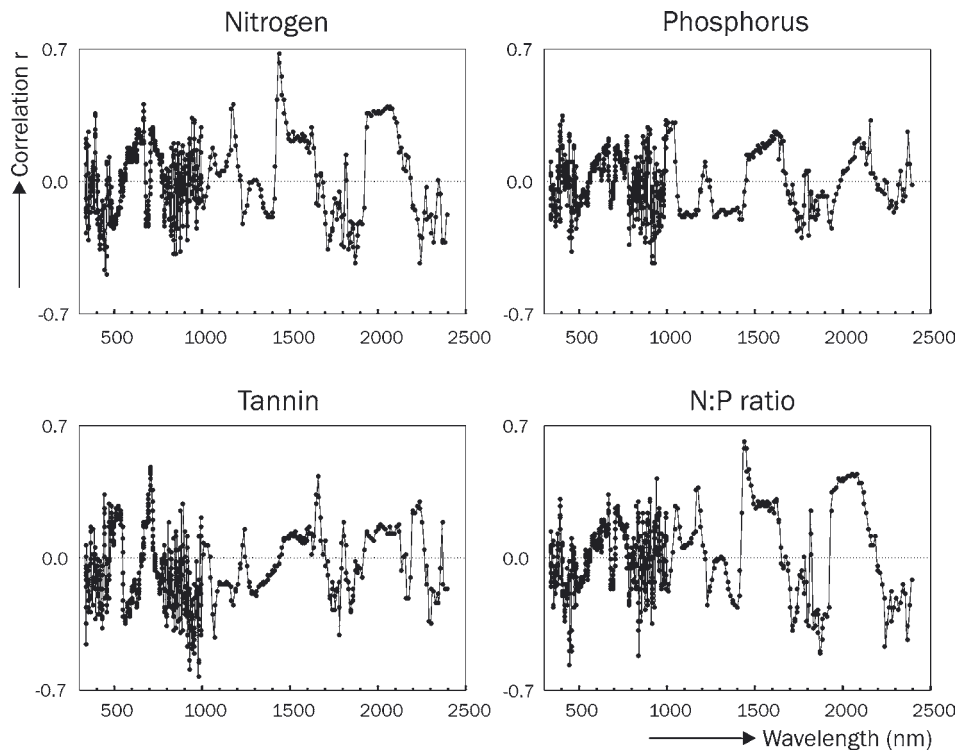
Differences occurred in the derivative spectra between plants grown on nitrogen rich and nitrogen poor soils (see figure 3.2). In the visible light, differences in derivative spectra occurred from 400 nm to 450 nm, and from 630 nm to 670 nm ( $p \leq 0.1$ ), with highest significance of the differences for bands between 665 nm and 672 nm ( $p \leq 0.01$ ). In the infrared, differences in derivative spectra occurred from 708 nm to 729 nm and at a number of reflectance maxima and minima between 800 nm and 1200 nm ( $p \leq 0.1$ ). Also between 1430 nm and 2114 nm several regions occur where derivative spectra are different ( $p \leq 0.1$ ).

Differences in derivative spectra between the two soil phosphorus treatments occur at a few individual wavebands (see figure 3.2). These bands are located at 456 nm, and at several bands between 700 nm and 800 nm, at 932 nm, 981 nm ( $p \leq 0.1$ ) and for a small number of bands between 1700 nm and 1800 nm ( $p \leq 0.1$ ).

The interactive effect of soil phosphorus and soil nitrogen on the derivative spectra resulted in a number of broad spectral regions where significant differences occur (see figure 3.2). These were located around 381 nm, from 520 nm to 536 nm, from 550 nm to 600 nm, from 800 nm to 830 nm, around 932 nm, from 1865 nm to 2052 nm, and from 2319 nm to 2347 nm ( $p \leq 0.1$ ). Also, a number of scattered bands showed significant differences.

#### *Relation with foliar chemical composition.*

Several wavebands showed strong correlation between the concentration of individual chemical components, and the derivative spectrum (see figure 3.4). For nitrogen these peaks of high correlation included: 454 nm ( $r=-0.49$ ), 669 nm ( $r=0.41$ ), 842 nm ( $r=-0.38$ ), 1434 nm ( $r=0.67$ ), 1177 nm ( $r=0.41$ ), 1870 nm ( $r=-0.47$ ), a number of bands from 1940 nm to 2080 nm, with a maximum at 2063 nm ( $R=0.40$ ), and at 2244 nm ( $r=-0.44$ ). The correlation between foliar phosphorus concentration and derivative reflectance peaks at 401 nm ( $r=0.35$ ), 454 nm ( $r=-0.38$ ), 903 nm ( $R=-0.43$ ), 932 nm ( $r=-0.43$ ), a range of points between 997 nm and 1040 nm ( $r=.31$ ), and 2156 nm ( $r=0.32$ ). For condensed tannin, maximum correlations with derivative spectra occur at 709 nm ( $r=0.48$ ), 978 nm ( $r=-0.62$ ), 1660 nm ( $r=0.44$ ), 1781 nm ( $r=0.40$ ) and 2234 nm ( $r=0.30$ ). The N:P Ratio has high correlations at a number of bands: 447 nm ( $r=-0.56$ ), 842 nm ( $r=-0.51$ ), 938 nm ( $r=0.42$ ), 1441 nm ( $r=0.62$ ), 1870 nm ( $r=-0.51$ ), a range of bands between 1940 nm and 2080 nm, and at 2244 nm ( $r=-0.47$ ). Some of these bands (447 nm, 842 nm, 1441 nm, 1870 nm, 1940 nm to 2080 nm and 2244 nm) are similar to bands with high correlation with foliar nitrogen alone (454 nm, 842 nm, 1434 nm, 1870 nm, 1940 nm to 2080 nm and 2244 nm). One band (938 nm) is similar to a peak correlation found for phosphorus (932 nm).



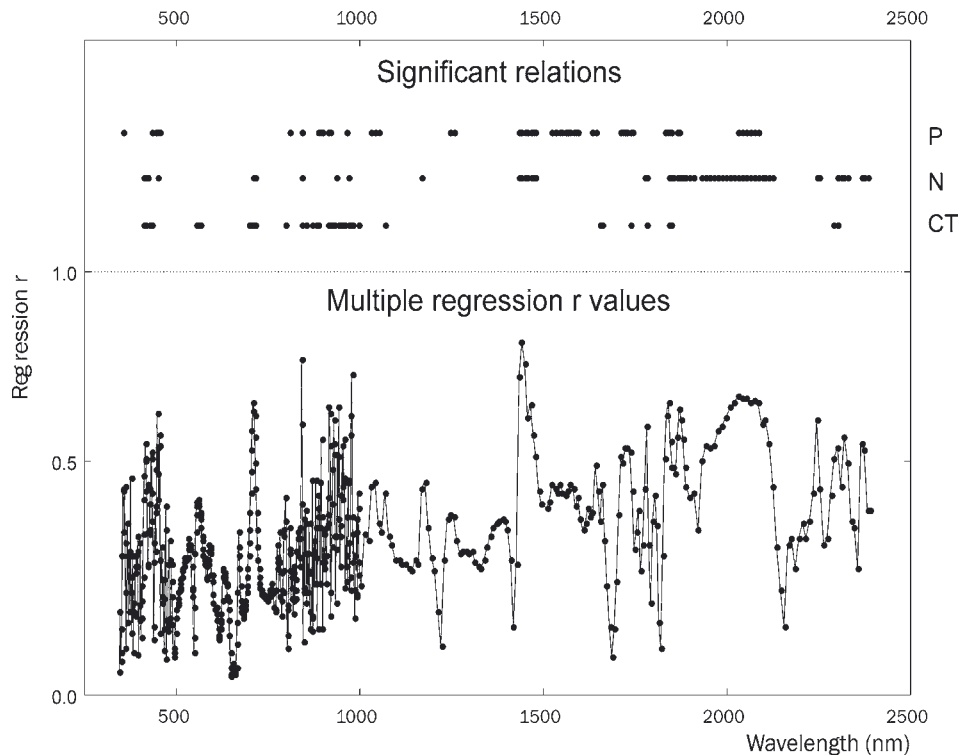
**Figure 3.4.** Correlation between the ratio of foliar nitrogen and phosphorus, the foliar concentration of nitrogen, phosphorus, condensed tannin and the derivative spectra of mopane plants.

#### Combined effect of chemical composition

The regression coefficient of determination between the three chemical elements and the derivative spectra show a number of maxima, each of which is related to one or more of the chemical components included in the model (see figure 3.5). These points are located at: 447 nm ( $r=0.60$ ), 710 nm ( $r=0.63$ ), 842 nm ( $r=0.72$ ), 978 nm ( $r=0.69$ ), 1441 nm ( $r=0.76$ ), 1840 nm ( $r=0.63$ ), 1870 nm ( $r=0.62$ ) and from 2020 nm to 2083 nm ( $r=0.63$  to  $r=0.64$ ).

Significant effects of the individual chemical components on the regression model occur in several spectral regions. For foliar nitrogen these are mainly located around 1400 nm and between 1800 nm and 2100 nm. For foliar phosphorus this is around 900 nm, 1050 nm, and several areas between 1400 nm and 2100 nm. For condensed tannin the main spectral regions where the derivative spectrum is affected is from 800 nm to 1000 nm and a few isolated bands between 1600 nm and 1900 nm and at 2301 nm.





**Figure 3.5.** Coefficient of determination for a multiple regression model between nitrogen, phosphorus and condensed tannin content and the derivative spectra of mopane plants. The wavebands for which the input of nitrogen, phosphorus and condensed tannin (CT) in the regression models are significant are marked above the overview.

### 3.4 Discussion

Many of the bands found in this study are related to known absorption features. For reflectance spectra, the regions with highly significant differences ( $p \leq 0.01$ ) included a number of known absorption features for protein (located at 1690 nm, 2140 nm and 2180 nm, (Curran 1989)), starch (located at 1530 nm, 1540 nm, 1580 nm, 1690 nm, (Curran 1989)), and cellulose (located at 1736 nm, 1780 nm, and 1820 nm, (Curran 1989)). These regions also included the bands with high correlation to actual nitrogen concentration and the ratio between nitrogen concentration and phosphorus concentration in the samples. This work therefore

confirms findings by other studies, and adds knowledge to the existing body of work on the detection nutrients in living vegetation, which eventually may enable us to develop robust nitrogen and phosphorus detection routines, which are valid across species and vegetation types (Ferwerda et al. 2002).

The correlation between the chemical composition and the individual reflectance values (and to a lesser extend the derivative spectra) are not as high as found in some other studies. In this regard it is important to note that the reflectance data were collected only for individual leaves. However, the chemical composition of the plant was determined for leaves from the whole canopy. And although the chemical content of each leaf is related to the chemical composition of the whole plant, variation exists between leaves (Close et al. 2004). This will result in inaccuracies in the relation between reflectance of one leaf, and the whole-plant nutrient status.

Chemical analysis of foliage showed strong effects of soil nitrogen on foliar nitrogen, foliar N:P-ratio, and the plant growth (Table 3.2). No effects of soil phosphorus on the plant chemical composition or size were found. This may indicate that phosphorus levels in the soil were not limiting plant growth (Chapter 2, Ferwerda et al. 2002). There are however differences in the derivative reflectance of plants growing on soil different phosphorus levels, in interaction with nitrogen availability. The main spectral region where differences occur is located between 1865 nm and 2030 nm. According to Curran (1989) the absorption features in this region are related to O-H bonds in water, lignin, protein, starch and cellulose at 1940 nm, O-H bonds in sugar and starch at 1960 nm, N-H bonds in protein at 1980 nm and O-H, C-O bonds in starch. We therefore hypothesize that young mopane plants may reduce investments in reserves (starch and sugars) and change cell-structure (water and cellulose) when nutrients become more limited. Eventually growth rates would be reduced as nitrogen and phosphorus become more limiting (Koerselman and Meuleman 1996, Sword et al. 1998).

Within the 1865 to 2030 nm spectral region an absorption feature related to lignin and tannin is found, located around 1870 nm (Soukupova et al. 2002). The absorption features of these components display an overlap in this spectral region, which may explain the absence of a high correlation between the derivative spectra of this band and condensed tannin concentration.

For derivative spectra the spectral regions with differences between treatments included a number of known absorption features. Chlorophyll a shows strong absorption around 430 nm, which affects the reflectance curve between 400 nm and 450 nm, and results in significant differences in derivative reflectance between nitrogen treatments in that region, and the peak in regression *r*-value, located at 447 nm. Wavebands between 630 nm and 670 nm and between 708 nm and 729 nm form the start and the centre of the spectral region known as the red edge. The shift in spectral position of this region is related to increases in chlorophyll

concentration in fresh vegetation (Dawson and Curran 1998) which explains this high correlation between nitrogen and the derivative spectrum at 669 nm. The combined regression model shows a peak at 710 nm, which is part of the red edge, and thus probably linked to chlorophyll concentration and functioning.

The differences in derivative spectra between 800 nm and 900 nm cannot directly be linked to specific absorption features related to the nutrient composition of vegetation. However, for the regression model between the derivative spectrum and the three measured chemical components in this region, mainly condensed tannin shows significant contributions. This indicates that the differences in derivative spectra in this region may be due to changes in concentration of phenolic compounds, which can be induced through a reduction of nutrient availability (Kraus et al. 2004b). The same is also the case for the effect of soil nitrogen, soil phosphorus and their interactive effect on the derivative spectrum at 931 nm and 978 nm. This is related to C-H bond stretching (at 930 nm, (Curran 1989)) and O-H bond stretching (at 970 nm, (Curran 1989)), which is confirmed by the regression model, with only significant relationships between condensed tannin concentration and the derivative reflectance at these spectral positions.

The band with peak correlation between leaf nitrogen concentration and the derivative spectrum, located at 1434 nm, also shows significant differences in slope between treatments. However, this band is located between two absorption features which are not directly related to nitrogen concentration (C-H bond stretch at 1420 nm and O-H bond stretch at 1450 nm, (Curran 1989)). The observed differences may reflect differences in cell structure of plants growing under different physiological conditions, resulting in differences in relative water content.

High correlation between foliar nitrogen concentration and derivative spectra, and significant differences in derivative spectra occur at 2063 nm, which is related to the protein/nitrogen absorption feature located at 2060 nm. This relationship is clearly observed in the regression model between leaf chemistry and derivative spectra.



# 4

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## Nitrogen Detection Across Plant Species

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*“It is not the possession of truth, but the success which  
attends the seeking after it, that enriches the seeker and  
brings happiness to him.”*

*M. Planck (1858-1947)*



**Abstract**

The main focus of recent studies relating vegetation leaf chemistry with remotely sensed data is the prediction of chlorophyll and nitrogen content using indices based on a combination of bands from the red and infrared wavelengths. The use of high spectral resolution data offers the opportunity to select the optimal wavebands for predicting plant chemical properties. In order to test the optimal band combinations for predicting nitrogen content, normalized ratio indices were calculated for all wavebands between 350 and 2200 nm for five different species. The correlation between these indices and the nitrogen content of the samples was calculated and compared between species. The results show a strong correlation between individual normalized ratio indices and the nitrogen content for different species. The spectral regions that are most effective for predicting nitrogen content are, for each individual species, different from the NDVI spectral region traditionally used to predict nitrogen content. By combining the areas of maximum correlation it was possible to determine the optimal spectral regions for predicting leaf nitrogen content across species. In a cross-species situation, NRI using the combination of reflectance at 1770 nm and at 693 nm may give the best relation to nitrogen content for individual species.

*In press as:*

Jelle G. Ferwerda, Andrew K. Skidmore & Onesimo Mutanga. *Nitrogen detection with hyperspectral NRI across multiple plant species*. International Journal of Remote Sensing.

## 4.1 Introduction

Nutrient levels in plants affect ecosystem processes such as nutrient cycling (Aber and Federer 1992) and grazing behaviour (Bernays et al. 1989). To better understand these processes, many attempts have been made to predict biophysical parameters through remotely sensed indices such as NDVI, TCARI and SAVI (Baret and Guyot 1991, Haboudane et al. 2004, Haboudane et al. 2002, Huete 1988, Maire et al. 2004). By using high spectral resolution data, detailed variation in the spectrum can be measured (Broge and Leblanc 2000), resulting in an opportunity to select optimal wavebands for predicting biochemical components, and many attempts using hyperspectral data to predict biochemical content have been published (Berardo 1997, Broge and Leblanc 2000, Curran et al. 2001, Gong et al. 2002, Gupta et al. 2001a, 2001b, Kokaly and Clark 1999, Mutanga et al. 2003, Zarco-Tejada et al. 2004).

To predict biochemical patterns at landscape scale, indices should preferably be general and species-independent, taking into account the species-specific differences in reflectance, scattering and internal structure (Peterson and Hubbard 1992). Some authors have incorporated a great number of species in their studies (Johnson 2001, Kokaly and Clark 1999, Peterson et al. 1988) to find optimal band combinations for predicting nutrient and chlorophyll content. Others have chosen to work on datasets with only a few species, usually chosen from a wide range of age classes or locations, thus ensuring a wide range in chemical content and reflectance (Berardo 1997, Gong et al. 2002, Haboudane et al. 2002, Tian et al. 2001). Often these studies focus on spectral regions known to respond to changes in nutrient levels or to spectral regions known to be affected by vegetation components from broadband remote sensing, such as the red and near infrared part of the spectrum (e.g., Broge and Leblanc (2000), and Gupta et al. (2001b)). Different approaches have been taken by Mutanga and Skidmore (2004b), Kabail et al. (2004), and Hansen and Schoerring (2003), who used indices based on all available wavebands for biomass estimation, land cover classification, and nitrogen detection, respectively. Of particular interest is the work by Mutanga and Skidmore (2004b), who showed that new combinations of high-resolution spectral bands can overcome the NDVI saturation problem for grass, and the work by Hansen and Schoerring (2003), who found that NDVI-based indices for reflectance at 440 nm and 573 nm have the strongest relation to foliar nitrogen concentration of winter wheat for all band combinations between 438 and 884 nm.

The NDVI is a normalized ratio index that uses the difference between high reflectance in the near infrared and low reflectance in the red part of the electromagnetic spectrum to predict biophysical parameters (Larsson 2002, Van der Meer et al. 2001, Scanlon et al. 2002, Sims and Gamon 2002). This difference is related to increased red-absorption by chlorophyll for denser vegetation, or vegetation with increased chlorophyll concentration, combined with the increased near infrared scattering by vegetation structural components, e.g. cell wall material, for denser vegetation. NDVI calculated from Landsat TM data has been tested, and proven to be a good predictor

for vegetation parameters such as biomass (Huete et al. 2002) and net primary production (Prince et al. 1995) at medium vegetation densities. However, this index is known to saturate at higher vegetation densities (Haboudane et al. 2004, Mutanga and Skidmore 2004b) and is invariable at low densities.

Nitrogen is an important factor affecting the concentration of chlorophyll in the leaf (Zhao et al. *In Press*). After testing a variety of common indices related to chlorophyll concentration on a dataset with a few species of different structures, we found them to have a weak overall correlation with nitrogen content (e.g., red edge position:  $R^2=0.03$ ; MSR705 (Sims and Gamon 2002)  $R^2=0.51$ ; MCARI (Haboudane et al. 2004)  $R^2=0.31$ ). Because of the strong relation between NDVI from Landsat TM data and photosynthetically active vegetation, it is expected to find good correlations between normalized difference vegetation indices based on band combinations from the NDVI spectral regions, and nitrogen. However, due to saturation of the index at higher vegetation densities, other waveband combinations than those from the NDVI region might be more effective at detecting nitrogen content, as was already shown by Hansen and Schoerring (2003) for the visible to near infrared part of the spectrum.

There have been no studies, to our knowledge, that systematically check the electromagnetic spectrum from 300 nm to 2500 nm for band combinations suitable for predicting nitrogen content across a limited number of species. This study compares normalized ratio indices (NRI) from across the incident solar spectrum for suitability for predicting nitrogen content. The NRI were calculated for all waveband combinations between 300 nm and 2500 nm, for a dataset consisting of samples of five plant species of different structures and from different eco-climatic regions under different light conditions.

## 4.2 Methods

### 4.2.1 Data collection / biochemical analysis

To find indices that are generally applicable, a number of species of different structures and from different climatic regions were selected. At a typical field site, a combination of trees, shrubs and grasses can be found. In this study, five areas dominated by trees (olive (*Olea europaea* L.), willow (*Salix cinérea* L.) and mopane (*Colophospermum mopane*)), grass (Blue Buffalo grass (*Cenchrus ciliaris*)) and shrubs (heather (*Calluna vulgaris* L.)) were included (Table 4.1).



**Table 4.1.** Description of plots and samples with collection date. Dominant species are listed for each plot. Mean nitrogen concentration was calculated for samples used in this study, and displayed as a percentage of dry biomass  $\pm$  the standard deviation.

Species	Location	Date	n	Nitrogen (%) $\pm$ St.Dev.
Olive ( <i>Olea europea</i> )	Alora Spain	07-2001	28	1.30 $\pm$ 0.22
Heather ( <i>Calluna vulgaris</i> )	Wageningen-Hoog the Netherlands	08-2001	10	1.06 $\pm$ 0.07
Willow ( <i>Salix cinerea</i> )	Wageningen the Netherlands	08-2001	10	2.20 $\pm$ 0.23
Mopane ( <i>Colophospermum mopane</i> )	Northern Kruger NP South Africa	04-2002	20	1.37 $\pm$ 0.20
Grass ( <i>Cenchrus ciliaris</i> )	Wageningen the Netherlands	08-2002	24	2.22 $\pm$ 1.10

Reflectance spectra of olive trees were collected over the summer of 2001 in olive plantations between Alora and Antequera, Spain (Table 4.1). Ten spectral reflectance measurements were taken for each sample at the sunlit side of each tree, at nadir from a distance of approximately 10 to 20 cm, using a GER 3700 field spectroradiometer (Geophysical and Environmental Research Corporation, Buffalo, New York) equipped with a fibre-optic cable of 1.5 m from the same company. Spectral measurements for each sample were taken at several locations of the tree crown, to average out variation in canopy structure and BRDF effects. In order to calculate absolute reflectance, the reflection of a calibrated Spectralon Reflectance Target (Labsphere, Inc, North Sutton, New Hampshire) was measured between readings of the canopy. Leaves were collected from the measured branches of the plants for chemical analysis. After being transported to the field station, the samples were sun-dried for 24 hours. Upon return to the Netherlands the samples were dried for an additional 24 hours at 70 degrees.

An additional 20 samples were collected from two sites in the Netherlands (Table 4.1). Ten samples were collected of heather shrubs and 10 of willow trees, using the same sampling design as for the olive trees. After spectral measurements had been taken, vegetation samples were collected and transported to the laboratory, where they were dried for 48 hours at 70 degrees.

In the late wet season of 2002, 24 samples were collected from mopane trees in the north of Kruger National Park, South Africa. Reflectance spectra and samples were collected using the same sampling design as described for olive trees. In the field camp an oven was available, where samples were dried at 70 degrees for 48 hours before transport to the Netherlands.

To increase the variation in the dataset as a result of variations in plant structures and light conditions, 24 samples of Blue Buffalo grass were included (Table 4.1). Unlike the other samples, this grass was grown in a greenhouse, and the reflectance of this grass was measured in a darkroom set-up, using an artificial light source (Mutanga and Skidmore 2004b).

After drying, all samples were stored at 20 degrees until laboratory analysis. Sub-samples were prepared with a mixture of sulphuric acid, selenium and salicylic acid (Novozamsky et al. 1983). Nitrogen was then measured with a Skalar San-Plus auto analyser.

#### 4.2.2 Data processing

To compensate for the effects of BRDF on the spectra, and to remove random noise from the data, the reflectance spectra of each sample were averaged. Areas which displayed high levels of atmospheric noise (1815 nm to 2000 nm) and noise due to reduced declined solar irradiance (2215 nm to 2500 nm) were removed from the data, resulting in a total of 598 spectral bands.

#### 4.2.3 Index calculation and correlation

NRI is defined as shown in equation (E.4.1) (Sims and Gamon 2002). NRI were calculated for each sample for all waveband combinations between 300 nm and 2500 nm. This resulted in a total of 178,503 indices ( $598 * 597 / 2$ ).

$$NRI_{band1, band2} = \frac{(R_{band1} - R_{band2})}{(R_{band1} + R_{band2})} \mid \lambda_{band1} > \lambda_{band2} \quad (E.4.1)$$

Correlation between each individual NRI and the nitrogen content of the samples was calculated ( $R^2_{NRI-N}$ ). This was done for each species individually, and for all species combined. To visualize the results of these correlations, the  $R^2_{NRI-N}$  values were plotted. The wavelengths of band 1 and band 2 were plotted on the x and y axes. The correlation between nitrogen and the NRI for each band combination was then plotted on a colour scale, where red corresponds to high correlation, yellow to intermediate, and green to low correlation (Figure 4.1). The spectral region defined by NDVI for Landsat TM (Wachtendonk and Root 2003) is indicated with a black box.

#### 4.2.4 Between species correlation

Two datasets were created for further processing. The first dataset was calculated using the distance between wavebands as the selection criterion. A minimum distance between band centres of 15 nm was used to select wavebands in order to reduce the number of redundant calculations caused by adjacent bands less than 15 nm apart. This resulted in a total of 41,830 selected NRI (dataset a). From

dataset a, a subset, dataset b was calculated, which contained indices based on bands from the spectral region of the Landsat TM NDVI (band 1 from 630 nm to 690 nm, band 2 from 760 nm to 900 nm, dataset b, n=359). For both datasets the correlation of  $R^2_{\text{NRI-N}}$  between each individual species and the other four species was calculated (termed 'the between species correlation' of  $R^2_{\text{NRI-N}}$  values).

#### 4.2.5 *Similarity of species*

Kappa statistics were used to analyse classified correlation data in order to overcome statistical problems with this large volume of data. Kappa statistics calculate the measure of agreement between two classified datasets (Cohen 1960). In remote sensing kappa statistics have typically been used to calculate whether two images are different – for example, whether the outputs of two classifiers are different. When the two images are exactly the same, kappa is one. When the images are unrelated, kappa is zero. However, when the two images show a less than random, or reverse relation, kappa is negative (Cohen 1960). We propose using kappa statistics to compare high-volume output of hyperspectral data. Kappa statistics require classified, non-continuous data, with matching classes between datasets (Cohen 1960). To compare species with different absolute values of  $R^2_{\text{NDVI-N}}$ , and to minimize the effect of outliers in the dataset, the  $R^2_{\text{NDVI-N}}$  values for each species were ranked (1 to 41,830) by their respective  $R^2$  value. Based on this ranking the data was classified into 10 equal-sized classes. Using error matrices, kappa was calculated for each species combination. Kappa statistics were used on this rank-classified data to test whether the different species show similar spectral regions of high and low correlation between NRI and nitrogen.

### 4.3 *Results*

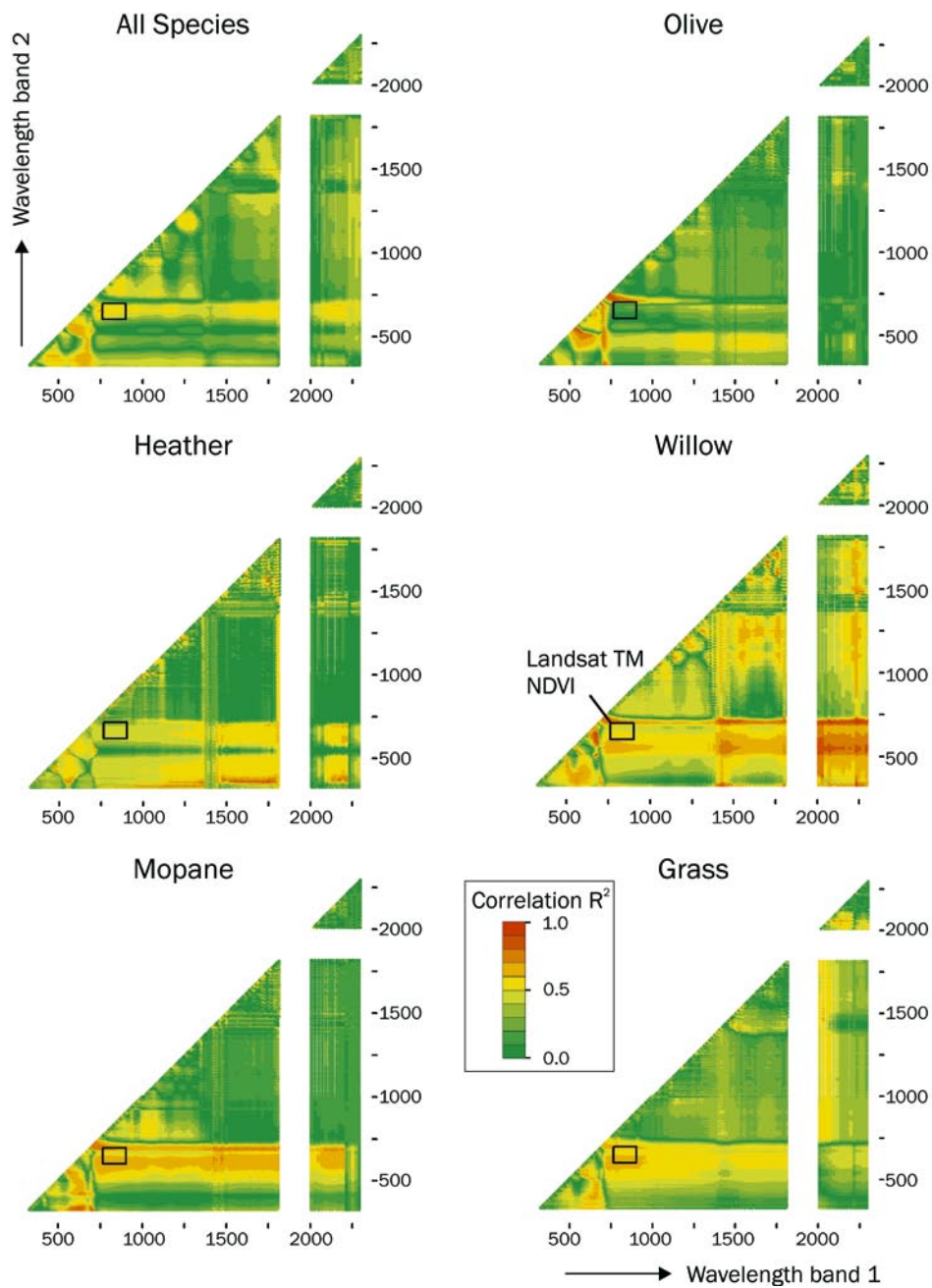
#### 4.3.1 *Correlation between NRI and nitrogen*

Although absolute correlation values between NRI and nitrogen vary, similar patterns appear between different species (Figure 4.1). High correlations occur for NRI where band 2 is based in the visible part of the spectrum. For olive a region of high correlation occurs between 300 nm and 700 nm. For mopane, grass and willow high correlation occurs when band 2 is chosen between 500 nm and 750 nm. For heather high correlation occurs when band 2 is chosen in both regions, from 300 nm to 700 nm and from 500 nm to 750 nm. The set of all species combined shows high correlations when band 2 is chosen between 500 nm and 750 nm.

For each species the highest correlation between an individual NRI and nitrogen content in dataset a (indices based on bands from the full spectrum) is higher than the maximum correlation found in dataset b (indices based on bands from the NDVI region, Table 4.2). Maximum correlations in set a range from 0.72 to 0.95. For set b the range in correlation is from 0.23 to 0.69 (Table 4.2).

**Table 4.2.** Maximum correlation ( $r$ ) between nitrogen content and NRI for indices of all band combinations and indices based on bands from the NDVI region of the spectrum.

	Whole dataset ( $n=41.830$ )	NDVI region ( $n=359$ )
All Species	0.72	0.61
Olive	0.85	0.23
Heather	0.93	0.47
Willow	0.95	0.58
Mopane	0.86	0.69
Grass	0.75	0.63



**Figure 4.1.** Classified scatterplot for the correlation between nitrogen content and the NRI for each band combination plotted on a color-scale. Red corresponds to high correlation, and green to low correlation.

#### 4.3.2 Between species correlation

The between species correlations of  $R^2_{NRI-N}$  values for indices from set b (indices based on bands from the Landsat TM NDVI region) are high, but often negative (Table 4.3). This implies an opposite effect of changes in nitrogen content on NRI for different species. In other words, indices that have a high correlation with nitrogen content for one species have a low correlation with nitrogen content for another species. For indices from dataset a (indices based on bands from the full spectrum) the between species correlation of  $R^2_{NRI-N}$  values is lower than for set b, but positive (Table 4.4). In other words, indices that have a higher correlation with nitrogen for one species also show a higher correlation with nitrogen for other species.

**Table 4.3.** Between species correlation of  $R^2_{NRI-N}$  values for NRI indices based on bands from the traditional NDVI region (band 1 between 760 nm and 900 nm, band 2 between 630 nm and 690 nm,  $n=359$ ).

	All Spec.	Olive	Heather	Willow	Mopane
Olive	.73				
Heather	.17	.60			
Willow	-.56	-.95	-.60		
Mopane	-.50	-.90	-.55	.94	
Grass	-.58	-.91	-.41	.95	.94

**Table 4.4.** Between species correlation of  $R^2_{NRI-N}$  values for NRI indices based on bands from the whole spectrum.

	All Spec.	Olive	Heather	Willow	Mopane
Olive	-.03				
Heather	.31	.39			
Willow	.12	-.12	.13		
Mopane	.34	.01	.30	.59	
Grass	.07	.08	.08	.21	.52

#### 4.3.3 Similarity of species

The kappa values show a low degree of similarity between different species. For NRI from dataset a (indices based on bands from the full spectrum) 14 of the 15 calculated kappa values are higher than 0, with most indices having a Kappa of less than 4% (Table 4.5). Only mopane and willow ( $\kappa = 9.9\%$ ) and mopane and grass ( $\kappa = 5.6\%$ ) have slightly increased similarity values (Table 4.5). The kappa statistics

for dataset b (indices based on bands from the NDVI region) show even lower kappa values for most combinations (Table 4.6). Only two of the 15 calculated values have a kappa value higher than 0 (Table 4.6). The best agreement is found between willow and olive, with a kappa of 9.9% (Table 4.6).

**Table 4.5.** Measure of similarity ( $k$ ) of ranked correlation indices between species, calculated for indices based on all bands of the full spectrum.

	All Spec.	Olive	Heather	Willow	Mopane
Olive	0				
Heather	0.011	0.032			
Willow	0.017	0.027	0.017		
Mopane	0.039	0.013	0.032	0.099	
Grass	0.02	0.001	0.004	0.035	0.056

**Table 4.6.** Measure of similarity ( $k$ ) of ranked correlation indices between species, calculated for indices based on bands from the NDVI part of the spectrum (band 1 between 760 nm and 900 nm, band 2 between 630 nm and 690 nm,  $n=359$ ).

	All Spec.	Olive	Heather	Willow	Mopane
Olive	0				
Heather	0	0			
Willow	0	0.099	-0.251		
Mopane	-0.046	0	0.061	-0.064	
Grass	-0.384	0	0	0	-0.157

#### 4.4 Discussion

NRI based on wavebands from other spectral regions than those used to calculate NDVI show higher correlation with nitrogen content for each species compared to indices based on band combinations from the NDVI region. For applications in field situations indices should preferably be universally applicable between species and locations. Optimal indices should therefore show a high correlation with the nitrogen content for each individual species as well as when all species are compared together. We can therefore conclude that NDVI is not the optimal ratio for detecting nitrogen.

Indices based on bands from the NDVI region of the spectrum show conflicting responses to changes in nitrogen. The between species correlation of  $R^2_{\text{NRI-N}}$  values is for some species positive, for others negative. This implies an opposite effect of changes in nitrogen content on NRI for different species. The positive between species correlations of  $R^2_{\text{NRI-N}}$  values for dataset a indicate similar spectral responses for all species to changes in nitrogen content. In other words, indices from dataset a that have a high correlation with nitrogen for one species, also show a high correlation with nitrogen for other species.

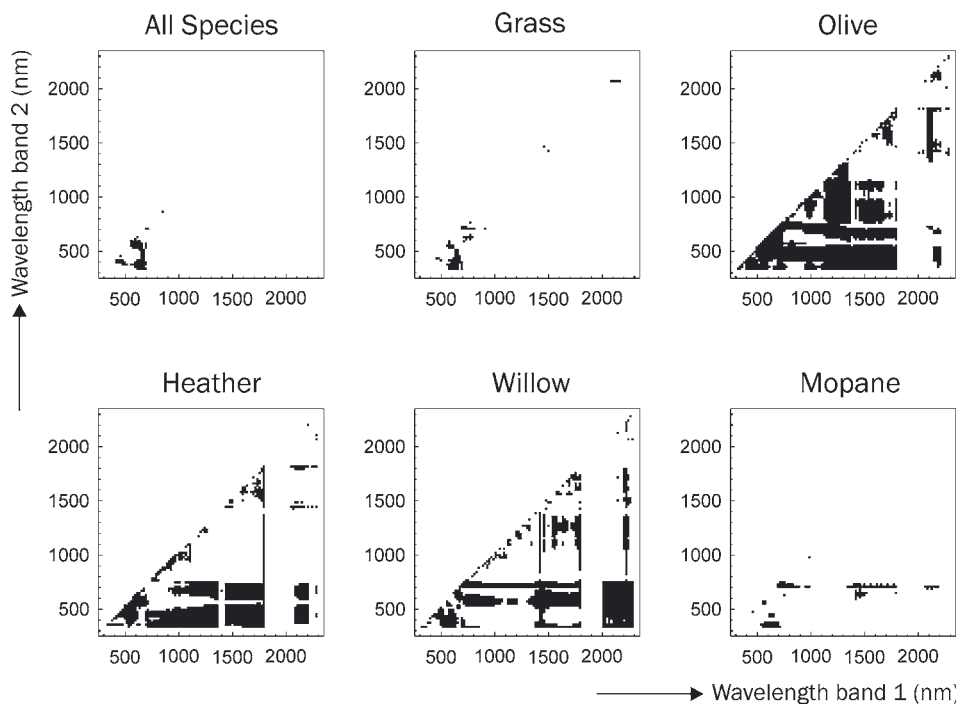
The dataset with all species combined may be seen as a representation of a real-world situation, where multiple species are mixed and result in averaged spectral responses. By comparing the kappa values between all species combined and the individual species, it is possible to assess whether an index that works well for all species combined results in similar responses for individual species. As for the results found for the between species correlation of  $R^2_{\text{NRI-N}}$  values, kappa statistics show that the overall correlation between nitrogen and NRI varies between species. The kappa values calculated for the NDVI region (Table 4.6) show that the agreements between all species combined and olive, heather and willow are completely random, and in the case of grass even less than random ( $\kappa < 0\%$ , Table 4.6). Therefore these band combinations are not optimal. Looking at dataset a (indices from the full spectrum), kappa is low for all combinations ( $\kappa < 5\%$ , Table 4.5). In other words, there are few NRI where for each species the correlation with nitrogen falls in the same class.

Different species show different spectral regions with an optimal response to changes in nitrogen content. Consequently band selection should be based on field spectra from a range of species in the target area. One way to select optimal regions is to compare the different correlation diagrams. Combining table 4.2 and figure 4.1 results in figure 4.2, with band combinations for which the NRI has a higher correlation with nitrogen content than the maximum value found in the NDVI region. Spectral regions with higher correlations occur for all species, except grass illuminated with artificial light, with band combinations of the red and the infrared part of the spectrum (Figure 4.2).

An overlay of the plots in figure 4.2 shows the waveband combinations for which the derived NRI has a higher correlation with nitrogen for each species than the maximum found in the NDVI region of the spectrum. There were no band combinations for which this was true, although when grass was excluded, a number of band ratios were the same for the four remaining species (Table 4.7). (Perhaps grass shows a different pattern because it was illuminated using artificial light, which might result in saturation problems due to lower light intensity.) These band combinations in particular could be generalized and used when no knowledge of the species is available. A number of indices used wavebands between 1430 nm and 1490 nm. Since reflectance around 1450 nm is strongly affected by atmospheric water content, the indices based on



bands from this region were not further investigate. The remaining band combinations are presented in table 4.7. These indices are composed of a combination of bands from the visible (627 nm to 640 nm and 690 nm to 700 nm) in combination with bands from the infrared (1516 nm to 1580 nm, 1770 nm, 1803 nm and 2196 nm).



**Figure 4.2.** Spectral regions where the correlation between the NRI based on band 1 and band 2 is higher than the maximum correlation for that species in the NDVI region.

The bands in the visible are related to absorption by chlorophyll a and b, centred at 660 nm and 640 nm, respectively (Curran 1989, Curran et al. 2001). This link is related to the strong influence of nitrogen on chlorophyll production and functioning. The absorption of chlorophyll may saturate at the absorption pit centre, and can therefore often better be detected at off-centre wavebands (Curran 1989). Gitelson and Merzlyak (1998) showed that reflectance at 700 nm is very sensitive to variation in chlorophyll content, which explains why indices that include bands between 690 nm and 700 nm perform well.

**Table 4.7.** Band combinations for which the indices for all species, except grass, have a stronger higher correlation with nitrogen than for all indices based on bands from the NDVI spectral region, and chemical components and bonds to which these features have been related. (Source for features: Curran 1989, Gitelson and Merzlyak 1998)

Band 1 centre (nm)	Band 2 centre (nm)	Absorption feature & chemical bond
430		Chl. a (430 nm)
	620	Chl. b (640 nm)
627, 629, 630, 639, 640 (693, 694)		Chl. b (640 nm)
	1516	N-H stretch (1510 nm)
631, 633, 636, 637		Chl. b (640 nm)
	1523	N-H stretch (1510 nm) O-H stretch (1530 nm)
631		Chl. b (640 nm)
	1809	O-H, C-H stretch (1820 nm)
633, 636, 637		Chl. b (640 nm)
	1523	N-H stretch (1510 nm) + O-H stretch (1530 nm)
	1809	
690		Chl. a (660 nm)
	2196	N-H stretch (2180 nm)
693		Chl. a (660 nm)
	1516, 1538, 1559	N-H stretch (1510 nm) + O-H stretch (1530 nm)
	1770	C-H, O-H stretch (1780 nm)
694		Chl. a (660 nm)
	1516, 1538, 1559, 1580	N-H stretch (1510 nm) + O-H stretch (1530 nm)
	1770	C-H stretch (1690, 1780 nm)
	1803	C-H, C-O, O-H stretch (1780, 1820 nm)
696		Chl. a (660 nm)
	1545, 1566	O-H stretch (1540, 1580 nm)

The 1500 nm to 1600 nm region is dominated by absorption features due to N-H bond stretching (located at 1510 nm, Curran, 1989), and O-H band stretching (located at 1530 nm, 1540 nm, and 1580 nm). The N-H bond is related to the amount of nitrogen present in protein. The O-H bond absorption features centred at these bands have been related to starch or cellulose content (Curran, 1989) and may reflect changes in the C:N-ratio as a result of varying nitrogen availability. The 1770 nm and 1803 nm

bands reflect the effect of the cellulose absorption feature (light absorption by C-H stretch, O-H stretch) located at 1780 nm and 1820 nm. The 2196 nm band reflects changes in protein content, and is related to protein absorption features centred at 2180 nm (N-H, C-H, C-O and C=O bonds) and 2240 nm (C-H bonds) (Curran 1989).

One index is based on two bands from the visible light: 430 nm combined with 620 nm, and is related to the absorption pits identified for chlorophyll a (430 nm) and chlorophyll b (640 nm) (Curran 1989). It is worth noting that absorption at 430 nm easily saturates, and as a result, the reflectance between 400 nm and 500 nm is insensitive to variation in chlorophyll concentration (Gitelson and Merzlyak 1998). This results in an index which uses the absorption by chlorophyll, standardised against a chlorophyll-insensitive part of the spectrum to detect nitrogen content. The 400 nm region is however affected by atmospheric scattering (Curran 1989), which may affect the usefulness when applied to hyperspectral images.

The infrared bands presented in this study are not located at the main water absorption features, centred at 1450 nm and 1900 nm (Ceccato et al. 2002). However, the reflectance at all wavelengths between 1100 nm and 2500 nm is sensitive to foliar water content (Ceccato et al. 2002). Of the identified indices in this study, those which include the 1770 nm absorption feature are least affected by foliar water content, and are preferred over the other indices presented.

In summary we can conclude that, although there were no NRI that gave a high correlation for all species, individual species have high correlations between NRI and nitrogen. In common with previous authors looking at vegetation density, chlorophyll concentration, nitrogen content, and biomass, we found that also nitrogen is not most effectively detected using bands from the NDVI spectral region. Since narrow wavelength regions show the highest correlation with nitrogen, broadband scanners may miss the absorption by nitrogen. In a cross-species situation, NRI using the combination of reflectance at 1770 nm and at 693 nm may give the best relation to nitrogen content for individual species. Finally, this study indicates that species-specific differences may have a big influence on the detection potential of indices for nitrogen content.



# 5

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## Detecting Foliar Nutrients and Trace Elements

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*“Part of the secret of success in life is to eat what you like  
and let the food fight it out inside”*

*M. Twain (1835-1910)*



### **Abstract**

This paper demonstrates the potential of hyperspectral remote sensing to predict the chemical composition (i.e., nitrogen, phosphorus, calcium, potassium, sodium, and magnesium) of three tree species (i.e., willow, mopane and olive) and one shrub species (i.e., heather). Reflectance spectra, derivative spectra and continuum-removed spectra were compared in terms of predictive power. This showed that the best predictions for nitrogen, phosphorus, and magnesium occur when using derivative spectra, and the best predictions for sodium, potassium, and calcium occur when using continuum-removed data. To test whether a general model for multiple species is also valid for individual species, a bootstrapping routine was applied. Prediction accuracies for the individual species were lower than prediction accuracies obtained for the combined dataset for all except one element / species combination, indicating that indices with high prediction accuracies at landscape scale are less appropriate to detect the chemical content of individual species.

#### *Submitted as:*

Jelle G. Ferwerda & Andrew K. Skidmore. *Can nutrient status of four woody plant species be predicted using field spectrometry?* ISPRS Journal of Photogrammetry and Remote Sensing .

## 5.1 Introduction

The use of remotely sensed data for detecting nutrients and trace elements has been developed, tested, and has proven effective for dried, ground samples under controlled conditions (Kokaly and Clark 1999, Rabkin 1987). Although this is cost-effective in projects with many samples to analyse, the potential of remote sensing is not fully exploited. Ideally the spatial variation in the chemical composition of the vegetation should be mapped with a minimal amount of field sampling. To achieve this, several studies have developed models for predicting nutrients at canopy level in fresh vegetation. Some of these have shown that it is possible to obtain high accuracies (Haboudane et al. 2002); others, however, have achieved only moderately accurate results (Gong et al. 2002).

Often these studies have focused on only a few chemicals, such as chlorophyll (Haboudane et al. 2002, Patel et al. 2001) and other pigments (Blackburn 1998) or nitrogen (Ebberts et al. 2002, Lilienthal et al. 2000). Pigments such as chlorophyll and carotenes are detectable, with accuracies of over 80 percent reached for field samples (Haboudane et al. 2002). Nitrogen content, which is related to chlorophyll functioning, can also be predicted quite accurately (Johnson 2001). The detection of other nutrients and trace elements (phosphorus, potassium, sodium, calcium and magnesium) in fresh vegetation has received less attention, although Mutanga et al. (2003) has carried out some research in this respect.

Many papers on the detection of biochemicals focus on their content in grass species (Haboudane et al. 2002, Hansen and Schoerring 2003, Mutanga et al. 2003, Strachan et al. 2002). The cultivated, often larger, species such as maize and sugarcane are important agricultural crops. The health status and yield are influenced by soil nutrient availability, which in turn affects leaf nutrient levels. Consequently the potential to detect the nutrient status in agricultural crops is of significant interest. Grasses are also a source of food for grazing herbivores, and the nutrient status of grasses influences the distribution of these herbivores. In the Tarangire ecosystem, the seasonal migration of wildebeest is determined by changes in the phosphorus content of grasses (Grant et al. 1995, Voeten 1999). The detection of nutrients in grasses is therefore useful, not only for species conservation and natural resources management, but also for precision agriculture and production forecasts.

For tree species, the direct applications of measuring leaf biochemistry are less clear. As a consequence, most studies looking at nutrients in trees have worked on nitrogen or chlorophyll content. However, many tree species also play an important role in conservation and agriculture. Olive production in Spain, for instance, which accounts for a major part of the worldwide olive production (Galan et al. 2004), is heavily dependent on nitrogen, phosphorus and potassium fertilisation (Lopez-Granados et al. *In Press*). It has been shown that a saving of over 80 percent in fertilisers can be achieved if the spatial variations in nutrient levels are determined before planning fertiliser programmes (Lopez-Granados et al. *In Press*). In savanna ecosystems,

knowledge of nutrient levels in trees and shrubs may help us to understand the spatio-temporal distribution of browsing herbivores. Large areas of southern Africa are covered with almost mono-specific stands of mopane (*Colophospermum mopane*). This legume forms shrubs or trees, depending on the substrate, and is only used by browsers at a specific time of the year, after the first leaves have appeared in early spring. Caterpillars of the emperor moth (*Imbrasia belina*) use mopane as their primary food source, and the so-called 'mopane worms' are traditionally a source of protein for people in the region. Major outbreaks occur twice a year (in November / December and March / April), coincident with the rainy season (Klok and Chown 1999), with extensive areas becoming denuded of leaves (*personal observation*). What determines the place of these outbreaks is unclear. Linking the spatial patterns of vegetation nutrients with outbreak occurrences may help in understanding the system behind these outbreaks.

Detecting nutrients in woody species is particularly difficult owing to differences in the physical structure of the canopies of different species. Internal scattering and mixing of bark signatures obscures the absorption signal of nutrients, which in turn is affected by incident sun angle. Two different techniques have been suggested to compensate for these effects. The first, derivative analysis (Tsai and Philpot 1998), assumes that differences in the absolute reflectance of individual samples do not affect the actual absorption pits. Therefore, by using the slope of the spectrum instead of the absolute reflectance, the same signal is produced for samples with different absolute reflectance but the same absorption features. The second method, continuum removal, which was introduced by Clark and Roush (1984) and further developed by Kokaly and Clark (1999), is based on a similar principle. Although the reflectance may vary for different illumination situations and angles, the absorption features will stay the same. By comparing the absorption feature against an approximation of the original curve, the absorption feature is enhanced (Clark and Roush 1984). This technique has been tested successfully for several biochemicals in dried, ground samples (Curran et al. 2001) and fresh grass samples (Mutanga et al. 2003).

This study aims at detecting nutrients and trace elements across a range of woody plant species. A comparison is made between absolute reflectance, derivative spectra and continuum-removed spectra for four different species, and the prediction accuracies for individual species using models based on a combined dataset are compared to assess the accuracy of across-species models at an individual species level.



## 5.2 Methods

### 5.2.1 Study areas

Four plant species from three different climatic regions were selected to maximise the range in internal physiology. The first study area is around Wageningen, the Netherlands, and is located in a temperate climatic region, with a cool and wet climate. The second region is in southern Spain, with a dry Mediterranean climate, and the samples were collected in olive plantations around Alora, just north of Malaga. The last set of samples was collected in the north of Kruger National Park, South Africa, which is located in the dry tropics.

### 5.2.2 Field sampling

In the Netherlands, samples were collected for heather (*Calluna vulgaris* L., n=10) and willow (*Salix cinera* L., n=10). In Spain olive trees (*Olea europaea* L., n=28) were sampled, and in South Africa mopane trees (*Cholophospermum mopane*, n=18). In all cases, sampling followed the same experimental set-up. From around the canopy of an individual tree, leaves were collected at a height of approximately 1.5 m and placed in a pile in the sun. Heather was the exception: reflectance was measured before clipping, and samples included young twigs. For each sample, 10 reflectance spectra were recorded using a GER 3700 spectrometer. In order to calculate absolute reflectance, a reference spectrum was measured from a Spectralon reference target between readings. After measuring the reflectance, samples were dried at 70 degrees for 24 hours and stored until analysis.

### 5.2.3 Chemical analysis

Subsamples were destructed with a mixture of sulphuric acid, selenium and salicylic acid (Novozamsky et al. 1983). The content of the elements nitrogen and phosphorus was measured with a Skalar San Plus auto-analyser. Sodium, potassium and calcium were measured with a flame photometer, and magnesium with an atomic absorption spectrometer.

### 5.2.4 Spectral processing

To remove random noise from the spectra, the ten reflectance spectra per sample were averaged. From the averaged spectra, derivative spectra were calculated using an adjusted version of the Savitzky-Golay smoothing technique (Savitzky and Golay 1964, Tsai and Philpot 1998). Instead of first smoothing the spectra and then calculating the derivative spectra from the smoothed spectra, the parameters of the fitted third-order, seven-band moving polynomial were used to directly calculate the derivative at the centre waveband of the moving spline window.

Continuum-removed spectra were calculated as described in Kokaly and Clark (1999), and the band depth was used in this study. Table 5.1 gives an overview of the absorption features used and the end points between which the continuum line was calculated.

Table 5.1 Definition of continuum end points for absorption features observed in the reflectance spectra of fresh leaves

Feature (nm)	Continuum line start (nm)	Continuum line end (nm)
680	551	716
1194	1132	1280
1460	1338	1646
1730	1652	1778
2100	2030	2218
2300	2238	2366

#### 5.2.5 Statistics

The datasets were randomly split into two equal-sized subsets, each containing half of the samples for each species. One subset was used for training models (n=33), the other for testing purposes (n=33). To enable comparison between reflectance, derivative data and band depth, the same division of samples into training and test data was used for all three datasets.

Through a stepwise regression routine, the bands with optimal fit to the content were selected for the three training subsets (Reflectance, Derivatives, Band Depth) for each chemical. The number of predictors was limited to four. The parameters of the fitted models were then used to predict the content of the test subset, and the correlation between predicted content and actual content was calculated.

To assess the certainty of the obtained correlation value, a bootstrap was applied. The training and test subsets were combined, and split by species. For each species individually and for all species together, the correlation between predicted and observed chemical content was bootstrapped with 1000 iterations. The distribution and mean correlation give an indication of the differences in model accuracy between species, and of the robustness of the model.

### 5.3 Results

There is a wide variety in chemical composition between the species, but there are no species with a clearly higher concentration of nutrients and trace elements overall (Table 5.2). The regression goodness of fit is high for almost all chemicals and all predictor sets, and ranges from  $R^2=0.42$  to  $R^2=0.77$  for sodium, and from  $R^2=0.81$  to  $R^2=0.89$  for nitrogen (Table 5.3).

The highest prediction accuracy for nitrogen content occurs with the model based on derivative spectra ( $R^2_{\text{max}}=0.86$ ;  $n=33$ ), followed by the models based on band depth and then reflectance. This is the same for phosphorus ( $R^2_{\text{max}}=0.51$ ;  $n=33$ ) and magnesium ( $R^2_{\text{max}}=0.35$ ;  $n=33$ ). Sodium, potassium and calcium are best predicted using continuum-removed spectra (Table 5.3). The bootstrapped data show the same results, except for sodium, which shows a higher average correlation for derivative data than for continuum-removed data. Histograms of the correlations between observed and predicted concentrations achieved for all samples in the bootstrapping routine were plotted for each species individually, and for all species combined (Figure 5.1). These figures show that, although for all elements the overall fit is high, for individual species the models perform less well. For none of the species the correlation is high for all elements.

**Table 5.2.** Chemical composition of the sample datasets categorised by species and training versus test data.

<b>Element Species</b>	<b>Training Mean (%)</b>	<b>Training St Dev (%)</b>	<b>Test Mean (%)</b>	<b>Test St Dev (%)</b>
<b>Nitrogen</b>				
Olive	1.296	0.242	1.311	0.204
Heather	1.062	0.072	1.058	0.068
Willow	2.114	0.174	2.276	0.270
Mopane	0.966	0.381	1.054	0.563
All	1.294	0.456	1.349	0.528
<b>Phosphorous</b>				
Olive	0.0823	0.0178	0.0825	0.0299
Heather	0.0864	0.0055	0.0828	0.0045
Willow	0.1682	0.0176	0.1790	0.0195
Mopane	0.0816	0.0503	0.0758	0.0308
All	0.0957	0.0421	0.0954	0.0441
<b>Sodium</b>				
Olive	0.0168	0.0034	0.0154	0.0033
Heather	0.0104	0.0022	0.0119	0.0040
Willow	0.0095	0.0023	0.0092	0.0013
Mopane	0.0078	0.0075	0.0052	0.0074
All	0.0122	0.0060	0.0111	0.0062
<b>Potassium</b>				
Olive	0.596	0.171	0.644	0.169
Heather	0.502	0.023	0.494	0.056
Willow	0.692	0.109	0.722	0.087
Mopane	1.048	0.135	1.059	0.187
All	0.720	0.250	0.746	0.252
<b>Calcium</b>				
Olive	1.956	0.402	1.761	0.376
Heather	0.416	0.036	0.410	0.042
Willow	0.866	0.214	0.954	0.130
Mopane	1.305	0.287	1.320	0.268
All	1.380	0.652	1.314	0.560
<b>Magnesium</b>				
Olive	0.220	0.044	0.210	0.045
Heather	0.151	0.003	0.139	0.009
Willow	0.277	0.041	0.277	0.022
Mopane	0.272	0.047	0.271	0.063
All	0.232	0.058	0.226	0.065

**Table 5.3.** Correlation between predicted and observed concentrations of nitrogen, phosphorus, sodium, potassium, calcium and magnesium for the training subset ( $TR R^2$ ;  $n=33$ ), the test subset ( $TE R^2$ ;  $n=33$ ), and the average  $R^2 \pm 95\%$  confidence interval after bootstrapping (1000 iterations) the full dataset ( $BS R^2$ ;  $n=66$ ), using reflectance spectra (A), derivative spectra (B) and band depth (C) for four plant species. Bands 1 to 4 refer to the central wavelengths in nanometres of the four bands selected in a stepwise regression.

(A)

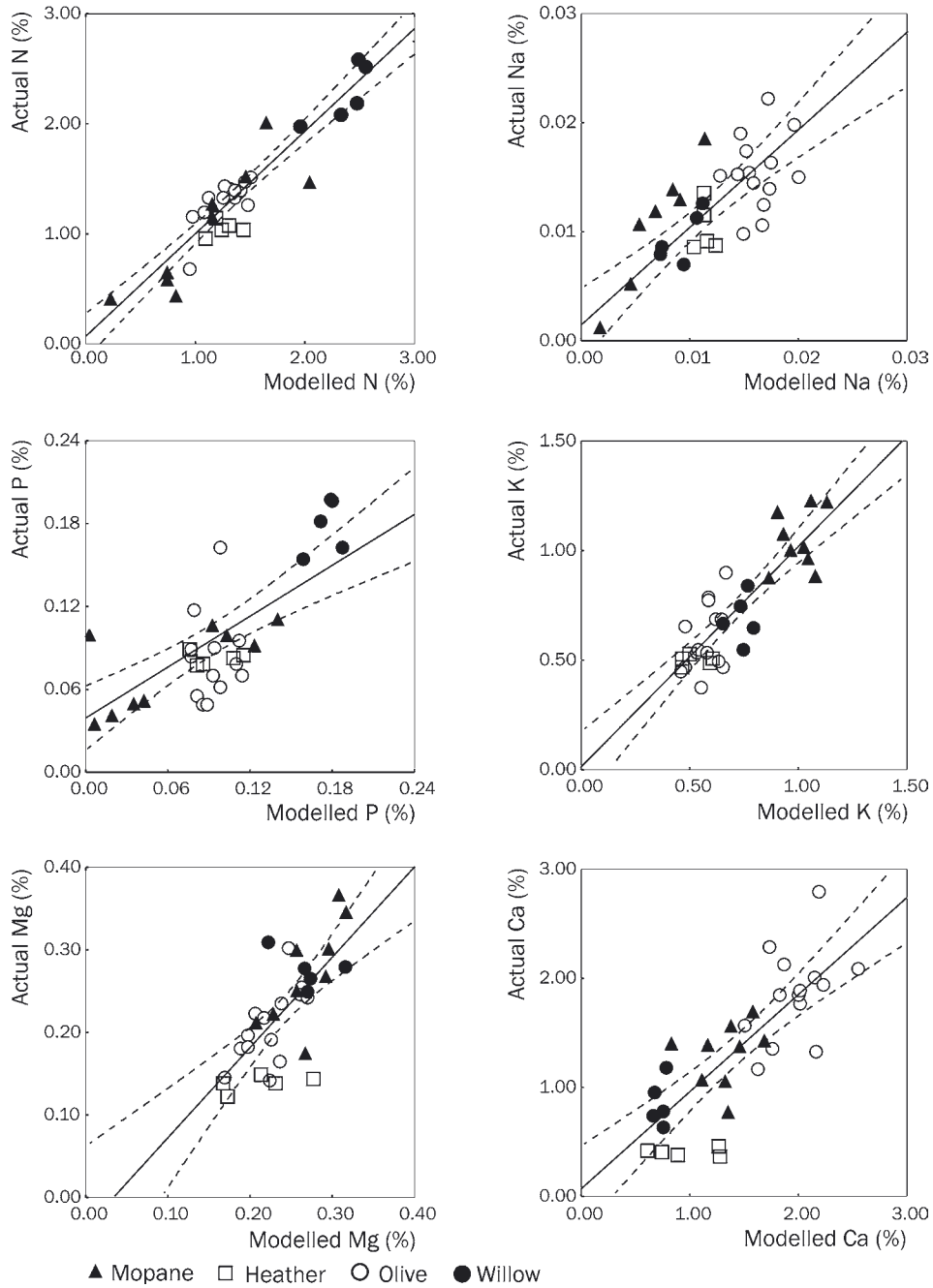
Element	$TR R^2$	$TE R^2$	$BS R^2$	Band 1	Band 2	Band 3	Band 4
N	0.81	0.70	$0.74 \pm 0.01$	683	1898	1516	700
P	0.87	0.38	$0.57 \pm 0.02$	674	1752	1607	1735
Na	0.58	0.28	$0.42 \pm 0.02$	724	459	513	-
K	0.76	0.63	$0.69 \pm 0.02$	729	852	947	1305
Ca	0.70	0.51	$0.59 \pm 0.02$	748	444	771	558
Mg	0.62	0.21	$0.39 \pm 0.02$	1814	1735	1752	1781

(B)

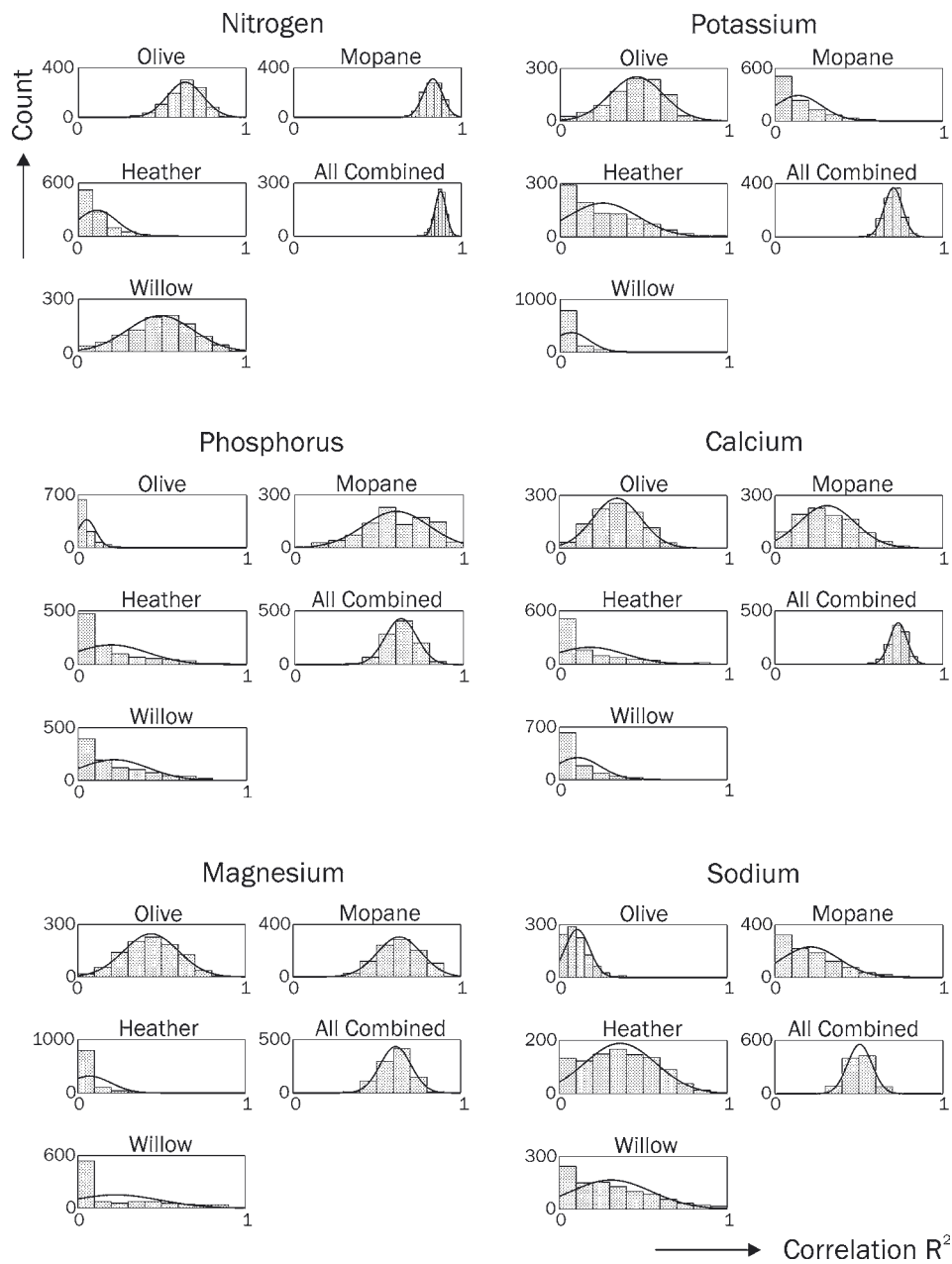
Element	$TR R^2$	$TE R^2$	$BS R^2$	Band 1	Band 2	Band 3	Band 4
N	0.89	0.86	$0.87 \pm 0.01$	732	1203	1792	955
P	0.83	0.51	$0.63 \pm 0.02$	731	1195	1449	1747
Na	0.77	0.51	$0.63 \pm 0.02$	1803	990	972	1050
K	0.81	0.50	$0.65 \pm 0.02$	548	1698	984	960
Ca	0.76	0.41	$0.56 \pm 0.02$	1464	1673	1371	753
Mg	0.77	0.49	$0.61 \pm 0.02$	1770	858	1141	763

(C)

Element	$TR R^2$	$TE R^2$	$BS R^2$	Band 1	Band 2	Band 3	Band 4
N	0.88	0.76	$0.81 \pm 0.01$	713	1410	2347	1246
P	0.85	0.50	$0.65 \pm 0.02$	614	2156	2320	713
Na	0.42	0.60	$0.50 \pm 0.02$	1379	1347	1141	553
K	0.73	0.68	$0.70 \pm 0.01$	1347	1764	2206	1387
Ca	0.86	0.62	$0.73 \pm 0.01$	713	1410	2347	1246
Mg	0.63	0.35	$0.44 \pm 0.02$	614	2156	2320	713



**Figure 5.1.** Scatterplots of predicted and observed concentrations of nitrogen, phosphorus, potassium, calcium, magnesium and sodium for the models with highest  $R^2$ . (N, P and Mg content was predicted using derivative spectra, K, Ca and Na content using continuum-removed spectra.)



**Figure 5.2.** Histograms of  $R^2$  values in a bootstrapped correlation (1 000 iterations) between predicted and observed nutrient content for four species individually, and all species together.

## 5.4 Discussion

Scatterplots of predicted against observed chemical content, categorised by species, show the samples clustered by species for all elements (Figure 5.2). This clustering is partly the result of differences in the range of chemical content between species (for instance, for nitrogen). However, for many plots this is not the case. Particularly for heather, and to a lesser extent willow, the variation in observed values does not match the predicted values (Figure 5.1). This shows that, although a model build for a range of different species and locations may result in good predictions of the chemical composition of the overall dataset, the prediction accuracy for individual species can be low.

Studies that use hyperspectral indices to predict chemical content usually give an overview of the prediction capabilities of the model by testing their model on a test dataset, with or without a bootstrapping routine to test the robustness of the model. Often these models are based on many different species (Kokaly and Clark 1999, Serrano et al. 2000), or just one species (Berardo 1997, Goel et al. 2003, Gong et al. 2002), and accuracy assessments are made for the full dataset. However, a model which is valid for a wide range of species may not be capable of accurately predicting chemical content for a single species alone. Thus, to understand the accuracy of these models, one has to keep in mind the actual aim of the model. Is it to accurately predict chemical content at individual plant level, at species level, or at landscape level? We tested whether there are differences in prediction accuracy between the combined dataset, and the individual species.

Significant differences in prediction accuracy occurred between the five groups (each species and all data combined) for all elements (Kruskal-Wallis analysis of variance,  $p \leq 0.05$ ). The best prediction for individual species occurs for mopane, with high average correlations between modelled and actual chemical content for nitrogen, phosphorus, calcium and magnesium (Group differences were determined using a multiple comparison Z-test, and an overall confidence level of five percent (Noether 1976)). The overall best prediction, however, occurs for the combined dataset for all elements, except for nitrogen, which is best predicted for the mopane samples. All groups have a different mean correlation between modelled and actual content, for all elements, except for phosphorus in olive and heather and potassium in heather and mopane.



# 6

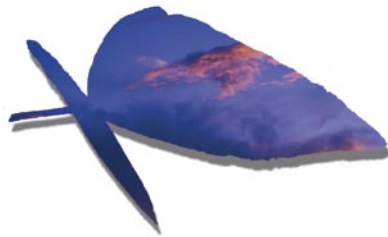
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## Detecting Foliar Condensed Tannins in Mopane

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*What is food to one, is bitter poison to others.*

*T. Carus (95-55 BC)*



### **Abstract**

Detection of hydrocarbons in plants with hyperspectral remote sensing is hampered by overlapping absorption pits, while the 'optimal' wavebands for detecting some surface characteristics (e.g. chlorophyll, lignin, tannin) may shift. We combined a phased regression with a bootstrap procedure to find wavebands related to the tannin content of fresh leaves, and compared the bands thus identified with the reflectance spectra of pure quebracho tannin. For both reflectance and derivative spectra, bands were identified that can be linked to known absorption features (1471, 1640, 1710, 2175 nm), or fall within the absorption regions identified from a reflectance curve of pure quebracho tannin. Furthermore, we confirmed the link suggested by other studies between tannin content and the derivative spectra around 800 nm. The study shows that by combining bootstrapping with phased regression it is possible to determine wavebands of which the reflectance is influenced by tannin.

#### *Submitted as:*

Jelle G. Ferwerda, Andrew K. Skidmore & Alfred Stein. *A Bootstrap Procedure to Select Hyperspectral Wavebands Related to Tannin Content*. International Journal of Remote Sensing.

## 6.1 Introduction

The ability of hyperspectral remote sensing to detect and map the chemical composition of fresh vegetation samples has been demonstrated for macronutrients and photosynthetically active components such as chlorophyll and pigments (Blackburn 1998, Coops et al. 2003, Haboudane et al. 2002, Sims and Gamon 2002, Strachan et al. 2002). The detection of other organic compounds in plants, on the other hand, is more difficult, owing to the overlapping absorption pits of chemical bonds, and their overtones (Curran 1989, Soukupova et al. 2002).

An important carbon-based compound in plants is tannin. Tannins are estimated to be the fourth most abundant biochemical produced by vascular plants (Hernes and Hedges 2000) and they play an important role in ecosystem processes. Recent studies have shown the effect that condensed tannins have on nitrogen cycling in forest ecosystems (Kraus et al. 2004a, Northup et al. 1995) and the effect of tannins in plants on herbivores: Tannins in plants influence herbivore health by controlling gut parasite infections (Min et al. 2004) and herbivore nutrient intake through the alteration of gut efficiency (Jones et al. 2001, Kondo et al. 2004, Mangan 1988, Mole 1989, Robbins et al. 1987a) and forage selection (Belovsky and Schmidt 1994, Class et al. 2003, Cooper et al. 1988, Matson et al. 2004). Knowledge about the content of tannin in vegetation, and the distribution over larger areas can therefore help us to understand ecosystem processes. Standard wet chemical techniques to determine tannin content are time-consuming and expensive, which makes attempts using these techniques to measure tannin content over large areas unrealistic. Therefore the potential to use remote sensing to map tannins was investigated.

Soukupova et al. (2002) give an overview of recent literature and wavebands identified for the detection of tannin. These bands are all located in the infrared part of the solar spectrum and have been linked to C-H and C-O bond stretching and their overtones (Ben-Dor et al. 1997, Curran 1989, Soukupova et al. 2002).

Tannin absorption pits show an overlap with lignin absorption pits (Soukupova et al. 2002). Both are based on one or more C<sub>6</sub> rings, with (C)-(O)-(H) groups attached to them, and both can form polymers (Van Soest 1987). This similarity in chemical structure results in overlapping absorption pits of both compounds and may result in a spectral shift of wavebands with maximum correlation to the compound of interest (Curran 1989). Also, studies have shown that wavebands at maximum absorption are usually not the best bands to use in prediction models, since they saturate at higher concentrations. Consequently, off-centre wavebands of the absorption feature may perform better, since they maintain their sensitivity in prediction models at higher concentrations (Curran 1989). As a consequence, a potential spectral shift of the optimal spectral bands to detect tannin occurs for different combinations of samples with variable chemical composition.

A sample dataset is assumed to be a representative subset of a real-world situation. This subset contains a random combination of concentrations of different chemical components. The bands that are selected as the optimal bands to predict the concentration of tannin in samples of the sample dataset might therefore not be the optimal bands to predict the tannin content for the whole population, and may vary between datasets. Ideally, the range of bands that are related to the concentration of the chemical of interest is estimated, so that the most robust band can be selected.

Such estimation can be achieved through bootstrapping. Bootstrapping (as defined by Efron and Tibshirani (1993), and Manly (1997)) is a resampling method that has traditionally been used to estimate statistical properties that are difficult to arrive at analytically, i.e. standard error, confidence intervals and sampling distributions of datasets. Specific biological applications of bootstrapping range from the estimation of population sizes through mark-recapture data (Buckland 1980, 1984) to the analyses of fisheries data (Swartzman et al. 1992). Recently, Mutanga et al. (In Press, 2003) used bootstrapping to estimate the correlation between reflectance parameters and the chemical content of tropical grasses. Bootstrapping simulates different combinations of samples (Efron and Tibshirani 1993, Pitt and Kreuzweiser 1998), and can help to estimate the variation in the best wavelengths to detect tannin concentration.

The aim of this study is to present a new band selection method that can overcome the problem of overlapping absorption pits. It analyses the variation in selected bands, and finds the optimal bands to detect tannins. Bootstrapping was used to estimate the variation in the spectral position of the maximum response to changes in foliar condensed tannin concentration in fresh mopane leaves, resulting from the variation in overlap between absorption features. From this distribution the most robust wavebands were selected.

## 6.2 *Methods*

### 6.2.1 *Data Collection / Study Area*

Two different datasets were used in this study. The first set is a reflectance spectrum of analytical grade pure quebracho tannin powder, measured in a darkroom, using a GER 3700 field spectrometer fixed on a tripod and fitted with a 3 degree field-of-view standard fore-optic. The GER field spectrometer (Geophysical and Environmental Research Corporation, Buffalo, New York) measures light reflectance between 350 and 2 500 nm in 646 bands, with a bandwidth of 3 nm in the visible and 10 nm in the infrared part of the spectrum. Multiple spectral measurements were taken

of the tannin, from which absolute reflectances were calculated using a calibrated Spectralon Reflectance Target (Labsphere, Inc, North Sutton, New Hampshire) as a reference. Spectra were averaged per sample and the averaged spectrum was used as a standard reflectance spectrum for tannins.

The second set of data consists of reflectance data of fresh mopane (*colophospermum mopane*) leaves, and the tannin content of these leaves. Mopane is a tree-species which occurs in near mono-specific stands over large areas in southern Africa (Mapaure 1994). Because of the importance of this tree species in these areas (Grundy 1999, Ben-Shahar 2002, Styles and Skinner 1997) it was decided to use this species for this study. Samples were collected in the Klaserie Game Reserve in South Africa, which is covered with dense mopane woodland. To maximize the range in foliar tannin content, samples were collected for plants in different phenological stages. Samples of mature green leaves, senescent leaves, dead leaves still on the plant, and brown litter were collected from multiple locations in the woodland. Piles, deep and optically dense enough to yield measurements of infinite reflectance (Gausman 1970) of these leaves were placed in the full sun in an open area of the forest where ten spectral reflectance measurements were taken of each pile, using a GER 3700 field spectroradiometer equipped with a fibre-optic cable of 1.5 m. A calibrated Spectralon reflectance target was used as reference. After the reflectances had been measured, the samples were dried and stored until laboratory analysis.

Condensed tannins were extracted from the samples according to (Hagermann 1988) and condensed tannin content of the extracts was determined using the improved acid butanol assay by (Porter et al. 1986). Tannin levels were standardized against a quebracho tannin solution of known concentration (Waterman and Mole 1994), and recalculated to quebracho tannin equivalents per gram of dried sample.

### 6.2.2 Processing of Spectra

To minimize the effect of BRDF (caused by variation in sensor angle, sun angle and target structure), the ten spectra of each sample were averaged. Bands between 1800 nm and 1970 nm and from 2200 nm to 2500 nm displayed high levels of noise, and were excluded. The resulting 545 wavebands were used for analysis. Derivative spectra are less sensitive to BRDF, and from the averaged spectra derivative spectra were calculated using an adjusted version of the seven band moving spline smoothing technique (Savitzky and Golay 1964, Tsai and Philpot 1998). Instead of smoothing the spectra first and then calculating the derivative spectra from the smoothed spectra, the parameters of the moving polynomial were used to directly calculate the derivative at the centre waveband of the moving spline window.

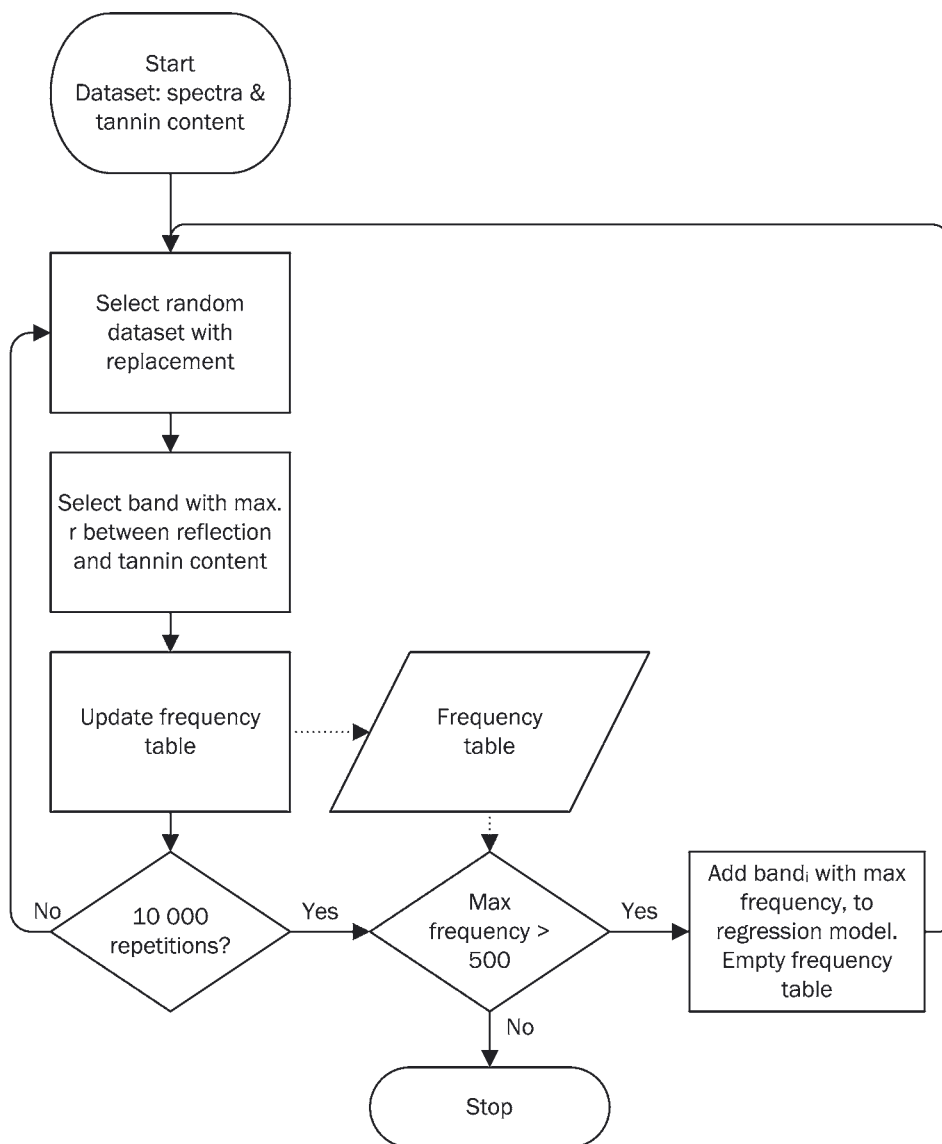
### 6.2.3 *Red Edge and 'Infrared Edge'*

The steep transition from low reflectance in the visible light to a high reflectance in the infrared present in vegetation reflectance spectra is known as the red edge, the characteristics of which (slope, position and reflectance) have been linked to absorption features of chlorophyll (Curran 1989). The position, slope and reflectance of the red edge were all included in the predictor dataset. The steep decrease in reflectance between 1200 and 1400 nm in vegetation spectra has been linked to water absorption at 1400 nm. In line with the term 'red edge', this decrease will here be referred to as the 'infrared edge', and is measured with similar characteristics. Thus the slope, inflection point, and reflection at the inflection point of the infrared edge were included in the analysis.

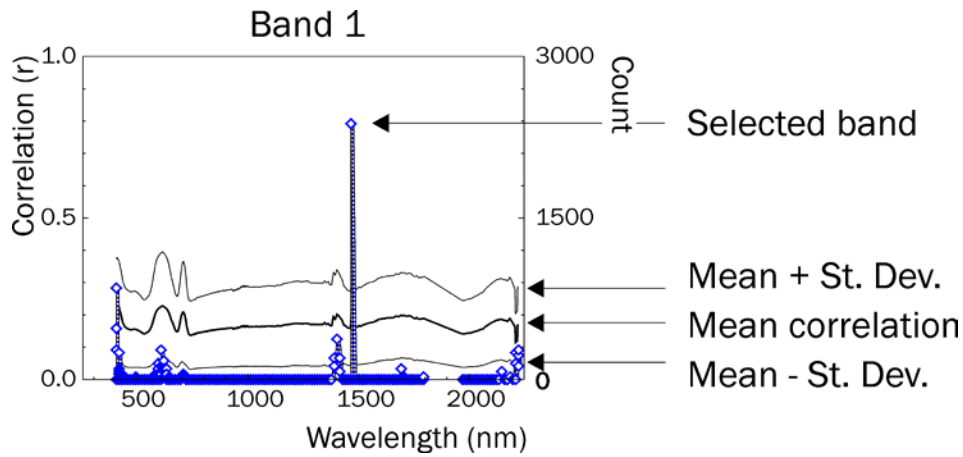
### 6.2.4 *Statistical Methods*

To select the most robust wavebands for detecting tannin content, bootstrapping was applied to the regression between tannin content and each of the wavebands. From the original dataset of 33 unique samples, a bootstrap dataset of 33 samples was selected, allowing duplicates to occur (Efron 1993). This was repeated 10 000 times (Figure 6.1), a number comparable to that suggested by (Potvink and Roff 1993) and (Pitt and Kreuzweiser 1998). For each repetition the waveband with maximum correlation to the tannin content was recorded, which resulted in a frequency table detailing the number of times that each waveband had maximum correlation with the tannin content. The band with the highest frequency after 10 000 iterations was selected, where the position of a band is defined as the central wavelength of that band.

This step was repeated in order to build a linear regression model with more than one predictor by calculating the regression goodness of fit between tannin content and the already selected band combined with each of the other bands, again selecting the band with the highest frequency of maximum correlation for 10 000 random datasets (Figure 6.1). This was repeated until the maximum number of any selected bands fell below 500 (=5%). This routine deviates from a full stepwise regression within each bootstrap repetition because the aim is to select the best waveband to use with respect to already selected wavebands.



**Figure 6.1.** Flowchart of the routine used to select wavebands. First, the correlation  $r$  between tannin content and each waveband was calculated for 10 000 random bootstrap datasets. The band that most often has the highest correlation with tannin was selected. A linear regression model between the selected band plus each of the other bands and the tannin content was calculated, and the next best band after 10 000 iterations was selected. This step was repeated until the maximum frequency of any selected band fell below 500.



**Figure 6.2.** Structure of the output plots of the bootstrapping routine: along the left y-axis the regression  $r$  between reflectance / derivative data and the tannin content (small markers: mean  $r \pm$  the standard deviation); along the right y-axis the number of times

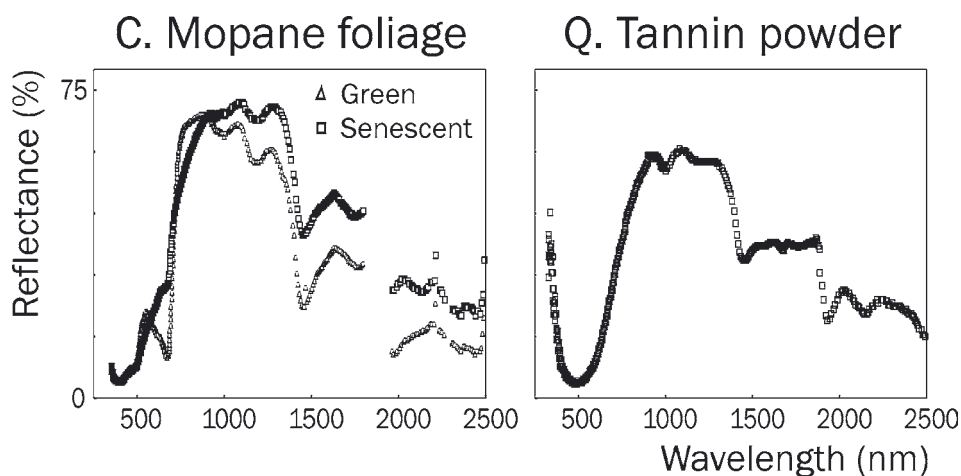
The frequencies with which individual wavelengths were selected in the bootstrap were visualized in a combined line chart with the regression goodness of fit and the standard deviation of  $r$  for each step and waveband (Figure 6.2).

A separate bootstrap routine with 10 000 iterations was applied to estimate the linear regression goodness of fit between the final bands and the tannin content of the samples.

### 6.3 Results

The reflectance of powdered quebracho tannin displayed a reflectance curve with low reflectance in the visible part of the spectrum, and a steep increase in reflection towards the infrared (Figure 6.3). Reflectance decreases between 1300 nm and 1470 nm, a transition earlier defined as the infrared edge. Distinct absorption features occur between 300 nm and 900 nm, between 950 nm and 1050 nm, from 1300 nm to 1530 nm, from 1640 nm to 1705 nm, between 1870 nm and 2020 nm, and from 2020 nm to 2230 nm (Figure 6.3).





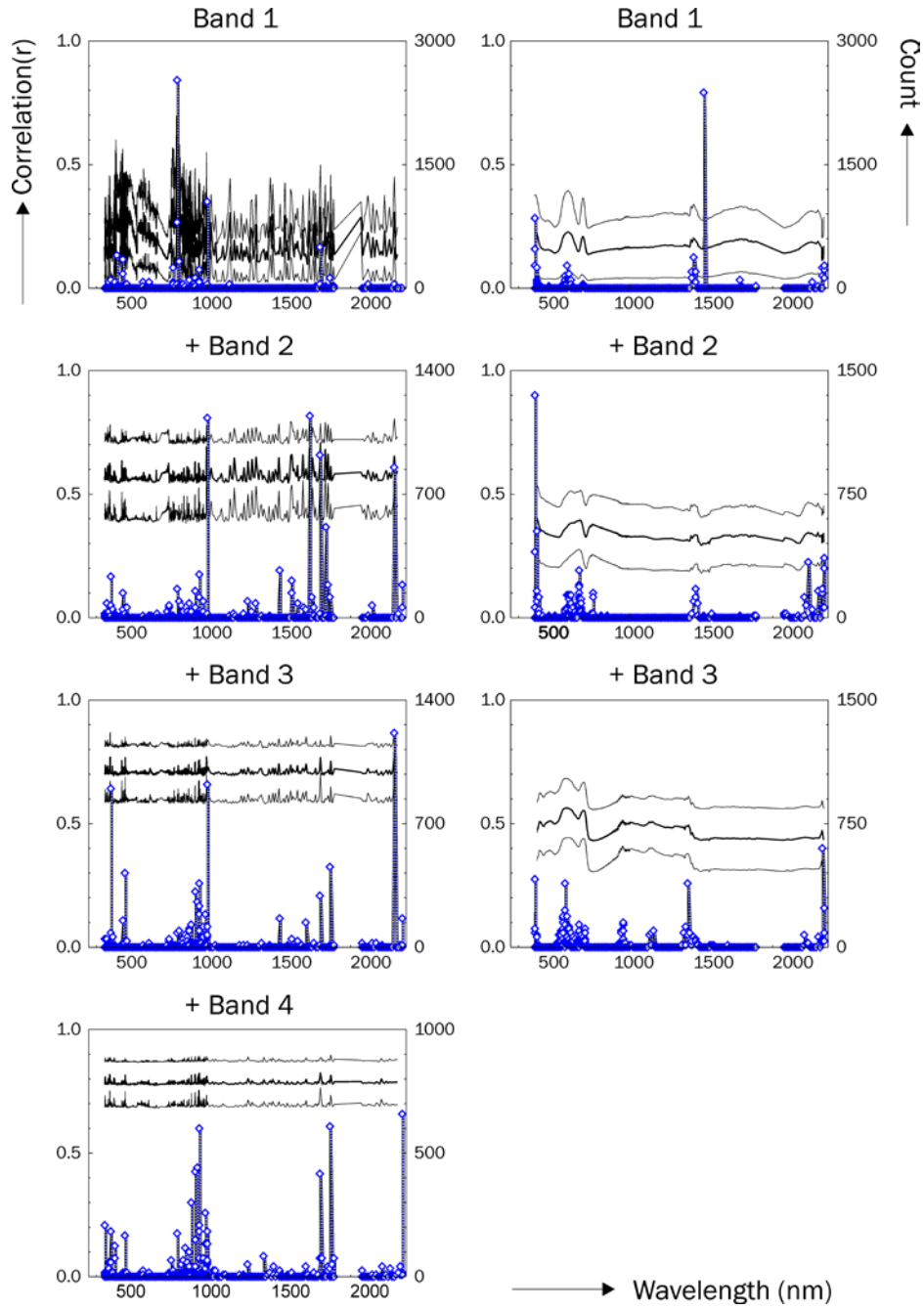
**Figure 6.3.** Mean reflectance spectra of quebracho tannin, green mopane leaves, and senescent mopane leaves.

**Table 6.1.** Mean condensed tannin content and 95% confidence limits in 'quebracho tannin equivalent grams per gram' for mopane samples in multiple phenological stadia.

State	<i>n</i>	Mean	95% CL
Green	23	0.283	0.033
Senescent	11	0.325	0.100

The condensed tannin content of mopane leaves is variable, and tannin content in senescent leaves is slightly higher, but this difference is not significant (Table 6.1).

Table 6.2 shows the selected bands for each model, the number of times each band was selected over 10 000 iterations, and the regression *r*. Chi<sup>2</sup>-tests for each selected band rejected the null-hypothesis that the frequencies of the bands with a maximum correlation were selected at random (Df = 544,  $p \leq 0.001$ ). Figure 6.4 gives an overview of the correlation between each waveband and tannin, and the frequency with which each band reached the maximum correlation over 10 000 repetitions. The derivative spectra give the best fit between the condensed tannin content of the samples and light reflection. The overall explained variance is 67% for models based on derivative spectra, using four predictor bands, while for models based on reflectance values this is 36%, for a model with three predictor bands.



**Figure 6.4.** Output plots of the bootstrapping routines for reflectance data (right column) and derivative data (left column), depicting the mean correlation value between tannin content and one band (top images), two bands (second row), three bands (third row) and four bands (bottom row) for all wavelengths.

**Table 6.2.** The selected wavebands in a bootstrapped phased regression of reflectance and derivative spectra on the concentration of condensed tannins. Bands marked with an asterisk have previously been found to be related to tannin content. (Count = number of times a band was selected as best predictor over 10 000 bootstrap iterations, RRE = reflectance at red edge position, RIRE = reflection at the infrared edge.)

<i>Input data</i>	<i>Band</i>	<i>Wavelength</i>	<i>Count</i>	<i>Model r</i>
Reflectance	$\lambda_1$	1471 *	2380	0.47±0.12
	$\lambda_2$	405	1355	
	$\lambda_3$	RRE	603	
Derivative	$\lambda_1$	803	2525	0.83±0.07
	$\lambda_2$	1640 *	1147	
	$\lambda_3$	2175 *	1212	
	$\lambda_4$	RIRE	656	

By looking at the number of times each individual band had the highest correlation with tannin content, it is possible to determine which bands, besides those selected for the model, have a strong relation with tannin content. Tables 6.3 and 6.4 give an overview of all wavebands that were selected in more than 5% of the iterations of each step in the band selection process, and the chemical bonds they have been identified with. For reflectance data, mainly wavebands that are influenced by electron transitions have been selected (Table 6.3). For derivative spectra, most bands are related to C-H, O-H or C=C bonds (Table 6.4).

**Table 6.3.** The selected wavebands (**bold**) and wavebands that were selected more than 500 times (5% of all instances) with highest response to tannin content for each phase of a bootstrapped phased regression routine using reflectance data.

<i>Wavelength</i>	<i>Count</i>	<i>Attributed to</i>	<i>Reference</i>
<b>1472</b>	2380	C-H stretch	Curran 1989
405	858	Electron transitions in chlorophyll A	Curran 1989
<b>406</b>	1355	Electron transitions in chlorophyll A	Curran 1989
414	523	Electron transitions in chlorophyll A	Curran 1989
<b>RRE</b>	603	Electron transitions in chlorophyll	

**Table 6.4.** The four selected wavebands (**bold**) and other wavebands that were selected more than 500 times (5% of all instances) with highest response to tannin content for each phase in a bootstrapped phased regression routine using derivative reflectance data.

<b>Wavelength</b>	<b>Count</b>	<b>Attributed to</b>	<b>Reference</b>
<b>803</b>	2525	Lignin / tannin	Ben-Dor 1997
993	1046	O-H stretch	Curran 1989
806	799	Lignin / tannin	Ben-Dor 1997
1711	502	C-H stretch	Ben-Dor 1997
<b>1641</b>	1147	C-H stretch, 1 <sup>st</sup> overtone	Curran 1989
993	1129	O-H stretch	Curran 1989
1711	927	C-H stretch	Ben-Dor 1997
2176	851	Aromatic C=C bond 3 <sup>rd</sup> overtone	Ben-Dor 1997
1741	518	C-H stretch, 2 <sup>nd</sup> overtone	Ben-Dor 1997
<b>2176</b>	1212	Aromatic C=C bond 3 <sup>rd</sup> overtone	Ben-Dor 1997
993	924	O-H stretch	Curran 1989
386	899	-	
<b>RIRE</b>	656	C-H deformation / stretch	
1775	611	C-H stretch 1 <sup>st</sup> overtone, O-H stretch	Curran 1989
947	602	C-H 3 <sup>rd</sup> overtone	Curran 1989

## 6.4 Discussion

Most of the bands identified by means of regression fall within the absorption regions identified in the standard reflectance curve of quebracho tannin, or can be linked to known absorption features (Table 6.3, Table 6.4). A wide variety of wavelengths at which the absorption of light energy occurs due to the stretching and bending of atomic bonds have been identified in literature (Ben-Dor et al. 1997, Curran 1989, Kokaly and Clark 1999, Soukupova et al. 2002). Six different bands in the range of 1635 nm to 1675 nm have been linked to the first overtone of C-H stretching alone (Ben-Dor et al. 1997, Curran 1989, Hernes and Hedges 2004, Kokaly and Clark 1999, Soukupova et al. 2002), which illustrates the variety in wavebands potentially related to absorption by one individual component.

In this study the first band selected using reflectance data is located at 1471 nm (Figure 6.4), which can be linked to the C-H deformation / stretching absorption peak located at 1450 nm, and falls in the centre of the 1300 nm to 1530 nm absorption feature identified in the quebracho tannin reflectance curve. Soukupova et al. (2002) found this band to be related to tannin, dissolved in 80% methanol. The two other bands (405 nm and the reflection at the red edge) cannot be directly linked to specific atomic bonds, but are related to energy absorption due to electron transitions (Curran 1989). Lignin shows absorption features in the visible part of the spectra (Elvidge 1990). The quebracho tannin curve also shows strong absorption in this region, which could explain the strong response in this region. Also, the tannin content of senescent leaves appears to be very variable (Table 6.1), which may result in increased responses to light absorption in the visible–near infrared, owing to the variation in visible reflectance for senescent leaves.

From the predictor set with derivative spectra, two bands were selected that are directly related to known absorption features. The waveband at 1640 nm is related to the first overtone of C-H atomic bond stretch. The band at 2175 nm is related to the third overtone of aromatic ring C=C bond stretching. The first band, located around 803 nm, is not directly related to a known absorption feature. However, it has the maximum correlation with tannin content for 2 525 of the 10 000 iterations, which is a very clear and strong response. Ben-Dor et al. (1997) found the slope around 800 nm to be influenced by compost maturity, and they suggested that this could be controlled through intense ultraviolet absorption by components such as lignin, tannin and pectin. Our study confirms the link between the slope of the VIS-NIR part of the spectrum and tannin content in organic matter. Reflection at the infrared edge is the fourth predictor for tannin content. This could be related to the bending of O-H bonds, which shows absorption at 1400 nm (Curran 1989). Tannins do have O-H bonds in the molecular structure, making this a realistic bond. Unfortunately reflectance in this region is influenced by water absorbance features, which makes this a less practical band to be used.

The bands selected in this study do not exactly match wavelengths identified by previous studies, but in most cases they are found within 20 nm distance of wavebands described in the overviews by Ben-Dor et al. (1997), Curran (1989), and Soukupova et al. (2002) (Tables 6.3 & 6.4). This difference may be caused by a combination of factors. Averaging and spline fitting result in the smoothing of the spectra, which can mask the signal of interest. Also all sensors experience a certain amount of sensor drift. The sensitivity and the wavelengths at which a sensor is sensitive, changes over time and with the temperature at which it is operated. This results in a slight mismatch between labels, the actual sensitivity, and the calibration files of the instrument. Only frequent re-calibration of the instrument can avoid the physical changes in sensitivity. Differences as a result of sensor temperature changes cannot be avoided.

Although for derivative spectra only four bands were included in the model, an additional seven bands were identified. Tables 6.3 & 6.4 show a number of wavebands that were not included in the initial models for detecting tannin content, but had a high frequency of select. In some of these cases the frequency difference between the selected band and non-selected bands is small. It is important to note that, although these bands were not included in the final model, they were detected by the bootstrapped phased regression as being of importance. For the reflectance data, these bands are mainly related to electron transitions in chlorophyll. But for the derivative spectra, all the bands except one (386.4 nm) are related to chemical bonds present in tannins, and have been described in earlier literature.

In this study bands from the visible part of the spectrum have been related to tannin content in leaves. To our knowledge only a few studies have included bands from this part of the spectrum (Ben-Dor et al. 1997, Elvidge 1990). This is probably because a direct link with absorption between C-O, O-H and C-H bonds is absent, and absorption in this region is related to electron transitions (Curran 1989). Spectra of purified tannin, however, show a strong absorption in this region (Figure 6.3). Therefore the use of these bands in models to predict tannin may be realistic.

The overlap in the basic shape of reflectance curves of pure tannin and vegetation is surprising (Figure 6.3). The deep absorption pit in the visible and the steep transition to the infrared part of the light spectrum have traditionally been linked to the presence of pigments and chlorophyll, this study shows a striking similarity in basic shape to a vegetation spectrum, which suggests that the presence of more structural organic components, such as tannin and lignin may influence the basic shape of foliar reflectance signatures.

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## Natural Variation in Mopane Biochemistry

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*"I have called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection"*

*C. Darwin (1809-1882)*



**Abstract**

Identification of the factors that determine the variation in browse quality, as determined by their chemical composition, is an important step towards understanding herbivore distribution patterns. Therefore the variation in leaf chemical composition (Digestibility lowering compounds: condensed tannin and total polyphenol concentration, and nutrients: nitrogen and phosphorous concentration) was related to geomorphology, vegetation structure, and fire history, in a stretch of mopane (*Colophospermum mopane*) open woodland in Kruger National Park. The results show that the principle drivers for foliar nitrogen, condensed tannins and total polyphenols differ from those for foliar phosphorus. Nitrogen, condensed tannin and total polyphenol concentrations are mainly determined by the effect of fire. The foliar concentration of phosphorus is mainly determined by parent material. This difference may be the result of differences in mobility of nitrogen and phosphorous in the soil.

*Submitted as:*

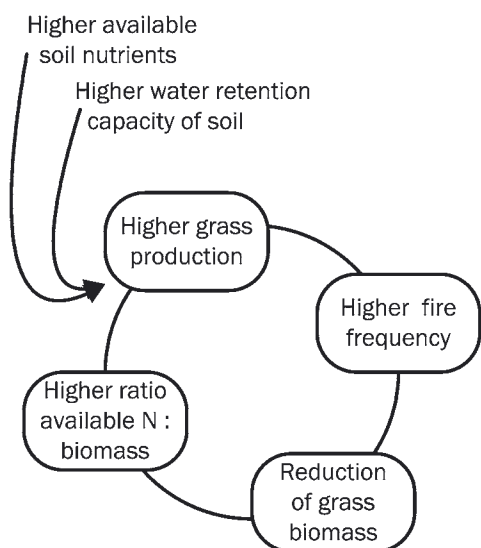
Jelle G. Ferwerda, Wouther Siderius, Sipke E. van Wieren, Rina C. Grant, Mike Peel, Andrew K. Skidmore & Herbert H.T. Prins. *Parent-material and fire as principle drivers of foliage quality in woody plants*. Ecology.



## 7.1 Introduction

By feeding on the leaves and young twigs of trees and shrubs, the nutrient intake of browsers is directly dependent on the chemical composition of these plants. Therefore, the identification of factors that determine the spatial variation of browse quality is a step towards understanding the distribution of herbivores. Here we show that the factors that drive the local variation in concentration of foliar nitrogen, condensed tannin, and total polyphenol may be fundamentally different from the factors controlling foliar phosphorus concentration in woody plants.

Major drivers of variation in vegetation structure and chemical composition have been identified, many of which operate and interact at different spatial scales. Climatic variables, such as temperature and precipitation, are known to affect vegetation quality at a landscape level (Van Soest 1987), but are not expected to vary significantly over a few kilometres, except in areas with very erratic rainfall. On this scale, surface properties such as slope and aspect are likely to be more important, affecting profile depth, water availability, and nutrient accumulation (Buol et al. 1973, Young 1976). The chemical and physical characteristics of the parent material of a soil, in combination with local climate and age of the soil, determine for a large part the soil properties, such as water retention capabilities, soil rootable depth and nutrient content (Jenny 1941), which in turn affect vegetation height and chemical composition. Plants on nutrient-rich soils are expected to display higher leaf nutrient content, and lower concentration of non-structural carbohydrates, than plants on nutrient-poor soils, as shown by Coley et al. (1985) and Kraus et al. (2004), but in contrast with results by Ferwerda et al. (Chapter 2, *In Press*).



**Figure 7.1.** The effect of soil nutrients and water availability on fire frequency through grass production. With increasing water retention capabilities or nutrient levels of soil, grass production increases, which results in higher fire frequencies, reduced standing grass biomass, and a higher available N:biomass-ratio.

The effect of greater availability of nutrients on richer soils translates into a higher production of grass, which is the main source of fuel for fire in savanna landscapes (Van Langevelde et al. 2003). As a consequence of the increased grass production, fire frequency and intensity on nutrient-rich soils are higher than on nutrient-poor soils, under the same rainfall conditions

(Fischer et al. 2003, Vigilante and Bowman 2004) (Figure 7.1). The expected effects of fire on plant chemical composition, plant growth and nutrient cycling are a reduction of aboveground biomass (Ben-shahar and Raphael 1998), accelerated nitrogen mineralization (Giardina and Rhoades 2001), increased post-fire growth (Rieske 2002), and tree mortality (Vigilante and Bowman 2004). The removal of aboveground biomass results in a general rejuvenation of the vegetation, which is expressed in short term increments in average vegetation nutrient content (Van de Vijver et al. 1999, Bennet et al. 2002), and temporal decreases in non-structural carbohydrates (Rieske et al. 2002).

Kruger National Park (KNP) in South Africa is a well-known African savanna reserve (Du Toit et al. 2003). Fire is an important tool for management, and the date, and extent of fire-events in KNP are registered in a geographical information system (GIS). The combination of a 'mosiac' burning policy, with unintentional fire events, caused by tourists or illegal immigrants, and natural fires caused by electric storms, results in a patchy distribution of fire events in both space and time. The north of KNP is dominated by open mopane woodland. The woody component of this vegetation is near mono-specific, consisting of mopane (*Colophospermum mopane*, J.Kirk ex Benth.) trees and shrubs (Venter et al. 2003). This tree species is an important browse species for elephants (Timberlake 1995), but is avoided by many herbivores, except at the end of the dry season, when refoiliation starts. The young foliage it is well sought after, probably because of the reduced foliar concentration of herbivore deterring chemicals (deterrents) such as polyphenols (e.g., condensed tannins) (Styles and Skinner 1997); Crude protein digestibility of browse is through to be reduced by condensed tannins, and browsers may avoid plants with high condensed tannin content (McLeod 1974, Provenza et al. 1990, Styles and Skinner 1996). This shift in foliage usage makes it a particularly interesting tree for studies on the factors that affect plant biochemistry.

Using an extensive field dataset, this study explains how foliar nutrient and deterrent concentration in a fire-dominated system are determined by landscape position (geology, slope, aspect, and elevation) and local fire history.

## 7.2 **Methods**

### 7.2.1 *Study area*

The study area covers 240 km<sup>2</sup> (eight by thirty km) in the north of KNP, laid out from west to east to cover the main north-south running geological formations. These are: (1) sandstone with quartzites and some andesite, (2) shales, and (3) basalts rich in olivine (Venter 1990, Venter et al. 2003). The sandstone-based areas are mainly flat; soils are deep to moderately deep, well-drained, medium and fine sands with

some admixture of coarse sand. The shale-based areas are slightly hilly and some rock outcrops occur. Soils are of variable depth and drainage, fine loamy to clayey and may show an enrichment of clay and cations in the subsoil. The area underlain by basalt is flat with poorly drained, shallow to moderately deep, vertic clays (Venter 1990).

### 7.2.2 *Data collection*

Samples of mopane were collected and dried (48 hours at 65 degrees) for chemical analysis in the peak of the growing season (From mid-January to mid-March 2003) when growth is at maximum. Plot locations were determined at random before entering the field. A plot of 15 by 15 meters to the north and east from each of the 59 plot-coordinates was laid out at each location. One foliar sample was collected of fully-grown mopane leaves for each plot, from multiple trees and shrubs of median height, at the sunlit side of the plant, between 1.50m - 2.00m. The maximum height of trees and shrubs, as well as average grass height, and total grass, shrub and tree cover were determined. The cover of herbs was generally low (< 5% of total cover) and the variation in this vegetation stratum was not analysed.

At each plot the height of ten random tufts was measured and averaged as grass height. The percentage grass and bare soil cover within a one by one metre frame, randomly thrown into the plot (Kent and Cooker 1992), was estimated by two observers. The estimates of three repetitions per plot were averaged into five percent interval classes.

Tree and shrub height were measured using a calibrated 'dimension meter' (Westfall and Panagos 1984, Smith 1996). The projected cover of trees (DBH > 2cm) and shrubs (DBH < 2cm) was visually estimated by two observers from each of the four plot corners, and averaged into ten percent interval classes. For an estimated projected cover of less than ten percent, classes of zero (no trees), one (one tree / shrub present, with low cover), and five percent (a few trees/shrubs with low cover) were included.

Parent material was determined from the geological map by Venter (1990) (datasource: KNP GIS database). Scale numbers (1 for sandstone and quartzite; 2 for shale; and 3 for basalt, were assigned based on typical weatherability and relative nutrient retention capabilities (Buol et al. 1973, Jenny 1941). The elevation, slope, and aspect were calculated from a 90-metre resolution digital elevation model.

The digital KNP fire record from 1978 to 2003 was used to calculate the fire return frequency and the number of days since the last fire for each plot using ArcGIS (ESRI).

### 7.2.3 *Chemical analysis*

Condensed tannin, total polyphenol, nitrogen, and phosphorus concentrations in ground, dried foliar samples were determined. Condensed tannin and total polyphenol were extracted following Hagerman (1998, 2002) and the condensed tannin concentration was determined following Porter et al. (1986). Total polyphenol concentration was measured following Graham (1992). Condensed tannin and total polyphenol levels were standardized against a quebracho tannin calibration series (Waterman and Mole 1994). After chemical destruction of dried samples following Novozamsky et al. (1983), N and P concentration were measured with a Skalar San-Plus auto analyser.

## 7.3 *Data analysis.*

### 7.3.1 *Data transformation*

The concentration of nitrogen, phosphorus, total polyphenol and condensed tannin was recorded as a fraction of the dry weight. Since these values are generally low (<10%), these concentrations were log-transformed to adjust for deviations of normality (Zar 1999) for ANOVA's and t-tests, after which groups did not deviate from normality (Shapiro wilks' W;  $p > 0.05$ ).

### 7.3.2 *Fire effects*

The effect of fire was assessed in two ways: Firstly the effect of individual fire events, measured as time passed since the last fire event, and secondly the effect of re-occurrences of fire, measured as fire frequency. Therefore the data was split: plots with frequent fires (more than 6 fire events in 25 years,  $n=27$ ) and less frequent fires (less than 6 fire events,  $n=32$ ). A t-test was used to test for differences in chemical content. A Spearman rank correlation coefficient between the tree height and cover, and fire return frequency was calculated to test for the effect of fire on vegetation height and density. Related to 'fire frequency' is the 'time passed since last fire'. The sample set was split: plots that were burnt after the 2002 growing season ( $n=34$ ), and the rest ( $n=25$ ). Using a t-test, the difference in condensed tannin, total polyphenol, nitrogen and phosphorus was determined.

### 7.3.3 *Parent material*

The effect of parent material on leaf chemical composition was tested with an ANOVA. We tested for the interaction between parent material (3 classes), and time passed since the last fire event (2 classes) and fire return frequency (2 classes).

#### 7.3.4 Relations

The correlation between foliar concentration of nitrogen, phosphorus, total polyphenol, and condensed tannin and the measured environmental factors was calculated. After transformation, several parameters (tree height, time since fire and fire frequency) deviated from normality (Shapiro Wilks' W test:  $p \leq 0.05$ ). Therefore a Spearman rank correlation test was used. The number of interacting variables was reduced using a Principle Component Analysis. Principle component transformation on the reduced dataset (grass, shrub, and tree height and cover, and bare soil percentage as active variables) determined the most important relations with the chemical composition of foliage. These relationships were combined into a path-diagram for nitrogen, total polyphenol and condensed tannin, depicting the strength of relations between individual chemical components and the environmental factors.

## 7.4 Results

**Table 7.1.** Effect of fire return frequency, and the time passed since the last fire event on the chemical composition of mopane tree leaves. Given values: mean concentration in % (nitrogen, phosphorus), and quebracho tannin equivalents  $g\ g^{-1}$  (total polyphenols and condensed tannins) and levels of significance of differences (\*:  $p \leq 0.05$ , \*\*:  $\leq 0.01$ , \*\*\*  $\leq 0.001$ , ns = not significant) as a result of a Tukey t-test on log-transformed data.

	Fire return frequency			Time since last fire		
	High	Low		<1 wet season	>1 wet season	
n	27	32		34	25	
Condensed Tannin	0.213	0.258	***	0.222	0.256	*
Total Polyphenol	0.031	0.046	***	0.034	0.046	***
Nitrogen	2.21	1.91	***	2.14	1.93	*
Phosphorus	0.186	0.205	ns	0.178	0.226	ns

#### 7.4.1 Fire effects

The effects of fire return frequency and the time passed since the last fire event were significant for all chemical components, except for phosphorus (Table 7.1). Foliar condensed tannin and total polyphenol concentration was higher on sites where fire frequency is low than where fire frequency is high, and lower on sites with more

recent fire events (Table 7.1). For nitrogen this relation was inverted (Table 7.1). The time passed since had a positive relation with grass, shrub, and tree height and cover, except for shrub cover (Table 7.2). For fire return frequency, this relationship was inverted (Table 7.2).

**Table 7.2.** Spearman rank correlation coefficients and, if significant, levels of significance (\*:  $p \leq 0.05$ , \*\*:  $\leq 0.01$ , \*\*\*:  $\leq 0.001$ ) between plot structural variables, continuous environmental variables and the concentration of nutrients and deterrents in mopane leaves. CT: Condensed tannin content, TP: Total polyphenol content, TSF: Time passed since the last fire event, FRF: Fire return frequency, Tree H: Tree Height, Tree C: Tree Cover.

	Tree H	Tree C	TSF	FRF	Elevation	Aspect	Slope
CT	0.31 **	0.35 **	0.38 **	-0.39 **	-0.05	0.10	0.04
TP	0.37 **	0.37 **	0.46 **	-0.54 ***	0.13	-0.06	-0.13
N	-0.17	0.25 *	-0.39 **	0.30 *	-0.03	-0.07	0.01
P	-0.47 ***	-0.48 ***	-0.06	0.34 **	-0.04	-0.05	-0.32
Grass	Height		0.43 **	-0.46 ***	0.10	-0.22	-0.03
	Cover		0.46 ***	-0.40	-0.03	-0.16	0.06
Shrub	Height		0.37 **	-0.36 **	-0.09	0.08	0.16
	Cover		-0.13 *	-0.19	0.03	0.03	0.09
Tree	Height		0.37 **	-0.59 ***	0.08	-0.04	0.26 *
	Cover		0.42 ***	-0.53 ***	-0.01	0.08	0.33 **
Bare	Soil		-0.59 ***	0.49 ***	-0.02	0.08	-0.22

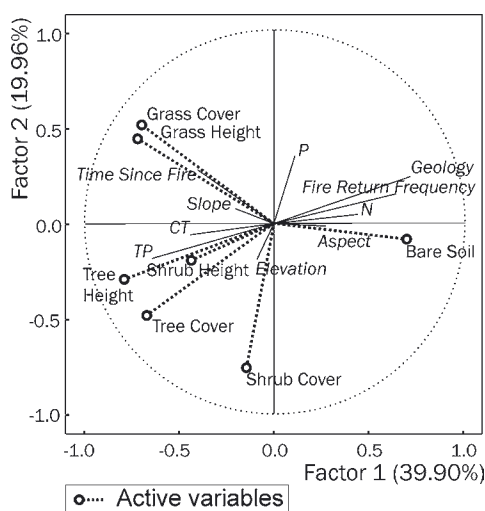
#### 7.4.2 Parent material

The effect of parent material, and the interaction with time since fire, were only significant for foliar phosphorus concentration (foliar phosphorus was higher on nutrient-rich (basalt) than on nutrient poor (sandstone, shale) soils, ANOVA;  $p \leq 0.05$ ; Foliar phosphorus was lower in trees on nutrient rich soils with recent fire than other trees on nutrient rich soils, ANOVA;  $p \leq 0.01$ ). The interaction between parent material and fire return frequency was biased (more samples on nutrient-rich sites with high fire frequency), and group differences could not be calculated.

#### 7.4.3 Relations

The correlation with the time since fire was positive for condensed tannin, total polyphenol and phosphorus, and negative for nitrogen. The correlation between the fire return frequency, and all measured chemical components was significant (Table 7.2); negative for condensed tannin and total polyphenol, and positive for nitrogen and phosphorus.

There is no significant correlation between the chemical constituents and elevation, aspect or slope of the plots, except for the relation between phosphorus and slope, which was negative. Tree height showed a significant correlation with condensed tannin, total polyphenol, and phosphorus content (Table 7.2) and tree cover with condensed tannin, total polyphenol, phosphorus, and nitrogen content (Table 7.2). For both factors the same relationship occurred: with increasing tree height and cover, leaf condensed tannin content and leaf total polyphenol content increase, while leaf nitrogen and phosphorus content decrease.

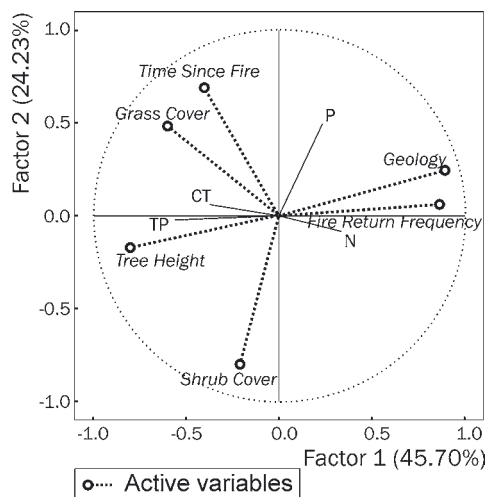


**Figure 7.2.1.** X-Y vector plot of factor loadings for the chemical composition of mopane samples, and each explanatory factor. Plot structure was used to define the principle component space (Active factors). The x-axes display factor loadings (correlations) for each explanatory factors along the first PCA factor, the y-axes display the factor loadings for the explanatory factors along the second PCA factor.

#### 7.4.4 Reduction of variables

Most of the variation in vegetation structure was captured by the first two principle component axes (Figure 7.2.1). The first axis shows a strong relation to tree height, cover and bare soil. The second axis was mainly defined by differences in grass and shrub cover. The effect of slope, aspect, and elevation on the structure of vegetation was minimal (Figure 7.2.1).

Slope, elevation, and aspect do not explain much of the plot structural variation (Figure 7.2.1); grass height, tree cover and, shrub height had a strong relation to grass-cover, tree-height and shrub cover (Figure 7.2.1) while bare soil was a pseudo variable, related to grass, shrub and tree cover. These variables were removed from the analysis. A new set of principle component axes was defined with vegetation structure and environmental variables as active variables (Figure 7.2.2). Foliar nitrogen had a weak but positive relation to geology and fire return frequency. For condensed tannin and total polyphenol this relation was reversed. Foliar phosphorus showed a weak relation to parent material and time since fire.



**Figure 7.2.2.** X-Y vector plot of factor loadings for the chemical composition of mopane samples, after reducing the number of explanatory factors. Plot structure, fire history and parent material were used to define the principle component space (active factors). The x-axes display factor loadings (correlations) for each explanatory factors along the first PCA factor, the y-axes display the factor loadings for the explanatory factors along the second PCA factor..

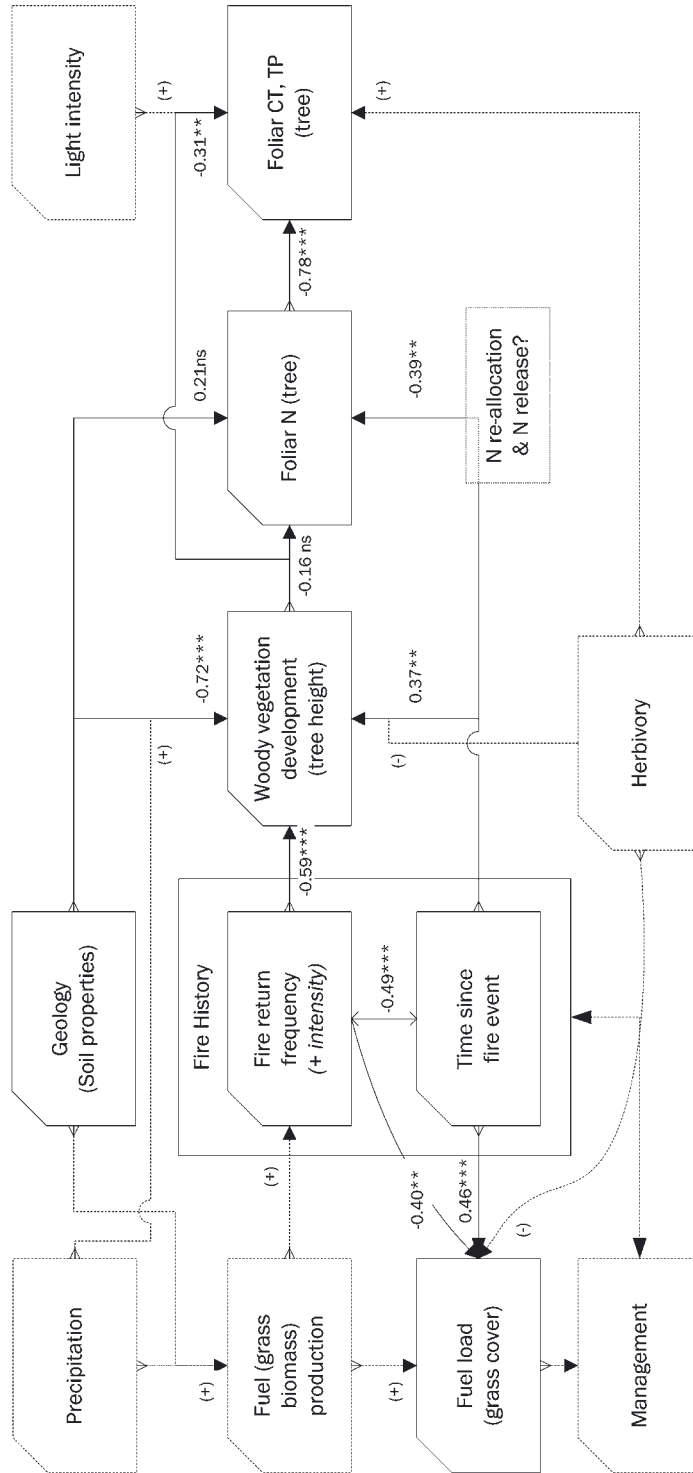
## 7.5 Discussion

This study used a snapshot observation of the actual processes involved, and agrees with studies that show, in post-fire vegetation, increments of foliar nitrogen (Rieske 2002) and decreases of the non-structural carbohydrates condensed tannin and total polyphenol (Rieske 2002). We find, in a PCA that links vegetation structure and environmental variation to foliar chemical concentration (Figure 7.2.2), that foliar P is not related to foliar nitrogen, condensed tannin and total polyphenol concentration. This indicates that variation in foliar phosphorus is driven by factors other than those controlling foliar nitrogen, condensed tannin and total polyphenol.

Individual fire events may result in a reduction of grass, shrub and tree height and cover, with increased foliar nutrient concentration. In this study however, the effect of fire frequency is stronger than that of individual fire event (Figure 7.2.2, Tables 7.1 & 7.2). An increase in fire frequency results in increased N concentration and decreased non-structural carbohydrate (total polyphenol and condensed tannin) concentrations (Tables 7.1 & 7.2). This carbohydrate decrease is most likely related to increased demand of carbon for biomass production as a result of increased nitrogen availability (Bryant et al. 1983, Coley et al. 1985, Coley 1988). This may result in a reduction of available carbon to produce condensed tannins and other phenolic compounds (Jones and Hartley 1999).

In an ANOVA, the effect of parent material on foliar condensed tannin, total polyphenol and nitrogen is not significant. However, fire frequency is related to geology (Figure 7.2.2). This is the result of increased grass production on nutrient-rich soils, which results in increased fire frequencies (Figures 7.1 & 7.3; Van Langevelde et. al., 2003).





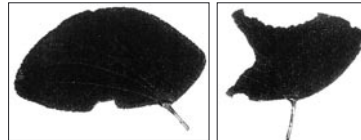
**Figure 7.3** . Overview diagram of the interaction between environmental factors, vegetation structure and mopane (*Colophospermum mopane*) chemical composition. The strength of relations was calculated using a spearman rank correlation test (levels of significance: \*\*\*:  $p \leq 0.001$ ; \*\*:  $p \leq 0.01$ ; \*:  $p \leq 0.05$ ; ns: not significant). Solid lines depict variables and relations which have been observed / calculated. Dashed lines depict other important factors affecting vegetation structure, fire or the chemical composition of mopane leaves, but have not been measured. Where possible the direction of the effect (positive: + or negative: -) has been included in the diagram.

Figure 7.3 shows that, although fire reduces woody plant height, this does not affect nitrogen content. There is however a direct effect of fire on foliar nitrogen. This may be related to increased nitrogen mineralization in the soil or a general rejuvenation of plant material.

Foliar phosphorus shows a different relation to environmental factors than foliar nitrogen, and to keep figure 7.3 clear, the relation with foliar phosphorus was not included. In contrast to nitrogen, foliar phosphorus is affected by parent material but not by the time since the last fire event. This probably reflects a difference in mobility of nitrogen and phosphorus in the soil. After mineralization of organic material, nitrogen mainly occurs in mobile  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , which is easily absorbed by plants (Sumner 1999). Soil phosphorus however is quickly immobilized by Al, Fe, Mn and Ca in the soil, a process which is strongly affected by soil properties (Sumner 1999).

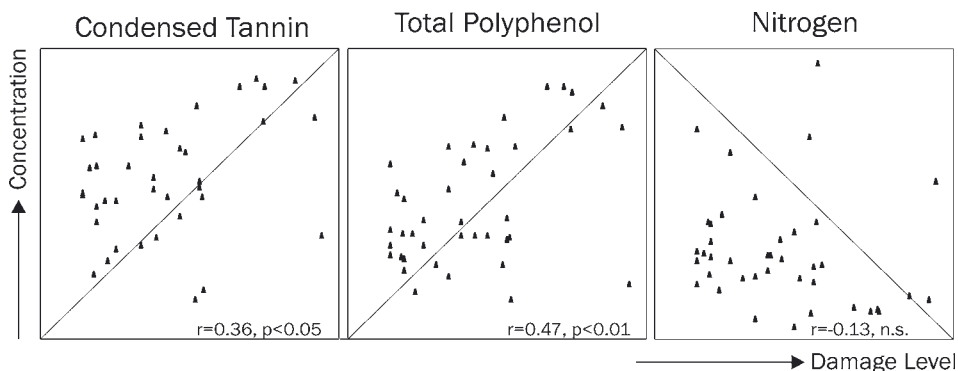
## Box 1. Herbivory and Plant Chemical Composition.

The concentration of phenolic compounds in vegetation is hypothesized to be induced by herbivory. These increased levels of phenolic compounds in heavily browsed vegetation are thought to reduce herbivory. To test whether induction takes place, the relation between post-herbivory chemical composition and the percentage of leaf surface removed by herbivores was therefore calculated for 38 samples from chapter 8.



**Figure b.1.** Damage levels of mopane foliage

For each sample, the percentage of leaflets in the sample of which more the 10% of the leaf surface was removed (Figure b.1) was calculated, and correlated to the foliar concentration of condensed tannin, total polyphenol, and nitrogen of the samples.



**Figure b.2.** Relation and correlation  $r$  between damage levels and foliar chemical concentration of mopane leaves.

### Results

A significant relation occurred between deterrent content, and the damage inflicted on leaves (Figure b.2). This may indicate a response to herbivory, as described in a number of previously mentioned publications. There is no significant relation between nutrient content, and damage on the foliage. These results suggest that a post-herbivory increase of phenols in mopane may occur, which is in contrast to results found in chapter 2. There leaves were removed, but relative little damage was done to the plant. Perhaps the amount of damage to the foliage is more important than the amount of foliage which is removed. This could result in a stronger response when damage is inflicted by insects, than when leaves are manually removed, as done in the experiment described in chapter 2.



# 8

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## Mapping Phenolic Compounds in Mopane Woodland

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*"It is during our darkest moments that we must focus to  
see the light"*

*A. Onassis (1906-1975)*



**Abstract**

Knowledge on the spatial variation in herbivore deterring compounds in foliage may help to understand herbivore distribution over the landscape. Therefore we selected wavebands related to condensed tannin and total polyphenol from established literature. This resulted in seven wavebands to be used for prediction of condensed tannin concentration in mopane from hyperspectral imagery. The centre of the wavebands that were used to predict condensed tannin and polyphenol concentration in mopane were located at: 710 nm, 803 nm, 1641 nm, 1714 nm, 1738 nm, 2146 nm and 2182 nm. The final map for condensed tannin concentration has a mean prediction accuracy (correlation  $R^2$ ) of  $0.54 \pm 0.26$ , based on a 5 000 iteration bootstrapping validation routine. For the total amount of polyphenols the optimal model resulted in a mean predicted  $R^2$  of  $0.51 \pm 0.17$ . In these maps clear differences between regions with high and low concentrations can be distinguished.

*In preparation as:*

Andrew K. Skidmore, Jelle G. Ferwerda, Karin Schmidt, Onesimo Mutanga & Herbert H.T. Prins. *Mapping vegetation quality. State of the art in remote sensing*. Nature.

Jelle G. Ferwerda, Herbert H.T. Prins & Andrew K. Skidmore. *Mapping non-structural carbohydrates in Mopane woodland using HyMap hyperspectral imagery*. Remote sensing of Environment.

## 8.1 Introduction

Knowledge on the spatial variation in chemical composition of vegetation may help to understand herbivore distribution. In most ecosystems overall herbivore density is not limited by resource quantity (Hairston et al. 1960). As a consequence, herbivores may select high quality forage, while avoiding other, less nutritious, plants. The quality of resources can be seen as the total concentration of nutrients available to the herbivore. This is on the one hand determined by the concentration of nutrients (N, P, Ca, Mg) in the vegetation. On the other hand, nutrient uptake may be hindered by digestibility lowering compounds such as condensed tannins and lignin.

Although it has been shown that herbivores may select for low concentrations of tannin in their diet (Class et al. 2003), higher concentrations of condensed tannins are known to reduce digestibility of plant material through the formation of chemical complexes with proteins (Van Soest 1987). Gut enzymes and bacteria are unable to digest these complex chemicals, and the protein may be lost through the faeces (Van Soest 1987). A number of studies have recently shown that some herbivores are capable of selecting resources with low concentrations of condensed tannins, and avoid forage with high concentration of condensed tannins (Albrechtsen et al. 2004, Matson et al. 2004, Provenza et al. 1990). Therefore, knowledge on the spatial variation of foliar tannin concentration may help to understand herbivore distribution. Although measuring the chemical composition of foliar samples using laboratory techniques is possible, it is not realistic to attempt to map the spatial variation of the individual concentrations at a high spatial resolution, over larger areas. Therefore new techniques are needed to analyze vegetation chemical properties over large areas.

Over the past decades, hyperspectral remote sensing has developed from a laboratory-based technique, used to measure the chemical composition of standardized, dried and ground samples (Marten et al. 1985), to an experimental technique used to measure vegetation characteristics from airplanes and satellites (Schmidtlein and Sassini 2004, Secker et al. 2001, Underwood et al. 2003, Williams and Hunt 2002). In a recent study, Mutanga and Skidmore (2004a) showed that it is possible to map variation in nitrogen content in savanna vegetation by using a neural network to relate grass nitrogen concentrations to HyMap imagery. In the same study, it was shown that neural networks are better at modelling the relation between reflectance data and the nitrogen content of vegetation than standard linear regression models, which can be explained by the ability of neural networks to model potential non-linear relationships between the chemical composition and the reflectance properties of the surface (Zhang et al. 2002).

One important step towards linking the chemical concentration of vegetation to remotely sensed data is the selection of meaningful wavelengths to include in the model. Commonly, studies that relate the chemical composition of foliage to hyperspectral data use stepwise regression techniques to select the optimal

wavebands to use (Curran 1989, Gastellu-Etchegorry and Bruniquel-Pinel 2001, Mutanga et al. 2003). A major drawback of this method is the potential lack of causality in the derived model (Curran 1989). By combining the known relationships between chemical composition and reflectance properties, generated in other studies, it is possible to select wavebands that may be more universal, and may represent causal relations. For example, Curran (1989) generated an overview of wavebands related to a wide range of chemical components in fresh vegetation, and the related chemical bonds. More recently, Soukupova et al. (2002) did the same, specifically for condensed tannins and lignin. Using a bootstrapping routine, Ferwerda (Submitted; Chapter 6) determined a number of bands related to condensed tannins in fresh foliage of *Colophospermum mopane*, and linked the selected bands to the overviews by Curran (1989) and Soukupova (2002). The combined information from these studies provide spectral regions that have a causal relation to condensed tannins in vegetation. Condensed tannins are part of a larger group of non-structural carbohydrates, the polyphenols (Levin 1971, Van Soest 1987). The compounds in this family contain the same chemical bonds, and share the same basic chemical structure (Van Soest 1987). As a consequence they may show the same absorption features, and it may be useful to simultaneously look at condensed tannins, and the total polyphenol concentration

Several studies have shown that normalizing the reflectance spectra improves the stability of the derived models (Curran et al. 2001, Elvidge and Chen 1995, Kokaly and Clark 1999). One way to normalize reflectance spectra is by calculating the derivative spectra (Tsai and Philpot 1998). The derivative spectrum reflects the rate of change in reflectance values from band to band, resulting in a signal of which the individual absorption features are enhanced (Tsai and Philpot 1998). In a previous study, derivative spectra showed a stronger relation to condensed tannin content in mopane than reflectance spectra (Chapter 6)

The aim of the current study is to test whether the neural network method (Mutanga and Skidmore 2004a, Secker et al. 2001, Underwood et al. 2003, Williams and Hunt 2002) can be extended to other compounds, and can be used to map foliar condensed tannin concentration in an open mopane woodland.

## 8.2 Methods

### 8.2.1 Study area

The study was performed as part of a wider study on the ecological and chemical characteristics of mopane (*Colophospermum mopane*) open woodlands in Kruger National Park, South Africa. The study area consists of an area of approximately eight by thirty kilometres, laid out from east to west across the north of Kruger National



Park, South Africa. In the east, the vegetation is dominated by dense shrubland (1-4 meters tall) with solitary trees on basalt-based soils. The western part of the study area is characterised by fragmented mopane woodland on granite and shale-based soils.

#### 8.2.2 *Validation*

At the peak of the 2003 growing season (between mid-January and mid-March), 63 samples were collected from the same number of plots in the study area. Plot locations were determined before entering the field, using a random number generator, linked to the fieldwork coordinate range. Using a compass and measuring tape, a plot of 15 m by 15 m was laid out to the north and east from each of the plot-coordinates. At each plot, one mixed sample was collected of fully-grown mopane leaves, from multiple trees at the sunlit side of the plant, at 1.50 m to 2.00 m height.

#### 8.2.3 *Chemical Analysis*

The collected foliar samples were dried for 48 hours at 70 degrees, and transported to the Netherlands for chemical analysis. Condensed tannins and total polyphenols were extracted from the samples according to Hagermann (1988) and condensed tannin content of the extracts was determined using the improved acid butanol assay by Porter et al. (1986). Total polyphenol concentration was measured using the modified Prussian blue assay for total polyphenols by Graham (1992). Condensed tannin and total polyphenol levels were standardized against a quebracho tannin solution of known concentration (Waterman and Mole 1994) and recalculated to grams quebracho tannin equivalents per gram of dried sample (QT in  $\text{g g}^{-1}$ ).

#### 8.2.4 *Image acquisition and processing*

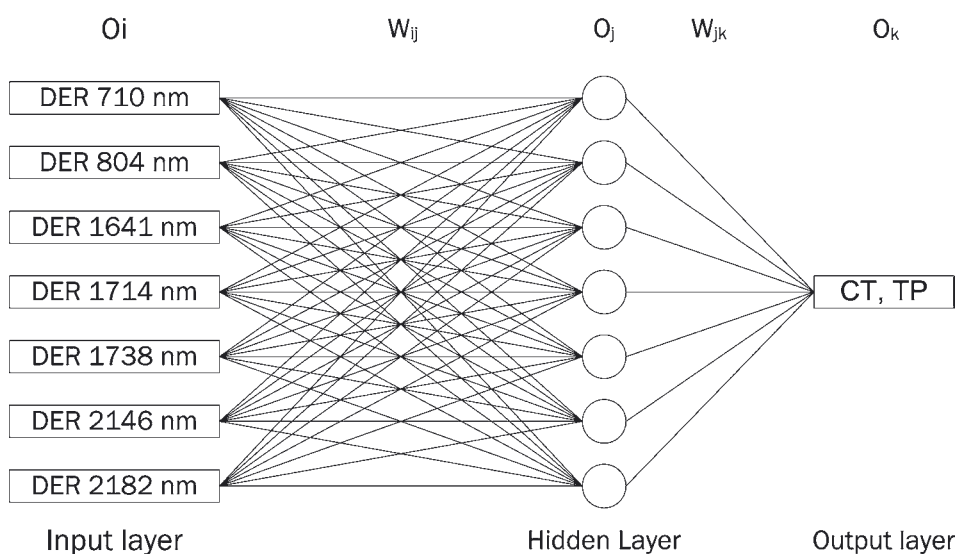
On the 18<sup>th</sup> of March 2003, HyMap MK1 hyperspectral imagery was acquired by 'De Beers' mining company (South Africa). The scanner was commissioned from an Australian consortium consisting of Auspace, CSIRO Division of materials, and Integrated Spectronics Ltd. To avoid problems with cloud cover, which at this time of year normally occurs around midday, the imagery (pixel size: 4.2 m x 4.2 m) was flown in the morning instead of at solar noon. The scanner was mounted on a Zeiss stabilized platform in order to minimize distortions due to yaw, pitch, and roll. To enable geometric correction, GPS and internal navigation gyros were attached to the system. 'De Beers' performed the required geometric and atmospheric corrections. Atmospheric correction was performed using the package ACORN (Atmospheric CORrection Now), which uses the molecular and aerosol scattering model Modtran 4 to transform the measured radiance at sensor into apparent surface reflectance (an approximation of actual surface reflectance).

### 8.2.5 Band selection

First derivative spectra were calculated from the reflectance spectra in the atmospherically corrected and geo-coded images using the ENVI spectral derivative tool. Although the neural network used in this study can select the bands with the strongest relation to the chemical components by adjusting weighting variables, and ignoring non-significant relations, this may result in the inclusion of bands, which have no causal relation to the component of interest. Therefore specific bands were extracted to be included in the neural network structure. The selected bands were based on known absorption features of condensed tannins or phenolic compounds as described in previous studies (Curran 1989, Soukupova et al. 2002, Windham et al. 1988, Ferwerda Submitted; Chapter 6).

### 8.2.6 Neural Network

Artificial neural networks are neurologically inspired statistical learning mechanisms (Rojas 1991), which have recently been applied in the retrieval of canopy variables (Abuelgasim et al. 1998, Atzberger 2004) and for classification routines (Augusteijn and Folkert 2002). Here we have used a one-layer feed-forward, error-back propagation artificial neural network implemented in the Interactive Data Language (IDL) (Skidmore et al. 1997), to predict condensed tannin and total polyphenol as continuous variables, using derivative spectra as input data (Figure 8.1).



**Figure 8.1.** The neural network structure that was used in this study. The input layers ( $O_i$ ) are connected to the hidden layer ( $O_j$ ) via weighted links ( $W_{ij}$ ). The output layer  $O_k$  is calculated by evaluating the weight values  $W_{jk}$  with the nodes in the hidden layer ( $O_j$ )

The following section is based on Rojas (1991) and Skidmore et al. (1997); see figure 8.1. The learning process of this network consists of two phases. During the first phase, the forward phase, the input values  $O_i$  (here the derivative reflectance values) of a set of training pixels are multiplied with the weight value  $W_{ij}$  for each node of the hidden layer (Equation E.8.1), and the resulting value  $Z_j$  for each node of the hidden layer is passed through a sigmoidal activation function, in order to add non-linearity to the model (Equation E.8.2).

$$Z_j = \sum_i w_{ij} * o_i \quad (\text{E.8.1})$$

$$O_j = \frac{1}{1 + e^{-(z_j + \theta)/\theta_0}} \quad (\text{E.8.2})$$

The values for each output node  $O_j$  in the hidden layer are multiplied by the weight values  $W_{jk}$  to calculate the output value,  $O_k$ . This represents the end of the feed-forward phase.

In the next phase, the back propagation phase, the root mean square error of the predicted value is calculated based on the difference between the desired (input) values, and the produced (output) values in the training dataset. This information is fed back into the system, and the weighting values are adjusted to reduce RMSE, after which the forward phase starts again. This full cycle is called one epoch or iteration, and is repeated until the systems' root mean square error falls below a predefined threshold, or until the maximum number of epochs is reached.

### 8.2.7 Optimizing neural net settings

The performance of a neural network depends strongly on the initial settings for the learning rate and momentum (Skidmore et al. 1997). If the learning rate is too high, the network may become unstable. Momentum is a term added to the learning rate to incorporate the previous changes in weight with the current direction of movement in weight space, which allows the system to learn faster, and reduces the risk of system instability (Skidmore et al. 1997). During training, the system error decreases, and training accuracy increases. However, predictive capabilities only increase until an optimum is reached, after which predictive capabilities decrease, and overtraining commences. Overtraining occurs when the network memorizes the details of the training dataset, but is unable to generalize the relations when applied to a different dataset. To find the optimal combination of learning rate, momentum, and number of epochs to predict condensed tannin and total polyphenol concentration, the neural network was run for a variation of combinations of number of epochs, learning rate, and momentum. The final output of a neural network training session is affected by the starting weights of the network, which are randomly assigned at the start of

each run. Therefore the network was run 25 times for each combination of settings, and the mean prediction accuracy, as well as the maximum prediction accuracy achieved, were compared to select the optimal combination of learning rate, number of epochs, and momentum.

As described in Skidmore (1997), the training speed of neural networks models can be improved by normalizing the input data between 0 and 1, according to formula (E.8.3), ( $C_{input}$  = normalized input concentrations,  $C_{original}$  = Measured concentration,  $C_{min}$  = Minimum observed concentration, and  $C_{max}$  = Maximum observed concentration).

$$C_{input} = \frac{C_{original} - C_{min}}{C_{max} - C_{min}} \quad (E.8.3)$$

To test the prediction accuracy of the neural network, the dataset was randomly split into two for each run, with 75% of the samples ( $n=48$ ) to train the network, and 25% of the samples ( $n=15$ ) to subsequently test the prediction accuracy of the trained network. This accuracy was calculated as the correlation  $R^2$  between the predicted and measured concentration of the samples. The optimal combination of training rate, number of iterations and momentum was subsequently used to calculate the concentration of condensed tannin and total polyphenol for each pixel in the image.

### 8.2.8 Masking

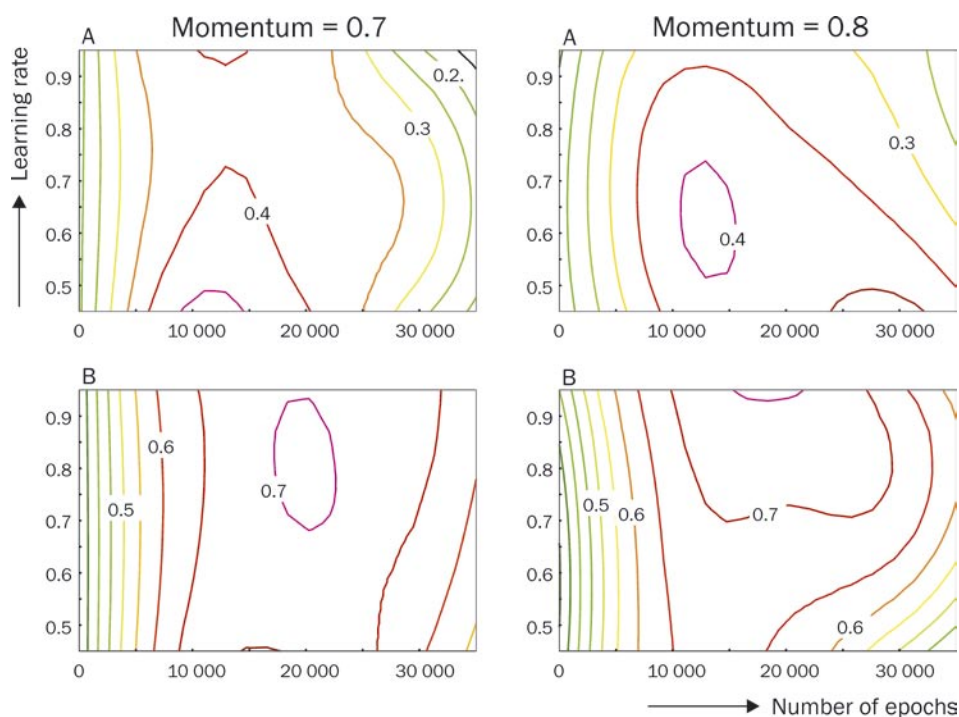
Previous studies have shown that the relation between reflectance properties and chemical composition is not uniform between species (Chapters 4 & 5). Therefore, the non-mopane pixels in the image were masked using a classified image, which was obtained through a maximum likelihood classifier routine using field-plot descriptions for validation.

## 8.3 Results

### 8.3.1 Band selection

Studies have shown that wavebands at maximum absorption are usually not the best bands to use in prediction models, because they may saturate at higher concentrations. Consequently, off-centre wavebands of the absorption feature may perform better (Curran 1989) resulting in selected wavebands that are not located

at the centre of the absorption features, but next to them. Therefore, and because the spectral resolution of the HyMap scanner does not match the spectral resolution of handheld scanners used in the published studies, slight deviations from the described wavebands occurred.

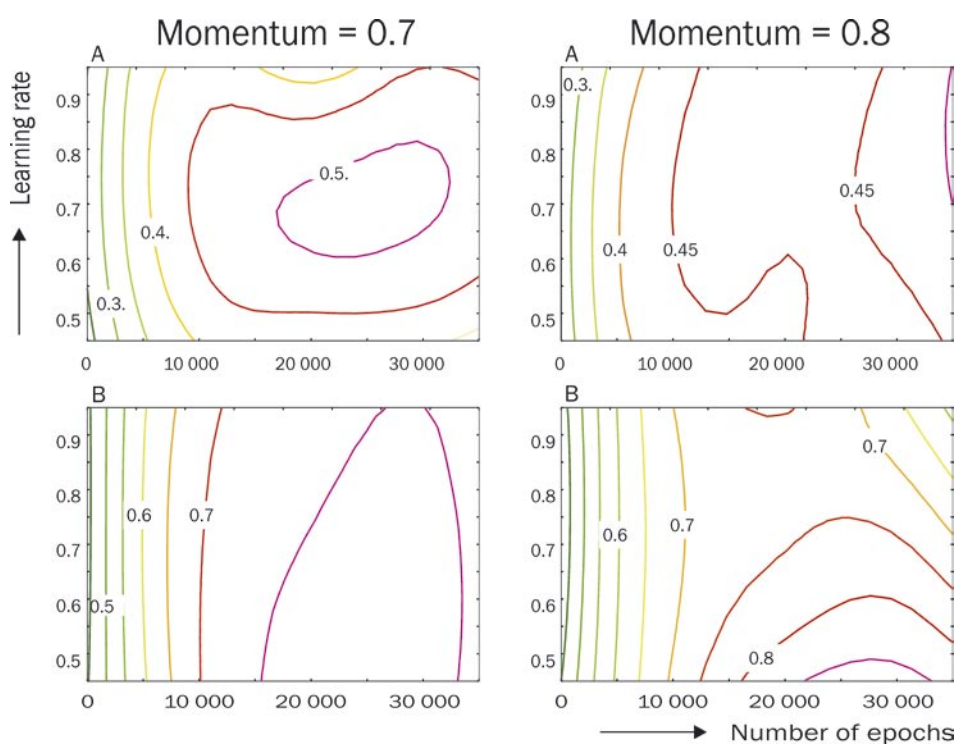


**Figure 8.2.** Relation between learning rate, number of epochs, momentum and obtained prediction accuracy of condensed tannin. Prediction accuracy was obtained after running the network with each combination of settings for 25 times, and calculating the mean (A) and maximum (B) correlation  $r$  between the actual concentration of an independent test dataset ( $n=15$ ) and the concentration predicted by the neural network

The following bands were selected:

- 710 nm** This is the centre of the Red Edge. This transition of red to infrared reflectance has in a previous study been found to have strong relation to condensed tannin (Ferwerda Submitted; Chapter 6).
- 804 nm** Ben-Dor et al. (1997) concluded that the reflection at 800 nm might be related to absorption by condensed tannins, and also in a previous study in the reflectance properties of mopane (Ferwerda Submitted; Chapter 6) this band was found to be related to condensed tannins.
- 1641 nm** Soukupova et al. (2002) described an absorption feature related to condensed tannins at 1660 nm, and at 1635nm. In a previous study we found a strong relation between 1641 nm and condensed tannin concentration in Mopane foliage (Ferwerda Submitted; Chapter 6).

- 1714 nm Soukupova et al. (2002) described absorption in the region around 1715 nm to be related to condensed tannin concentration.
- 1738 nm This band is located between the two main absorption features for C-H bonds, located at 1690 nm and 1780 nm (Curran 1989).
- 2146 nm Soukupova et al. (2002) found a strong relation between absorption 2143 nm to 2167 nm and condensed tannin concentration.
- 2182 nm Absorption at 2179 nm was described by Soukupova et al. (2002), and Ferwerda (Submitted; Chapter 6) found the derivative at 2175 nm to have good relation to condensed tannin concentration.



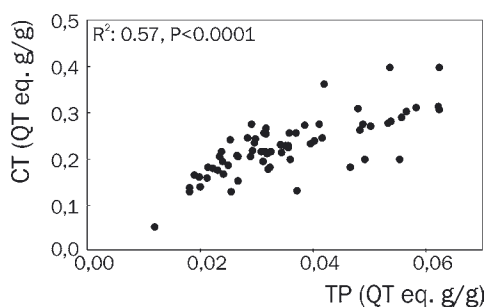
**Figure 8.3.** Relation between learning rate, number of epochs, momentum and obtained prediction accuracy of total polyphenol. Prediction accuracy was obtained after running the network with each combination of settings for 25 times, and calculating the mean (A) and maximum (B) correlation  $r$  between the actual concentration of an independent test dataset ( $n=15$ ) and the concentration predicted by the neural network.

### 8.3.2 Optimizing neural network settings

By combining the settings that result in optimal mean and maximum prediction accuracies (Figure 8.2) the optimal settings to detect phenolic compounds can be derived. This resulted in a combination of a momentum of 0.8, a learning rate of 0.75 and 16 000 epochs to calculate the final condensed tannin map. For models that predict total polyphenol concentration a combination 25 000 epochs, with a learning rate of 0.7 and a momentum of 0.7 was used (Figures 8.2 & 8.3).

### 8.3.3 Polyphenol and condensed tannins

Condensed tannins are part of a larger group of chemical components, called polyphenols. To understand the relation between the concentration of condensed tannins with the concentration with total polyphenol concentration, the correlation between those was calculated, and visualized in a scatterplot (Figure 8.4). This shows a strong ( $R^2=0.57$ ) correlation between condensed tannin and total polyphenol concentration in the foliage.

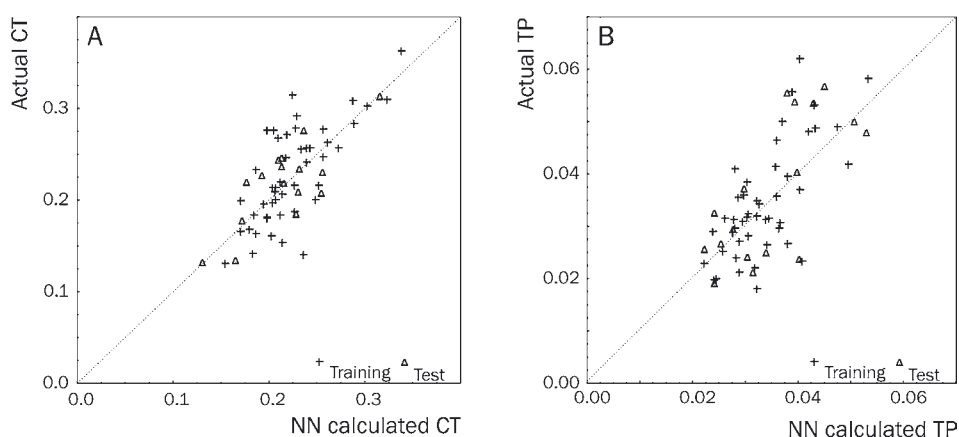


**Figure 8.4.** Relation between condensed tannin (CT) and total polyphenol (TP) concentration in mopane leaves.

### 8.3.4 Prediction accuracy

The predicted concentrations of foliar condensed tannin and total polyphenols, as predicted by the neural network, were plotted against the actual concentrations in the samples, for both the training and the test dataset (Figure 8.5). The predicted values in the test dataset showed a stronger correlation with the actual concentration, than the training dataset. For total polyphenol this difference was small ( $R^2_{\text{test}} = 0.52$ ,  $R^2_{\text{training}} = 0.48$ ), but for condensed tannins this difference was substantial ( $R^2_{\text{test}} = 0.67$ ;  $R^2_{\text{training}} = 0.53$ ). This may be the result of a small cluster (7) of samples, with an observed concentration of  $0.3 \text{ g QT g}^{-1}$  and a predicted concentration of approximately  $0.2 \text{ g QT g}^{-1}$  (Figure 8.5). In the test dataset there is only one sample with this effect (Figure 8.5). This may result in a lower correlation for the training dataset, than for the test dataset. For total polyphenol the distribution of predicted and observed values are the same for both the training as the test dataset, which indicates that the model used is stable and reliable.

To test whether the correlations for the training datasets are higher than expected a bootstrapping routine was applied. Bootstrapping (Efron and Tibshirani 1993, Manly 1997) is a resampling method that has traditionally been used to estimate statistical properties that are difficult to arrive at analytically, e.g., standard error, confidence intervals and sampling distributions of datasets. Bootstrapping simulates different combinations of samples (Efron and Tibshirani 1993, Pitt and Kreuzweiser 1998), resulting in a histogram of the distribution of the mean and range of the statistic of interest. Here, the training and test subsets were combined, and the correlation between modelled and actual chemical concentration of a random subset of 15 samples, drawn with replacement, was calculated for a bootstrap with 5 000 iterations. Correlation  $R^2$  values are recorded on a scale between 0 and 1. These data typically require an arcsine transformation to adjust for deviations of normality (Zar 1999). The mean and standard deviation of the transformed correlation values give an indication of the robustness of the neural network output. With a one-tailed Z-test the probability of obtaining a correlation  $R^2$  of 0.67, using this dataset, was calculated.



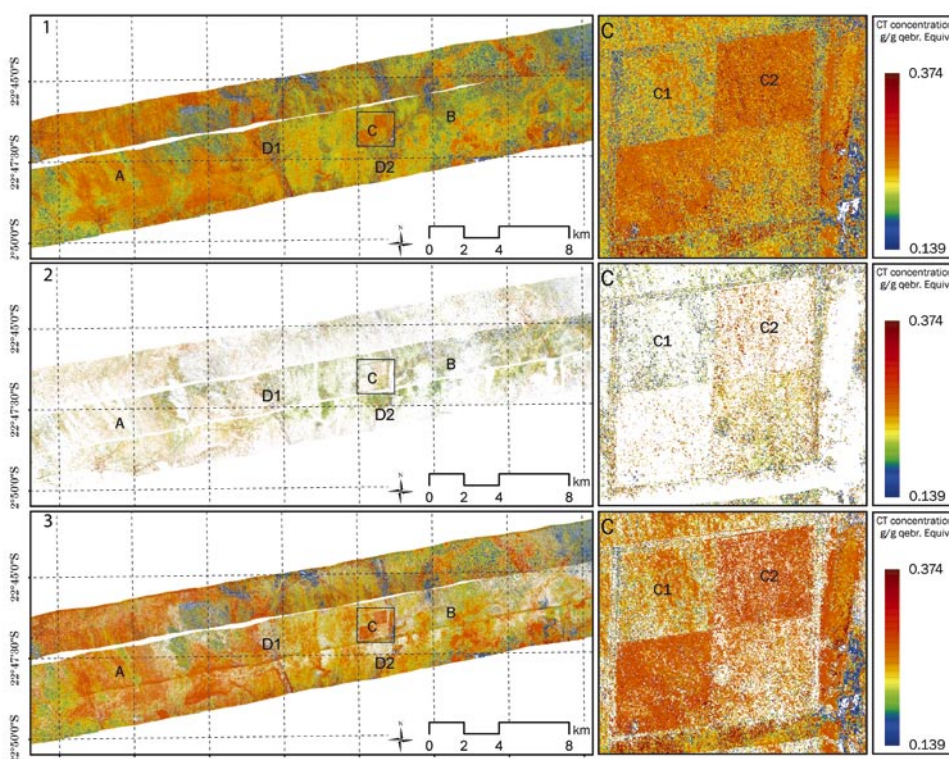
**Figure 8.5.** Relation between predicted and observed condensed tannin concentration (CT, plot A) and total polyphenol concentration (TP, plot B), both measured as quebracho tannin equivalents in gram per gram.

For condensed tannin, the arcsine-transformed data followed a normal distribution with a mean of 0.829 and a standard deviation of 0.220. The one-tailed Z-statistic, with  $1-(\text{cumulative } p)$  shows that the obtained predicted correlation of  $r^2=0.67$  is not exceptionally high for this dataset ( $p \leq 0.75$ ). For polyphenol, the distribution of bootstrapped  $R^2$  values has a mean of 0.79, with a standard deviation of 0.187. The probability of obtaining a correlation of 0.52 is  $p \leq 0.51$ . Therefore, for both tannin and polyphenol, the correlation values for the test datasets are realistic, and the models reliable.

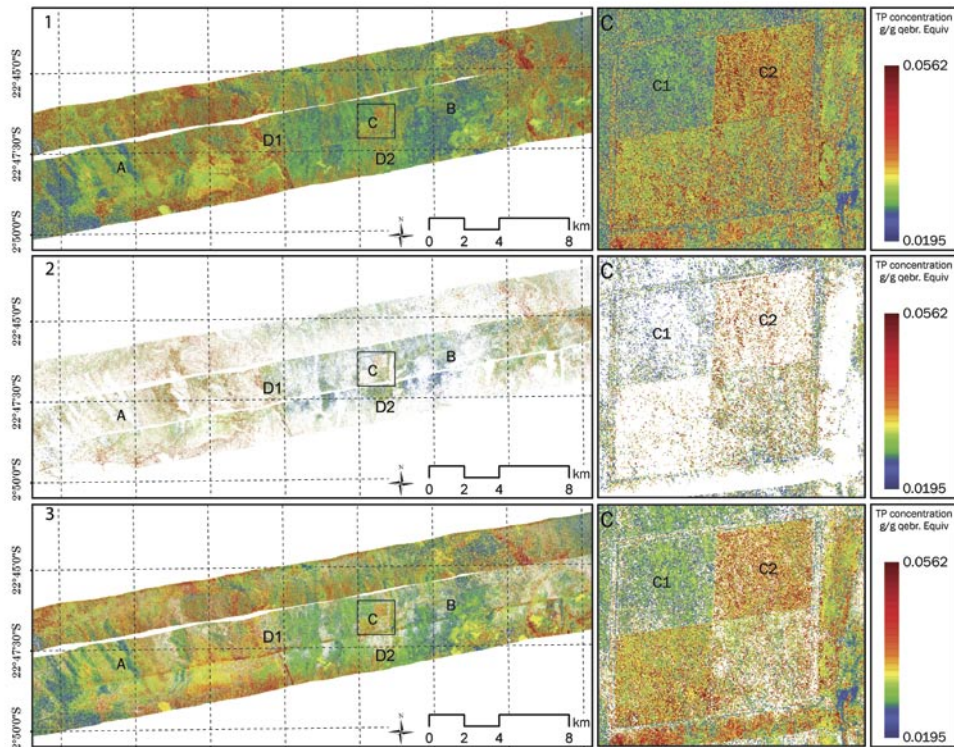


### 8.3.5 Spatial patterns

For both the concentration of foliar condensed tannins and total polyphenols the spatial patterns are partially masked by the effect of grass, although this effect is strongest for condensed tannins (Figures 8.6.1 & 8.7.1). When the “non-mopane pixels” are masked, the trends become visible (Figures 8.6.2 & 8.7.2): In the nutrient-poor eastern part of the study area (A), tannin concentrations are higher than in the west, where nutrient rich basalts occur (B). A roan-breeding enclosure, consisting of four separate burning sections, is located within the study area, on the basaltic soils (Location ‘C’). The north western section (Location ‘C1’) shows a lower concentration of non-structural carbohydrates than the other sections (Location ‘C2’).



**Figure 8.6.** Distribution of condensed tannin concentration, as calculated with a neural network from HyMap derivative reflectance images. The output of the neural network for the whole image (1) was split into tree-dominated pixels (2) and non-tree pixels (3) to enhance spatial patterns in trees, for which the final model was calibrated. See also appendix 3.



**Figure 8.7.** Distribution of total polyphenol concentration, as calculated with a neural network from HyMap derivative reflectance images. The output of the neural network for the whole image (A) was split into tree-dominated pixels (B) and non-tree pixels (C) to enhance spatial patterns in trees, for which the final model was calibrated. See also appendix 4

## 8.4 Discussion

Visual inspection of the patterns of calculated total polyphenol concentration and condensed tannin concentration show a striking similarity between these two. The main differences occur for non-mopane pixels. However, the model used in this study was only calibrated for the tree fraction of the vegetation. It was already shown that as a result of between-species differences in reflectance species-specific models should be used when predicting the chemical composition of foliage through remote sensing (Chapter 5). Therefore these differences will not be discussed here.

#### 8.4.1 Complete images

In the full images (Figures 8.6.1 & 8.7.1) the trends in polyphenol and condensed tannin concentration across the landscape are difficult to interpret. As mentioned before, different chemical components have overlapping absorption features, and different plant species display different spectral responses to variation in chemical composition. As a result, the calculated spatial patterns of condensed tannins and, to a lesser extent total polyphenol concentration in mopane are obscured by the effect of grass reflectance (Figures 8.6.1 & 8.7.1). Two drainage lines cross the study-area from north to south. These drainage lines stand out in the very low calculated condensed tannin concentration. One drainage line runs along the division of two geological units, with on the east shales and quartzites (typically nutrient-poor (Buol et al. 1973)), and in the west basalts (typically nutrient-rich (Buol et al. 1973), (Location D<sub>1</sub> Figures 8.6 & 8.7). The second drainage line consists of two merging branches on the basalt-based plains (Location D<sub>2</sub> Figures 8.6 & 8.7).

#### 8.4.2 Masked images

To retrieve the main patterns in condensed tannin and total polyphenol concentration in Mopane trees, the pixels which were not classified as predominantly mopane were masked. To the east of the study area, on relatively nutrient-poor quartzites and shales, tannin concentration is higher than in the west, where nutrient rich basalts occur (Buol et al. 1973, Venter 1990, Chapter 7). A roan-breeding enclosure, consisting of four sections separated by fire breaks, is located within the study area, on the basaltic soils. This enclosure has a different burning regime than the rest of the study area. The north-western quadrant (Location C<sub>1</sub> Figures 8.6 & 8.7) shows a clearly lower foliar concentration of tannins than the rest of the camp (Location C<sub>2</sub> Figures 8.6 & 8.7). The difference here is the result of differences in fire regime: The north-western quadrant was subjected to burning in May 2002 for management purposes. The rest of the roan camp has not been burnt since September 1999. Fire reduces canopy-height, which may increase growth and nutrient concentration in the plant. As a result, the concentration of phenolic compounds is lower in vegetation that recently burnt than in vegetation that has not been burnt for some time (Chapter 7)

Trees growing around the wetland in the west of the study area (Location A<sub>2</sub> Figures 8.6 & 8.7) show local spots of high and phenolic compound (condensed tannin and total polyphenol) concentrations. Many salt patches occur alongside the wetland (personal observation). These areas are frequently visited by herbivores, and on some of these patches intense grazing occurs, resulting in an almost complete removal of aboveground grass biomass (personal observation). It has been suggested that herbivores frequently visit these sites to match their sodium requirements (Grant et al. 2002). Possibly the concentration of herbivores on these natural saltlicks also results in an increased herbivore pressure on trees. The result of this increased herbivore pressure may be an increased concentration of phenolic compounds in woody plant foliage (Stock et al. 1993).

#### 8.4.3 *Between flight-line differences*

When comparing the three flight lines, there is an apparent difference in the density of trees in the different strips. This is the result of the automated approach of classifying land cover for each pixel directly from the remotely sensed image: All strips were classified simultaneously, using absolute reflectance values. Reflectance values are strongly affected by sun angle, through bi-directional reflectance effects, which may affect classification accuracies (Pellikka et al. 2000). Since the images were flown in the morning, instead of at solar noon, differences in BRDF effects occur between the different strips, and across the individual flight lines. Across the flight line, BRDF effects occur as a result of differences in view-angle, with a specific sun angle. Between strip differences occur as a result of changes in sun angle: the time needed to align the airplane between strips results in differences in sun-angle between the first and last strip. For the calculation of condensed tannin and polyphenol concentration, derivative spectra were used. When comparing the strips in the non-masked images, there are no distinct differences in predicted concentration between the images. This indicates that the derivative spectra are less sensitive to changes in reflectance than reflectance spectra, as was already indicated by previous studies ((Tsai and Philpot 1998), Chapter 6). However, when comparing the south-western areas of overlap for the two bottom strips in the masked images, slight differences in predicted concentration for both condensed tannin and total polyphenol occur between the two bottom strips, which is most likely a result of across-track BRDF effects.

#### 8.4.4 *Relevance for ecology & management*

The current study is an example of the potential of remote sensing to detect changes in the chemical composition of vegetation. To be really useful for the management of large game reserves, such as the Kruger National Park, these images arguably should be collected over a larger area, and with a reasonable frequency (e.g., annually at the end of the rainy season). Then it is possible to follow the chemical composition of the vegetation over time, and assess how different management policies affect the vegetation nutrient and deterrent status.

The three images, which in total cover an area of approximately 240 km<sup>2</sup>, give insight in regional differences in plant chemical composition. Differences between soil types, soil salinity and fire regimes are quickly spotted on the maps, providing insight in the effects of locally changing factors on vegetation chemical composition. Individual samples may give a similar answer, but one can never be sure that the sampled sites are in fact representative of the wider area. By using remote sensing to measure the spatial variation in chemical composition it is possible to move away from spatial interpolation techniques, and start to answer ecological questions using data which has been collected spatially, and not been made spatially by interpolation techniques.

This study has shown the potential of hyperspectral remote sensing to detect subtle changes in chemical composition of vegetation, and map these over larger areas. When this technique becomes available over larger areas, this may provide park managers and ecologists with a powerful tool to assess the influence of management policies on the quality of vegetation for herbivores. In a wider perspective, this technique can be expanded to detect an array of chemical components, and become useful in mapping nutrient status of agricultural crops. Farmers may then use this information to decide on appropriate fertilisation schemes for their crop, and adjust their fertiliser-needs to match local depletion levels.



# *Synthesis*

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## **Hyperspectral Remote Sensing and Vegetation Chemical Composition: Looking towards the Future**

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*"I am enough of an artist to draw freely upon my  
imagination. Imagination is more important than  
knowledge: Knowledge is limited. Imagination encircles  
the world."*

*A. Einstein (1879-1955)*







## 9.1 Introducing the synthesis

One of the main ecological research challenges is to answer the question ‘What determines the density and diversity of animals at a given location at any given moment?’ Many theories have been formulated, ranging from the balance between immigration and emigration (MacArthur and Wilson 1967) and the effect of body mass on nutrient requirements (Demment and Van Soest 1985) to the effect of landscape fragmentation (McNaughton 1988, McPherson et al. 2004). A common factor in many theories is the role of resources: the distribution in the landscape and the variation over time of quantity, as well as quality, of resources is thought to be of high importance.

Most of the world around us is green, and even though herbivores constantly reduce the amount of foliar biomass, in most locations their combined effect is not enough to completely remove all green biomass. This indicates that resource quantity *per se* is not generally limiting herbivore population density (MacArthur and Sanson 1993). It is more likely that the effect of foliar nutrient concentration, foliar deterrent concentration and their combined density distribution over the landscape are the driving forces that determine which species can meet their nutritional requirements at specific locations, and the densities at which this is possible.

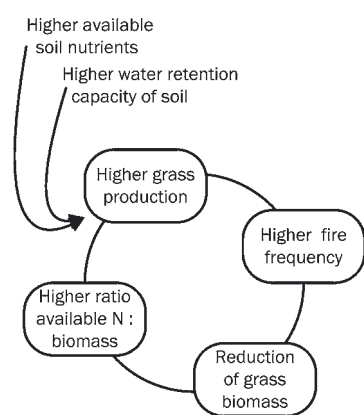
Both the quantity and quality of resources show an uneven distribution across the landscape, and point-based samples do not fully capture their distributions. To predict the distribution of herbivores based on the availability of resources, a more continuous measure of the variation in chemical composition of vegetation across the landscape is needed. Therefore, in this thesis I set out to develop a method to measure chemical characteristics of vegetation across the landscape. Spatial variation in plant chemical composition is the result of the interaction between several environmental factors (e.g., soil properties, local climate, and herbivory). To understand the observed patterns in vegetation chemical composition, the effect of factors that may determine the variation in foliar nutrient and deterrent concentration was evaluated.

For this study I focused on the mopane veld in South Africa. The woody component of mopane veld consists primarily of one plant species: *Colophospermum mopane* (mopane). Most mammalian herbivores avoid this plant species for food during most of the year, probably because of the high concentration of condensed tannin in mopane foliage. To map the chemical properties of plants at a landscape scale, I used hyperspectral remote sensing. By using an area with only one tree species, the variation in reflectance signatures that results of differences between species is reduced. Changes in plant chemical composition result in changes in reflectance signatures of vegetation. Measuring these changes in reflectance signature is assumed to be possible with the modern imaging spectrometers, that have a high spatial and spectral resolution. This technique results in detailed maps, depicting the distribution of plant chemical composition across the landscape.

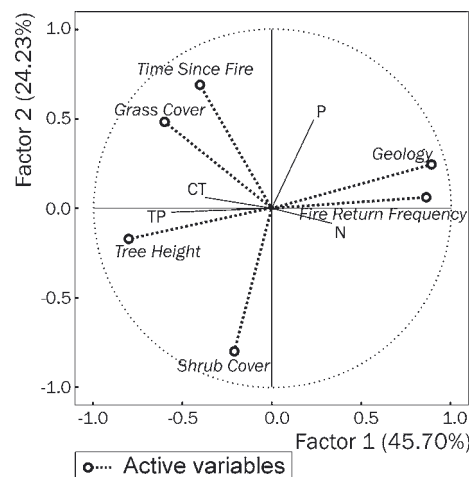
The variation in chemical composition of plants is related to the characteristics of the soil these plants grow on. To increase the variation in chemical composition of foliage, the study area was placed in Kruger National Park, where three types of parent material occur within a 30 km range.

### 9.1.1 Understanding chemical composition of foliage

To test whether the chemical composition of mopane leaves is indeed affected by changes in soil nutrient levels, young mopane plants were grown under controlled conditions in a greenhouse (Chapter 2). This confirmed that foliar nitrogen concentration is affected by soil nitrogen. However, under these controlled conditions foliar phosphorus and condensed tannin concentrations were not affected.

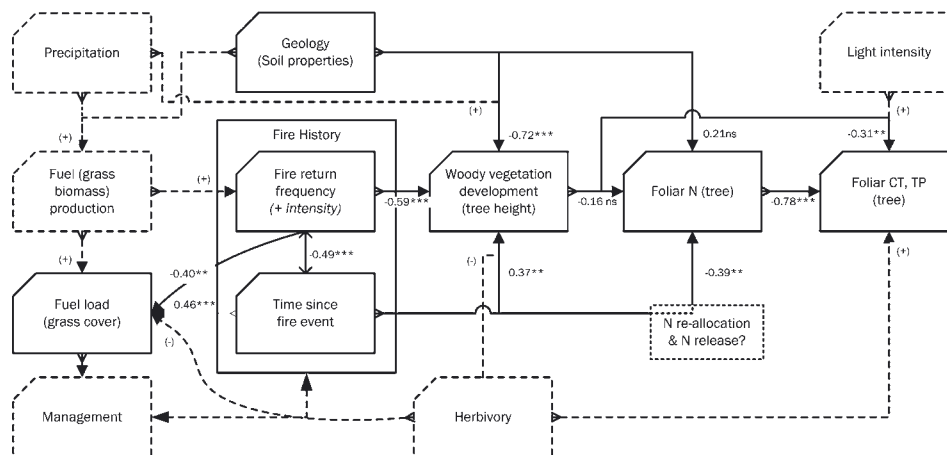


**Figure 9.1.** The effect of soil nutrients and water availability on fire frequency through grass production. With increasing water retention capabilities or nutrient levels of soil, grass production increased, which results in higher fire frequencies, which reduces standing grass biomass, and a higher available N:biomass ratio.



**Figure 9.2.** X-Y vector plot of factor loadings for the chemical composition of mopane samples, and a reduced set of explanatory factors. The x-axis displays the factor loadings (correlation) for each explanatory factor and the first principle component factor, the y-axis displays the factor loadings for the explanatory factor and the second principle component factor. Plot structure, fire history and parent material were used to define the principle component space (active factors).

To understand which factors determine the chemical composition of mopane foliage in the north of Kruger National Park, foliar samples of mopane that were collected in the park were analysed, and the chemical composition of these samples was related to the data on vegetation structure collected at each sample site, and to secondary information, such as fire history and parent material. This showed that

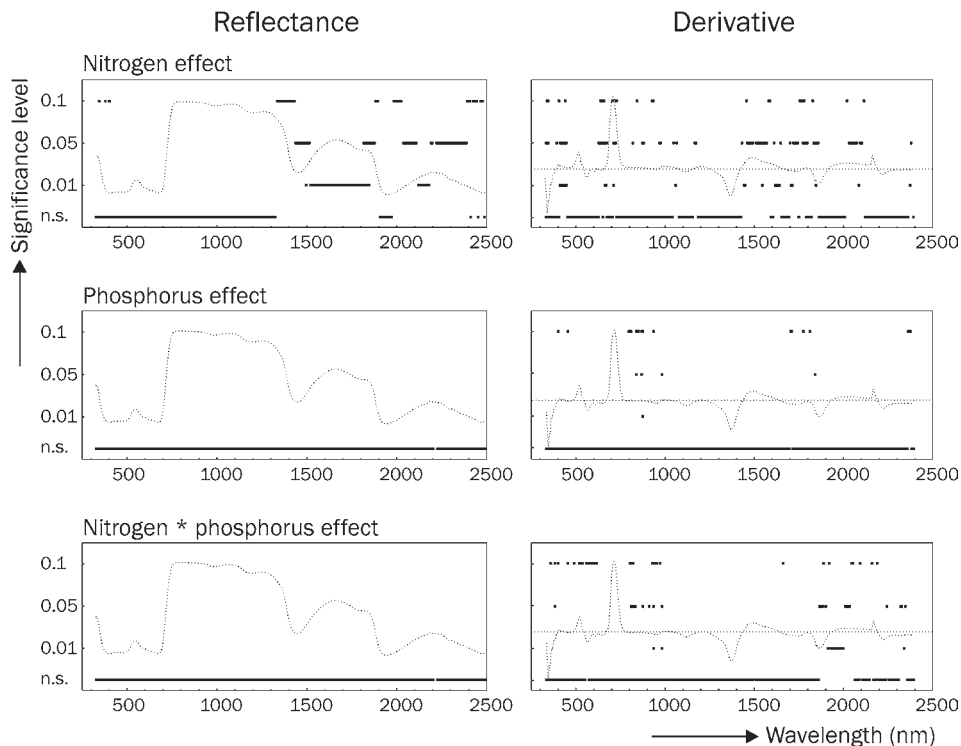


**Figure 9.3.** Path diagram of the interaction between environmental factors, vegetation structure and mopane (*Colophospermum mopane*) chemical composition. The strength of relations was calculated using a spearman rank correlation test (levels of significance: \*\*\*:  $p \leq 0.001$ ; \*\*:  $p \leq 0.01$ ; \*:  $p \leq 0.05$ ; ns: not significant). Solid lines depict variables and relations which have been observed / calculated. Dashed lines depict other important factors affecting vegetation structure, fire or the chemical composition of mopane leaves, but these have not been measured. Where possible the direction of the effect (positive: + or negative: -) has been included in the diagram

fire, in interaction with soil properties, plays an important role in the composition of this savanna system (Chapter 7). Fire frequency is closely linked to soil nutrients and water availability (Figure 9.1), and is one of the main factors that controls the chemical composition of mopane foliage in Kruger National Park (Chapter 7, Figures 9.2 & 9.3).

### 9.1.2 Measuring chemical composition of foliage

In the greenhouse study, only the concentration of three chemical components (nitrogen, phosphorus and condensed tannin) in the foliage was measured. But reduced levels of available nitrogen and phosphorus in the soil may affect physiological processes in the plant before the concentration of nitrogen and phosphorus in the foliage shows significant changes. Therefore reflectance spectra of the young mopane plants were tested for differences between different levels of soil available phosphorus and nitrogen (Chapter 3, Figure 9.4). Significant differences in derivative reflectance spectra were found between plants grown on different soil nitrogen and soil phosphorus levels. The bands that showed differences in reflectance between the soil treatments were related to known absorption features for protein, starch and cellulose (Chapter 3).



**Figure 9.4.** Overview of ANOVA results, testing for differences in reflectance spectra and derivative spectra between plants grown on high and on low concentrations of soil nitrogen, soil phosphorus, and the interaction between these two. The significance levels were grouped: 0.01:  $p \leq 0.01$ , 0.05:  $p \leq 0.05$  and 0.1:  $p \leq 0.1$ .

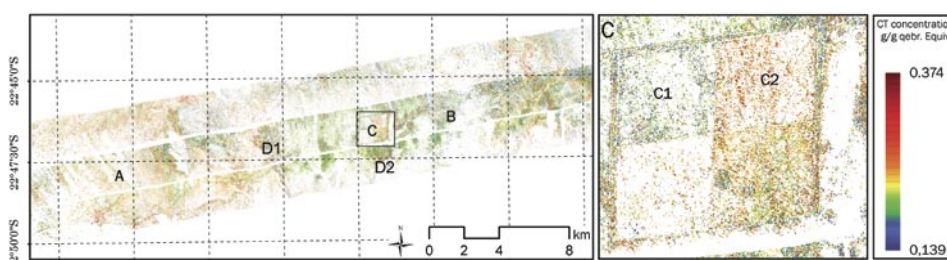
From several remote sensing studies it is known that reflectance signatures vary between species. Actually, this is the basis for the classification of vegetation using remote sensing. And although the spectral regions used to predict foliar nutrient (e.g. nitrogen, phosphorus, magnesium) concentration are linked to absorption features of compounds that are related to the chemical components of interest, differences in the depth and shape of absorption features may occur between species. When we upscale measurements from an individual sample to a plot level, reflectance signatures of different species are mixed, and reach the sensor as a composite signal. Therefore I tested in a number of studies whether one model can be used to predict the concentration of foliar components in different species. I found that models calibrated to predict nutrient content across species, are poor predictors of content within the species (Chapter 4, 5). When it is not possible to use species-specific models, an NDVI-type index, based on reflectance at 1770 nm and 693 nm, may be the best index to predict nitrogen content across woody plant species (Chapter 4). This difference between species was also observed in a study by Mutanga (2004), who worked on the relation between the reflectance spectra of different grass species and their sodium content. His models gave the best prediction accuracies

when they were calibrated for individual species. This indicates that the differences between species might be a universal trait, which needs to be taken into account when dealing with a multi-species situation, such as generally present in remotely sensed imagery.

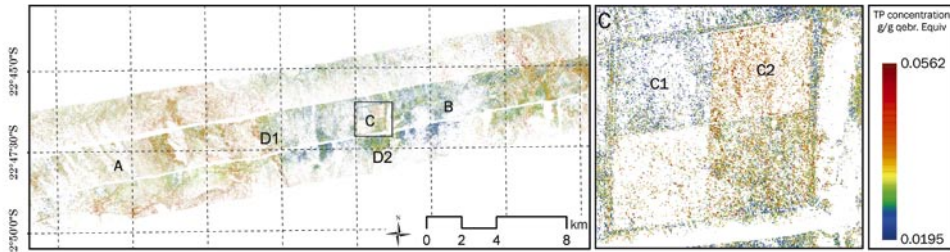
From a herbivore's point of view, not only nutrients are of importance. Also the concentration of compounds that lower the digestibility of nutrients is important. Using stepwise regression in combination with a resampling technique (bootstrapping) it was possible to determine wavebands related to condensed tannins. The bands detected in this study were compared to absorption features described in other studies. Subsequently these were used in a neural network routine to predict condensed tannin concentration and the total concentration of phenolic compounds in mopane woodland from hyperspectral imagery (Figures 9.5 & 9.6).

### 9.1.3 Mapping chemical composition of foliage

The information on absorption features of condensed tannins derived in chapter 6 was combined with information from a number of published studies. This resulted in seven wavebands to be used for prediction of condensed tannin concentration in mopane from hyperspectral imagery. The spectral resolution of the HyMap images was lower than the spectral resolution of the GER 3700 field spectrometer used in this thesis. Therefore, not all the absorption peaks, as detected in previous studies, could be used. Also, since absorption features may saturate at higher concentrations of the chemical of interest, the used bands do not match the exact centre of absorption features as they appear in literature. The centre of the bands that were used to predict condensed tannin and polyphenol concentration in mopane were located at: 710 nm, 804 nm, 1641 nm, 1714 nm, 1738 nm, 2146 nm and 2182 nm. The final map for condensed tannin concentration has a mean prediction accuracy (correlation  $R^2$ ) of  $0.54 \pm 0.26$ , based on a 5 000 iteration bootstrapping validation routine. For the total amount of polyphenols the optimal model resulted in a mean predicted  $R^2$  of  $0.51 \pm 0.17$ .

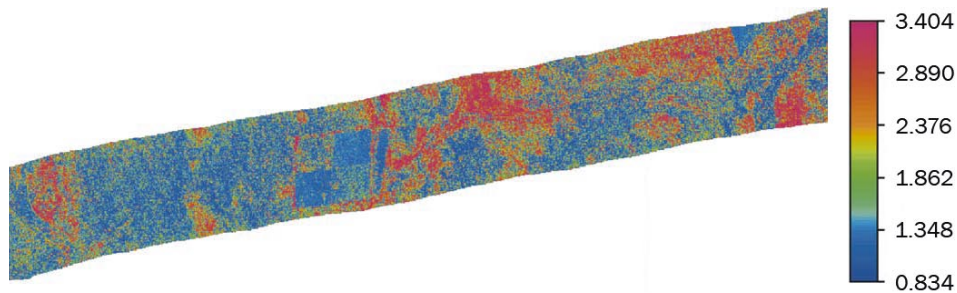


**Figure 9.5.** Distribution of condensed tannin concentration, as calculated with a neural network from HyMap derivative reflectance images. Because the neural net was trained for mopane reflectance properties only, the output of the neural network for the whole image was masked to only display tree-dominated pixels.



**Figure 9.6.** Distribution of total polyphenol concentration, as calculated with a neural network from HyMap derivative reflectance images. Because the neural net was trained for mopane reflectance properties only, the output of the neural network for the whole image was masked to only display tree-dominated pixels.

So far I have shown that the chemical composition of foliage is affected by soil properties and fire events. The resulting variation can be statistically linked to reflectance properties of foliage. Based on this relation it was possible to map the concentration of condensed tannins and total phenols in mopane foliage with a self-learning neural network.



**Figure 9.7.** Foliar concentration of nitrogen, as calculated with a neural network from HyMap derivative reflectance images. (Source: Mutanga, 2004b)

My study links up with a study by Mutanga (2004b), who used our field-data to map foliar nitrogen content in the study area in Kruger National Park (Figure 9.7). This shows that potentially a variety of chemical components may be mapped using hyperspectral remote sensing. The next step is now logical. As mentioned in paragraph 9.1, one of the main questions in herbivore ecology is: 'What determines the density and diversity of herbivores at a given moment and location'. Can we use the knowledge obtained in this thesis to help answering this question?

## 9.2 *Plant-herbivore systems*

From the previous chapters we have learned that the chemical composition of forage varies across the landscape. The combination of chemical compounds in forage is unique at each location and usually not optimal for foraging herbivores. Optimal foraging theory (Stephens and Krebs 1986) pre-supposes a perfect knowledge by herbivores of the distribution of available resources. Therefore, one of the main questions in herbivore ecology is: What is the decision mechanism that determines whether a herbivore stays at a specific feeding station, or moves away, in search of the next suitable patch of forage? Herbivores have to optimise their foraging strategies to simultaneously fulfil their daily requirements for energy (Van Wieren 1996) and nutrients (Behmer et al. 2002, Owen-Smith and Novellie 1982), while avoiding plants with high levels of anti-herbivore chemicals (Behmer et al. 2002, Cornelissen and Fernandes 2001, Furstenburg and Hoven 1992, Ginane et al. 2005 *In Press*, Matson et al. 2004, Provenza et al. 1990) or spines and thorns (Cooper and Owen-Smith 1986, Dunham 1980, Gowda et al. 2003, Rooke et al. 2004). Herbivores face this challenge in an environment, which is heterogeneous in both time and space (Cooper et al. 1988, McNaughton 1983, McNaughton 1988, 1990, McPherson et al. 2004). A number of studies have suggested explanations for the different optimization strategies of herbivores, where specific feeding strategies are related to local conditions. (Bradshaw et al. 2002, Fortin 2002, Lewison and Carter 2004, Marell et al. 2002, Pellew 1984, Prins 1996, Senft 1989, Turner et al. 1993).

These studies have shown the importance of spatial variation in quantity and quality of forage (Parsons and Dumont 2003, Seagle and McNaughton 1992), the size and distribution of individual suitable feeding patches (Johnson et al. 2001), previous sampling knowledge (Fortin 2002) and the size of the herbivore (Demment and Van Soest 1985), in combination with predation-risk at a certain location and time (Marin et al. 2003, Prins 1996), when studying local-scale herbivore distribution and foraging decisions. However, when looking at the distribution at landscape level, the foliar chemical composition may be an important factor that determines where relative herbivore densities are highest (McNaughton 1988, 1990, Seagle and McNaughton 1992) and which species can meet dietary requirements (Voeten and Prins 1998).

To test the different hypotheses on foraging strategies of herbivores, a good quantification of the factors that may explain herbivore distribution across the landscape is needed (Augustine 2003), and the appropriate scale to study these processes should be determined (Bradshaw et al. 2002, Johnson et al. 2001, Murwira 2003, Said 2003). This is where the work presented in this thesis becomes important. Although determining the variation in chemical composition across the landscape is possible using geo-located, point-based sampling, and subsequent interpolation techniques, the derived data is a coarse estimation of the actual patterns in the vegetation. In this thesis I have shown that it is possible to predict the chemical composition of foliage using hyperspectral imagery. This gives more

detailed information on the spatial variation in foliar chemical composition. The thesis has also shown distinct differences in chemical composition between different soil types in interaction with fire history (Chapter 7). By combining this information with herbivore foraging-paths, and the observed feeding time at different locations, we can test theories on foraging strategies of herbivores.

In the rest of this chapter, when referring to the fitness of a herbivore, the following definition is used (after Haldane 1937): “The fitness of an individual herbivore is the contribution of that individuals’ genetic material to the future population, relative to the rest of the current population”. The fitness of an individual can therefore be measured as the number of offspring capable of reproduction, produced over the course of that individuals’ life, relative to the offspring capable of reproducing produced by the other individuals of the population. Consequently, any activity that increases the potential reproduction of an individual herbivore compared to that of the population can be considered to have a positive effect on the fitness of the individual, and activities which reduce an individuals’ potential reproduction compared to the population can be considered to have a negative effect of that individuals’ fitness.

In the next sections I will consider the effects of different foraging strategies of an individual herbivore, while the rest of the population does not show this response. Let us assume that the nutritional status of a herbivore is directly linked to the potential reproduction of this herbivore (Gaillard et al. 1998), then any strategy that improves the nutritional status of that individual herbivore while other external factors are kept equal, results in an increased fitness.

### 9.2.1 *Foraging strategies in the landscape*

Several optimisation strategies that may be applied by herbivores have been suggested, but falsification is difficult without knowledge on the actual distribution of plant chemical composition across the landscape. Hyperspectral remote sensing enables us to increase understanding of these chemical landscapes. By directly mapping the concentration of individual chemical compounds in leaves, we have the ability to ‘see’ plant chemical composition. Instead of observing the landscape as a combination of discrete vegetation types, and individual palatable or unpalatable species that occur in discrete or semi-continuous units in the landscape, we can start to understand the landscape as gradients of herbivore nutrition, and high and low quality. This insight can help to understand the spatial distribution of herbivores, and test different optimisation models.

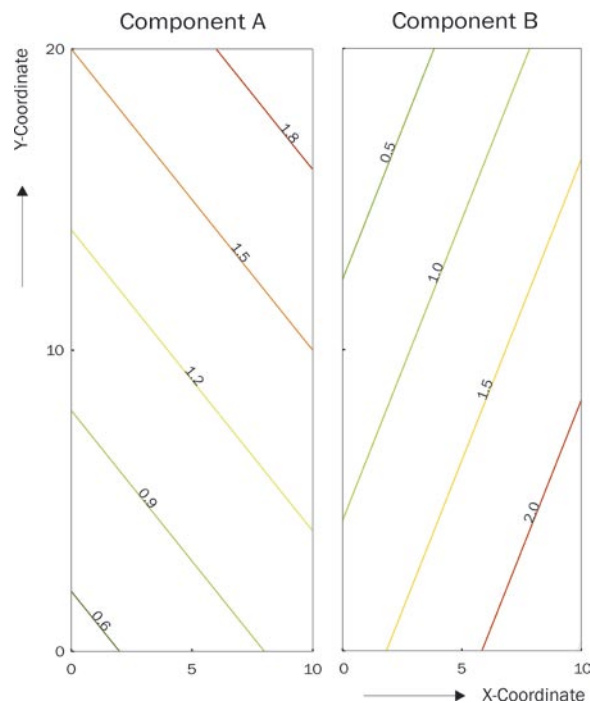
The food on offer, in the form of foliage, forms continuous multidimensional landscapes in which nutrient, deterrent, energy, and fibre concentration vary in space. Herbivores move through these chemical-composition landscapes in a search for food. These herbivores have to simultaneously cover the requirements for nutrients and minerals to maintain body tissue and skeleton, and energy needed for locomotion, maintenance and as a buffer to cover lean periods (e.g., the dry season



in African savanna systems) (Prins and Van Langevelde *In Press*). Herbivores have specific requirements for the concentration of nutrients in forage (e.g., in a balanced diet of a 500 kg African buffalo cow, the crude protein concentration should be around 9% (Prins 1996)). Ideally, all vegetation used by a herbivore contains all minerals, amino acids and energy in a perfect balance as required by the herbivore. In practice, however, the foliar concentrations of the required elements are highly variable in both space and time and deviate from what is needed. Therefore a herbivore needs to migrate through the landscape and combine forage from different locations. This way, a herbivore can blend the different concentrations, and obtain the optimal concentration for each component. Using a metaphor, a herbivore actively ascends and descends along the slopes of the different chemical-composition landscapes, in order to obtain the optimal intake concentration for each component. The success with which a herbivore can maintain a balanced diet will determine the nutritional status and health, in turn affecting reproductive success.

### 9.3 Conceptual model

Let us consider two chemical components -component A and B- that change along a continuous gradient (Figure 9.8), in a landscape the size of Kruger National Park. Component A has a high concentration in the northeast of the artificial study area, and decreases towards the southwest. For component B, the concentration decreases from the southeast to the northwest.



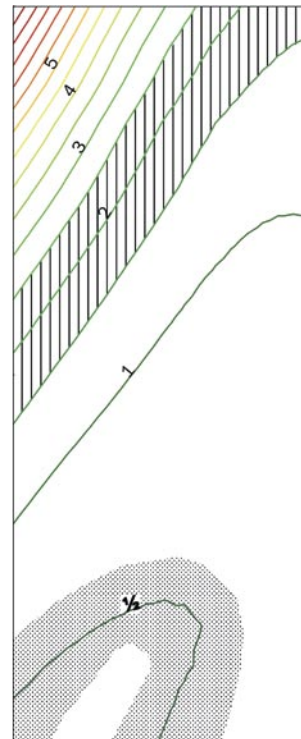
**Figure 9.8.** Spatial distribution of two chemical components in forage, across an artificial landscape

### 9.3.1 Foraging herbivore

Let us now consider a hypothetical large herbivore, capable of migration over long distances, foraging in this landscape. To reduce complexity, we assume for now that in this system predation, water availability and all other factors that may affect fitness are homogeneously distributed across the landscape, and affect herbivore fitness at different locations in the same way. In other words: from a herbivore's points of view, these factors are everywhere equal, and thus irrelevant. Also, we assume that herbivore densities are low, so that competition does not influence forage selection. Consequently, the only factor affecting herbivore fitness in this situation is the quality of forage, and the capability of a herbivore to maintain a well-balanced diet. Herbivores require energy to maintain body temperature, locomotion, and control internal processes. We can therefore assume that herbivores will focus their foraging efforts on patches of forage that resemble the ideal diet as closely as possible, in order to minimize energy costs while foraging.

### 9.3.2 Balancing intake ratios

In the first situation, herbivores have one foraging constraint: to maintain the ratio of two components at a specific level. E.g., the ratio between Ca and P concentration in mammals is 2:1 (Robbins 1993 in: Prins & Van Langevelde *In Press*). Lactating mammals maintain the intake ratio of Ca:P at approximately 2 to 1, in order to avoid milk-fever (Oetzel 1991). In forage, this ratio can vary from 6:1 in red clover, to 1:8 in peanuts (Robbins 1993 in: Prins & Van Langevelde *In Press*). If we take component A and B as Ca and P, we can calculate the areas where the foraging effort required to maintain a balanced diet with regards to Ca and P is lowest, e.g., the areas where the ratio between Ca and P approximates 2:1 (Figure 9.9). Clearly, if we take component A as Ca and component B as P, the preferred regions are different than if component A is P, and component B is Ca. Mixing forage with a higher and lower Ca:P-ratio than preferred can result in an optimal Ca:P-ratio in the diet, but moving from one location to the next requires spending energy on locomotion. Also, with the increasing time needed to move between foraging sites, mixing of plant material of different quality in the gut becomes less feasible. Therefore we can expect herbivores to concentrate in an area where forage contains the ideal ratio of Ca to P (Figure 9.9).

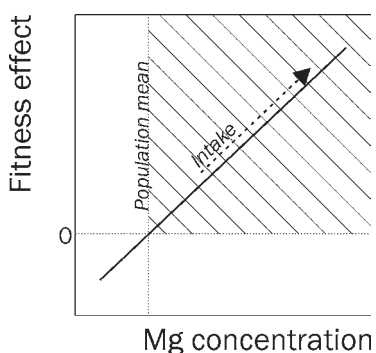


**Figure 9.9.** Ratio between foliar concentration of component A and B in an artificial landscape, with component A and B as Ca and P. Lactating cows need to maintain a Ca:P-ratio of 2:1 in forage. This concentration is closely matched when component A = Ca and component B = P (Hatched areas) or component A = P and component B = Ca (Dotted areas).

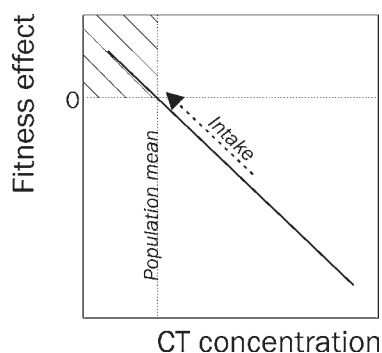
### 9.3.3 Intake strategies

As mentioned before, herbivores maintain specific intake concentrations of foliar nutrients and minerals (Behmer 2003, Simpson 2004), and may display different responses to different chemical components. Three basic strategies can be distinguished: Components with a predominantly positive effect on meeting diet requirements; components with a predominantly negative effect on meeting diet requirements; and components where the an intermediate concentration has an optimal effect on meeting diet requirements.

Some components occur in sub-optimal concentrations in forage (e.g., Mg and P, required for the skeleton). Herbivores may actively search food sources with elevated concentrations of these components (Grant et al. 2002, Klaus et al. 1998), maximizing intake where possible. In this case we consider increments of concentration to positively affect the individuals' fitness (Figure 9.10).



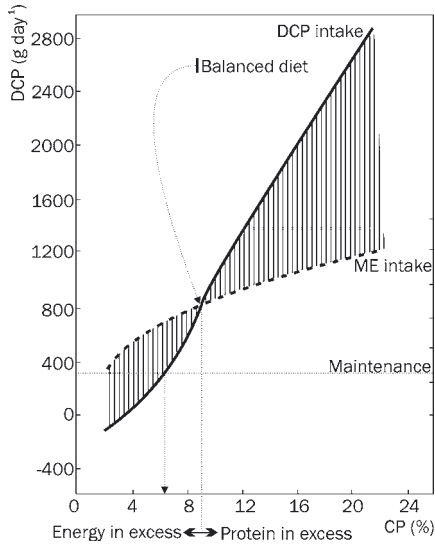
**Figure 9.10.** Intake maximisation. Increased Mg concentration results in a higher fitness when intake is higher than the population mean.



**Figure 9.11.** Intake minimisation. Decreased CT concentration results in a better fitness when intake is lower than the population mean.

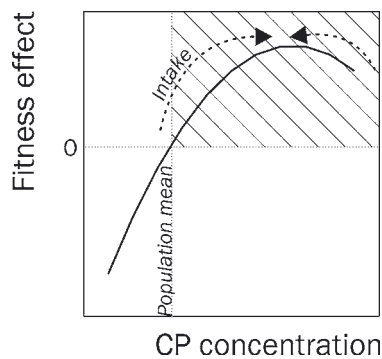
Other components may be beneficial at low concentrations, but have a negative effect at lower concentration. As an example we can think of condensed tannins (CT), the focus of this thesis. Although some ruminants select for low concentrations of condensed tannin in forage, which results in a higher crude protein update (Kondo et al.), higher concentrations of condensed tannin result in a reduced digestion (Robbins et al. 1987), which has a negative effect on the nutrient intake of herbivores, and subsequently fitness (Figure 9.11). For these components, we assume that reducing concentration increases the herbivore's fitness, and a herbivore may actively try to minimize intake of these components (Figure 9.11).

Some other components may be beneficial above a certain threshold, reach a maximum positive effect, and have a decreased positive or even negative effect on diet balance at higher concentrations. Here we can think of protein concentration. In a study on African buffalo around Lake Manyara, Prins (1996) combined field data with a literature review, and showed that buffalo in the Manyara area do not



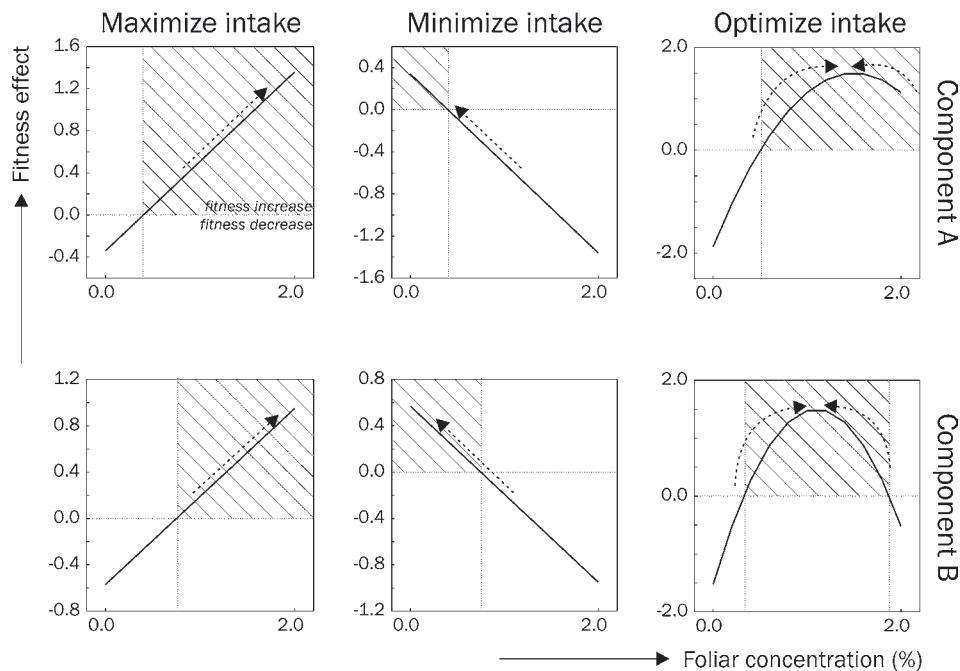
**Figure 9.12.** Intake of digestible crude protein (DCP; g day<sup>-1</sup>) and of metabolizable energy (ME; kcal day<sup>-1</sup>) as a function of the crude protein concentration (CP; %) of roughage feed. The right-hand scale is constructed so that 24 g DCP stands for 1000 kcal ME, which gives a balanced diet for a 500 kg cow at maintenance. The lines describe the daily intake of DCP (in g) and ME (in kcal) as function of the crude protein concentration of the food, taking into account the reduction in voluntary food intake when quality decreases. Reproduced from: Prins, 1996)

maximize the concentration of crude nitrogen in their diet, and avoid food of which nitrogen concentration is too high. This was then related to the link between protein and energy: With increasing crude protein (CP) concentration, the metabolizable energy increases too. (Van der Honing, 1977 in: Prins, 1999). However, any CP intake in excess of requirement needs to be digested. The metabolic cost for digestion of amino acids is high, which results in a reduced energy availability. Therefore, the best CP concentration in the diet is located around an optimum, which for non-lactating Manyara adult female buffalo can be found around 9% CP (around 1.75% N) (Figure 9.12). For these components, herbivores maintain a fairly consistent intake concentration, located around an optimum level (Figure 9.13).



**Figure 9.13.** Balancing intake concentration. Crude protein concentration in forage is balanced around a specific concentration. At lower and higher concentrations CP and ME are unbalanced.

Generalisation of these strategies for component A and B results in the two series in figure 9.14, which depict maximized, minimized and optimal intake strategies. For both components the basic strategies are the same, but with different threshold levels where the obtained intake levels match the preferred diet.



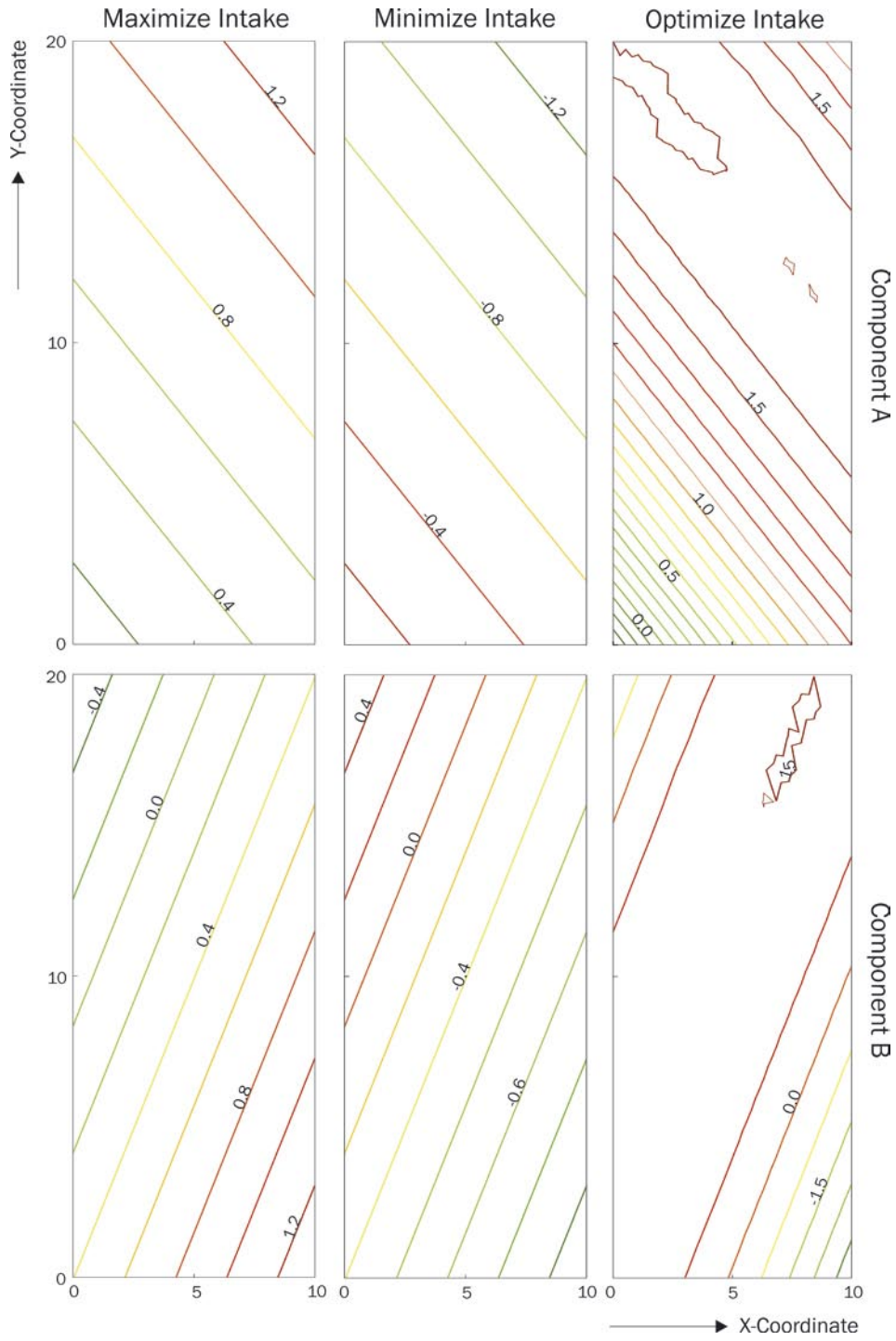
**Figure 9.14.** Different effects of component A and B on herbivore fitness, with resulting fitness strategies. When increased concentration of the component always results in an increase of fitness, an herbivore is expected to maximise intake, and forage mainly on vegetation with high concentration of the component. For components where an increase in concentration results in a decreased fitness, an herbivore is expected to minimize intake. For most components a specific concentration results in maximum positive effect on nutritional status. In this case intake is focussed on maintaining a diet with that concentration.

#### 9.3.4 One-component intake control

A combination of the foliar concentration maps for component A and B (Figure 9.8) with the three effects on fitness, results in forage suitability maps (see Figure 9.15). These maps depict the expected fitness effect for the herbivore, when compiling the diet on vegetation from each location in the artificial study area. As expected: For the minimized intake and maximized intake strategies of component A and B, we find optimal foraging areas in the corners of the study areas. For the optimised intake strategy, optimal feeding grounds are located somewhat away from the edges, and herbivores are expected to focus foraging efforts on intermediate foliar concentrations located towards the centre of the study area.

#### 9.3.5 Two-component intake control

In general, if a herbivore is capable of detecting and regulating intake of specific chemical components in forage, it is not likely that a herbivore will regulate the intake of only one component. More likely, the herbivore will optimise intake of a variety of

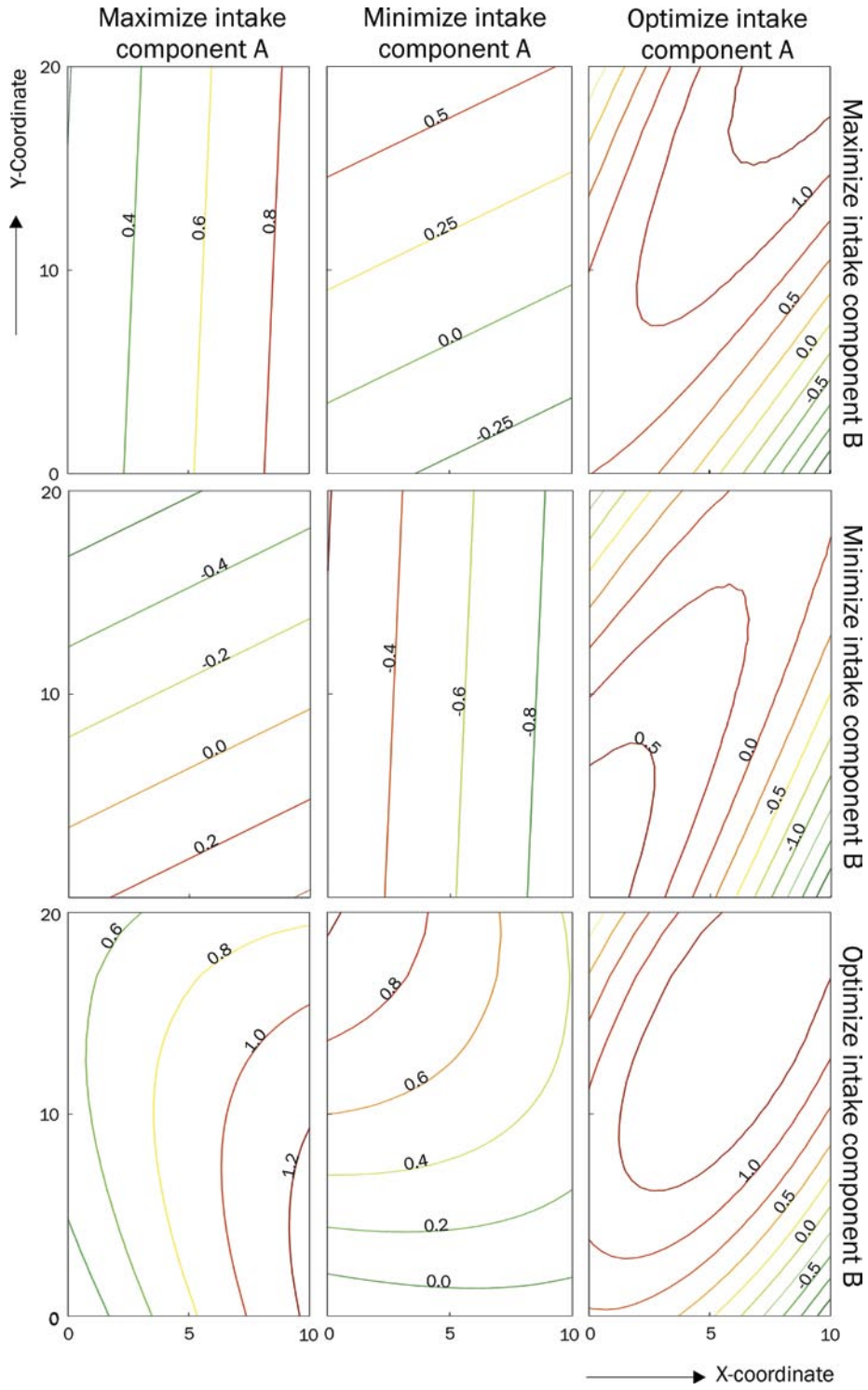


**Figure 9.15.** Fitness landscapes for an herbivore foraging on vegetation consisting of gradients in chemical composition (Figure 9.8), based on three strategies: Minimize intake, maximise intake and optimize intake of a specific concentration (Figure 9.14). (Previous page)

chemical components in his forage (Simpson et al. 2004, Villalba et al. 2004), and ensure intake of each component on a fairly regular basis (Behmer et al. 2003). The effect this has on forage suitability for herbivores can be illustrated by combining the forage suitability maps under different strategies for component A (Figure 9.15) with those for component B (Figure 9.15). The resulting nine combinations (Figure 9.16) show a wide variety of expected effects on fitness levels for herbivores foraging using different optimisation strategies in the artificial study area.

When the two components represent two beneficial components, for which intake are maximized, herbivores are expected to be able to meet intake requirements throughout the study area, with highest herbivore densities in the east. When we move towards minimized intake of component A, and maximized intake of component B, a better than average reproduction is expected in the north, and a less than average reproduction in the south. For a herbivore aiming at a specific (optimized) intake of component A, and a maximized intake of component B, we see that most of the study area results in a better than average reproduction, with only a negative effect on fitness in the southeast (Figure 9.16). Combining the optimum intake strategy for both components results in an oval region where maximum reproduction can be achieved, and herbivores are expected to forage in the north-centre of the study area.

The two simple landscapes of chemical component A and B (Figure 9.8), combined with three forage suitability maps for each component (Figure 9.15), resulted in a wide range of possible preferred foraging sites for my hypothetical herbivore (Figure 9.16). Figure 9.16 and Figure 9.17 may help to understand how regional species-richness is influenced by the spatial arrangement and size of patches of homogeneous resources. Figure 9.17 shows a small area in the western part of the produced foliar tannin concentration map (Figure 8.6). In figure 8.6 I found landscape-level patterns in forage chemical concentration, with high concentrations of tannins in the west, and lower concentration in the nutrient rich east. Based on this it was expected that more herbivores can meet nutrient requirements in the east, than in the west. When we look at a subsection of the map, this shows a highly variable distribution of tannin concentration at patch-level (Figure 9.16). This fragmented distribution may enable a herbivore to maintain low concentrations of condensed tannin in the diet, while foraging in an area with relatively high foliar tannin concentration.







**Figure 9.17.** Subsection of Figure 8.6, showing pixels with low (blue) and high (red) concentration of condensed tannin within an area with high concentrations of foliar condensed tannin

Increased homogenisation of resource chemical composition across the landscape (A reduction in variation of foliar chemical composition, and/or an increase of patch sizes) increases the distance to required resources, and between suitable feeding sites. Therefore the distance between required resources, in combination with the frequency at which intake of individual components is needed, limits the range at which herbivores may occur, and, at a landscape scale, the range over which herbivores may migrate over the course of a season (As was found for wildebeest in the Tarangire-Simanjoro migration system, where phosphorus requirements explain migration to the wet-season feeding grounds, and water-requirements explain the return to the dry-season feeding grounds, (Voeten 1999)). On the other hand, with decreasing homogenisation of resource chemical composition, landscape becomes less predictable for herbivores, and search-time to locate high quality patches is increased, at the expense of feeding-time. Also, the size of smaller patches may be large enough for smaller herbivores to locate and use effectively, but for larger herbivores these patches may become too small to effectively use, rendering a chemically extremely heterogeneous landscape unsuitable for larger herbivores.

The arguments I have presented in this last chapter illustrate the importance of knowledge on the distribution of plant chemical components across the landscape. The combination of some very simple patterns in the landscape may result in complex distribution patterns of the dependent herbivores. Trying to predict or even explain herbivore distribution without knowing the vegetation chemical composition, is like trying to find the two prime-numbers which, when multiplied, give a specific large number. By trying, this is next to impossible. However, when given one of the prime numbers, understanding the multiplication is easy.

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**Figure 9.16.** Forage suitability landscapes for an herbivore foraging on vegetation containing two chemical compounds, while applying three different intake optimisation strategies. (Previous page)



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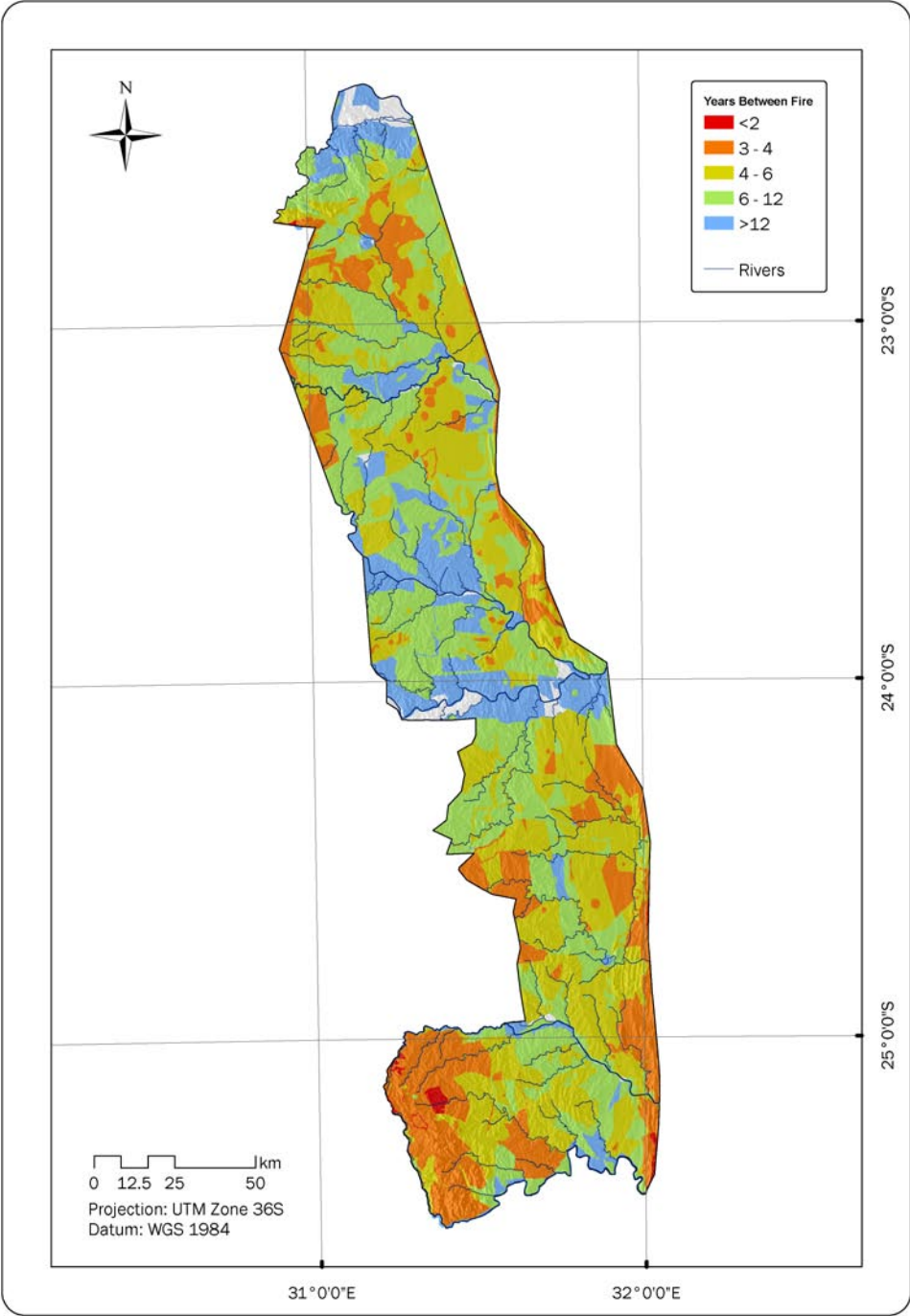


# Appendices

*Appendices*



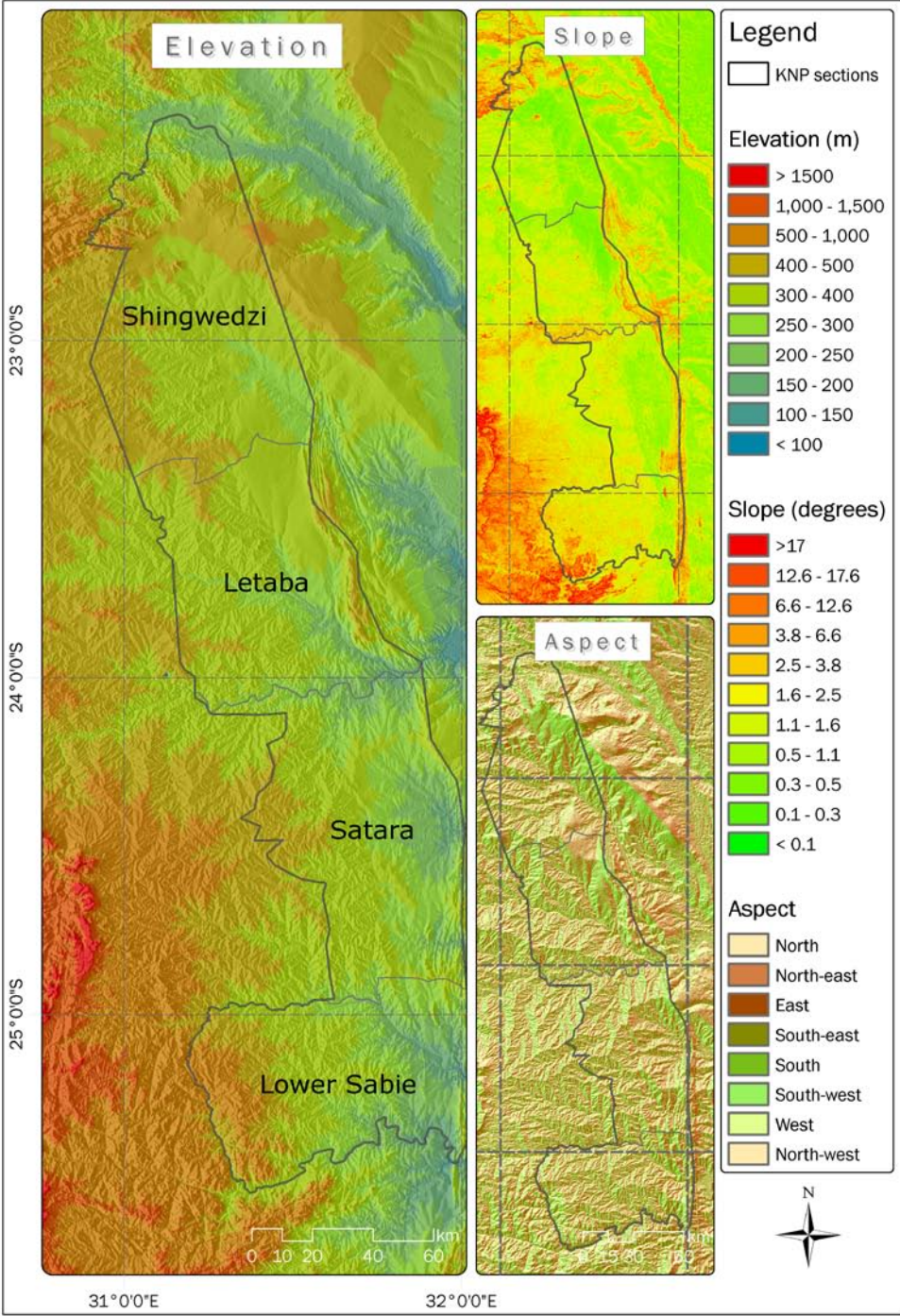
# Appendix 1. Kruger National Park fire frequency map



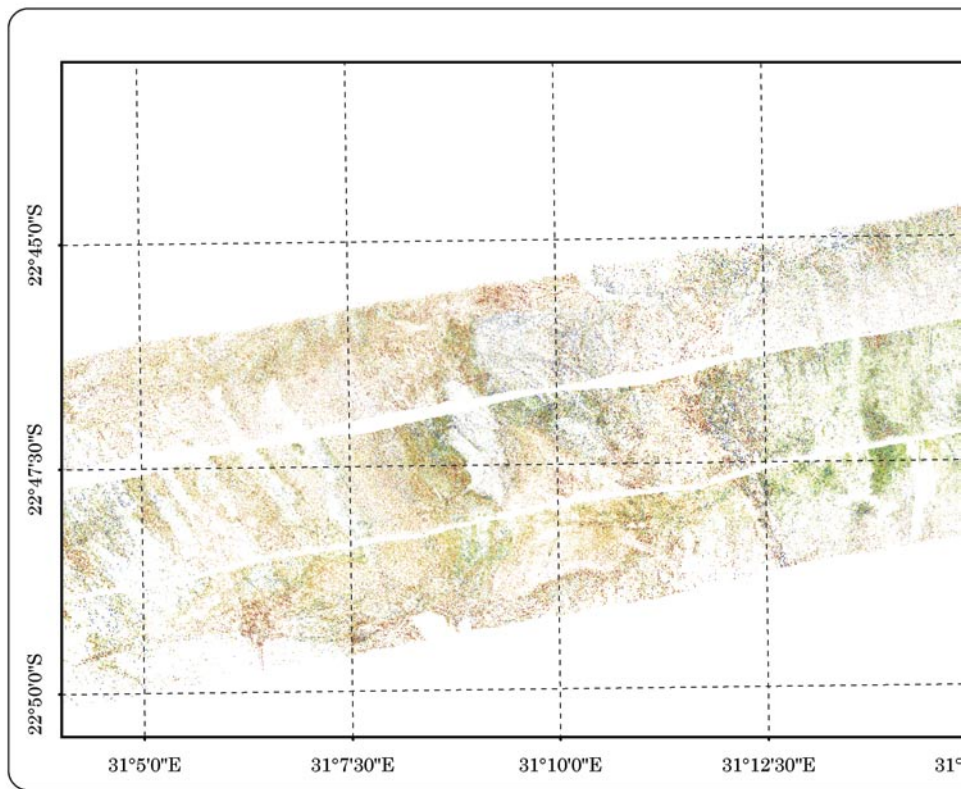
Appendices



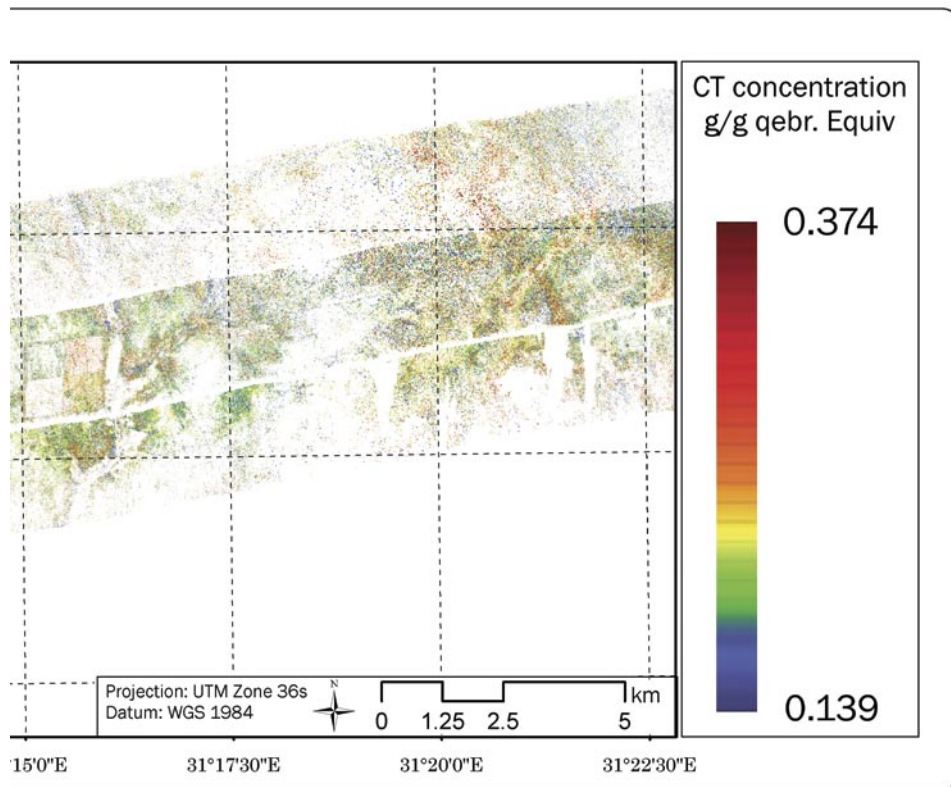
# Appendix 2. Kruger National Park geography maps.



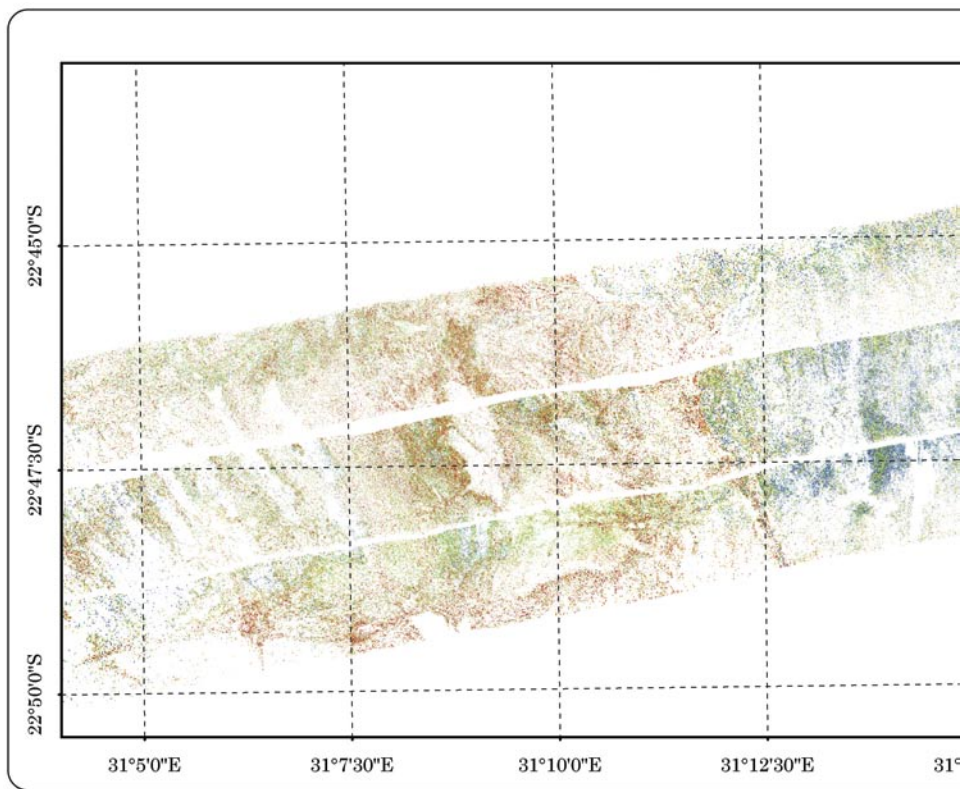
Appendices



### Appendix 3. Foliar Condensed Tannin in Mopane.

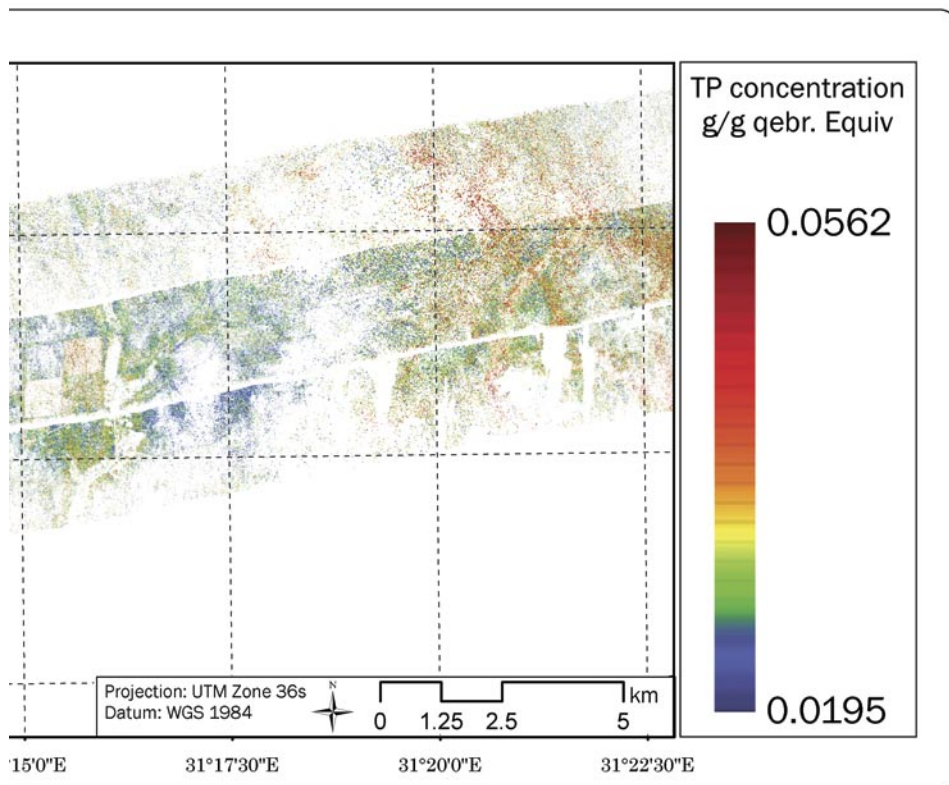


Appendices





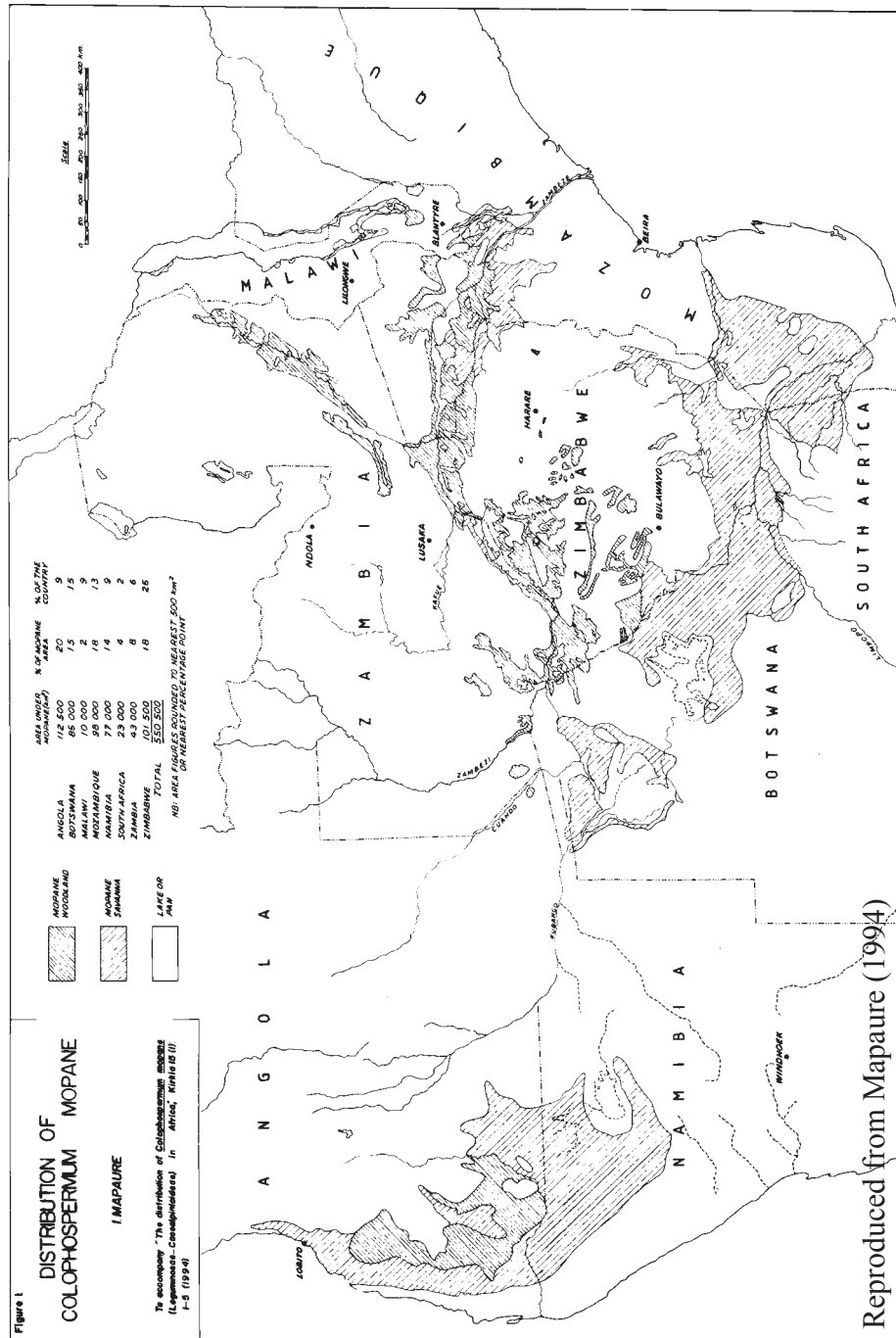
## Appendix 4. Foliar Total Polyphenol in Mopane.



Appendices



## Appendix 5. Distribution of *Colophospermum mopane*.



Appendices



## Résumé

Jelle Ferwerda was born on August 4<sup>th</sup>, 1976 in Den Helder, the Netherlands, and grew up in Gouda, the Netherlands. After graduating from the 'VWO' in 1994 he started his MSc in Wageningen University. He passed the propedeutic examination in 1995, and worked on four MSc projects in the period between 1998 and 2000. He received his MSc (Ir.) degree in November 2000.

During his MSc work, he spent 2 months traveling in West Africa for the International Association of Agricultural Students (IAAS) and 11 months in the Americas where he worked on conservation projects for the Costa Rican government and Earthwatch. His final MSc project was funded with three additional scholarships and was carried out in the mangroves of Darwin, Australia, where he lived for 11 months. After finishing his MSc, Jelle worked for half a year as logistics manager to set up the logistical framework for the government health institute LCIG, through the consulting firm 'Wedman en Partners'. Around the same time, he founded a photography and web-design company (bio-vision.nl), which he continued to work for throughout his PhD. In June 2001, he was awarded a full AIO position with ITC and Wageningen University, which resulted in this thesis.



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