

**Seasonal and inter-annual variations of leaf-level
photosynthesis and soil respiration in the
representative ecosystems of the Okavango Delta,
Botswana**

Khanyisa Brian Mantlana

Promotor

Prof. dr. F. Berendse
Hoogleraar in het Natuurbeheer en de Plantenecologie
Wageningen Universiteit

Co-promotoren

Dr A. Arneth
Centre for GeoBiosphere Science
Lund University, Sweden

Dr. E. M. Veenendaal
Universitair Hoofddocent,
Leerstoelgroep Natuurbeheer en Plantenecologie
Wageningen Universiteit

Promotie commissie

Prof. dr. J. Grace
Edinburgh University

Prof. dr. J. Rozema
Vrije Universiteit, Amsterdam

Prof. dr. ir. H. J. Bouwmeester,
Wageningen Universiteit

Dr. T.L. Pons
Universiteit Utrecht

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To: My parents, bhuti Mphuthumi and sisi Nokubonga.

ABSTRACT

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Seasonal and inter-annual leaf-level photosynthesis and soil respiration measurements were conducted in representative ecosystems of the Okavango Delta, Botswana, that differ in their long-term soil water content: the permanent swamp, the seasonal floodplain, the rain-fed grassland and the mopane woodland. CO₂ fluxes (both leaf-level and soil respiration) at all the sites showed pronounced seasonality with highest and lowest values observed during the wet season and the dry season, respectively. Lowest values of soil respiration at the permanent swamp were a result of the surface flood, while low soil moisture content led to low soil respiration values at the rain-fed grassland and also at the upper lying areas of the seasonal floodplain. Soil temperature alone was a poorly related to soil respiration, while a simple model combining soil water content and soil temperature provided strong correlations with soil respiration.

Species dominating wetter habitats showed highest stomatal sensitivity to vapour pressure deficit. Grasses at the seasonal floodplain showed high water use efficiency and strong stomatal limitation of CO₂ assimilation as the dry season progressed. During the wet season, high CO₂ assimilation among the grasses at the permanent swamp was associated with high nitrogen use efficiency, while at the rain-fed grassland high CO₂ assimilation was associated with high leaf nitrogen and leaf phosphorus values. Within each species and across the sites leaf phosphorus appeared to exert stronger influence on leaf-level photosynthesis than leaf nitrogen. Taken together, the results of this study contribute toward understanding the role of environmental factors (soil water content, soil temperature, leaf nutrients) in controlling ecosystem functioning of tropical grassland and savanna ecosystems.

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CHAPTER 1: GENERAL INTRODUCTION

Climate change in Southern Africa

Most of Africa's continental landmass is classified as arid or semi arid with sixty percent of the population living in zones with mean annual runoff of 300 mm or less, and 40% in areas with less than 100 mm of runoff (Vörösmarty *et al.*, 2005). Despite this reality, almost 80% of African agricultural land is used for grazing (Seo & Mendelsohn, 2008). However, agricultural production and lack of access to safe water are some of the key vulnerability areas facing the continent in light of climate change (Solomon *et al.*, 2007).

In Southern Africa, predicted changes in precipitation point to a general pattern of increased aridity in the arid western parts, while the eastern part of the subcontinent could receive substantially more rain (IPCC, 2007). Most climate models predict that not only climatic means are expected to change, but so too might the variance. This implies an increase in the frequency of rare events such as cyclones, floods, and extremely low temperatures, with potentially negative effects on terrestrial vegetation (IPCC, 2007). In addition, economic development, increased urbanization and rapid population growth are likely to reduce per capita water availability throughout southern Africa and climate change is expected to exacerbate this situation, particularly in the seasonally dry areas (Le Blanc & Perez, 2008).

Agriculture in Southern Africa is predicted to be particularly severely affected by climate change, while primary production of large lakes, e.g. Lake Tanganyika, is projected to decline by the 2050s with negative impacts on populations in the surrounding countries (IPCC, 2007). Forecasts of the impacts of climatic change on the vegetation of south-central Africa suggest that woodlands, such as wet miombo vegetation and the savanna biome, will persist in the face of climatic change (von Maltitz & Scholes, 2008). Increased climatic variation would tend to favor ruderal strategists exemplified by many *Acacia* species, which are typically found in disturbed habitats or areas of low and temporally variable rainfall throughout much of south-central Africa (Fuller & Prince, 1996). In South Africa, the fynbos and the succulent karoo

ecosystems are predicted to be the most vulnerable to projected climate changes in the 21st century (von Maltitz & Scholes, 2008).

Southern African countries are among those that are most vulnerable to climate change impacts because they have fewer resources that can be used to adapt: socially, technologically and financially. These countries also share a number of common environmental challenges that may be exacerbated as a result of climate change. These include water scarcity, threats to biodiversity, land degradation, desertification and deforestation (UNEP, 2008). Vulnerability to climate change is likely to be increased by other factors like the expected large increases in the region's population in the coming decades (UN, 2007), and also substantial pressures to accelerate economic development (UN, 2006).

During the last two decades the carbon cycle has received increased attention among researchers because of its crucial role in influencing climate change. The growing recognition of the need of biophysical and biogeochemical descriptions of land surface-atmosphere exchange processes, especially in global circulation models, has led to increased efforts of quantifying rates of water vapour and CO₂ exchange between terrestrial ecosystems and the atmosphere as well as to increase understanding of these processes on all scales.

However, one of the challenges facing global integration of studies on terrestrial CO₂ and water fluxes is that the global distribution of these studies is concentrated in the northern hemisphere. Consequently, some of the most dominant vegetation types in tropical and sub-tropical regions may have significant effects on the global climate system without being comprehensively studied. So, there is an urgent need for detailed studies of the effects of these plant communities on the exchange of carbon and water with the atmosphere.

Tropical grassland ecosystems

Grassland ecosystems cover nearly one-fifth of the world's land surface ($24 \times 10^6 \text{ km}^2$), and contain > 10% of global C stocks (Figure 1; Eswaran *et al.*, 1993). They are an important habitat for wildlife, humans and their domestic livestock. Tropical grasslands occupy 15 million km^2 square kilometers (as much as tropical forests). In terms of both land area and biomass, tropical grasslands are second only to tropical forests in importance (Long *et al.*, 1989). On the African continent, tropical grasslands and savannas are more extensive than tropical forests (Scurlock & Hall, 1998). In addition, periodically inundated grasslands, a form of wetland ecosystem, occupy vast areas of South America but also Africa, for example the Sudd in southern Sudan.

Tropical grasslands store approximately 26% of the carbon sequestered by terrestrial ecosystems (Long *et al.*, 1989), and some studies have shown that tropical savannas can become modest carbon sinks (Parton *et al.*, 1995) with native savannas seasonally sequestering carbon (Miranda *et al.*, 1997). However, these regions are experiencing substantial changes in land cover and land use which may reverse this potential, leading to carbon losses and oxidation, instead of the desirable carbon sequestration.

Areas where grasslands naturally occur have a semi-arid climate (Long *et al.*, 1989) with rainfall that is irregular both in distribution and in intensity as one of the most prominent features. Greater variability of rainfall, leading to a greater frequency of periods of severe water stress during the growing season, is predicted for the Southern Africa region (Solomon *et al.*, 2007). Moreover, the IPCC (2007) showed that there were very few studies in Africa observing the changes caused by climate change in physical systems (*e.g.* hydrology and coastal processes) and biological systems (*i.e.* terrestrial, marine and freshwater). However, recently increased efforts have been undertaken to determine carbon and nutrient turnover

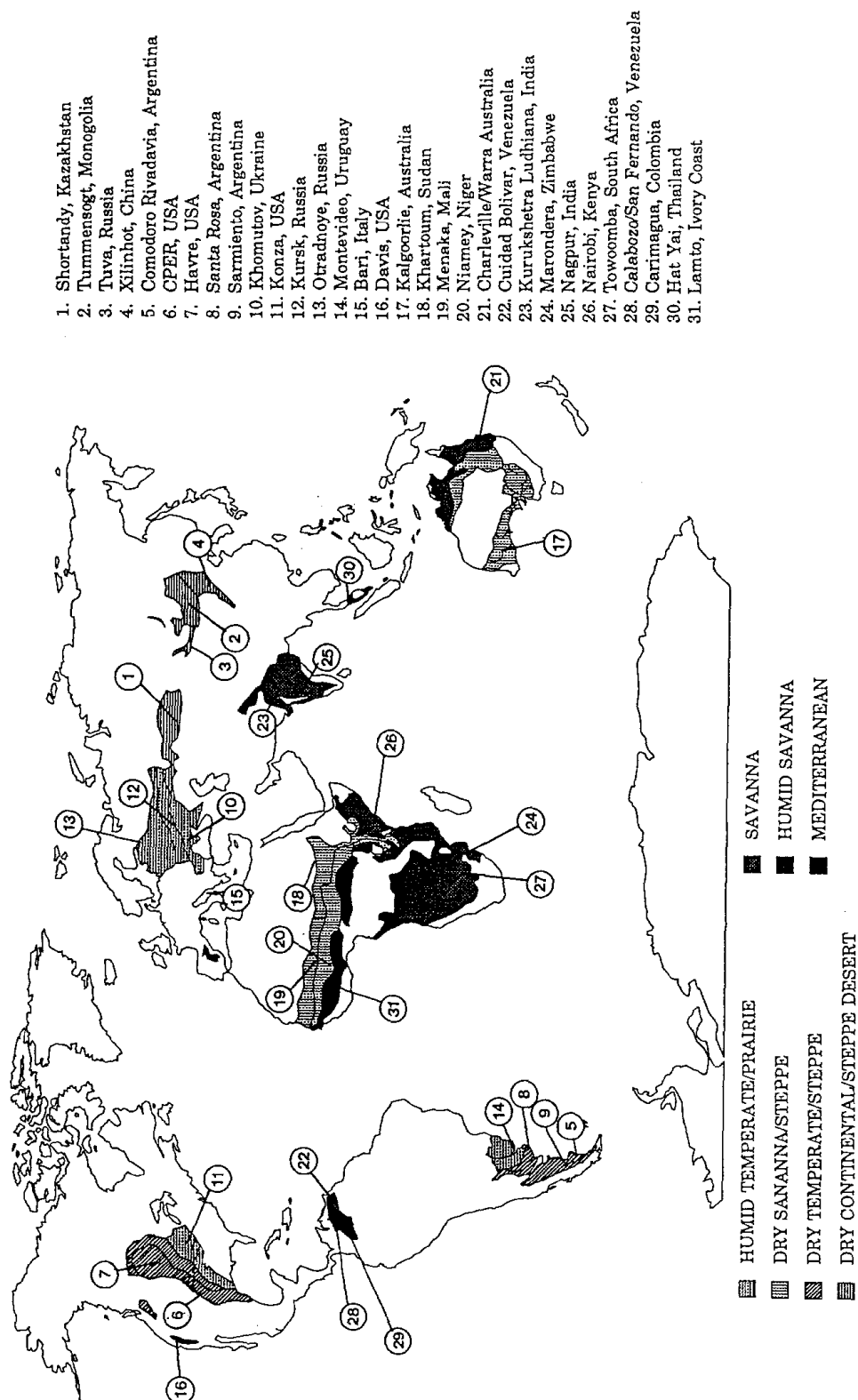


Figure 1: Map showing location of grassland sites and boundaries of Bailey eco-regions representing grasslands worldwide (Figure taken from Parton *et al.*, 1995).

in African savanna grasslands and woodlands or the factors regulating these fluxes (e.g., Simioni *et al.*, 2004; Veenendaal *et al.*, 2004; Arneeth *et al.*, 2006; Meir *et al.*, 2007; Werner *et al.*, 2007). Such studies are likely to make a major contribution toward improving the construction of global carbon budgets, since they provide the required detailed information at the local scale from a diverse range of ecosystems and climate zones.

The effects of global climate change on plant communities and ecosystems are likely to be determined by responses of individual species, with the dominant species of primary importance (Huxman *et al.*, 2004). Similar responses among species would make changes in composition and productivity straightforward to predict, but species-specific or functional group specific responses are more likely, rendering predictions of future productivity and diversity more complex. Added to this, how vegetation changes interact with precipitation and nutrient (nitrogen and phosphorus) variability to affect fluxes of semi-arid ecosystem carbon and water is not well understood. Therefore, an accurate assessment of CO₂ and water fluxes of tropical C₄ dominated ecosystems is indispensable to establish a baseline against which the effects of future changes in global climate may be assessed.

C₄ photosynthesis

Grasslands in tropical and subtropical areas are dominated by C₄ species (Baruch, 1996). The C₄ photosynthetic pathway is strongly represented in the Poaceae (grass) family, comprising about 47% of total grasses (Sage *et al.*, 1999). The most important modern environmental characteristic that favour C₄ plants include aridity for C₄ dicots and strong seasonal precipitation (that is, warm-season precipitation) coinciding with high minimum temperatures during the growing season for C₄ grasses (Sage *et al.*, 1999). Dry winters and wet summers also promote C₄ species expansion (Ehleringer & Monson, 1993). At the global scale, increasing variability of seasonal rainfall has been shown to have accelerated the expansion of C₄ grassland in Northern America, China and Africa (Pagani *et al.*, 1999).

C₄ photosynthesis involves at least three different biochemical pathways that differ in the mechanisms used to transport CO₂ to the bundle sheath cells and also to regenerate phosphoenol pyruvate (Hatch, 1987).

These biochemical pathways are NAD-malic enzyme species (NAD-ME), NADP-malic enzyme species (NADP-ME) and PEP carboxykinase species (PCK). Previous studies have associated the distribution of these pathways with precipitation, where NAD-ME and PCK are predominantly found in drier environments and NADP-ME dominates wetter environments (Ellis *et al.*, 1980; Hattersley, 1983).

It is now well established that C₄ plants can obtain high photosynthetic rates even under conditions of low resource (water and nitrogen) availabilities, that they possess a CO₂ concentrating mechanism that increases the carboxylation rate of Rubisco, decrease inhibitory effects of photorespiration and reduce both intercellular CO₂ concentration and stomatal conductance (Knapp & Medina 1999; von Caemmerer, 2000). However, in communities where C₃ and C₄ species co-exist, consistent competitive advantage due to the presence of CO₂ concentrating mechanism has been difficult to prove in experiments (Ehleringer & Monson, 1993; Sage *et al.*, 1999; Ghannoum *et al.*, 2001).

Previous studies have shown differences in gas exchange behaviour between the C₄ photosynthetic subtypes. These include variations in quantum yields (Ehleringer & Pearcy, 1983), water use efficiency (Ghannoum *et al.*, 2001), in the initial slopes of the A-c_i response curves, carboxylation efficiency, (Pooley *et al.*, 1992), leaf nitrogen content (Ghannoum *et al.*, 2002) and whole-plant and photosynthetic nitrogen use efficiencies (Ghannoum *et al.*, 2005).

Moreover, there is expanding interest in understanding the response of C₄ species to many environmental variables. Some of these are responses to varying levels of CO₂ concentration (Owensby, *et al.*, 1993; Wand *et al.*, 2001), vapour pressure deficit (Maroco *et al.*, 1997), leaf nutrient content (Simioni *et al.*, 2004), fire and seasonal re-translocation of leaf nitrogen (Baruch & Bilbao, 1999), phenological diversity (Goldstein & Sarmiento, 1987). All these factors are projected to affect the future physiology and community ecology of C₄ plants. However, the extent to which one can generalize about ecological and physiological responses of C₄ plants to their

environmental factors, particularly in light of global climate change, still remains unclear.

Soil respiration

The flux of carbon from soils to the atmosphere occurs primarily in the form of CO₂, and is a result of 'soil respiration'. Soil respiration is one of the major pathways of flux in the global carbon cycle, second only to gross primary productivity (Raich *et al.*, 2002). Therefore, even a small change in soil respiration could considerably exacerbate or mitigate increases of atmospheric CO₂ concentration, with consequent feedbacks to climate change.

The gas exchange between the soil and the atmosphere depends on numerous complex and non-linear relationships, like physiological, biochemical, chemical, ecological and meteorological conditions. Consequently, the rates of soil respiration vary by ecosystem (Raich & Schelsinger, 1992) and are also highly spatially variable within one site (Law *et al.*, 2001). A positive co-variation between soil temperature and soil water content is commonly observed in tropical regions where the dry season is often cooler than the wet season (Miranda *et al.*, 1997; Epron *et al.*, 2004). These and other studies have shown that interactions of soil temperature and soil water content are such that the effect of one variable can depend on the range of the other (Chen *et al.*, 2002; Kiese & Butterbach-Bahl 2002; Epron *et al.*, 2004; Doff Sotta *et al.*, 2004). Furthermore, soil CO₂ efflux is influenced by other factors like substrate amount (Zak *et al.*, 2000), soil pH (Andersson & Nilsson, 2001), the activity of the vegetation (Raich & Schlesinger, 1992), litter fall, decomposition dynamics and the amount and timing of rainfall (Davidson *et al.*, 2000).

Despite the importance of tropical and subtropical ecosystems to the global carbon cycle, we have only a limited understanding of the magnitude of soil respiration within and across most tropical and subtropical ecosystems. This is particularly the case regarding African tropical and subtropical ecosystems, where the available information is often too sparse to serve as a basis for making reliable predictions about the existence and the strength of a possible terrestrial sink.

The study area

This study was done in the Okavango Delta in north-western Botswana. The Okavango Delta is the world's largest inland delta and is supplied by the Okavango River, which never reaches the ocean, and whose two main headwater tributaries (the Cubango and the Quito) arise in humid highlands of central Angola where annual precipitation exceeds 1000 mm. Below the Cubango - Quito junction, the Okavango River flows southeast into the arid and semi-arid Kalahari Desert. Despite the aridity, these inflows enable the Delta to host a diverse range of geomorphological and sedimentary features that defy simple classification as abiotic (hydroclimatic, sedimentological and tectonic) factors combine with biotic (plant and animal) factors to shape the character of this wetland.

The Okavango River enters the Delta through a narrow corridor called the Panhandle (Figure 2). In the Panhandle, the Okavango is an actively meandering, largely single-thread river. The Okavango River then divides into a number of straighter, more laterally stable, distributary channels (e.g. Thaoge, Jao/Boro) that disperse across a broader depression that is considered to be a type of an alluvial fan, and thus is referred to as a 'Fan'. In both the Panhandle and Fan, channels are typically well defined, with banks formed largely of luxuriant stands of sedges (e.g. *Cyperus papyrus*) and emergent grasses (e.g. *Phragmites* spp.; *Miscanthus junceus*) rooted in a layer of peat up to ~4 m thick that contains variable amounts of clay.

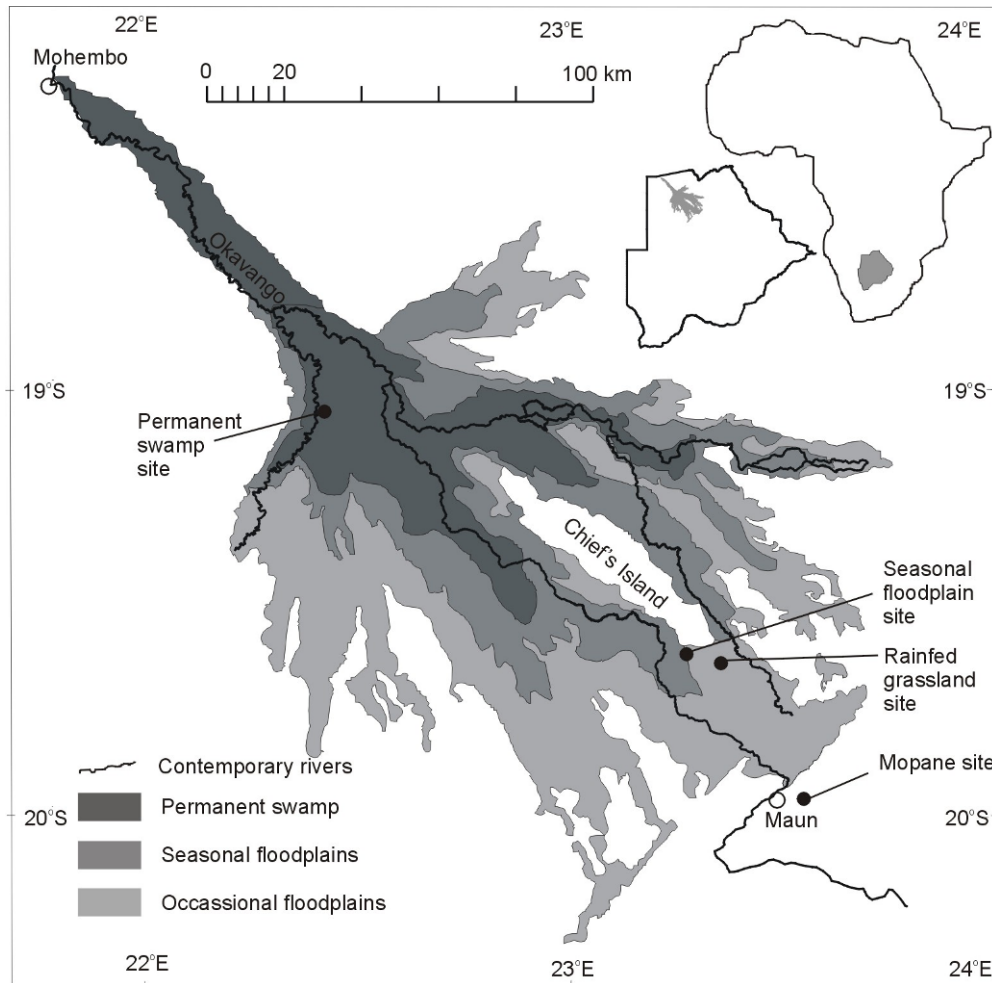


Figure 2: The map of the Okavango Delta showing the area covered by the permanent swamp, seasonal floodplains and the river channels. Also indicated in the map are the locations of the study sites: permanent swamp, seasonal floodplain, rain-fed grassland and the Mopane woodland.

The vegetated channel margins are commonly breached by animal trails, particularly by hippopotami (*Hippopotamus amphibius* L.) that move from the channels to the surrounding areas for nocturnal grazing (McCarthy *et al.*, 1998) thus creating additional pathways for water flow. It is in these permanent swamps that we chose one of our study sites (Figure 2 and Figure 3), situated at 19° 00'71" S, 022° 24' 18" E. Peak flow in the Panhandle occurs late in the summer wet season (March to April) but the passage of the seasonal flood wave is slowed by vegetation in the permanent swamps and by filling of depressions in the seasonal floodplains. The vegetation cover and species abundances across seasonal floodplains exhibit a marked zonation

pattern (Figure 3). Here species composition is primarily determined by the extent and the duration of the flood regime (Thompson, 1985; Tooth & McCarthy, 2007). In addition, subtle local edaphic conditions (soil type, salinity) and local factors (e.g. grazing pressure and fire frequency) also become significant determinants of species sequencing across the gradient of the seasonal floodplains (Thompson, 1985). The low-lying ground where inundation is frequent (more or less annually) is typically dominated by sedge species, e.g., *Schoenoplectus corymbosus* and *Cyperus articulatus* co-existing with grass species, e.g. *Oryza longistaminata* and *Leersia hexandra*, while grass species, e.g. *Cynodon dactylon* and *Imperata cylindrica*, dominate the upper lying areas. The seasonal floodplains have approximately two to four months of surface flow, with peak flows during the atmospherically dry season, June –September. Rainfall (mean annual precipitation of ~500 mm) over the panhandle and Fan is low but nevertheless is an important factor contributing to ground water recharge. Here we chose our second site area in a seasonal floodplain that is close to the Chief's Island at the southern tip of the Moremi Game Reserve (S 19°36'33" E 23°16'44"; Figure 2 and Figure 3).

Periodic shifts in water distribution on the lower Fan result from changes in upstream channel courses. In areas that become deprived of seasonal floodwaters, the water table falls, and precipitation gradually flushes soluble salts from the soils, leaving silica and calcium carbonate. Our third study site was a rain-fed semi-arid grassland (19° 39' 35" S, 023° 20' 92" E; Figure 2 and Figure 3), situated in an area that has not received flooding for several years, possibly for decades. Here, a forb, *Pechuel loechea*, co-existed with an annual grass, *Urochloa trichopus*, and perennial grasses, *Cynodon articulata* and *Eragrostis lehmanniana*. A range of trees, e.g. *Acacia sp.*, *Lonchocarpus sp.* and *Phoenix sp.*, formed the edge around the study area. the Okavango Delta provides an opportunity to study effects of widely differing soil moisture regimes within the same climate zone. That way I could concentrate on effects of soil moisture-vegetation interactions on the processes that govern leaf and soil carbon fluxes in their natural environment. This study was part of a broader effort to determine the carbon sink-source capacity of the C₄ dominated representative ecosystems of the Okavango Delta, in Botswana. The objectives of my study were:

- To investigate the environmental and physiological controls of the photosynthesis of C₄ grass and sedge species under field conditions.
- To determine whether there are any systematic effects of long term hydrological regimes on the photosynthetic traits and leaf nutrient levels (nitrogen and phosphorus) of the dominant C₄ species found in natural environments that differ in long-term water availability.
- To elucidate leaf-level gas exchange characteristics of little known, but commonly found, C₄ grass species in the Okavango Delta under optimum growth conditions.
- To study the seasonal variation in leaf-level gas exchange characteristics and leaf nutrient levels in the dominant woody savanna species, *Colophospermum mopane*, in its natural habitat.
- To characterize spatial and temporal variation in soil respiration fluxes to examine the effects of soil water content and soil temperature within each site and across sites.

Thesis structure

This study presents results from leaf-level photosynthesis and soil respiration measurements conducted between December 2001 and December 2003 in three distinct tropical grassland ecosystems in the Okavango Delta, Botswana. It also reports the analysis of leaf gas exchange measurements in dominant savanna tree species *Colophospermum mopane* in a woodland located just south of the Delta, taken between 2000 and 2001. In the following chapters we report on findings of this study. Chapter two focuses on the seasonal

Chapter 1





Figure 3 : Representative ecosystems of the Okavango Delta, from top to bottom (start on opposite page): the aerial photograph of the seasonal floodplain, the permanent swamp showing extensive dominion by *Cyprus papyrus*, a seasonal floodplain with its characteristic dominant communities along a hydrological gradient, a rain-fed grassland showing co-existence of C_4 grasses with a C_3 forb, and mono-specific stands of the mopane woodland.

Geographically, all these sites are within relatively close proximity from each other, and they experience the same climatic conditions. Yet, since this area represents one of the most striking gradients of moist/ dystrophic to

arid/eutrophic savanna landscapes in Africa, it provides a unique opportunity for a field study on carbon and nutrient fluxes.

Objectives of the study

Despite the large geographical extent of Africa, few leaf eco-physiological and soil CO₂ emissions data are available for this continent, making any data set in these topics of particular interest. More importantly, the hydrological variability in floodplain and compares the spatial and seasonal variation of photosynthetic capacities and leaf nitrogen concentration in three dominant C₄ species, *Imperata cylindrica*, *Panicum repens* (grasses) and *Cyperus articulatus* (sedge). Chapter three compares leaf photosynthetic capacity, as estimated by the light and CO₂ response curves, and the role of leaf nitrogen and leaf phosphorus, in regulating this capacity under optimum growth conditions in the three different ecosystems, that is, permanent swamp, seasonal floodplain and a semi-arid rain fed grassland.

Chapter four explores the presence of any adaptive leaf-level gas exchange traits that allow different grass species a comparative advantage to occupy wet and dry habitats. Chapter five focuses on the seasonal variation of leaf-level gas exchange and leaf nitrogen in the two growth forms of Mopane in Maun, Botswana. Chapter six quantifies the soil respiration in representative ecosystems (rain-fed semi arid grassland, seasonal floodplain and in a permanent swamp) of the Okavango delta, characterize spatial and temporal variation of soil respiration within each ecosystem and examines the relationship between environmental factors and soil respiration. Chapter seven presents the conclusions and provides a context within which the results of this study fit with the existing body of literature on ecosystem functioning in savanna and tropical grasslands. This chapter also highlights the contribution of this study to the small body of measurements of leaf physiological properties and soil respiration in savanna and grasslands ecosystems in tropical Africa.

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CHAPTER 2

Seasonal and inter-annual photosynthetic response of representative C₄ species to soil water content and leaf nitrogen concentration across a tropical seasonal floodplainⁱ

Abstract: We examined the seasonal and inter-annual variation of leaf-level photosynthetic characteristics of three C₄ perennial species, *Cyperus articulatus*, *Panicum repens* and *Imperata cylindrica*, and their response to environmental variables, to determine comparative physiological responses of plants representing particular microhabitats within a seasonal tropical floodplain in the Okavango River Delta, Botswana. Five measurement campaigns were carried out over a period of 2 y which covered two early rainy seasons, two late rainy seasons and one dry season. For all three species, light-saturated net photosynthetic rates (A_{sat}) and stomatal conductance (g_{sat}) decreased with decreasing soil water content with a seasonal range for A_{sat} of approximately 5–45 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and for g_{sat} of 0.03–0.35 $\text{mol m}^{-2} \text{s}^{-1}$. The species representing the wettest microhabitat (*Cyperus*) had the highest g_{sat} at low leaf-to-air vapour pressure deficits (D_i), the highest ratio of intercellular to ambient CO₂ concentration (C_i/C_a), as well as the highest degree of variation in C_i/C_a from season to season. We interpret this as being indicative of its adaptation to a moist growth environment allowing for non-conservative water use strategies as soil moisture is usually abundant. For all three species there was significant variation in photosynthetic fluxes from one year to another that was related to variation in leaf nitrogen and phosphorus. This study shows that when assessing the role of savanna stands in large-scale carbon balance models, the remarkable inter-annual variation in leaf photosynthesis reported in this study should be taken into account.

INTRODUCTION

C₄ plants are most abundant in semi-arid tropical and subtropical regions. This is usually attributed to the C₄ pathway being competitive at high-light environments and allowing for higher temperature optimum of photosynthesis compared to C₃ plants (Edwards *et al.* 1985, Ehleringer *et al.* 1997). The CO₂ concentrating mechanism of C₄ plants allows them to attain high nitrogen and water use efficiencies (NUE and WUE respectively).

In (semi-)arid environments one benefit of having a high WUE should be a lengthening of the period of potential photosynthetic carbon gain during periods of water shortage. Only a few exceptions to the pattern of high WUE in C₄ species have been reported; this being for very highly productive stands of *Echinochloa*

ⁱK. B. Mantlana; A. Arneth; E. M. Veenendaal; P. Wohland; P. Wolski; O. Kolle and J. Lloyd (2008) *Journal of Tropical Ecology* 24: 201–213

polystachya, *Paspalum repens* and *Paspalum fasciculatum* growing along river floodplains in tropical South America (Piedade *et al.* 1991). C₄ plants also have a relatively low nitrogen requirement in order to sustain any given level of carbon uptake (Sage & Pearcy 1987). This may also be of benefit in savanna environments that are nutrient poor (Simioni *et al.* 2004).

Despite the fact that C₄ grasses account for 20-30% of global photosynthetic productivity (Lloyd & Farquhar 1994), there have been only a few studies on the photosynthetic properties, nutrient content and water relations and modulation of these factors by the environment for C₄ grasses growing in their natural environment (Anten *et al.* 1998, Baruch & Bilbao 1999, Simioni *et al.* 2004). Moreover, we still lack to date a comprehensive study of changes in photosynthetic characteristics of C₄ plants over a growing season or year in a tropical semi-arid environment, especially in relation to the underlying internal (physiological) or external (environmental) causes.

In order to help understand the underlying environmental and physiological controls of C₄ grass and sedge species we studied spatial and seasonal variation in photosynthetic capacities and stomatal conductances of C₄ species growing in the Okavango River Delta, Botswana. We studied three species, *Imperata cylindrica* (L.) P. Beauv., *Panicum repens* L. (grasses) and *Cyperus articulatus* L. var. *nodosus* (Humb. & Bonpl. ex Willd.) Kük. (sedge) that grow in distinct areas microhabitats characterized by markedly different hydrological regimes. We hypothesised that differences in leaf-level gas exchange properties between these species will vary across the period of measurements and this variation will be most pronounced during the dry season, reflecting different ecological traits of the studied species.

METHODS

Study site

The Okavango River Delta, situated in north-western Botswana, is one of the world's largest inland deltas with an estimated area of 40 000 km² (McCarthy & Ellery 1994). The area has a semi-arid climate. Rainfall occurs mainly in November to April, averages ca. 500 mm y⁻¹, but is irregular both in spatial distribution and in

intensity. Precipitation is greatly exceeded by potential evapotranspiration, (1580 mm y⁻¹; McCarthy & Ellery 1994) but in many areas of the Delta rainfall is supplemented by annual floodwaters from the Angolan highlands that enter through the Okavango River. The work described here was undertaken at a seasonal floodplain located at the distal end of the Delta where the period of maximum inundation occurs during the climatically dry season, June-September (Ellery & McCarthy 1994). In this part of the Delta a mix of seasonal floodplains and of dry, rain-fed 'islands' at slightly higher elevations is typical.

Apart from clayey soils (Vertisols), extensive soil formations in northern Botswana, including the Okavango Delta, derive from Kalahari sands (Arenosols) with a low agronomic potential (Almendros *et al.* 2003). The vegetation cover of the Okavango Delta seasonal floodplains exhibits a marked zonation with different species occupying microhabitats characteristic of systematic variation in both frequency and length of inundation (Bonyongo *et al.* 2000). At the study location (S 19°36'33" E 23°16'44") a typical floodplain community occurred on low-lying ground where inundation lasts typically for several weeks to months during any given flood season. The dominant species in this area were a C₄ sedge, *Cyperus articulatus* var. *nodosus* growing alongside the C₃ sedge *Schoenoplectus corymbosus* (Roth ex Roem. & Schult.) Raynal. *Cyperus articulatus* is known to tolerate extended periods of standing water (Abd El-Ghani & Fahmy 2001). The soil material here is clay and, when wet, becomes blackish in colour. On the higher parts of the floodplain where plant water availability is almost always dependent on rainfall, and the soils are more sandy in character, sedges are all but absent and the perennial and rhizomatous grass *Imperata cylindrica* dominates. *Imperata cylindrica* belongs to the Nicotinamide-Adenine Dinucleotide Phosphate-malic enzyme (NADP-ME) photosynthetic subtype. This species is found throughout tropical regions, but has spread to warm temperate zones worldwide (Bryson & Carter 2004). It is one of the most intensively studied weed species worldwide but surprisingly limited data exists on its ecophysiological characteristics in its natural habitat. Few animals, other than hippopotami (*Hippopotamus amphibus* L.) will attempt to graze this plant (Thompson 1985). Lastly, what we refer to as the 'intermediate zone' (between the lower and upper floodplain) is characterised by a large year-to-year variation with respect to presence or absence, and height and duration of flooding. Here the pasture grass, *Panicum repens*, which belongs to the C₄ phosphoenolpyruvate

carboxykinase (PCK) photosynthetic type, was the dominant species. Globally, *P. repens* is not confined to intermediate floodplains, its native range extends from terrestrial, wetland and aquatic environments of tropical and North Africa, Mediterranean, South America and Asia and it is considered a weed in Northern America (Gibbs Russell *et al.* 1991).

Gas exchange measurements

Between December 2001 and March 2003 five field campaigns were conducted to study the response of plant gas exchange to seasonally varying environmental conditions. Light and CO₂ response curves were measured, between 08h00 and 13h00, on attached, fully expanded leaves randomly selected for each of the three species growing in their characteristic different microhabitats. We used a portable gas exchange system (Li-6400, Li-Cor Inc., Nebraska, USA) with one leaf from at least four plants or separate tillers sampled for each species. The leaf temperature inside the 6-cm² leaf chamber was not controlled, the only exception being in March 2003. This was to avoid temperatures in excess of 40 °C, the temperature above which C₄ net photosynthetic assimilation may become inhibited (Edwards & Walker 1983). The red/blue LED light source inside the chamber allowed for automatic changes of photon flux density (*I*).

For light response curves, measurements were made at *I* = 2000, 1800, 1500, 1000, 700, 500, 300, 100 and 0 μmol m⁻² s⁻¹ with the CO₂ mole fraction inside the chamber maintained at 370 μmol mol⁻¹. For the CO₂ response curves, measurements were made with *I* = 1600 μmol m⁻² s⁻¹. Varying CO₂ concentrations were obtained from portable CO₂/air mixture tanks and automatically controlled by a CO₂ injector fitted onto the Li-6400 system. In situ CO₂ assimilation rates were measured at 370, 300, 200, 100, 50, 400 (twice), 500, 600 and 700 μmol mol⁻¹. During measurements both the leaf-to-air vapour pressure deficit, *D*_{*l*}, and leaf temperature, *T*_{*l*}, in the chamber did not always exactly match the ambient conditions because these two parameters are affected by leaf transpiration rate (Beale *et al.* 1999); *T*_{*l*} typically exceeded air temperature at time of measurements by not more than 1 to 2 °C.

For every leaf sampled, light and CO₂ response curves were fitted individually by a non-linear regression (SPSS 11.0 for Windows) to the hyperbolic function $y = a (1 - e^{-b-cx})$, where y is the rate of CO₂ exchange, x is the independent variable (I or C_i), b and c are constants, with values set to 0.1 and 0.002, respectively (Causton & Dale 1990). In the case of light response curves, coefficient a gives the light-saturated rate of CO₂ exchange (A_{sat}), b/c gives the compensation point, $a(1-e^{-b})$ gives the dark respiration, and apparent quantum yield (the slope, or derivative of the curve at the light compensation point) is given by ace^b . In the case of the $A-C_i$ curve, a represents the light and CO₂-saturated rate of CO₂ exchange (A_{pot}), the CO₂ compensation point is again calculated from b/c , and the carboxylation efficiency (the slope, or the derivative of the curve at the CO₂ compensation point) is given by ace^b (for all see: Causton & Dale 1990). This simple equation has been widely used to analyse light and CO₂ response curves of a variety of species (Kgope 2004, Midgley *et al.* 1999, Wand *et al.* 2001) and fitted our data well ($r^2 \geq 0.9$). Light-saturated values of stomatal conductance (g_{sat}) and C_i/C_a values were obtained from the light response curves at $I > 1600 \mu\text{mol m}^{-2} \text{s}^{-1}$.

After completion of the gas exchange measurements the leaves were scanned and their area determined afterwards using WinFOLIA software (Regents Instruments Inc., Quebec, Canada). Leaf dry weight was obtained after oven-drying at 70 °C for 24 h and C and N concentration measured using a Vario EL (Elementar Americas, Inc. Mt. Laurel, New Jersey, USA). Leaf phosphorus concentration was measured after a nitric acid digestion using ICP-AES (Atomic Emission Spectrometry with Inductively Coupled Plasma, Perkin-Elmer, Norwalk, USA). Nitrogen use efficiencies (NUE) were determined by dividing A_{sat} by leaf N and expressed on a leaf area basis. Water use efficiency (WUE) is expressed here as $A_{\text{sat}}/g_{\text{sa}}$.

Meteorological variables and soil characteristics

Half-hourly rainfall, air temperature and water vapour saturation deficit at *ca.* 3 m height were measured at a nearby eddy-covariance flux tower, using a tipping bucket rain gauge (Young; Model 52202, R.M. Young Company, Traverse City, USA), temperature probe (HMP45A, Vaisala, Helsinki, Finland) and RPT 410

Barometric Sensor (Druck, New Fairfield, CT, USA), respectively. Volumetric soil water content (θ) was measured at each microhabitat within the floodplain, at 0-5 cm, 5-10 cm and 10-15 cm soil-depth intervals using a battery-powered hand-held soil moisture sensor (Moisture Meter type HH2 with Theta probe, Delta T Devices, Cambridge, UK) for each measurement campaign.

For each microhabitat, four soil samples were randomly collected at intervals of 0–5, 5–10, 10–20, 20–30 and 30-50 cm. These were then oven dried at 40 °C before being analysed for nutrients using Vario MAX (Elementar Americas, Inc. Mt. Laurel, New Jersey, USA). Soil nutrient data reported here are for the upper 10 cm of the soil profile. Soil pH was measured, from similar depths as for soil nutrients, in mixtures of 10 g of soil with 25 ml of distilled water with a pH meter (pH 538 WTW, Germany).

RESULTS

The soils of this seasonal floodplain were alkaline, pH ranged from 7.7 to 9.8, being highest in the upper parts of the floodplain (Table 1). Bulk densities in the sandiest (upper) parts were somewhat higher (1.01 g cm^{-3}) compared to the flooded parts (c. $0.7\text{-}0.8 \text{ g cm}^{-3}$), but differences were not significant (ANOVA, $n = 6$ for each microhabitat, $F = 5.29$, $df = 17$, $P = 0.097$). Between the rainy and dry season December 2001 to June 2002, soil N, soil C and soil C:N remained almost constant within each microhabitat. In March 2003 soil C and N were higher (upper floodplain) and lower (intermediate floodplain) than during the previous campaigns but remained unchanged in the floodplain. Overall, the sandy soils of the upper floodplain had lowest N and C content. With the exception of sulphur, the floodplain displayed the lowest concentrations of micronutrients (not shown).

Generally, air saturation deficit (D , Figure 1) was highest just before and at the onset of the rainy season. Towards the dry season T_a and air temperature (T_a) decreased, but even then average daily values were around 25 mbar and 25°C, respectively. Cooler days with high cloud cover (not shown), particularly during the days when measurements were made, led to substantially lower chamber leaf-to-air vapour pressure deficit (D_l) during the December 2001 campaign when compared

Table 1: Mean (\pm SD) of soil N, soil C and soil C:N ratio, together with some soil physical characteristics of the three microhabitats at the seasonal floodplain. Soil pH and bulk density (BD) were determined in March 2003. Data shown represent the 0-10-cm soil layer, and were tested with ANOVA and grouped with the post hoc Tukey test. Different letters in a column indicate means are significantly different ($P < 0.05$) when testing for differences in each variable within each microhabitat.

	Soil N (%)	Soil C (%)	Soil C:N	BD (g cm ⁻³)	pH
Upper floodplain					
December 2001	0.04 \pm 0.01a	0.62 \pm 0.13a	14.7 \pm 0.38a		
March 2002	0.04 \pm 0.01a	0.68 \pm 0.17a	16.2 \pm 1.54a		
June 2002	0.06 \pm 0.04a	0.84 \pm 0.54a	14.6 \pm 0.99a		
March 2003	0.12 \pm 0.03b	1.58 \pm 0.49b	13.5 \pm 1.95a	1.01 \pm 0.10	9.8 \pm 0.33
Intermediate floodplain					
December 2001	0.10 \pm 0.04b	1.47 \pm 0.56b	14.5 \pm 0.99b		
March 2002	0.12 \pm 0.03b	1.65 \pm 0.48b	14.1 \pm 0.79b		
June 2002	0.11 \pm 0.03b	1.48 \pm 0.44b	13.7 \pm 0.91ab		
March 2003	0.04 \pm 0.01a	0.52 \pm 0.06a	11.9 \pm 0.98a	0.73 \pm 0.06	9.1 \pm 0.24
Floodplain					
December 2001	0.10 \pm 0.06a	1.36 \pm 0.91a	13.7 \pm 0.92b		
March 2002	0.11 \pm 0.09a	1.26 \pm 0.32a	13.9 \pm 0.77b		
June 2002	0.15 \pm 0.07a	2.19 \pm 0.91ab	14.6 \pm 0.49b		
March 2003	0.10 \pm 0.07a	1.16 \pm 0.86a	11.1 \pm 0.89a	0.78 \pm 0.27	7.7 \pm 0.59

to the rest of the measurement periods. However, D_i remained similar across the rest of measurement periods and also between species (Table 2).

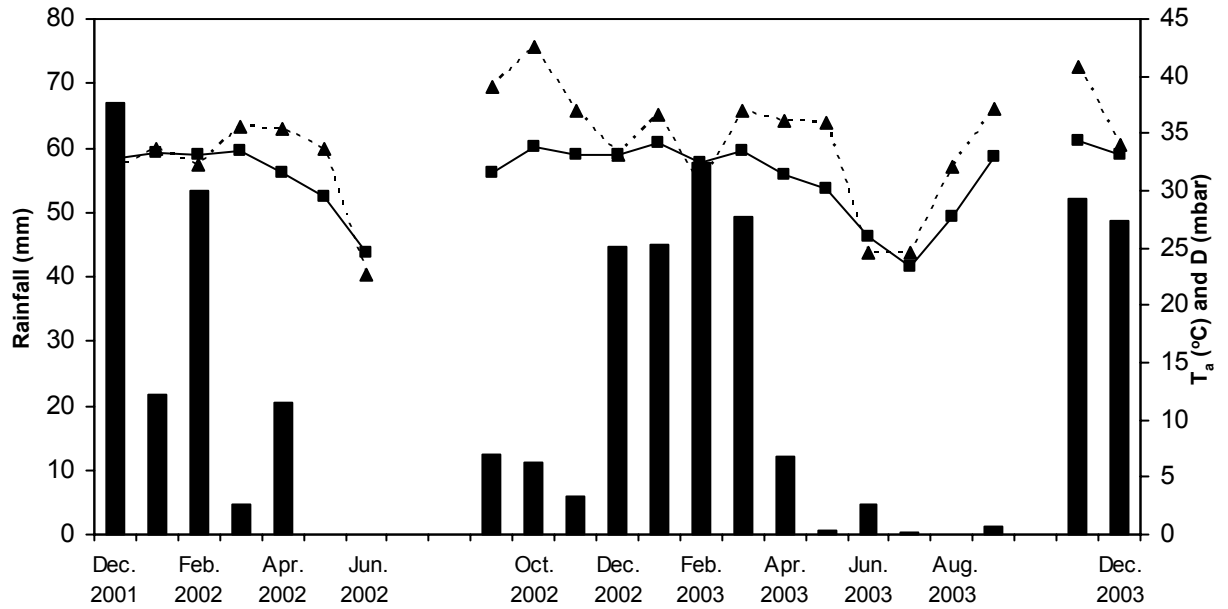
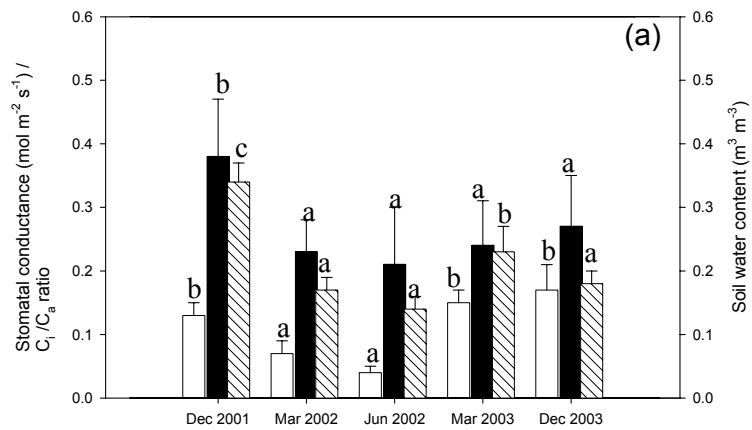


Figure 1: Monthly rainfall (bars), mean maximum monthly air temperature (solid line) and mean maximum vapour pressure deficit (dotted line) at the seasonal floodplain measurement site, located in the Okavango River Delta in north-western Botswana. Five measurement campaigns were conducted between December 2001 and December 2003.

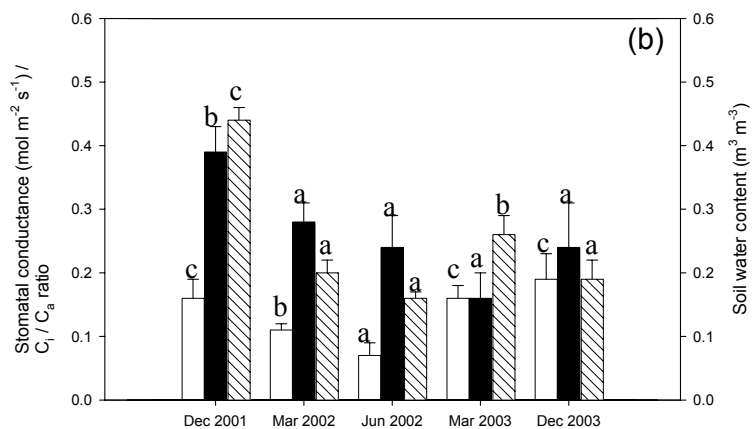
Table 2: Leaf-to-air vapour pressure deficit (D_i), water use efficiency (WUE), nitrogen use efficiency (NUE) obtained from the A-c_i response and light response curves and specific leaf area (SLA). WUE and NUE were determined as $A_{\text{sat}}/g_{\text{sat}}$ and $A_{\text{sat}}/\text{leaf N}$, respectively. Values are mean \pm SD of at least four measurements on randomly selected leaves of each species. All data were tested with ANOVA and grouped with the post hoc Tukey test. Different letters in a column indicate means are significantly different ($P < 0.05$) when testing for differences in each variable within each microhabitat.

	D_i (kPa)	WUE ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$)	NUE ($\text{CO}_2 \text{ mol (N)}^{-1} \text{ s}^{-1}$)	SLA ($\text{cm}^{-1} \text{ g}^{-1}$)
Upper floodplain				
<i>Imperata cylindrica</i>				
December 2001	1.7 \pm 0.1a	156 \pm 14.9a	671 \pm 146c	176 \pm 11.1b
March 2002	4.9 \pm 1.1b	173 \pm 12.3a	340 \pm 22.9a	188 \pm 14.8b
June 2002	4.2 \pm 1.4b	180 \pm 21.7a	365 \pm 37.3a	158 \pm 17.6b
March 2003	4.8 \pm 0.2b	178 \pm 24.5a	522 \pm 69.8bc	116 \pm 13.2a
December 2003	4.4 \pm 0.4b	159 \pm 20.1a	482 \pm 65.8bc	181 \pm 10.3b
Intermediate floodplain				
<i>Panicum repens</i>				
December 2001	2.3 \pm 0.2a	151 \pm 28.2a	745 \pm 64.5c	156 \pm 13.6ab
March 2002	3.8 \pm 0.4b	163 \pm 5.2a	391 \pm 35.4a	162 \pm 21.0ab
June 2002	3.9 \pm 1.3b	176 \pm 12.3a	625 \pm 27.2b	167 \pm 11.7ab
March 2003	5.1 \pm 0.8b	183 \pm 11.6ab	479 \pm 26.3b	132 \pm 18.1a
December 2003	4.1 \pm 0.9b	170 \pm 29.7a	489 \pm 48.9b	171 \pm 12.4ab
Floodplain				
<i>Cyperus articulatus</i>				
December 2001	2.5 \pm 0.2a	113 \pm 38.7a	900 \pm 91.9b	156 \pm 9.6ab
March 2002	5.3 \pm 1.3b	94.9 \pm 15.5a	443 \pm 68.9a	155 \pm 13.0ab
June 2002	5.1 \pm 0.8b	106 \pm 26.9a	455 \pm 78.7a	158 \pm 15.6ab
March 2003	3.9 \pm 0.5b	142 \pm 26.1ab	1053 \pm 35.5c	126 \pm 15.1a
December 2003	5.1 \pm 0.6b	122 \pm 25.3a	505 \pm 153a	168 \pm 10.8b

Imperata cylindrica (upper floodplain)



Panicum repens (intermediate floodplain)



Cyperus articulatus (lower floodplain)

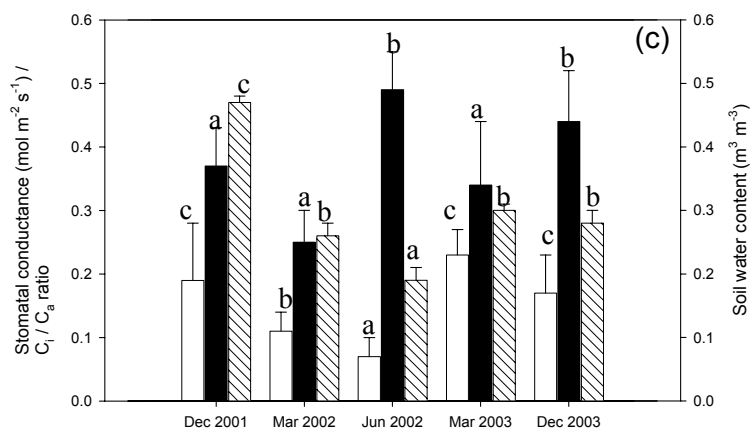


Figure 2: Stomatal conductance (white), C_i/C_a ratio (black) and soil water content (shaded) of the three species during the measurement periods. Data are mean (\pm SD) and were tested with ANOVA and grouped with the post hoc Tukey test. Different letters indicate means are significantly different ($P < 0.05$) when testing for between-season differences in each variable within each microhabitat. *Imperata cylindrica* (a) grows on the driest parts in the floodplain, *Panicum repens* (b) grows on the intermediate part of the floodplain, while *Cyperus articulatus* (c) grows on the (lower) wettest parts in the floodplain.

For all three species, light-saturated stomatal conductances, g_{sat} , were highest during the wet season (December and March) with mean values ranging from 0.15 and 0.23 mol m⁻² s⁻¹ (Figure 2), and in each campaign increasing from the driest microhabitat to the wettest microhabitat. For all three species g_{sat} were lowest during the June 2002 campaign when θ was the lowest.

Figure 2 shows reductions in C_i/C_a in all microhabitats towards the end of the rainy season (December 2001 vs. March 2002). However, as the dry season progressed mean C_i/C_a of the sedge, *C. articulatus*, increased substantially (June 2002). By contrast, the mean C_i/C_a of the grass species continued to decline. Overall, differences in C_i/C_a across the floodplain were most pronounced in the absence of surface flooding, i.e. in June 2002, March 2003 and December 2003 (Figure 2). During these periods, C_i/C_a in *C. articulatus* was consistently higher than those measured at the drier microhabitats (ANOVA, $n = 6$ for each microhabitat; June 2002: $F = 27.2$, $df = 17$, $P < 0.001$; March 2003: $F = 7.08$, $df = 17$, $P = 0.008$; December 2003: $F = 9.36$, $df = 17$, $P = 0.001$).

With the exception of March 2003, which showed a tendency of low specific leaf area (SLA), no significant differences were found in the values of mean SLA within each microhabitat (ANOVA; *I. cylindrica*: $n = 26$, $F = 22.2$, $df = 25$, $P > 0.05$; *P. repens*: $n = 25$, $F = 29.2$, $df = 24$, $P > 0.05$; *C. articulatus*: $n = 22$, $F = 19.1$, $df = 21$, $P > 0.05$) and across the species (ANOVA, $n = 15$, $F = 0.366$, $df = 14$, $P = 0.701$) (Table 2). Foliar N of the grass species was highest in the wet season just after the onset of the annual rains and declined progressively towards the dry season, reaching their lowest values in June (Figure 3).

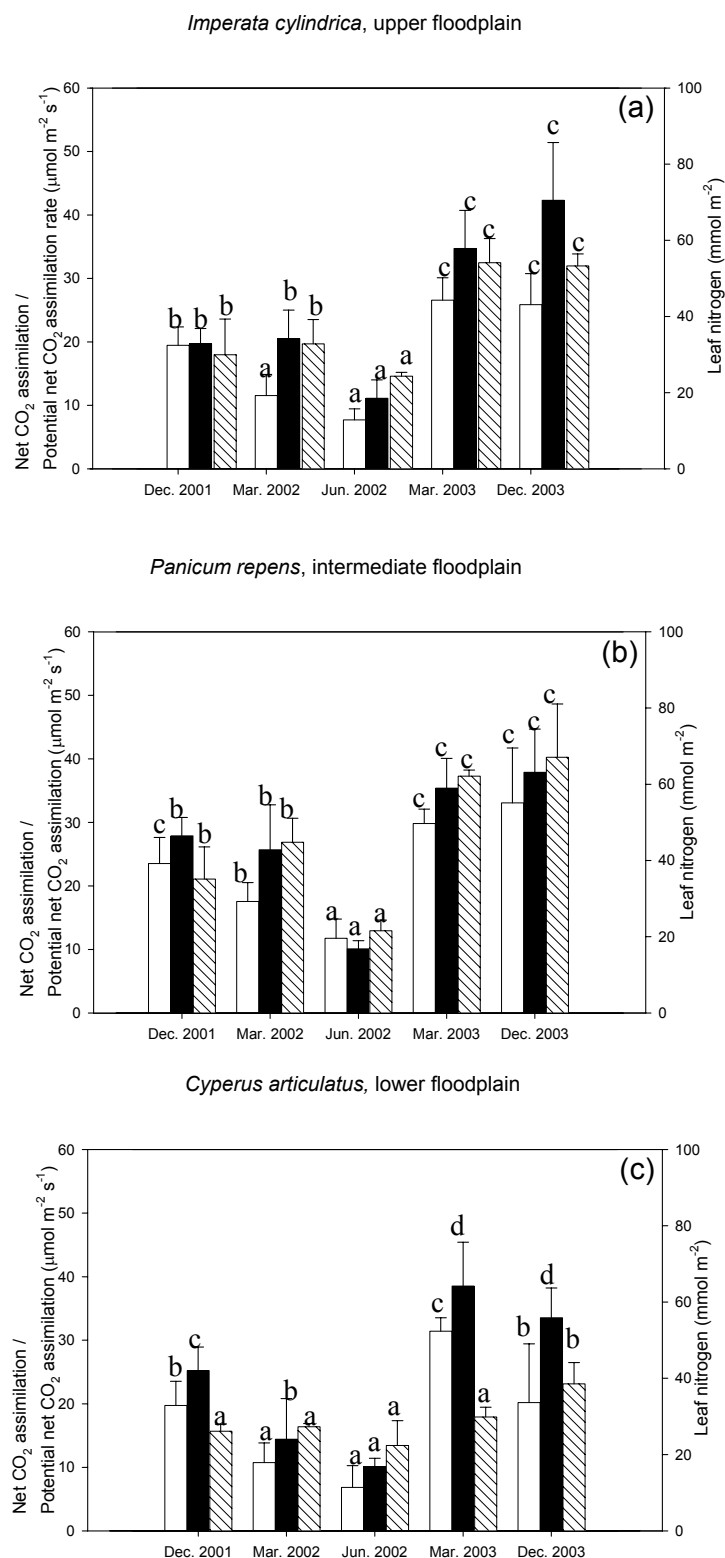


Figure 3: Light-saturated net CO₂ assimilation (white), potential net CO₂ assimilation rate (black) and leaf nitrogen content (shaded) for *Imperata cylindrica* (a), *Panicum repens* (b) and *Cyperus articulatus* (c) during the measurement periods. Data are mean (\pm SD) and were tested with ANOVA and grouped with the post hoc Tukey test. Different letters indicate means are significantly different ($P < 0.05$) when testing for between-season differences in each variable within each microhabitat.

Values measured in December 2003 were in all species significantly higher (t-test, *I. cylindrica*: $t = 13.4$, $n = 13$, $df = 12$, $P < 0.05$; *P. repens*: $t = 9.2$, $n = 11$, $df = 10$, $P < 0.05$; *C. articulatus*: $t = 9.2$, $n = 11$, $df = 10$, $P < 0.05$) than in December 2001. With the exception of the dry season, mean leaf N values in *P. repens* were always higher than those of the other species, but the difference was not always statistically significant. Moreover, leaf N values of *P. repens* varied seasonally by a factor of three (67 to 22 mmol N m⁻²) contrasting with a much lower variation in leaf N for *C. articulatus*. Leaf phosphorus data were available only for the last two wet-season measurement campaigns, March and December 2003. No species showed significant differences (t-test; *I. cylindrica*: $t = 15.3$, $n = 8$, $df = 7$, $P = 0.074$; *P. repens*: $t = 25.7$, $n = 8$, $df = 7$, $P = 0.46$; *C. articulatus*: $t = 7.62$, $n = 8$, $df = 7$, $P = 0.065$) in mean leaf P between March 2003, ranging from 1.04 to 1.84 mmol m⁻², and December 2003, ranging 1.49–1.86 mmol m⁻². During both periods, the two grasses had higher mean leaf P than the sedge, although this difference was significant (ANOVA, $n = 4$ for each species, $F = 29.12$, $df = 11$, $P < 0.05$) only in March 2003.

As was the case with leaf N and g_{sat} , A_{sat} and A_{pot} were highest during the wet season when mean values ranged between 20 and 31 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 19 and 42 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Figure 3). The species dominating the intermediate floodplain, *P. repens*, had the highest mean A_{sat} overall (except for March 2003) and highest mean A_{pot} values from December 2001 to June 2001. However, these differences in A_{sat} and A_{pot} were not always significantly different. From December 2001 to June 2002, both A_{sat} as well as g_{sat} (Figure 2 and Figure 3) decreased by approximately 60–70% across the entire floodplain, while A_{pot} was less affected. The seasonal decline of A_{sat} was smallest in *I. cylindrica* and largest in *C. articulatus*. Over a broad range of D_l and during the drying sequence December 2001–June 2002, the C₄ sedge (in the wettest microhabitat) consistently had lower A_{sat} at similar or higher g_{sat} when compared to the grasses (representing the drier microhabitats). Thus the slope of the linear A_{sat} versus g_{sat} relationship was considerably lower (data not shown). As a consequence, mean water use efficiency (WUE) was lowest in *C. articulatus*, ranging from 95 to 142 $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$ (Table 2), whereas WUE of *I. cylindrica* and *P. repens* were similar, ranging between 152 and 183 $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$. Largest and significant differences

between the mean WUE of the grass species and those of sedge species were found during the late rainy (ANOVA; March 2002: $n = 19$, $F = 34.9$, $df = 18$, $P < 0.01$), and dry season (ANOVA; June 2002: $n = 27$, $F = 34.0$, $df = 26$, $P < 0.01$).

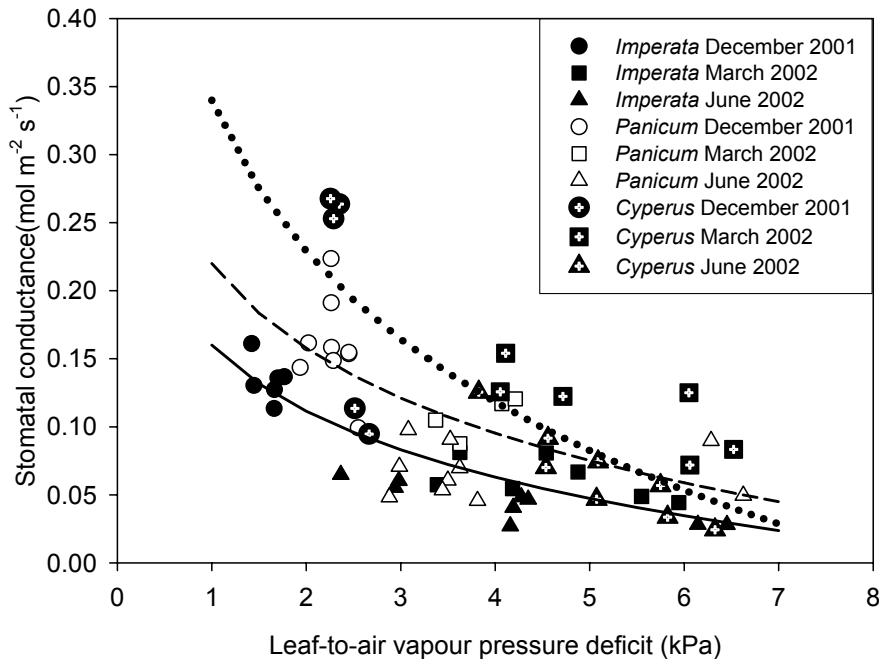


Figure 4: Relationship between leaf-to-air vapour pressure deficit and stomatal conductance of *Imperata cylindrica*, *Panicum repens* and *Cyperus articulatus*. Data shown were obtained during the soil drying cycle (December 2001 to June 2002). Each datum point represents measurements taken from an individual leaf. All data points were obtained at high light ($\geq 1600 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient CO_2 concentration ($370 \mu\text{mol mol}^{-1}$). Lines were fitted using the logarithmic Lohammar-type relationship.

All species showed the typical curvilinear decline of g_{sat} with D_l at non-limiting light conditions (Figure 4) which can be described by using for instance, the logarithmic ‘Lohammar-type’ relationship $g_s = -m \ln D_l + b$; where m is the slope and b the intercept. There was some scatter in the data, but in general g_{sat} at a given D_l in the wettest microhabitat (*C. articulatus*) was higher than in the driest microhabitat (*I. cylindrica*) irrespective of time of year. *Cyperus articulatus* also showed the highest stomatal sensitivity, indicated by the highest initial decline of g_{sat} with D_l .

For the two grasses, a strong positive relationship between leaf N and A_{pot} , was discernible across all experimental campaigns as can be seen for instance when comparing the response found for *I. cylindrica* in March 2002 vs. June 2002

(Figure 5a). When merging data from all measurement periods there was an overall increase of A_{pot} with N in *P. repens* and *I. cylindrica*, which was notably steeper for the latter. By contrast, no significant relationship existed between A_{pot} and leaf N in *C. articulatus*, except for the December 2003 campaign. These patterns were similar in A_{sat} (not shown).

Leaf N in the sedge was at the lower end of the range observed in the floodplain, and varied seasonally only little between ca. 20–40 mmol N m⁻². This translated into higher mean NUE in the wet season when compared to the plants in the other microhabitats (Table 2). Across the entire floodplain, NUE values were higher, but not significantly different (ANOVA, $n = 30$, $F = 0.931$, $df = 29$, $P = 0.76$), during the rainy season December 2001, when compared to the dry season, June 2002. The species and seasonal response of g_{sat} to leaf N was similar to that of A_{pot} and A_{sat} to leaf N (data not shown).

For the two periods where leaf phosphorus data were available, all three species expressed a strong linear relationship of leaf P with A_{pot} with greatly lessened difference between species and microhabitats that was the case for N (Figure 5d). Values obtained for *C. articulatus* in March 2003 were however exceptional, as A_{pot} of a similar magnitude to those observed in another species were obtained at much lower foliar phosphorus concentrations.

DISCUSSION

On islands and dry floodplain areas of the Okavango Delta soil pH values above 9, as found in this study, frequently indicate the development of sodic soils that turn hard and crusted when dry (Bonyongo & Mubyana 2004, McCarthy & Ellery 1994, Tooth & McCarthy 2007). The range of A_{sat} (20–45 $\mu\text{mol m}^{-2} \text{s}^{-1}$) observed when soil moisture levels were high is similar to values reported for C₄ species elsewhere under well-watered field conditions (15–60 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Long 1985). Values for rainy season g_{sat} , however, were at the lower end of the spectrum (0.14–0.23 mol m⁻² s⁻¹ versus 0.2–0.7 mol m⁻² s⁻¹; Lawlor 2001, Long 1985), and as such the C_i/C_a ratios were also relatively low (0.16–0.49), with the highest values generally found in the sedge.

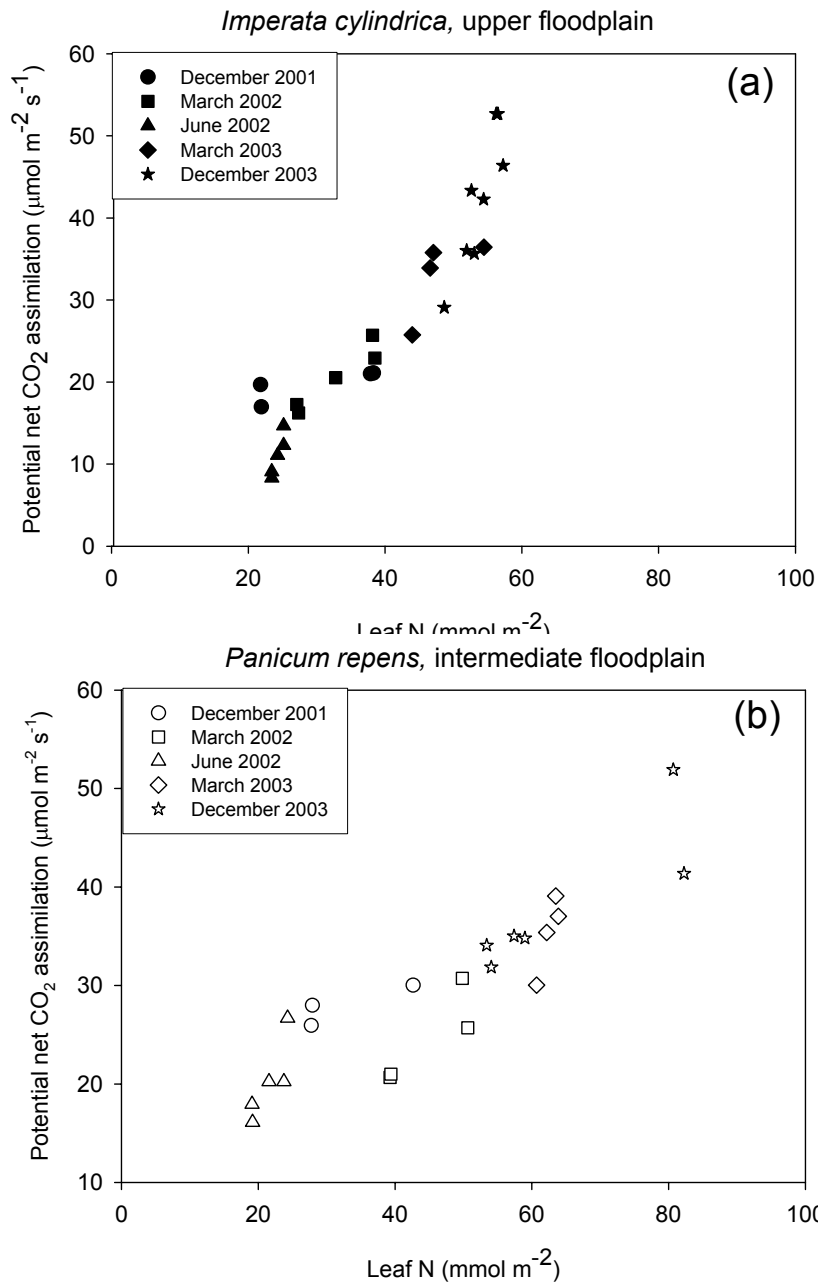


Figure 5: (a & b) The relationship between leaf nitrogen and potential net CO₂ assimilation for *Imperata cylindrica* (a), *Panicum repens* (b) and *Cyperus articulatus* (c) throughout the entire measurement periods. Figure 5(d) shows the relationship between leaf phosphorus and potential net CO₂ assimilation at the three microhabitats species during the rainy season campaigns of March and December 2003.

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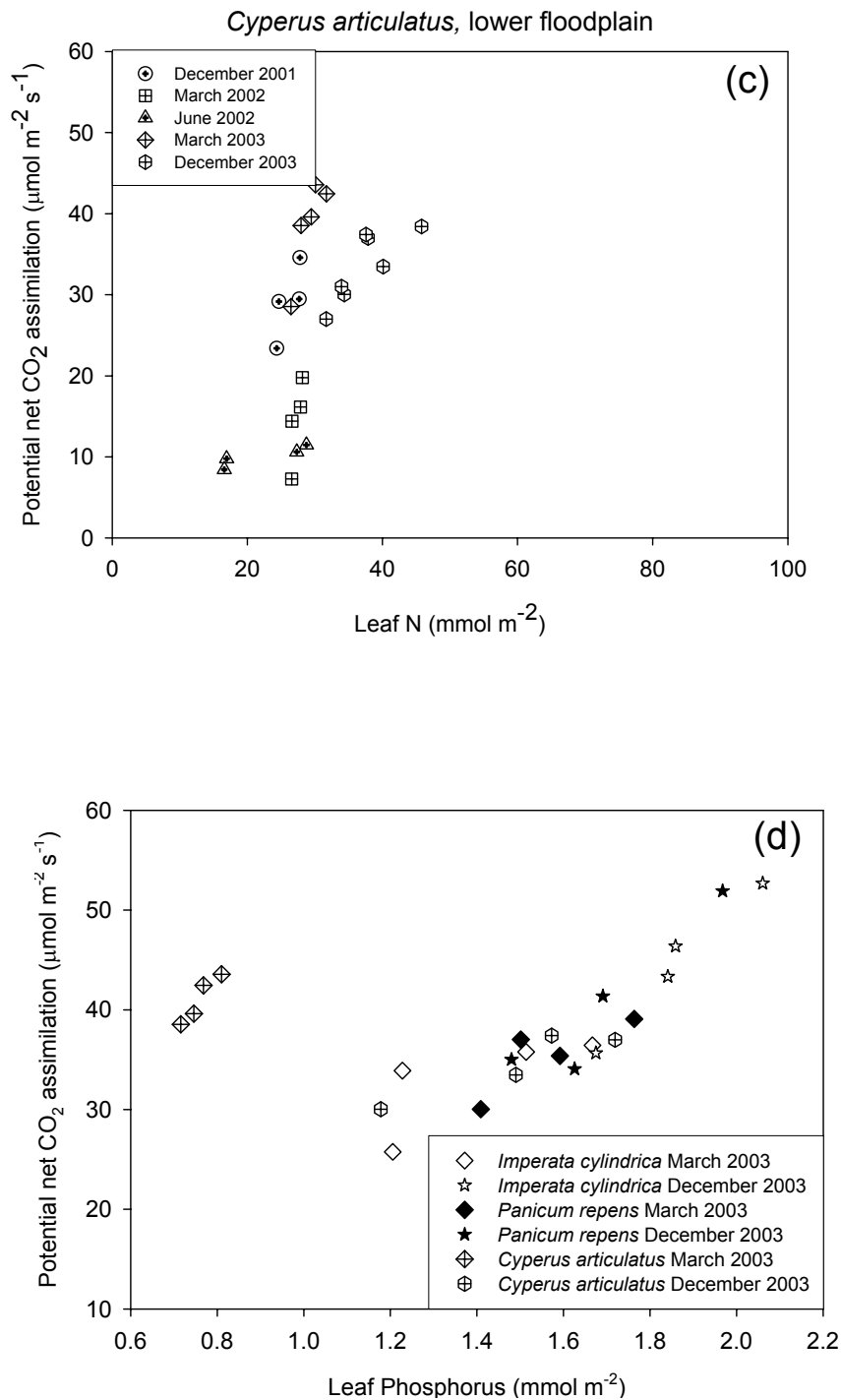


Figure 5: (c & d) The relationship between leaf nitrogen and potential net CO₂ assimilation for *Imperata cylindrica* (a), *Panicum repens* (b) and *Cyperus articulatus* (c) throughout the entire measurement periods. Figure 5(d) shows the relationship between leaf phosphorus and potential net CO₂ assimilation at the three microhabitats species during the rainy season campaigns of March and December 2003.

Mean WUE of *C. articulatus* (95–142 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) was comparable to that found in two *Cyperus* species, 100 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, in a tropical wetland at Kahawa Swamp, Kenya (Jones 1988), while mean WUE of the two grasses (151–183 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$) was higher than that found in the C_4 grasses of a tallgrass prairie, 96 $\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$, in Kansas, USA (Knapp 1993). In the grass species a more efficient stomatal function relative to that of the sedge allowed carbon uptake at minimal loss of water but structural differences between the grass and sedge species may also have played a role in the observed gas exchange differences. It is likely that the root system of the sedge is deeper and larger than that of the grass species, so as to provide support during periods of inundation and more importantly giving it access to larger amounts of water to support its relatively high water demand even in the dry season.

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CHAPTER 3

Photosynthetic properties of C₄ plants growing in an African savanna/wetland mosaicⁱⁱ.

Abstract: We analyzed photosynthesis rates and photosynthesis-leaf nutrient relationships of nine tropical grass and sedge species growing in three different ecosystems: a rain-fed grassland, a seasonal floodplain and a permanent swamp; located along a hydrological gradient in the Okavango Delta, Botswana. These investigations were conducted during the rainy season, at a time of the year when differences in growth conditions between the sites were relatively uniform. At the permanent swamp, we found largest variation of area-based leaf nitrogen contents, from 20 to 140 mmol m^{-2} , nitrogen use efficiencies (*NUE*), from 0.2 to $2.0 \text{ mmol (C) mol}^{-1} \text{ (N) s}^{-1}$, and specific leaf areas (*SLA*) from 50 to $400 \text{ cm}^2 \text{ g}^{-1}$. For the vegetation growing at the rain-fed grassland we found highest leaf gas exchange rates, high leaf nutrient levels, low ratio of intercellular to ambient CO₂ concentration and high carboxylation efficiency. Taken together, these observations indicate a very efficient growth strategy that is required for survival and reproduction during the relatively brief period of water availability. The overall lowest values of light-saturated photosynthesis (*A_{sat}*) were observed at the seasonal floodplain; around 25 and $30 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$. To place our observations into the broader context of functional leaf-trait analysis, we plotted relationships of photosynthesis rates, specific leaf area and foliar nutrient levels, in the same way as was done for previously published “scaling relationships” that are based largely on C₃ plants, noting the differences in the analyses between this study and that of Wright *et al.* 2004. The within and across species variation in both *A_{sat}* and *SLA* appeared better predicted by foliar *P* (dry mass or area basis) rather than by foliar *N* concentrations, possibly because the availability of phosphorus is even more critical than availability of nitrogen in the studied relatively oligotrophic ecosystems.

Abbreviations:

A = net photosynthesis

α = carboxylation efficiency;

A_{pot} = potential net photosynthetic rate

A_{sat} = light-saturated photosynthetic rate,

C_i/C_a = ratio of intercellular to ambient CO₂ concentration;

D = vapour pressure deficit;

D_l = leaf-to-air vapour pressure deficit,

g_s = stomatal conductance;

g_{sat} = light-saturated stomatal conductance;

leaf *N* = leaf nitrogen content

leaf *P* = leaf phosphorus content

ⁱⁱ K. B. Mantlana, A. Arneth, E. M. Veenendaal, P. Wohland, P. Wolski, O. Kolle, M. Wagner and J. Lloyd: *Journal of Experimental Botany*. Accepted

L_g = gas phase limitation to photosynthesis

NUE = photosynthetic nitrogen use efficiency;

$PEP-C$ = phosphoenol pyruvate carboxylase;

PCR = photosynthetic carbon reduction cycle

Rubisco = Ribulose biphosphate carboxylase oxygenase;

θ = soil water content;

T_a = air temperature;

T_l = leaf temperature;

WUE = water use efficiency.

Γ = CO_2 compensation point

INTRODUCTION

Plants of the C_4 photosynthetic mode are capable of high photosynthetic rates at low intercellular CO_2 concentrations (Hatch and Osmond, 1976), having high temperature optima (Long, 1985) and being highly efficient in assimilating carbon when exposed to full sunlight (Pearcy and Ehleringer, 1984; Piedade *et al.*, 1991). It is now well established that C_4 plants can obtain high photosynthetic rates even under conditions of low resource (water and nitrogen) availabilities (Knapp and Medina, 1999) and they tend to dominate in hot environments characterised by seasonal soil water deficits (Hattersley, 1983; Collatz *et al.*, 1998). Within the tropics, C_4 grasses also dominate in permanent and seasonally waterlogged environments where tree maintenance and establishment is presumably not possible (Piedade *et al.*, 1994; Long, 1999).

Due to the differing ecophysiological requirements of C_3 and C_4 plants, global climate change related factors have the potential to shift the dynamic equilibria in ecosystems dominated by C_3 - C_4 interactions (Ehleringer *et al.*, 1997; Bond and Midgley, 2000). The major factors that constrain the relative abundances of C_3 vs. C_4 species are water, nutrients, fire and biotic stress. However, it remains difficult to quantify their overall contribution as the relative importance of each of these factors differs regionally (Sankaran *et al.*, 2005). In broadest terms, warmer growth conditions have been shown to favour C_4 species over woody C_3 species (Collatz *et al.*, 1998; Sage *et al.*, 1999). This difference has been attributed to the elimination of photorespiration by the C_4 species, thus making their energy requirement for CO_2 assimilation

independent of temperature (Long, 1999). High atmospheric CO₂ concentrations should improve the water status of both photosynthetic types through reduced stomatal conductance, but effects will be more marked for C₃ types (Wand *et al.*, 2001). Reduced ecological benefits in terms of water use efficiency (*WUE*) of C₄ plants over C₃ plants might therefore, in grasslands, shift the probabilities of establishment towards C₃ woody seedlings.

Nevertheless, surprisingly little is known about the short-term and small-scale response to environmental factors of C₄ species growing across a range of natural ecosystems, even within a single region. Such data may be useful for the validation of processes in models that simulate land-surface fluxes (Collatz *et al.*, 1992; von Caemmerer and Furbank, 1999). Since the seasonality of water and nutrient availability are the two major factors that constrain C₄ gas exchange (Knapp and Medina 1999), we characterised gas exchange, leaf nitrogen and leaf phosphorus content of C₄ species growing in natural environments that differ in their long-term water availability ranging from permanent swamp to rain-fed grassland. The chief objective was to determine whether the dominant species that grow in these very different habitats would, under non-limiting soil water conditions at the time of measurement, differ in terms of their leaf photosynthetic capacity, as estimated by the light and CO₂ response curves, and how photosynthetic capacity changes with leaf *N* and leaf *P*. These data were used to determine if there were systematic effects of long-term hydrological regime on the photosynthetic traits of the characteristic C₄ species found at a particular location.

MATERIALS AND METHODS

Study area

We selected three study sites of different hydrology within the Okavango River Delta, Botswana. The Okavango River flows from the Angolan highlands into Botswana where it spreads into a complex, dynamically changing mosaic of perennial swamps, seasonal swamps, floodplains, and rain-fed grasslands

and savannas. The herbaceous types of vegetation which dominate much of the Delta are dominated by C₄ grasses and sedges but do also contain a number of C₃ species, especially in the moister areas (Ellery *et al.*, 1992).

Although the rainy season in the southern part of Africa typically lasts from November to March, the Delta is sustained by rainfall collected in its Angolan catchment which usually reaches its upper parts (dominated by permanent swamps) shortly after the rainy season ceases, around April or May, then reaching its distal parts (dominated by seasonally flooded grasslands, dry grasslands, and savannas) around July or August when the dry season is near its peak. The level and area of surface flooding varies distinctly between the northern and the southern parts, as well as on a micro-scale within any area of more than 1 km² or so.

In the perennial swamps, some of the typical plant communities are formed by *Cyperus papyrus* and *Phragmites australis* together with *Miscanthus junceus*, *Typha latifolia* and *Imperata cylindrica* as co-dominants. One of our study areas was chosen in the central region of these perennial swamps, close to the Jao distributary channel (S19° 01.18' E22° 24.03'). In this area, peat has gradually accumulated, indicating a prevalence of inundated conditions.

The second study area (S19° 36.06' E23° 16.07'), represented a typical seasonal floodplain with the sedges *Schoenoplectus corymbosus* and *Cyperus articulatus* growing in its lowest parts. The clay, representing the predominant soil material here, becomes blackish in colour when wet. *Panicum repens* dominated slightly higher areas with less seasonal inundation, and *Imperata cylindrica* was found on the upper, drier areas of the floodplain (Mantlana *et al.*, 2008). *P. repens* and *I. cylindrica* were found in areas that have soils with a sandy-loam character.

The third area investigated was a rain-fed grassland (S19° 39.06' E23° 21.53'), located in an area that had not received flooding for several years, and possibly for decades. Here, the top 30 cm of the soil consisted predominantly of sand. Here, the vegetation was dominated by annual and perennial grasses, *Urochloa trichopus*, *Cynodon dactylon* and *Eragrostis lehmanniana*, together with a forb, *Pechuel loechea*. Around the edge of the

study area, there were trees of the genera *Lonchocarpus*, *Acacia* and *Phoenix*. An overview of the species measured in this study and some relevant characteristics are found in Table 1.

Table 1: List of the *C₄* species that were measured in this study, their physiological and growth classification and the soil type within each site.

Site	Species	Physiology	Growth form	Soil type
Permanent swamp	<i>Miscanthus junceus</i>	C4-NADP-ME	perennial	Peat
Permanent swamp	<i>Imperata cylindrica</i>	C4-NADP-ME	perennial	Peat
Permanent swamp	<i>Cyperus papyrus</i>	C4-NADP-ME	perennial	Peat
Seasonal floodplain	<i>Imperata cylindrica</i>	C4-NADP-ME	perennial	Sandy-loam
Seasonal floodplain	<i>Panicum repens</i>	C4-PCK	perennial	Sandy-loam
Seasonal floodplain	<i>Cyperus articulatus</i>	C4 unknown	perennial	Clay
Rain fed grassland	<i>Cynodon dactylon</i>	C4-NAD-ME	perennial	Sandy
Rain fed grassland	<i>Eragrostis lehmanniana</i>	C4-NAD-ME	Perennial	Sandy l.
Rain fed grassland	<i>Urochloa trichopus</i>	C4-PCK	annual	Sandy

Gas exchange measurements

Measurements were carried out during the second half of the rainy season undertaken in February and March 2003 (Table 2), providing the opportunity to study plant gas exchange of the various species present in the different areas under close to optimum soil moisture conditions and to investigate whether clear differences emerge that are related to the different growth conditions at the sites. Steady state leaf gas exchange measurements were made using an open gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA). Within each study area, at least four to five individuals of the dominant species were selected at random for measurements on fully expanded leaves. Measurements were made between 09h00 and 16h00 and were recorded

only after photosynthetic rate and stomatal conductance were considered constant and at equilibrium with the ambient conditions within the gas exchange cuvette. For each measured leaf, light-saturated photosynthetic rate, A_{sat} , stomatal conductance, g_{sat} , and ratio of internal to ambient CO_2 concentration, C_i/C_a , were first obtained as average of three measurements at high photon irradiance (1600, 1800 and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and at an ambient $[\text{CO}_2]$ of 380 $\mu\text{mol mol}^{-1}$. Subsequently $A : C_i$ response curves were determined at high photon irradiance ($> 1600 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at different chamber $[\text{CO}_2]$ in the sequence ambient, 300, 200, 100, 50, ambient, 600, 800 and 1000 $\mu\text{mol mol}^{-1}$. For every leaf sampled, light and CO_2 response curves were fitted individually by a non-linear regression (SPSS 12.0 for Windows) to the hyperbolic function, $y = a (1 - e^{-b-cx})$, (Causton and Dale, 1990) where y is the rate of CO_2 exchange, x is the independent variable (I or C_i), b and c determine the slope of the curve and were allowed to vary for each curve fitting procedure. In the case of light response curves, coefficient a gives the light saturated rate of CO_2 exchange (A_{sat}), b/c gives the compensation point, $a(1-e^{-b})$ gives the dark respiration, and apparent quantum yield (the slope, or derivative of the curve at the light compensation point) is given by ace^b . In the case of the $A : C_i$ curve, a represents the light and CO_2 saturated rate of CO_2 exchange (A_{pot}), the CO_2 compensation point, Γ , is again calculated from b/c , and the carboxylation efficiency (the slope, or the derivative of the curve at the CO_2 compensation point) is given by ace^b (for all see: Midgley *et al.* 1999; Causton and Dale, 1990). This simple equation has been widely used to analyse light and CO_2 response curves of a variety of species (Kgope, 2004; Wand *et al.*, 2001; Midgley *et al.*, 1999) and fitted our data well ($r^2 \geq 0.9$; Table 2). Gas exchange characteristics of *C. papyrus* were determined from its umbel section, since it is the most productive part of the plant (Jones, 1988). Gas phase limitation to photosynthesis, L_g , was estimated from $[(A_{\text{pot}} - A_{\text{sat}}) / A_{\text{pot}}]$ (Farquhar and Sharkey, 1982; Long, 1985).

Table 2: Values of b and c together with the r^2 of the hyperbolic function, $y = a(1 - e^{-b \cdot cx})$, that was fitted in the data shown in Fig. 2.

	b	c	r^2
Permanent swamp			
<i>M. junceus</i>	0.61	0.04	0.97
<i>I. cylindrica</i>	0.11	0.01	0.97
<i>C. papyrus</i>	0.13	0.01	0.98
Seasonal floodplain			
<i>I. cylindrica</i>	0.32	0.02	0.98
<i>P. repens</i>	0.32	0.05	0.98
<i>C. articulatus</i>	0.06	0.01	0.98
Rain-fed grassland			
<i>C. dactylon</i>	0.36	0.06	0.91
<i>E. lehmanniana</i>	0.58	0.09	0.97
<i>U. trichopus</i>	0.94	0.17	0.94

After completion of the gas exchange measurements the leaves were scanned and their area calculated afterwards using WinFOLIA software (Regents Instruments Inc., Quebec, Canada). Leaf dry weight was obtained after oven-drying at 70 °C for 24 hours and C and N concentration measured using a Vario EL (Elementar Americas, Inc. Mt. Laurel, New Jersey, USA; Mantlana *et al.*, 2008). Specific leaf area (SLA) was determined as the ratio of the measured leaf surface area divided by leaf dry weight. Leaf phosphorus concentration was measured after a nitric acid digestion using ICP-AES (Atomic Emission Spectrometry with Inductively Coupled Plasma, Perkin-Elmer, Norwalk, USA; Mantlana *et al.*, 2008). Nitrogen use efficiencies (NUE) were determined by dividing A_{sat} by leaf N and expressed on a leaf area basis. Bivariate relationships between foliar N and P concentrations (both dry weight and area basis), A_{sat} and SLA were evaluated using Standardised Major Axis Regression, SMA (Warton *et al.*, 2006) using the program SMATR (Falster *et al.*, 2006). Standardised Major Axis Regression is a regression method preferred when one is more interested in the true slope of a relationship, rather than predicting values for a dependent variable from a

predictor variable. It is thus commonly used to establish allometric scaling relationships, especially when the two variables are not measured on comparable scales (Warton *et al.* 2006).

Meteorological and soil variables

At the seasonal floodplain, half-hourly rainfall, air temperature and air water vapour saturation deficit at ca. 3m height were measured at a nearby eddy-covariance flux tower, using a tipping bucket rain gauge (Young; Model 52202, R.M. Young Company, Traverse City, USA), temperature probe (HMP45A, Vaisala, Helsinki, Finland) and RPT 410 Barometric Sensor (Druck, New Fairfield, CT, USA), respectively. At the perennial swamp and the semi-arid rain-fed grassland similar meteorological data, at approximately 7m and 3m height, respectively, were collected at a nearby mobile tower using similar equipment as in the seasonal floodplain. Volumetric soil water content (θ) was measured at each microhabitat within the floodplain, at 0-5 cm, 5-10 cm and 10-15 cm soil-depth intervals using a battery-powered hand-held soil moisture sensor (Moisture Meter type HH2 with Theta probe, Delta T Devices, Cambridge, UK) during each measurement campaign.

For each site, 12 – 15 soil samples were collected at intervals of 0 – 5, 5 –10, 10 –20 and 20 – 30 cm. These were then air dried (sandy soils from the rain-fed grassland), or oven dried at 40 °C (loam, clay and peat soils from seasonal floodplains and perennial swamp, respectively) before being analysed for carbon and nitrogen using Vario MAX (Elementar Americas, Inc. Mt. Laurel, New Jersey, USA). Soil nutrient data reported here are for the upper 10 cm of soil.

To test the significance of differences among the species in leaf traits and gas exchange parameters, data were analysed with univariate analysis of variance (ANOVA), using Tukey's HSD test or the t-test. Statistical analyses were performed using the SPSS (SPSS 12.0 for Windows) statistical package.

RESULTS

Environmental conditions during measurements

Daily mean maximum air temperatures (T_a) during the study period were similar across the three habitats, ranging from 33°C to 35°C (Table 3). Nevertheless, mean vapour pressure deficit (D) at the rain-fed grassland exceeded those at the permanent swamp by about 0.7 kPa. The measurement periods at the rain-fed grassland and, to a lesser extent, the seasonal floodplain were associated with unusually high rainfall events. In one case more than 200 mm of rain fell in one day. Still, mean volumetric soil water content (θ) at 10 cm soil depth were lowest at the rain-fed grassland ($0.18 \text{ m}^3\text{m}^{-3}$) and highest at the permanent swamp ($0.42 \text{ m}^3\text{m}^{-3}$) with the seasonally flooded grassland intermediate ($0.30 \text{ m}^3\text{m}^{-3}$), reflecting the different soil physical properties. Soil C : N, at 0 – 10 cm depth, showed no significant difference between the sites (ANOVA, $n = 52$, $F = 2.75$, $P = 0.74$) and was 13.9 at the swamp, 14.9 at the seasonal floodplain, and 11.8 at the rain-fed grassland (Table 3)

Table 3. Means and standard errors of environmental conditions at the three sites during the measurement period. T_a = daily mean maximum air temperatures; D = vapour pressure deficit and θ = soil water content, measured at 0– 10 cm depth. All data were tested with ANOVA and grouped with the post hoc Tukey test. Different letters in a column indicate means are significantly different ($P < 0.05$) when testing for differences in each variable across the three study sites.

	Date	Soil C/N	T_a °C	D kPa	θ m ³ m ⁻³
Permanent swamp	05 -17 Feb	13.9 (0.4)a	33.3 (0.4)a	3.3 (0.2)a	0.42 (0.008)c
Seasonal floodplain	01 - 12 Mar	14.9 (0.5)a	33.9 (0.4)a	3.5 (0.2)a	0.30 (0.004)b
Rain fed grassland	20 - 28 Feb	11.8 (0.2)a	34.6 (0.5)a	4.0 (0.2)a	0.18 (0.02)a

The different environmental conditions encountered during measurements were also reflected in the leaf chamber conditions. Leaf temperatures (T_l) at the rain-fed grassland and seasonally flooded grassland were typically around 38°C, slightly higher than at the permanent swamp (35°C) (Table 4). Similarly, mean leaf-to-air vapour pressure deficit (D_l) was between 4 and 5.3 kPa at the two grass dominated sites, while those at the permanent swamp were significantly lower (ANOVA, $n = 60$, $F = 23.9$, $P < 0.01$) at 3.7 kPa (Table 4). These differences in microclimate were unavoidable as the three sites were sufficiently far from each other to preclude any measurement strategy covering all sites on just one day. There were also other logistical (eg. vehicular) constraints on the measurement strategies possible. However, we do believe the observed differences to be small enough not to affect the results or conclusions presented below more than just marginally.

Gas exchange parameters at ambient conditions

Photosynthetic rates and maximum stomatal conductances differed significantly, both between species at a given site, but also across sites. The species growing at the permanent swamp displayed the largest within-site differences with mean A_{sat} of *M. junceus*, 52 $\mu\text{mol m}^{-2} \text{s}^{-1}$, being almost twice that of the proximally growing *C. papyrus*, 27 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig.1).

Inter-specific variations at the seasonal floodplain were smaller with A_{sat} ranging from 25 to 31 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Significant differences at this site (ANOVA, $n = 19$, $F = 4.19$, $P = 0.03$) in A_{sat} were found between *I. cylindrica* and *C. articulatus* only. At the rain-fed grassland, the annual grass, *U. trichopus*, had the highest mean A_{sat} , 47 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which was significantly higher (ANOVA, $n = 19$, $F = 5.17$, $P = 0.018$) than that of the co-existing perennial grass, *C. dactylon* (36 $\mu\text{mol m}^{-2} \text{s}^{-1}$), only.

Table 4. Means and standard errors (in brackets) of leaf nitrogen (leaf N), leaf temperature (T_l) and leaf-to-air vapour pressure deficit (D_l) across the three study sites. All data were tested with ANOVA and grouped with the post hoc Tukey test. Different letters in a column indicate means are significantly different ($P < 0.05$) when testing for differences in each variable within each site.

	Leaf N $\text{mol m}^{-2} \text{s}^{-1}$	T_l $^{\circ}\text{C}$	D_l kPa
Permanent swamp			
<i>M. junceus</i>	55.6 (3.65)b	34.5 (0.68)a	3.7 (0.14)a
<i>I. cylindrica</i>	28.7 (2.42)a	36.3 (0.39)a	3.6 (0.14)a
<i>C. papyrus</i>	124.2 (4.65)c	35.3 (0.35)a	3.7 (0.12)a
Seasonal floodplain			
<i>I. cylindrica</i>	48.4 (2.84)a	37.7 (0.95)a	4.7 (0.09)a
<i>P. repens</i>	62.1 (0.80)a	38.9 (1.14)a	5.1 (0.39)a
<i>C. articulatus</i>	60.5 (5.01)a	36.9 (0.39)a	4.7 (0.16)a
Rain-fed grassland			
<i>C. dactylon</i>	57.4 (3.74)a	38.1 (0.96)a	5.3 (0.29)b
<i>E. lehmanniana</i>	73.6 (2.82)a	37.8 (0.35)a	5.1 (0.16)b
<i>U. trichopus</i>	63.0 (4.80)a	38.0 (0.41)a	4.0 (0.19)a

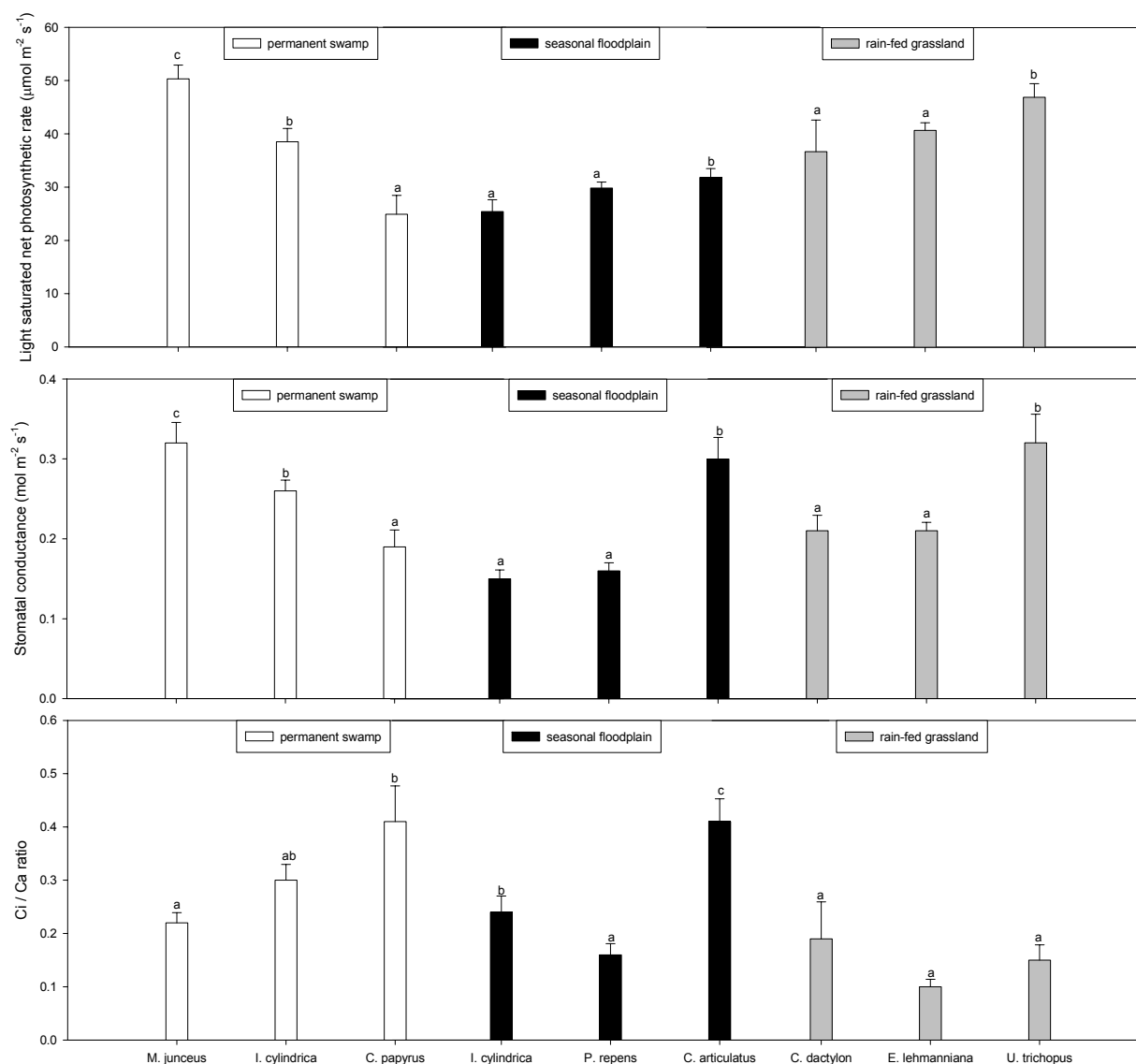


Figure 1: Means and standard errors of light saturated photosynthetic rate, stomatal conductance and ratio of intercellular to ambient CO_2 (C_i/C_a ratio) of all the species that were measured at the three sites. All data were tested with ANOVA and grouped with the post hoc Tukey's test. Different letters indicate means are significantly different ($P < 0.05$) when testing for differences within one site.

At all three sites, there were also significant differences in mean g_{sat} between species. At the permanent swamp the observed pattern was similar to the one for A_{sat} : *M. junceus* had the highest mean value, $0.32 \text{ mol m}^{-2} \text{s}^{-1}$ and *C. papyrus* had the lowest, $0.22 \text{ mol m}^{-2} \text{s}^{-1}$ (Fig. 1). Despite A_{sat} varying little for the three species examined for the seasonal floodplain, mean g_{sat} of the sedge, *C. articulatus*, $0.23 \text{ mol m}^{-2} \text{s}^{-1}$, was about 25% greater than that of

the two perennial grass species growing close by. At the rain-fed grassland, g_{sat} was within the same range as that at the seasonal floodplain ($0.2 - 0.3 \text{ mol m}^{-2} \text{ s}^{-1}$) and values were similar among the perennial grasses ($0.21 \text{ mol m}^{-2} \text{ s}^{-1}$) but these values were significantly lower (ANOVA, $n = 19$, $F = 6.88$, $P < 0.05$) than those found in the annual grass, *U. trichopus*. Mean chamber D_i during the measurement period (Table 3) did not vary at a given site, with the exception of data collected for *U. trichopus* where D_i was significantly lower (ANOVA, $n = 19$, $F = 15.55$, $P < 0.01$) than for the other two species.

The ratio of intercellular to ambient CO_2 , C_i/C_a , reflects the changes in the relationship between stomatal conductance and rate of net CO_2 assimilation. Light saturated ratios at the permanent swamp differed significantly (t test, $t = 7.8$, $n = 16$, $P < 0.01$) between the highest values (*C. papyrus*, 0.41), and lowest values in *M. junceus*, 0.22 (Fig. 1). This range in C_i/C_a was similar to that observed at the seasonal floodplain where mean C_i/C_a of the sedge *C. articulatus*, 0.4, was almost two-fold higher than that found in the co-existing *P. repens* (0.16) and almost twice that of *I. cylindrica*, 0.24. Among the species at the rain-fed grassland, mean C_i/C_a were lower when compared to the other sites, and showed no significant difference (ANOVA, $n = 19$, $F = 1.8$, $P = 0.19$) despite varying by a factor of two on average (0.10 to 0.19).

A : C_i response curves

CO_2 response curves of all the species yielded very low CO_2 compensation points (Γ ; Table 5), which were similar for the species growing at the permanent swamp and in the seasonal floodplain ($8 - 11 \text{ } \mu\text{mol mol}^{-1}$) and three times as high as those observed for the species growing in the rain-fed grassland ($3-6 \text{ } \mu\text{mol mol}^{-1}$; Table 5). The species at the latter site also had the steepest initial slope of CO_2 response curves (α), ranging on average from 3.2 to $3.6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} (\mu\text{mol/ mol}^{-1})$, indicating highest efficiency of CO_2 utilization at low C_i (Table 5). These values exceeded those of the species at the permanent swamp and the seasonal floodplain by a factor of four to six, where average α ranged from 0.5 to $1.6 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1} (\mu\text{mol/ mol}^{-1})$.

Table 5. Gas exchange parameters obtained from A-c_i response curves. Γ = compensation point; α = carboxylation efficiency; A_{pot} = light and CO₂ saturated net photosynthetic rate; L_g = Gas phase limitation to photosynthesis. Data are means and (in brackets) standard errors and were tested with ANOVA and grouped with the post hoc Tukey test. Different letters in a column indicate significant difference ($P < 0.05$) when testing for differences across the three study sites.

	Γ ($\mu\text{mol mol}^{-1}$)	α ($\mu\text{mol m}^{-2} \text{s}^{-1}$. $\mu\text{mol mol}^{-1}$)	A_{pot} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	L_g %
Permanent swamp				
<i>M. junceus</i>	10.1 (2.6)b	1.6 (0.3)ab	56.4 (1.9)d	14.2(3.7)ab
<i>I. cylindrica</i>	11.2 (2.9)b	0.8 (0.1)a	47.0 (6.2)cd	12.7 (3.6)ab
<i>C. papyrus</i>	11.4 (1.8)b	0.7 (0.3)a	37.5 (4.4)ab	26.9 (5.2)b
Seasonal floodplain				
<i>I. cylindrica</i>	10.2 (3.0)b	0.6 (0.2)a	33.9 (1.7)ab	16.9 (6.5)ab
<i>P. repens</i>	7.8 (3.2)b	0.6 (0.1)a	35.4.(3.4)ab	8.4 (0.4)ab
<i>C. articulatus</i>	9.9 (4.4)b	0.5 (0.1)a	38.5 (3.9)ab	26.3 (2.9)b
Rain fed grassland				
<i>C. dactylon</i>	5.6 (0.3)a	3.2 (1.5)b	39.9 (1.7)ab	10.2 (1.7)a
<i>E. lehmanniana</i>	3.9 (1.4)a	3.6 (0.7)b	50.4 (2.8)cd	7.8 (3.8)a
<i>U. trichopus</i>	3.3 (1.2)a	3.5 (1.1)b	52.9 (3.9)cd	5.2 (3.2)a

Overall C_i at the rain fed grassland site did not exceed $300 \mu\text{mol mol}^{-1}$, even at high chamber C_a , of 800 to $1000 \mu\text{mol mol}^{-1}$ (Fig. 2), whereas C_i at the floodplain and the permanent swamp were approximately $400 \mu\text{mol mol}^{-1}$ at high C_a .

The range in average net photosynthesis rates at saturating light and CO₂ concentration, A_{pot} , at the permanent swamp and at the rain-fed grassland was nearly identical, ranging from 38 to $56 \mu\text{mol m}^{-2} \text{s}^{-1}$, while species from the seasonal floodplain had overall lower A_{pot} , approximately $34 - 39 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 5). Short-term increases of chamber CO₂ concentration above ambient levels led to increased photosynthetic rates in all species, as

indicated by the estimation of gas phase limitation to photosynthesis, L_g . The higher the L_g of a particular species, the further its A_{sat} operates from A_{pot} (Long, 1985). Perennial grasses at both the permanent swamp and the seasonal floodplain were within the same range of L_g , 8 – 17 %, and lower than the co-existing sedge species, ca. 27 %. Despite tending to operate at a lower C_i/C_a , the species at the rain-fed grassland showed weakest response to short-term increase of CO_2 as indicated by the low range of mean L_g of 5 - 10 %, but overall there was no statistical difference (ANOVA, $n = 12$, $F = 0.71$, $P = 0.52$) at this site.

Net CO_2 assimilation rates in relation to foliar nitrogen and phosphorus concentrations

Fig. 3a shows the relationship between A_{sat} and leaf nitrogen expressed on a leaf area basis (N_a), the latter of which varied considerably across the study, from 16 to 166 mmol m^{-2} . Variation in N_a between species for the plants growing in the permanent swamp was large and highly significant (ANOVA, $n = 18$, $F = 251.6$, $P < 0.01$) and this was also reflected in the large variation in NUE which was much smaller in the sedge (0.26 $\text{mmol mol}^{-1} \text{s}^{-1}$ on average) than in the co-existing grasses, *M. junceus* and *I. cylindrica* (0.94 and 1.64 $\text{mmol mol}^{-1} \text{s}^{-1}$, respectively; Fig. 4). Leaves of all plants sampled on the seasonal floodplain had relatively low N_a (43-78 mmol m^{-2}), and NUE varying from 0.46 to 0.78 $\text{mmol mol}^{-1} \text{s}^{-1}$ (Fig. 4). These values were only slightly lower than those determined at the rain-fed grassland site (N_a : 52-120 mmol m^{-2} ; NUE : 0.49 to 0.65 $\text{mmol mol}^{-1} \text{s}^{-1}$). By contrast, N_a showed no significant differences among the co-existing species for either the rain-fed grassland (ANOVA, $n = 16$, $F = 2.68$, $P = 0.11$) or the seasonal floodplain (ANOVA, $n = 17$, $F = 1.69$, $P = 0.22$).

Fig. 3a suggests that within a given species there is a positive relationship between A_{sat} and N_a (and in a similar way A_{pot} , not shown). However, across all species investigated, the overall relationship was poor and not significant ($r^2 = 0.02$; $P = 0.45$; standardised major axis (SMA) regression).

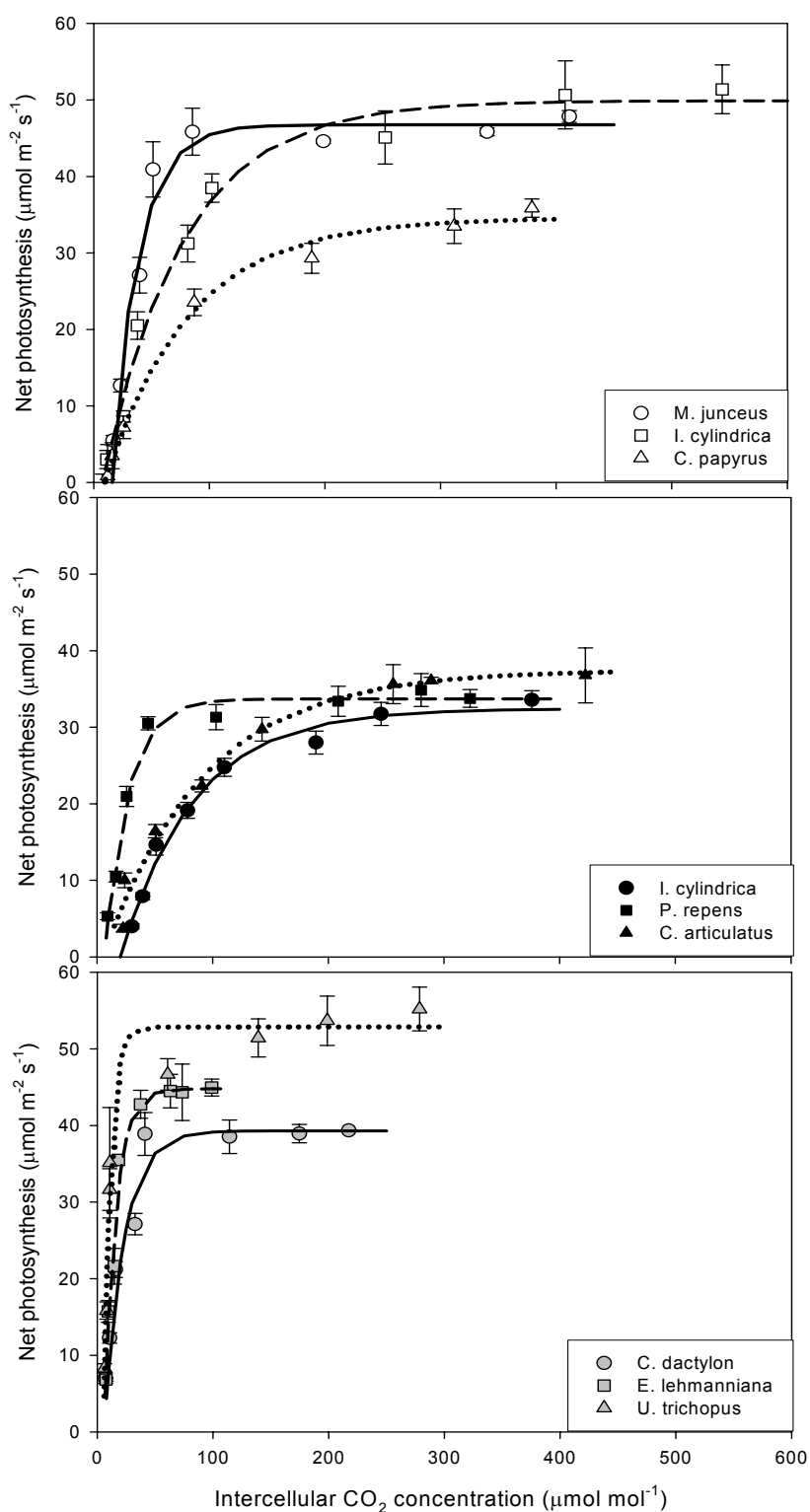


Figure 2: Responses of net photosynthesis (A) to intercellular CO₂ concentration (C_i) for all the species. Each curve shows the means of A and C_i for individual species on at least four different leaves. For clarity of the figure, only standard errors for A are shown. Standard errors for C_i were in the same order of magnitude as those of A. The fitted curves are non-linear regressions of the hyperbolic function, $y = a(1 - e^{-b \cdot x})$ (see Materials and Methods). All measurements were done at irradiance above 1600 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

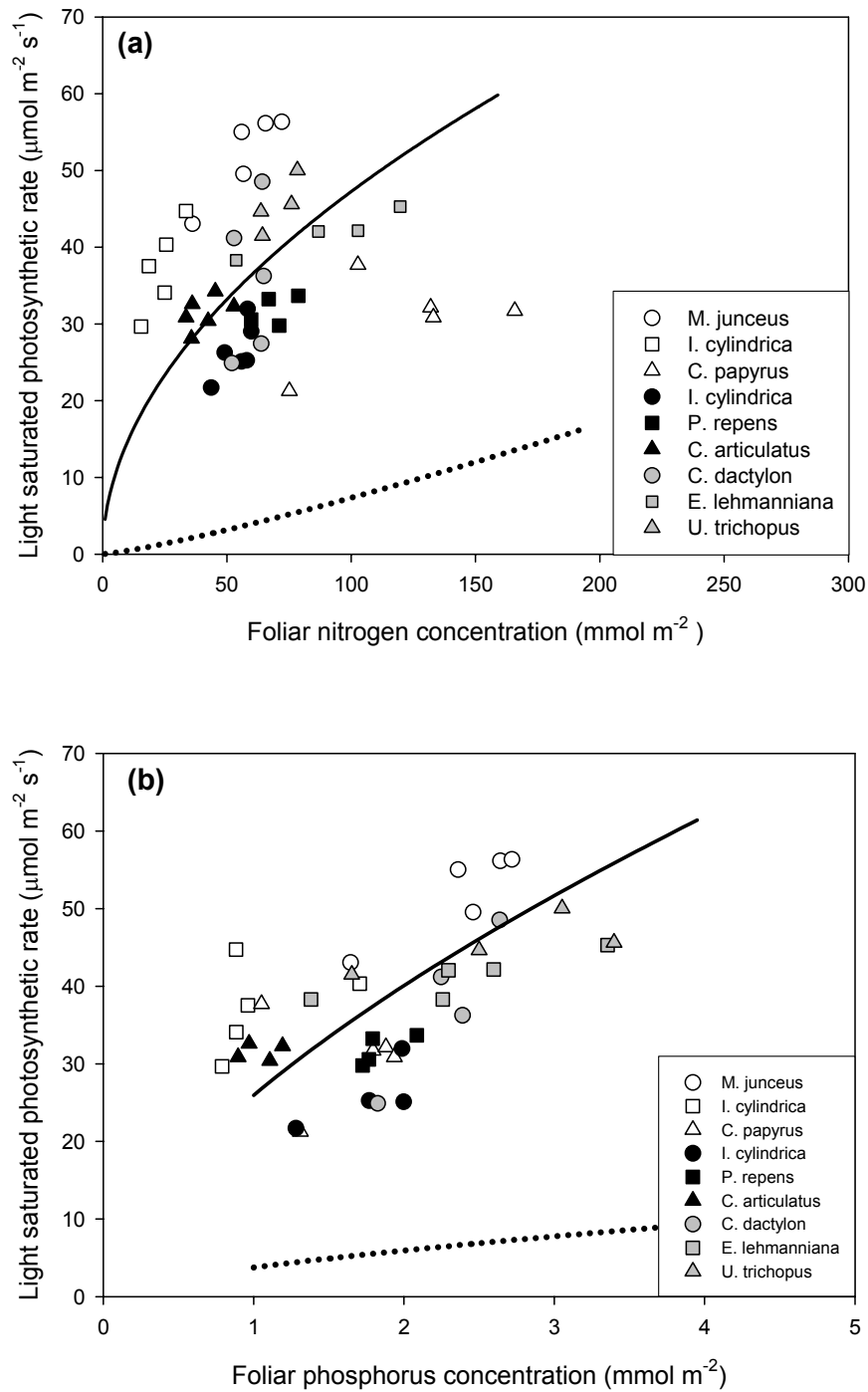


Figure 3: Relationship between light saturated photosynthetic rates and (a) foliar nitrogen and (b) foliar phosphorus concentrations expressed on a leaf area basis. The fitted curves are of the “scaling” form used by Wright *et al.* (2004) with the dashed line showing their best fit equation for the C_3 plant economic spectrum (see Materials and Methods).

Variations in A_{sat} were, however, closely related to variations in foliar phosphorus when expressed on a leaf area basis (P_a), not just within a given

species, but also across species within a given site and between sites ($r^2 = 0.24$; $P = 0.002$; SMA regression; Fig. 3b). Nevertheless, as observed for N_a , it was also the case that *M. junceus* and *I. cylindrica* at the permanent swamp had highest A_{sat} at any given P_a than the other species examined. The large variation in A_{sat} at the permanent swamp (Fig. 1) would therefore seem to be well accounted for on the basis of variations in P_a , and the generally lower A_{sat} for the seasonally flooded grassland, especially in comparison to the rain-fed grassland (Fig. 1) appear also accountable in terms of the significantly higher (t-test, $t = 13.8$, $n = 25$, $P < 0.01$) P_a of the latter.

The general C_3 plant scaling relationships for A_{sat} versus N_a and P_a as given by Wright *et al.* (2004) also based on using SMA regression, are shown as dashed lines in Fig. 3a and Fig. 3b. In both cases our data show a drastically different response with all C_4 species having a much sharper response than is typically observed for C_3 plants. Photosynthetic rates at a given N_a or P_a were 5-10 fold higher than would be predicted from the generalized relationship given by Wright *et al.* (2004). We, however, note that our analyses were made using replicates of species while those of Wright *et al.*, (2004) were established on species means. We also note that our results merge intra-specific, inter-specific, and inter-site variation. While our data point to a possibly different response of C_4 plants it is clear, also when considering the variability between the C_4 species encountered in our study, that a larger number of samples from a wider range of C_4 environments is required to ascertain this observation.

General aspects of the “leaf economic spectrum”

Fig. 5 illustrates pair-wise relationships between foliar properties, allowing a more general evaluation of leaf property differences among the species studied here and comparing the relationships found with established relationships previously established by Wright *et al.* (2004).

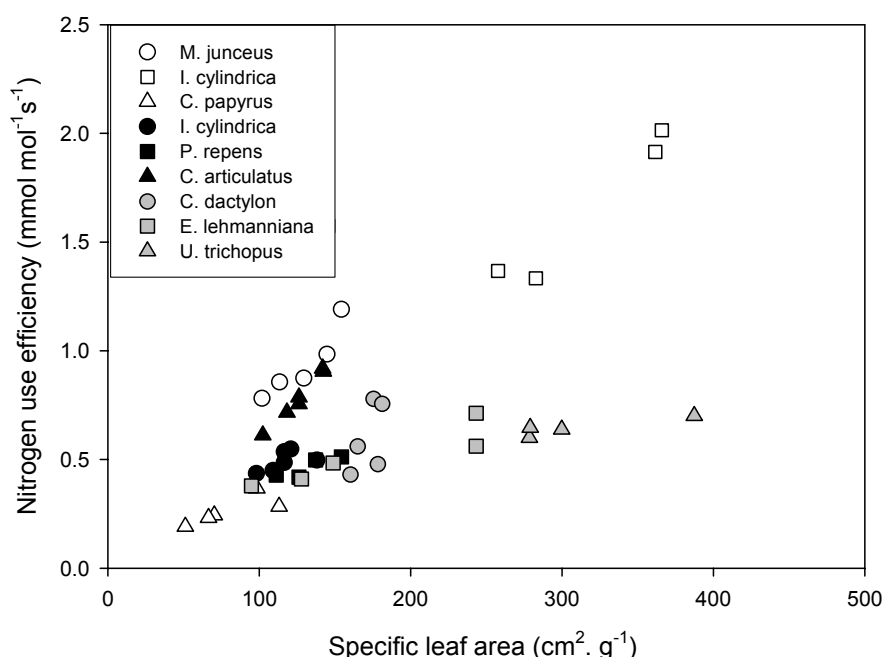


Fig. 4: Relationship between specific leaf area and nitrogen use efficiency of all the measured species. Different species are represented by different symbols.

Independent of being expressed on an area or dry weight basis, phosphorus emerges as a better predictor of A_{sat} than nitrogen ($r^2 = 0.66$ versus $r^2 = 0.23$). Observed values of A_{sat} at any given foliar N or P concentration were much higher in the current study than would be predicted from the relationships established by Wright *et al.* (2004). However, the general relationship between foliar N and P on a dry weight basis (Fig. 5c) was similar to the one postulated by Wright *et al.* (2004).

Fig. 5d and Fig. 5e show the relationship between SLA and leaf N and P (again on a dry weight basis). In both cases the C_4 grasses investigated here had significantly higher SLA than would be expected for C_3 plants characterized by similar foliar N or P concentrations. As for A_{sat} , phosphorus proved to be a markedly better predictor of SLA than did nitrogen (r^2 of 0.51 and 0.18 respectively; SMA regression with SLA^{-1} as the y variable as in Wright *et al.*, 2004).

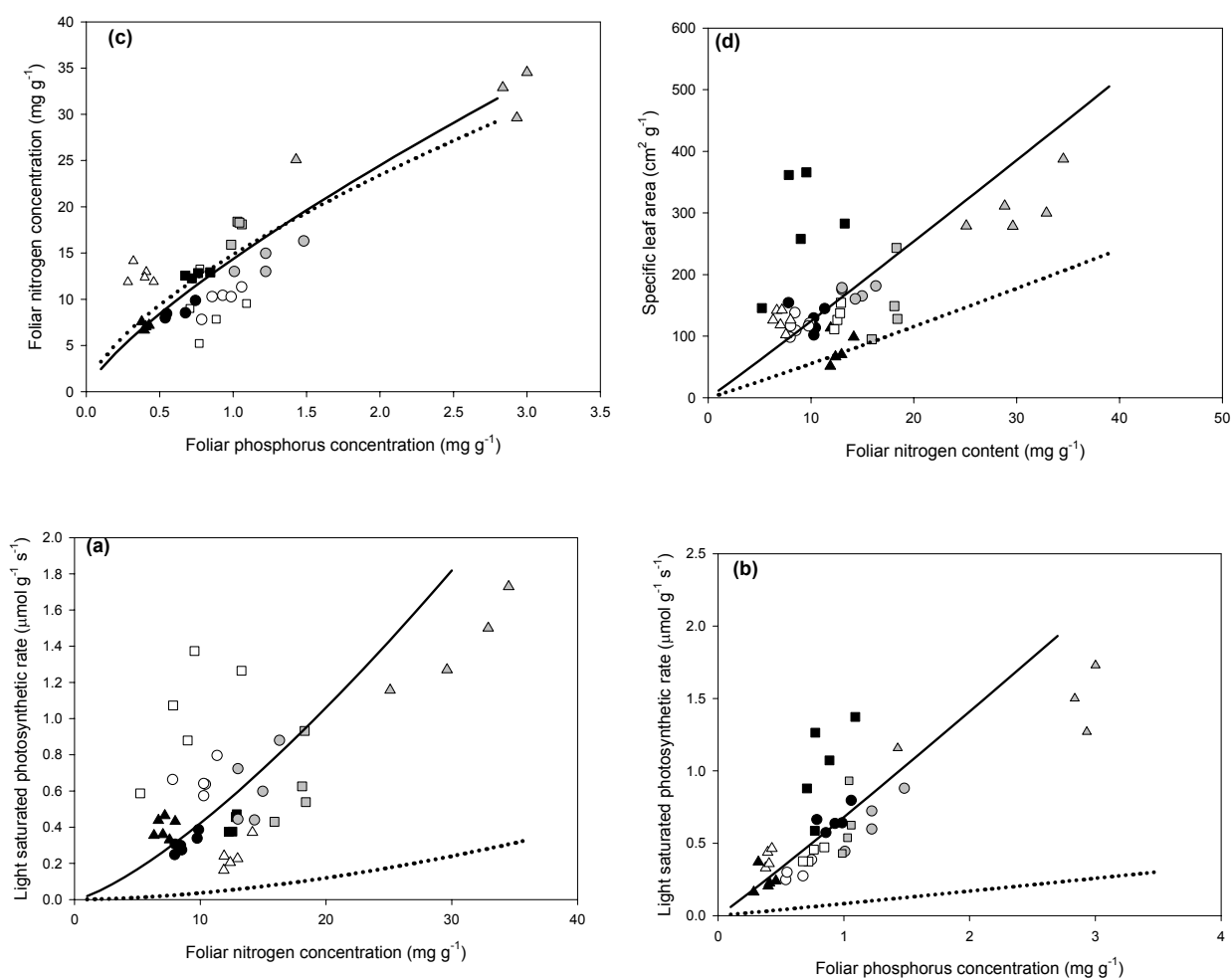
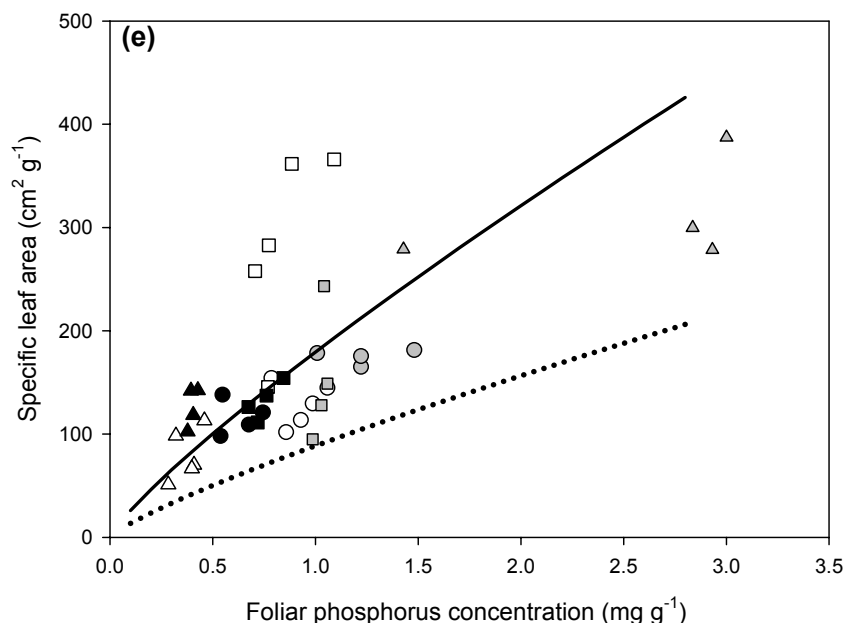


Fig. 5: Scaling relationships for (a) light saturated photosynthetic rate versus leaf nitrogen; (b) light saturated photosynthetic rate versus leaf phosphorus; (c) foliar nitrogen versus foliar phosphorus; (d) specific leaf area versus leaf nitrogen; (e) specific leaf area versus leaf phosphorus. The fitted curves are of the “scaling” form used by Wright et al. (2004) with the dashed line showing their best fit equation for the C_3 plant economic spectrum (see Materials and Methods). All values are expressed on a leaf dry weight basis. Symbols as in Fig. 3,

Figure 5 continued.



DISCUSSION

Leaf-level photosynthetic rates at light saturation, *ca.* 25 to 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$, were comparable to those obtained for other C_4 tropical plants growing in their natural environment under optimum light, water and nutrient conditions (30 to 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Long, 1985; Baruch, 1996). Similarly, the range of g_{sat} observed in this study, 0.15 to 0.32 $\text{mol m}^{-2} \text{s}^{-1}$, was well within that of C_4 species under optimum growth conditions, from 0.2 - 0.4 $\text{mol m}^{-2} \text{s}^{-1}$ (Lawlor, 2001).

Under saturating light and low D_i leaves of C_4 plants commonly show a range of C_i/C_a from 0.25 to 0.4 (Lawlor, 2001). High C_i/C_a values of 0.41 - 0.45 as found for the two sedge species in this study may be typical for tropical C_4 species that dominate wet habitats (Jones, 1988; Piedade *et al.*, 1994). This suggests that a low C_i/C_a among C_4 tropical species growing in their natural habitat is not necessarily a universal phenomenon (Grace *et al.*, 1998). Still, the low g_{sat} taken together with low C_i/C_a in the grasses of the rain-fed grassland and the seasonal floodplain, suggest that these species would retain significantly higher daily WUE than their co-existing sedge species, or grasses growing in the permanent swamp. The relatively non-conservative

WUE of sedges vs. grasses is probably retained throughout most of the year, as was the case at the seasonal floodplain (Mantlana *et al.*, 2008). The ecological benefit for higher *WUE* in this semi-arid environment would be the lengthening of the daily period of carbon acquisition and growth, as it maintains high CO₂ assimilation at reduced water loss.

The low compensation points found in this study are typical of C₄ plants (Lawlor, 2001). At the rain-fed grassland in particular the markedly high α indicate a very efficient re-fixation of photorespiratory CO₂ by phosphoenolpyruvate carboxylase (*PEP-C*) as has been observed in laboratory studies (von Caemmerer and Furbank, 1999). In addition, the high α found at the rain-fed grassland implies that species in this site had either more efficient *PEP-C* or higher levels of the enzyme when compared to species from the two other habitats (von Caemmerer, 2000). Previous studies have mentioned that in C₄ plants maximal *PEP-C* activity affect primarily the initial slope of the CO₂ response curves (Polley *et al.* 1992; Pfeffer and Peisker, 1998; Shenton *et al.* 2006). These results may be a reflection of the slightly higher leaf *N* found in this habitat, as *PEP-C* content is positively related to leaf *N* (von Caemmerer, 2000) while nitrogen deficiency decreases both Rubisco amount and activity and also carboxylation efficiency in C₄ plants (Long *et al.* 1985; Von Caemmerer, 2000). In a similar way as discussed for the C_i/C_a, our results suggest that under well-watered field conditions, C₄ species from different natural habitats differ significantly in their efficiency of CO₂ utilization at low C_i, with species from the dry habitats showing higher efficiency than those from the wet habitats.

The grasses were operating closer to A_{pot} than co-existing sedge species, as indicated by their lower gas phase limitation to photosynthesis, L_g . Relatively higher C_i/C_a in the sedges, as discussed above, was thus associated with relatively higher L_g , suggesting that factors other than CO₂ demand in the stomata were responsible for the observed high L_g .

Previous field studies have also reported high values of *NUE* in C₄ species (Baruch *et al.*, 1985; Anten *et al.*, 1998; Simioni *et al.*, 2004). Given the high *NUE* of C₄ plants, it is therefore not surprising that the photosynthesis scaling relationship observed for the C₄ species in our study showed a

significantly greater slope than in the “general” C₃ case. Since the higher *NUE* of C₄ plants is generally associated with a more efficient use of Rubisco, this suggests that under natural conditions these plants would have an ecological advantage in situations of nitrogen limitation (Long, 1999), for instance by allocating more carbon to tissues involved in acquisition of the resources that are most limiting to plant growth (Sage *et al.*, 1987).

Although high *A*_{pot} among the grasses at the permanent swamp can be explained by the combination of a fast turnover rate of Rubisco giving rise to a high nitrogen use efficiency as is commonly found amongst NADP species (Ghannoum *et al.*, 2005), overall photosynthetic rates were best correlated with leaf *P* contents, which, along with N, may play a central role in the regulation of the photosynthetic carbon reduction (*PCR*) cycle (von Caemmerer, 2000). Phosphorus supply is potentially limiting in approximately 30% of terrestrial ecosystems, including boreal forests, tropical forests and savannas (von Uexkull and Mutert, 1995). Phosphorus supply rate directly affects CO₂ fixation (Campbell and Sage, 2006; Jacob and Lawlor, 1991) as was demonstrated by a decrease in photosynthesis in phosphate-deficient leaves of sunflower, maize and wheat plants (Jacob and Lawlor, 1991). At forest sites in Hawaii, addition of *P* increased above-ground productivity at *P* limited sites stronger than at *N* limited sites, although foliar *P* concentration was similar at both sites (Harrington *et al.*, 2001) and at a rainforest in Cameroon leaf gas exchange responded somewhat stronger to phosphorous than to nitrogen, although the difference was small (Meir *et al.*, 2007).

For savannas, inter-specific differences have been observed in the nutritional requirement of C₄ grasses from different ecological regions, with nitrogen requirements for production of organic matter generally increased by addition of phosphorus (Bilbao and Medina, 1990). One explanation for the generally low foliar *P* levels of the plants growing in the seasonal floodplain, is a reduced availability of phosphorus under aerobic soil conditions when the plants are actively growing, for example due to the precipitation of ferrous phosphate (vivianite, Fe₃(PO₄)₂·8H₂O) (Zachara *et al.*, 1998). It is also likely that overall low rates of mineralization under anaerobic conditions in both the seasonally and permanently flooded grasslands may have resulted in increasingly large amounts of soil *P* being bound in less plant accessible

forms (Chacón *et al.* 2005). Thus, while an increasing number of data from tropical species suggest an equally strong, perhaps stronger effect of phosphorous on gas exchange or growth the exact mechanism is not yet clear.

Our data set is limited by using replicates of a small number of species but the overall range encountered in our data is comparable to the GLOPnet data set used in Wright *et al.* (2004). For instance, the latter included a log LMA = 1.2 to 3.2, log N_{mass} = -0.6 to 0.8, and log P_{mass} = -0.2 to -2.1 whereas our data covered log LMA = 1.4 to 3.3, log N_{mass} = -0.3 to 0.5, and log P_{mass} = -0.5 to -1.5. The observed leaf N vs. SLA relationships showed the pattern typically observed in relation to leaf economy (Wright *et al.*, 2004): Long lived leaves tend to have low N_{mass} at low SLA , a pattern that was also observed for the perennial species in our study. The opposite is found short-lived leaves and, accordingly, we observed highest SLA and highest foliar N concentration in the annual grass *Urochloa*. Across the two locations where *Imperata* was measured, its SLA and A_{sat} varied widely while consistently having relatively low foliar N levels. This adaptable leaf economy of *Imperata* may be one of the reasons for its success as an invasive species under many environmental conditions.

The well known relationship between foliar nitrogen contents (dry weight basis) and SLA , are attributed to accommodate for higher photosynthesis rates (area basis) at given N_a if foliar N (DW) contents are high (Reich *et al.*, 1999). It is therefore interesting that in the current study foliar P seems to correlates somewhat better with SLA than does foliar N . The reasons for this are unclear, but as for photosynthesis, it does suggest that phosphorus, as opposed to nitrogen was the most critical element modulating variations in plant ecophysiological characteristics of the species studied here. A study comparing a cultivated African grass species with a South American pasture grass showed that the African species was more dependent on P supply, than on N supply, for maximal growth (Bilbao and Medina, 1990). These results suggest that there may be a differentiation among the studied wetland-savanna C_4 species according to their P and N requirements for growth and their capability to exploit soil nutrient sources. Moreover, the general tendency for lower soil fertility to be negatively correlated with levels of

dominance of C₄ grasses over C₃ trees and shrubs in savanna ecosystems (eg. Goodland and Pollard, 1973; Lopes and Cox, 1977) may at least be partly explainable in terms of the much lower photosynthetic nutrient use efficiencies of the latter.

In conclusion, our results showed that since species at the rain-fed grassland experience high D even during the height of the rainy season that may lead to high transpiration rates, they possess strategies (low C_i/C_a and high leaf N) that allow them to reduce water loss and achieve light saturated photosynthesis close to their potential rates that are at least as high as those of permanent swamp and seasonal floodplain species. However, the gas exchange behaviour that we observed would occur only during periods of adequate soil water content for plant growth. Our data do not imply that throughout the year the grasses from the rain-fed grassland would fix similar rates of CO₂ per unit loss of water compared to species from the permanent swamp and seasonal floodplain. In fact, the above-ground living biomass is greatly reduced during the dry months, and the overall efficient physiology during the rainy season reflects an optimum use of resources during a short active season. Moreover, the cost of the water-conservation strategy of the grasses at the rain-fed grassland was apparent in their lower NUE compared to grasses from the permanent swamp. Our results suggest that leaf P plays an important role in determining the ecological performance of C₄ tropical grasses, perhaps through modulating the degree to which these grasses respond to nitrogen acquisition. Because of their role in supporting carbon assimilation in plants, understanding of the metabolic demand for leaf N and leaf P is crucial in improving our knowledge of C₄ plant growth in natural ecosystems.

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CHAPTER 4.

Biomass and leaf-level gas exchange characteristics of three African savanna C₄ grass species under optimum growth conditionsⁱⁱⁱ.

Abstract

C₄ savanna grass species, *Digitaria eriantha*, *Eragrostis lehmanniana* and *Panicum repens*, were grown under optimum growth conditions with the aim of characterizing their above- and below-ground biomass allocation and the response of their gas exchange to changes in light intensity, CO₂ concentration and leaf-to-air vapour pressure deficit gradient (D_l). *D. eriantha* showed the largest above- and below ground biomass, high efficiency in carbon gain under light-limiting conditions, high water use efficiency (WUE) and strong stomatal sensitivity to D_l ($P = 0.002$; $r^2 = 0.5$). *P. repens* had a high aboveground biomass and attained high light saturated photosynthetic rates (A_{sat} , $47 \mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance, (g_{sat} , $0.25 \text{ mol m}^{-2} \text{s}^{-1}$) at relatively high WUE. *E. lehmanniana* had almost half the biomass of other species, and had similar A_{sat} and g_{sat} but were attained at lower WUE than the other species. This species also showed the weakest stomatal response to D_l ($P = 0.19$, $r^2 = 0.1$). The potential ecological significance of the contrasting patterns of biomass allocation and variations in gas exchange parameters among the species are discussed.

INTRODUCTION

The main constraints on plant and ecosystem productivity in the seasonally dry tropical savanna-wetland mosaic of the Okavango Delta in north-western Botswana are low soil water availability during the dry season, periodic flooding, generally low soil fertility, fire and grazing. The wet season (November – April) in this environment, when over 90% of the 400 mm annual rain falls, coincides with high leaf-to-air vapour pressure deficit (D_l ; $>4.5 \text{ kPa}$) and leaf temperatures (T_l) may exceed 35°C .

A main factor that is supposed to regulate the adaptation of plants to environmental constraints is plasticity in the pattern of resource allocation (Lambers and Poorter, 1992). Plants that originate from infertile soils frequently display greater allocation of biomass to fine roots, and in order to increase the absorbing area (Baruch, 1994), invest less biomass to produce

ⁱⁱⁱ KB Mantlana, EM Veenendaal, A Arneth, V Grispen, CM Bonyongo, IG Heitkonig & J Lloyd, *African Journal of Ecology*, Accepted

root length and produce fine roots with greater specific root length (Lambers and Poorter, 1992). For grasses in the Okavango Delta, below ground accumulation of carbon and nutrient reserves would also allow for quick recovery of photosynthetic biomass after fires (Baruch *et al.*, 1985). Previous studies have reported contrasting patterns in below-and above-ground biomass allocation in tropical and temperate grasses (Veenendaal *et al.*, 1993; Baruch, 1994; Rhyser and Lambers, 1995), which suggests amplitude for resource partitioning.

C₄ species constitute a high proportion of the grass flora in the Okavango Delta. C₄ photosynthesis involves at least three different biochemical pathways that differ in the mechanisms used to transport CO₂ to the bundle sheath cells and also to regenerate phosphoenol pyruvate (Hatch, 1987). These biochemical pathways are NAD-malic enzyme species (NAD-ME), NADP-malic enzyme species (NADP-ME) and PEP carboxykinase species (PCK). Plants that use C₄ metabolism possess a CO₂ concentrating mechanism that enables them to have high nitrogen use efficiency, high water use efficiency, preference of high light intensities and high optimum leaf temperatures for CO₂ uptake. Such adaptations make these plants particularly adapted for survival in the infertile and seasonally dry tropical ecosystems that often experience high vapour pressure deficits.

The aim of this work was to quantify the biomass allocation and to elucidate important information about the ecophysiology of little known, but commonly found, C₄ grass species under optimum growth conditions. In the Okavango Delta, these species are found in different habitats: *Digitaria eriantha* Steud occurs in a wide-range of drier often partially shaded habitats in woodlands and islands and, *Eragrostis lehmanniana* Nees dominates dry, open and disturbed habitats, while *Panicum repens* L. dominates moist seasonally flooded areas of floodplains (Gibbs Russel *et al.*, 1990). This was the first study to investigate gas exchange characteristics of C₄ grasses from the Okavango Delta. Growing these plants under similar growth conditions (optimum water and nitrogen levels, high air temperature and high vapour pressure deficit) that mimic a period of high productivity with plant material sourced from their natural environment could provide insights into their potential responsiveness in natural conditions.

MATERIALS AND METHODS

Growth conditions

Vegetative cuttings of a tillering perennial, *Digitaria eriantha* Steud (NADP-ME), a tufted perennial *Eragrostis lehmanniana* Nees (NAD-ME), and a rhizomatous perennial *Panicum repens* L. (PCK), were collected from a floodplain in the Okavango Delta. *D. eriantha* is native to Southern Africa. It is regarded as drought tolerant, and considered to have low shade tolerance, but high fire tolerance (Gibbs Russel *et al.*, 1990; Bogdan, 1977). When collected from the field *D. eriantha* was growing under or very near to trees. *E. lehmanniana* is a seed-banking species and following fire, it re-germinates when soil water content becomes favourable. It dominates disturbed well drained sandy soils (Gibbs Russel *et al.*, 1990). When it was collected, *E. lehmanniana* was growing in open areas. Tropical and north Africa and the Mediterranean are the native range of *P. repens* but is now widespread throughout the tropics (Gibbs Russel *et al.*, 1990). It favours open sunny conditions on moist, often sandy soil, and can withstand occasional flooding, but its rhizomes can stand prolong dry periods. *P. repens* was collected in the middle of a seasonal floodplain. Common characteristic among these species is that they are grazed extensively by herbivores (Gibbs Russel *et al.*, 1990).

The plants were then planted into large bags (three cuttings per bag, 19 x 7.5 cm) to provide for ample rooting space, and kept outside a shade house to allow full exposure to light and ambient air temperatures which ranged from 35 – 39 °C. The bags were filled with sandy soil from Lake Ngami and watered daily. After five weeks, the cuttings were transferred (one vegetative cutting of one species per bag) into new bags filled with uniform river sand. After transplanting, each plant received a fertiliser application. The fertiliser was applied as 10 ml liquid plant food (Supranure, containing 11 % N, 7.3 % P and 3.7 % K) diluted in 5 litres of tap water. Each bag was given 200 ml of this solution. Fertiliser was re-applied after 3.5 weeks before the start of the photosynthesis measurements.

Harvest

After eight weeks of growth and photosynthesis measurements had been completed, plants were harvested. The soil of roots of all individual plants was carefully rinsed with water, to minimize root damage or loss. Subsequently, each plant was separated into roots and shoots, and in the case of *P. repens*, also rhizomes. Sub samples of five leaves per shoot were taken to determine leaf area with a leaf area meter (model 3100 area meter, Li-Cor Inc., Lincoln, NE, USA). Sub samples of the roots were taken to determine specific root length, using a ruler. All plant samples (roots and shoots) were oven-dried for 48 hours at 80°C to determine dry weight. Total biomass, shoot-root ratio (*SRR*), leaf area ratio (*LAR*) and specific leaf area (*SLA*) were determined according to Beadle (1993). Specific root length (*SRL*) was determined by dividing the length of the root sample by the dry weight.

Photosynthesis characteristics

Steady state leaf gas exchange measurements were made using an open gas exchange system (LI-6400; Li-Cor Inc., Lincoln, NE, USA) on youngest and fully expanded leaves from at least four randomly selected plants or separate tillers of each species. Measurements were made between 08h00 and 10h00, to avoid midday stomatal depression. The leaf temperature inside the 6cm² leaf chamber was not controlled, but did not exceed 40 °C, the temperature threshold above which C₄ net photosynthetic assimilation may become inhibited (Lawlor, 2001). During the gas exchange measurements, all plants experienced similar leaf temperature and leaf-to-air vapour pressure deficit (*D_i*) values (approximately 37 °C and 4.3 kPa, respectively). Leaf areas were calculated using leaf dimensions of the enclosed leaf sections in the chamber (Anderson *et al.*, 2001). Measurements were recorded only after the photosynthetic rate and stomatal conductance were considered constant and at equilibrium with the ambient conditions within the gas exchange cuvette.

During measurements both the leaf-to-air vapour pressure deficit, *D_i*, and leaf temperature, *T_i*, in the chamber did not always exactly match the ambient conditions because these two parameters are affected by leaf transpiration rate (Beale *et al.*, 1999); *T_i* typically exceeded air temperature at

time of measurements by not more than 1 to 2 °C. The red/blue LED light source inside the chamber allowed for automatic changes of light intensity (I) of 2000, 1500, 1000, 500, 200, 100, 50, 20, and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Apparent light use efficiency (ϕ) was estimated as the slope of the first four points in the linear part of the light response curve. The qualification, apparent, is used since the estimate is based on incident and not absorbed photon flux (Lawlor, 2001). Light-saturated photosynthetic rate, A_{sat} , light-saturated stomatal conductance, g_{sat} , the ratio of ambient to internal CO_2 concentration ($[\text{CO}_2]$), C_i/C_a ratio, and water use efficiency, WUE , were obtained at high photon irradiance ($> 1500 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at an ambient $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$. WUE was determined as $A_{\text{sat}} / g_{\text{sat}}$.

CO_2 response curves were then determined at high photon irradiance ($2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at different chamber $[\text{CO}_2]$ in the sequence ambient, 380, 300, 200, 100, 50, 400, 400, 600, 700, and $800 \mu\text{mol mol}^{-1}$. The different CO_2 concentrations were obtained by means of a portable CO_2 /air mixture tanks and automatically controlled by a CO_2 injector. From each CO_2 response curve we determined the CO_2 compensation point, Γ , the initial slope, α , and the light and CO_2 saturation rate of photosynthesis (A_{pot}). Γ was determined by extrapolating the linear portion of the CO_2 response curve to intercept the x-axis, and carboxylation efficiency (α ; the initial slope of the CO_2 response curve) was determined as the slope of the first four points in the linear part of the CO_2 response curve (Polley *et al.*, 2002). Gas phase limitation to photosynthesis, L_g , was estimated from the CO_2 response curves as $[(A_{\text{pot}} - A) / A_{\text{pot}}] * 100$, (Farquhar and Sharkey, 1982; Ripley *et al.*, 2007), where A is net photosynthesis at ambient chamber $[\text{CO}_2]$, $380 \mu\text{mol mol}^{-1}$. To test the significance of differences among the species in growth parameters and gas exchange parameters, data were analysed with univariate analysis of variance (ANOVA), using Tukey's HSD test. Statistical analyses were performed using the SPSS statistical package.

RESULTS

Plant growth

D. eriantha and *P. repens* had almost twice as much biomass, dry weight, as that of *E. lehmanniana*. Specific leaf area (SLA) realized with this biomass was highest in *D. eriantha* and similar between the other two species (Table 1). As was the case with biomass, the ratio of leaf area to total plant weight (LAR) of *D. eriantha* and *P. repens* was significantly higher ($P < 0.05$) than that of *E. lehmanniana*. Root length per unit root biomass (SRL) of *D. eriantha* was significantly higher ($P < 0.05$) than that of the other two species. *D. eriantha* had similar shoot: root ratio (SRR) with *E. lehmanniana* that were almost double that of *P. repens*.

Table 1: C₄ photosynthetic subtypes and means and standard deviations of biomass allocation characteristics of the three savanna grass species. (SRR = shoot root ratio; SRL = specific root length; LAR = leaf area ratio; SLA = specific leaf area). Different superscripts in each column indicate significant differences at $P = 0.05$; Tukey HSD test.

Species	C ₄ subtype	<i>n</i>	Biomass	SRR	SRL	LAR	SLA
			g	g.g ⁻¹	cm g ⁻¹	cm ² g ⁻¹	cm ² g ⁻¹
<i>D. eriantha</i>	NADP-ME	8	39.4 ^b	3.8 ^b	326 ^b	90 ^c	245 ^b
<i>E. lehmanniana</i>	NAD-ME	11	21.2 ^a	3.7 ^b	211 ^a	36 ^a	191 ^a
<i>P. repens</i>	PCK	12	40.0 ^b	1.8 ^a	170 ^a	63 ^b	191 ^a

Photosynthetic characteristics

Typical of C₄ species, net photosynthesis in all the species was not light saturated (Figure 1). *D. eriantha* had highest mean apparent light use efficiency (ϕ ; 0.067 mol (CO₂) mol⁻¹(incident photon)) which was significantly higher ($P = 0.021$) than that of *P. repens* (0.056 mol (CO₂) mol⁻¹ (incident photon); Table 2).

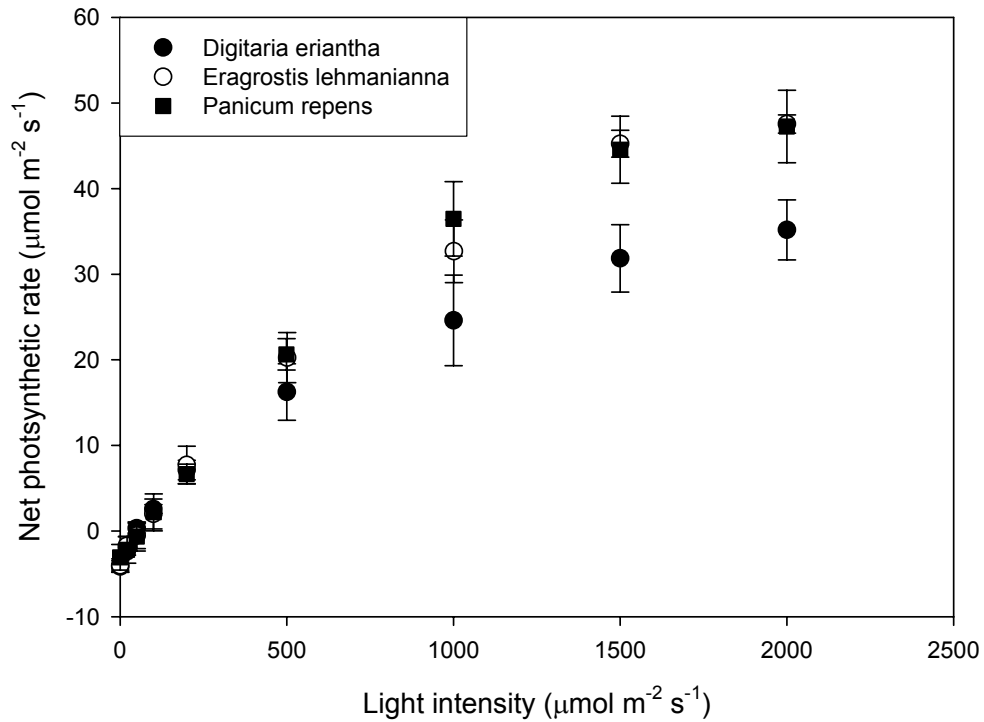


Figure 1: Means and standard deviations of the response of net CO₂ assimilation to photon irradiance in leaves of three C₄ grasses. Each datum point is a mean of at least four measurements. For clarity of the figure, only standard deviations for *A* are shown.

E. lehmanianna and *P. repens* displayed similar mean A_{sat} and mean g_{sat} values (47 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 0.27 $\text{mol m}^{-2} \text{s}^{-1}$, respectively) that were significantly higher ($P < 0.05$) than in *D. eriantha* (Table 3). Mean WUE values were similar between *D. eriantha* and *P. repens* (approximately 190 $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$) and were significantly higher ($P < 0.05$) than those of *E. lehmanniana* (Table 3). The rank order of the species remained the same even when WUE was determined as A/E (net photosynthesis / transpiration) (data not shown).

Table 2: Means and standard deviations of carboxylation efficiency (CE), CO₂ compensation point (Γ), light use efficiency (ϕ), dark respiration (R_d), gas phase limitation to photosynthesis (L_g) for the three grass species. Different superscripts in each column indicate significant differences at $P = 0.05$; Tukey HSD test.

	CE	Γ	ϕ	L_g
	$\mu\text{mol m}^{-2} \text{s}^{-1}$ ($\mu\text{mol mol}^{-1}$) ⁻¹	$\mu\text{mol m}^{-2} \text{s}^{-1}$	mol mol^{-1}	%
<i>Digitaria eriantha</i>	2.19 (0.84) ^a	6.96 (1.19) ^a	0.067 (0.01) ^b	22.14 (4.44) ^b
<i>Eragrostis lehmanniana</i>	1.66 (0.33) ^a	5.98 (3.34) ^a	0.063 (0.01) ^{ab}	14.44 (3.06) ^a
<i>Panicum. repens</i>	2.59 (1.08) ^a	6.91 (1.31) ^a	0.056 (0.02) ^a	17.47 (2.39) ^{ab}

D. eriantha and *P. repens* displayed similar mean Ci/Ca ratio (0.11 and 0.12 respectively) that were significantly lower ($P < 0.05$) than that of *E. lehmanianna* (0.18; Table 3). Consistent with these observations, there were similar slopes of the relationship between A_{sat} and g_{sat} in *D. eriantha* and *P. repens* (Figure 2). This relationship was linear and close to proportional in *D. eriantha* and *P. repens*, in contrast to that of *E. lehmanianna* which was non-linear. In *E. lehmanianna*, A_{sat} increased with g_{sat} until values of approximately $0.3 \text{ mol m}^{-2}\text{s}^{-1}$, above which a further stomatal opening did not lead to any evident further increase in A_{sat} .

Despite considerable scatter in the data, g_{sat} and Ci/Ca ratio decreased with an increase in D_i in all the species (Figure 3). The strongest and significant linear correlations between g_{sat} vs D_i and Ci/Ca ratio vs D_i relationships were found in *P. repens* ($P < 0.05$, $r^2 = 0.5$ and 0.6 , respectively). By contrast, both relationships were weak and non-significant ($P > 0.05$, $r^2 = 0.1$) in *E. lehmanianna*. In all the species, the relationship between A_{sat} and D_i followed a similar pattern to that of g_{sat} vs D_i . (data not shown).

Table 3: Means and standard deviations of light saturated net photosynthetic rate (A_{sat}), light and CO₂ saturated net photosynthetic rate (A_{pot}), light

saturated stomatal conductance (g_{sat}), the ratio between ambient and intercellular CO_2 (C_i/C_a) and water use efficiency (WUE) for the three species at saturating light ($1600 - 2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$. Different superscripts in each column indicate significant differences at $P = 0.05$; Tukey HSD test.

	A_{sat} $\mu\text{mol m}^{-2} \text{s}^{-1}$	A_{pot} $\mu\text{mol m}^{-2} \text{s}^{-1}$	g_{sat} $\text{mol m}^{-2} \text{s}^{-1}$	C_i/C_a	WUE $\mu\text{mol CO}_2$ $\text{mol}^{-1} \text{H}_2\text{O}$
<i>Digitaria eriantha</i>	35.28 (6.76) ^a	41.23 (4.37) ^a	0.19 (0.05) ^a	0.11 (0.04) ^a	190.3 (14.2) ^b
<i>Eragrostis lehmanniana</i>	47.47 (4.04) ^b	52.33 (2.25) ^b	0.31 (0.08) ^b	0.18 (0.05) ^b	161.8 (28.6) ^a
<i>Panicum repens</i>	47.33 (6.86) ^b	51.30 (8.07) ^b	0.26 (0.05) ^b	0.12 (0.03) ^a	184.2 (12.9) ^b

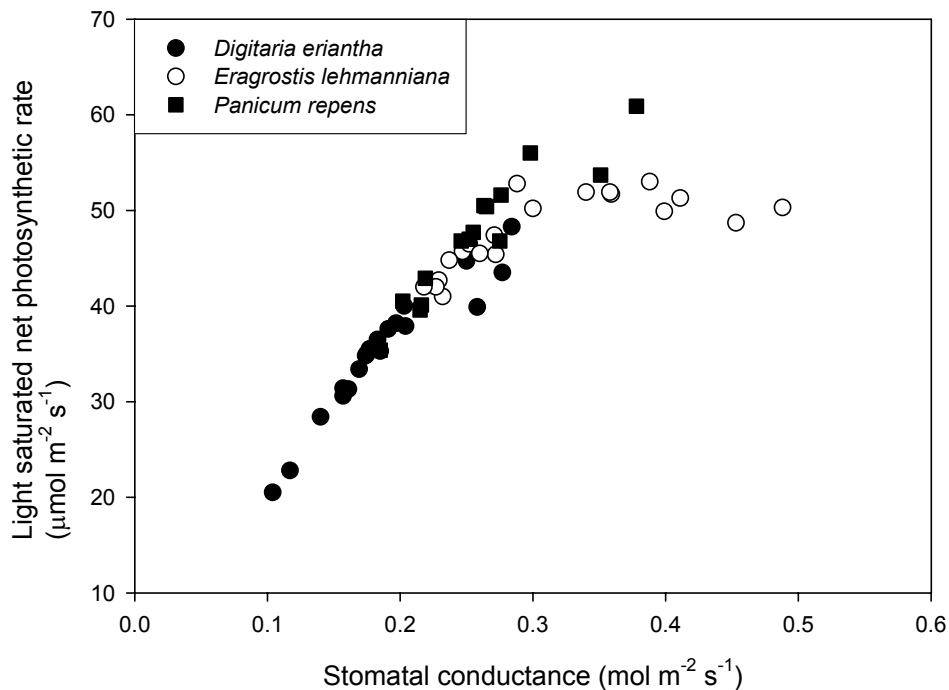


Figure 2: Relationship between light saturated net CO_2 assimilation and stomatal conductance of leaves of C_4 species. Each point represents an individual measurement at saturating light ($1600 - 2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$.

Consistent with C_4 photosynthesis, CO_2 assimilation rates increased rapidly, in response to increasing internal CO_2 concentrations, C_i , up to C_i

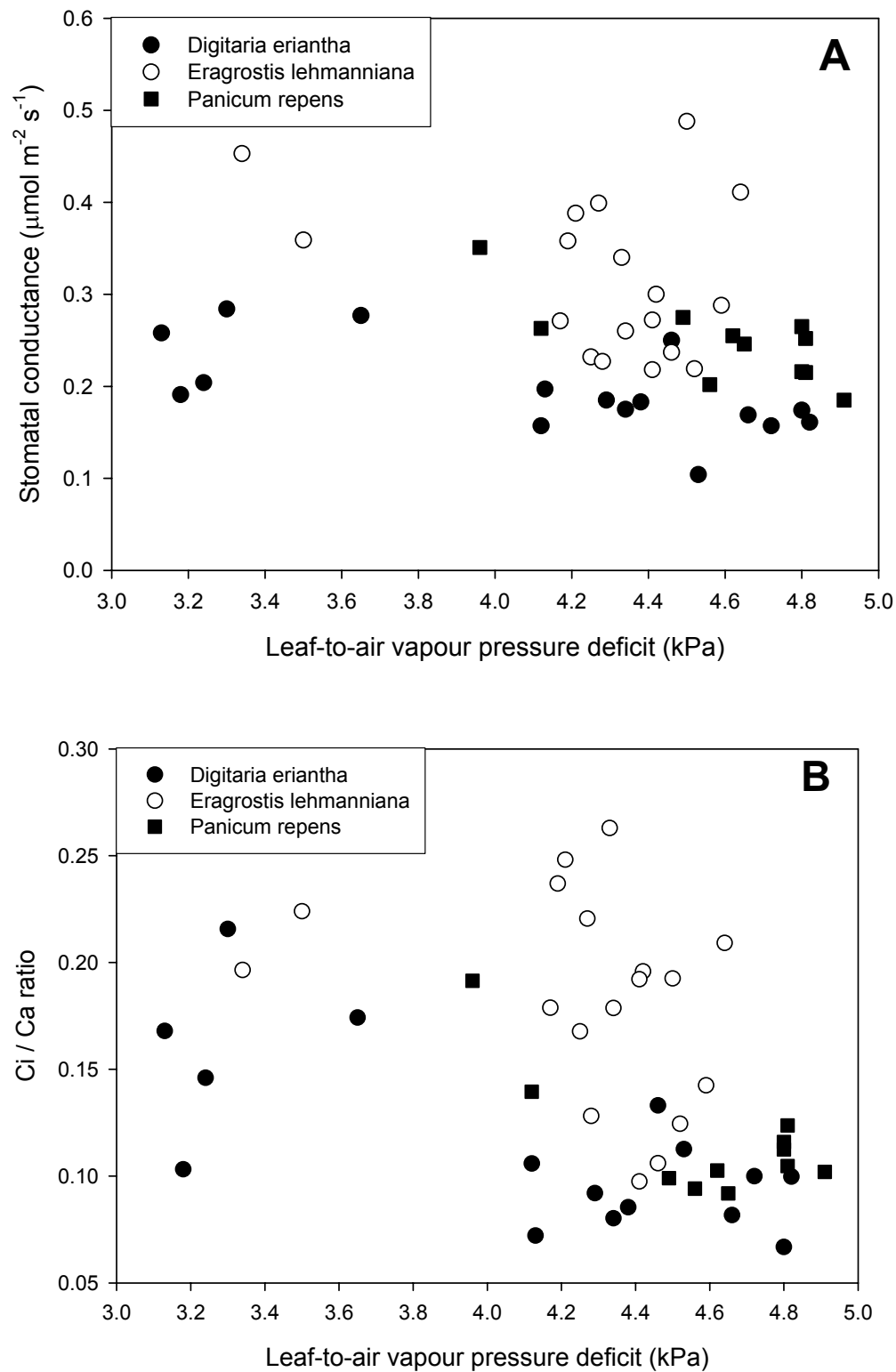


Figure 3: The response of stomatal conductance (A) and C_i/C_a ratio (B) to leaf to air vapour pressure deficit of the three species. Each point represents an individual measurement at saturating light ($1600 - 2000 \mu\text{mol m}^{-2} \text{s}^{-1}$) and at ambient $[\text{CO}_2]$ of $380 \mu\text{mol mol}^{-1}$.

values of 50 to 80 $\mu\text{mol mol}^{-1}$, before becoming saturated at high chamber $[\text{CO}_2]$. (Figure 4).

Also, typical of C_4 plants, CO_2 compensation points of all species were low, approximately 6 $\mu\text{mol m}^{-2} \text{s}^{-1}$, with no significant differences ($P > 0.05$) between the species (Table 2). Initial slopes of the CO_2 response curves (α), showed no significant differences ($P = 0.306$) between the species, ranging from 1.7 to 2.6 $\mu\text{mol m}^{-2} \text{s}^{-1}(\mu\text{mol mol}^{-1})^{-1}$ (Table 2).

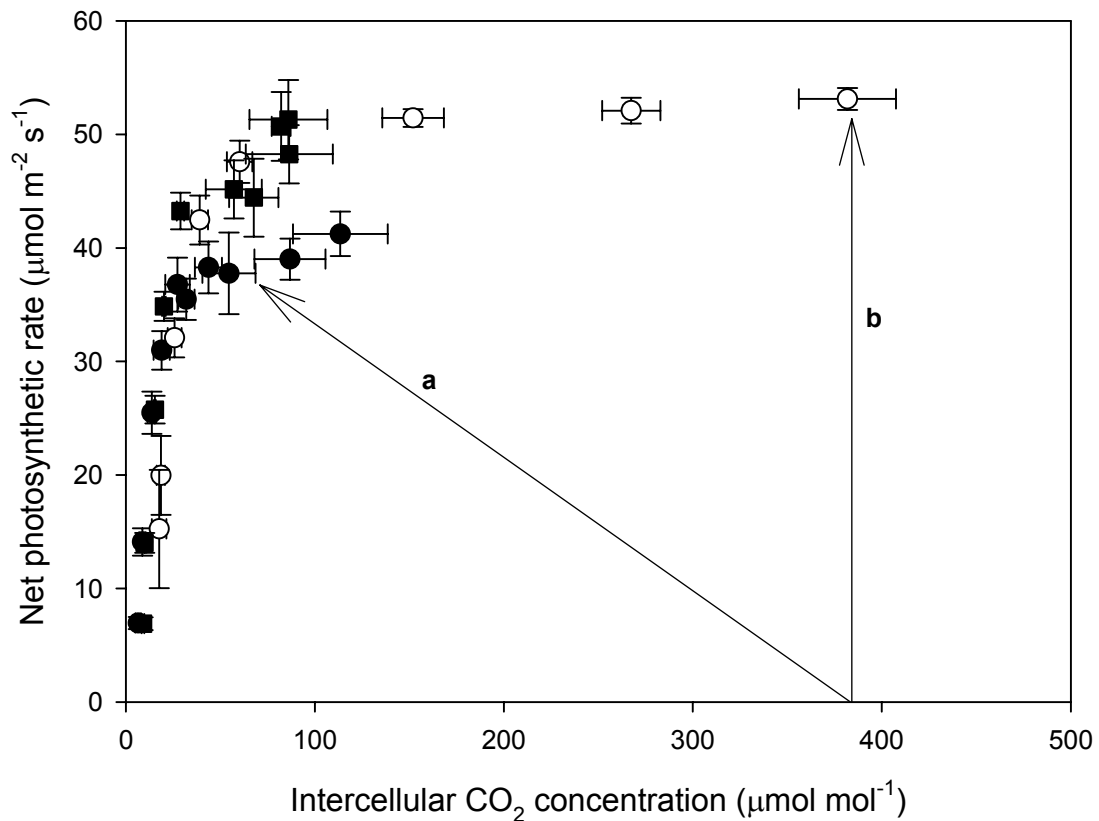


Figure 4: Means and standard errors of the response of net CO_2 assimilation to CO_2 in leaves of three C_4 grasses. Each datum point is a mean of at least four measurements. The vertical line, denoted *b*, represents photosynthetic rate at ambient $[\text{CO}_2]$ if resistance to diffusion was zero and that denoted *a*, represents the limitation on net photosynthesis imposed by CO_2 diffusion. Line *a* has a slope set by the stomatal conductance and both lines intercept the C_i axis at ambient $[\text{CO}_2]$. Symbols are the same as those in Figure 1.

In *D. eriantha* and *P. repens*, raising chamber C_a values above ambient $[\text{CO}_2]$ led to a relatively small increase in C_i , up to 120 $\mu\text{mol mol}^{-1}$ (Figure 4). By contrast, at high C_a , C_i of *E. lehmanianna* increased to almost 400 $\mu\text{mol mol}^{-1}$. Similar to A_{sat} , highest mean light and CO_2 saturated photosynthetic

rates (A_{pot}) values were found in *E. lehmanniana* and *P. repens* (approximately $52 \mu\text{mol m}^{-2} \text{s}^{-1}$) and were significantly higher ($P < 0.05$) than that found in *D. eriantha* ($41 \mu\text{mol m}^{-2} \text{s}^{-1}$). Mean gas phase limitation (L_g) values, which indicates the magnitude of increase of photosynthesis at ambient CO_2 concentration relative to A_{pot} , were highest in *D. eriantha* (22 %) while *E. lehmanianna* had lowest values (14 %; Table 2).

DISCUSSION

The aim of this study was to characterize plant biomass and leaf-level gas exchange of commonly found C_4 grasses with the aim of improving our knowledge in predicting their field performance in natural environments. In the long term, our objective was to identify representative species that may be used to gather information that would be useful when constructing the carbon budget in the ecosystems of the Okavango Delta.

Although caution is necessary, when comparing allocation patterns in plants when only single harvests are taken (Lambers and Poorter, 1992), the long growth period and the similar order of magnitude of the final biomass of the plants at the end of the experiment suggests that some differences can still be considered to reflect true differences allocation patterns. For instance; the larger biomass in *D. eriantha* was associated with high *SRL*. Increased *SRL* results from longer root length (and hence larger root surface area) per unit carbon invested (Eissenstat, 1991), and indicates potential for high rates of root growth in favourable soil conditions. Further, the high *LAR* displayed by *D. eriantha*, which was probably due to its high *SLA*, suggests a physiological trait to optimize photon harvesting. The advantage of a high *SLA* is a higher photosynthetic rate per unit leaf weight (Roderick and Cochrane, 2002). Indeed, our results showed that in spite of lowest area based photosynthetic rates in *D. eriantha*, mass based photosynthetic rates between the species were similar. Taken together, the above-and below ground allocation of biomass in *D. eriantha* suggests high potential of this species to exploit the seasonal pulses in nutrient and water availability and to tolerate different levels of shading that it encounters under natural conditions, hence its

occurrence in a wide range of habitats within the Okavango Delta and throughout Southern Africa (Gibbs Russel *et al.*, 1990).

The low shoot: root ratio found in *P. repens* indicates a strong priority for shoot growth. A trait particularly important in wetlands where under more stable moisture conditions shoot competition may be more important. The high productivity of *P. repens* makes it an important grazing resource for game and domestic livestock (Gibbs Russel *et al.*, 1990; Van Bommel *et al.*, 2006). However, the small root allocation in this species could imply low capacity to tolerate dry conditions and also reduced capacity for soil water and nutrient uptake when growing under natural conditions.

The range of mean A_{sat} and g_{sat} , 35–48 $\mu\text{mol m}^{-2}\text{s}^{-1}$, 0.19 – 0.31 $\text{mol m}^{-2}\text{s}^{-1}$, respectively, observed in this study was comparable to that reported for other C_4 species grown under optimum conditions (25 and 60 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and 0.2 – 0.4 $\text{mol m}^{-2}\text{s}^{-1}$ respectively) (Lawlor, 2001). The positive curvilinear relationship between g_{sat} and A_{sat} found in *E. lehmanniana* implies more stress on g_{sat} than on photosynthetic capacity (Hetherington and Woodward, 2003), while the close to proportional and almost linear relationship in *D. eriantha* and *P. repens*, if maintained would result in a constant C_i (Jones, 1987). However, this relationship did not pass through the origin; therefore C_i could not be constant.

The range of mean C_i/C_a values of these species, 0.11 – 0.18, was on the lower side of those previously reported at moderate vapour pressure deficits and well-watered C_4 species (0.2 – 0.4; Lawlor, 2001). Such low C_i/C_a values were probably a reflection of both the direct response of stomata to high D_l and also the relatively high photosynthetic capacity (Condon *et al.*, 2002). The C_i/C_a ratio is determined by the balance between the supply of CO_2 to the leaf interior and the demand for CO_2 (Farquhar and Sharkey, 1982). The low C_i/C_a ratio and high WUE displayed by *D. eriantha* and *P. repens* indicates that carbon gain occurred at minimal loss of water. However, our results suggest that the low C_i/C_a ratio in *P. repens* might have been due to high photosynthetic capacity as indicated by its high A_{sat} , by contrast, the low C_i/C_a ratio of *D. eriantha* might have been a result of lower stomatal conductance, as indicated by the low A_{sat} .

The slope of the relationship between g_{sat} and D_l has been used in C_4 species to indicate stomatal sensitivity to D_l (Bunce, 1983; Kawamitsu *et al.*, 1993; Maroco *et al.*, 1997). The lower sensitivity of g_{sat} to D_l and the weaker response of A_{sat} to D_l displayed by *E. lehmanniana* suggest better adaptation to seasonally dry environments where high D_l is associated with the wet season. High stomatal sensitivity, as found in *P. repens* and *D. eriantha* might be ecologically disadvantageous, since plants might miss the opportunity for photosynthesis when there is available soil water to meet the transpirational demand. A study on the three Sahelian C_4 grass species attributed pronounced stomatal sensitivity to drought resistance, while a lack of stomatal sensitivity was associated with drought escaping species (Maroco *et al.*, 1997).

Well-established theory suggests that, on average, NADP-ME species have higher ϕ than NAD-ME species with PCK species being intermediate (Ehleringer and Pearcy, 1983). This pattern was not apparent in this study and may have been masked by the use of only one species per C_4 subtype. However, the high ϕ found in *D. eriantha* indicates higher efficiency of leaf photosynthesis in light-limiting conditions (Ehleringer and Pearcy, 1983). Functionally, this may be a useful trait since this species is sometimes found in savanna communities with higher leaf area indices (Gibbs Russel *et al.*, 1990). Because of the presence of a CO_2 -concentrating mechanism, photorespiration is suppressed and photosynthesis operates at near CO_2 -saturation in well-watered C_4 plants (von Caemmerer, 2000). Consistent with this theory, our results showed Γ of less than $10 \mu\text{mol mol}^{-1}$ and a relatively small increase, 14 - 22 %, in photosynthesis at short-term exposure to high $[\text{CO}_2]$.

In conclusion, substantial differences in above-and below-ground biomass allocation may potentially contribute to their different abilities to extract water and nutrients, and likely reflect the adaptations to the different habitats they dominate. *D. eriantha* produced high above-and below-ground biomass, showed high WUE and high adaptation to low light conditions. If this pattern holds in the field, it would suggest that both morphological (biomass accumulation) and physiological (gas exchange characteristics) play a role in

facilitating this species relative abundance in a wide range of habitats within the savanna ecosystem. Gas exchange parameters in *E. lehmanniana* showed the weakest response to high evaporation demand, indicative of better adaptation to seasonally dry environments and consistent with the dry, open and disturbed habitats dominated by this species. The high above ground biomass allocation of *P. repens* may be indicative of a mechanism to cope with herbivory. On the other hand, high stomatal sensitivity suggests a physiological basis for this species greater abundance in wet areas of the Okavango Delta.

The experimental design of this study prevented the detection of plant-plant interactions which would be found under natural conditions. Moreover, since these plants were grown under ambient [CO₂] and exposed only for short periods to high CO₂ chamber environment, it was not possible to compare our results to other C₄ grasses grown under high [CO₂] (e.g. Roumet & Joy, 1996). A more complete comparative assessment of the morphological and leaf-level photosynthetic characteristics of these species awaits the incorporation of species-specific leaf nitrogen content. We suggest that the adaptive traits observed here could be tested over a larger number of species and growing them over a longer period of time, combined with manipulation of water and/or fertilization regimes, to allow for a further identification of evolutionary pressures that best reflect selection for functionally adaptive morphological and physiological characteristics. Taken together, our results make a significant contribution to the understanding needed to predict the spatio-temporal distribution of these species and may also be useful in determining gross primary productivity of this savanna-wetland mosaic.

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CHAPTER 5

Growth form and seasonal variation in the leaf gas exchange of the savanna tree *Colophospermum mopane* in northwest Botswana^{iv}

Summary

We studied photosynthetic capacity of physiognomically different forms of the mopane tree (*Colophospermum mopane* Kirk ex J. Leonard (Kirk ex Benth) growing on Kalahari sands above calcrete duricrusts in response to variations in climatic and edaphic factors. Stands growing on deeper soils were taller in height (5.5 m *cf.* 1.6 m) with much lower leaf to fine root biomass ratios, *viz* 1:20 for tall growth form and 1: 6 for the short growth form. Both forms had similar leaf area indices (0.9 – 1.0 m² m⁻²).

Maximum net assimilation rates (A_{sat}) occurred during the rainy season (March – April 2000 and January – February 2001) and were similar for both stands at this time (15 – 22 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) before declining to less than 10 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ at the end of the rainy season in late April. Leaf nitrogen concentrations varied between 18 mg g⁻¹ and 27 mg g⁻¹ and were consistently around 20% higher in tall mopane compared to the shorter form. A_{sat} declined rapidly as the dry season progressed with soil water, pre-dawn leaf water potential and nitrogen all declining concurrently during this time. Prior to leaf abscission pre-dawn leaf water potential was more negative for the short mopane form (-3.5 MPa) than for the tall mopane (-2.8 MPa). Stomatal regulation of water use and carbon assimilation differed between years with the taller growth form having a consistently more conservative water use strategy as the dry season progressed.

INTRODUCTION

The amount and seasonality of soil water availability plays an important role in structuring patterns of activity and growth of seasonally dry forests and woodland savanna plants (Holbrook et al. 1995, Duff et al. 1997, Choat et al. 2006) and it is important to investigate how dryland trees obtain, transport, lose and store water. The pattern and depth to which trees are rooted and the sources of water taken up by these roots may be particularly important in influencing the structure and dynamics of dry forest and savanna ecosystems (Medina 1995, Canadell et al. 1996, Veenendaal and Swaine 1998, Ares and Fawnes 1999).

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Colophospermum mopane Kirk ex J. Leonard (Kirk ex Benth) is a deciduous leguminous tree often occurring in valley bottoms on heavy, calcareous and often sodic soils in Southern Africa (Mapaure 1994). It is also widespread on Kalahari sands where it is generally found as nearly pure monospecific stands (Wild and Fernandes 1967). Within Southern Africa it often exists as two readily identifiable forms: multiple stemmed shrubs with a modal height of about 1–2 m or single stemmed trees up to 6 m in height. The existence of these two physiognomic forms has been associated with differences in soil physical and/or chemical properties. The multiple stemmed (“short”) form tends to occur on heavier soils derived from basic materials with the single stemmed tree (“tall”) form tending to occur on sandier soils (Fraser et al. 1987). Le Roux (1980) attributed these variations more specifically to variations in soil nitrogen and magnesium; the dwarf form tending to occur where soil N is low and soil Mg high. Nevertheless, alternative explanations for the short form of mopane also exist. For example, both browsing by elephants (Smallie and O’Connor 2004) or high fire frequencies (Mlambo and Mapaure 2006) have been invoked as possible causes.

Working at the site which formed the basis of this study, Mantlana (2002) observed that “tall” stands occurred on a sand profile 5 m deep on top of a calcium-rich duricrust layer, with the interspersed patches (islands) of “short” (<2 m) multi-stemmed shrubs on a sand layer with a similar calcium duricrust layer only 2 m below. We decided to utilize this variation in physiognomic form at closely proximal locations to study seasonal variations in leaf gas exchange and water relations. We hypothesized that the differences observed in structure and leaf gas exchange characteristics of the two growth forms would be directly linked to the way in which they are able to exploit water in the soil profile.

MATERIALS AND METHODS

Study Area

The study was conducted about 20 km east of Maun, Botswana (23° 33' E, 19° 54' S, 960 m elevation) in a typical Kalahari sandveld environment

(Skarpe 1996, Moore and Attwell 1999). Rainfall is about 450 mm per annum but highly variable (Moore and Attwell 1999), falling mainly between November and April.

The woody layer was almost exclusively dominated by *Colophospermum mopane* Kirk ex J. Leonard (Kirk ex Benth) but with *Acacia nilotica* (L.) Willd. Ex Delille, *A. erioloba* E. Mey., *Terminalia sericea* Burch. Ex DC., *Grewia flava* DC., and *Ximenia Americana* L. also occurring.

Two sites about 300 m apart were chosen; one with the tall and the other with the short mopane growth form. Soil electrical conductivity varied from 0.04 mS.cm⁻¹ at the surface to 0.25 mS.cm⁻¹ at 5 m and pH between 7 and 8.5 indicating no evidence of salinity at either location. At both sites soil bulk density varied between 1.6 and 2 kg dm⁻³ in the first 1.0 m of the profile, and varied similarly with depth. Fire had not played a primary role in influencing the vegetation structure due to a lack of combustible undergrowth. The general area was not browsed by elephants (Veenendaal et al. 2004) and the grass cover at both sites was sparse (Herbaceous LAI < 0.1)

Gas exchange measurements

CO₂ assimilation rate (*A*) and incident photosynthetic photon flux density (*PPFD*) were measured using a LI-6200 Portable Photosynthesis System (Li-Cor INC. Lincoln, NE. USA) with a standard 1L leaf chamber (McDermitt et al. 1989). A *PPFD* quantum sensor was fitted outside the chamber. The gas analyser was calibrated before each measurement day with a gas of known [CO₂] and the quantum sensor cross-checked against a newly factory calibrated sensor. At monthly intervals five trees were selected at random from each site with two healthy looking, sun-facing leaves per tree selected for gas exchange measurements. Measurements were taken from dawn (06h00) to mid afternoon (15h00), generally at two-hour intervals. To prevent excessive increases in leaf temperature measurements were taken at fixed time intervals of 22 seconds (McDermitt et al. 1989). Leaf orientation was maintained whilst enclosed in the chamber and all measurements were made at ambient [CO₂]. Measurements were made between March and August 2000, and again when new leaves appeared in January 2001 until March

2001. In all cases the two sites were measured on consecutive days. Light saturated CO₂ assimilation rates were not obtainable in March 2000 at the tall mopane site because of persistent cloud cover.

Leaf water potential

Predawn leaf water potentials (Ψ_l) were measured using a Scholander-type pressure chamber (Skye instruments Plant moisture System, Powys, Wales). Two randomly selected healthy leaves per tree were measured on the same trees at the same time as the gas exchange measurements.

Soil water content

Soil water content was measured gravimetrically on soil cores at both sites from May 2000 to March 2001 at 25 cm intervals over the first meter and then every 50 cm to 2.0 m in the short form and to 5.0 m in the tall form. There were three replicates per site.

Leaf nitrogen concentrations

Ten to 15 leaves per tree were harvested monthly from five randomly selected trees on both sites, dried at 70 °C to constant weight and ground to a very fine powder. Total nitrogen concentration of the ground tissue was analysed with an Elemental Analyser 1110 CHNS-O (ThermoQuest Carlo Erba Instruments, Wigan, UK) (Pregitzer et al. 1998). All measurement sequences included standard reference samples.

Root distribution and root structure

At the end of the growth season in May 2001, single monoliths of soil 1 m by 1 m, to a depth of 1 m were removed from the stem outward from 3 randomly selected trees, in 5 layers of 20 cm each. Fine-mesh sieves (0.2 mm) were used to separate the roots by gently wet sieving the soil. The roots from each soil layer were sorted into 2 classes, <1 mm (fine roots) and >1 mm (large roots).

Canopy leaf areas

Leaf area index (LAI) was measured using a Sunscan canopy analysis system (Delta T Devices, Cambridge, UK.) for both stands in April 2000 (toward the end of the wet season).

Meteorological data

Precipitation daily totals and average afternoon temperature and maximum vapour pressure deficits (average of measurements between 12.00 h and 18.00 h.) were obtained from a meteorological station on a nearby eddy covariance tower (Veenendaal et al. 2004) except for the period September through October 2000 when they were obtained from the long term weather station at nearby Maun airport.

RESULTS

Stand characteristics

Mean tree height was 5.5 m for the tall form and 1.6 m for the short form (Table 1). Average crown diameter was 4.1 m for the tall and 1.6 m for the short form with the stand density of the short mopane form being about 4 times that of the tall. This is similar to the variation in densities reported from other mopane woodlands in Botswana (Tietema et al. 1991). Although both sites had not known recent fire or grazing disturbance, many short-form plants had several dead stems of approximately the same height as the leaf-bearing stems. The average stem circumference (ankle height) for the tall form was 29.9 cm. An equivalent measure was not possible for the multi-stemmed short form (Table 1). There was no significant difference in average leaf size, specific leaf area (SLA) or LAI; the latter being 1.0 for the tall form and 0.9 for the short form. When measured in March 2001 (towards the end of the wet season) SLA was 9.8 and 9.6 m² kg⁻¹ (tall and short forms respectively).

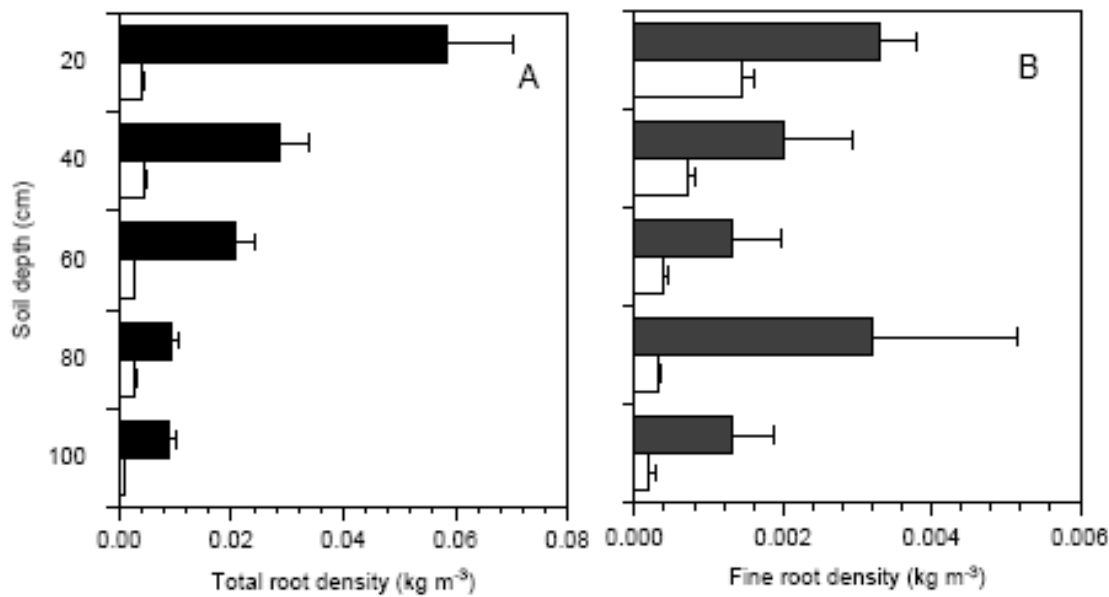


Figure 1: Distribution of roots under the canopy of tall (filled bars) and short (open bars) mopane with depth, (A) total roots and (B) roots < 1 mm in diameter. Error bars indicate 1 standard error.

Total tall mopane root density at the end of the growing season was 8.5 times higher than the short mopane; 25 g dm^{-3} versus 3 g dm^{-3} (Figure 1a). For both forms total root density was highest in the first 20 cm of the soil profile. Fine roots (<1 mm) also showed the highest density in the upper 20 cm for both forms, but then declined consistently with depth for the short form only (Figure 1). In contrast, the tall form had the second highest fine root density at the 60 – 80 cm soil layer (Figure 3b). The short form had no roots below 1 m soil depth, whereas observations at a nearby site which was being cleared for construction purposes showed the roots of the tall form to reach at least 3 m. Similarly, during gravimetric soil moisture measurements, root fragments were observed in soil samples obtained from as deep as 3 m at the tall mopane site. Integrating over the first meter of the soil profile gives average fine root density values of 2 g dm^{-3} and 0.6 g dm^{-3} for the tall and short forms respectively. From the LAI and SLA data, we estimate leaf-to-fine-root biomass ratios of 1:20 for the tall mopane and to 1: 6 for the short mopane.

Table 1: Morphological characteristics of tall and short mopane in the study area. Values are means with standard deviations in brackets. Leaf surface area, specific leaf area and leaf area index were determined in March 2001.

Stand characteristic	Tall mopane	Short mopane
Number of trees per (ha ⁻¹)	100	390
Height (m)	5.5 (0.6)	1.6 (0.1)
Crown diameter (m)	4.1 (0.3)	1.7 (0.1)
Stem circumference (m)	0.3 (0.1)	-
Mean leaf surface area (cm ²)	25.4 (2.1)	23.5 (2.1)
Specific leaf area (m ² kg ⁻¹)	9.8 (0.1)	9.6(1.1)
Leaf area index (m ² m ⁻²)	0.9 (0.1)	1.0(0.1)

Meteorology, soil water and leaf water potential

Patterns of precipitation (mean afternoon temperature, T , and mean afternoon vapour pressure deficit (VPD), along with variations in gravimetric soil water content, (θ_g) and predawn leaf water potential (ψ_{pd}) illustrate a strong seasonality (Figure 2). Precipitation patterns show less than 10 mm of rainfall per month from May through October with marked differences in wet season rainfall between years. The total precipitation for January to March 2001 (towards the end of the experiment) was 118 mm, this being only 36% of the 321 mm observed one year earlier.

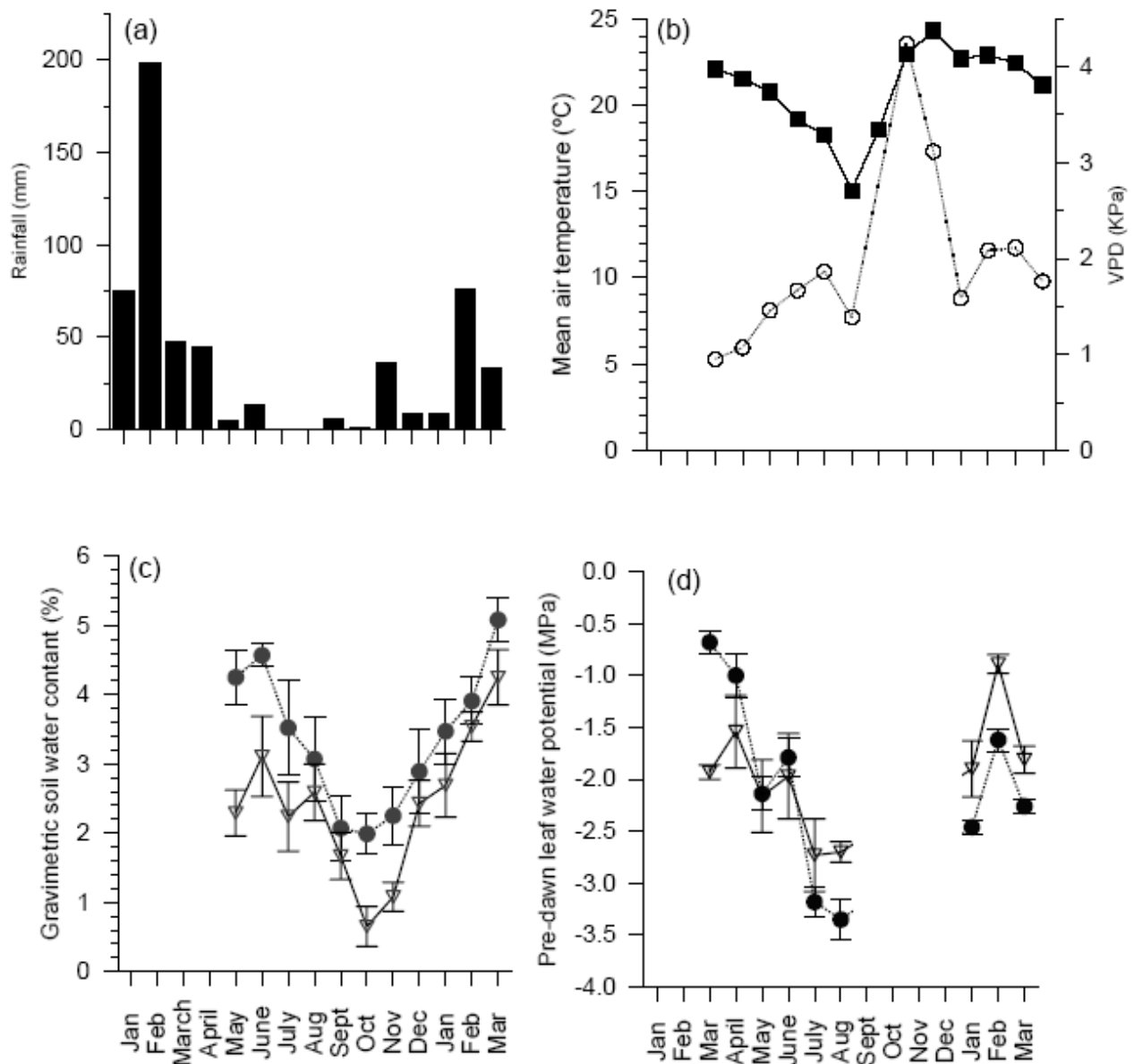


Figure 2: (a) Monthly rainfall; (b) Mean maximum afternoon (12.00h – 18.00 h) temperature (closed squares) and Vapour pressure deficit (open circles); (c) Gravimetric Mean soil water content (0-50 cm) and (d) Pre-dawn leaf water potential (c and d; closed circles data from short mopane; open triangles, data from tall mopane). Error bars indicate 1 standard error.

Mean daily air temperatures also showed a strong seasonal variability, being markedly lower during the dry season (Austral winter) than for summer (Figure 2b). Mean daytime VPD were also nearly two fold higher at the peak of the dry season than during the wet season (Figure 2b). As for rainfall, there was marked variation in wet period VPD between the two study years. Average values in March 2001, for example, were 1.87 kPa, this being nearly twice the 0.95 kPa observed for March 2000. Thus it is important to note that as well as being exposed to very different precipitation regimes in the early wet season for the two years studied, trees also experienced large variations in atmospheric water demand.

As the dry season progressed in 2000, differences in the relative water extraction of the two growth forms were observed. This was especially the case from May through September with θ_g integrated over the entire soil profile showing a 50 % decline, from 4.2 to 2.1 %, for short form but with a decline of only 27 % (from 2.3 to 1.7 %) for the tall form. This suggests that the tall form must have been able to extract water from deeper levels. Indeed, under the short form, moist soil (i.e. $\theta_g > 5\%$) could always be found below 1.5m depth. This contrasted with the tall mopane for which θ_g was always depleted below 5% to at least 4 m depth by the end of the dry season.

Both sites showed similar increases in soil water levels with the onset of the wet season, with θ_g in March 2001 being significantly higher than when measurements had commenced in May 2000. Irrespective of time of year, the soil underneath the short mopane form always had the higher θ_g , perhaps as a consequence of subtle differences in clay content.

Although initially less negative when sampled in March 2000, a much sharper decline in ψ_{pd} occurred for the shorter form from March through August (Figure 2d) with ψ_{pd} declining from -0.7 MPa in March to -3.4 MPa as opposed to from -1.9 to -2.7 MPa for the taller form. For the tall form ψ_{pd} in March 2001 were quite similar to those of March 2000, but this was not the case for the short form where ψ_{pd} for 2001 was substantially

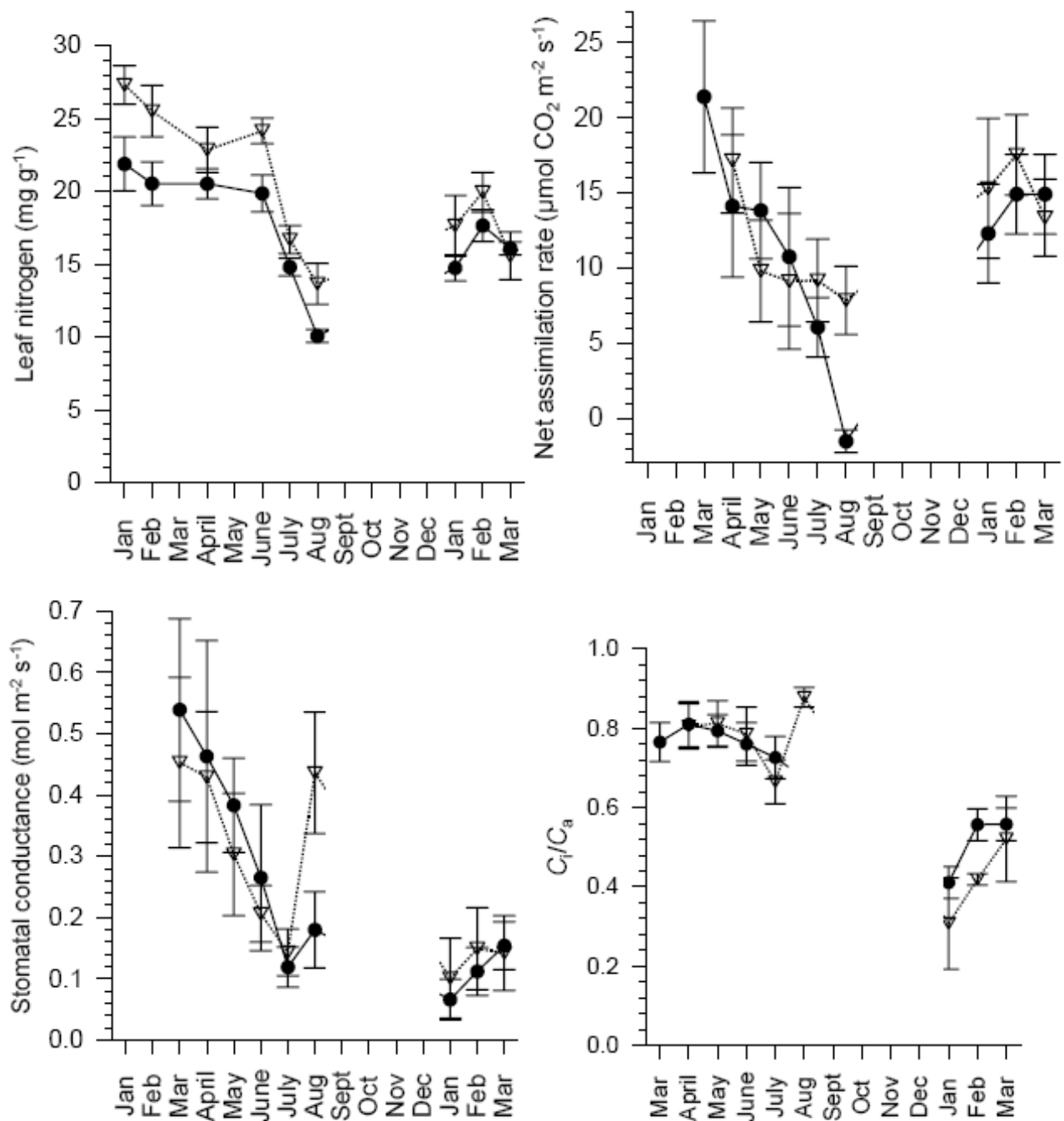


Figure 3: Seasonal variation in (a) Leaf nitrogen; (b) Net light saturated assimilation rate; (c) Stomatal conductance and (d) C_i/C_a ratio (open triangles, data from tall mopane, closed circles data from short mopane). Error bars indicate 1 standard error. Gas exchange Data for the tall form is not available for March 2000 due to cloudy weather conditions.

more negative than the previous year (-2.1 MPa versus -0.7 MPa).

Photosynthetic properties and leaf nitrogen

Leaf nitrogen concentration [N] varied little for both growth forms from January through July 2000, but declined rapidly thereafter with the tall form consistently having higher [N] than the short form (Figure 3a). New leaves the following season showed similar differences but values were about 30% lower than the year before (Figure 3a). Leaf senescence and abscission had commenced in late August for the short form, with this occurring two weeks later (mid-September) for the tall form.

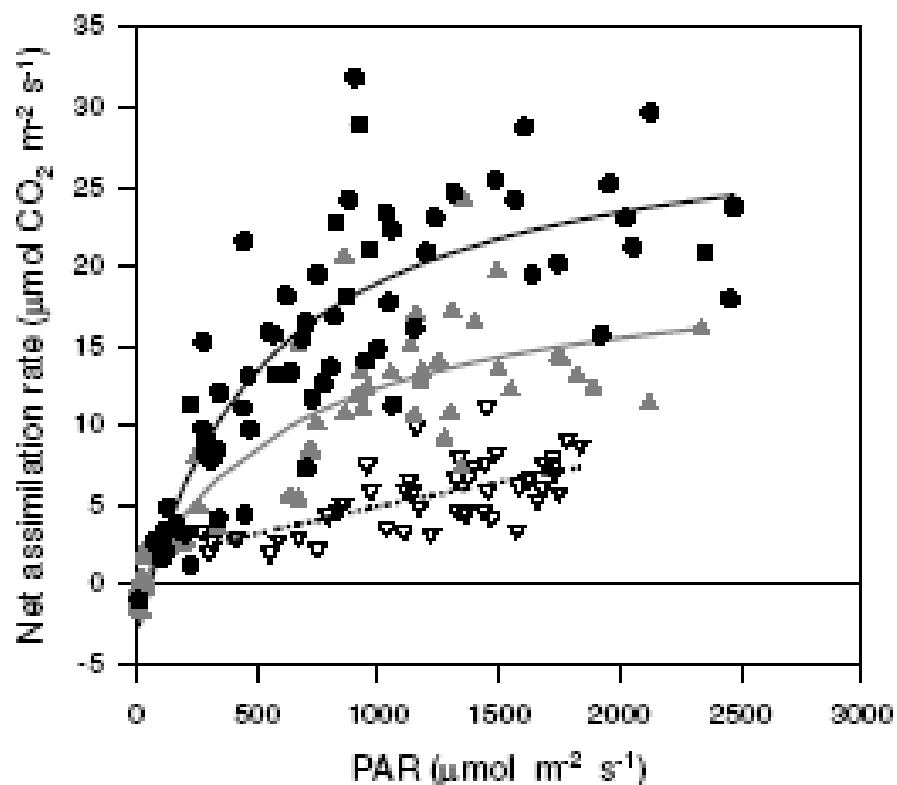


Figure 4: Seasonal change in response of net assimilation rate to photosynthetically active radiation (*PPFD*) in Short mopane (Closed symbols dark line; March 2000; Grey symbols grey line, May 2000; open symbols dotted line. July 2000). Curves were fitted through the data using a rectangular hyperbola (Causton and Dale 1990)

Seasonal variations in the light saturated rate of photosynthesis, A_{sat} ($\text{PPFD} > 1000 \mu\text{mol m}^{-2}\text{s}^{-1}$) are shown in Figure 3b. Maximum A_{sat} , Stomatal conductance, g_s and C_i/C_a were typical for mopane under field conditions at the height of the rainfall season (Midgley et al. 2004). A_{sat} declined for both

forms as the season progressed but the extent of decline was much greater for the short form, especially after June 2000. Stomatal conductance, g_s , also measured at high light, showed similar significant declines as the dry season progressed. Nevertheless, the short form had a slightly higher g_s throughout the entire period, the only exception being August 2000, which was close to the commencement of leaf fall. At this time g_s were higher than the previous month, especially so for the tall mopane, perhaps as a result of an unseasonal rain shower two weeks earlier.

Although there were significant decreases in both A_{sat} and g_s from March through July, C_i/C_a were similar for both growth forms and remained relatively stable around 0.8. Both A_{sat} and g_{sat} were however markedly lower after leaf flush when measured in the second wet season.

Typical seasonal variations in light response curves are shown in Figure 4. This shows both the initial slope of the light response curve as well as the maximal (light saturated rate) to have declined as the dry season progressed. It is unclear, however, whether light saturation was observed for the measurements in July 2000. Nevertheless, A showed a clear tendency to saturation only for PAR above about $1000 \mu\text{mol m}^{-2}\text{s}^{-1}$ under field conditions, characteristic for savanna tree species in this region (Midgley et al. 2004). Co-variations between the seasonal decline in pre-dawn leaf water potential, photosynthetic parameters and $[N]$ are illustrated in Figure 5. Here regression slopes account for the known (estimated) variances in both dependent and independent variables (Ripley and Thompson 1987). This shows a close linear decline of both A_{sat} and $[N]$ with ψ_{pd} , also being similar for growth forms. No such relationship existed for g_s , which also showed a substantially greater variation (Figures 3 and 5). Figure 3 shows that the strongest decline in ψ_{pd} was observed in June and July which was also the time at which the most marked decline in $[N]$ occurred.

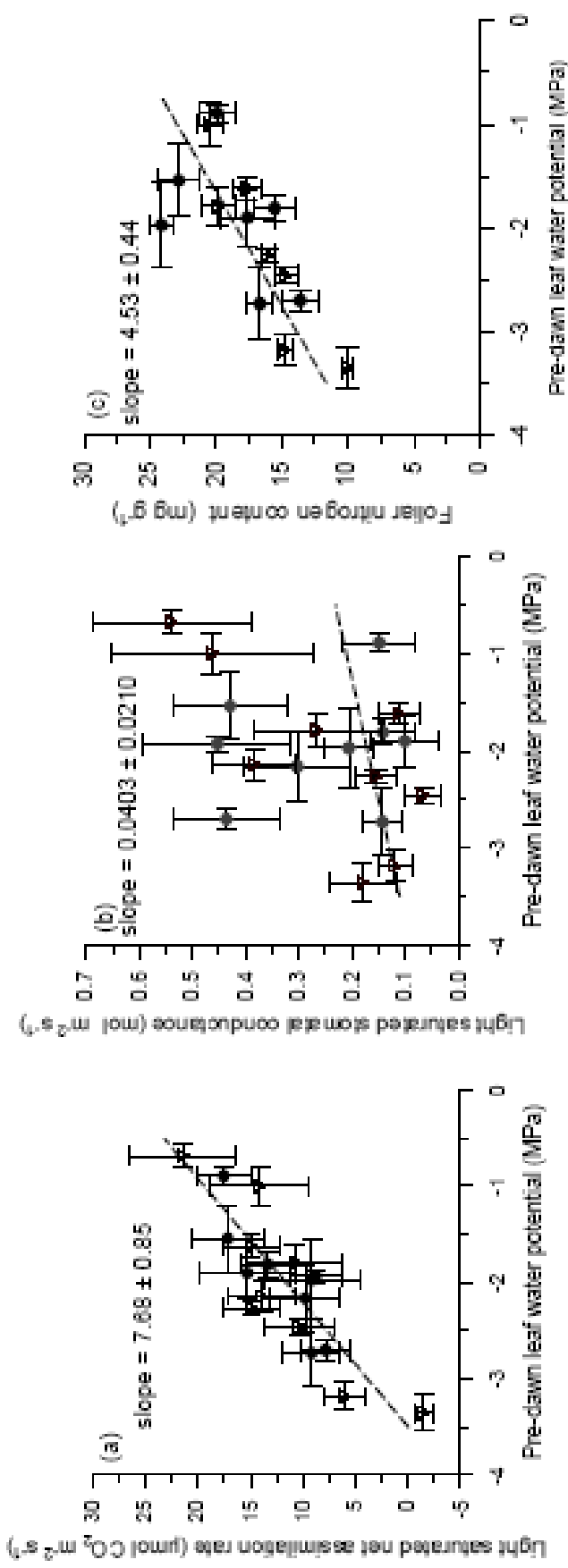


Figure 5: Variation in Light saturated Net assimilation rate, Light saturated Stomatal conductance and Foliar nitrogen concentration in relation to pre-dawn leaf water potential. (symbols as in figure 2). Error bars indicate 1 standard error.

Stomatal regulation of water use and carbon gain

The relationship between photosynthesis, stomatal conductance and vapour pressure was analysed using the approach of Lloyd and Farquhar (1994), who showed that stomata respond to optimise water use for carbon gain (Cowan 1977), then if the Lagrange operator λ which describes this effect relates the physiological and environmental parameters together according to:

$$g_s = \frac{A * \lambda^{0.5}}{(D_l (C_i - \Gamma)/1.6)^{0.5}} \quad (\text{eqn 1})$$

where D_l is the leaf-to-air vapor pressure deficit and Γ is the CO_2 compensation point. Eqn (1) is applied here not so much in an attempt to demonstrate that λ , the marginal evaporative cost of plant carbon gain, is indeed constant over a day or so (as required by the theory of Cowan 1977), but rather to use λ as a descriptor of stomatal behavior that partitions out, for example, simple day-to-day variation in D_l on photosynthesis, stomatal conductance and C_i/C_a . The utility of this approach is shown in Figure 6 where g_s is plotted against $A/(D_l (C_i - \Gamma)/1.6)^{0.5}$ the slope of which equals $\lambda^{0.5}$. This slope was very different for the two different years with values in early 2001 being less than 50% those observed in 2000 for both growth forms. This indicates a much more conservative water use strategy in the early wet season 2001 as compared to the middle-late dry season 2000 for both growth forms. It is also worth noting that such an effect is also consistent with the large differences in C_i/C_a between the two years.

Similarly, eqn 1 suggests that the decline in stomatal conductances as the dry season progressed in 2000 was not simply due to increasing D_l . Rather, intrinsic changes in stomatal behavior must also have been involved. This is because λ declined throughout the dry season, especially for the tall form. The short form showed a much smaller reduction over the same period (Table 2).

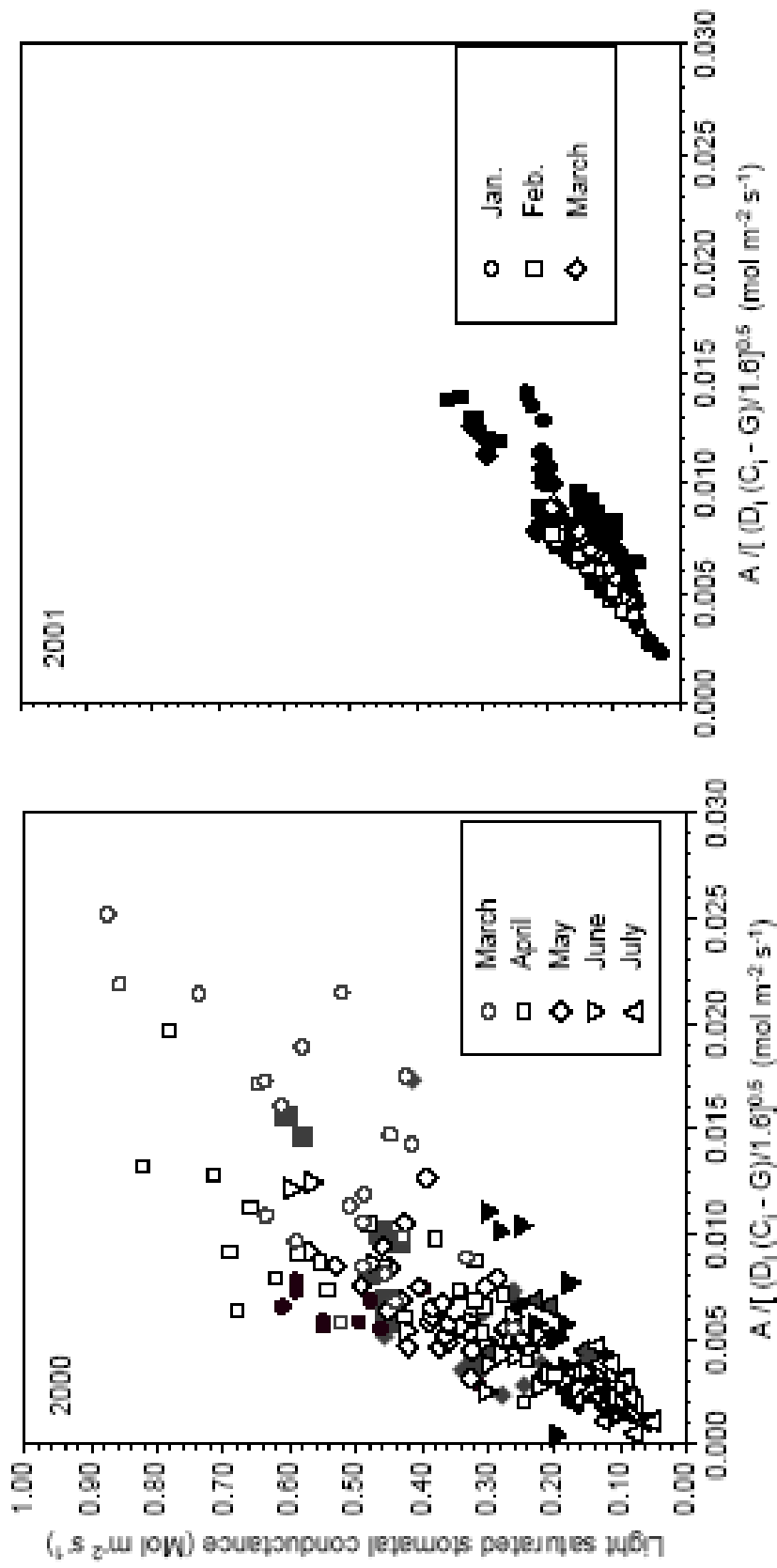


Figure 6. The response of stomatal conductance to a multiplicant involving net saturated CO₂ assimilation (A), leaf-to-air vapour pressure difference (D_l), internal CO₂ concentration (C_i) and the CO₂ compensation point (Γ). Open symbols: data from short mopane, filled symbols: data from tall mopane).

Discussion

There was a more marked decline in ψ_{pd} for the short versus the tall mopane form as the dry season progressed. This may have been attributable to large differences in rooting patterns and densities (Figure 1), which occurred despite similar LAI. Limited exploitation of the available soil volume by the short form may also explain that the decline in ψ_{pd} was more rapid than for the tall form despite soil water contents initially being higher, and with soil water always being available within 2 m from the soil surface, even during the dry season. A positive correlation between pre-dawn leaf water potential and tree height for saplings was also observed for both the evergreen *Eucalyptus tetrodonta* and the deciduous *Terminalia ferdinandiana* by Prior and Eamus (1999). Working in a northern Australian savanna they similarly attributed differences to less well developed root systems for the smaller plants. As discussed below, differing responses of stomata, in particular an apparent insensitivity of the short form to the more rapidly increasing soil water deficits during the dry season may also have been responsible in our case.

Although the two growth forms showed similar seasonal declines in foliar [N], concentrations were consistently lower for the short form (Figure 3). This did not, however, lead to a consistently lower A_{max} . Significant declines in A_{max} were also observed as early as April/May before any significant in [N] occurred in June (Figure 3). This suggesting that a decline in [N] prior to leaf abscission was not the primary or sole cause of decreased photosynthetic capacity as the season progressed (Figure 5c). Nevertheless, for both growth forms, maximum assimilation rates were similarly related to predawn leaf water potentials, both within and across years (Figure 4a). Pre-dawn leaf water potentials are often considered to give a good surrogate measurement of plant soil water availability (Sala et al. 1981, Irvine, et al. 1998, Le Roux and Bariac 1998, Tardieu and Simonneau 1998, Stratton, et al. 2000). It thus seems reasonable to conclude that increasing leaf water deficits (Tuzet et al. 2003) and/or root mediated signals (Blackman and Davies 1985) were the prime drivers giving rise to the declining photosynthetic capacity as the dry season progressed. Nevertheless, this decline was also accompanied by

decreases in foliar [N] as is often observed for deciduous tropical tree species prior to leaf abscission (eg. Martin et al.1994).

Table 2. Inter-annual and seasonal variation in the marginal evaporative cost of carbon gain for the tall and short forms of mopane in 2000 and 2001. Values are means with 95% confidence intervals given in brackets. Units are mol H₂O mol⁻¹ CO₂.

	April 2000	May 2000	June 2000	July 2000
Tall	3674 (2613-4915)	3837 (2404-5602)	2120 (1384-3012)	1580 (1399-1773)
Short	3725 (2793-4792)	3685 (3103-4423)	3613 (2939-4356)	2788 (2372-3236)

	January 2001	February 2001	March 2001
Tall	228 (198-252)	336 (277-402)	368 (337-401)
Short	328 (308-349)	556 (516-598)	415 (384-446)

The tall, deeper rooting growth form remained physiologically active for a longer period of time into the dry season, with the later leaf fall probably being a reflection of this and associated with the ability of the tall form to access water stored in deeper soil layers (Sultan et al. 1998, Laclau et al. 2001). We attribute the unexpectedly higher stomatal conductances for remaining leaves of the tall form in August after a short unseasonal rainfall to the already documented loss of stomatal control with age as tropical tree leaves senesce (Reich and Borchert 1988).

The similar LAI values but more than two-fold difference in the density of fine roots gave rise to considerable differences in the estimated fine root to leaf biomass of the two growth forms. This is surprising as one would expect, particularly in a semi-arid environment, some form of homeostasis in the balance between water-acquiring and water-dissipating organs (Cairns et al. 1997, Caldwell et al. 1998). Data from temperate habitats suggest that trade-offs in allocation of captured resources determine root: shoot ratios (Greco and Baldocchi 1996). In particular, when light is limiting, there should be higher allocation to shoots, whereas on dry soil allocation to roots should theoretically be higher (Blum and Sullivan 1997). The year 2000 season was wetter than average, and this may have permitted greater development of canopy in the short form prior to the onset of water stress. Nevertheless, the contrast in root: shoot ratios was large and, as discussed above, provides some explanation for the different seasonal patterns in physiological characteristics observed for the two forms.

Estimates of λ showed that the taller form has an increasingly conservative water use strategy (Table 2), suggesting that the decline in stomatal conductance as the dry season progressed, was not simply a consequence increasing D_i . This effect was much reduced for the shorter form. Such an effect may have occurred, for example, through root signals reducing stomatal conductances (Blackman and Davies 1985) consistent with the different behaviour of the two growth forms. This is because the significantly lower fine root to leaf biomass ratios for the short form would have reduced the efficacy of any such signal.

We also observed λ to be considerably reduced for early 2001 as compared to the much wetter 2000. Again this suggests that stomatal behaviour was significantly affected by soil water status and that the lower C_i/C_a in early 2001 were not simply due to higher D_i than for the same period in 2000. Perhaps soil moisture conditions during leaf development in the early wet season may be critical for subsequent stomatal behaviour over the rest of the growth period.

Various explanations have been proposed to account for the short form of mopane including browsing by elephants (Smallie and O'Connor

2004) fire effects (Mlambo and Mapaure 2006) and adverse soil conditions (Fraser et al. 1987) Our data clearly point towards edaphic factors being important, with limited root development in the short form of mopane being associated with greater water stresses as the dry season progresses. This results in reduced carbon assimilation rates and an earlier leaf abscission. Our data do not, however, explain the multi-stemmed structure of the short form. Nevertheless, we point out, however, that the short form could be more susceptible to drought and frost because of the reduced rooting depth and lowered ψ_{pd} throughout much of the year. Thus it is possible that periodic severe droughts and/or frosts kill aboveground parts of these plants. Subsequent coppicing would then give rise to the multi-stemmed nature of this form (Holdo 2005, Mlambo and Mapaure 2006). Additional data on soil physical and chemical conditions associated with the two growth forms is, however, required in order to explain the inability of the short form to deploy roots into what appears to be a substantially under utilized soil rooting volume. One possibility is that calcrete duricrust may have lead to increased hard setting of the soil, restricting fine root growth (Townend et al 1996).

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CHAPTER 6.

Seasonal and inter-annual variation of soil CO₂ efflux in representative ecosystems of the Okavango Delta, Botswana.^v

Abstract

We determined seasonal and inter-annual variation of soil CO₂ efflux (F_{CO_2}) of three different ecosystems: a rain-fed grassland, a seasonal floodplain and a permanent swamp; located along a hydrological gradient in the Okavango Delta, Botswana. We found pronounced seasonality in F_{CO_2} at all the sites, with highest average values ($8 \mu\text{mol m}^{-2} \text{s}^{-1}$) and lowest average values ($0.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) at the permanent swamp and rain-fed grassland, respectively. At all sites, highest F_{CO_2} were associated with the wet season. Lowest F_{CO_2} values at the permanent swamp and at the lower floodplain of the seasonal floodplain were associated with seasonal flood and with extreme dry soil conditions at the rain-fed grassland. Our results indicated pronounced sensitivity of these ecosystems to the inter-annual variability of the timing of the rainy season, particularly the rain-fed grassland. At all the sites, soil temperature alone was a very poor predictor of F_{CO_2} . However, a simple, multiplicative model that combined effects of soil temperature and soil moisture on F_{CO_2} could account for a considerable amount of variation at the rain-fed grassland and at the permanent swamp. F_{CO_2} was also poorly correlated with maximum leaf-level photosynthesis at all the sites, but was strongly correlated with seasonal variation of mean leaf nitrogen at the seasonal floodplain. Our results add to the small body of measurements of soil carbon properties in natural tropical ecosystems in Africa and make a useful contribution in understanding carbon dynamics of the ecosystems in semi-arid tropical areas.

INTRODUCTION

After photosynthesis, total soil respiration represents the second most important flux of carbon dioxide from terrestrial systems to the atmosphere (Reichstein *et al.*, 2003; Raich *et al.*, 2002). Global estimates suggest that 75–80 Pg of carbon are produced in soils annually (Raich *et al.*, 2002; Schlesinger, 1977), in contrast to approximately 6 Pg C from fossil fuel emissions and approximately 2 Pg C from land-use change (Houghton *et al.*, 2001). Therefore, any factors that affect the magnitude of soil respiration could significantly alter atmospheric CO₂ concentrations and thus play an

^v K. B. Mantlana, A. Arneth, E. M. Veenendaal, P. Wohland, P. Wolski, O. Kolle and J. Lloyd. In preparation for submission to *Plant and Soil*.

important role for our understanding of terrestrial carbon cycle feedbacks on atmospheric composition and future climate change.

The major components of soil respiration (F_{CO_2}) are autotrophic (root) and microbial and fungal heterotrophic respiration. Short-term variation in heterotrophic respiration is mainly driven by soil temperature and soil water content (e.g., Arneth *et al.*, 2006; Reichstein *et al.*, 2003; Shibistova *et al.*, 2002; Arneth *et al.*, 1998; Norman *et al.*, 1992). Autotrophic respiration is additionally affected by above-ground photosynthesis and plant physiological processes (Högberg *et al.*, 2001) but the vegetation carbon cycle and canopy composition also affect heterotrophic soil respiration, through production and quality of litter, and by affecting the soil microclimate (Elberling *et al.*, 2003; Raich & Tufekcioglu, 2000;). The latter are difficult to quantify from field observations alone since changes in a variety of processes that all contribute to total soil respiration might occur in different directions, resulting in little overall response of the observed F_{CO_2} (Raich & Tufekcioglu, 2000).

Grassland ecosystems cover nearly one-fifth of the world's land surface ($24 \times 10^6 \text{ km}^2$), and contain > 10% of global C stocks (Eswaran *et al.*, 1993). Tropical grasslands store approximately 26% of the carbon sequestered by terrestrial ecosystems (Long *et al.*, 1989). On the African continent, tropical grasslands and savannas are more extensive than tropical forests (Scurlock & Hall, 1998). But despite the importance of tropical and subtropical grassland ecosystems in the global carbon cycle, we know comparatively little about the spatial and temporal distribution of carbon sources and sinks.

Grassland and savanna ecosystem carbon fluxes are tightly linked with precipitation and water availability (McCulley, *et al.*, 2007; Arneth *et al.*, 2006; Chen *et al.*, 2002; Scholes & Walker, 1993). Some published analyses of soil respiration measurements in tropical savanna ecosystems demonstrated that during the dry season, soil temperature has little influence (Pinto *et al.*, 2002; Chen *et al.*, 2002; Davidson *et al.*, 2000; Malhi & Grace, 2000; Zepp *et al.*, 1996; Scholes & Walker, 1993; Holt *et al.*, 1990), since at that time of year heterotrophic activity is limited by the low soil water content and vegetation is inactive or dead. Seasonally, variations in savanna soil respiration therefore were best explained by changes in soil water content (Epron *et al.*, 2004; Zepp *et al.*, 1996; Holt *et al.*, 1990) and wet season root respiration (Chen *et*

al., 2002, Medina *et al.*, 1980), a dry season-wet season variation that is enhanced by the dry season temperatures being often cooler than wet season temperatures (Epron *et al.*, 2004; Miranda *et al.*, 1997).

For tropical regions, the projected temperature increases as a result of climate change are less than those anticipated for higher latitudes (Solomon *et al.*, 2007). However, greater variability of rainfall, leading to a greater frequency of periods of severe water stress during the growing season, is predicted for the Southern Africa region (Solomon *et al.*, 2007). High variability of rainfall will affect the rates of decomposition; therefore, changes in water balance resulting from altered precipitation patterns will need to be taken into account when envisaging possible changes in the seasonal patterns of respiration (Scholes, 1990).

We studied temporal and spatial variation of soil CO₂ efflux at three sites that differ in their hydrological regime, that is, a permanent swamp, a seasonal floodplain and a rain-fed grassland in an inland tropical wetland, the Okavango Delta, in Botswana. Our objectives were (1) to characterize spatial and temporal variation of soil respiration within each site (2) to examine the relationship between environmental factors and soil respiration within each site (3) and to determine the differences in soil CO₂ efflux among the sites. We hypothesised that the prominent hydrological variability that characterises the Okavango Delta would be the dominant feature that will constrain variation in soil respiration. This would result in a large variation of soil respiration both between and within the three representative ecosystems of this Delta. Although we expect temperature and soil moisture to be the main short-term drivers, the shape of the response to these will vary in a site-specific pattern, and reflect dominant vegetation and vegetation productivity at each site.

MATERIALS AND METHODS

Site description

The Okavango Delta, situated in the Kalahari Desert in north-western Botswana, is one of world's largest inland deltas with an estimated area of 40 000 km² (McCarthy & Ellery, 1994). The area has a semi-arid climate, with a

distinctly seasonal rainfall pattern. Rainfall is irregular both in distribution and in intensity (500 mm a⁻¹ on average) and falls mainly in the summer months, November to April, and is greatly exceeded by potential evapotranspiration (1580 mm a⁻¹, Wilson & Dincer, 1976). Average minimum and maximum temperatures range from 20°C to 37°C in the wet season and from 4°C to 26°C the dry season (June to September). The Delta has an extremely small elevation gradient, the difference between the upper-reaches and the outflow at the south-eastern end is 65 m over a distance of 240 km.

The Okavango River enters the Delta through a narrow corridor known as the Panhandle, where it is an actively meandering, largely single-thread river. It then divides into a number of relatively well defined distributary channels surrounded by permanent swamps. These are characterised by stands of sedges (e.g. *Cyperus papyrus*) and emergent grasses (e.g. *Phragmites* spp.; *Miscanthus junceus*) that are rooted in a layer of peat up to ~4 m thick that contains variable amounts of clay. It is in these permanent swamps that we chose one of our study sites, situated at 19° 00' 71" S, 022° 24' 18" E (Mantlana *et al.*, 2008 a, b).

One of the main characteristics of the Delta is the annual flood, which varies both in extent and timing, and reaches its period of maximum inundation during the climatically dry season (Ellery & McCarthy, 1994). A mix of seasonal floodplains and purely rain-fed islands characterise the distal end of the Delta. A second study location (S 19° 36' 33" E 23° 16' 44") was in a seasonal floodplain located south of Chief's Island, one of the largest dry parts of the Delta. Typically, the vegetation cover across these seasonal floodplains exhibits a marked zonation, defined by a distinct distribution of plant species that occupy the characteristic microhabitats in response to the frequency and length of inundation (Bonyongo *et al.*, 2000). Areas flooded for the longest period are dominated by communities of sedge species, *Cyperus articulatus*, *Schoenoplectus corymbosus* and the wild rice *Oryza longistamata*, while those areas flooded for short durations are dominated by grasses like *Panicum repens* and *Sorghastrum friesii*. Higher-lying areas which do not receive flood waters formed a zone dominated by *Imperata cylindrica* and *Cynodon dactylon* (Mantlana *et al.*, 2008 a, b).

Periodic shifts in water distribution on the distal end of the Delta result from changes in the course of upstream channels (Tooth & McCarthy, 2007). In areas that become deprived of seasonal floodwaters, the water table falls, and precipitation gradually flushes soluble salts from the soils, leaving silica and calcium carbonate (Tooth & McCarthy, 2007). Our third study site was such a rain-fed semi-arid grassland (19° 39' 35" S, 023° 20' 92" E), situated in an area that has not received flooding for several years, possibly for decades. Here a forb, *Pechuel loechea*, co-existed with an annual grass, *Urochloa trichopus*, and perennial grasses, *Cynodon articulata* and *Eragrostis lehmanniana*; while large patches that were more or less free of vegetation were found in the vicinity of termite mounds (Mantlana *et al.*, 2008 a, b). An overview of the three sites with their microhabitats, dominant species and soil types is provided in Table 1.

Soil CO₂ efflux, temperature and moisture measurements

A set of five measurements were conducted between December 2001 and January 2004 at the seasonal floodplain; and three sets of measurements were conducted at the permanent swamp and the rain-fed grassland between March 2002 and March 2003. Soil CO₂ efflux was measured using a LI-6400 with LI-6000-9 soil chamber (LI-COR inc, Lincoln, NE, USA). To avoid surface disturbance during repeated measurements, and to ensure repetition at identical locations, PVC collars with sharpened edges were installed to exactly fit the chamber's outer diameter. The collars were inserted into the soil surface, leaving approximately 2 cm protruding above the soil surface, a day before the first measurement. We used collars of two different lengths (10.7 cm diameter, 15 cm high or 10.7 cm diameter, 30 cm high) depending on presence or absence of flood conditions. In places where the area inside the collar included notable amounts of live vegetation, *e.g.*, at the *Imperata cylindrica* microhabitat (permanent swamp, see below), the soil surface within the collars was kept free by clipping at the soil surface. Across the study area, each site was divided into two or three microhabitats to account for small-scale differences in hydrology and dominant vegetation, and at least four collars were randomly distributed within each microhabitat.

At the permanent swamp, soil CO₂ efflux measurements were made along a 40m long transect that represented three microhabitats. These were dominated by *C. papyrus* closest to the river channel, a grass-covered area that was made up of almost entirely by the perennial and rhizomatous grass *Imperata cylindrica*, while stands of *Miscanthus junceus* grew furthest away from the river channel. A fire had passed through the area in March 2003, but re-sprouting/re-germination of the grass vegetation was already taking place at the time of the measurement 2003 campaign.

Similar to the permanent swamp site, measurements at the seasonal floodplain were located along a hydrological gradient within the site, spaced over a distance of ca. 50 m. Three microhabitats were differentiated. An upper floodplain, where seasonal flood waters do not reach, consisted of a homogenous community of *I. cylindrica*. An intermediate floodplain, subject to intermittent flooding of large year-to-year variations, was dominated by a perennial grass, *P. repens*. The lowest-lying parts, where some-week long inundation occurs more or less annually was dominated by sedge species, *Cyperus articulatus* and *Schoenoplectus corymbosus*. Soils of upper and intermediate floodplains were sandy loams while at the lower floodplain soils had high clay content and, when wet, became blackish in colour.

At the rain-fed grassland, soil CO₂ efflux measurements were made over a distance of approximately 30 metres between two termite mounds. The area in the vicinity of these mounds was bare, while grass species, *Urochloa trichopus*, *Cynodon articulata* and *Eragrostis lehmanniana*, dominated the remainder of the site.

With each respiration measurement, soil temperature (T) was recorded using a soil temperature probe placed next to the collar at 5 cm depth. At the seasonal floodplain, from June 2002, volumetric soil water content (θ) was measured at each spot on a daily basis at 0-5 cm and 5-10 cm soil depth using a battery-powered hand-held soil moisture sensor (Moisture Meter type HH2 with Theta probe, Delta T Devices, Cambridge, UK). This sensor was not available for the earlier campaigns, and soil water content was taken from equivalent micro-sites close to an eddy covariance tower (Wohland, unpublished) or determined gravimetrically close to each soil collar and

converted to volumetric data using soil bulk density. A side-by-side comparison of hand-held probe and gravimetric measurements during March 2003 gave a conversion factor of 0.75 between the two. Gravimetric samples were also available from the measurement campaigns in March and June 2002 at the permanent swamp and rain-fed grassland.

During each field campaign, measurements were made at the three locations within one month. Under optimum environmental conditions, it took approximately one hour to complete a daily cycle of ca. 12 measurements. Environmental conditions that led to very low soil CO₂ efflux, *e.g.*, very dry or flooded soils, resulted in longer duration of each measurement, thus making it impossible to make a large number of measurements per day. Depending on access to the site or other constraints, measurements were generally made between 08h00 and 17h00.

Auxiliary soil information

Soil bulk density was determined in March 2003 by taking soil cores of known volume at 0 - 5 cm, 5 - 10 cm, 10 - 20 cm, 20 - 30 cm and 30 - 50 cm intervals. For each site, three cores were taken approximately 1 m away from of the collars used for soil CO₂ efflux measurements. Soils from the three cores were mixed and sub-samples were removed for determining soil organic carbon and soil nitrogen. Samples taken for soil nutrients were air dried (sandy soils from the rain-fed grassland), or oven dried at 40 °C (soils from the seasonal floodplain and the perennial swamp) before being analysed for carbon and nitrogen (Vario MAX, Elementar Americas, Inc. Mt. Laurel, New Jersey, USA). Soil nutrient data reported here are for the upper 10 cm of the soil (Table 1). Cores for determination of root mass were taken in March 2002 and 2003 following the same procedure. Roots were obtained by elution with water, oven dried (40 °C) and weighed. No attempt was made to separate live and dead roots.

Data analysis

The seasonal variation in soil respiration was analysed by non-linear curve-fitting (SigmaPlot 9.0, Systat Software Inc. 2004) of the observations to a combined, multiplicative soil-temperature (T) and soil moisture (θ) model:

$$F_{CO_2} = f(T) \times f(\theta) \quad (1)$$

with

$$f(T) = R_{25} \exp [E_0^* (1/(T_{ref} - T_0) - 1/(T - T_0))] \quad (1a)$$

and

$$f(\theta) = \exp[-0.5 \cdot (\ln(\theta_{rel}/\theta_1)/b)^2] \quad (1b).$$

Equation (1a) is the Lloyd and Taylor temperature response (Lloyd & Taylor, 1994), where R_{25} is F_{CO_2} at a reference soil temperature at 5cm depth (T_{ref}), taken here as 298.15K (25°C), chosen to reflect the high soil temperatures at the tropical measurement sites. E_0 is a constant (308.56K) and T_0 was set either to the value suggested by Lloyd and Taylor (227.13K) or determined from the fitting procedure. In equation (1b) θ_{rel} is θ relative to its observed average maximum value (θ_{sat} , Table 2). The coefficient θ_1 is equivalent to the value of θ_{rel} when $f(\theta)$ reaches unity while b determines the steepness of the respiration response to low and high soil moisture levels. For fitting, only data with adequate moisture measurements were included.

RESULTS

Variation in environmental factors

Soil T varied most typically around 25-30°C during the rainy season campaigns and 15-20°C at dry season campaigns (Figures 1a, 2a and 3a). Temperatures were highest overall at the rain-fed grassland location where values above 45°C were measured (March 2002). During this period, mean \bar{T} (43.3 °C) was significantly higher (ANOVA, $n = 195$, $df = 192$, $F = 72.59$, $P < 0.001$) than at the permanent swamp (31.6 °C) and at the seasonal floodplain (29.1 °C). During the absence of surface flooding, average maximum 5cm soil

moisture content (θ) was $0.4 \text{ m}^3\text{m}^{-3}$ at the permanent swamp and $0.25 - 0.3 \text{ m}^3\text{m}^{-3}$ at the other sites (Figures 1b, 2b and 3b). The largest seasonal variation in θ was found at the rain-fed grassland, with mean values ranging between close to zero during the dry season and $0.26 \text{ m}^3 \text{ m}^{-3}$ (March 2003; Figure 3b). This site was also the most illustrative for the possibly large inter-annual variability that can be encountered at a given time of the year with soil moisture levels differing by a factor of six at the two measurement campaigns in March 2002 and March 2003. By contrast to the rain-fed grassland site, not surprisingly, soils at the permanent swamp were relatively moist even when water levels dropped below the surface at the end of the rainy season (*i.e.*, March 2002 and March 2003, Figure 2b), before annual floods reached the site.

Comparison made across the different sites showed that the permanent swamp had highest values of soil C and soil N, ranging between 1.3 to 1.6 % and 20 to 23.4 %, respectively, that were at least 16 times higher than those found at the other sites (Table 1). Lowest C and N values were measured at the driest micro-sites (*i.e.*, at the upper floodplain of the seasonal floodplain site and at the rain-fed grassland). C to N ratios, however, were relatively similar at all sites with a tendency to lower values at the rain-fed grassland.

Table 1: Sites, micro-sites, dominant vegetation and soil type of the study area. Data are mean and standard deviation (in brackets) of soil nitrogen (N, g g⁻¹), carbon (C, g g⁻¹) C:N ratio, and root mass (g m⁻²), all determined at 0-10 cm depth, and were measured in March 2002. Letters represent statistical significance ($P < 0.05$) within each micro-site.

Location	Micro-sites	Dominant vegetation	Soil type	N	C	C:N	Root mass
Permanent	<i>Papyrus</i>	<i>Cyperus papyrus</i>	Peat	0.013 (0.0006) ^a	0.20 (0.005) ^a	15.04 (0.55) ^a	0.009 (0.003) ^a
Swamp	<i>Imperata</i>	<i>Imperata cylindrica</i>	Peat	0.016 (0.0009) ^a	0.23 (0.015) ^a	14.73 (0.31) ^a	0.012 (0.002) ^a
	<i>Miscanthus</i>	<i>Miscanthus junceus</i>	Peat	0.015 (0.0015)	0.22 (0.026)	14.63 (0.52) ^a	0.011 (0.003) ^a
Seasonal	upper	<i>Imperata cylindrica</i>	Sandy-loam	0.0004 (0.0001) ^a	0.007 (0.002) ^a	16.19 (0.37) ^a	0.003(0.0003) ^a
Floodplain	intermediate	<i>Panicum repens</i>	Sandy-loam	0.0012 (0.0003) ^b	0.017 (0.005) ^b	14.12 (0.24) ^a	0.008 (0.003) ^{ab}
	lower	<i>Cyperus articulatus</i>	Clay	0.0011 (0.0009) ^b	0.016 (0.016) ^b	13.89 (0.19) ^a	0.011 (0.008) ^b
Rain-fed	Bare-soil	none	Sand	0.0008 (0.00037)	0.093 (0.0048)	11.83 (1.28) ^a	0.003 (0.0008) ^a
Grassland	Grass-covered	<i>Cynodon dactylon</i>	Sand	0.0098 (0.01587)	0.012 (0.0056)	12.18 (0.77) ^a	0.003 (0.0008) ^a
		<i>E. lehmanniana</i>					
		<i>U. thichopus</i>					

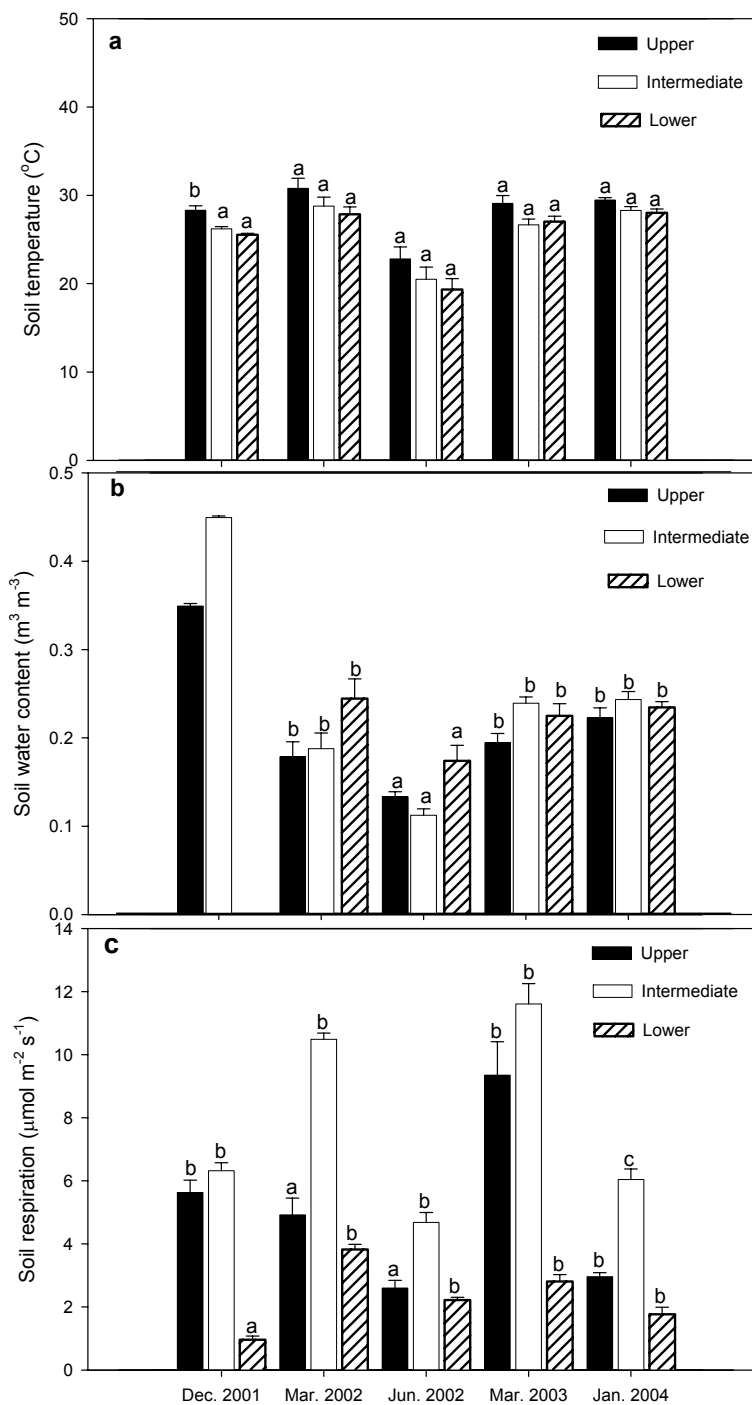
Significant differences (ANOVA, $n = 67$, $df = 65$, $F = 33.692$, $P < 0.001$) were observed in root mass in 0 – 10 cm soil depth, between the sites with highest and lowest values found at the permanent swamp and at the rain-fed grassland, respectively, while the seasonal floodplain had intermediate values. Values in the Table 1 are from the March 2002 campaign and were found to be representative for most of the other measurement campaigns (not shown).

Seasonal variation of soil respiration

Average F_{CO_2} values were typically lowest during the dry season (June), when the measurement location was either flooded (permanent swamp) or when the soils were dry (rain-fed grassland, seasonal floodplain: intermediate and upper (Figures 1c, 2c and 3c). Only at the low lying areas of the seasonal floodplain (lower floodplain) were lowest rates of soil respiration observed in December 2001, under conditions of standing water as during that campaign the annual flood had not fully receded (Figures 1c). Average F_{CO_2} ranged overall from $0.3 \mu\text{mol m}^{-2}\text{s}^{-1}$ (rain-fed grassland, grass-covered microhabitat, June 2002) to $11.6 \mu\text{mol m}^{-2}\text{s}^{-1}$ (seasonal floodplain, intermediate microsite, March 2003).

On average, highest F_{CO_2} were observed in the rainy season campaign in March 2003 at all locations, although the difference compared to measurements taken in March 2002 was small at the permanent swamp (Figure 2c). The measurements during this period coincided with the arrival of pronounced rainfall events of that year's (rather late) wet season, by contrast to March 2002, which was more drier as it was towards the end of the rainy season. During the March 2003 campaign, we observed also very high rates of light saturated photosynthesis (A_{sat} ; Figures 1d, 2d and 3d) and high levels of leaf nutrients (Mantlana *et al.*, 2008a, b), but high A_{sat} and high leaf nutrients were also found during the December 2003 campaign, when F_{CO_2} values were lower. The average maximum F_{CO_2} were similar at the permanent swamp and seasonal floodplain location, 8.9 and $8.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, and approximately three-fold higher than F_{CO_2} at the rain-fed grassland during that same campaign, $2.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 1c, 2c and 3c).

The average value for the latter location includes data measured at the grass-covered microhabitat directly after a heavy rain event (Figures 3c, Figure 6).



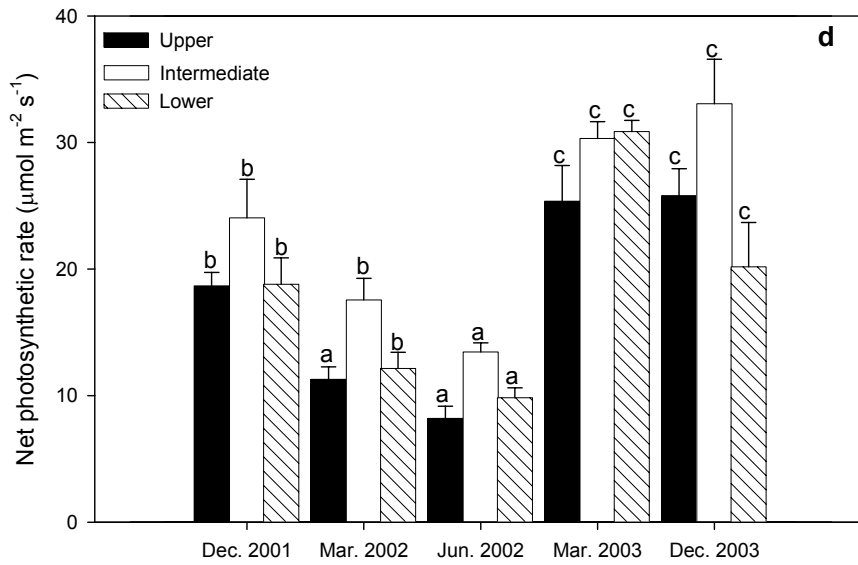
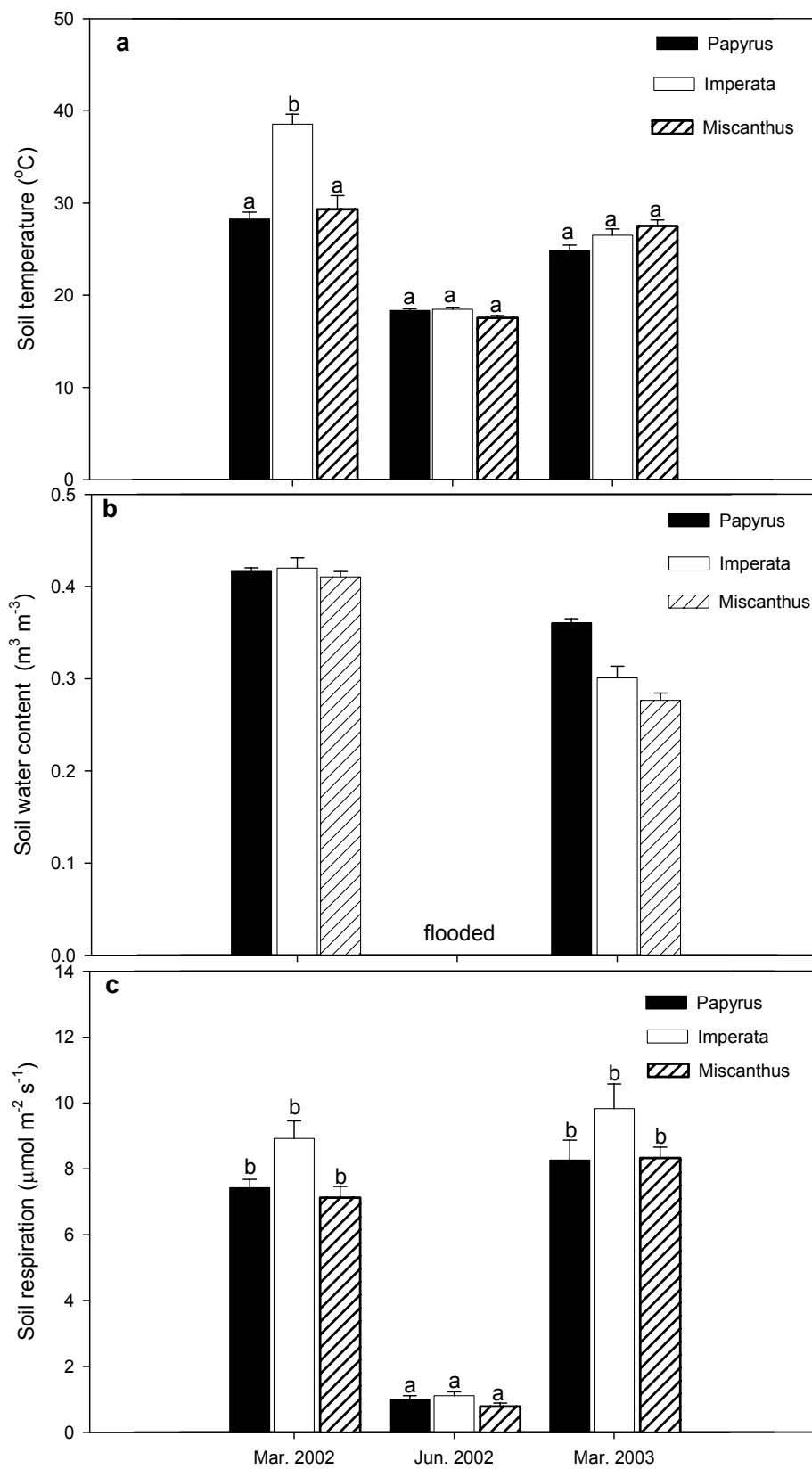


Figure 1: Measured soil temperature at 0 – 5 cm depth (Soil T , °C), soil moisture content at 0 – 5 cm depth (θ m³ m⁻³), soil CO₂ efflux (F_{CO_2} , μmol m⁻² s⁻¹) and net photosynthetic capacity at the seasonal floodplain. Data are averages and (in brackets) standard error for five measurement campaigns and are separated by the three distinct microhabitats at this floodplain that differ in their hydrology and plant cover. Small letters indicate significant difference ($P < 0.05$) within each measurement period. The lower floodplain was flooded during December 2001. Statistical analysis did not include soil moisture data taken in December 2001 since it was obtained from an eddy flux tower, while the other θ data were obtained using a hand-held TDR probe.



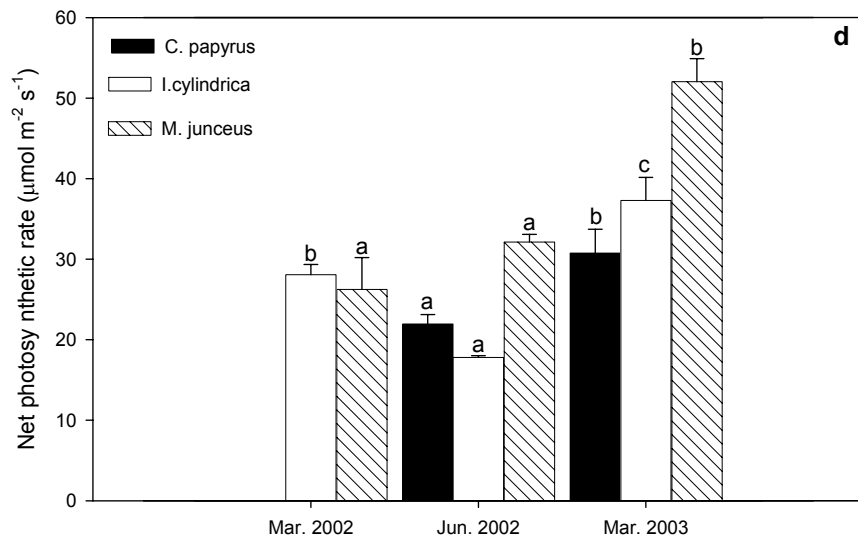
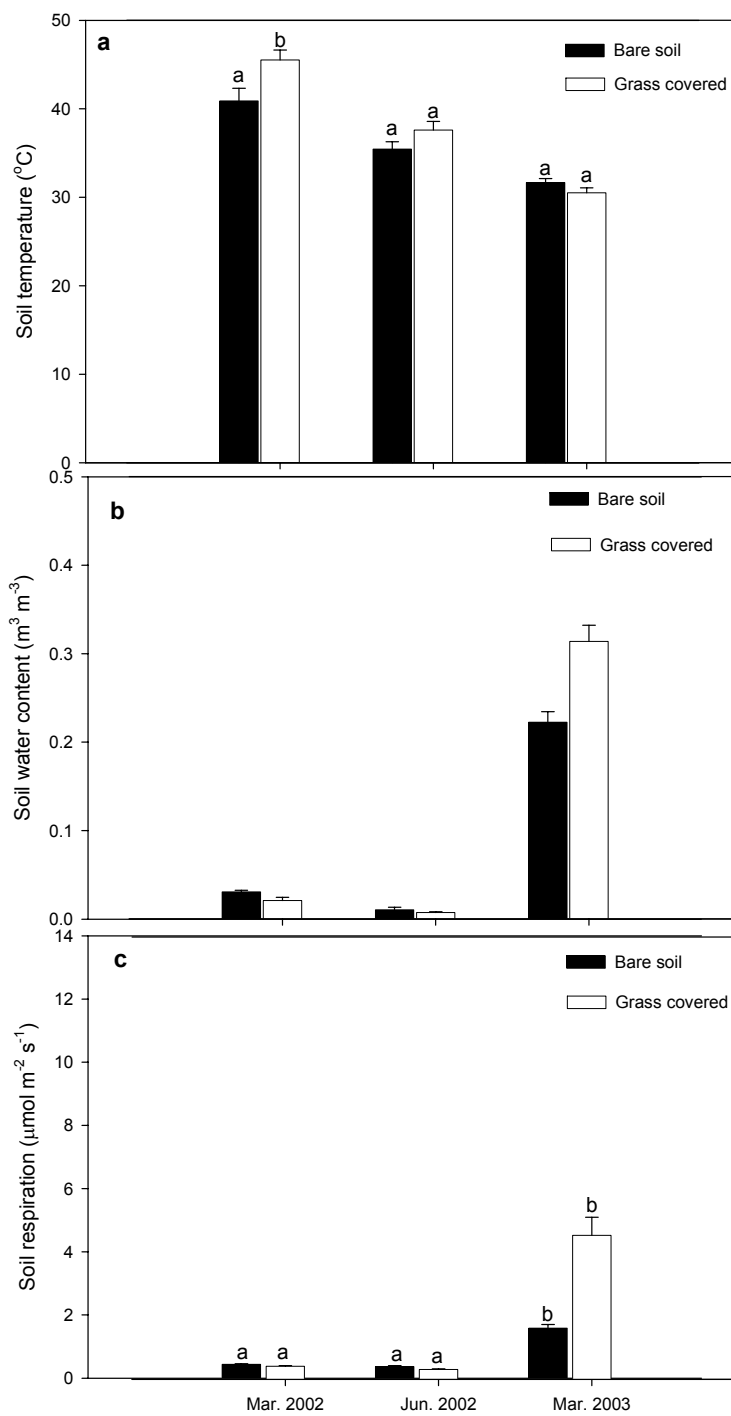


Figure 2: As in Figure 1, at the measurement site located in the permanent swamp area. At this site too, three microhabitats could be distinguished by their dominating vegetation. In March 2002, data for θ were obtained from gravimetric samples, while in March 2003 θ were obtained using a hand-held TDR probe; the site was flooded during the June 2002 campaign.



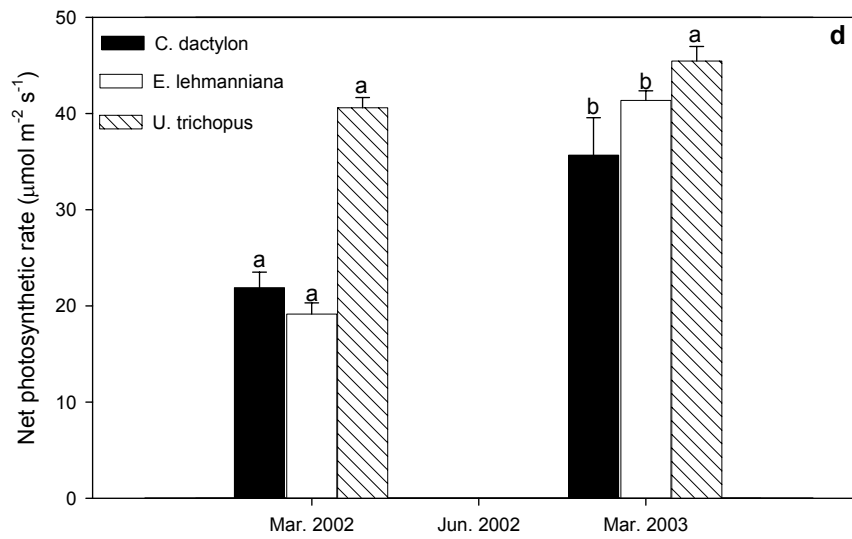


Figure 3: As in Figure 1, at the measurement site located at the rain-fed grassland. At this site, two microhabitats could be distinguished, one covered by a range of C₄ grasses and the other had no vegetation (bare). In March 2002 and June 2002, data for θ were obtained from gravimetric samples, while in March 2003 θ were obtained using a hand-held TDR probe. Gravimetric samples were taken once during each measurement period. Grass vegetation had withered away in June 2002; therefore, there were no net photosynthetic measurements during this period. Statistical analyses were done for θ data collected within one measurement campaign.

Within site variation of F_{CO_2}

Variation between micro-sites at a given location was relatively small compared to the large seasonal variation in average F_{CO_2} , soil temperature and θ that existed between the three sites (Figures 1 - 3), with the exception of the seasonal floodplain (Figure 1). At this site, F_{CO_2} were consistently lowest at the lower floodplain although, when compared to the higher-lying areas of the floodplain, the differences were not always statistically significant (Figure 1c). Generally, T decreased from the upper to the lower floodplain, which also had highest values of θ values for large parts of the year but the variation in F_{CO_2} across the floodplain were larger than those observed in microclimate. The F_{CO_2} pattern at the seasonal floodplain was also reflected to some degree in leaf level A_{sat} (Figure 1), e.g., the *Panicum*-dominated intermediate areas that had highest F_{CO_2} had (with the exception of the March 2003 campaign) also highest rates of photosynthesis. On the other hand, F_{CO_2} values at the lower floodplain were notably more reduced compared to the other two microhabitats than the A_{sat} at this location (Figure 1d).

Neither at the permanent swamp nor at the rain-fed grassland did F_{CO_2} , T , θ or A_{sat} consistently differ between micro-sites. F_{CO_2} at *Imperata* micro-site of the permanent swamp were somewhat higher than at the other two microhabitats, but the difference was not statistically significant. Soil temperatures at that microhabitat were notably warmer only in March 2002 (ANOVA, $n = 102$, $df = 100$, $F = 41.3$, $P < 0.001$; Figure 2a, 5), while A_{sat} was consistently highest at the *Miscanthus*-dominated location. The considerable differences in θ (and F_{CO_2}) at the grass-covered locations of the rain-fed grassland observed in March 2003 were caused by measurements taken after a heavy rainfall that were restricted to the grass-covered micro-sites (Figures 3b, Figure 6).

Environmental regulation of soil CO_2 efflux

As expected, F_{CO_2} was lowest during the dry season when not only soil water content at most locations was minimum but also temperatures relatively cool (June 2002, Figures 1 – 3). Temperature alone, therefore, was a very poor predictor of F_{CO_2} (Figures 4 – 6). This became particularly apparent at

the rain-fed grassland, where T varied between 25 and 45°C without having any visible effect on F_{CO_2} during the March and June 2002 measurements (Figure 6). Soil T also did not visibly affect F_{CO_2} at the lower floodplain micro-site (Figure 4) during the (dry season) June 2002 and (flooded period) December 2001 campaigns. At the permanent swamp microhabitats as well as at the higher-lying areas of the seasonal floodplain F_{CO_2} increased with T , but neither for a given campaign nor when pooling all observations did we find the clear exponential pattern that is so typical for respiration-temperature relationship (Figures 4, and 5).

The simple, multiplicative model (equation 1) that combined effects of temperature and soil moisture on soil respiration could account for a considerable amount of variation at the rain-fed grassland and at the permanent swamp, with r^2 values of 0.55 and above (Table 2). Detailed soil moisture measurements that were taken together with each respiration measurement were not available for all campaigns due to instrumental problems which prevent closer investigation of some of the observed variation. Moreover, physiological activity might be an additional factor to be taken into account (see seasonal floodplain, below): at the permanent swamp, where F_{CO_2} values measured in March 2002 were, at a given T and at each micro-site, visibly lower compared to the March 2003 campaign (Figure 5) A_{sat} were only approximately two-thirds of the rates observed one year later (Figure 2d). At this location the obtained R_{25} were quite similar for the three micro-sites, but notable differences emerged for the shape of the moisture response (e.g., coefficient b). The values of the latter suggest for the *Papyrus* micro-site a steep increase and decrease of F_{CO_2} around a well defined optimum moisture level.

At the rain-fed grassland, the two microhabitats were indistinguishable in terms of the respiration response to T and θ . R_{25} at this site was more than six times lower than at the permanent swamp. The model was not able to reproduce the very high efflux rates measured at the grassland micro-sites directly after a heavy rain event (Figure 6, encircled), indicating that this rain-pulse possibly included a physical component (e.g., replacement of air in soil pores with water

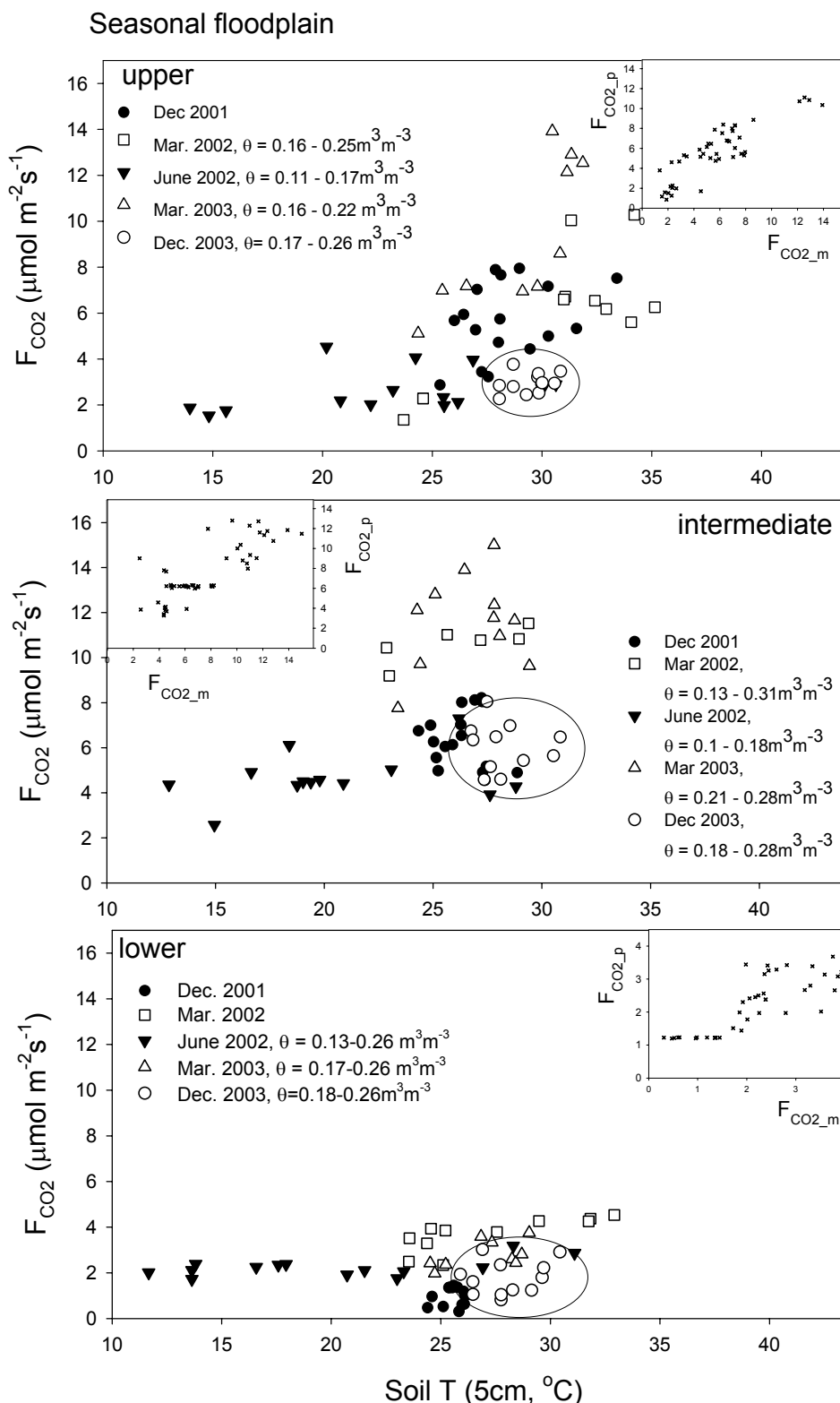


Figure 4: F_{CO_2} at the seasonal floodplain, measured at five campaigns between December 2001 and January 2004. Data are separated by the three distinct microhabitats at this floodplain. The range of 5cm soil moisture (θ) is indicated for campaigns when this information was available at each measurement spot. Insets show agreement between measured ($F_{CO_{2_m}}$) and predicted ($F_{CO_{2_p}}$) F_{CO_2} , in $\mu\text{mol m}^{-2} \text{s}^{-1}$, including effects of leaf nitrogen (see Figure 7). For the fitting, data from Dec. 2003 (encircled) were excluded.

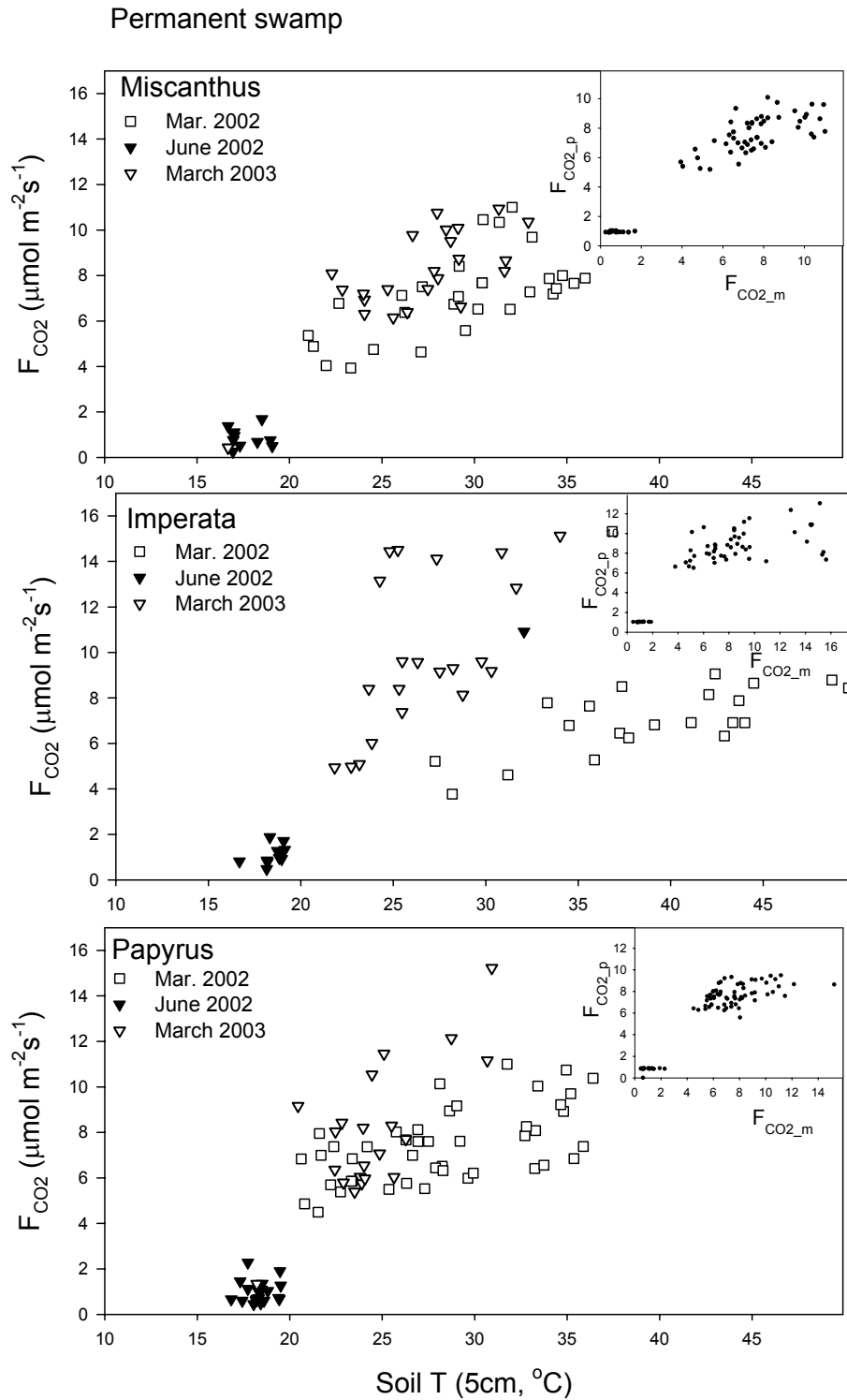


Figure 5: As in Figure 4, at the measurement site located in the permanent swamp area. At this site too, three microhabitats could be distinguished by their dominating vegetation. The site was flooded during July; daily θ data for the campaign in March 2003 were 0.24-0.39 $\text{m}^3 \text{m}^{-3}$ (*Miscanthus* microsite), 0.21-0.46 $\text{m}^3 \text{m}^{-3}$ (*Imperata* micro-site) and 0.33-0.42 $\text{m}^3 \text{m}^{-3}$ (*Papyrus* micro-site; see Figure 2). In March 2002, soil moisture was between 0.3-0.35 $\text{m}^3 \text{m}^{-3}$ and did not vary much between micro-sites.

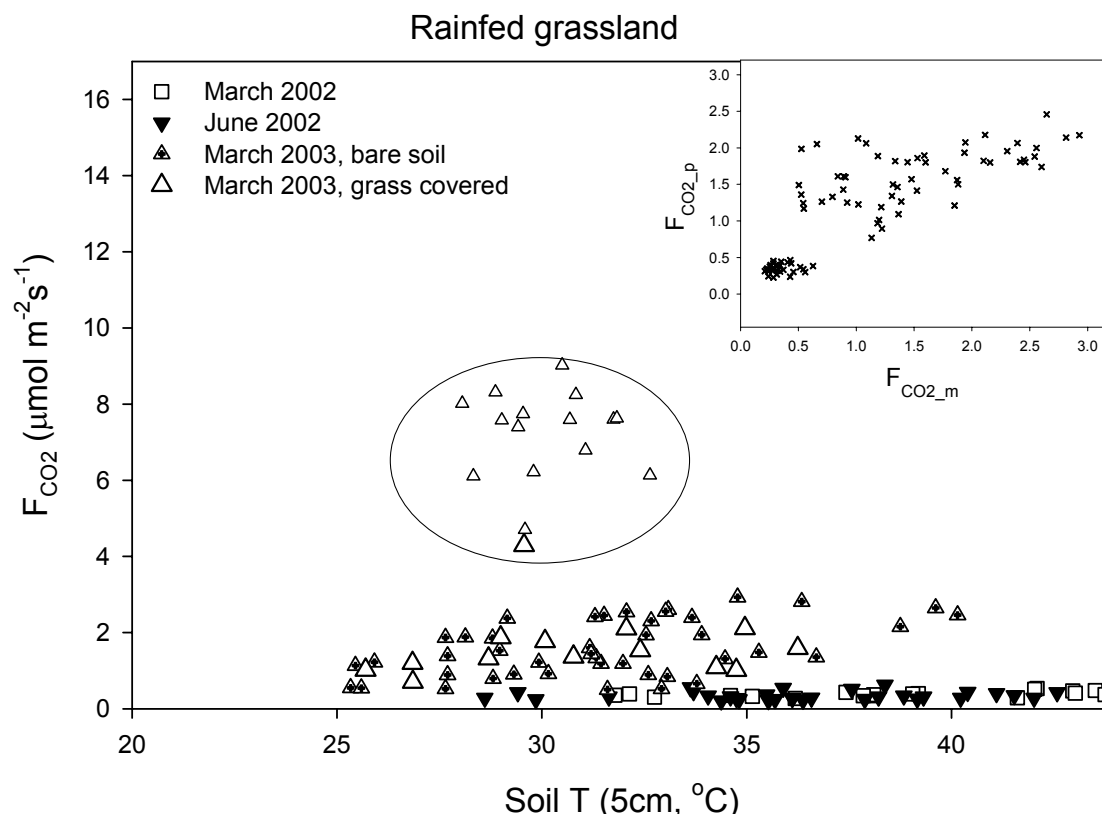


Figure 6: As in Figure 4, the rain-fed grassland site. Measurements were done at grass covered and bare micro-sites. There was, in general, no clear difference in F_{CO_2} at the two microhabitats in terms of the temperature and moisture response. Soil moisture content was below $0.05 \text{ m}^3 \text{ m}^{-3}$ during the dry season campaign in June. Daily soil moisture data during the campaign in March 2003 were generally $0.11\text{--}0.33 \text{ m}^3 \text{ m}^{-3}$ (bare soil micro-site) and $0.12\text{--}0.34 \text{ m}^3 \text{ m}^{-3}$ (grass-covered micro-site) except for F_{CO_2} at the grass-covered micro-site following immediately after a heavy rainfall even ($\theta > 0.34 \text{ m}^3 \text{ m}^{-3}$; encircled). No observations at the bare microhabitat were available for this same period. During March 2002, average soil moisture was $0.03 \text{ m}^3 \text{ m}^{-3}$, obtained once, F_{CO_2} from this campaign and from the March 2003 “rain-pulse” was excluded from fitting equation 1 to the data.

and/or pressure-driven release; Arneth *et al.*, 1998) in combination with a very rapid biological response (Lee *et al.*, 2004).

By contrast to the permanent swamp and the rain-fed grassland, values obtained from the non-linear fitting at the seasonal floodplain differed substantially between the micro-sites (Figure 4). Values of R_{25} reflected the pattern observed for the measured average respiration rates (Figure 1d), being notable higher at the intermediate floodplain when compared to the upper and lower lying areas of the floodplain. Agreement between fitted and

measured F_{CO_2} were poorest at this site, with r^2 ranging from 0.21 to 0.4. In particular, respirations rates during the December 2003 campaign (Figure 4, encircled) were lower than in the previous wet-season campaigns, although the soil temperatures and moisture encountered was not unusual. When excluding this data from the non-linear curve fitting, r^2 improved to 0.47-0.51 (not shown).

A number of studies have demonstrated a link between vegetation physiological activity and growth and soil respiration (Tjölker *et al.*, 2005; Reichstein *et al.*, 2003; Ekblad & Högberg, 2001; Raich & Nadelhoffer, 1989). One possible variable that can be explored in this context is assimilation rate but across the studied ecosystems no clear relationship emerged between average light-saturated photosynthesis of the species dominating a microhabitat, and average F_{CO_2} (Figure 1d, 2d and 3d). However, at the seasonal floodplain site we found F_{CO_2} to increase linearly with leaf nitrogen concentration (Figure 7). At this site there was also a tendency for F_{CO_2} to increase with A_{sat} , but this relationship was much poorer (not shown). What is more, while F_{CO_2} during the December 2003 campaign were much lower at similar or even higher leaf N concentrations compared to previous periods the data still indicate a linear relationship (Figure 7, dashed line), albeit with a much lower slope.

To investigate, whether accounting for leaf nitrogen at the seasonal floodplain increases the amount of variability that can be explained by the soil respiration model we therefore added an additional linear relationship to equation (1) which became:

$$F_{CO_2} = f(T) \times f(\theta) \times f(N_{leaf}) \quad (2),$$

with $f(N_{leaf}) = m N_{leaf}$

When fitting this version of the model to the seasonal floodplain data, omitting observations from December 2003, r^2 between observed and fitted F_{CO_2} increased to between 0.61 and 0.76 (Table 2).

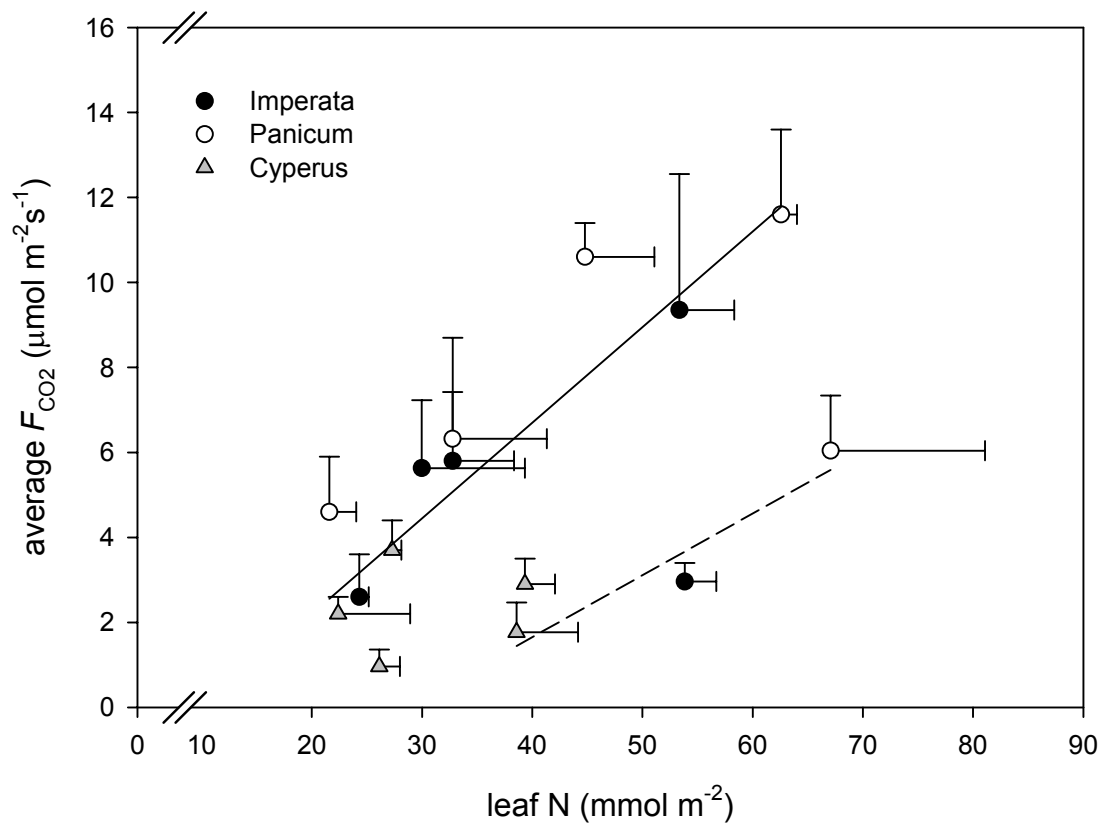


Figure 7: Average F_{CO_2} at the seasonal floodplain vs. leaf nitrogen for each of the five measurement campaigns. The data imply a linear relationship between these two variables with a possibly notably lower slope in the December 2003 (dashed line) campaign compared to the data obtained in the previous four campaigns.

Table 2: Values for R_{25} ($\mu\text{mol m}^{-2}\text{s}^{-1}$; respiration at a 5cm-soil temperature of 25°C), T_0 (K; temperature coefficient at the Lloyd Taylor equation), b (describing steepness of 5cm-soil moisture response) and θ_1 (m^3m^{-3} ; the relative 5cm soil water content when the moisture response reaches unity; $\theta_1 \leq 1$) determined by non-linear curve-fitting of equations 1 and 2 to the measured F_{CO_2} . θ_{sat} is the average measured maximum soil moisture in the absence of surface flooding (0-5cm; m^3m^{-3}). Values were determined when adequate soil moisture measurements were available: for the rain-fed grassland this included two campaigns in June 2002 and March 2003, for the permanent swamp three campaigns in March 2002, June 2002 and March 2003, and at the seasonal floodplain, additional data from December 2003 were available. For the seasonal floodplain, parameter values are also listed after accounting for the observed effect of leaf nitrogen concentration (equation 2), m is the slope of the respiration-leaf nitrogen relationship; these results are given for observations excluding the December 2003 data (Figure 7). At the rain-fed grassland, the goodness of fit did not change when the bare-soil and grass-covered microhabitat were separated. The data immediately after a heavy rain event at the rain-fed grassland (see Figure 6) were excluded for parameter-determination.

	microsite	R_{25}	$R_{25} \text{ m}^{-1}$	T_0	b	θ_1	r^2	θ_{sat}
Seasonal floodplain	Upper	5.3		227	0.58	0.85	0.4	0.25
	intermediate	8.8		148	0.54	0.76	0.21	0.25
	lower	2.7		152	0.99	0.55	0.37	0.3
As above, include leaf N (Dec01-Mar03 only)	Upper		5.8	227	0.56	0.88	0.76	0.25
	intermediate		8.1	148	0.96	0.61	0.65	0.25
	lower		5.8	157	0.71	0.63	0.61	0.3
Rain-fed grassland	bare soil & grass covered	1.2		230	0.91	0.99	0.55	0.3
Permanent swamp	<i>Miscanthus</i>	8.1		208	0.41	0.74	0.87	0.4
	<i>Imperata</i>	10.9		179	0.45	0.62	0.63	0.4
	<i>Papyrus</i>	8.3		193	0.15	0.93	0.76	0.4

Discussion

Seasonal and within-site variation of F_{CO_2}

Large variation in respiration rates, soil temperatures and moisture content were observed on all scales covered in this study: between the three study sites, between micro-sites at a given site, seasonally from one campaign to the next, and inter-annually during campaigns that were

conducted at approximately similar times of the year. Overall, the pattern of rainfall and the flood regime were the dominating constraints. The highly variable F_{CO_2} during the course of the year is characteristic of the semi-arid regions that receive seasonal rainfall as was shown for a Mopane woodland located south of the Okavango Delta (Arneth *et al.*, 2006), tropical savanna in Australia (Chen *et al.*, 2002), in pastures and forests of southwestern Amazonia (Salimon *et al.*, 2004) and in forests and cattle pastures of Eastern Amazonia (Davidson *et al.*, 2000); but it is also a prominent feature of summer-dry temperate or boreal ecosystems (Tang & Baldocchi, 2005; Shibistova *et al.*, 2002). The associated processes are manifold and likely reflect a direct response of microbial activity as well as possible peaks of root respiration resulting from active root growth at the height of the plant growing season.

The rainy season in the Okavango Delta is a period of intense biological activity reflected by maximum leaf CO_2 uptake and a peak in plant growth (Mantlana *et al.*, 2008a). Average wet-season F_{CO_2} at the permanent swamp and at the seasonal floodplain (approximately $8 \mu\text{mol m}^{-2} \text{s}^{-1}$) were at the upper end of previously reported rates in semi-arid tropical regions of Africa, Australia and South-America, that appear to be closer to $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Epron *et al.*, 2004; Salimon *et al.*, 2004; Chen *et al.*, 2002; Fan *et al.*, 1990).

Soil respiration also displayed the additional sharp, pulse-like response immediately after rainfall that is typically observed in dry environments with irregular patterns of precipitation (e.g., Arneth *et al.*, 1998, 2006; Lee *et al.*, 2004), which was seen clearly, for instance, in a tripling of F_{CO_2} observed at the rain-fed grassland immediately after a heavy rain-event. Zepp *et al.*, (1996) found that F_{CO_2} following artificial addition of water persisted 2 to 3 days after watering in a dry African savanna woodland soils. These pulses of CO_2 efflux may be the combination of a physical flushing of the soil pores as CO_2 -rich air is replaced by water and a fast response of microbial activity that is possible from a persistent pool of enzymes capable of tolerating extended periods of desiccation (Borken *et al.*, 2003; Gallardo & Schlesinger 1992).

Minima in F_{CO_2} were observed at the permanent swamp in June 2002, and at the seasonal floodplain (lower floodplain) in December 2001, as a result of the seasonal flood that is characteristic of the Okavango Delta. High

soil water content limits oxygen diffusion through the soil porosity and therefore microbial activity (Xu & Qi, 2001; Davidson *et al.*, 1998). At the other locations, lowest F_{CO_2} occurred during the dry season when microbial activity was reduced owing to low soil moisture content combined with dormancy or death of the aboveground biomass and decreased root activity. Results from a study in Brazilian savannas showed considerable seasonal changes in leaf area index between wet season (1.00 ± 0.06) and dry season (0.42 ± 0.15 ; Miranda *et al.*, 1997). At the Okavango Delta sites, pronounced changes in leaf area index between the dry and wet seasons (Wohland, unpublished) and decline in photosynthetic capacity (Mantlana *et al.*, 2008a, b) make it plausible that seasonal variation of root activity that may have contributed to the decline of F_{CO_2} . This asks for further investigation of the dynamics and phenology of roots among the dominant grass and sedge species in this savanna-wetland mosaic which remain largely unknown.

Modelling soil CO₂ efflux

Numerous field and laboratory studies have pointed to the interacting limitation by soil temperature and moisture on soil CO₂ efflux in dry environments (e.g., Conant *et al.*, 2004; Huxman *et al.*, 2004, and references therein). We hypothesised that a maximum-type model would be most suitable for our locations, rather than a linear or hyperbolic response (e.g., Reichstein *et al.*, 2003; Arneth *et al.*, 1998; Norman *et al.*, 1992) since we encountered the full range of very dry to very wet and flooded conditions. Agreement between measured and modelled F_{CO_2} was variable, the model performed overall well at the rain-fed grassland and swamp sites, but less so at the seasonal floodplain.

Field observations of soil respiration are known to be spatially highly heterogeneous and model analyses are therefore typically more robust when a large range of an environmental variable is encountered. The relatively small temperature range at this tropical location, typically only around 10 degrees during the rainy season, thus further confounds the model analysis. This small temperature range may be one reason for the improved goodness of fit at many of the measurement locations when T_0 was allowed to vary, with fitted values being lower compared to using the fixed value (227.13K) in Lloyd

& Taylor (1994), but it should be also noted that the temperature sensitivity of soil respiration declines under water-limiting conditions (Reichstein *et al.*, 2002).

F_{CO_2} is regarded as a good indicator of total below-ground allocation of carbon, photosynthetic activity or ecosystem productivity (Reichstein *et al.*, 2003; Ekblad & Högberg, 2001; Raich & Nadelhoffer, 1989). While there was no significant relationship between F_{CO_2} at the seasonal floodplain and A_{sat} , we found it to vary with the level of leaf nitrogen of the dominating vegetation, albeit with a possibly very different slope between one rainy season and the next. From our data we cannot specify whether the observed positive response of F_{CO_2} with leaf nitrogen indicates stimulation of microbial activity fostered by litter quality, assuming that higher nitrogen concentration levels in living leaves corresponds to a relatively larger soil N input via leaf litter, or a direct response to vegetation physiological activity, or both.

Respiration of roots, specifically maintenance respiration is known to be linked to tissue nitrogen concentration, and furthermore, measured rates of root respiration in a number of grass species has been found to be higher in fast-growing species (Scheurwater *et al.*, 1998), although the difference to slow growing species may be relatively small (Ryan *et al.*, 1997). But also new photo-assimilates can be available for soil autotrophic and heterotrophic respiration already within few days (Ekblad & Högberg, 2001). Although for the seasonal floodplain the relationship between A_{sat} and F_{CO_2} was much weaker than the one with leaf N, we nonetheless found the typical increase of A_{sat} with foliar nitrogen at this floodplain and across all ecosystems included in this study (Mantlana *et al.*, 2008b).

Owing to the limited data we cannot comment in depth whether the notably lower slope linking F_{CO_2} to foliar nitrogen concentration observed in the December 2003 campaign is a typical inter-annual feature of the floodplain ecosystems. Possibly, the lower rates of soil CO_2 efflux in December 2003 at the seasonal floodplain may be affected by the timing of arrival of the wet season rainfall. The rainy season 2003/2004 had commenced late, with the first rain observed in Maun in mid-December 2003. Our observations might therefore reflect a lag between leaf physiological activity and soil CO_2 efflux

with above-ground plant growth and photosynthesis responding more rapidly to the onset of the rains.

Tjölker *et al.* (2005) identified a positive response of fine root respiration and nitrogen in fine root tissue across a number of grassland and savannah species that was valid for a larger regional flora. Moreover, they identified a number of analogue trait relationships that were valid for leaves and roots. Without data on microbial or root activity and nutrients we may speculate that in the absence of surface flood, the permanent swamp displayed higher rates of F_{CO_2} , compared to the seasonal floodplain, because this site is dominated by plants that have high photosynthetic capacity (Mantlana *et al.*, 2008b) and larger root biomass. Under non-flooded conditions, root respiration and microbial decomposition of dead roots and the production of plant detritus would be expected to be high at the permanent swamp and this would result in increased respiration.

Summary & Conclusion

We have presented soil T , θ , A_{sat} , and F_{CO_2} for representative ecosystems of a tropical savanna-wetland mosaic that has a strong seasonal climate which is associated with a seasonal flood pulse. To our knowledge there has been no other study that has determined seasonal and inter-annual variation of F_{CO_2} at different ecosystems of the Okavango Delta. Lowest fluxes were observed during the dry season and also during the flooded periods whereas maximum fluxes were found during the wet season. The rain-fed grassland had lowest F_{CO_2} compared to the seasonal floodplain and the permanent swamp. F_{CO_2} values were considerably reduced by flooding and were strongly enhanced by a rainfall event. At a given location and/or between campaigns no clear relationship was found between average A_{sat} and average F_{CO_2} across the three study areas. However, the observed increase with leaf nitrogen levels demonstrate the influence of physiological activity and litter quality. This highlights the need for more research to understand how much additional variance can be explained by biotic factors including microbial biomass. These results can be useful when designing field experiments for establishing soil or ecosystem carbon balance or to elucidate relationships between soil carbon balance and species diversity. Furthermore, the

correlations found in this study may contribute to more mechanistic models of F_{CO_2} .

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CHAPTER 7

General Discussion: Controls of gas exchange and soil respiration in the representative ecosystems of the Okavango Delta, Botswana.

INTRODUCTION

Increasingly more information is becoming available on determinants of gas exchange in African savanna ecosystems as researchers undertake to quantify the fluxes of greenhouse gases in these ecosystems. However, uncertainties still remain on a number of aspects related to the general patterns that determine savanna ecosystem function. This study focused on understanding leaf-level gas exchange patterns of dominant C₄ species in representative ecosystems of the Okavango Delta, the seasonal variation of gas exchange characteristics of monotonous stands of woody C₃ species and the seasonal and inter-annual variation of soil CO₂ efflux in representative ecosystems of the Okavango Delta. The study focused on representative ecosystems and representative species because of the enormity of the task of assessing individual ecosystems and species responses, especially in natural ecosystems. The objective of this chapter is to highlight some of the important findings of this study and also to explore general patterns that were found and put these in context with already existing information on the gas exchange patterns in semi-arid tropical ecosystems.

Seasonal patterns of leaf-level gas exchange and soil CO₂ efflux in the Okavango Delta.

The principal factors influencing the gas exchange of this savanna-wetland ecosystem include pronounced seasonality of precipitation, frequent fires, low soil fertility, high temperatures and low humidities. In addition, the low relative humidity and high daytime temperatures impose a consistently high evaporative demand during the prolonged dry season (Mantlana *et al.*, 2008a). During the dry season, water in upper soil layers is severely depleted as evidenced by the dieback of grasses (Mantlana *et al.*, 2008a), while deep-rooted

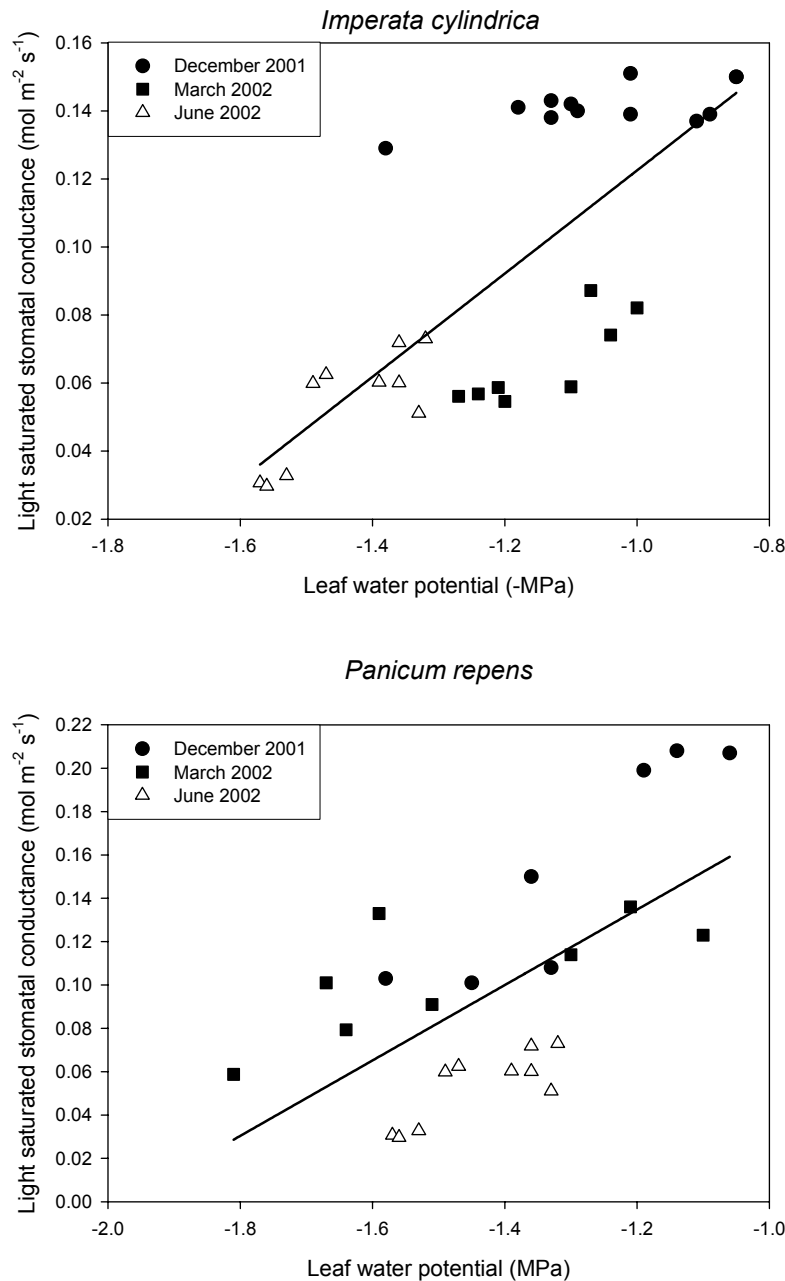


Figure 1: Relationship between light saturated stomatal conductance and morning leaf water potential of *I. cylindrica* and *P. repens*. Different symbols indicate measurements taken at different seasons (December 2001 – June 2001). Each dot represents an individual measurement. The fitted lines are linear regressions, for *I. cylindrica*, $y = 0.1517 + 0.2743x$, $r^2 = 0.56$ and for *P. repens*, $y = 0.1585 + 0.3167x$; $r^2 = 0.39$.

trees are able to supply enough water to maintain transpiration and CO_2 assimilation longer into the dry season (Veenendaal *al.*, 2008).

There was the typical seasonal decline of light saturated net photosynthetic rate (A_{sat}), light saturated stomatal conductance (g_{sat})

(Mantlana *et al.*, 2008a) and morning leaf water potential (Ψ_m) (Figure 1) among the dominant species in the seasonal floodplain and also at the mopane woodland (Veenendaal *et al.*, 2008). This observation is consistent with previous studies in seasonally dry environments (Franco *et al.*, 2005; Tezara *et al.*, 1998; Eamus & Cole, 1997; Sobrado, 1986; Le Roux & Mordelet, 1995) and it is a function of declining soil water content and increasing leaf-to-air vapour pressure deficit (D_l ; Eamus & Prior, 2001).

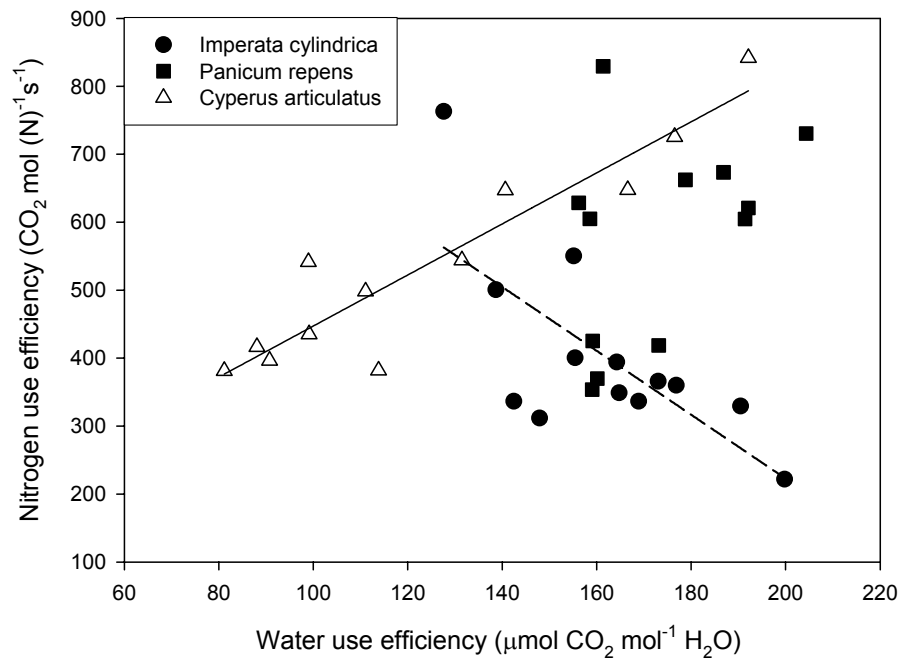


Figure 2: Relationship between intrinsic water use efficiency and nitrogen use efficiency in the dominant C_4 species at the seasonal floodplain. Different symbols indicate species. Data pooled from December 2001 to June 2002. Each dot represents an individual measurement. Dotted line is the linear regression for *I. cylindrica* ($y = -4.710 + 1164.2x$, $r^2 = 0.56$) and the solid line is the linear regression for *C. articulatus* ($y = 3.76 + 71.55x$, $r^2 = 0.87$). This relationship very poor correlations for *P. repens* (not shown for clarity).

Similarly, soil CO_2 efflux displayed strong seasonal variation in all the three ecosystems that were studied, with largest seasonal variation found at the semi-arid grassland (Chapter 6). The remarkable seasonal difference in soil CO_2 efflux at this site could be explained by a rainfall event. Flooded soil conditions at the permanent swamp and also at the low-lying area of the seasonal floodplain led to low soil CO_2 efflux; while dry soil conditions were

responsible for the lowest values of soil CO₂ efflux at the rain-fed grassland and also at the upper-lying areas of seasonal floodplain (Chapter 6). Despite a similar seasonal pattern in maximum and minimum leaf-level photosynthesis and soil CO₂ efflux, particularly at the seasonal floodplain, no significant relationship was found between light saturated net photosynthetic rate (A_{sat}) and soil CO₂ efflux (Chapter 6).

Another important finding was the effect of the timing of the rainfall on the soil CO₂ fluxes and also on leaf-level photosynthesis. The first rains at the beginning of the wet season stimulated soil CO₂ efflux proportionally more than A_{sat} at the rain-fed grassland; while there was proportionally more increase of A_{sat} than soil CO₂ efflux at the seasonal floodplain (Chapter 6). This pattern at the seasonal floodplain was further indicated by a significant correlation between leaf nitrogen and soil CO₂ efflux (Chapter 6). At this site, the dry season resulted in a more pronounced decline of soil CO₂ efflux than A_{sat} . These findings stress the sensitivity of the ecosystems of the Okavango Delta to inter-annual variability in rainfall patterns, not only as a result of natural year-to-year variability but also as a consequence of global climate change.

Soils of savanna ecosystems are characterized by high bulk density and low porosity due to compaction caused by trampling of the herbivores. The stronger seasonal reduction of Ψ_m observed in *I. cylindrica* (Figure 1) and in the short form mopane (Veenendaal *et al.*, 2008) should play an important role in increasing water uptake ability from the soil to the leaf (Ishida *et al.*, 1992). On the other hand, the low g_{sat} , and the resultant low transpiration values, which is largely unaffected by Ψ_m , as found in *P. repens*, could be a water saving strategy for tolerating the progressive seasonal decline of soil water content. A similar strategy has been reported for a C₄ grass, *Digitaria adscendens* (Kobayashi & Hori, 2000).

Despite a common pattern of an exponential decline in g_{sat} with increasing D_i , species at the seasonal floodplain ((Mantlana *et al.*, 2008a) and also those grown under controlled conditions (Mantlana *et al.*, 2008c) differed in their stomatal sensitivity; with strongest stomatal sensitivity to D_i found in species typical found in wetter habitats. High sensitivity of stomatal sensitivity to D_i indicates poor adaptation to seasonally dry environments (Mantlana *et*

al., 2008c) as carbon assimilation of such species would be substantially affected by the increasing seasonal decline of soil water content, which is essential to support their water spending strategy.

Results of this study also showed more conservative water use strategy in the tall form of mopane than in the short mopane form as the dry season intensified (Veenendaal *et al.*, 2008). Similarly results from the seasonal floodplain showed higher intrinsic water use efficiency (WUE_i ; A_{sat}/g_{sat}) among the grass species dominating the higher-lying drier microhabitats than the sedge species at the low-lying microhabitat (Mantlana *et al.*, 2008a). A study along a precipitation gradient in Southern Africa reported that the magnitudes of WUE at a given vapour pressure deficit were dissimilar for the individual sites and were found to be stratified according to the position of the sites along the long-term aridity gradient (Scanlon & Anderson, 2004).

There were contrasting relationships between WUE_i (A_{sat}/g_{sat}) and nitrogen use efficiency (NUE ; $A_{sat}/\text{leaf } N$) along the hydrological gradient between the wet and dry season (Figure 2). The positive linear correlation between NUE and WUE_i found at the wettest microhabitat was attributable to the larger relative decline of g_{sat} than A_{sat} as the dry season progressed. Such positive correlation between NUE and WUE_i indicates that NUE may have components that were poorly explained by variation in either leaf nitrogen or C_i/C_a ratio (Field *et al.*, 1983). Consistent with this hypothesis, our results showed the smallest seasonal variation of leaf N in the species at the floodplain and an increase in C_i/C_a ratio during the dry season (Mantlana *et al.*, 2008a). Meanwhile, the negative relationship found at the upper floodplain indicates that this species has adapted to relatively dry conditions by conserving water, through lowering g_{sat} , resulting in high WUE_i , at the cost of low returns on leaf N , which results in low NUE .

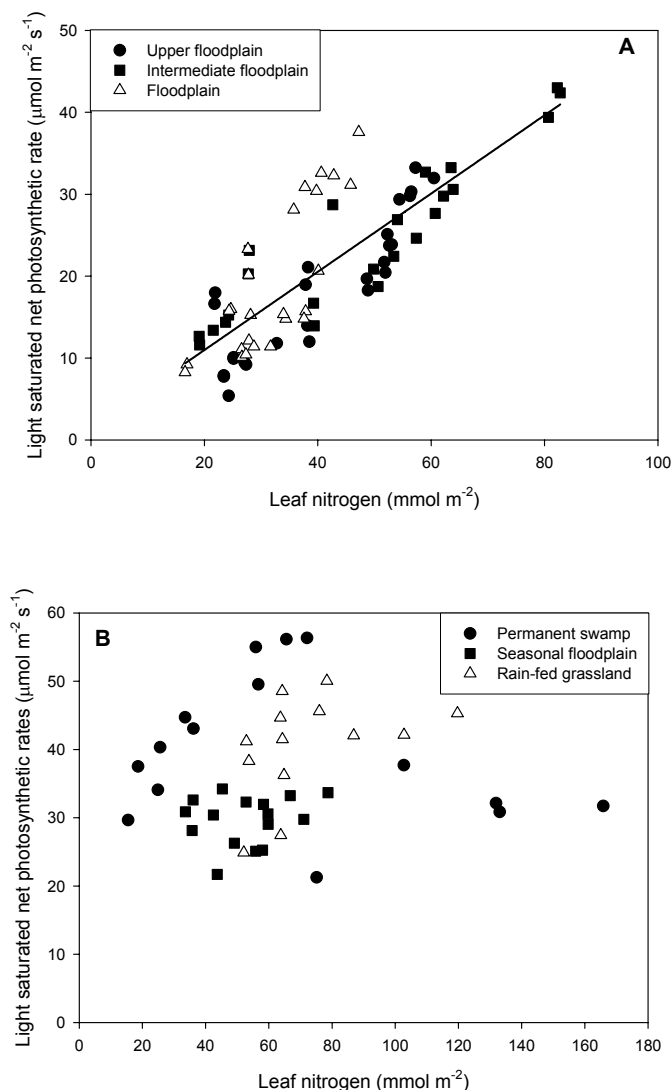


Figure 3: Relationship between leaf nitrogen and light saturated net photosynthetic rate of the species at the seasonal floodplain (A) and those from three different ecosystems (B). At the seasonal floodplain, measurements were taken at different seasons (December 2001 – June 2002), while at the three different ecosystems measurements were taken during the wet season (March 2003). The fitted line in (A) is a linear regression for the pooled data set ($y = 0.4778 + 1.4188x$; $r^2 = 0.68$). Each dot represents an individual measurement.

The role of leaf nitrogen (leaf *N*) in regulating net photosynthesis (A) of C₄ species

Photosynthesis is strongly affected by nitrogen availability (Field & Mooney, 1986). This reflects a combination of the larger proportional investment of nitrogen in photosynthetic machinery (Evans, 1989) and the widespread nitrogen limitation in natural ecosystems (Vitousek & Howarth, 1991). Leaf *N* content provides a simple and crude estimate of nutrient

availability in an ecosystem, and numerous studies have shown that the photosynthetic capacity of tropical grass and tree species under greenhouse and natural conditions is linearly correlated with total leaf *N* content (Simioni *et al.*, 2004; Anten *et al.*, 1998; Le Roux & Mordelet, 1995; Baruch *et al.*, 1985). Similarly to these studies, results taken at different seasons at the seasonal floodplain showed a strong significant linear relationship between A_{sat} and leaf *N* (Figure 3A).

The results obtained during a wet season measurement period and from the three different ecosystems showed that the A_{sat} vs leaf *N* relationship can vary substantially among co-existing species that have different life forms (perennial grasses vs annual grass) and growth forms (grasses vs sedges) (Figure 3B). These results are comparable to the findings of Evans (1989), using C_3 species, where he pointed out that when both *A* and leaf *N* content are expressed on a unit leaf area basis, this relationship shows considerable variation among different species and life forms adapted to different environments.

Previous studies have suggested an increase in nitrogen limitation toward the wettest extreme of the hydrological gradient (Paruelo *et al.*, 1999; Burke *et al.*, 1997). In addition, African swamps have been shown to be nitrogen-limited (Mladenov *et al.*, 2005; McCarthy & Ellery, 1994; Gaudet & Muthuri, 1981). Results from this study showed remarkably high A_{sat} by the perennial grasses at the permanent swamp despite low leaf *N* (Figure 3B), thus indicating considerably high *NUE*. Similarly, using C_4 species in a garden experiment, Oyarzabal *et al.*, (2008) found highest *NUE* values at the wettest extreme of a hydrological gradient. The considerably high *NUE* found in the perennial grasses at the permanent swamp would play an important role in increasing *N* investment into roots and development of additional leaf area (Sage & Pearcy, 1987).

The relationship between carboxylation efficiency and leaf *N* followed a similar pattern to that of A_{sat} vs leaf *N*, with strong linear relationship at the seasonal floodplain where data was obtained at different seasons; while no relationship was found on data collected from three different ecosystems and during one wet season (Figure 4). Taken together, results from this study indicate that generalizations regarding effect of leaf *N* on photosynthetic

capacity in C_4 plants cannot be made without long-term field studies that will cover different seasonal and inter-annual variations.

The role of leaf phosphorus (leaf P) in regulating net photosynthesis (A) of C_4 species

Previous studies on the effects of leaf P in C_4 species have concentrated on the maize species, and have reported that growth and photosynthesis of maize are greatly dependent on P nutrition (Khamis *et al.* 1990; Jacob & Lawlor, 1992; Usuda & Shimogawara, 1991). Under optimum growth conditions, results of this study showed higher carboxylation efficiency and lower relative mesophyll limitation of photosynthetic rate in species that had higher leaf P (at the rain-fed grassland) than in species with lower leaf P levels (at the seasonal floodplain; Mantlana *et al.*, 2008b). Similar observations have been reported for sunflower (*Helianthus annuus* L. cv Asmer) and maize (*Zea mays* L. cv Eta) plants that were grown under controlled environmental conditions Jacob & Lawlor (1992).

The characteristic shape of the $A-C_i$ response curve is due to the presence of a CO_2 concentrating mechanism in C_4 leaves. Results of this study suggest that lower leaf P content reduces the efficiency of the CO_2 concentrating mechanism in C_4 leaves. Similarly, using C_4 tropical grasses, Ghannoum *et al.*, (2008) concluded that leaf P deficiency interferes with efficient operation of the CO_2 concentrating mechanism in C_4 leaves, possibly by limiting the supply of inorganic phosphate to the C_4 cycle enzymes.

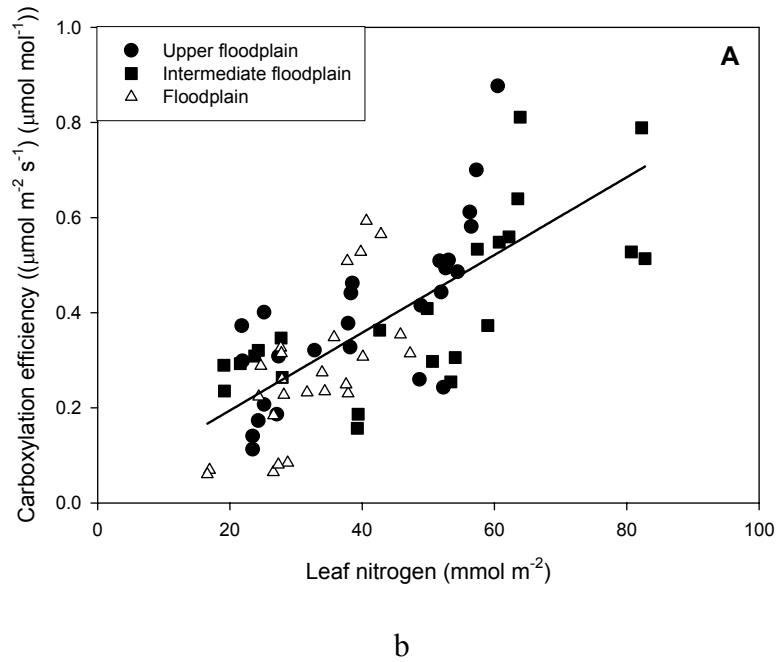


Figure 4: Relationship between leaf nitrogen and carboxylation efficiency (A) at the seasonal floodplain, data shown for all five measurement periods (December 2001 – December 2003); and (B) at the three different ecosystems during the wet season in March 2003. Each dot represents an individual measurement. The fitted line in (A) is a linear regression for the pooled data set ($y = 0.082 + 0.031x$; $r^2 = 0.53$).

Reduced carboxylation efficiency may reduce ecosystem productivity since low carboxylation efficiency also results in low A_{sat} values (von Caemmerer, 2000).

The generality of the relationship between leaf P and A_{sat} together with that of leaf P and carboxylation efficiency across different ecosystems (Figure 5) suggests that leaf P limit photosynthetic capacity more than leaf N in this savanna-wetland ecosystem (Mantlana *et al.*, 2008b). Using seven woody (C_3) species from humid rain forest in Cameroon, Meir *et al.*, (2008) found that leaf gas exchange at this site was more limited by leaf P than leaf N . Although not determined in this study, recent evidence implies that it is possible that in addition to the benefits of high WUE and NUE , the adoption of the C_4 pathway provides advantages over the C_3 pathway in coping with low P supplies (Ghannoum *et al.*, 2008; Ghannoum & Conroy, 2007).

Structural and functional plant traits of grasses

Plant eco-physiological traits related to leaf morphology, nitrogen, phosphorus, carbon content and *WUE* should respond to variation in resource availability (Wright *et al.*, 2004; Oyarzabal *et al.*, 2008). Previous descriptions of graminoid traits made along a precipitation gradient were done by Coughenour *et al.*, (1984, 1985) for a small group of species and recently by Oyarzabal *et al.*, (2008) in a common garden experiment. As a result, we scarcely know how grass traits vary along broad environmental gradients, and specifically, we are frequently not able to distinguish between the effects of resource supply and the effects of other specific site factors (Oyarzabal *et al.*, 2008). The additional challenge is to minimize site effects when describing plant traits from different areas.

This study compared leaf physiological traits at three different sites during the wet season, and the results have showed that independent of being expressed on an area or dry weight basis, phosphorus emerges as a better predictor of A_{sat} than nitrogen (Mantlana *et al.*, 2008b). This pattern was found not just within a given species, but also across species within a given site and between sites.

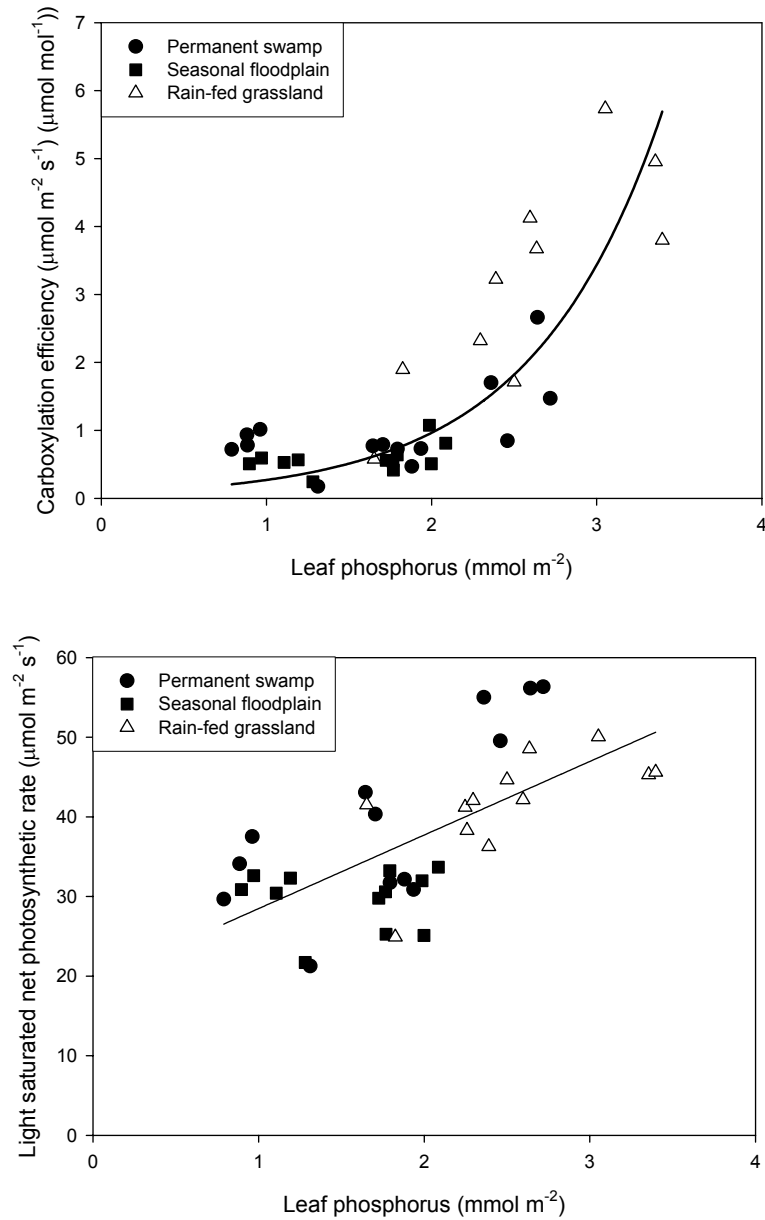


Figure 5: Relationship between leaf phosphorus and carboxylation efficiency at the seasonal floodplain (A), data are for March 2003 and December 2003; and at the three different ecosystems during the wet season in March 2003 (B). The fitted line in (A) is a linear regression for the pooled data set ($y = 250.31 + 22.825x$; $r^2 = 0.35$) and in (B) it is an exponential curve ($y = 0.1915e^{0.098x}$; $r^2 = 0.74$). Each dot represents an individual measurement.

Similarly, phosphorus proved to be a markedly better predictor of *SLA* than did nitrogen (Mantlana *et al.*, 2008b).

The observed leaf N vs. SLA relationships showed the pattern typically observed in relation to leaf economy (Wright *et al.*, 2004): long lived leaves tend to have low N_{mass} at low SLA , a pattern that was also observed for the perennial species in our study (Mantlana *et al.*, 2008b). The opposite was found for short-lived leaves as highest SLA and highest foliar N concentration were observed in the annual grass, *Urochloa*, at the rain-fed grassland.

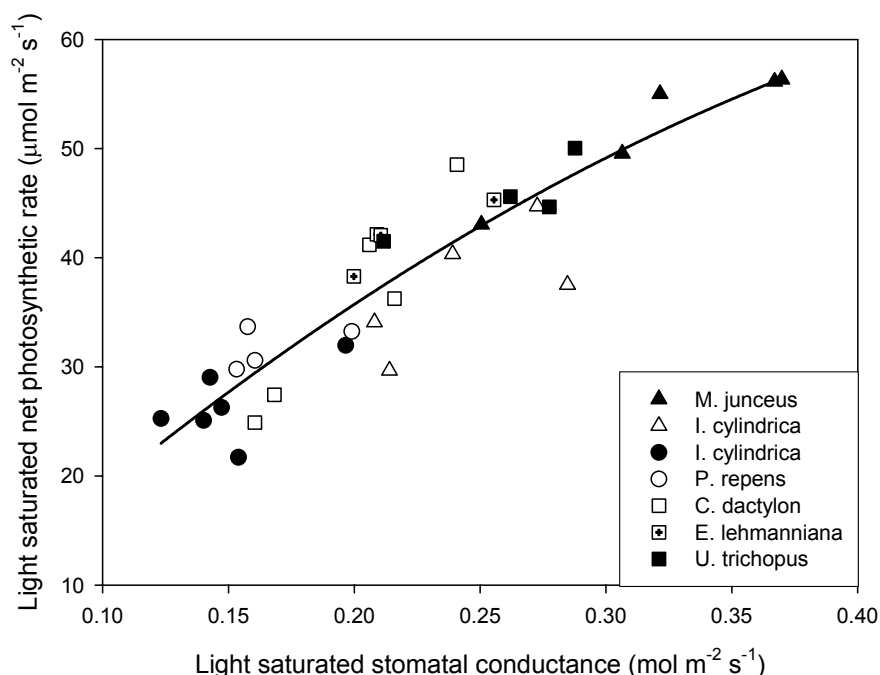


Figure 6: Relationship between light saturated stomatal conductance and light saturated net photosynthetic rate of the grass species in three different ecosystems during the wet season (March 2003). The fitted line is a non-linear regression ($y = 0.02233 e^{-0.0178x}$; $r^2 = 0.84$).

Convergence of relationships in processes related to water and carbon economy in C_4 grass species.

At the leaf level, general relationships among fundamental leaf traits such as A_{sat} vs leaf N (Field *et al.* 1983), and A_{sat} and leaf life-span (Chabot & Hicks 1982) have been known for some time. Consistent with these examples of general relationships in the regulation of carbon economy at the leaf level, this study displayed that A_{sat} and g_{sat} co-vary in a similar manner among seven C_4 grass species that are dominant at three different ecosystems (Figure 6).

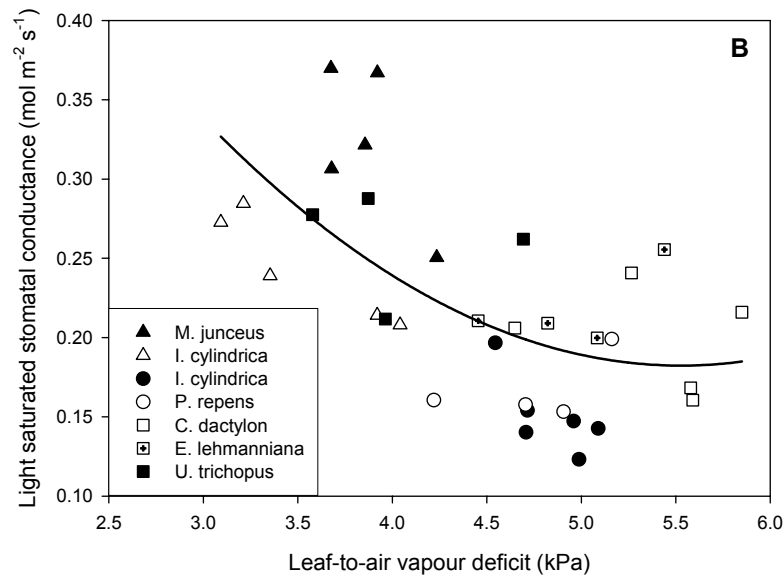


Figure 7: Relationship between light saturated stomatal conductance and morning leaf water potential (A) and with leaf-to-air vapour pressure deficit (B) during the wet season (March 2003) of the grass species from three different ecosystems. The fitted lines are (A) linear regression ($y = 0.0864 + 0.332x$; $r^2 = 0.59$) and non-linear regression ($y = 0.269 e^{-0.0244x}$, $r^2 = 0.4$).

Moreover, this study also showed strong co-variation of A_{sat} and g_{sat} three dominant species across different seasons (data not shown, but see Mantlana *et al.*, 2008a). Similarly, Meinzer (2003) reported convergence in the regulation of carbon economy for nine C_4 species which represented all of three known C_4 metabolic subtypes. Figure 7 further suggests that there is considerable convergence of relationships in processes related to water and carbon economy in C_4 grass species. These results point to highly conserved coupling between gas exchange characteristics at leaf-level and suggest that C_4 species may share common response mechanisms to multiple environmental conditions. However, because the work on which these relationships are based has been carried out in few co-occurring species, the degree to which they are universal remains uncertain. In addition, the existence of widespread functional convergence should not be taken to imply that species do not matter.

Value of this study

The overall objective of this study was to understand the processes that determine the functioning of key components of the carbon cycle in representative ecosystems of the Okavango Delta; with a goal of improving our knowledge on how the Okavango delta carbon cycle functions. Therefore, this effort can be seen as a response to the increasing need to understand the contribution of the major African ecosystems to global carbon fluxes, and also to develop a clearer understanding of the spatial and temporal variations of these fluxes in the representative ecosystems of the Okavango Delta.

This study makes a direct contribution to the advances in our understanding of grassland ecology and eco-physiology; and also contributes to the advances in our understanding of the integrated functioning of C₄ plants in diverse environments. At the same time, descriptions and analyses of structural and functional plant traits of savanna C₄ species reported in this study provide a quantitative description to develop mechanistic hypotheses of processes operating at ecosystem level (e.g. limitation by phosphorus vs limitation by nitrogen). In addition, data from this study may be used to improve the calibration of simulation models of tropical grassland ecosystem functioning and could also be used to recommend a sampling protocol to improve capture of variation in leaf-level photosynthesis.

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Seasonal and inter-annual variations of leaf-level photosynthesis and soil respiration in the representative ecosystems of the Okavango Delta, Botswana.

SUMMARY

Seasonal and inter-annual leaf-level photosynthesis and soil respiration measurements were conducted in representative ecosystems of the Okavango Delta, Botswana, that differ in their long-term soil water content: the permanent swamp, the seasonal floodplain, the rainfed grassland and the mopane woodland. In addition, an experiment was conducted using four C₄ grasses that were collected from the Okavango Delta and grown under optimum soil water content conditions.

Maximum light saturated net photosynthetic rates (A_{sat}) and light saturated stomatal conductance (g_{sat}) were found during the wet season and were comparable to those that have been previously reported for C₃ and C₄ species in the field and in controlled conditions. These parameters co-varied proportionally during both the dry and wet season at the seasonal floodplain and also at the mopane woodland. A_{sat} and g_{sat} showed small seasonal variation at the permanent swamp, contrasting pronounced seasonal variation of A_{sat} and g_{sat}

found at the seasonal floodplain, the rain-fed grassland and the mopane woodland. Similarly, pre-dawn and morning leaf water potential at the mopane woodland and the seasonal floodplain decreased as the dry season progressed.

Results from the seasonal floodplain suggest that the grass species, at the higher-lying microhabitats, are better adapted to the seasonally dry conditions that typically occur in this region than the sedge species, at the low-lying microhabitat. This was indicated by the higher water use efficiency of the grass species compared to that of the sedge species. In addition, relatively high photosynthetic rates were found in the grass species at the intermediate floodplain even during the dry season, while the sedge species showed the strongest seasonal decline of A_{sat} as the dry season progressed.

Leaf-to-air vapour pressure (D_l) strongly influenced gas exchange of all the species that were studied as indicated by the significant relationships between D_l and g_{sat} , with species dominating wetter habitats displaying highest stomatal sensitivity to D_l . Results from the seasonal floodplain indicated that low stomatal sensitivity to D_l may lengthen the period of CO_2 assimilation well into the dry season. Similar to A_{sat} and g_{sat} , leaf nitrogen at the mopane woodland and that of the grass species at the seasonal floodplain was highest in the wet season just after the onset of the annual rains and declined progressively towards the dry season, reaching their lowest values during the dry season. This pattern was in contrast with a much lower seasonal variation in leaf nitrogen for the sedge species at the seasonal floodplain. In addition, the strong positive relationship between leaf nitrogen and A_{sat} found among these grass species did not occur in the sedge species. These results indicate that at the seasonal floodplain, seasonal changes of soil water content, D_l and leaf nitrogen exert different effects on the gas exchange characteristics of the dominant species found along the hydrological gradient.

During the wet season, on average, highest A_{sat} and CO_2 saturated photosynthetic rates values were found at the permanent swamp and also at the rain-fed grassland. The A_{sat} among the grasses at the permanent swamp was associated with high nitrogen use efficiency, while the sedge species at this site, similar to sedge species in the wettest microhabitat at the seasonal floodplain, had the highest ambient to intercellular CO_2 concentration (C_i/C_a) ratio. High A_{sat} values at the rain-fed grassland were associated with high leaf nitrogen, high leaf phosphorus, lower C_i/C_a ratio and highest carboxylation efficiency. At this site, these parameters were significantly higher in the annual grass species when compared to the coexisting annual grass species. The benefits of these characteristics at the rain-fed grassland are interpreted as part of a rapid growth strategy during the relatively brief period of water availability.

Within each species and across the sites during the wet season, leaf phosphorus

Summary

(whether expressed on a dry mass or a leaf area basis) appeared to exert stronger influence on leaf-level photosynthesis than leaf nitrogen. Moreover, leaf phosphorus appeared to correlate somewhat better with specific leaf area (SLA) than do leaf nitrogen. These results suggest that phosphorus availability, as opposed to nitrogen, may exert the more critical control on the functioning of herbaceous vegetation in this wetland-savanna mosaic.

Soil CO₂ fluxes at all the sites showed pronounced seasonality with highest and lowest values observed during the wet season and the dry season, respectively. On average, highest and lowest soil CO₂ fluxes were found at the permanent swamp and rain-fed grassland, respectively. The permanent swamp also had considerably high soil carbon values. Lowest values of soil CO₂ fluxes at the permanent swamp and at the low-lying microhabitat of the seasonal floodplain were a result of the surface flood, while low soil moisture content led to low soil CO₂ fluxes at the rain-fed grassland and also at the upper lying microhabitats of the seasonal floodplain.

Soil temperature alone was poorly related to soil respiration, while a model combining soil water content and soil temperature provided strong correlations with soil respiration. Results of this study showed that CO₂ fluxes (both soil CO₂ fluxes and leaf-level photosynthesis) are highly sensitive to the timing of the rainfall. With the onset of wet season, soil CO₂ fluxes increased remarkably at the rain-fed grassland; while leaf-level photosynthesis at the seasonal floodplain was more strongly stimulated than soil CO₂ fluxes. Taken together, the results of this study contribute toward understanding the role of environmental factors (soil water content, soil temperature, leaf nutrients) in controlling ecosystem functioning of tropical grassland and savanna ecosystems.

Seasonal and inter-annual variations of leaf-level photosynthesis and soil respiration in the representative ecosystems of the Okavango Delta, Botswana.

SAMENVATTING

In dit proefschrift is onderzoek gedaan naar seizoensgebonden en jaarlijkse variatie in fotosynthese en bodem respiratie. Metingen zijn gedaan in representatieve ecosystemen in de Okavango Delta in Botswana. Deze ecosystemen kunnen worden onderverdeeld in permanent moeras, seizoensgebonden overstromingsvlakten, door regen gevoed grasland en Mopane bossavanne. Ze verschillen in hun waterhuishouding. Daarnaast is er een experiment uitgevoerd met vier C_4 grassen onder optimale bodemwater condities.

Maximale lichtverzadigde fotosynthese snelheden (A_{sat}) en lichtverzadigde stomatale geleiding (g_{sat}) werden waargenomen tijdens het regenseizoen. De waarden waren vergelijkbaar met eerder gepubliceerde gegevens voor C_3 and C_4 soorten in het veld en onder experimenteel gereguleerde omstandigheden. De twee paramaters varieerden sterk en op dezelfde manier in het droge en natte seizoen in de overstromingsvlaktes en de Mopane bossavanne. In tegenstelling tot deze systemen was er veel minder seizoensvariatie van A_{sat} en g_{sat} in het permanente moeras. Net als bij A_{sat} en g_{sat} , nam, naarmate het droge seizoen vorderde, ook de blad waterpotentiaal, gemeten vlak voor of tijdens zonsopgang af in de overstromingsvlaktes en de mopane bossavanne.

Uit metingen in de seizoensgebonden overstromingsvlakten blijkt dat grassoorten die zich in hoger gelegen microhabitats bevinden, beter aangepast zijn aan normaal voorkomende droge omstandigheden dan de zeggesoort in de lager gelegen microhabitat. Dit blijkt in het bijzonder uit de verhoogde efficiëntie van het watergebruik in grassen in verhouding tot de zegge. Daarnaast werden in de middengedeeltes van de overstromingsvlaktes naar verhouding hogere waarden voor A_{sat} gemeten, zelfs gedurende het droge seizoen. De zegge vertoonde juist een relatief

sterke daling van A_{sat} naarmate het droge seizoen vorderde. Het tekort op de verzadigde waterdampspanning tussen blad en lucht (D_l) had een sterke invloed op de uitwisseling van gassen tussen blad en buitenlucht voor alle soorten in deze studie. Dit blijkt uit de gevonden significante relaties tussen D_l and g_{sat} . Soorten in de nattere habitats vertoonden de sterkste stomatale response ten opzichte van D_l . Resultaten in de seizoensgebonden overstromingsvlakten geven aan dat een lage gevoeligheid van de stomata voor D_l van de daar groeiende plantensoorten, de periode waarin CO_2 assimilatie kan plaatsvinden tot ver in het droge seizoen verlengen.

Net zoals voor de seizoensvariatie in A_{sat} en g_{sat} , was ook het gehalte in bladstikstof in de Mopane bossavanne en in de grassoorten in de seizoensgebonden overstromingsvlakten het hoogst in het begin van het regenseizoen, vlak na de eerste regens. Het bladstikstofgehalte nam steeds verder af naarmate het droge seizoen dichterbij kwam en de laagste waarden werden gemeten gedurende het droge seizoen. Dit patroon contrasteerde sterk met de veel lagere seizoensvariatie in het bladstikstof in de zegge soort in de seizoensgebonden overstromingsvlakten. Ook werd er in deze soort een veel minder sterke relatie tussen bladstikstof en A_{sat} gevonden in vergelijking tot de grassoorten. De resultaten laten zien dat in de seizoensgebonden overstromingsvlakten, de seizoensgebonden variatie in de hoeveelheid beschikbaar bodemwater, D_l en bladstikstof, de gasuitwisseling van de verschillende dominante plantensoorten langs de hydrologische gradiënt op een verschillende manier beïnvloeden.

In het permanente moeras en in het door regen gevoede grasland werden de hoogste waarden voor A_{sat} en CO_2 -verzadigde fotosynthese waargenomen tijdens het regenseizoen. De hoogste waarden van A_{sat} in het permanente moeras werden gevonden in grassen met een hoge efficiëntie van het stikstof gebruik. De zeggesoort in het permanente moeras vertoonde net als de zeggesoort in het natste microhabitat van de seizoensgebonden overstromingsvlakte de hoogste waarde voor de verhouding tussen de concentratie in de buitenlucht en de intercellulaire CO_2 concentratie (C_i/C_a).

Hoge waarden voor A_{sat} werden in het regen gevoede grasland waargenomen in combinatie met hoge bladstikstof- en bladfosforwaarden, een lagere Ci/Ca ratio en een hoge carboxylatie-efficiëntie. In dit habitat vertoonde het eenjarige gras voor de eerder genoemde parameters significant hogere waarden dan de meerjarige grassen. Het voordeel van deze eigenschappen voor het eenjarige gras in het door regen gevoede grasland worden geïnterpreteerd als onderdeel van een snelle groei strategie gedurende de relatief korte periode van water beschikbaarheid.

Voor elke soort en ook op alle plekken werd gedurende het natte seizoen de trend waargenomen, dat het bladfosfor gehalte, of het nu berekend is op basis van drooggewicht of op basis van bladoppervlakte, een sterkere invloed heeft op fotosynthese op blad niveau dan het bladstikstof gehalte. Bovendien was er een wat sterkere correlatie tussen het gehalte aan bladfosfor en de specifieke bladoppervlakte (SLA) dan met het bladstikstof gehalte. De resultaten suggereren dat de beschikbaarheid van fosfor nog meer controle uitoefent over het functioneren van de kruidlaag in het mozaïek van natte en droge gebieden in de delta dan bladstikstof.

Fluxen van CO_2 uit de bodem lieten een duidelijke seizoensgebonden variatie zien, waarbij de hoogste waarden werden waargenomen tijdens het regenseizoen en de laagste waarden tijdens het droge seizoen. De gemiddeld hoogste waarden voor bodemfluxen van CO_2 werden gevonden in het permanente moeras en de gemiddeld laagste waarden in het door regen gevoede grasland. In het permanente moeras werden ook hoge waarden gevonden voor bodemkoolstof. De laagste waarden voor CO_2 bodemfluxen in het permanente moeras en de laag gelegen delen van de seizoensgebonden overstromingsvlakten werden gevonden tijdens periodes van overstroming terwijl een lage bodemvochtigheid de oorzaak was voor lage waarden in het door regen gevoede grasland en de hoger gelegen delen van de seizoensgebonden overstromingsvlakten. Bodemtemperatuur op zich was maar matig gecorreleerd met bodemrespiratie. Een model, dat bodemtemperatuur en bodemvocht combineert liet juist een sterke correlatie zien met bodemrespiratie

De resultaten in dit proefschrift laten zien dat CO₂ fluxen (zowel van de bodem als van fotosynthese op blad niveau) sterk gevoelig zijn voor regenval patronen. Met het begin van het natte seizoen stegen bodem CO₂ fluxen opmerkelijk in het door regen gevoede grasland, terwijl in de seizoensgebonden overstromingsvlakte de fotosynthese juist sterker werd gestimuleerd dan de CO₂ bodemfluxen.

De resultaten in dit proefschrift zijn van waarde voor het ontwikkelen van een beter begrip van de milieufactoren (e.g. bodem water gehalte, bodemtemperatuur, blad nutriëntengehalten) die controle uitoefenen op het functioneren van ecosystemen van tropische graslanden en savannes.

Ke a Leboga, Thank You, Danke, Bedankt!

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CURRICULUM VITAE

Khanyisa Brian Mantlana was born on 20th September 1976 in Lady Frere, Eastern Cape, South Africa. After attending a number of primary and secondary schools, he went to Cathcart High School where he completed his Matric in 1994. He completed his Bachelor of Science (B. Sc.) degree in 1998 and B. Sc Honours in 1999 at the University of Durban-Westville. He completed his Masters of Science (M. Sc.) in 2002 at the University of Natal (Durban campus). Field work for the M. Sc thesis was done in Maun, Botswana, in collaboration with and funded by the University of Botswana and the university of Natal. During this period, the author was based in Maun, at the Harry Oppenheimer Okavango Research Centre (HOORC). In 2001 he received a scholarship from the Max Planck Institute for Biogeochemistry, in Jena, Germany; and was based there from 2002 to 2004 while continuing to work in the collaborative research programme with the HOORC. In 2002 he registered as a PhD student with the Nature Conservation and Plant Ecology Group, Wageningen University, and lived in Wageningen from July 2004 to May 2005, funded by Wageningen University. Since June 2005 he has been working in Cape Town at the South African National Biodiversity Institute in the Climate Change and Bio-adaptation Division.

PUBLICATIONS

- Mantlana KB**, A. Arneth; E. M. Veenendaal; P. Wohland; P. Wolski; O. Kolle; M. Wagner and J. Lloyd. Inter-site and species specific differences in photosynthetic properties of C₄ plants growing in an African savanna/wetland mosaic. Accepted, *Journal of Experimental Botany*.
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Propositions

1. The extent and timing of the flood pulse in the Okavango Delta plays an important role in regulating soil-plant-atmosphere carbon dioxide fluxes.

this thesis

2. Phosphorus is a major factor limiting leaf-level gas exchange of C₄ species in the Okavango Delta.

this thesis

3. The insight that contrasting forest types have a different influence on climate only serves to underline the importance of leaf level physiological research under field conditions.

G.B. Bonan (2008) Science, 320: 1444-1449.

4.. The post 2012 Climate Change regime under the UNFCCC should not include legally binding national emissions reduction targets for developing countries with already low per capita energy consumption.

5. Cleverly packaged media messages are often more important in communicating the message of climate change, than well researched scientific publications.

Al Gore, An Inconvenient Truth.

6. Modeling results can be inspiring but should afterwards be subjected to the rigor of reality.

W.J. Bond, & G.F. Midgley (2000) Global Change Biology 6:865-869; J.A. Van Vegten (1983) Vegetatio 56: 3-7.

7. The observation that in cars the rear-view mirror is clearer than the windshield should serve as a constant lesson and reminder for experimentalists.

8. The global banking crisis suggests that a cattle post can be a better investment than a savings account.

Propositions accompanying the PhD thesis “Seasonal and inter-annual variations of leaf-level photosynthesis and soil respiration in the representative ecosystems of the Okavango Delta, Botswana” by Khanyisa Brian Mantlana to be defended on November 21th, 2008.