Spatial variation in functional traits and grazing value of savanna grasses at the community and species level

by

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I, Arend Jacobus de Beer declare that the thesis/dissertation, which I hereby submit for the degree Master’s in Plant Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Dissertation summary

Compositional and functional plant community characteristics can strongly govern some ecosystem processes. These community characteristics, may, in turn, be related to multiple environmental variables, including edaphic, climatic, and topographic conditions. However, there is still an incomplete understanding of how environmental conditions affect plant community composition and functioning and, therefore, how species composition and trait expression potentially link habitat conditions to ecosystem processes. This is particularly true for grasses, despite this taxon having considerable ecological and economic importance. The grass family (Poaceae) is a species-rich taxon, with close to 10 000 species occurring worldwide, and is chiefly responsible for providing the key ecosystem service of grazing provisioning since grass species make up the bulk of the herbaceous plant growth in grasslands and savannas. The grazing quality of grasses is assumed to vary strongly between species, with intra-specific variation in grazing quality often ignored, resulting in grass species often uniformly being categorized as being of high or low grazing quality. There is, however, an increasing debate about the validity of this approach since many grass characteristics (including plant functional traits; PFTs) vary intra-specifically along environmental gradients. Therefore, this study examined the relationship between 19 environmental factors and community composition and cover, community-weighted mean (CWM) trait values, intra-specific trait variation and the grazing quality in two C4-dominated savanna grass assemblages.

This study demonstrated that the relationships between grazing quality and PFTs and environmental variables are typically weak and highly idiosyncratic at both the community- and species-level. Cover and soil (particularly soil nutrients) variables were most consistently influential environmental variables. Grazing quality (i.e. nutritional value) differed significantly between grass species, with large proportions of the variation in all grazing quality components being explained by the identity of species. However, within species, few environmental variables explained intra-specific variation in grazing quality. Additionally, plant functional traits were also weakly related to intra-specific variation in grazing quality. Therefore, these findings suggest that grass grazing quality and PFTs do not respond consistently to environmental variables frequently quantified in ecological studies and that, contrary to results from C3-dominated temperate grasslands, leaf dry matter content, specific leaf area and force to tear are not useful proxies of grazing quality. More broadly, this study highlights that there may be considerable intra-specific variation in grass grazing quality, but that this within-species variation is not related to commonly recorded environmental conditions or easily measured plant traits, and, therefore, remains challenging to predict.

Chapter 1: General introduction

The grass family (Poaceae) is a species-rich taxon with members of this family growing worldwide (Moffett, 1997) and close to 10 000 species being documented globally and c. 1 000 species in southern Africa (Fish et al., 2015). All modern grass species evolved from a common ancestor in the upper Cretaceous period between 50 – 80 million years ago (Crepet and Feldman, 1991; Bennetzen, 2007). Grasses evolved with grazing mammals and therefore developed a strong mutualistic relationship with grazers (Owen and Wiegert, 1981) and grasses are now an important component of all terrestrial grazing systems (McGranahan and Yurkonis, 2018). All species of grass grow herbaceously, except for bamboo (Van Oudtshoorn, 1999; Fish et al., 2015), and only a handful of species have been identified as possessing phenolic compounds for deterring herbivores (Coughenour, 1985). Grasses all share the same basic growth form, with similar morphological structures (Van Oudtshoorn, 1999; Fish et al., 2015). Furthermore, grasses have been shown to have very little genetic difference between species (Bennetzen, 2007). However, variation exists both between species (i.e. inter-specifically) and between individuals of the same species (i.e. intra-specifically). For example, grass species display differences in inflorescence shapes (Van Oudtshoorn, 1999; Fish et al., 2015), plant height (Sandel and Dangremond, 2012) and functional traits such as specific leaf area (SLA, leaf area per dry leaf mass ; Sandel et al., 2021). Grass species can have different growth statures such as short creeping- or tall tufted species and at the same time grasses can display considerable variation in their grazing value (Van Oudtshoorn, 1999; Hempson et al., 2015). Equally, different individuals of the same species can display variation even under similar conditions (e.g. *Digitaria. eriantha* can exhibit two different growth forms, clumped and stoloniferous within the same site; personal observation, 2019 - 2020).

Plant functional traits (PFT's; Pérez-Harguindeguy et al., 2013) are measurable attributes an individual plant possesses which can undergo change and, in turn, affect the fitness of the individual (Violle et al., 2007), however, these traits can vary greatly not only between species but also within species (Albert et al., 2011). Plant functional traits provide insights into plant life-history strategies (Sandel and Low, 2019), and into variation in growth, survival, and reproduction (Weiher et al., 1999). At broader scales, plant functional traits can also act as proxies for ecosystem services (Zirbel et al., 2017), such as primary production (Pérez-Harguindeguy et al., 2013) and carbon and nitrogen cycling (Garnier et al., 2004). Plant functional traits are affected by environmental conditions and provide an easily monitored indication of the response of plants to changes in environmental conditions (Violle et al., 2007; Pérez-Harguindeguy et al., 2013). The trait values a species or individual possesses reflects the functional needs for survival and persistence under local environmental conditions (Díaz and Cabido, 1997). For example, low specific leaf area(SLA) in grasses globally is correlated to high temperatures

(Roybal and Butterfield, 2018), since plants with low SLA generally have thicker cell walls which allow for survival in extreme temperatures (Lambers et al., 2008). Additionally, at a more local scale, SLA can be positively correlated to grazing intensity, with high SLA grasses being more palatable (Moreno García et al., 2014). However, the drivers and extent of inter-and intra-specific variation in PFTs are poorly understood for grasses (Roybal and Butterfield, 2019) and especially in southern Africa (Roybal and Butterfield, 2018).

Functional traits can vary on two levels: between different species (inter-specific variation; BTV) or between individuals of the same species (intraspecific variation; ITV). Functional traits typically vary more strongly inter-specifically, with, for example, up to 75% of the variation in plant functional traits being contributed by inter-specific trait variation at the community level (Siefert et al., 2015). In theory, environmental conditions exert selective pressures on species and filter out those with unsuitable trait values (Keddy, 1992). Consequently, environmental conditions limit the range of values of each trait present (i.e. BTV in the community; Lavorel and Garnier, 2002). Since functional traits are generally quite different between species, the influence of environmental factors driving within-species trait variation (i.e. ITV) is typically overwhelmed by differences between species (i.e. species turnover; Pescador et al., 2015).

At the same time, functional traits also vary between individuals of the same species (Andrade et al., 2014), with ITV contributing substantially towards trait variation observed within plant species (25-32% at the community level; Siefert et al., 2015; Roybal and Butterfield, 2018). Intra-specific variation represents the plasticity and genetic variation in individuals of a single species, with phenotypic plasticity impacting the ability of these individuals to respond to local environmental conditions through non-genetic changes in their morphology and physiology (Wellstein et al., 2013). Intra-specific variation, therefore, allows for the survival and reproduction of a species under different environmental conditions (Byars et al., 2007). Hence, increased ITV potentially allows for optimum species performance, under a greater range of environmental conditions, and as a result supporting a well-functioning ecosystem (Albert et al., 2010). The inclusion of ITV allows for better predictions regarding the environmental drivers of trait variation at the community level (e.g. response of communities to extreme drought; Jung et al., 2014), and could, for example, influence predictions of food web dynamics (Bolnick et al., 2011). By using community-level trait values, our understanding of how much each species contributes to the community-level trait variation (i.e. ITV + BTV, weighted by species abundance) could be improved (Garnier et al., 2004). Community weighted means (CWM) could also potentially reveal the most important environmental drivers of functional traits at the community level and offer new insights into how communities respond to environmental changes (Jung et al., 2014).

Intra-specific variation in functional traits has been found to be strongly correlated with some environmental variables, including soil type, moisture availability, mean annual rainfall and temperature (Witkowski and Lamont, 1991; Wright et al., 2004; Roybal and Butterfield, 2019). Intra-specific trait variation is strongly impacted by small scale changes in environmental conditions such as temperature, aridity, light availability, as well as biotic relationships including herbivory (Westerband et al., 2021). Responses in the ITV of traits related to the size of plants in reaction to grazing has also been demonstrated recently, but PFTs related to plant growth and water usage show inconsistent relationships with grazing (Whitworth-Hulse et al., 2016). Moreover, environmental variables in general show inconsistent patterns as to how they are related to PFTs and how strong their relationships are with PFTs (Gerdol, 2005; Albert et al., 2010; Kichenin et al., 2013; Westerband et al., 2021).

A diversity of PFTs can be measured but only a handful are considered to be clearly ecologically relevant across different scales and between habitats (Kattge et al., 2020), with traits such as SLA and leaf dry matter content (LDMC, dry mass of a leaf divided by the wet mass of the leaf) being considered the most suitable for the study of variation in resource use in plants (Wilson et al., 1999a). Both SLA and LDMC are variable at the inter-and intra-specific level (Garnier et al., 2001), with SLA, generally being more variable than LDMC (Wellstein et al., 2013). With higher SLA values it is expected that a plant would display higher growth rates, higher photosynthetic rates, and decreased investments into secondary metabolites, and, as a result, decreased leaf lifespan (Wilson et al., 1999a; Pérez-Harguindeguy et al., 2013), and increased herbivory (Coley et al., 1985). The increased growth rates can also allow for rapid regrowth after herbivory events allowing the plant to tolerate grazing (Coughenour, 1985; Adler et al., 2004). In contrast, species with high LDMC values typically display the opposite trend with lower growth rates, lower photosynthetic rates, longer leaf lifespan and reduced herbivory rates expected (Pérez-Harguindeguy et al., 2013). Along environmental gradients, contrasting trends are generally observed between SLA and LDMC, with changes in environmental conditions leading to an increase in the values of one trait while decreasing the other trait's values (Wellstein et al., 2013). Likewise, high SLA values are expected from individuals in highly productive environments with high water and nutrient availability, while high LDMC values are typically common in disturbed and unproductive environments (Wilson et al., 1999a; Pérez-Harguindeguy et al., 2013). However, while SLA and LDMC influence the same biological features these traits can be impacted differently by changes in environmental conditions (Schöb et al., 2012). Further, since these traits are not directly correlated with each other, they provide different insights into the drivers of variation in PFTs (Pérez-Harguindeguy et al., 2013).

Other traits to consider, particularly in terms of herbivory, are leaf thickness (LT), leaf width (LW) and force to tear (FT, force in N required to break leaf by pulling force divided by the width of the leaf). These traits are avoidance traits which prevent excessive biomass being lost to herbivores (Adler et al., 2004). Force to tear (i.e. toughness) is a strong avoidance trait since generally, a strong negative relationship exists between palatability and the toughness of foliar biomass (Cornelissen et al., 1999). Leaf thickness is often measured as an integrative metric of the physical properties of leaves and is a key factor influencing SLA, with thicker leaves having lower SLA (Pérez-Harguindeguy et al., 2013). Thick leaves allow for optimization of photosynthesis in dry, hot, and nutrient-poor environments, since in thicker leaves the gains obtained through photosynthesis outweigh losses experienced due to respiration and transpiration (Pérez-Harguindeguy et al., 2013). Leaf thickness, LW and FT are used to express the physical strength of leaves and the ability of leaves to survive mechanical damage such as herbivory (Pérez-Harguindeguy et al., 2013).

The grazing value of grasses can be defined as the advantage obtained by a grazing animal when feeding on a specific grass individual. The grazing value of grasses is an important component in grazing systems which is influenced by environmental variables such as temperature, precipitation, and atmospheric CO_2 concentrations as well as biological factors, such as the developmental stage of grass plants (Duru et al., 2008; Craine et al., 2010; McGranahan and Yurkonis, 2018). An exact grazing value is however not often calculated since the grazing value of grass can be impacted by various plant components, including the grass' nutritional value, ability to regrow after grazing, acceptability by grazers, leaf production and digestibility of grass biomass (Van Oudtshoorn, 1999), palatability and grazing capacity (Barnes et al., 1984). However, grazing quality (i.e. a term used synonymously with grass nutritional value in this dissertation) can be measured and is often summarized by three key components: digestibility, crude protein (CP) and fibre content (Khaled et al., 2006). Digestibility (measured *in vitro)* is the most often studied of the three key components (Tasset et al., 2019), and is considered to be the best measurement of grazing quality (Habermann et al., 2019). Crude protein which consists of amino nitrogen, nucleic acids, nitrates, and nitrites (Habermann et al., 2019), is important for good body condition and growth of herbivores and is positively related to the digestibility of grass biomass (Hughes et al., 2014). The fibre content of grass biomass is made up of neutral detergent fibre (NDF) which consists of hemicellulose, cellulose and lignin and acid detergent fibre (ADF) which consists of cellulose and lignin (Habermann et al., 2019). Higher levels of NDF and ADF results in reduced digestibility (Habermann et al., 2019), which in turn reduces the grazing quality of the grass biomass (Ball et al., 2001). During the wet season, the crude protein levels, and digestibility of grass biomass peak (Hughes et al., 2014) and generally decreases as the grasses reach maturity (Rosser et al., 2013; Grev et al., 2017). The fibre and lignin content on the other hand peaks during the dry season (Hughes et al., 2014). Traditionally, expensive, and time-consuming wet chemical analyses were the only method available for determining the grazing quality of grass samples. However, near-infrared reflectance spectroscopy (NIRS) offers an accurate, time- and cost-efficient (Corson et al., 1999) method for determining CP and fibre content fractions, which can provide accurate estimates of digestibility. By obtaining accurate grazing quality values a better understanding of how environmental conditions impact the grazing quality could be achieved. Furthermore, the importance of the different components of grazing quality could be revealed and a better understanding developed of how these components interact, which will allow for effective management of grazing regimes in grassy ecosystems.

In contrast to PFTs, grazing quality is typically only considered to vary at the species level, ignoring potential intra-specific variation (Van Oudtshoorn, 1999). Traditionally in South Africa, grass species have been assigned a categorical grazing value (i.e. low, average, or high; Fig. 1) with the emphasis being placed on inter-specific differences in grazing value (i.e. BTV in grazing quality; Van Oudtshoorn, 1999). Consequently, some grass species are considered good for grazing and others not, with all individuals of a species assumed to have the same grazing quality irrespective of local environmental conditions and ecotypes (Van Oudtshoorn, 1999; Truter and Venter, 2017; Van Oudtshoorn, 2019). However, there is an increasing debate about the validity of this approach as several grass characteristics (including PFTs and morphology) do vary strongly with environmental conditions (e.g. soil conditions, water availability and temperature; Roybal and Butterfield, 2018; Van Oudtshoorn, 2019). Indeed, environmental conditions are also expected to drive intra-specific variation in the grazing quality of grasses, since, for example, the crude protein levels of *Panicum maximum* increase under water-stressed conditions (Marais, 2005). Therefore, it is expected that the grazing quality of grasses varies at both the inter-and intra-specific levels (Fig. 1.1). For example, a common grass found in Limpopo province of South Africa, *Schmidtia pappophoroides*, is classified as having a high grazing quality (Van Oudtshoorn, 1999), despite this grass also exhibiting low grazing quality in nutrient-poor areas (personal communication Truter, 2019). Exploring the relationships between PFTs and grazing quality could alter our understanding of spatial variation in, and environmental drivers of, grazing quality in grassy environments. Functional traits are easy to measure, and the development of new Near-Infrared Reflectance Spectroscopy (NIRS) technology allows for rapid and affordable measurements of the grazing quality of grass material and has been widely used for the measurement of various factors impacting the digestibility of forages (Corson et al., 1999). However, NIRS technology has predominantly been used for livestock forage quality analysis (Park et al., 1998; Nousiainen et al., 2004) with very few studies examining the grazing quality of natural vegetation. Thus, the combination of these methods opens the door to novel studies, including the possibility of using PFTs as time- and cost-efficient proxies of grazing quality.

Various authors have demonstrated a negative link between LDMC and grazing quality at the species (Khaled et al., 2006; Pontes et al., 2007; Duru et al., 2008; Andueza et al., 2010; Tasset et al., 2019) and community-level (CWM; Gardarin et al., 2014). However, these studies have all been conducted in French grasslands, which represent a small fraction of the world's grassy ecosystems and are dominated by C3 grasses whereas African savannas are dominated by C4 grasses (Ratnam et al., 2011). Therefore, it is important to know the extent to which PFTs can be used as proxies for the grazing quality of grasses in other ecosystems. Specific leaf area and LDMC have the greatest potential as proxies for grazing quality as these traits directly relate to primary production and resource use (Garnier et al., 2001). It is generally expected that high SLA and low LDMC correspond to high grazing quality due to low concentrations of tough cell wall components such as fibre, cellulose, and hemicellulose (Wilson et al., 1999a; Cornelissen et al., 2003; Khaled et al., 2006; Pontes et al., 2007; Whitworth-Hulse et al., 2016). Specific leaf area has a negative correlation with the bulk density of leaves which is determined by the abundance of structural components (i.e. lignin, cellulose, and hemicellulose) in cell walls relative to the volume of cell contents (Wilson et al., 1999a; Cornelissen et al., 2003; Whitworth-Hulse et al., 2016), which theoretically should allow for the prediction of grazing quality from SLA (Duru et al., 2004). A positive relationship is known to exist between SLA and digestibility of grasses (Duru et al., 2004; Pontes et al., 2007), as well as between leaf area and digestibility of *P. maximum* in a pasture system (Da Silva et al., 2019). Indeed, it has also been shown that the relationship between SLA and grazing intensity (i.e. the level of herbivory in an area) is positive while the relationship between LDMC and grazing intensity is negative (Peco et al., 2005; Saatkamp et al., 2010). This suggests that plants with high SLA are prone to grazing and that these plants compensate for high defoliation rates through increased growth rates (Westoby, 1998).

Leaf thickness and FT are positively related to the toughness of leaves and are thus expected to be negatively related to grazing quality, as tougher leaves offer greater resistance to mechanical damage (Pérez-Harguindeguy et al., 2013). However, these traits are not often linked to the grazing quality of grasses (i.e. CP, digestibility, and fibre content) even though the toughness of leaf material can be considered as one of the most important factors impacting grazing quality, through the general acceptability by grazers (Theron and De V. Booysen, 1966). For a grazer to be impacted by CP, digestibility, and fibre content, of grass material, the ingestion of grass material must take place. Therefore, it is vital that assessments of grazing quality additionally take this potentially important component of grazing quality into account. The combination of growing interest in the variation of grazing quality, and PFTs, as well as the relatively recent availability and validation of NIRS, elicited a novel question: is there a link between PFTs and the grazing quality of grasses (Fig. 1.1)?

Dissertation aims and structure

The main objective of this study was to investigate and compare the environmental drivers of leaf functional traits, grass cover, grass species richness and grazing quality (i.e. grass characteristics) at the community and species level using savanna grasses as the focus taxonomic group. In chapter 1, I have reviewed the literature on these grass characteristics and how the variation in these characteristics is related to environmental conditions at the species- and community-level. In Chapter 2 I investigate what drives spatial variation in grass assemblages, both at the species- and community-level, with a focus on cover, composition and PFTs at Lapalala Wilderness Nature Reserve (LWNR; Fig. 1.1). The different grass characteristics were expected to show variable relationships with environmental variables between the species- and community-levels as well as between different species. The knowledge gained from this study will result in an improved understanding of influential environmental variables of grass characteristics. Chapter 3 builds on Chapter 2's focus on PFTs, expanding it to also include grazing quality, by asking what environmental factors are related to variation in grass PFTs and grazing quality at the inter-and intraspecific level at Welgevonden Game Reserve (WGR). Additionally, in this chapter, I examine which environmental factors influence the variation in PFTs and grazing quality at the species- and community-level. Finally, in this chapter, I test if PFTs are strongly related to grazing quality (Fig. 1.1). Chapter 4 synthesises the results from Chapters 2 and 3.

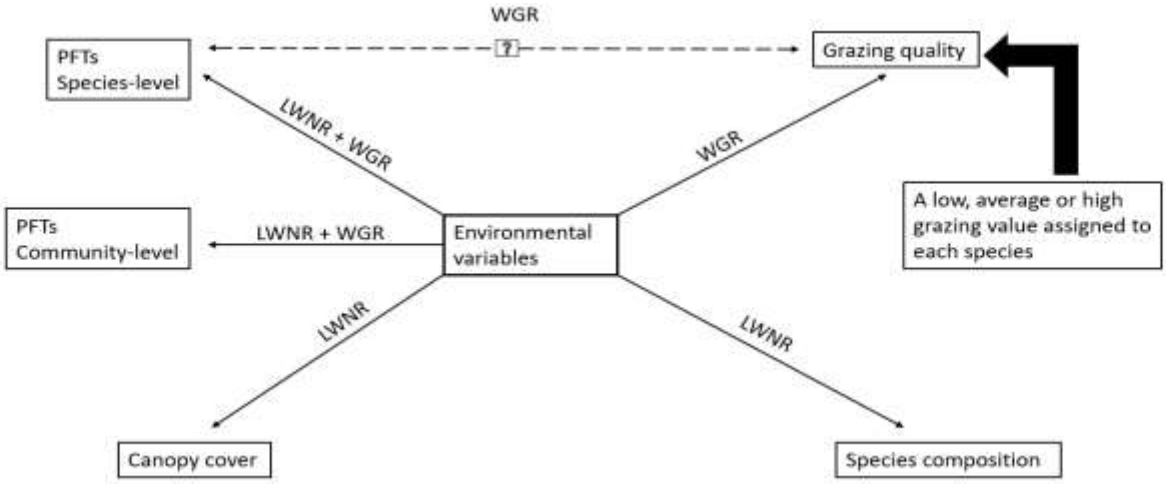

Figure 1.1 Links between the different components of this study and which dataset was used. Solid lines indicate well-established relationships in the literature and dashed lines show speculative relationships. LWNR = Lapalala Wilderness Nature Reserve, WGR = Welgevonden Game reserve and PFTs = Plant functional traits.

This study took place at two nature reserves, LWNR and WGR, situated in the Waterberg biosphere reserve in the Limpopo province of South Africa (Fig. 1.2). Lapalala Wilderness Nature Reserve is a 45,000-ha reserve (23°51'S 28°18'E), and Welgevonden Private Game reserve is a 36,000-ha reserve located 60 km SW of LWNR (24°10'S 27°45'E). These reserves share similar patterns in annual precipitation levels, with predominantly summer rainfall (Hulsman et al., 2010; Zwerts et al., 2015). The main vegetation type of both reserves is classified as Waterberg Mountain Bushveld, however, WGR also has large areas of Waterberg Magaliesberg Summit Sourveld (Mucina and Rutherford, 2006), resulting in more open vegetation in portions of WGR. Both reserves support various grazers such as blue wildebeest (*Connochaetes taurinus*), plains zebra (*Equus quagga*), eland (*Tragelaphus oryx*), Cape buffalo (*Syncerus caffer*), white rhinoceros (*Ceratotherium simum*) and African elephant (*Loxodonta africana).*

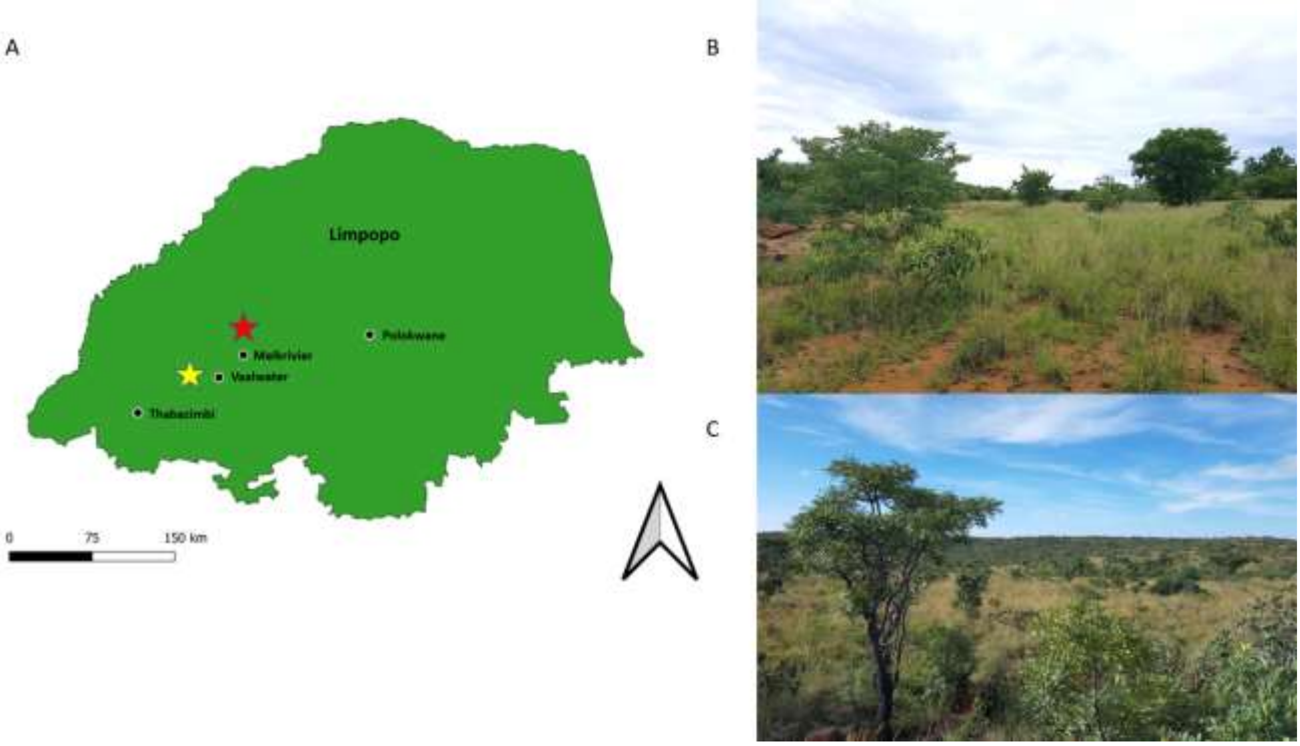

Figure 1.2 Limpopo province of South Africa, with the locations of the two study sites (A). Red star = Lapalala Wilderness Nature Reserve, Yellow star = Welgevonden Game Reserve. Landscape pictures of the vegetation in LWNR (B) and WGR (C).

Chapter 2: Limited consistency in drivers of savanna grass characteristics at both the community and species-level[1]

Abstract

Compositional and functional plant community characteristics can strongly govern some ecosystem processes. These community characteristics, may, in turn, be related to multiple environmental variables, including edaphic, climatic, and topographic conditions. However, there is still an incomplete understanding of how environmental conditions affect plant community composition and functioning and, therefore, how species composition and trait expression potentially link habitat conditions to ecosystem processes. This is particularly true for grasses, despite this taxon having considerable ecological and economic importance. Therefore, this study examined the relationship between 19 environmental factors and community composition and cover, community-weighted mean (CWM) trait values, and intra-specific trait variation in a savanna grass assemblage. However, in this study, several different environmental variables were related to variation in grass characteristics at the community and the species level, but only a small subset of variables related to grass characteristics in multiple analyses. Soil potassium content was the only variable that was consistently influential across multiple species and all characteristics, having, for example, a positive effect on (CWM_{LDMC}) and a negative effect on (CWM_{SLA}). Soil pH, sand fraction, bare ground cover, woody cover and woody debris cover were also strongly related to grass assemblage characteristics, while variables related to moisture had very little influence on these characteristics. Inconsistencies between the response of dominant grass species compared to the responses of the entire grass assemblage to environmental conditions suggest that shifts in individual species are not necessarily matched by community-level shifts along abiotic gradients. These results, therefore, highlight the challenges of forecasting the impacts of changing environmental conditions on grass assemblages, and the complexity of designing management plans for this ecologically and economically important taxon.

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Introduction

Various compositional and functional plant community characteristics can strongly govern ecosystem services and functions (including, e.g. primary production, pollination, nutrient cycling and erosion control; Schmitz et al., 2015; Faucon et al., 2017; Zirbel et al., 2017). These plant community characteristics may, in turn, be related to multiple environmental variables, including edaphic (e.g. soil nutrient levels), climatic (e.g. temperature and precipitation) and topographic conditions (e.g. slope and aspect; Kardol et al., 2010; Faucon et al., 2017; Zirbel et al., 2017; Bouchenak-Khelladi et al., 2020). Traditional proxies of ecosystem structure, such as species composition and canopy cover, may therefore provide easily measured surrogates for some ecosystem processes (Kardol et al., 2010; Zirbel et al., 2017) since they are intrinsically/causally linked to both environmental conditions and ecosystem-level functioning. For example, environmental gradients may be correlated with variation in species composition, which, in turn, affects primary production (Tilman, 1996; Symstad et al., 1998; Balmford et al., 2001; Gaitan et al., 2014), as well as other ecosystem functions and services (Egoh et al., 2008). Similarly, grass canopy cover, which is strongly influenced by abiotic habitat conditions, is also typically strongly correlated with primary production and is therefore also potentially a proxy for several ecosystem processes (e.g. soil respiration and erosion control; Symstad et al., 1998; Maestre et al., 2005; Gaitan et al., 2014; Van Oudtshoorn, 2019). As a result, an improved understanding of the environmental influences on the species composition and cover of grass communities could allow for enhanced predictions of ecosystem services and functioning.

Plant functional traits (PFTs) can provide a mechanistic link between species responses and environmental conditions, and how variation within communities and species affect aspects of ecosystem functioning compared to using only species composition or measures of plant cover (Zirbel et al., 2017). Plant functional traits are any quantifiable features an individual plant possesses which can vary along environmental gradients and, in turn, affect the fitness of the individual (Violle et al., 2007; Dong et al., 2020). As a result, PFTs indicate the physiological response of plants to changes in environmental conditions (Violle et al., 2007; Pérez-Harguindeguy et al., 2013), and can provide insights into plant life-history strategies (Sandel and Low, 2019) and variation in growth rates, survival, and reproduction (Weiher et al., 1999). Additionally, PFTs allow a comparison of patterns along environmental gradients even for communities with non-overlapping species composition (e.g. Kemppinen et al., 2021). Due to plant functional traits' connection with physiological processes, these traits can also act as proxies for ecosystem services, such as primary production (Pérez-Harguindeguy et al., 2013) and carbon and nitrogen cycling (Garnier et al., 2004). As a result, PFTs have the potential for improving our understanding and monitoring of key ecosystem processes. However, before such an approach can be applied, either at local or global scales some key aspects of functional trait ecology still require investigation (e.g. Kemppinen et al., 2021).

Specific leaf area (SLA) and leaf dry matter content (LDMC) are two of the most commonly investigated PFTs (Kattge et al., 2020), and are variable at the inter-and intra-specific level (Garnier et al., 2001). Both SLA and LDMC are related to growth rate, palatability (Pontes et al., 2007) and resource use (Wilson et al., 1999a), but can be impacted differently by changes in environmental conditions (Schöb et al., 2012). Recent studies, focus more on the environmental drivers of SLA compared to LDMC, with edaphic (e.g. soil nutrient- and moisture levels), climatic (e.g. precipitation) and topographic variables (e.g. slope and aspect) being considered to be the strongest drivers of variation in these traits (Wellstein et al., 2013; Sandel and Low, 2019; Bouchenak-Khelladi et al., 2020; Jardine et al., 2020).

Functional traits vary at two levels: interspecifically (between-species variation; BTV) or intra-specifically (between individuals of the same species; ITV). Functional traits typically vary more strongly inter-specifically than intra-specifically, with up to 75% of the variation in PFTs being contributed by inter-specific trait variation at the community level (Siefert et al., 2015). Since environmental conditions may filter species by traits (or trait levels; Keddy, 1992), the habitat conditions acting on species composition, likely determine the expression of BTV within a community (Lavorel and Garnier, 2002). In consequence, across large geographical areas changes in species composition (via, e.g. species turnover) increase the level of BTV, with ITV often being masked by the large differences in PFTs between species (Albert et al., 2011; Pescador et al., 2015).

Intra-specific trait variation allows for the survival and reproduction of a species (Byars et al., 2007) under changing environmental conditions through adaptation by microevolution over generations (Lajoie and Vellend, 2018; Dong et al., 2020), as well as plastic responses in morphology and physiology (Wellstein et al., 2013; Lajoie and Vellend, 2018). However, ITV is often assumed to be negligible in community-level studies since trait differences between species are thought to have a stronger influence on a community than differences within species (McGill et al., 2006). Intra-specific trait variation may, nonetheless, still contribute substantially to trait variation of communities (25-32 % of total variation; Albert et al., 2010; Siefert et al., 2015; Roybal and Butterfield, 2018; Mitchell et al., 2020) and impact on community assembly (Jung et al., 2010) and ecosystem processes (Albert et al., 2011). Moreover, ITV can account for a greater proportion of variation in leaf traits than BTV between some communities, suggesting that not only species identity but also variation within a species is important to understand the community and ecosystem dynamics (Bouchenak-Khelladi et al., 2020). Indeed, the inclusion of ITV allows for better predictions regarding the environmental drivers of trait variation at the community level (e.g. response of communities to extreme drought; Jung et al., 2014), and could, for example, influence predictions of food web dynamics (Bolnick et al., 2011). Intra-specific variation in functional traits has been correlated with various environmental variables (Jung et al., 2010), including soil type, moisture availability and temperature (Witkowski and Lamont, 1991; Roybal and Butterfield, 2019), but no consistent patterns have yet been observed regarding which environmental drivers are most strongly related to ITV.

Grass assemblages provide an excellent study system to investigate the drivers of variation in plant characteristics within and between species and at the community level. The Poaceae are fundamental to several ecosystem services which are ecologically and economically important, including carbon sequestration (Egoh et al., 2009; Wigley et al., 2020), erosion control, and surface water supply and regulation (Van Oudtshoorn, 1999; Egoh et al., 2009; Parr et al., 2014). Since grasses are a dominant life form in multiple biomes and can rapidly produce large amounts of biomass, the Poaceae are one of the most economically valuable plant families (Van Oudtshoorn, 1999; Bouchenak-Khelladi et al., 2020). A range of environmental variables, such as edaphic (e.g. soil nutrients) and climatic (e.g. temperature and precipitation) conditions, act as filters of grass species composition (Sandel and Tsirogiannis, 2016; Bouchenak-Khelladi et al., 2020), and grass cover often shows clear relationships with fire frequency and soil moisture (Symstad et al., 1998; Zirbel et al., 2017). However, in contrast to species composition and cover, the drivers and extent of inter-and intra-specific variation in grass PFTs, especially in southern Africa, are poorly understood (Roybal and Butterfield, 2018; Roybal and Butterfield, 2019), despite, for example, grass traits being linked to various key ecosystem services and functions (Egoh et al., 2009; Wigley et al., 2020).

This study investigated a species-rich C4 grass assemblage within a South African savanna system, identifying the environmental variables related to variation in (a) grass canopy cover, (b) grass species composition, and SLA and LDMC at the (c) community level and (d) species level for grass species. Considering the constrained morphological variation and the large geographic distribution of South African savanna grass species (Van Oudtshoorn, 1999; Fish et al., 2015), it is expected that a similar set of environmental variables will be relatively consistently related to community- and species-level variation in the grass assemblage.

Materials and methods

Study Area

This study was performed in the Lapalala Wilderness Nature Reserve (LWNR, Fig A5), which is a 45,000-ha reserve situated in the Melkrivier district of Limpopo South Africa (23°51’S and 28°18’E; Fig. 2.1). Lapalala Wilderness Nature Reserve is located within the savanna biome with vegetation comprising a near-continuous ground layer dominated by C4 grasses and scattered woody plants forming fragmented taller vegetation. Grass species contribute most of the biomass of the ground layer vegetation in savannas generally (and as can be seen at this study site) and can provide considerable forage to grazing herbivores (Ludwig et al., 2004; de Lima et al., 2018; Guo et al., 2018). Lapalala Wilderness Nature Reserve supports various grazers and mixed feeders such as Blue Wildebeest (*Connochaetes taurinus*), Plains Zebra (*Equus quagga*), Eland (*Tragelaphus oryx*), Cape Buffalo (*Syncerus caffer*), White Rhinoceros (*Ceratotherium simum)*, and African Elephant (Loxodonta africana; Cillié and Kruger-Burger, 1997).

The reserve is dominated by sandy soils produced from the Kranskop subgroup of the Waterberg group which are low in nutrients, and some clay soils are found close to the main rivers (including, e.g. primary production, pollination, nutrient cycling and erosion control; Ruwanza, 2018). Rainfall at LWNR ranges between c. 400 – 600 mm per annum (Hulsman et al., 2010) and the vegetation of LWNR is classified as Waterberg Mountain Bushveld (Mucina and Rutherford, 2006).

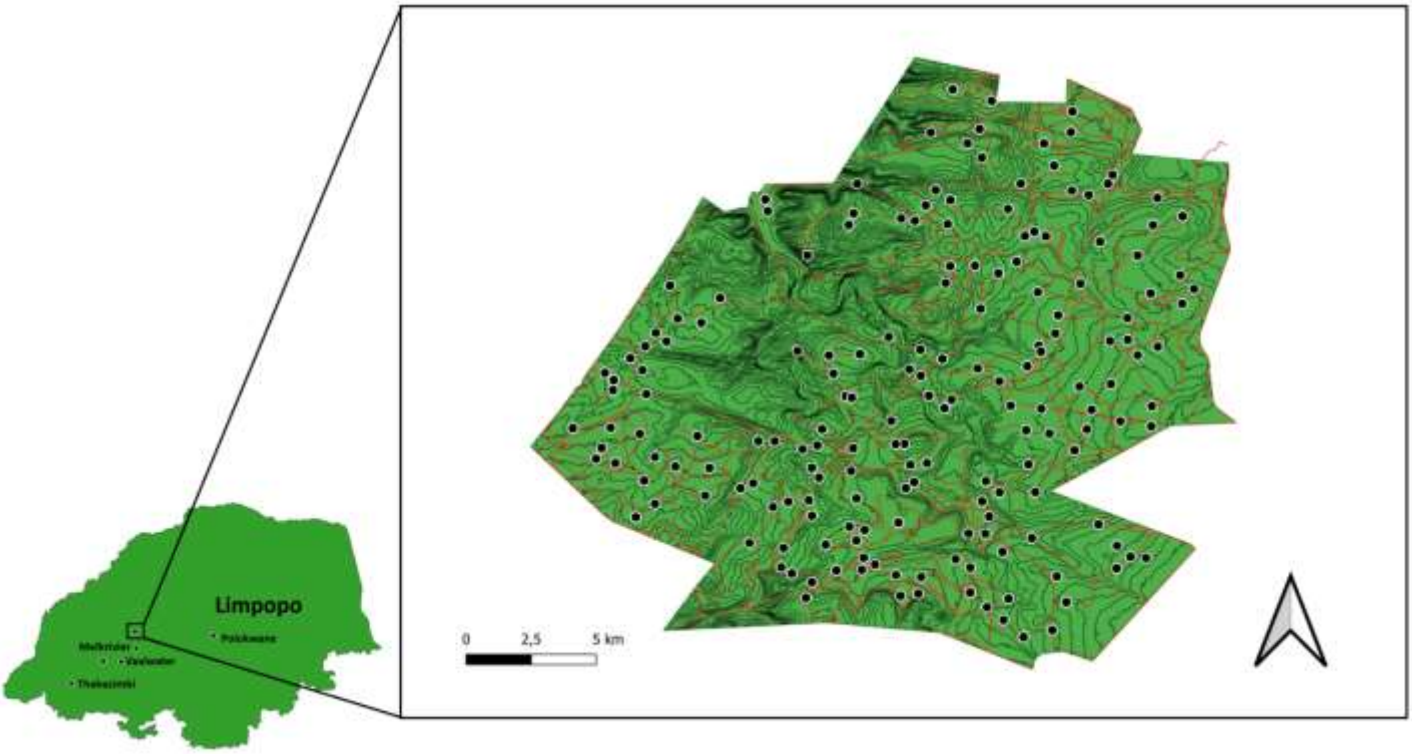


Figure 2.1 Lapalala Wilderness Nature Reserve is located within the Limpopo province of South Africa. Black symbols indicate relevés locations within the reserve's borders (n = 180), with grey lines indicating contours (at 20 m intervals) and orange lines indicating the reserve's road network.

Environmental data collection

The location of one hundred and seventy 20 × 20 m sampling relevés was predetermined through a random stratification process to ensure sampling under a wide range of different environmental conditions. Each relevé was additionally constrained to be > 50 m from any human disturbance. An additional 10 relevés were located within LWNR on an ad hoc basis to sample unusual conditions not captured by the other, randomly located, relevés. Within each relevé, vascular plant species composition, and canopy cover (%) for each species was visually estimated by one observer to allow for consistency. The cover (%) of bare ground, leaf litter, woody debris, rock cover and woody plant species was visually estimated for each relevé. The geographical coordinates as well as altitude of each relevé was recorded with a handheld GPS. The day of year that the sampling took place was calculated as the number of days from 1 January 2019. Data from all 180 relevés were used for the analysis of grass cover and species composition drivers (see Womack et al., 2022 for full details on relevé positioning and establishment).

Functional trait data were simultaneously collected from a subset of 111 relevés. Specific leaf area and LDMC were collected from three individuals of each of the five most dominant (based on cover) grass species per relevé (following: Pérez-Harguindeguy et al., 2013).

Four additional GIS-derived landscape variables, curvature, topographical wetness index (TWI), slope and potential solar radiation (PSR) were calculated for each relevé from a digital elevation model (DEM: NASA/METI/AIST/Japan Space Systems and U.S./Japan ASTER Science Team. ASTER Global Digital Elevation Model. 2009, distributed by NASA EOSDIS Land Processes DAAC, https://doi.org/10.5067/ASTER/ASTGTM.002; Table A1), using ArcGIS (ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute). The northness of each relevé was calculated (following: Bader and Ruijten, 2008) from the aspect measurement that was recorded in the field.

Soil samples were collected from the top 10 cm of soil near the four corners of each relevé (after removing the organic matter at the soil surface). When soil samples could not be collected at these locations due to the presence of rocks, fallen trees, or other natural features, soil samples were collected from a different location within the relevé. The soil from these four samples was pooled, forming a sample of c. 1 kg of soil per relevé. Soil samples were air-dried at room temperature and then analysed in the University of Pretoria's Soil Laboratory for calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), and phosphorous (P) using the Mehlich 3 extraction protocol (Ziadi and Tran, 2007a). The pH of each soil sample was determined using the $CaCl_2$ method (Carter and Gregorich, 2008) and carbon content was determined through titration (following: Ziadi and Tran, 2007b). Finally, the texture of each soil sample was determined by using the Hydrometer method (Kroetsch and Wang, 2008).

Plant functional trait data and community weighted means

The three most dominant grass species within each of the 111 relevés, based on species cover within individual relevés, were sampled by collecting leaf material from three individuals per species by collecting a culm with leaves attached. Leaf material was then stored in water-tight plastic bags and transported to the laboratory in a dark cooler box within 8 hours. From each grass culm, a mature leaf blade with no herbivore or pathogen damage was subsequently selected and a *c.*5 cm long section was cut from the middle of each leaf blade. For grasses with leaves smaller than 5 cm in length, the entire leaf was used without the ligule. The leaf sections were then weighed using a Precisa XB 160M balance (Precisa Switzerland; accurate to 1 mg) to obtain the fresh weight of each leaf section. Each leaf section was then scanned along with a 5 cm calibration bar using a Canon MX 424 scanner (Canon, U.S.A; optical resolution, 1200 × 2400 dpi). ImageJ (Fiji software; Abràmoff et al., 2004) was used to calculate the fresh leaf area of each leaf segment after calibration using the calibration bar in each scanned image. All leaf material was dried at 72°C until a constant dry weight was obtained (typically 48 hours). The dry weight of each leaf segment was determined by reweighing each segment using a Precisa XB 160M balance or a Radwag XA 250.4Y analytical balance (accurate to 1 mg). Subsequently, the SLA of each leaf segment was calculated as the fresh leaf section area divided by the leaf section dry mass ($mm^2\ mg^{-1}$), and the LDMC of each leaf segment was calculated as the leaf section dry mass divided by the leaf section fresh mass ($mg\ g^{-1}$).

The community weighted mean (CWM) for each trait was determined for each relevé by multiplying the relative cover of each species within each relevé by the trait value of each species calculated as the average trait value of each species across LWNR. Grass species for which no trait data were collected (15 species across LWNR), were assigned trait values obtained from the average for the genus, tribe, or subtribe of the species using the lowest possible taxonomic rank, sampled in this study (Table A2). These 15 species were rare, found only in an average of 1.9% of the 111 relevés, and had a mean cover < 1% in the relevés in which they were present.

Statistical analysis

Highly correlated environmental variables were excluded before statistical analyses (i.e. see Table A1 for a list of all variables, see Fig A1 for correlation matrix of all variables after highly correlated predictors were excluded). To identify the environmental variables driving variation in species- and community-level grass characteristics, a two-step model building process was followed. First univariate generalized linear models were used to test the relationships between each predictor variable and each response variable (i.e. the species-level and CWM values for SLA and LDMC, and grass canopy cover). Quadratic terms were included for all predictor variables where the more complex model gave a better fit based on the generalized linear models (Tables A4, A5).

Second, all combinations of predictor variables that were significantly related to response variables in univariate models were subsequently examined using a best subset modelling approach for each response variable, implemented using generalized linear models. This model-building approach compares all possible combinations of predictor variables, with the best combination(s) of predictors identified based on Akaike information criterion (AIC) scores. All models with a ΔAIC < 2 (difference between the AIC of the top-ranked model and each subsequent model) were considered to have substantive support (Burnham et al., 2011). As a fairly low number of variables were significant in univariate analysis (i.e. 9 – 11 environmental variables), other environmental variables considered as biologically relevant were additionally selected to be initially included in model building for each response variable. Woody debris was included as a potential predictor for LDMC, and TWI for SLA and grass canopy cover since these predictor variables were significant for other grass characteristics in univariate models. Additionally, slope was included as a predictor initially for SLA and LDMC, specifically as an additional proxy for moisture availability (which is related to some PFTs; Wellstein et al., 2013; Jung et al., 2014; Chen et al., 2019) Northness was included for SLA, LDMC and grass canopy cover since a strong relationship between grass traits and this variable were recently demonstrated (Sandel and Low, 2019). Finally, altitude was also included as a predictor variable for SLA, LDMC and grass species composition since a relationship between these characteristics and altitude has also been previously demonstrated for grasses (Cabido et al., 1997; Gonzalo-Turpin and Hazard, 2009; Fontana et al., 2017). All relationships were modelled using generalized linear models, with cover data analysed using a binomial distribution, and CWM_{SLA} and CWM_{LDMC} data analysed using a Gaussian distribution.

Community composition, weighted by plant cover, was analysed using non-metric multidimensional scaling (NMDS) ordination and permutational multivariate ANOVA (PERMANOVA) tests to assess relationships between environmental variables and species composition based on the presence or absence of each grass species.

The relationship between intra-specific variation in SLA and LDMC and environmental variables was tested for the nine most common grass species (i.e. species sampled in at least 10 relevés, with FT values from > 30 individuals) using multivariate linear mixed-effect models. In these models, relevé was included as a random effect to account for up to three samples. The same model building approach was used for the analyses of BTV. Univariate analysis was conducted at the species level and the environmental drivers found to have a significant effect on the PFTs of a species were included in the multivariate analysis for each species (Table A6). However, as few univariate drivers were significantly related to the PFTs of most species (Table A6), variables found to be the most important drivers in univariate and multivariate models of CWM_{SLA} (leaf litter, woody plant cover, bare ground, woody debris, % sand and soil K) and CWM_{LDMC} (bare ground, rock cover, day of year, woody plant cover, soil K and soil P) were therefore also included as predictor variables for all species during model building. Quadratic terms were included where they provided the best fit in univariate models at the species level and where the community-level models included quadratic terms (Table A6). The best multivariate mixed-effect models were selected based on AIC.

All statistical analyses were conducted using R version 4.0.2 (R Core Team, 2020), including functions from the car (version 3.0-9; Fox and Weisberg, 2011), lme4 (version 1.1-23; Bates et al., 2015), MuMIn (version 1.43.17; Barton, 2020), lmerTest (version 3.1-2; Kuznetsova et al., 2017) and vegan (version 2.5-6; Oksanen et al., 2019) libraries.

Results

Community-level characteristics

A total of 60 grass species were recorded at LWNR (see Table A3), and SLA and LDMC were collected from 45 species, with the number of samples per species ranging from 3 to 174 (full dataset available via the TRY data portal: https://www.try-db.org/de/Datasets.php Data set ID: 643). Community-level, variation in SLA and LDMC (Fig. A2 and A3) were significantly related to nine and eleven predictor variables respectively (Table A4) in the univariate analyses. However, these relationships were generally weak, with the best univariate predictors being soil pH (having a positive relationship with CWM_{SLA}; $R^2 = 0.13$; Fig. 2.2-A) and soil K (having a negative relationship with CWM_{LDMC}; $R^2 = 0.06$; Fig. 2.2-B). The top-ranked multivariate models contained five variables each and explained a considerably greater proportion of the variation in CWM_{SLA} ($R^2 = 0.25$; Table A8) and CWM_{LDMC} ($R^2 = 0.25$; Table A9).

Grass canopy cover was significantly related to nine predictors in univariate models (Table A5; Fig. A4), with increasing grass canopy cover being observed along time (i.e. day of the year explaining the greatest variation in grass cover, $R^2 = 0.26$; Fig. 2-C). The top-ranked multivariate model explained considerably more variation in grass canopy cover ($R^2 = 0.64$) and included five predictors (Table A12).

Grass species composition was significantly related to six environmental variables (Fig. 2.3; Table A7). Day of year ($R^2 = 0.35$) was the strongest driver, while the influence of three other environmental variables (soil pH, K, and sand fraction) was strongly correlated (i.e. forming a key axis of variation in species composition). Rock cover ($R^2 = 0.23$) represented a third key axis of variation (Fig. 2.3).

Soil K was the only predictor found to consistently relate to all community-level characteristics (Table 2.1). However, bare ground and woody plant cover were consistently included in models for three of the four community-level characteristics (i.e. CWM_{SLA}, CWM_{LDMC} and grass cover) and day of year was included in the top-ranked models for CWM_{LDMC}, grass cover and species composition (Table 2.1).

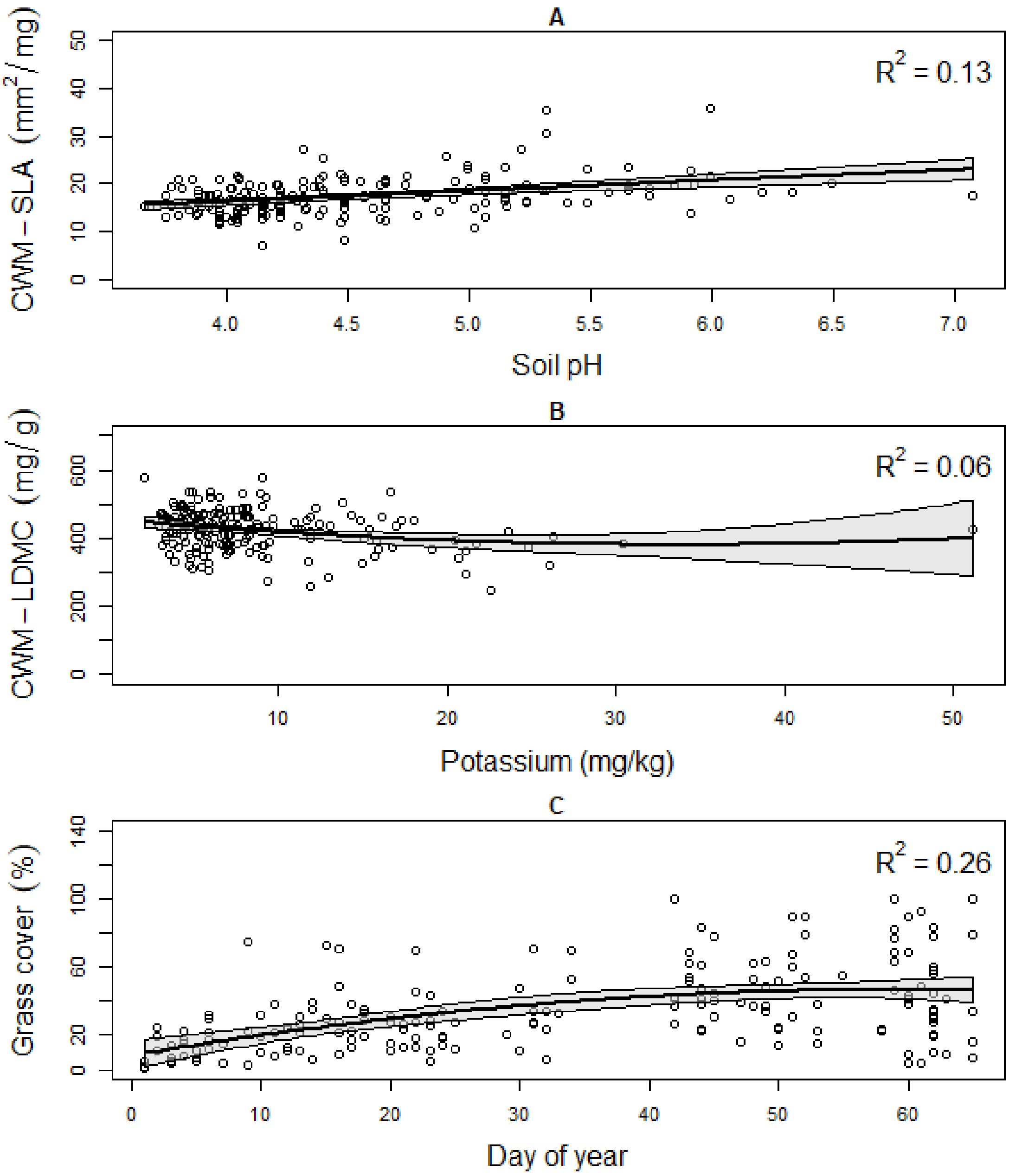


Figure 2.2 In univariate models, (a) soil pH, (b) soil potassium (K) and (c) day of year explained the greatest proportion of variation for CWM_{SLA}, CWM_{LDMC} and grass cover, respectively. The line in each panel represents the best fit from the univariate generalized linear model.

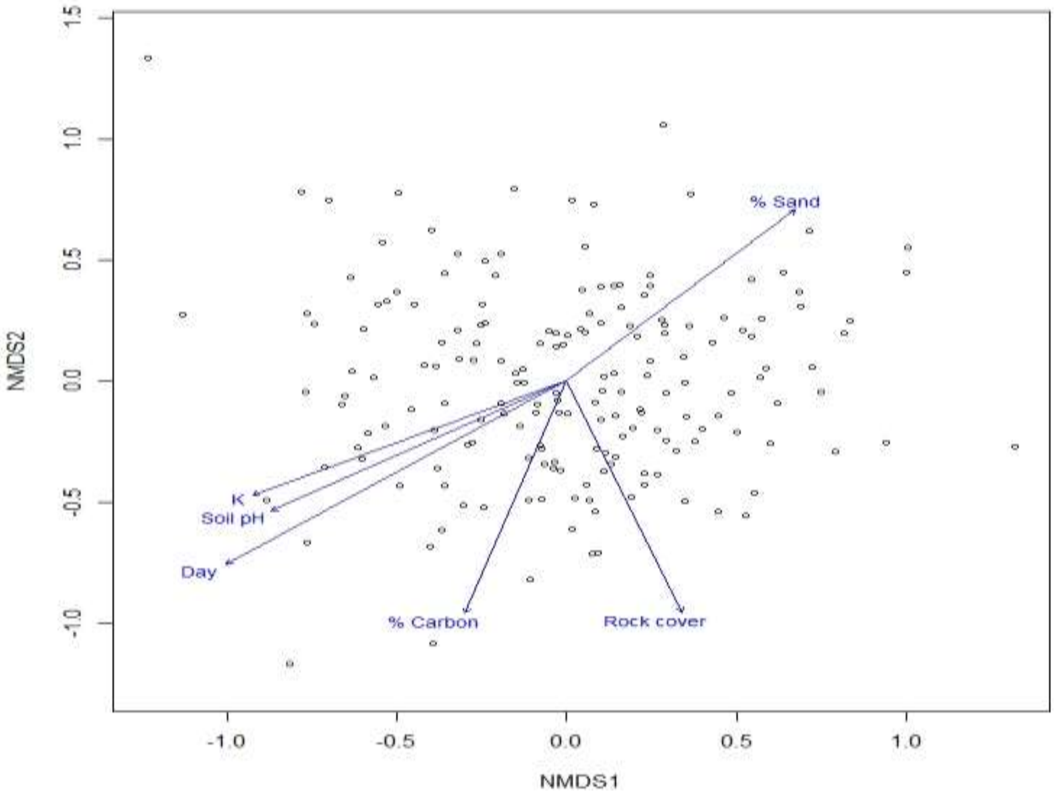


Figure 2.3 Non-metric multidimensional scaling ordination of relevés based on the grass species composition across Lapalala Wilderness Nature Reserve. Only the variables correlated strongly with species composition (R^2 value > 0.2; all $p < 0.05$) are plotted. Three dimensions were applied to the NMDS to obtain a good representation (stress = 0.182) of the data, with the first two axes plotted here. K = Soil potassium (K) content and Day = Day of year.

Species-level functional traits

Intra-specific variation in SLA was generally relatively well explained by multivariate models (range of marginal R^2 = 0.1- 0.6; Table A10). However, ITV in SLA for two species, *Eragrostis rigidior* and *Schmidtia pappophoroides* (the latter being the most common and widespread grass species across LWNR) was not related to any of the environmental variables, with the null model containing only the random effect being ranked as the best model. Environmental variables which were included in the top models for SLA at the species level always had the same relationship with all species. For example, the relationship between woody debris and SLA was positive for *Aristida congesta* and *E. rigidior* (Table 2.1). Indeed, woody debris was the only variable that was related to SLA for multiple species and was also related to CWM_{SLA} (with positive effects on SLA at both the species and community levels; Table 2.1).

Models for ITV in LDMC on average included more variables than the models for SLA and performed slightly better (range of marginal R^2 = 0.11 – 0.72; Table A11). Four environmental variables (i.e. bare ground, rock cover, day of year and soil K) were related to LDMC for multiple species and $\mathrm{CWM}_{\mathrm{LDMC}}$ (although only day of year showed a consistently positive effect across the species- and community-level models; Table 2.1). Across species, the effect of environmental variables on LDMC was not as uniform as for SLA. For example, the relationship between rock cover ITV in LDMC for *A. congesta, Aristida stipitata, E. rigidior,* and *P. maximum* was positive but negative for *L. simplex and Setaria sphacelata* (Table 2.1).

Table 2.1 Summary of predictor variables included in the top-ranked multivariate model for each response variable at the community-and intra-specific level. Green shading indicates variables that affected all community-level response variables. Yellow shading indicates variables that impacted SLA at the community- (CWM$_{SLA}$) and species-level. Blue shading indicates variables that impacted LDMC at the community- (CWM$_{LDMC}$) and species-level. Only variables included in the top-ranked model for each response are shown. TWI = Topographical wetness index, PSR = Potential solar radiation, Soil K = soil Potassium content, Soil P = soil Phosphorous content, Soil C = soil Carbon content,% Sand = Sand fraction, U = valley-shaped quadratic relationship, ∩ = hump-shaped quadratic relationship, - = negative linear relationship, + = positive linear relationship. Ari con = *Aristida congesta,* Ari sti = *Aristida stipitata,* Dig eri = *Digitaria eriantha,* Era gum = *Eragrostis gummiflua,* Era rig = *Eragrostis rigidior,* Lou sim = *Loudetia simplex,* Pan max = *Panicum maximum,* Sch pap = Schmidtia pappophoroides and Set sph = *Setaria sphacelata.*

	TWI	**Curvature**	**Slope**	**PSR**	**Bare ground**	**Rock cover**	**Woody debris**	**Woody cover**	**Day of year**	**% sand**	**Soil K**	**Soil P**	**Soil C**
Response							**Community-level**						
CWM$_{SLA}$					+		+	+		U	U		
CWM$_{LDMC}$					-	U		U	+		∩		
Grass cover					-	-		-	+		+		
							Intra-specific level						
SLA													
Ari con							+						
Ari sti		+									-		
Dig eri	+												
Era gum													
Era rig							+						
Lou sim	+											-	
Pan max							U		-				
Sch pap													
Set sph							+						
LDMC													
Ari con					-	+	-	+	+		U	+	
Ari sti						+		-	+		U	+	
Dig eri	-		+	-					+	-	+	-	-
Era gum									+		-	+	
Era rig						+	-		+		-	-	
Lou sim			+			-			+		-	+	+
Pan max					-	+		-	+	+	U	-	+
Sch pap			+		+						∩	U	
Set sph						-	-		+		∩	+	

Discussion

This study demonstrates that a limited set of environmental conditions are consistently influential for multiple grass characteristics at both the community- and species-level, and that the majority of predictor variables considered in this study (all of which are ecologically relevant) are not widely or consistently related to variation in a savanna grass assemblage. These findings also demonstrate how community- and species-level variation in PFTs can show opposite responses to certain environmental variables, illustrating how trends in the community-level expression of traits may be comprised of several contrasting species-level patterns. Specific leaf area and LDMC tended to show opposite responses to environmental variables (in agreement with, e.g. Wellstein et al., 2013).

Drivers of community-level characteristics

Soil K was the only consistently influential predictor of variation in all four community-level characteristics (CWM_{SLA}, CWM_{LDMC}, grass cover and species composition). Potassium is an important macro-nutrient required for photosynthesis (Prajapati and Modi, 2012) and the positive relationship between soil K and CWM_{SLA} observed here likely reflects how SLA is directly associated with photosynthetic rate (Wellstein et al., 2013), while the negative relationship with CWM_{LDMC} may be due to lower LDMC values being associated with a reduced need for resource conservation under improved photosynthetic rates (Van Arendonk and Poorter, 1994; Wilson et al., 1999a; Pérez-Harguindeguy et al., 2013). The availability of K to plants is a limiting factor to primary production of grasses (Hejcman et al., 2013; da Silveira Pontes et al., 2015), with soil K also being positively related to grass canopy cover.

In addition to soil K, there were two other soil properties, soil pH and sand fraction, that were strongly related to grass characteristics. Soil pH is a good proxy for soil nutrient availability (Alam et al., 1999) which may impact various leaf traits (Dwyer et al., 2014; Dong et al., 2020). Soil pH was strongly related to grass species composition in this study and exhibited a positive relationship with CWM_{SLA} and a negative correlation with CWM_{LDMC}. This agrees with findings for grass species globally (Jardine et al., 2020), Australian woody plants (Dwyer et al., 2014), and across central African vegetation types (only for LDMC; Schellenberger Costa et al., 2017). However, despite soil pH being most strongly correlated with CWM_{SLA} during univariate analysis, it was not included in any of the best subsets models after the multivariate analysis, possibly due to being fairly strongly correlated with sand fraction. Sand fraction can also be a proxy for soil nutrient availability and soil water retention (Nath, 2014), and the negative relationship between CWM_{SLA} and sand fraction observed here agrees with the findings of (Jardine et al., 2020) for grasses at a global scale. These results, therefore, again reflect the importance of considering soil properties, including nutrient availability, when examining spatial variation in grass assemblages (Nixon and McMillan, 1964; Hufford et al., 2014; Jardine et al., 2020).

The date of sampling was related to CWM_{LDMC}, grass cover and species composition. Due to a short growing season in the summer of 2018/2019, sampling started soon after the first rains and continued until the grasses started to senesce. The increase in CWM_{LDMC} through the growing season was therefore expected since tissue density (which is positively correlated with LDMC; Duru et al., 2008) increases in grasses until the full expansion of a plant's inflorescence. The date of sampling also had a positive relationship with grass canopy cover, reflecting the accumulation of grass biomass through the growing season, and was the strongest driver of grass species composition, potentially reflecting species-specific growth rates at the start of the growing season.

Bare ground and rock cover were consistent predictors of three community characteristics each: bare ground = CWM_{SLA}, CWM_{LDMC}, canopy cover; rock cover = CWM_{LDMC}, canopy cover and species composition. Bare ground relates to open space, possibly opened through disturbances, and available resources and it is, therefore, expected that species growing in areas with high bare ground cover would display high SLA and low LDMC to allow for fast growth and utilization of resources (Wilson et al., 1999a; Pérez-Harguindeguy et al., 2013). However, the opposite may equally be true where bare ground results from stressful environmental conditions, where tough leaves would allow for survival (Pérez-Harguindeguy et al., 2013; Wellstein et al., 2013; Deléglise et al., 2015). Our results, specifically the positive correlation between bare soil and CWM_{SLA}, favour the former interpretation. In contrast, higher rock cover appears to be an indication of more stressful environments (e.g. via thinner soils and more extreme microclimatic conditions; Li et al., 2014), as rock cover was positively related to CWM_{LDMC}. Interestingly, rock cover had an independent effect from the other significant predictors (and explained the greatest proportion of variation) on grass species composition, suggesting that high rock cover is possibly associated with unique micro-habitat conditions that support unique combinations of grass species. Woody plant cover was the final consistent driver of three grass community characteristics (CWM_{SLA}, CWM_{LDMC} and canopy cover), and the positive relationship between woody cover and SLA was expected since thinner leaves (i.e. higher SLA) provide a competitive advantage in low light conditions (Wellstein et al., 2013). Low light conditions under the canopy of taller woody species would also be expected to decrease biomass production and, therefore, grass canopy cover.

Water availability was expected to be related to PFTs (particularly SLA; Dwyer et al., 2014; Jung et al., 2014; Chen et al., 2019), but none of the variables in this study that are proxies for water availability (including TWI and curvature) showed any significant relationships with CWM_{SLA}. This is in contrast to research that, for example, has shown that across broad scales precipitation is positively related to CWM_{SLA} of native grass species (Sandel and Low, 2019). However, several variables potentially related to lower evaporative rates and greater soil water-holding capacity were, nonetheless, positively related to $\mathrm{CWM}_{\mathrm{SLA}}$. Woody plant cover and woody debris could influence the soil water holding capacity, potentially leading to their positive relationship with $\mathrm{CWM}_{\mathrm{SLA}}$ (Dwyer et al., 2014; Jardine et al., 2020). In contrast, higher soil carbon content would be expected to enhance soil water holding capacity, however, it was not found to have a significant effect on $\mathrm{CWM}_{\mathrm{SLA}}$. These results suggest an incomplete understanding of how moisture-related variables impact community-level grass characteristics and point to the value of collecting soil moisture data whenever possible (Fay and Schultz, 2009).

Drivers of ITV

Intra-specific trait variation of SLA was consistently influenced by only one environmental variable, there was however consistency in the lack of multiple environmental variables being influential drivers of ITV in SLA. The ITV of LDMC was consistently influenced by multiple environmental variables which showed to have more idiosyncratic relationships when compared to ITV of SLA. Specific leaf area is a very plastic trait, which could explain the weak correlation between SLA and environmental variables at the species level (comparable with the results of; Jardine et al., 2020). Woody debris was the only environmental variable related to the intraspecific variation in SLA of at least two species in the community and the community-level SLA, with the relationship being positive in all three cases. Interestingly, some of the environmental variables that were related to community-level grass characteristics (e.g. bare ground, woody plant cover and potassium content) were not related to SLA at the species level. Conversely, there was a positive relationship between SLA and TWI for two of the most common grass species, *Digitaria eriantha* and *Loudetia simplex*, but no relationship between TWI and SLA at the community level, suggesting that the relationship observed for these two dominant species does not scale up to community-level.

The ITV of LDMC, in contrast, was correlated with more environmental variables and in more species. However, unlike the relatively uniform responses of ITV in SLA, the nature of LDMC-environmental relationships frequently differed between species (in agreement with, for example, Zhang et al., 2020). For example, soil K and P had varied relationships with LDMC (e.g. soil K was positively correlated with ITV in LDMC for *D. eriantha*, but negatively for *L. simplex*), but both of these soil variables were included in all top-ranked ITV models. Indeed, the sign of LDMC-environmental relationships was only consistent for slope (positive in three species) and day of year (positive in eight species).

Comparing LDMC-environment relationships at the community and species levels showed that only one variable, day of year, had the same relationship at both levels, with a constant increase in LDMC as time continues. This pattern is expected since high LDMC is characterized by having large amounts of structural tissues (Dong et al., 2020), which increases in grass leaf material as the growing season continues (Duru et al., 2008). Some other variables were also influential at both the species- and community-level but did not show consistent relationships. For example, there was a positive relationship between *S. pappophoroides* and bare ground, but a negative relationship with CWM_{LDMC} (similar patterns were observed for rock cover and soil K). These inconsistencies between the response of dominant grass species to environmental conditions compared to the responses of the grass assemblage as a whole suggest that community-level shifts along environmental gradients are not necessarily matched by changes in trait expression by all dominant species.

Conclusion

The species-specific relationships grass species show with environmental conditions observed in this study demonstrate the challenge of predicting how grass assemblages vary spatially. Specifically, the lack of consistent drivers of SLA at the species- and community-level points to the need for a more complete understanding regarding the drivers of this trait, particularly due to SLA's relationship with key ecosystem services. More generally, these results also demonstrate that community-level trait variation along a gradient may be comprised of multiple independent (and even inverse) species-level trait responses. As a result, investigating any individual species does not necessarily describe the community-level pattern, but understanding how individual species react to changes in environmental conditions could aid in making predictions of how species, and therefore communities, vary along environmental gradients.

This study provides limited empirical evidence of consistently influential environmental drivers of grass compositional and functional characteristics at the community- and species-level (Kuppler et al., 2020). These findings, therefore, suggest that the environmental variables that are consistently related to grass characteristics (e.g. soil K) should be prioritized when examining grass communities in future studies to determine if this pattern scales up beyond just this study site to other grass-dominated ecosystems. More generally, this study also highlights those measures of soil nutrient availability may need to be considered in future studies focusing on explaining spatial variation in grass characteristics.

Considering that grasses are one of the most widespread plant taxa and contribute strongly to numerous ecosystem services (Gibson, 2009), a deeper understanding of how spatial variation in environmental conditions affect grass species and grass communities is of considerable practical value. Furthermore, the relationship between traits, grass cover and species composition, and ecosystem functioning and services (de Bello et al., 2010; Kardol et al., 2010; Faucon et al., 2017; Zirbel et al., 2017), demonstrates the importance of additionally understanding the relationships between these characteristics and environmental conditions for informing effective management of grassy systems globally. For example, understanding if the correlation between SLA, LDMC and the grazing quality of grasses (see Khaled et al., 2006; Pontes et al., 2007) varies depending on soil conditions, could influence decisions about grazing regimes in grassy ecosystems. Species-specific trait-environment relationships have implications for the role different grass species play within a community and how different dominant species contributes to grazing, forage quality, flammability, and regrowth (Khaled et al., 2006; Pontes et al., 2007; Ripley et al., 2015; Osborne et al., 2018). Ignoring species- and community-level variation in grass assemblages may lead to inaccurate estimates of important ecosystem functions and, therefore, poor implementation of management and conservation efforts in many terrestrial ecosystems. This study and others that quantify similar trait-environment relationships, thus help to provide the understanding necessary for the future practical application of functional traits and other grass community characteristics in the fields of ecosystem functioning monitoring, rehabilitation of degraded land, and grass-dominated habitat conservation.

Appendix A

Table A1 All environmental variables initially considered in this study with bolded variables being used in univariate analyses after the exclusion of highly correlated variables.

Environmental variable	Collection or calculation method	
Altitude	Meters above sea level (handheld GPS)	Recorded during sampling
Bare ground	Visually estimated	Recorded during sampling
Leaf litter	Visually estimated	Recorded during sampling
Woody debris	Visually estimated	Recorded during sampling
Rock cover	Visually estimated	Recorded during sampling
Curvature	Calculated from Digital Elevation Model (DEM). Positive values = convex, negative values = concave and 0 = flat	Calculated after sampling
Topographical Wetness Index (TWI)	Calculated from DEM	Calculated after sampling
Northness	Calculated from aspect1 = North, -1 = South and 0 = East or West	Calculated after sampling
Slope	Calculated from DEM as degrees from horizontal	Calculated after sampling
Potential solar radiation (PSR)	Calculated from DEM	Calculated after sampling
% sand	Sand fraction present in each soil sample (texture determination)	Determined during soil analysis
Soil Clay	Clay fraction present in each soil sample (texture determination)	Determined during soil analysis
Soil pH	The pH of the soil in deionized water with $CaCl_2$	Determined during soil analysis
Soil K	% in soil (i.e. total content)	Determined during soil analysis
Soil P	% in soil (i.e. total content)	Determined during soil analysis
Soil Na	% in soil (i.e. total content)	Determined during soil analysis
Soil C	(% by weight of soil)	Determined during soil analysis
Woody plant cover	Calculated as total the woody plant cover per relevé	Calculated after sampling
Day of year	Calculated as numbers of days from the beginning of the year to date of sampling	Calculated after sampling

Table A2 Rare species with trait data obtained from genus, tribe, or sub-tribe. The mean cover represents the average cover of the species in the relevés where it was present.

Species name	**SLA assigned**	**LDMC assigned**	**Relevés present**	**Mean cover**	**Taxonomic level at which trait was estimated**	**Total individuals**
Andropogon huillensis	17.876	383.706	1	5.750	From genus	27
Anthephora pubescens	20.936	319.146	1	0.125	From sub-tribe	174
Aristida adscensionis	15.875	444.812	1	0.050	From genus	91
Aristida junciformis	15.875	444.812	1	0.017	From genus	91
Digitaria longiflora	20.936	319.146	14	2.463	From genus	174
Digitaria monodactyla	20.936	319.146	1	0.175	From genus	174
Digitaria ternata	20.936	319.146	1	0.025	From genus	174
Elionurus muticus	19.008	391.129	10	0.493	From tribe	51
Eragrostis capensis	15.712	405.550	1	0.075	From genus	178
Eragrostis digitaria	15.712	405.550	2	0.925	From genus	178
Eragrostis inamoema	15.712	405.550	1	0.650	From genus	178
Eragrostis nindensis	15.712	405.550	1	0.050	From genus	178
Eragrostis rehmannia	15.712	405.550	1	0.050	From genus	178
Eragrostis superba	15.712	405.550	11	0.493	From genus	178
Urochloa nigrescens	18.018	355.919	2	0.138	From genus	27

Table A3 All grass species recorded across LWNR, including the number of individuals sampled for PFT's.

Species	Species Abbreviation	Individuals sampled
Andropogon chinensis	And chi	24
Andropogon eucomus	And euc	3
Andropogon huillensis	And hui	0
Anthephora pubescens	Ant pub	0
Aristida adscensionis	Ari ads	0
Aristida congesta	Ari con	36
Aristida diffusa	Ari dif	55
Aristida junciformis	Ari jun	0
Arundinella nepalensis	Aru nep	3
Brachiaria deflexa	Bra def	12
Brachiaria eruciformis	Bra eru	3
Brachiaria serrata	Bra ser	13
Cenchrus ciliaris	Cen cil	12
Chloris virgata	Chl vir	3
Chrysopogon serrulatus	Chr ser	6
Cymbopogon pospischilii	Cym pos	6
Cynodon dactylon	Cyn dac	12
Digitaria eriantha	Dig eri	174
Digitaria longiflora	Dig lon	0
Digitaria monodactyla	Dig mon	0
Digitaria ternata	Dig ter	0
Diheteropogon amplectens	Dih amp	6
Elionurus muticus	Eli mut	0
Enneagopogon cenchroides	Enn cen	12
Enteropogon macrostachyus	Ent mac	6
Eragrostis capensis	Era cap	0
Eragrostis digitaria	Era dig	0
Eragrostis gummiflua	Era gum	80
Eragrostis heteromera	Era het	2
Eragrostis inamoema	Era ina	0

Eragrostis nindensis	Era nin	0
Eragrostis patentipilosa	Era pat	6
Eragrostis racemosa	Era rac	1
Eragrostis lehmannia	Era leh	0
Eragrostis rigidior	Era rig	86
Eragrostis superba	Era sup	0
Eustachys paspaloides	Eus pas	6
Helictotrichon turgidulum	Hel tur	1
Heteropogon contortus	Het con	21
Loudetia simplex	Lou sim	164
Melinis nerviglumis	Mel ner	1
Melinis repens	Mel rep	14
Microchloa caffra	Mic caf	12
Panicum deustum	Pan deu	9
Panicum maximum	Pan max	32
Perotis patens	Per pat	5
Schmidtia pappophoroides	Sch pap	165
Setaria sphacelata	Set sph	40
Sorghum halepense	Sor hal	3
Sporobolus festivus	Spo fes	6
Sporobolus ioclados	Spo ioc	3
Sporobolus panicoides	Spo pan	12
Sporobolus stapfianus	Spo sta	3
Themeda triandra	The tri	9
Tragus berteronianus	Tra ber	21
Urochloa mosambicensis	Uro mos	12
Urochloa nigrescens	Uro nig	0
Urochloa oligotricha	Uro oli	6
Urochloa panicoides	Uro pan	9

Table A4 Univariate modelling results for CWM response variables. Only the univariate environmental variables that significantly explain the variation in CWM trait values are shown (n = 111).

Response	Predictor	Best model	Model R^2	Model p
CWM_{LDMC}	Bare ground	Linear	0.044	0.005
	Rock cover	Quadratic	0.058	0.008
	Topographical wetness index	Linear	0.022	0.048
	Potential solar radiation	Linear	0.024	0.039
	% Sand	Quadratic	0.054	0.007
	Soil pH	Linear	0.044	0.005
	Soil K (%)	Quadratic	0.062	0.003
	Soil P (%)	Linear	0.025	0.035
	Soil Na (%)	Quadratic	0.038	0.032
	Woody plant cover	Quadratic	0.042	0.022
	Day of year	Linear	0.040	0.007
CWM_{SLA}	Bare ground	Quadratic	0.038	0.031
	Leaf litter	Linear	0.044	0.005
	Woody debris	Quadratic	0.054	0.007
	Potential solar radiation	Linear	0.038	0.008
	Sand	Quadratic	0.077	0.001
	Soil pH	Linear	0.133	<0.001
	Soil K (%)	Quadratic	0.103	<0.001
	Soil Na (%)	Linear	0.024	0.037
	Woody plant cover	Linear	0.081	<0.001

Table A5 Univariate modelling results including only the environmental variables which significantly explain the variation in grass percentage cover (n = 180).

Response	**Predictor**	**Best model**	**Model R^2**	**Model p**
Cover	Bare ground	Linear	0.155	<0.001
	Leaf litter	Linear	0.085	<0.001
	Rock cover	Linear	0.033	0.014
	Woody debris	Quadratic	0.049	0.011
	% Sand	Linear	0.034	0.013
	Soil K (%)	Linear	0.080	<0.001
	Soil Na (%)	Quadratic	0.118	<0.001
	Woody plant cover	Quadratic	0.085	<0.001
	Day of year	Quadratic	0.258	<0.001

Table A6 Univariate drivers of SLA and LDMC at the species level from

Species	**Significant**	**Marginal R^2**	**Significant univariate**	**Marginal R^2**
Aristida congesta	Woody debris	0.37	Woody debris	0.30
	Day of year	0.33	Day of year	0.42
			Sand fraction	>0.001
Aristida stipitata				
Digitaria eriantha	Topographical	0.10	Northness	0.007
			Slope	0.11
Eragrostis gumiflua			Day of year	0.35
Eragrostis rigidior	Woody debris	0.15		
Loudetia simplex	Topographical	0.09	Slope	0.09
			Woody plant cover	0.04
Panicum maximum	Soil K (%)	0.05	Soil C	0.45
	Day of year	0.47		
Schmidtia pappophoroides			Bare ground	0.07
Setaria sphacelata	Woody debris	0.4	Woody debris	0.26
	Soil K (%)	0.01		

Table A7 Permutational multivariate analysis of variance (PERMANOVA) results testing the relationship between grass species composition and environmental variables. TWI = topographical wetness index, PSR = Potential solar radiation.

Predictor	**R^2**	**P-value**
Rock cover	0.23	0.001
% Sand	0.21	0.001
Soil pH	0.23	0.001
Soil K (%)	0.24	0.045
Soil C (%)	0.23	0.028
Day of year	0.35	0.001
Altitude	0.07	0.003
Bare soil	0.16	0.001
Leaf litter	0.10	0.001
Curvature	0.02	0.261
Woody debris	0.02	0.138
TWI	0.12	0.001
Northness	0.00	0.832
Slope	0.18	0.001
PSR	0.04	0.02
Soil P (%)	0.01	0.582
Soil Na (%)	0.18	0.001
Woody cover	0.17	0.001

Table A8 All best subset models based on a difference of 2 AIC values for CWM-SLA, all models are significant. + indicates a positive effect and - a negative effect by predictor variables on the response variables. TWI = Topographical wetness index.

Model rank	Predictor	Altitude	TWI	Slope	Bare ground	Bare ground ^{2}	Leaf litter	Woody debris	Woody debris ^{2}	Woody plant cover	% Sand	% Sand ^{2}	Soil K (%)	Soil K^2	Model AIC	Model R^2
	Response CWM$_{SLA}$															
1					+			+		+	-	+	+	-	975.2	0.248
2					+			+		+			+	-	975.3	0.230
3		+			+			+		+	-	+	+	-	976.1	0.252
4		+			+			+		+			+	-	976.2	0.235
5			+		+			+		+	-	+	+	-	976.5	0.250
6					+		+	+		+			+	-	976.6	0.233
7				-	+			+		+	-	+	+	-	976.6	0.250
8					+					+	-	+	+	-	976.6	0.233
9			+		+			+		+			+	-	976.7	0.233
10					+		-	+		+	-	+	+	-	976.7	0.250
11				-	+			+		+			+	-	976.8	0.233
12					+			+	-	+	-	+	+	-	976.8	0.249
13					+			+	-	+				-	976.9	0.232
14					+	+		+		+	-	+	+	-	976.9	0.249

Table A9 All best subset models based on a difference of 2 AIC values for CWM-SLA, all models are significant. + indicates a positive effect and - a negative effect by predictor variables on the response variables. ^{2} represents the quadratic term of the predictor.

Model rank	**Predictor**	**Bare ground**	**Rock cover**	**Rock cover ^{2}**	**Woody plant cover**	**Woody plant cover ^{2}**	**Day of year**	**Soil K (%)**	**Soil K ^{2}**	**Soil P (%)**	**Model AIC**	**Model R^2**
	Response											
	CWM$_{LDMC}$											
1		-	+	-	+	-	+	-	+		1952.8	
2		-	+		+	-	+	-	+		1953.5	
3		-	+	-	+	-	+	-	+	+	1953.9	

Table A10 All best subset models based on a difference of 2 AIC values of SLA at the species level, + indicates a positive effect and - indicates a negative effect on each species SLA. The conditional R^2 is being indicated by the Model R^2 (con) and the marginal R^2 by the Model R^2 (mar) column for each species and model. TWI = Topographical wetness index, ^{2} represents the quadratic term of the predictor. For *E. gummiflua* and *S. pappophoroides*, the null model was the best-ranked model. See Table A3 for full species names.

Model rank	**Predictor**	**TWI**	**Curvature**	**Slope**	**Bare ground**	**Woody debris**	**Woody debris ^{2}**	**Day of year**	**% Sand**	**Soil K (%)**	**Soil K ^{2}**	**Soil P (%)**	**Soil C (%)**	**Model R^2 (con)**	**Model R^2 (mar)**	**Model AIC**
	Response															
	SLA															
1	Ari con					+								0.46	0.37	187.9
2	Ari con					+			-					0.48	0.41	189.3
1	Ari sti		+							-				0.42	0.20	225.2
2	Ari sti		+											0.40	0.17	225.4
3	Ari sti			+						-				0.41	0.22	225.5
4	Ari sti		+	+						-				0.44	0.26	226.1
5	Ari sti			+										0.40	0.17	226.2
6	Ari sti		+	+										0.42	0.22	226.7
7	Ari sti		+							-	+			0.45	0.22	226.8
1	Dig eri	+												0.57	0.10	1065.2
2	Dig eri	+							+					0.58	0.13	1066.09
3	Dig eri	+			+									0.58	0.16	1066.8
1	Era gum													0.47	0.00	382.7
2	Era gum									-				0.49	0.00	386.49
1	Era rig					+								0.42	0.15	553.3

1	Lou sim	**+**					**-**		0.27	0.11	987.4
2	Lou sim	**+**					**-**	**-**	0.28	0.11	987.6
3	Lou sim	**+**						**-**	0.28	0.10	987.8
4	Lou sim	**+**							0.27	0.09	988.5
1	Pan max		**+**	**-**	**-**				0.60	0.60	196.13
2	Pan max		**+**	**-**	**-**	**+**			0.61	0.61	197.95
1	Sch pap								0.21	0.00	950.4
2	Sch pap				**-**				0.22	0.05	952.9
1	Set sph		**+**						0.72	0.40	225.2

Table A11 All best subset models based on a difference of 2 AIC values of SLA at the species level of LDMC at the species level, + indicates a positive effect and - indicates a negative effect on each species LDMC. The conditional R^2 is being indicated by the Model R^2 (con) and the marginal R^2 by the Model R^2 (mar) column for each species and model. TWI = Topographical wetness index, PSR = Potential solar radiation, TWI = Topographical wetness index, ^{2} represents the quadratic term of the predictor. See Table A3 for full species names.

Model rank	Predictor	TWI	Slope	PSR	Bare ground	Rock cover	Woody debris	Woody plant cover	Day of year	% Sand	Soil K (%)	Soil K ^{2}	Soil P (%)	Soil P ^{2}	Soil C (%)	Model R^2 (con)	Model R^2 (mar)	Model AIC
	Response																	
	LDMC																	
1	Ari con				-	+	-	+	+		+	-	+			0.48	0.48	354.1
2	Ari con				-	+	+		+		+		+			0.48	0.48	354.4
3	Ari con				-	+	-	+	+		-		+			0.49	0.49	356.0
1	Ari sti					+		-	+		+	-	+			0.37	0.21	389.5
2	Ari sti							-	+		+	-	+			0.34	0.23	389.5
3	Ari sti					+			+		+	-	+			0.36	0.18	390.2
4	Ari sti								+		+	-	+			0.32	0.19	390.2
5	Ari sti							-	+			-	+			0.30	0.24	390.3
6	Ari sti							-	+		+	-	+			0.33	0.23	390.6
1	Dig eri	-	+	-					+	-	+		-		-	0.69	0.55	1936.1
2	Dig eri	-	+	-					+	-	-	+	-		-	0.69	0.56	1936.5
3	Dig eri	-	+	-				-	+	-	+		+		-	0.69	0.56	1936.8
4	Dig eri	-	+	-		+			+	-	+		-		-	0.69	0.55	1936.9
5	Dig eri	-	+	-		+		-	+	-	+		-		-	0.69	0.56	1937.1
6	Dig eri	-	+	-				-	+	-	-	+	-		-	0.69	0.56	1937.3
7	Dig eri	-	+	-	-				+	-	+		-		-	0.69	0.55	1937.3
8	Dig eri	-	+	-		+			+	-	-	+	-		-	0.69	0.56	1937.4
9	Dig eri	-	+	-	+				+	-	-	+	-		-	0.69	0.55	1937.7

10	Dig eri	-	+	-		+		-	+	-	-	+	-		-	0.69	0.56	1937.8
11	Dig eri	-	+	-	+			-	+	-	+		+		-	0.69	0.55	1937.7
1	Era gum								+		-		+			0.82	0.37	795.4
2	Era gum					+			+		-		+			0.82	0.37	795.9
3	Era gum				-				+		-		+			0.82	0.37	796.0
4	Era gum								+		-	-	+			0.82	0.36	796.4
5	Era gum				-	+			+		-		+			0.83	0.36	796.5
6	Era gum					+			+		-	-	+			0.83	0.36	796.7
1	Era rig					+	-		+		-		-			0.63	0.34	966.0
2	Era rig				+	+	-		+		-		-			0.64	0.34	966.9
1	Lou sim		+			-			+		-		+		+	0.42	0.30	1938.7
2	Lou sim		+		-	-			+		-		+		+	0.42	0.30	1938.8
1	Pan max					+		-	+	+	+	-	-		+	0.71	0.71	332.0
2	Pan max					+			+	+	-	+	-		+	0.70	0.70	333.2
3	Pan max					+		-	+	+	+		-		+	0.72	0.72	333.4
1	Sch pap		+		-						-	+	+	+		0.15	0.13	1977.7
2	Sch pap		+		-						+		+	+		0.15	0.11	1978.8
3	Sch pap		+		-				+		-	+	+	+		0.16	0.13	1979.0
4	Sch pap		+	+	-						-	+	+	+		0.16	0.13	1979.3
1	Set sph				+	-	-		+		-	+	+			0.54	0.36	436.8
2	Set sph			+	+	-	-		+		-	+	+			0.55	0.38	436.8
3	Set sph				+	-	-		+		-		+			0.52	0.35	437.2
4	Set sph			+	+	-	-		+		-		+			0.53	0.37	437.4
5	Set sph					-	-		+		-	+	+			0.52	0.37	437.6
6	Set sph			+		-	-		+		-	+	+			0.52	0.40	437.6

Table A12 All best subset models based on a difference of 2 AIC values of SLA at the species level for grass canopy cover. + = a positive effect, - = a negative effect by predictor variables on the response variables.

Model	**Predictor**	**Northness**	**Bare ground**	**Rock cover**	**Woody plant cover**	**Day of year**	**Day of year ^{2}**	**% Sand**	**Soil K (%)**	**Model R^2**	**Model AIC**
	Response										
1	Grass cover		-	-	-	+			+	0.640	155.4
2			-	-	-	+	-		+	0.669	155.4
3			-		-	+	-		+	0.624	156.3
4			-		-	+			+	0.592	156.4
5		-	-	-	-	+			+	0.647	156.6
6			-	-	-	+		+	+	0.644	156.6
7			-	-	-	+	-	+	+	0.672	156.7
8		-	-	-	-	+	-		+	0.675	156. 8
9			-	-	-	+	-			0.635	156.8

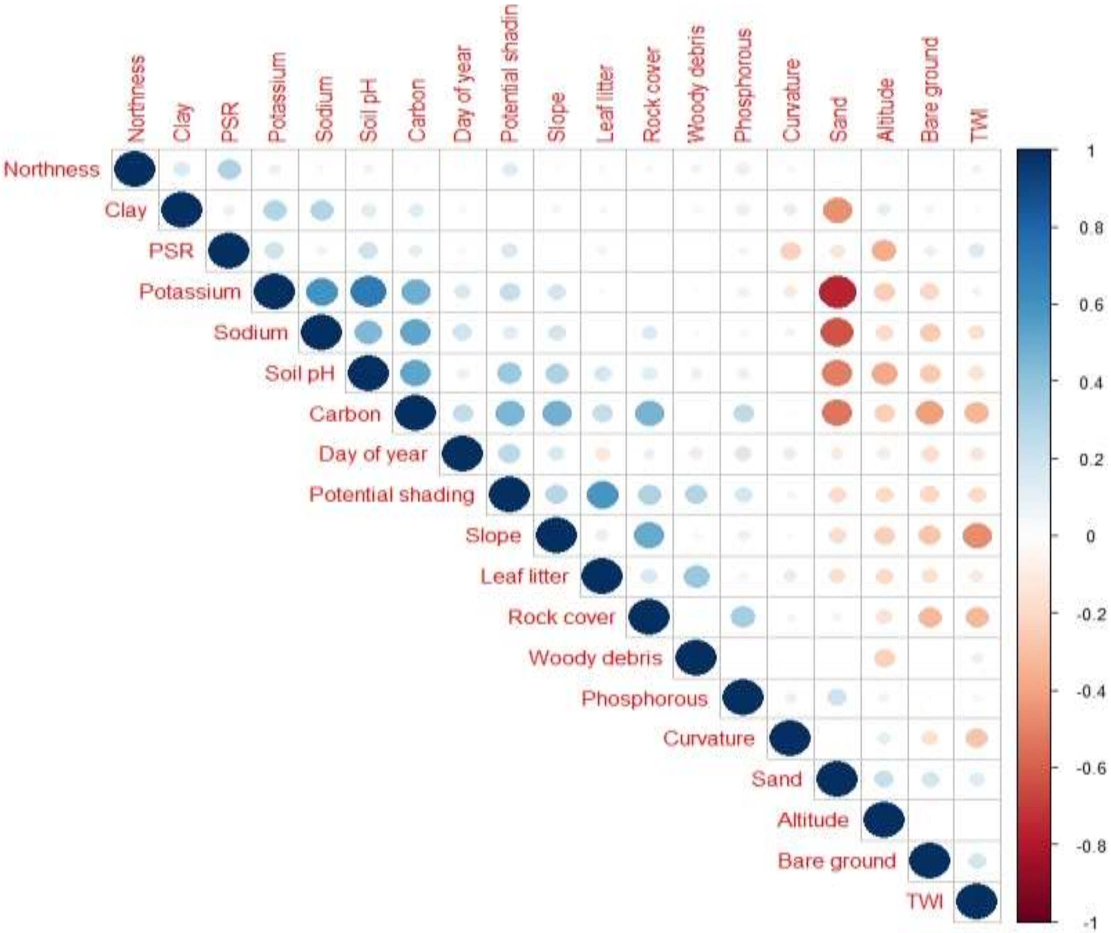


Figure A1 Correlation matrix of all variables used after the removal of highly correlated variables. PSR = Potential solar radiation, Potassium = soil K (%), Sodium = soil Na (%), Carbon = soil C (%), Phosphorus = soil P (%), TWI = Topographical wetness index.

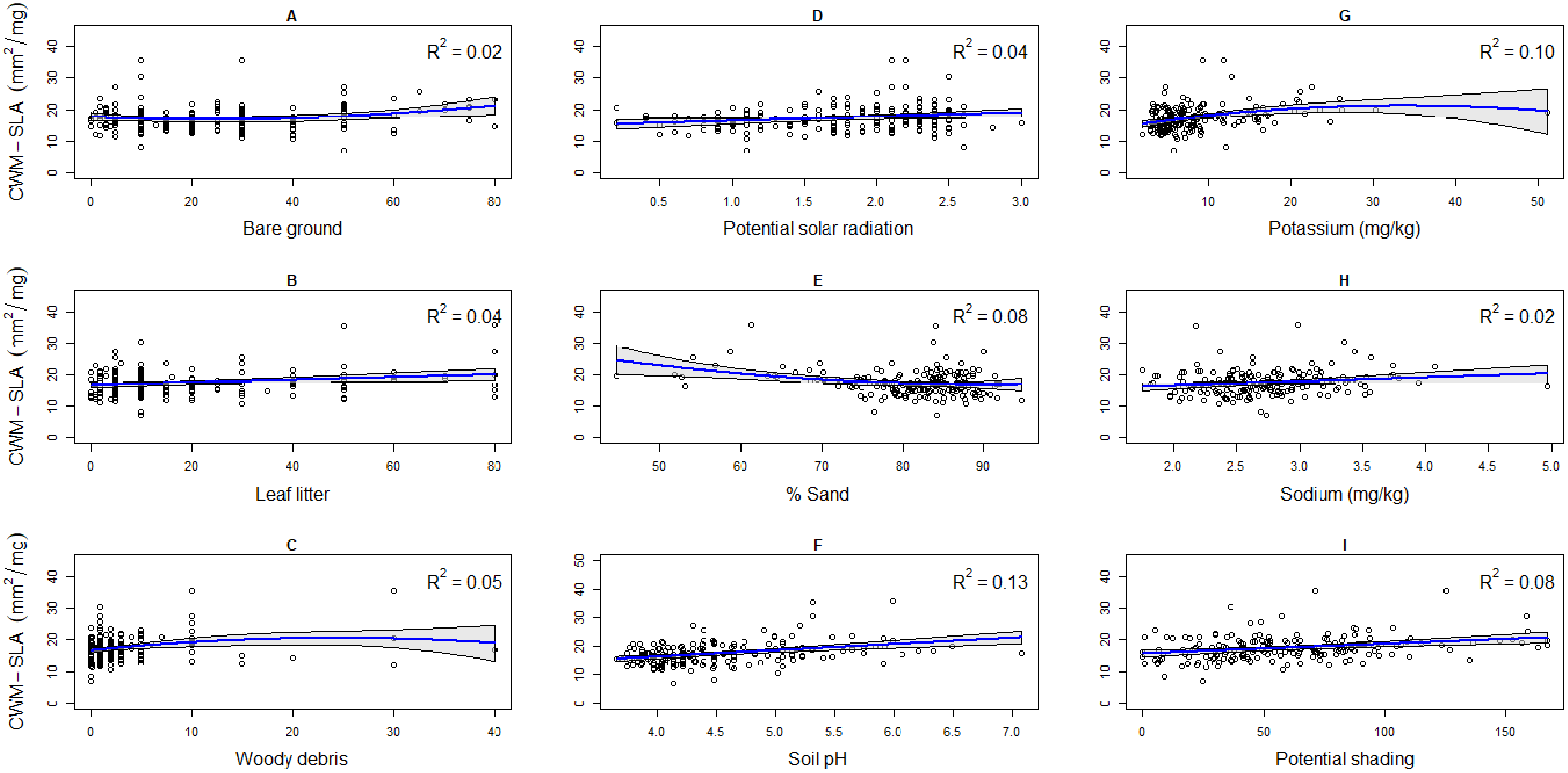


Figure 2A All significant univariate environmental drivers of SLA at the community level. Shaded areas indicate the 95% confidence interval

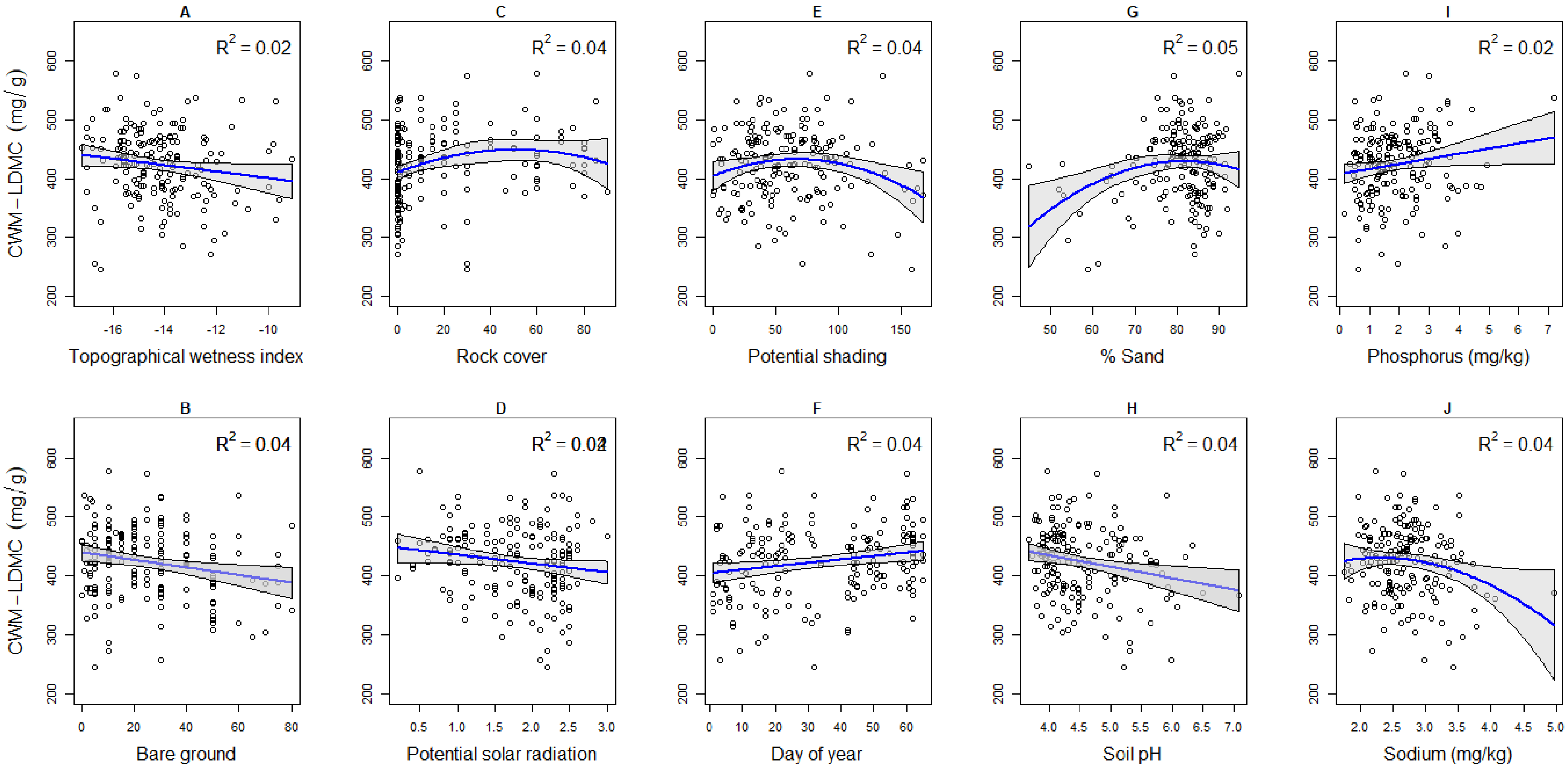


Figure 3A All significant univariate environmental drivers of LDMC at the community level. Shaded areas indicate the 95% confidence interval.

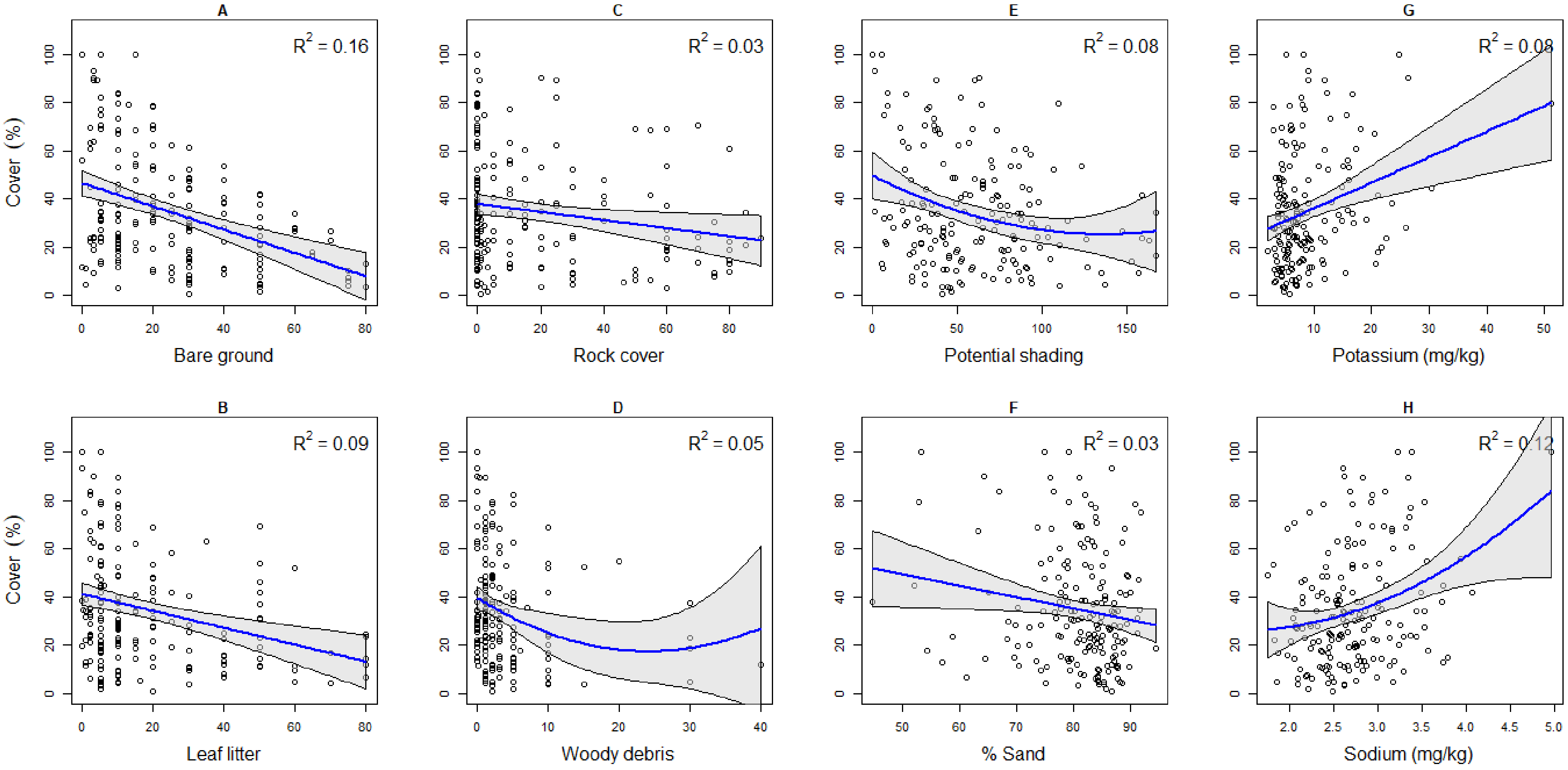


Figure 4A All significant univariate environmental drivers of grass cover at the community level. Shaded areas indicate the 95% confidence interval.

Chapter 3: Lining up variation: community- and species-level drivers of grazing quality and leaf functional traits of savanna grasses

Abstract

Grazing provisioning is a key ecosystem service, largely provided by the grass species that make up the bulk of the herbaceous plant growth in grasslands and savannas. The grazing quality of grasses is typically assumed to vary strongly between, but not within, species and, as a result, species are often uniformly categorized as being of high or low grazing quality. There is, however, an increasing debate about the validity of this approach since many grass characteristics (including plant functional traits; PFTs) vary intra-specifically along environmental gradients. This study, therefore, investigated (a) the inter-specific variation in grazing quality components, (b) the environmental drivers of intra-specific variation in grazing quality, (c) the environmental drivers of variation in grass leaf traits at the species- and community-level and (d) the relationships between PFTs and grazing quality of dominant C4 savanna grasses. This study demonstrated that the relationships between PFTs and environmental variables are typically weak and highly idiosyncratic at both the community- and species-level. Grazing quality differed significantly between grass species, with large proportions of the variation in all grazing quality components being explained by the identity of species. However, within species, few environmental variables explained intra-specific variation in grazing quality. Additionally, plant functional traits were also weakly related to intra-specific variation in grazing quality. This study demonstrates that grass grazing quality and PFTs do not respond consistently to environmental variables frequently quantified in ecological studies and that, contradictory to results from C3-dominated temperate grasslands, leaf dry matter content, specific leaf area and force to tear are not useful proxies of grazing quality. More broadly, this study highlights that there may be considerable intra-specific variation in grass grazing quality, but that this within-species variation is not related to commonly recorded environmental conditions or easily measured plant traits, and, therefore, remains challenging to predict.

Introduction

Grazing provisioning is an important ecosystem service provided by herbaceous primary producers (Augustine and McNaughton, 1998; Van Oudtshoorn, 1999; Bouchenak-Khelladi et al., 2020). Grasses make up the bulk of the herbaceous plant growth in grasslands and savannas and have developed growth forms and structures which allowed these plants to survive defoliation by herbivores (Van Oudtshoorn, 1999; Fish et al., 2015). The relative allocation of resources by grasses into growth (i.e. slow or fast growth rate), biomass production and defence mechanisms affect the grazing quality of grasses (Van Oudtshoorn, 1999; Massey et al., 2007; Fish et al., 2015). Traditionally, grass species have been assigned categorical grazing values (i.e. low, average, and high; Fig. 3.1), with the emphasis being placed on inter-specific differences in grazing quality (i.e. variation between species; Van Oudtshoorn, 1999). Consequently, some grass species are considered “good” for grazing and others not, with all individuals of a species, in general, being assumed to have the same grazing quality irrespective of local environmental conditions and ecotypes (Van Oudtshoorn, 1999; Truter and Venter, 2017; Van Oudtshoorn, 2019). However, there is an increasing debate about the validity of this approach as several grass characteristics (including plant functional traits; PFT’s, and morphology) vary strongly with environmental conditions (e.g. soil conditions, water availability and temperature; Roybal and Butterfield, 2018; Van Oudtshoorn, 2019; Sandel et al., 2021). Therefore, it may be expected that the grazing quality of gasses will also vary intra-specifically along environmental gradients. Indeed grazing quality across species can change along temperature and precipitation gradients and may additionally be influenced by biological factors, such as the developmental stage of grasses (Duru et al., 2008; Craine et al., 2010).

The grazing value of grasses is determined by various factors, including the grass’ nutritional value, ability to regrow after grazing, palatability, leaf production and digestibility of grass biomass (Van Oudtshoorn, 1999). However, grazing quality can typically be expressed by the nutritional value of grasses, often exemplified by three key components: protein content, fibre content and digestibility (Khaled et al., 2006), with digestibility being most often studied (Tasset et al., 2019). The protein content of grass biomass is important for well-maintained herbivore body condition and high protein content is often considered to be easily digested by herbivores (Habermann et al., 2019). The fibre content of grass biomass consists of two sub-components, acid detergent fibre (ADF) which is made up of cellulose and lignin, and neutral detergent fibre (NDF) which is made up of hemicellulose, cellulose, and lignin (Habermann et al., 2019). Neutral detergent fibre is an important component required by ruminants (Ravhuhali et al., 2019), but high concentrations of fibre (both ADF and NDF) reduce the digestibility of grasses (Habermann et al., 2019). At the same time, the protein content of grasses generally decreases with higher fibre content which increases the cut retention time and reduces the voluntary intake of grass material by herbivores (Adler et al., 2004).

Two levels of variation may be expected in grazing quality, with differences in protein and fibre content expected both within- and between species. While the partitioning of the variation in grazing quality between these two components is poorly understood, these two components of variation have been more intensively examined for a different set of plant attributes, plant functional traits (PFTs). Variation in PFTs can be split into differences between species (inter-specific variation; BTV) and differences between individuals of the same species (intra-specific variation; ITV; Siefert et al., 2015) with the former typically accounting for more variation at the community-level than the latter. Plant functional traits such as specific leaf area (SLA) and leaf dry matter content (LDMC) are known to be variable at the inter-and intra-specific level (Garnier et al., 2001), with SLA and LDMC typically showing a negative correlation, and with SLA generally being more variable than LDMC (Wellstein et al., 2013). Spatial variation in grass SLA has recently been linked to climatic (Sandel and Tsirogiannis, 2016; Roybal and Butterfield, 2018; Sandel et al., 2021) and edaphic variables (Jardine et al., 2020). Spatial variation in other less often investigated PFTs for example, force to tear (FT) have also shown relationships with soil nutrient levels (Bouchenak-Khelladi et al., 2020).

Given the high cost of measuring grazing quality components directly (e.g. via laboratory-based chemical analyses), identifying easily measured grass characteristics that can act as proxies for grazing quality would be of practical value. Given the utility of PFTs for predicting a range of species- and community-level ecological characteristics and processes (Sandel et al., 2021; Westerband et al., 2021), it might be possible that plant leaf traits could be correlated with variation in grazing quality (at the intra- and/or inter-specific level). However, despite some initial studies, it is still unclear whether grass PFTs are consistently related to grass grazing quality. Relationships between digestibility, LDMC and leaf nitrogen content (LNC) of grasses at the inter-specific (Khaled et al., 2006; Pontes et al., 2007; Duru et al., 2008; Andueza et al., 2010; Tasset et al., 2019) and community-level (Gardarin et al., 2014) have been demonstrated. However, these studies have all been conducted in wetlands and French grasslands, both of which represent a small fraction of the world's grassy ecosystems and are dominated by C3 grasses whereas tropical and subtropical savannas and grasslands are dominated by C4 grasses (Ratnam et al., 2011). These studies focused on the relationships between PFTs, digestibility and palatability, while little attention has been given to grazing quality components such as protein content and fibre content.

Specific leaf area and LDMC have the greatest potential to act as proxies for grazing quality and has previously been linked with the digestibility of grasses (Khaled et al., 2006; Pontes et al., 2007; Duru et al., 2008; Andueza et al., 2010; Tasset et al., 2019). For example, plants with high SLA (and low LDMC) values have increased digestibility in French grasslands (Khaled et al., 2006; Pontes et al., 2007; Duru et al., 2008; Andueza et al., 2010; Tasset et al., 2019). Additionally, SLA and LDMC are often correlated with resource use of plants (Garnier et al., 2001), with species that have high SLA and low LDMC expected to have high grazing quality due to low concentrations of fibre, cellulose, and hemicellulose (Wilson et al., 1999a; Wright et al., 2001; Khaled et al., 2006; Pontes et al., 2007; Pérez-Harguindeguy et al., 2013; Whitworth-Hulse et al., 2016). Indeed, it has also been shown that there is a positive relationship between SLA and grazing intensity (i.e. the level of herbivory in an area), while the relationship between LDMC and grazing intensity is negative (Peco et al., 2005; McIntyre, 2008; Saatkamp et al., 2010; Niu et al., 2016). This suggests that plants with high SLA and low LDMC are prone to grazing, but that the high growth rates associated with these trait levels enable these plants to compensate for high defoliation rates (Westoby, 1998).

Another trait potentially related to grazing quality is the mechanical strength of leaves, which can be measured as the force required to tear a leaf (FT). Force to tear is positively related to the physical strength of leaves and the ability of leaves to resist mechanical damage (such as herbivory; Wright and Cannon, 2001; Pérez-Harguindeguy et al., 2013). Additionally, high leaf toughness is generally positively related to fibre content and therefore, decreased nutritional value to herbivores (Coley, 1983). Indeed, leaf toughness (as measured through FT) has recently been related to decreased grazer densities (Bouchenak-Khelladi et al., 2020) and increased selectivity for less tough individuals by invertebrate herbivores and sheep (Cornelissen et al., 1999; Cingolani et al., 2005). However, FT has not often been linked to the grazing quality of grasses (i.e. protein content, digestibility, and fibre content).

Therefore, this study will investigate (a) inter-specific variation in grazing quality components of 12 dominant savanna grass species (b) the environmental drivers of variation in the grazing quality of these grasses, (c) the environmental drivers of species- and community-level variation in grass leaf traits, and (d) the relationships between PFTs and the components of grazing quality.

Methods

Study area

Welgevonden Game Reserve (WGR) is a 36,000-ha reserve situated in the Vaalwater district (24°10’S 27°45’E, Fig. 3.1; Zwerts et al., 2015) in the Limpopo province of South Africa. The topography of WGR consists of hills with occasional flat plateaus and open plains (Thompson et al., 2016). The geology of WGR is made up of the Waterberg group consisting mainly of coarse red clastic strata (Bumby, 2000). Welgevonden Game Reserve receives an annual rainfall of c. 620 mm, with the majority of this rain falling during the summer months (Zwerts et al., 2015). The reserve has an elevation gradient spanning from 1200 m a.s.l. in the north to 1450 m a.s.l. in the south. The vegetation of WGR is largely classified as Waterberg Mountain Bushveld (i.e. savanna vegetation), although WGR also contains areas of more open Waterberg Magaliesberg Summit Sourveld (i.e. grassland) at higher elevations (Mucina and Rutherford, 2006). The ground layer vegetation of WGR consists mainly of tall-growing grass which is considered to have low nutrient levels due to the acidic soils of the reserve (Thompson et al., 2016). However, WGR does support various large-bodied grazers and mixed feeders such as Plains Zebra (*Equus quagga*), Blue Wildebeest (*Connochaetes taurinus*), Eland (*Tragelaphus oryx*), Cape Buffalo (*Syncerus caffer*), White Rhinoceros (*Ceratotherium simum*) and African Elephant (*Loxodonta africana).*

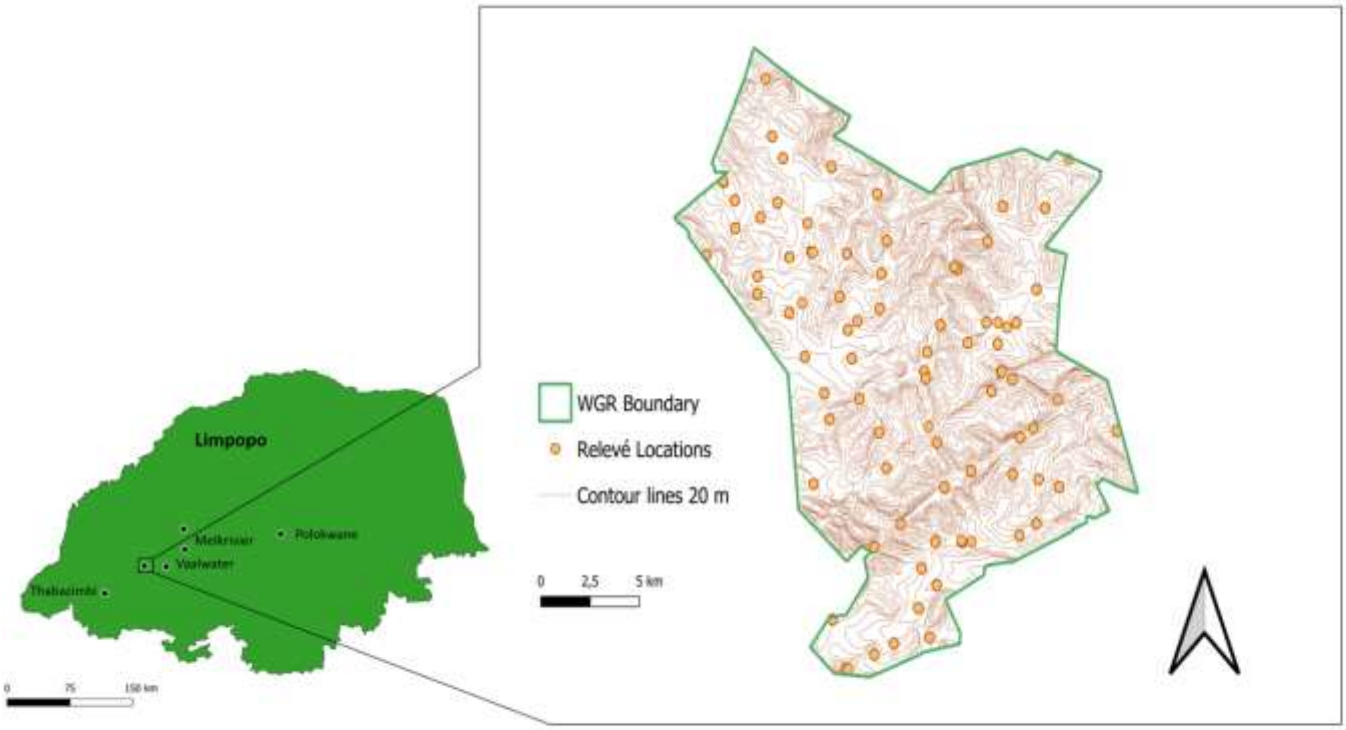


Figure 3.1 Location of Welgevonden Game Reserve (WGR) in the Limpopo province of South Africa, with the inset showing the border of the reserve and the locations of all the study’s relevés (n = 65). Contour lines are shown at 20 m intervals.

Data collection

Of the 120 permanent transects used by WGR management for vegetation monitoring, a subset of 65 locations were selected by spatial thinning (i.e. excluding c. half of the transect lines that were closest to each other), repeating this process within each of the six vegetation units identified by WGR management. The number of transects surveyed per vegetation type was proportional to the cover of each vegetation type in the reserve. Non-metric multidimensional scaling (i.e. NMDS = stress 0.25) was subsequently used to analyse grass species composition data collected in 2019 by WGR management from each of these transects. Transects underwent a second selection process where transects with very similar species composition were additionally excluded (to maximize the range of environmental conditions sampled). The final selection of transects provided a good representation of the grass species compositional space (Fig. B1), and these transects were then identified as locations to survey the relevés used in this study. Four ad hoc sites were additionally surveyed based on observations in the field which suggested that these sites were different in terms of species composition and/or environmental conditions compared to the other sites.

At each of the 61 selected transects, as well as at the four ad hoc sites, a 20 × 20 m relevé was surveyed with the GPS coordinates for the transect being used as the southwestern corner of the relevé (Fig. 3.1). Coordinates of each corner and the centre of the relevé were recorded using a Garmin eTrex 30 (Garmin International, Kansas City USA) handheld GPS. Soil samples were collected close to the four corners of each relevé. After the removal of organic material at the soil surface, soil from the four corners was pooled to make up a c. 1 kg sample per relevé. Soil depth was recorded as the average depth that a metal rod with a 1 cm diameter could be inserted into the ground by hand at three random locations within each relevé.

All grass and tree species rooted within each relevé were identified and the % canopy cover of each species was visually estimated. Additional environmental variables were recorded for each relevé in the field with rock cover, bare soil cover, woody debris cover, leaf litter cover and woody canopy cover (i.e. %) being visually estimated (Table B1). Dung counts of bulk grazers (White rhino and Plains Zebra) were conducted in each relevé, and a grazing score was assigned to each relevé based on the level of grazing observed on the grasses in the relevé (score of 0 = no signs of grazing and 5 = very high levels of grazing observed). Both of these parameters were used as proxies for the level of grazing taking place in each relevé but were weakly correlated (Spearman rank coefficient = 0.19, p = 0.13; Fig. B2). The sampling date for each relevé was calculated as the number of days since the first day of sampling (1 February 2021 = 1, 9 March =36).

GIS and soil data

The ASTER Global Digital Elevation (DEM) Model V003 with a 30 m resolution (https://doi.org/10.5067/ASTER/ASTGTM.003) was used to calculate GIS-derived variables for WGR through the use of QGIS 3.12 (QGIS.org, 2021). The elevation of the centre coordinates for each relevé was extracted from the DEM. The Topographic Wetness Index (TWI) was calculated using the Topographic Wetness Index tool (SAGA-GIS; Conrad, 2003), and the slope, aspect, and curvature of each relevé were calculated using the Slope Aspect Curvature tool (SAGA-GIS; Conrad, 2001). The northness of each relevé was calculated by using the aspect obtained from the DEM (following; Bader and Ruijten, 2008). Potential Direct Incidence Radiation (PDIR) was calculated from each relevés latitude, slope, and aspect (following; McCune and Keon, 2002; McCune, 2007).

Soil samples were air-dried and passed through a 2 mm sieve. Each soil sample was then analysed to determine texture through the standard hydrometer method (following; Kroetsch and Wang, 2008), pH using the H_2O method (following; AgriLASA, 2004), concentrations of Ca, K, Mg, Na, and P through the Melich-3 extraction protocol (following; Ziadi and Tran, 2007a), and soil carbon (C) content via titration (following; Ziadi and Tran, 2007b).

Grazing quality and PFT data

Grass species in each relevé with at least 5 % canopy cover were sampled for grazing quality analysis, and species with at least 12 replicates across WGR were subsequently selected (resulting in 183 biomass samples from 12 species). Entire individual plants were cut at c. 7 cm above the ground, and culms and inflorescence were excluded from each sample by cutting the culms above the foliar biomass. Biomass samples were collected per species and dried at 60˚C for 48h (following; Deléglise et al., 2015), after which each sample was milled to 1 mm particle size using a Retsch cyclone mill (Retsch GmbH, Haan Germany). All samples were subsequently subjected to four Near-infrared Reflectance Spectrophy (NIRS) scans using a Perten DA 7250 NIR analyser (PerkinElmer Billerica, Massachusetts USA) operating at 750 – 2000 nm. Average values for the grazing quality components: % ADF, % NDF, protein content, % Ca, % P, and % fat was calculated from the four scans. Eight samples per species were used to conduct wet chemistry analysis performed by the African Forage, Fodder, Feed and Food Quality Reference Laboratory (AF_4RICA LAB) at the University of Pretoria to confirm the values obtained for grazing quality components by the NIRS analysis. The filter bag technique was used for the determination of ADF (Ankom, 2017) and NDF (Ankom, 2014). Protein content was determined from N content (obtained by the Dumas method; Horneck and Miller, 1997). The % Ca and % P was obtained by following the (perchloric acid method; Miller and Kalra, 1998). The % fat was determined using the high-temperature solvent extraction procedure (AOCS, 2009). Of the six grazing quality metrics, three were considered as core proxies of grazing quality (NDF, ADF and protein content) based on previous studies (Khaled et al., 2006; Tasset et al., 2019).

Grass species with at least 5% cover in each relevé were also sampled for the measurement of leaf functional traits. Individuals of rarer species were also opportunistically sampled, resulting in a final data set with PFT measurements for 1020 individuals of 62 species (Table B2). Three individuals per species were sampled within a relevé for PFT's by collecting leaves from the basal biomass of each individual, although, for annual grass species (e.g. *Setaria pumila*) leaf material was collected from the grass culm. Leaf material was wrapped in a damp tissue paper at the base of the leaf sheath and stored in a dark cooler box to allow for rehydration and maintained freshness (following; Pérez-Harguindeguy et al., 2013). Two leaf blades per individual with no clear pathogen or herbivore injury were selected, with a section of approximately 5 cm in length cut from the centre of one leaf blade per individual used for the measurement of PFT's. For grass species with leaves smaller than 5 cm in length (e.g. *Microchloa caffra*), the entire leaf was used (excluding the ligule and the sharp tip to allow for more accurate area calculations. The leaf section cut from the first leaf blade of each individual was weighed using a Precisa 12100 D SuperBal-series (Precisa gravimetrics, Dietikon Switzerland) balance (1 mg accuracy) to determine fresh mass. A one-sided scan of each leaf section was conducted using an HP OfficeJet Pro 8710 (HP development company, Palo Alto CA USA) flatbed scanner (1200 dpi resolution) to determine the fresh leaf area using ImageJ (Abràmoff et al., 2004). Each leaf section was then dried at 60˚C for 48h (i.e. until a constant weight was reached), and the oven-dried weight of each segment was subsequently obtained using a Precisa XB 160M (Precisa gravimetrics, Dietikon Switzerland) balance (1mg accuracy). Some leaf segments (c. 40 out of 1020) were reweighed with a more sensitive Sartorius M-power (Sartorius AG, Goettingen Germany) balance (0.1 mg accuracy) due to the sample's dry mass being< 1 mg. For each leaf segment, LDMC was calculated as the oven-dried weight divided by the fresh weight (mg g^{-1}) and SLA which was calculated as the fresh leaf area divided by the oven-dried weight (mm mg^{-1}). The second leaf blade of each individual was used to measure the leaf width (LW) at approximately the middle point of the leaf blade using digital vernier callipers. Each leaf blade was subsequently subjected to a longitudinal pulling force to obtain the peak breaking force measured by a Sauter RS-232 FH 10 (Sauter international, Balingen Germany) force gauge (maximum force 10 N). Samples that broke at a force greater than the upper limit of the force gauge (c. 385 out of 1020) were assigned the highest peak breaking force (i.e. 10.97 N) obtained for a leaf blade before the upper limit of the force gauge was reached. The force to tear was calculated as the peak breaking force divided by the leaf width (i.e. N mm^{1}). All PFT's were measured and calculated following standard protocols (Pérez-Harguindeguy et al., 2013).

Community-level metrics calculations

The community weighted mean (CWM) was calculated for each PFT in each relevé by multiplying the average trait value of each grass species by the relative cover of each species for each relevé. For grass species for which no trait data were collected (17 species across WGR), trait values were estimated from the average trait value of the genus, tribe, subtribe, or subfamily the species belongs to (using the lowest possible taxonomic rank possible; Table B3). These 17 species were found in only 2 % of the 65 relevés and had a mean cover of 1.2 % in these relevés (Table B3).

Statistical analysis

Highly correlated environmental variables and highly correlated grazing quality components were identified and excluded from further analyses (Fig. B2 and Fig. B3). Linear models were used to test the relationships between all grazing quality components and species identity. A principal component analysis (PCA) was conducted to visually compare the grazing quality of the 12 most common grass species.

Due to a large set of potential predictor variables (24 variables; Table B1), a two-step model building process was used to identify environmental variables most strongly correlated to variation in grazing quality and leaf traits at the community- and species-level. Univariate generalized linear mixed effect models were first used to test the relationship between each environmental variable (Table B1) and the core grazing quality components (i.e. ADF, NDF and protein content). Species identity was included as a random effect (to account for multiple samples of the same species being collected). Univariate generalized linear mixed effect models were also used to test the relationship between each environmental variable (Table B1) and PFTs (i.e. LDMC, SLA and FT) for the 10 most common grass species (i.e. each with at least 30 leaf trait measurements across WGR) and each environmental variable (Table B1). The relevé identity was set as a random effect (to account for multiple samples being collected from relevés). For each univariate analysis, the null model (including only the random effect), a linear model and a quadratic model were compared for each environmental predictor variable, with the best model (i.e. null, linear, and quadratic) identified by the lowest Akaike's Information Criterion (AIC) score. Multivariate generalized linear mixed effect models were used next to test the relationships between all the variables where a linear or quadratic model performed better than the null model and each grazing quality component or PFT (with quadratic terms included for predictors based on the univariate analysis results). Due to a large number of variables being selected as influential predictors of grass protein content during univariate analyses, variables with R^2 values less than 0.013 were additionally excluded from multivariate models.

This same two-step model building approach was also used to analyse the relationships between the environmental variables and the CWM values of each leaf trait (using generalized linear models due to the lack of random effects in these models).

Finally, linear mixed-effect models were used to test the relationships between core grazing quality components and PFTs (LDMC, SLA and FT), while including species identity as a random effect. All statistical analyses were conducted using R version 4.0.2 (R Core Team, 2020), including functions from the car (version 3.0-9; Fox and Weisberg, 2019), lme4 (version 1.1-23; Bates et al., 2015), MuMIn (version 1.43.17; Barton, 2020), lmerTest (version 3.1-2; Kuznetsova et al., 2017), ggplot2 (version 3.3.5; Wickham, 2011), ggfortify (version 4.12; Tang et al., 2016), ggpubr (version 0.4.0; Kassambara and Kassambara, 2020) and vegan (version 2.5-6; Oksanen et al., 2019) libraries.

Results

Grazing quality differs between grass species

The core grazing quality components differed significantly between species (Table 3.1), with large proportions of variation being explained by species identity (R^2 = 0.51 – 0.95). However, when considering all grazing quality components simultaneously, most grasses did not differ strongly in terms of grazing quality components with most of the species being clustered together with similar grazing qualities (Fig. 3.3). Of the 12 dominant species examined, four species were distinct in terms of their grazing quality: *Cynodon dactylon* had higher fat content and lower fibre content than the other species, while *Digitaria eriantha* had high protein, Ca, and P content. In contrast, *Eragrostis gummiflua* and *Schmidtia pappophoroides* displayed higher ADF and NDF content than the majority of the other species.

Grazing quality is weakly related to environmental variables

Environmental variables generally had weak relationships with the core grazing quality components. The best multivariate models of grass ADF and NDF included just soil C which explained only 0.5 % and 1.8 % of the variation in ADF and NDF, respectively (Table 3.2; see Table B4 for univariate model results). The best multivariate model for grass protein content included four environmental variables (soil pH, soil C, soil Ca and clay content), with only soil Ca having a positive relationship with protein content and soil pH, soil C and clay content having negative relationships with protein content . This model explained 10.6 % of the variation in this component of grazing quality. None of the core grazing quality components had significant relationships with any of the PFTs measured in this study at the species level (Fig. 13B).

Table 3.1 Grazing quality components differed between species. ADF = acid detergent fibre and NDF = neutral detergent fibre.

Predictor	Response	F value	R^2	P-value
Species identity	ADF	159.2	0.905	<0.001***
	NDF	38.1	0.692	<0.001***
	Protein content	21.1	0.549	<0.001***

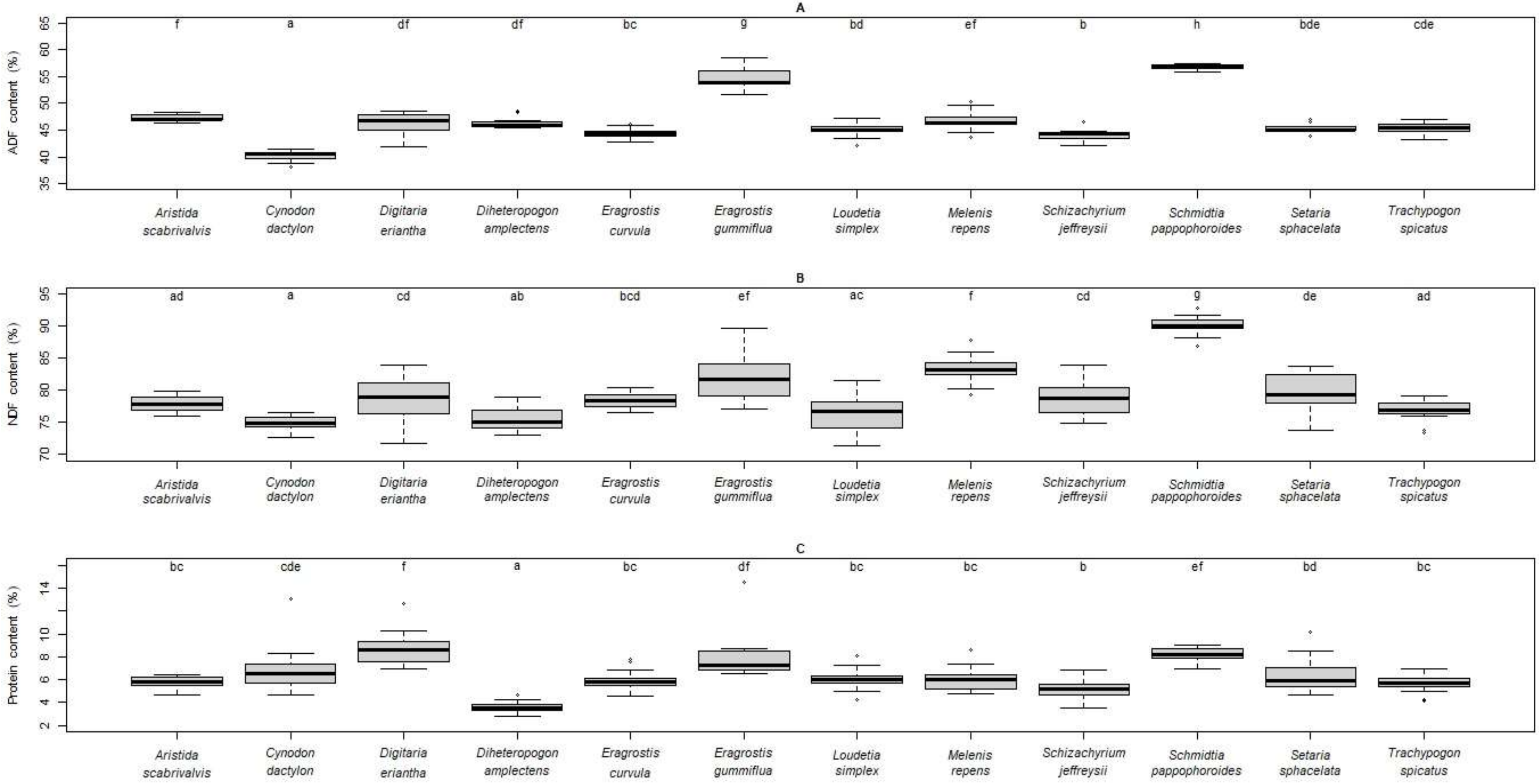


Figure 3.2 Variation in the core grazing quality components between species: A) ADF = acid detergent fibre, B) NDF = neutral detergent fibre, and C) protein content. Species not sharing matching letters differ significantly.

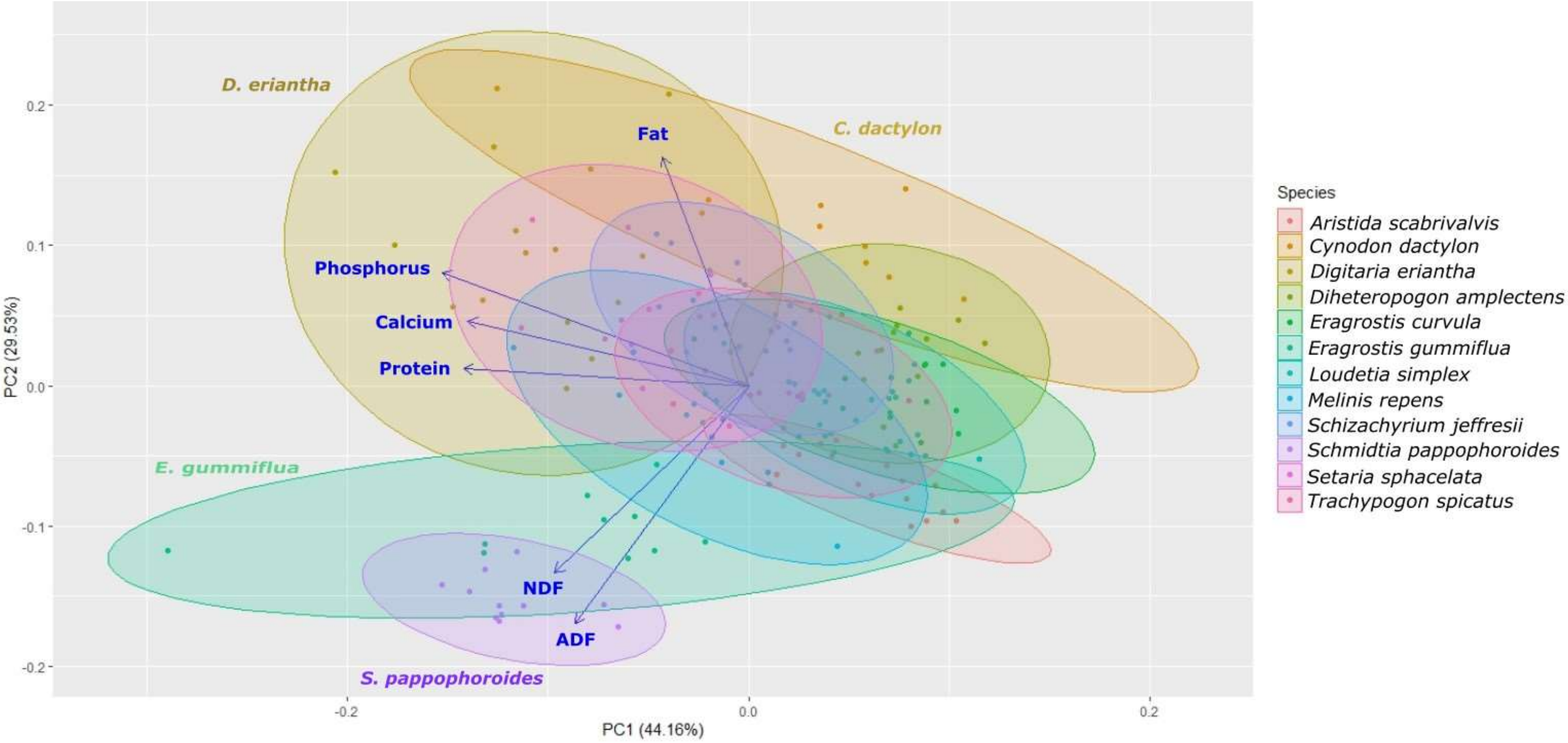


Figure 3.3 Principal component analysis (PCA) of the grazing quality of the 12 dominant grass species based on six grazing quality components (blue arrows). The PCA explains 44.2 % of the variation in grazing quality on the first axis and 29.5 % on the second axis. Ellipses indicate the 95 % confidence interval of each species, ADF = acid detergent fibre and NDF = neutral detergent fibre.

Table 3.2 Top-ranked multivariate model for each grazing quality component. Symbols indicate the nature of the effect each environmental variable has on the corresponding response variable (+ = positive relationship, - = negative relationship, ∩ = humped-shape relationship, U = valley-shape relationship. See Table B5 for detailed results.

	Soil pH	Soil C	Soil K	Soil Ca	Clay fraction	Model R^2
ADF				∩		0.005
NDF				∩		0.018
Protein content	-	-		+	U	0.106

PFTs show idiosyncratic relationships with some environmental variables

For each species-trait combination, there were different predictors included in the best-fit models, with marginal R^2 values ranging between 0.02 and 0.51 (Tables B6 – B9). In the species-level multivariate models of LDMC, the marginal R^2 values ranged between 0.08 and 0.46 (Table 3.3). Six environmental variables (woody debris, northness, TWI, soil C, soil K and sampling date) were included in models of LDMC of at least three of the dominant species (Table 3.3). Only three environmental variables (leaf litter, dung count and soil Na) were related to LDMC at both the community- and species-level.

There were generally fewer environmental variables related to SLA than to LDMC, at the species level (Table 3.3). Marginal R^2 values for the multivariate SLA models ranged between 0.03 and 0.46, with only two variables (PDIR and curvature) being included in models of SLA of at least three species. Soil pH was the only environmental variable that was related to SLA at both the community- and species-level (Table 3.3). Force to tear had no environmental variables which were found to be influential drivers across multiple species or at both the community- and species-level. The marginal R^2 values of the multivariate models of FT ranged between 0.14 and 0.27 at the species level (Table 3.3). However, for five species (*Digitaria eriantha, Eragrostis curvula, Melinis repens, Schizachyrium jeffreysii* and *Trachypogon spicatus*), no environmental variables were included in the top-ranked model, suggesting a lack of a clear environmental influence on variation in leaf strength.

Table 3.3 Top-ranked multivariate model for all traits at the community- and species-level. Shading highlights consistently present relationships: blue = influential driver at both the community level and species level for a trait, yellow = influential drivers of at least two traits at the community level, green = variables that were influential drivers for at least three species. CWM = community weighted mean, LDMC = leaf dry matter content, SLA = specific leaf area, FT = force to tear, Soil Ca = soil Calcium content, Soil Mg = soil Magnesium content, Soil Na = soil Sodium content, + = positive linear relationship, - = negative linear relationship, U = valley shaped quadratic relationship and ∩ = humped shaped quadratic relationship. *Ari sca = Aristida scabrivalvis, Dig eri = Digitaria eriantha, Dih amp = Diheteropogon amplectens, Era cur = Eragrostis curvula, Lou sim = Loudetia simplex, Mel rep = Melinis repens, Sch jef = Schizachyrium jeffreysii, Sch pap = Schmidtia pappophoroides, Set spa = Setaria sphacelata, Tra spi = Trachypogon spicatus,* Null = Null model is the top-ranked model for the species trait combination and NA = no variables were found to be influential at the univariate level.

	Rock cover	Bare soil	Woody debris	Leaf litter	Woody cover	Elevation	Slope	PDIR	Curvature	Northness	TWI	Dung count	Grazing	Soil depth	Soil pH	Soil C	Soil K	Soil Ca	Soil Mg	Soil Na	Soil P	Sand fraction	Clay fraction	Sampling date	Marginal R^2
LDMC - CWM_{LDMC}				-								+		U						U					0.45
Ari sca	-		-			-			-		∩		U		∩	+								-	0.19
Dig eri											-										+			+	0.12
Dih amp			∩	-	-			+								U								-	0.26
Era cur		U	-							+															0.46
Lou sim			-	∩					-							U	-			+					0.42
Mel rep										∩							-					+	-	-	0.17
Sch jef		-					+																	-	0.10
Sch pap			U					-		+		-											-		0.26
Set spa			-																			+			0.08
Tra spi	-		+								∩					U	-	-							0.28
SLA - CWM_{SLA}		+										-		U	-			+	-						0.62
Ari sca								U							-										0.24
Dig eri								-		∩						+									0.17
Dih amp			+										-								-		+		0.40
Era cur	-								U	∩					∩										0.46
Lou sim															U					+		U			0.39
Mel rep																						+			0.07
Sch jef									-																0.03
Sch pap								U		-															0.28
Set spa									+														U		0.21
Tra spi																									

Table 3.3 continued

	Rock cover	Bare soil	Woody debris	Leaf litter	Woody cover	Elevation	Slope	PDIR	Curvature	Northness	TWI	Dung count	Grazing	Soil depth	Soil pH	Soil C	Soil K	Soil Ca	Soil Mg	Soil Na	Soil P	Sand fraction	Clay fraction	Sampling date	Model R^2
FT - CWM$_{FT}$												+		∪			-	-							0.51
Ari sca															∩					-					0.23
Dig eri																									Null
Dih amp								∩																	0.20
Era cur (NA)																									Null
Lou sim					-																				0.21
Mel rep																									Null
Sch jef																									Null
Sch pap								-							∪										0.14
Set spa									∪	∩															0.27
Tra spi																									Null

PFTs – generally explained little variation at the community-level

Relatively little variation in CWM_{LDMC}, CWM_{SLA} and CWM_{FT} was explained by individual predictor variables (R^2 range = 0.03 – 0.32, mean ± SD =0.12 ± 0.08; Table B9). Soil Ca was the strongest predictor of CWM_{LDMC} (R^2 = 0.21) and CWM_{FT} (R^2 = 0.28) with both traits being negatively related to soil Ca. Woody cover was the strongest predictor of variation in CWM_{SLA} (R^2 = 0.32; Fig. 3.4), with a positive relationship with SLA.

Four environmental variables (dung count, soil depth, soil Ca and soil Na) were influential drivers of multiple leaf traits at the community-level (Table 3.3 – yellow shading), explaining 20 – 63 % of the variation in PFTs and having impacts across the majority of traits. The best model for CWM_{LDMC} (R^2 = 0.45; Table 3.3) included four environmental variables (leaf litter, dung count, soil depth and soil Na) with all predictors, except leaf litter, being positively related with CWM_{LDMC}. The best model for CWM_{SLA} (R^2 = 0.62; Table 3.3) included six environmental variables (bare soil, dung count, soil depth, soil pH, soil Ca and soil Mg), with three variables (bare soil, soil depth and soil Ca) having a positive relationship with CWM_{SLA}. For CWM_{FT} the best model (R^2 = 0.51; Table 3.3) included four environmental variables (dung count, soil depth, soil K and soil Ca), of which two variables (dung count and soil depth) had positive relationships with CWM_{FT}.

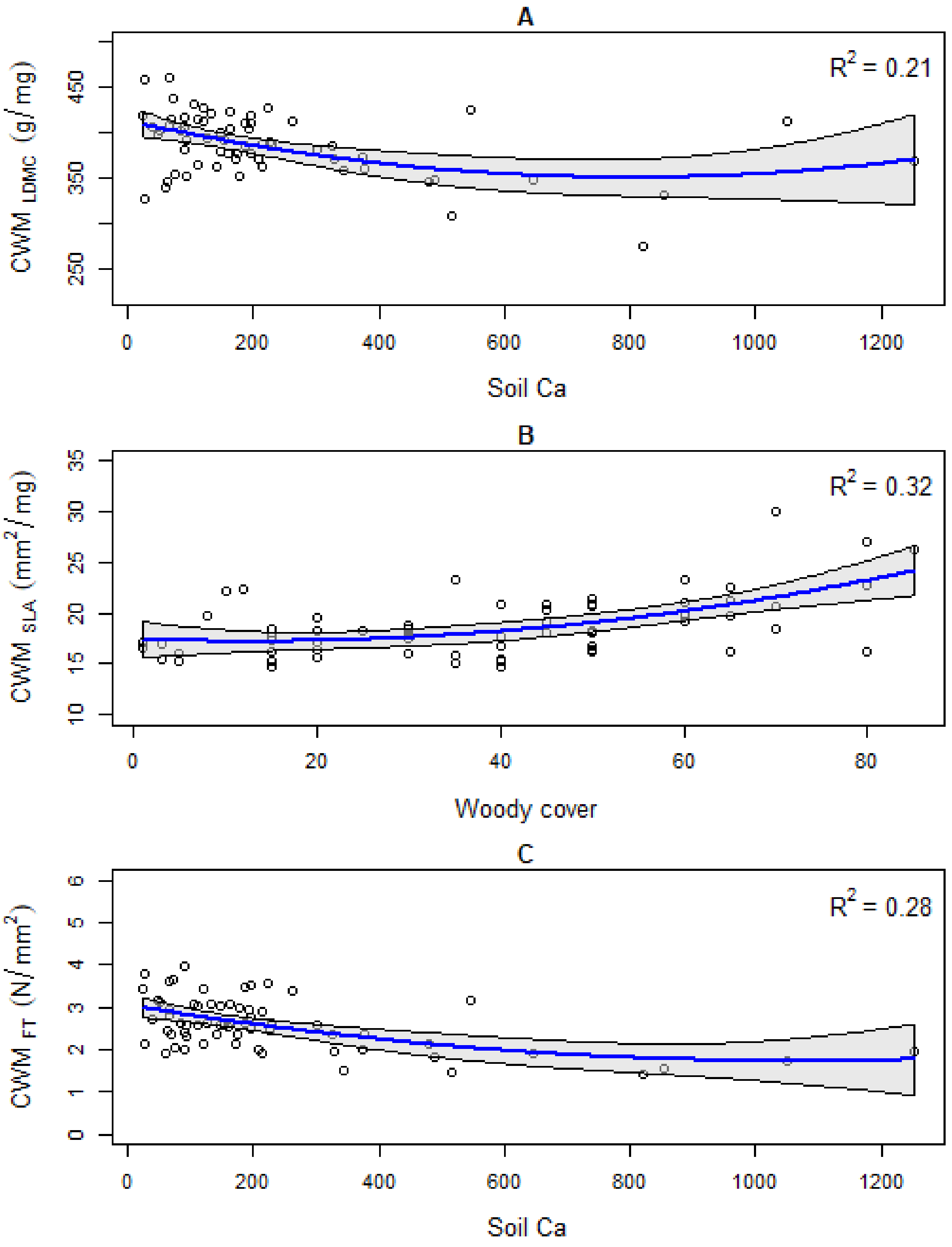


Figure 3.4 Most influential univariate predictor variables for each community-level response variable, shaded area indicate the 95% confidence interval. CWM_{LDMC} = community weighted mean leaf dry matter content, CWM_{SLA} = community weighted mean specific leaf area, CWM_{FT} = community weighted mean force to tear, and Soil Ca = soil Calcium content.

Discussion

Considerable variation in grazing quality was observed at the inter- and intra-specific levels, with the intra-specific variation being poorly explained by environmental conditions. For functional traits and core grazing quality components in this study, the general trend was highly idiosyncratic and mostly weak trait-environment and grazing quality-environmental relationships, at the species- and community-level (in agreement with Albert et al., 2010; Jessen et al., 2020; Sandel et al., 2021). Additionally, the PFTs investigated in this study show no potential as proxies for grass grazing quality.

The magnitude of inter-specific variation in the grazing quality of wild grass species in this study agrees with previous research (e.g. Beyene and Mlambo, 2012; Ravhuhali et al., 2019). The large proportions of variation in the core grazing quality components explained at the inter-specific level (up to 91 %) indicate that species identity alone can provide a good estimate of the grazing quality of grasses (at least at the landscape scale). The grass species in this study generally had high ADF and NDF values and low protein content, which is in common with data from other mature African C4 grasses (Mlay et al., 2006; Beyene and Mlambo, 2012; Ravhuhali et al., 2019). Grass species with low protein content and high fibre content are expected to be difficult for herbivores to digest (Ravhuhali et al., 2019) and, therefore, have lower overall grazing quality. Moreover, while leaf Ca and P are not core grazing quality components, both are important macrominerals required by grazers (McDowell and Arthington, 2005). All grass species in this study had Ca contents lower than the critical threshold level for ruminants and only two grass species, *D. eriantha* and *S. sphacelata,* reached the minimum critical level of P for ruminants (McDowell and Arthington, 2005).

In terms of grazing quality, four grass species were distinct from the other species in this study. *Cynodon dactylon* and *D. eriantha* are expected to be of high grazing quality due to the high protein content of these species, while *E. gummiflua* and *S. pappophoroides* appear to be of low grazing quality due to their high ADF and NDF. This study, however, shows that there is no simple trade-off between protein content and fibre content, revealing potentially more complex relationships between grazing quality components. This finding cautions against the assignment of grazing quality based on just a single grazing quality component (e.g. considering a species to have good grazing quality based solely on protein content) and highlights that multiple grazing quality components need to be considered simultaneously.

Intra-specific variation in the core grazing quality components was poorly related to environmental variables, with only soil variables being moderately influential. Soil nutrient levels are known to impact the protein content of plants (Buxton, 1996) and the pH of soil is a good proxy for soil nutrient availability (Alam et al., 1999). Surprisingly, however, soil pH had a negative relationship with protein content. This was an unexpected relationship since the soils at WGR are acidic and better nutrient availability would be expected under more neutral conditions. Light intensity, diurnal temperature range and water availability may also impact the protein content of grasses (Buxton, 1996; Da Silva et al., 2019), but no influence of PDIR or TWI, which are proxies of solar radiation and water availability, on grass protein content was observed. Temperature is also an important factor impacting protein content since lower temperatures decrease the maturation rate of grasses and are, therefore, expected to increase the protein content in plants (Buxton, 1996). Finally, time of harvest will typically also impact the grazing quality of grasses (Rosser et al., 2013; Hughes et al., 2014; Grev et al., 2017), but in this study, no relationship was found between sampling date and any of the grazing quality components. This likely reflects that the grasses in this study were all in their mature growth phase throughout the sampling period (Rosser et al., 2013). These results add to our understanding of which environmental conditions may influence intra-specific variation in grass grazing quality, but overall this issue remains poorly explored in the literature, despite the potential for, for example, changing environmental conditions to alter local and landscape-scale grazing quality.

The PFTs investigated in this study had no relationships with any core grazing quality components. Therefore, for C4 savanna grasses LDMC, SLA and FT are not useful proxies of grazing quality. The forages selected by herbivores often have high SLA along with low LDMC and leaf toughness, a trend likely linked to the high palatability (i.e. grazing value Elger and Willby, 2003; Adler et al., 2004; Cingolani et al., 2005) and not necessarily to the grazing quality of the plant material. Therefore, alternative PFTs should be investigated for their potential to act as proxies for grazing quality.

PFTs at the species- and community-level

Highly idiosyncratic relationships between environmental variables and PFTs were observed within species, a trend recently demonstrated for grasses elsewhere (Roybal and Butterfield, 2019; Sandel et al., 2021) and other plant growth forms (Albert et al., 2010). This result was, nonetheless, still somewhat unexpected since closely related species are expected to have similar trait-environment relationships (Gerdol, 2005). Leaf dry matter content was correlated with more environmental variables than the other PFTs across the 12 dominant species, but only northness and soil K had consistent relationships with LDMC across species. Leaf dry matter content is a good measure of resource use strategies in plants (Wilson et al., 1999b; Wellstein et al., 2013) and is negatively related to plant growth rate (Albert et al., 2010). The overall negative relationship between the LDMC of species and soil K (which is an important macronutrient required for photosynthesis and growth; Prajapati and Modi, 2012), possibly reflects grasses exhibiting faster growth rates under higher nutrient concentrations.

Equator-facing slopes receive higher levels of solar radiation, making these slopes generally hotter and drier (Sandel and Low, 2019). Relevés with north facing aspects (i.e. northness) had higher LDMC (i.e. reflecting increased structural components) and lower SLA would be beneficial to species since it would lower water loss rates (Jung et al., 2014; Deléglise et al., 2015). However, TWI, which is a measure of potential soil moisture content, was mostly negatively related to LDMC. Topography also impacts other underlying environmental variables such as nutrient availability, temperature, and wind exposure, which could ultimately alter the relationships between LDMC and topography (Moeslund et al., 2013). Higher LDMC was also observed for species in relevés with higher sand fractions, possibly reflecting that sandy soils typically have lower water holding capacity (Nath, 2014). In contrast, soil carbon content, litter cover, and woody debris, which are typically associated with greater soil moisture-holding capacity, had highly variable relationships with LDMC at the species level (Nath, 2014).

Sampling date was negatively related to LDMC in several species, despite being expected to have a positive relationship with this trait since structural tissues typically increase in grass leaf blades through the growing season (Duru et al., 2008; Dong et al., 2020; see also Chapter 2). This unexpected result may, however, reflect that trait values can shift from season to season depending on the change in conditions (Sandel et al., 2021). For example, the onset and duration of the rainy season would impact the growth stage and growth rate of grasses during sampling which, in turn, could affect the rate of change in PFTs. Cooler, wetter periods are expected to delay the maturation of grasses (Buxton, 1996) and, as a result, the structural tissues in leaf blades can remain low, resulting in more consistent PFT values being observed throughout the sampling season.

Soil pH is an important factor affecting the availability of soil nutrients (Alam et al., 1999; Dong et al., 2020), as well as impacting various plant traits (Dwyer et al., 2014; Dong et al., 2020). Specific leaf area was negatively correlated with soil pH for two species (in contrast to grass species globally having a positive relationship of SLA with soil pH; Jardine et al., 2020). Although not all species exhibited the same environment- functional trait relationships, SLA and FT always exhibited opposite relationships with the same variables. Force to tear was not related to environmental conditions in several species. This agrees with the findings of Jardine et al. (2020) where the FT of grasses globally was only weakly related to soil nutrients. Interestingly relationships between environmental variables at the community-level for SLA and FT were always opposite, a relationship recently demonstrated for other grass species (Jardine et al., 2020). This result likely reflects how higher SLA is associated with rapid growth and utilization of soil nutrients, and how higher FT (i.e., toughness) is associated with leaf longevity and resource conservation (Diaz et al., 2004; Pérez-Harguindeguy et al., 2013; Jardine et al., 2020).

Few environmental variables were consistently related to leaf traits, but a subset of predictor variables explained variation in community-level trait expression relatively well for all leaf traits. The best models at the community-level generally included fewer variables compared to the species level models and explained between 3 and 59 % more variation in PFTs. This potentially indicates that predictions of assemblage characteristics are more accurate than species-level characteristics. High LDMC and FT are related to leaf toughness and the ability to withstand physical damage such as herbivory (Augustine and McNaughton, 1998; Adler et al., 2004; Pérez-Harguindeguy et al., 2013), and the positive relationship between dung count (a proxy of grazing) and these traits at the community-level support this. However, there was no relationship between the grazing level of relevés and these traits, suggesting that in this system dung count may potentially reflect a different form of physical stress like trampling.

Conclusion

This study shows that variation in grazing quality chiefly occurs at the species-level and that grazing management actions based on dominant species are likely to represent most of the variation in grazing quality. Intra-specific variation was, nonetheless, observed for grazing quality components, although it is unclear to what degree environmental conditions drive this variation within species. This finding suggests that some key environmental variables were potentially not measured in this study. Moreover, grass leaf traits were not related to core grazing quality components, at least at the landscape scale studied here. Therefore, future studies focusing on this topic should aim to sample along broader gradients, since, for example, temperature and precipitation have been shown to impact protein and fibre content (Buxton, 1996). Additionally, other traits such as leaf nitrogen content (Deléglise et al., 2015; Tasset et al., 2019), plant size traits (Pérez-Harguindeguy et al., 2013) and growth form (Whitworth-Hulse et al., 2016) should likely also be investigated if searching for trait-grazing quality relationships that might be useful for implementation in the grazing regime management, for example, allowing for quick determination of an areas' grazing capacity.

Only a limited number of environmental variables were consistently related to PFTs at the community- and species-level, with idiosyncratic trait-environmental relationships typically observed in this study. This demonstrates the difficulty of understanding which environmental variables influence variation in PFTs (in agreement with Albert et al., 2010; Kichenin et al., 2013; Sandel et al., 2021). Nonetheless, this study has contributed to addressing the current knowledge gaps about the trait- and grazing quality-environmental relationships (Albert et al., 2010; Kichenin et al., 2013; Westerband et al., 2021), demonstrating how soil variables are most often influential in these relationships in this system. This new knowledge suggests that some trait- and grazing quality-environmental relationships may be relatively more robust to use in guiding management actions (e.g. soil variables are more consistently related to traits and grazing quality compared to other environmental variables). Finally, while this study was focused at a fairly local scale, expanding the study area to a regional- or even continental-scale would allow for larger variation in environmental variables to be observed, and it might then be clearer to identify the conditions that drive variation in grazing quality and PFTs.

Appendix B

Table B1 All environmental variables included in the initial univariate analyses after highly correlated variables were excluded.

Environmental variable	Collection or calculation method
Rock cover	Visually estimated (%)
Bare soil cover	Visually estimated (%)
Woody debris cover	Visually estimated (%)
Leaf litter cover	Visually estimated (%)
Woody cover	Visually estimated (%)
Elevation	Calculated from Digital Elevation Model (DEM), in m above sea level
Slope	Calculated from DEM as degrees from horizontal
PDIR	Calculated from DEM, scale = 0.81 – 1.16, higher values = higher potential radiation
Curvature	Calculated from DEM, - values = concave, positive values = convex
Northness	Calculated from aspect 1 = North, -1 = South and 0 = East or West
TWI	Calculated from DEM scale = 0.7 – 13.8, Smaller values = less wet
Dung count	Dung pads of Plains Zebra, White Rhinoceros and Cape Buffalo counted per relevé
Grazing level	Visually estimated 1 = few signs of grazing to 5 = high levels of grazing
Soil depth	Measured using a 1 cm in diameter, 60 cm long metal rod
Soil pH	Obtained using the H_2O method
Soil C	Obtained through titration (mg/kg
Soil K	Obtained through the Melich-3 extraction protocol (mg/kg)
Soil Ca	Obtained through the Melich-3 extraction protocol (mg/kg)
Soil Mg	Obtained through the Melich-3 extraction protocol (mg/kg)
Soil Na	Obtained through the Melich-3 extraction protocol (mg/kg)
Soil P	Obtained through the Melich-3 extraction protocol (mg/kg)
Sand fraction	Obtained through the standard hydrometer method (%)
Clay fraction	Obtained through the standard hydrometer method (%)
Sampling date	First day of sampling = 1 and last day of sampling = 36

Table B2 The grass species recorded in this study and the number of individuals sampled for the measurement of plant functional traits(PFTs) and grazing quality (GQ).

Species name	Individuals sampled (PFTs)	Individuals sampled (GQ)	Species name	Individuals sampled (PFTs	Individuals sampled (GQ)
Andropogon chinensis	24	NA	*Eragrostis viscosa*	6	NA
Andropogon schirensis	30	NA	*Eustachys paspaloides*	0	NA
Aristida adscensionis	6	NA	*Fingerhuthia africana*	0	NA
Aristida canenscens	21	NA	*Heteropogon contortus*	27	NA
Aristida congesta barbicollis	9	NA	*Hyparrhenia filipendula*	21	NA
Aristida congesta congesta	27	NA	*Hyparrhenia hirta*	12	NA
Aristida diffusa	27	NA	*Loudetia pediculata*	12	NA
Aristida scabrivalvis	39	13	*Loudetia simplex*	85	26
Aristida stipitata	8	NA	*Melinis nerviglumis*	1	NA
Aristida transvaalensis	0	NA	*Melinis repens*	60	17
Bewsia biflora	0	NA	*Microchloa caffra*	3	NA
Brachiaria brizantha	9	NA	*Miscanthus junceus*	3	NA
Brachiaria deflexa	0	NA	*Monocymbium ceresiiforme*	3	NA
Brachiaria nigropedata	3	NA	*Panicum coloratum*	3	NA
Brachiaria serrata	3	NA	*Panicum ecklonii*	0	NA
Chloris virgata	0	NA	*Panicum maximum*	18	NA
Cymbopogon nardus	3	NA	*Panicum natalense*	3	NA
Cynodon dactylon	30	13	*Perotis patens*	6	NA
Digitaria brazzae	0	NA	*Pogonarthria squarrosa*	6	NA
Digitaria eriantha	59	15	*Schizachyrium jeffreysii*	78	21
Digitaria longiflora	6	NA	*Schizachyrium sanguineum*	24	NA
Digitaria monodactyla	3	NA	*Schmidtia pappophoroides*	33	11
Digitaria sanguinalis	0	NA	*Setaria lindenbergiana*	3	NA
Diheteropogon amplectens	51	15	*Setaria pumila*	6	NA
Eleusine coracana	2	NA	*Setaria sphacelata*	33	11
Enneapogon cenchroides	0	NA	*Sporobolus africanus*	0	NA
Eragrostis acrea	2	NA	*Sporobolus festivus*	6	NA
Eragrostis bicolor	3	NA	*Sporobolus fimbriatus*	0	NA
Eragrostis chloromelas	18	NA	*Sporobolus ioclados*	0	NA
Eragrostis curvula	45	14	*Sporobolus pyramidalis*	6	NA
Eragrostis gummiflua	30	10	*Sporobolus stapfianus*	3	NA
Eragrostis inamoena	3	NA	*Themeda triandra*	3	NA
Eragrostis lapula	3	NA	*Trachypogon spicatus*	60	20
Eragrostis lehmanniana	3	NA	*Tragus berteronianus*	0	NA
Eragrostis patentipilosa	0	NA	*Trichoneura grandiglumis*	2	NA
Eragrostis racemosa	3	NA	*Triraphis andropogonoides*	0	NA
Eragrostis rigidior	9	NA	*Tristachya leucothrix*	3	NA
Eragrostis supurba	0	NA	*Urochloa mosambicensis*	4	NA
Eragrostis trichophora	3	NA	*Urochloa panicoides*	3	NA

Table B3 Species for which no leaf functional trait data were collected, the number of relevés each of these species was present in, the mean cover each species in each relevé where it was present, and the taxonomic level used for the estimation of traits for each species (subsequently used in the calculation of CWM values for each relevé.

Species	Relevés present	Mean cover in relevés where present	Taxonomic level used for trait estimation prior to CMW calculation
Aristida transvaalensis	1	3.0	From genus
Brachiaria deflexa	1	2.0	From genus
Bewsia biflora	1	0.5	From tribe (Cynodonteae)
Chloris virgata	3	0.5	From subtribe (Eleusininae)
Digitaria brazzae	1	1.0	From genus
Digitaria sanguinalis	1	1.0	From genus
Enneapogon cenchroides	1	3.0	From tribe (Eragrostideae)
Eragrostis patentipilosa	4	0.6	From genus
Eragrostis superba	3	2.0	From genus
Eustachys paspaloides	1	0.5	From subtribe (Eleusininae)
Fingerhuthia africana	1	0.5	From tribe (Eragrostideae)
Panicum ecklonii	1	2.0	From genus
Sporobolus africanus	3	1.2	From genus
Sporobolus fimbriatus	1	0.5	From genus
Sporobolus ioclados	1	1.0	From genus
Tragus berteronianus	7	0.9	From tribe (Cynodonteae)
Triraphis andropogonoides	3	1.5	From subfamily (Chloridoideae)

Table B4 Univariate model results for each grazing quality component × predictor combination, presenting only the linear or quadratic models which performed better than the associated null model. ADF = acid detergent fibre, NDF = Neutral detergent fibre, Lin = Linear model, Quad = Quadratic model, Soil C = Carbon content Soil K = Potassium content, Ca = Calcium content, Mg = Magnesium content, Soil Na = Sodium content, Soil P = Phosphorous content, PDIR = Potential Direct Incidence Radiation, TWI = Topographic wetness index, - = negative linear relationship, + = positive linear relationship, U = valley shaped quadratic relationship, ∩ = humped shaped quadratic relationship.

Response	Predictor	Best model	AIC null model	AIC linear model	AIC quadratic model	Marginal R^2	Relationship
ADF	Soil C	Quad	655.2	655.1	649.0	0.005	∩
NDF	Bare soil	Quad	888.1	889.6	887.4	0.008	U
	Woody debris	Lin	888.1	885.8	887.5	0.007	+
	Leaf litter	Lin	888.1	885.9	88709	0.007	+
	Woody cover	Lin	888.1	884.6	886.3	0.009	+
	Soil depth	Lin	888.1	884.6	885.7	0.015	-
	Soil C	Quad	888.1	886.4	882.6	0.018	∩
Protein	Bare soil	Quad	618.5	613.9	611.2	0.036	∩
	Leaf litter	Lin	618.5	614.5	616.1	0.017	-
	Woody cover	Quad	618.5	619.9	609.1	0.034	U
	Elevation	Quad	618.5	618.4	617.6	0.012	U
	Slope	Quad	618.5	620.3	617.0	0.013	∩
	Curvature	Quad	618.5	611.1	608.4	0.032	U
	TWI	Lin	618.5	617.1	618.7	0.008	-
	Soil pH	Lin	618.5	615.1	616.8	0.016	-
	Soil C	Lin	618.5	615.7	616.8	0.013	+
	Soil Ca	Quad	618.5	614.9	607.4	0.035	U
	Soil Mg	Quad	618.5	619.0	609.9	0.029	U
	Soil Na	Quad	618.5	619.5	616.8	0.013	∩
	Soil P	Lin	618.5	606.7	606.9	0.033	+
	Sand fraction	Quad	618.5	620.3	609.3	0.030	U
	Clay fraction	Quad	618.5	618.1	597.3	0.056	U

Table B5 Best subset of multivariate models for protein content based on AIC scores (i.e. models with AIC scores differing less than 2). Soil C = Soil Carbon content Soil P = Phosphorus content, Soil Ca = Soil Calcium content, + = positive linear relationship, - = negative linear relationship, U = valley shaped quadratic relationship.

Predictor	Leaf litter	Soil pH	Soil C	Soil P	Soil Ca	Clay fraction	Model AIC	Model R^2	Model ranking
Response									
Protein content		-	-		+	U	608.2		1
		-		+		U	608.6		2
		-		+		-	608.9		3
					U	-	609.4		4
				+		U	604.4		5
	-	-			+		604.4		6

Table B6 Species level univariate model results, best model LDMC × species × predictor combination presenting only the linear or quadratic models which performed better than the associated null model. Lin = Linear model, Quad = Quadratic model, Soil C = Carbon content Soil K = Potassium content, Ca = Calcium content, Mg = Magnesium content, Soil Na = Sodium content, Soil P = Phosphorous content, PDIR = Potential Direct Incidence Radiation, TWI = Topographic wetness index, - = negative linear relationship, + = positive linear relationship, U = valley shaped quadratic relationship, ∩ = humped shaped quadratic relationship, LDMC = leaf dry matter content.

Response	Predictor	Best model	AIC null model	AIC linear model	AIC quadratic model	Marginal R^2	Relationship
LDMC							
Aristida scabrivalvis	Rock cover	Quad	479.9	480.4	478.2	0.13	∩
	Woody debris	Lin	479.9	478.5	479.6	0.09	+
	Elevation	Quad	479.9	481.5	479.5	0.11	U
	Curvature	Lin	479.9	478.7	480.4	0.08	+
	TWI	Lin	479.9	478.2	478.9	0.09	-
	Grazing	Quad	479.9	481.5	479.1	0.11	∩
	Soil pH	Lin	479.9	477.76	478.7	0.10	+
	Soil C	Lin	479.9	477.8	477.8	0.10	-
	Sampling date	Quad	479.9	481.3	481.3	0.14	∩
Digitaria eriantha	TWI	Lin	671.1	669.9	671.8	0.05	-
	Soil K	Lin	671.1	670.5	671.6	0.05	+
	Soil P	Lin	671.1	669.1	670.3	0.07	+
	Sampling date	Lin	671.1	670.4	672.2	0.05	+
Diheteropogon amplectens	Woody debris	Quad	569.1	569.3	567.3	0.11	∩
	Leaf litter	Lin	569.1	563.5	565.4	0.15	-
	Woody cover	Quad	569.1	568.0	565.7	0.14	∩
	Elevation	Lin	569.1	569.1	570.6	0.04	+
	PDIR	Lin	569.1	567.8	569.8	0.07	+
	Soil C	Quad	569.1	568.2	567.0	0.12	∩
	Soil K	Lin	569.1	568.9	570.6	0.05	+
	Soil Ca	Lin	569.1	568.9	570.5	0.05	+
	Sampling date	Lin	569.1	566.1	566.7	0.10	+
Eragrostis curvula	Bare soil	Quad	566.3	566.6	566.0	0.14	∩
	Woody debris	Lin	566.3	560.9	562.9	0.23	-
	Northness	Lin	566.3	565.8	567.3	0.09	+
Loudetia simplex	Woody debris	Lin	1042.3	1037.4	1039.3	0.11	-
	Leaf litter	Quad	1042.3	1042.5	1037.3	0.14	U
	Woody cover	Lin	1042.3	1038.8	1039.5	0.09	-
	Elevation	Quad	1042.3	1043.5	1041.8	0.08	∩
	Curvature	Lin	1042.3	1042.3	1044.1	0.04	-
	TWI	Lin	1042.3	1038.9	1040.0	0.09	-
	Soil depth	Quad	1042.3	1038.0	1040.0	0.15	U
	Soil C	Quad	1042.3	1042.1	1036.4	0.08	U
	Soil K	Lin	1042.3	1040.3	1041.6	0.07	-
	Soil Ca	Lin	1042.3	1036.4	1042.3	0.13	-
	Soil Mg	Lin	1042.3	1035.6	1037.6	0.14	-
	Soil Na	Lin	1042.3	1040.4	1041.5	0.07	-
	Sand fraction	Quad	1042.3	1042.7	1041.3	0.08	∩

	Sampling date	Quad	1042.3	1042.3	1042.2	0.07	U
Melinis repens	Elevation	Lin	712.8	707.6	709.6	0.11	+
	Northness	Quad	712.8	714.6	711.8	0.08	∩
	Soil K	Lin	712.8	707.2	708.8	0.12	-
	Soil Ca	Lin	712.8	712.7	712.1	0.05	-
	Soil Mg	Quad	712.8	714.1	712.1	0.07	∩
	Sand fraction	Lin	712.8	712.18	713.1	0.04	-
	Clay content	Lin	712.8	711.8	713.5	0.05	-
	Sampling date	Lin	712.8	711.8	713.3	0.05	-
Schizachyrium jeffreysii	Bare soil	Lin	935.5	935.0	936.8	0.03	-
	Slope	Lin	935.5	933.6	935.6	0.05	+
	Sampling date	Quad	935.5	935.9	933.0	0.08	U
Schmidtia pappophoroides	Woody debris	Quad	414.1	415.9	411.1	0.18	U
	PDIR	Lin	414.1	409.2	410.2	0.19	-
	Northness	Lin	414.1	411.0	412.9	0.14	-
	Dung count	Quad	414.1	415.4	414.0	0.11	U
	Clay content	Lin	414.1	413.2	414.2	0.07	-
Setaria sphacelata	Woody debris	Lin	397.6	397.4	399.3	0.06	-
	Sand fraction	Lin	397.6	397.4	398.3	0.06	+
Trachypogon spicatus	Rock cover	Lin	694.7	694.2	696.1	0.04	+
	Woody debris	Lin	694.7	694.0	694.6	0.05	+
	TWI	Quad	694.7	695.5	692.5	0.10	∩
	Soil depth	Quad	694.7	693.7	693.6	0.09	U
	Soil C	Quad	694.7	696.4	694.4	0.07	∩
	Soil K	Lin	694.7	691.4	692.3	0.09	-
	Soil Ca	Quad	694.7	695.4	694.0	0.08	U
	Soil Mg	Quad	694.7	695.2	691.5	0.12	U

Table B7 Species level univariate model results, best model SLA × species × predictor combination presenting only the linear or quadratic models which performed better than the associated null model. Lin = Linear model, Quad = Quadratic model, Soil C = Carbon content Soil K = Potassium content, Ca = Calcium content, Mg = Magnesium content, Soil Na = Sodium content, Soil P = Phosphorous content, PDIR = Potential Direct Incidence Radiation, TWI = Topographic wetness index, - = negative linear relationship, + = positive linear relationship, U = valley shaped quadratic relationship, ∩ = humped shaped quadratic relationship, SLA = specific leaf area.

Response	Predictor	Best model	AIC null model	AIC linear model	AIC quadratic model	Marginal R^2	Relationship
SLA							
Aristida scabrivalvis	Woody debris	Lin	244.3	243.1	243.5	0.11	-
	Leaf litter	Quad	244.3	246.3	243.9	0.14	∩
	Elevation	Quad	244.3	246.0	244.0	0.14	∩
	Slope	Lin	244.3	243.1	244.5	0.11	+
	PDIR	Quad	244.3	246.0	243.2	0.15	U
	TWI	Lin	244.3	244.3	246.3	0.07	+
	Dung count	Lin	244.3	242.8	244.7	0.11	+
	Grazing	Quad	244.3	242.4	231.6	0.38	U
	Soil pH	Lin	244.3	240.6	241.3	0.18	-
	Soil P	Lin	244.3	243.6	245.4	0.09	+
	Clay content	Lin	244.3	242.6	243.3	0.13	-
Digitaria eriantha	Rock cover	Lin	419.8	418.6	419.8	0.05	+
	PDIR	Lin	419.8	419.2	420.4	0.04	+
	Northness	Quad	419.8	418.6	416.6	0.11	∩
	Dung count	Quad	419.8	420.5	414.7	0.02	U
	Soil C	Lin	419.8	419.7	421.6	0.04	+
	Soil Mg	Quad	419.8	420.1	419.2	0.08	U
	Sand fraction	Lin	419.8	419.3	419.4	0.04	+
Diheteropogon amplectens	Rock cover	Quad	310.3	310.1	308.9	0.15	U
	Woody cover	Lin	310.3	309.1	310.6	0.10	+
	Dung count	Quad	310.3	311.4	308.9	0.15	∩
	Grazing	Lin	310.3	310.3	311.9	0.06	-
	Soil P	Lin	310.3	309.2	309.3	0.09	-
	Clay fraction	Lin	310.3	309.4	311.4	0.09	+
Eragrostis curvula	Rock cover	Lin	248.8	248.5	249.9	0.09	+
	Woody debris	Quad	248.8	249.9	248.4	0.15	∩
	Curvature	Quad	248.8	249.9	242.1	0.31	U
	Northness	Quad	248.8	249.3	243.5	0.28	∩
	Soil depth	Lin	248.8	248.4	250.0	0.09	-
	Soil pH	Quad	248.8	249.9	248.4	0.15	∩
Loudetia simplex	Bare soil	Lin	488.0	487.9	489.6	0.03	+
	Woody debris	Lin	488.0	482.7	483.9	0.11	+
	Woody cover	Lin	488.0	487.4	488.5	0.04	+
	Elevation	Quad	488.0	488.4	486.0	0.09	U
	TWI	Lin	488.0	487.2	489.0	0.05	-
	Grazing	Lin	488.0	486.9	488.3	0.05	-
	Soil pH	Quad	488.0	487.1	475.4	0.22	U
	Soil K	Quad	488.0	486.0	484.2	0.12	U
	Soil Ca	Quad	488.0	472.0	468.2	0.28	U
	Soil Mg	Quad	488.0	470.2	465.8	0.30	U
	Soil Na	Lin	488.0	487.2	489.2	0.05	+

	Soil P	Quad	488.0	484.3	478.0	0.19	U
	Sand fraction	Quad	488.0	486.2	466.6	0.30	U
	Clay fraction	Quad	488.0	488.3	483.1	0.13	U
Melinis repens	Woody cover	Lin	408.1	406.7	408.7	0.07	+
	Elevation	Lin	408.1	401.4	403.4	0.16	-
	Soil K	Lin	408.1	403.8	405.6	0.12	+
	Soil Ca	Lin	408.1	406.9	407.6	0.07	+
	Soil Mg	Quad	408.1	409.9	407.9	0.08	U
	Sand fraction	Lin	408.1	406.9	408.5	0.07	+
	Clay fraction	Lin	408.1	407.7	409.4	0.05	+
Schizachyrium jeffreysii	Bare soil	Quad	530.2	530.8	529.9	0.05	∩
	Leaf litter	Lin	530.2	528.9	530.9	0.04	+
	Woody cover	Lin	530.2	526.5	528.2	0.07	+
	Elevation	Lin	530.2	530.1	532.0	0.03	-
	Slope	Lin	530.2	527.5	529.1	0.06	-
	Curvature	Lin	530.2	529.9	530.0	0.03	-
	Soil P	Quad	530.2	529.6	527.3	0.08	U
	Sampling date	Quad	530.2	532.2	528.0	0.08	∩
Schmidtia pappophoroides	Rock cover	Quad	256.8	257.4	256.6	0.11	U
	Elevation	Quad	256.8	255.3	251.4	0.24	U
	PDIR	Quad	256.8	252.2	249.8	0.27	U
	Northness	Lin	256.8	255.3	257.2	0.10	+
	Soil depth	Quad	256.8	257.6	254.5	0.17	∩
	Clay content	Lin	256.8	256.4	257.2	0.07	+
Setaria sphacelata	Leaf litter	Quad	201.9	203.9	200.9	0.14	U
	Elevation	Lin	201.9	195.8	197.8	0.22	-
	Curvature	Lin	201.9	198.5	200.4	0.15	+
	Soil Ca	Lin	201.9	201.6	203.0	0.07	+
	Soil Na	Lin	201.9	199.4	201.3	0.13	+
	Sampling date	Quad	201.9	203.9	199.6	0.17	U
Trachypogon spicatus	Woody cover	Quad	342.2	341.5	341.5	0.07	∩
	TWI	Quad	342.2	344.1	341.4	0.07	U
	Soil Ca	Quad	342.2	342.0	337.1	0.14	∩
	Soil Mg	Quad	342.2	343.6	338.9	0.11	∩

Table B8 Species level univariate model results, best model FT × species × predictor combination presenting only the linear or quadratic models which performed better than the associated null model. Lin = Linear model, Quad = Quadratic model, Soil C = Carbon content Soil K = Potassium content, Ca = Calcium content, Mg = Magnesium content, Soil Na = Sodium content, Soil P = Phosphorous content, PDIR = Potential Direct Incidence Radiation, TWI = Topographic wetness index, - = negative linear relationship, + = positive linear relationship, U = valley shaped quadratic relationship, ∩ = humped shaped quadratic relationship, FT = force to tear.

Response	Predictor	Best model	AIC null model	AIC linear model	AIC quadratic model	Marginal R^2	Relationship
FT							
Aristida scabrivalvis	Elevation	Quad	169.8	168.7	168.5	0.15	U
	TWI	Quad	169.8	171.5	169.4	0.13	∩
	Soil pH	Quad	169.8	170.3	169.5	0.13	∩
	Soil C	Lin	169.8	168.0	170.0	0.12	-
	Soil K	Quad	169.8	165.3	162.9	0.27	∩
	Soil Mg	Lin	169.8	168.3	169.6	0.11	-
	Soil Na	Lin	169.8	169.3	170.0	0.08	-
Digitaria eriantha	Rock cover	Quad	82.8	81.4	75.0	0.25	∩
	Woody debris	Quad	82.8	84.4	82.6	0.10	∩
	Leaf litter	Quad	82.8	80.9	80.5	0.15	∩
	Woody cover	Lin	82.8	81.5	83.1	0.08	+
	Dung count	Lin	82.8	82.8	82.9	0.05	+
	Grazing	Quad	82.8	82.7	82.0	0.12	∩
	Soil depth	Lin	82.8	80.1	82.1	0.12	-
	Soil pH	Lin	82.8	81.2	82.3	0.09	+
	Soil C	Quad	82.8	80.5	79.7	0.17	∩
Diheteropogon amplectens	Rock cover	Lin	4.0	4.0	5.8	0.07	-
	Slope	Quad	4.0	-4.7	-7.2	0.34	∩
	PDIR	Quad	4.0	5.4	0.4	0.20	U
	Soil pH	Lin	4.0	3.3	5.1	0.08	+
	Clay content	Lin	4.0	3.8	4.5	0.07	-
Eragrostis curvula	NA						
Loudetia simplex	Bare soil	Quad	248.2	247.8	247.8	0.06	U
	Woody debris	Lin	248.2	242.5	244.4	0.11	-
	Woody cover	Lin	248.2	233.2	235.2	0.21	-
	Elevation	Lin	248.2	245.1	245.9	0.07	+
	TWI	Lin	248.2	247.4	248.2	0.04	+
	Grazing	Quad	248.2	250.0	248.1	0.06	∩
	Soil C	Lin	248.2	247.9	249.4	0.03	-
	Soil Ca	Lin	248.2	241.3	241.7	0.12	-
	Soil Mg	Lin	248.2	241.9	243.6	0.12	-
Melinis repens	Leaf litter	Quad	118.8	117.8	114.9	0.12	∩
	Soil P	Lin	118.8	118.4	119.7	0.04	+
Schizachyrium jeffreysii	Woody debris	Lin	128.0	127.6	129.0	0.03	-
	Grazing	Lin	128.0	127.6	127.6	0.02	+
	Soil C	Lin	128.0	127.5	128.4	0.03	-
	Soil Ca	Lin	128.0	127.5	127.9	0.03	-
	Soil Mg	Lin	128.0	127.4	128.8	0.03	-
	Sampling date	Quad	128.0	129.4	122.0	0.12	∩

Schmidtia pappophoroides	Elevation	Quad	107.9	108.9	105.7	0.17	U
	PDIR	Lin	107.9	105.4	106.5	0.13	+
	Soil depth	Quad	107.9	108.8	107.1	0.13	∩
	Soil pH	Lin	107.9	107.6	109.5	0.07	+
	Soil C	Lin	107.9	107.4	108.4	0.07	-
	Sand fraction	Quad	107.9	109.8	107.6	0.12	∩
Setaria sphacelata	Rock cover	Lin	94.4	92.5	94.5	0.16	+
	Woody debris	Quad	94.4	95.4	94.2	0.16	∩
	Leaf litter	Quad	94.4	92.2	90.3	0.28	∩
	Curvature	Lin	94.4	92.6	94.5	0.16	-
	Northness	Quad	94.4	96.1	94.1	0.16	U
	TWI	Quad	94.4	96.1	90.4	0.28	U
	Soil depth	Lin	94.4	92.5	97.1	0.16	-
	Soil P	Lin	94.4	93.7	95.6	0.11	+
Trachypogon spicatus	Bare soil	Quad	146.0	146.8	144.1	0.09	U
	Leaf litter	Lin	146.0	145.6	147.1	0.04	-
	Dung count	Lin	146.0	145.8	147.1	0.04	+
	Grazing	Lin	146.0	144.6	146.6	0.06	+
	Soil depth	Quad	146.0	147.6	145.7	0.07	U
	Soil Na	Lin	146.0	143..8	145.2	0.07	+

Table B9 Community weighted mean univariate model results, best model for each trait CWM and predictor combination presenting only the linear or quadratic models which performed better than the associated null model. Lin = Linear model, Quad = Quadratic model, Soil K = Potassium content, Ca = Calcium content, Mg = Magnesium content, Soil Na = Sodium content, Soil C = Carbon content, Soil P = Phosphorous content, PDIR = Potential Direct Incidence Radiation, - = negative linear relationship, + = positive linear relationship, U = valley shaped quadratic relationship, ∩ = humped shaped quadratic relationship.

Response	Predictor	Best model	AIC null model	AIC linear model	AIC quadratic model	Marginal R^2	Relationship
CWM_{LDMC}	Leaf litter	Lin	647.50	647.46	649.01	0.031	-
	Woody cover	Lin	647.50	645.52	645.70	0.059	-
	Dung count	Lin	647.50	641.02	642.99	0.122	+
	Soil depth	Quad	647.50	646.00	642.51	0.129	∩
	Soil pH	Lin	647.50	646.88	648.88	0.040	-
	Soil K	Lin	647.50	644.86	644.93	0.069	-
	Soil Ca	Quad	647.50	638.60	635.83	0.214	∩
	Soil Mg	Lin	647.50	635.18	636.23	0.198	-
	Soil Na	Quad	647.50	648.88	644.86	0.097	∩
	Sand fraction	Lin	647.50	645.59	647.54	0.058	-
	Clay fraction	Lin	647.50	641.85	642.74	0.111	-
CWM_{SLA}							
	Bare soil	Lin	335.13	330.54	331.55	0.096	+
	Woody debris	Quad	335.13	329.77	329.27	0.141	U
	Woody cover	Quad	335.13	316.40	313.76	0.323	∩
	Elevation	Lin	335.13	320.25	322.08	0.229	-
	Dung count	Lin	335.13	324.50	325.14	0.177	-
	Grazing	Lin	335.13	334.89	335.84	0.034	-
	Soil depth	Quad	335.13	335.96	325.81	0.185	U
	Soil pH	Quad	335.13	333.93	333.75	0.079	∩
	Soil K	Lin	335.13	334.31	336.17	0.042	+
	Soil Ca	Quad	335.13	315.42	314.67	0.314	U
	Soil Mg	Lin	335.13	325.31	326.48	0.166	+
CWM_{FT}							
	Rock cover	Quad	123.13	120.62	114.07	0.182	U
	Woody cover	Quad	123.13	121.89	118.33	0.126	U
	Elevation	Lin	123.13	121.58	123.58	0.053	+
	PDIR	Lin	123.13	122.48	124.58	0.034	-
	Northness	Quad	123.13	122.96	119.44	0.112	∩
	Dung count	Lin	123.13	115.58	117.04	0.137	+
	Soil depth	Quad	123.13	114.92	106.82	0.268	∩
	Soil pH	Quad	123.13	115.06	114.20	0.180	U
	Soil K	Lin	123.13	114.76	115.38	0.147	-
	Soil Ca	Quad	123.13	105.66	105.23	0.286	∩
	Soil Mg	Lin	123.13	106.50	107.44	0.249	-
	Soil Na	Lin	123.13	122.39	124.10	0.041	-
	Soil P	Quad	123.13	124.44	122.81	0.064	U
	Sand fraction	Lin	123.13	122.03	123.80	0.046	-
	Clay fraction	Lin	123.13	118.96	120.14	0.091	-

Table B10 Best subset of multivariate models for CWM_{LDMC} based on AIC scores (i.e. models with AIC scores differing less than 2). CWM_{LDMC} = community weighted mean leaf dry matter content, Soil K = Potassium content, Soil Mg = Magnesium content, Soil Ca = Calcium content, Soil Na = Sodium content, + = positive linear relationship, - = negative linear relationship, U = valley shaped quadratic relationship.

Predictor	Leaf litter	Woody cover	Dung count	Soil depth	Soil pH	Soil K	Soil Mg	Soil Ca	Soil Na	Sand fraction	Clay content	Model AIC	Model R^2	Model ranking
Response CWM_{LDMC}	-		+	∩					∩			622.9	0.45	1
	-		+	∩			-		∩			623.5	0.46	2
	-		+	∩					∩			623.5	0.43	3
	-		+	∩					∩		-	623.6	0.44	4
	-		+	∩					∩		-	624.3	0.45	5
	-		+	∩			-		∩			624.4	0.45	6
	-		+	∩					∩	-		624.5	0.43	7
	-		+	∩					∩			624.5	0.43	8
	-	+	+	∩			-		∩			624.6	0.45	9
	-		+	∩			-		∩			624.7	0.45	10
	-		+	∩					∩	-		624.9	0.45	11
	-		+	∩			-		∩		-	624.9	0.46	12

Table B11 Best subset of multivariate models for CWM_{SLA} based on AIC scores (i.e. models with AIC scores differing less than 2). CWM_{SLA} = community weighted mean specific leaf area, Soil K = Potassium content, Soil Mg = Magnesium content, Soil Ca = Calcium content, Soil Na = Sodium content, + = positive linear relationship, - = negative linear relationship, U = valley shaped quadratic relationship.

Predictor	Bare soil	Woody debris	Woody cover	Dung count	Grazing level	Soil depth	Soil pH	Soil K	Soil Ca	Soil Mg	Model AIC	Model R^2	Model ranking
Response CWM_{SLA}	+			-		∩	-		+	-	288.2	0.62	1
	+		U	-				+	+	-	288.3	0.62	2
	+		+	-		∩			+	-	288.5	0.62	3
	+	∩	U	-		∩			+	-	288.6	0.63	4
	+		U	-	+				+	-	289.1	0.62	5
	+		U	-				+	+	-	289.3	0.60	6
	+		+	-		∩	-		+		289.5	0.61	7
	+		+	-		∩			+		289.7	0.60	8
	+		+	-		∩		+	+	-	289.7	0.61	9
	+		+	-	+	∩			+		289.9	0.61	10
	+	∩	U	-				+	+	-	289.9	0.62	11
	+		U	-		∩			+		289.9	0.61	12
	+		+	-		∩	-	+	+		290.0	0.61	13
	+	-	U	-				+	+	-	290.0	0.62	14
	+		+	-		U			+		290.1	0.58	15
	+		U	-			-	+	+	-	290.1	0.61	16

Table B12 Best subset of multivariate models for CWM$_{FT}$ based on AIC scores (i.e. models with AIC scores differing less than 2). CWM$_{FT}$ = community weighted mean force to tear, Soil K = Potassium content, Soil Mg = Magnesium content, Soil Ca = Calcium content, Soil Na = Sodium content, + = positive linear relationship, - = negative linear relationship and ∩ = humped shaped quadratic relationship.

Predictor	Woody debris	Dung count	Soil depth	Soil pH	Soil K	Soil Ca	Soil Na	Clay fraction	Model AIC	Model R^2	Model ranking
Response CWM$_{FT}$		+	U		-	-			87.3	0.51	1
			U			-	-		88.4	0.48	2
		+	U			-	-		88.4	0.49	3
			U		-	-			88.5	0.48	4
	-		U			-	-		88.5	0.50	5
			U			-			88.6	0.46	6
	-		U		-	-			88.7	0.50	7
			U			-			88.9	0.48	8
			U			-	-	-	89.0	0.49	9
		+	U			-			89.0	0.48	10
			U			-	-		89.1	0.49	11
			U	+	-	-			89.1	0.49	12
			U	+		-	-		89.1	0.49	13
	-	+	U		-				89.1	0.49	14

Table B13 Linear mixed effect models result predicting the relationships between PFT's and grazing quality components. LDMC = leaf dry matter content, SLA = specific leaf area, FT = force to tear, ADF = acid detergent fibre, NDF = neutral detergent fibre.

Predictor	Response	Model F	Model P	Marginal R^2
LDMC	Protein content	0.24	0.626	0.01
	ADF	0.63	0.427	0.00
	NDF	0.46	0.499	0.00
SLA	Protein content	2.65	0.105	0.01
	ADF	0.51	0.474	0.00
	NDF	0.34	0.558	0.00
FT	Protein content	2.57	0.111	0.02
	ADF	1.03	0.311	0.00
	NDF	0.00	0.989	0.00

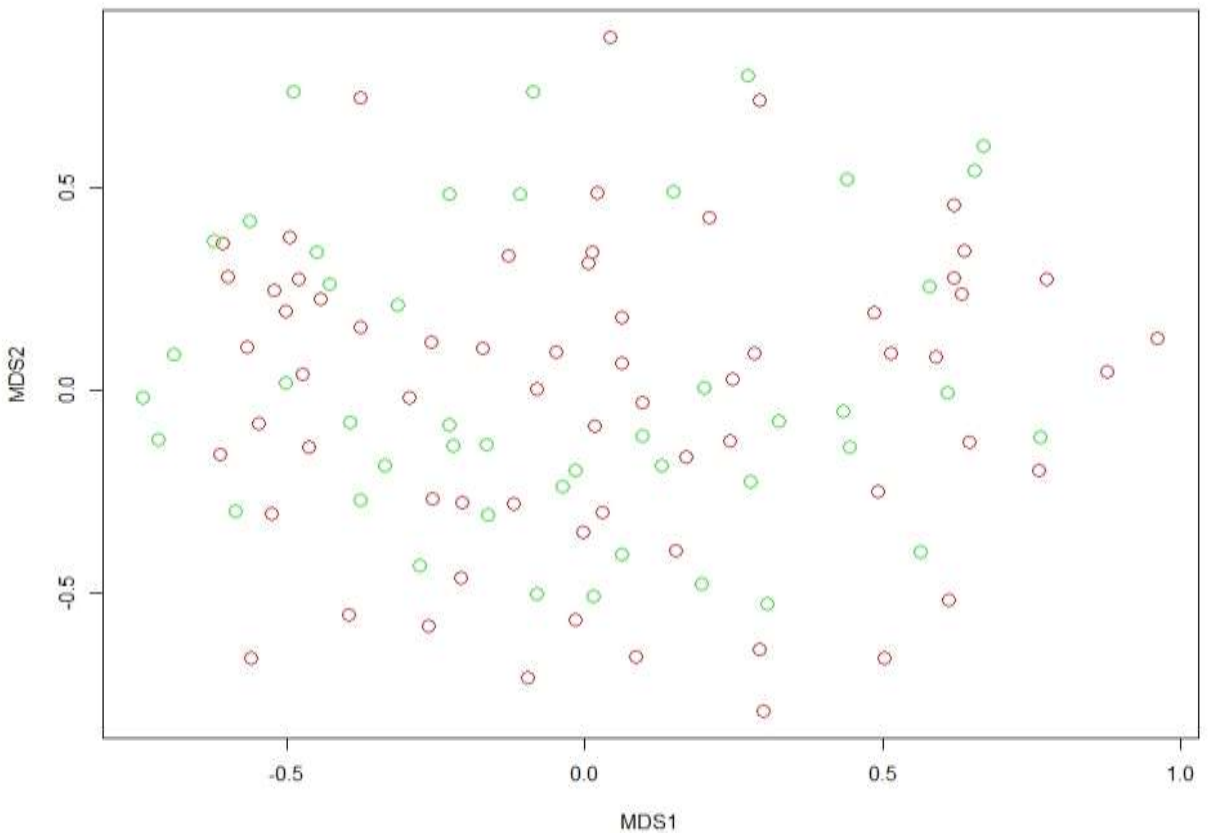


Figure B1 Non-metric multidimensional scaling based on grass species composition and cover used for the stratification process of the WGR transect lines. Red symbols indicate the final 65 selected transects and green symbols the excluded transects.

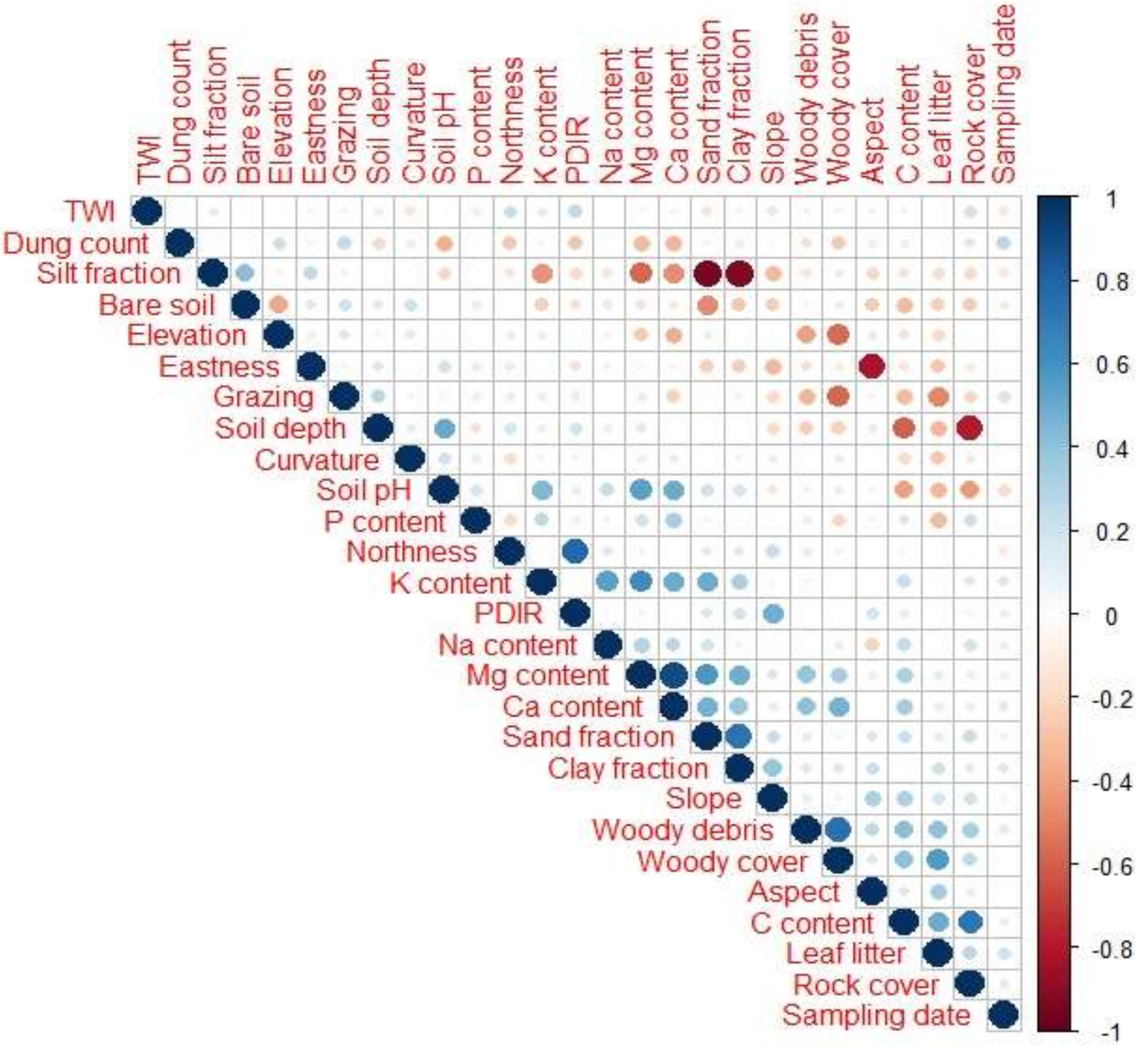


Figure B2 Correlation matrix of all environmental variables TWI = topographic wetness index, PDIR = potential direct incoming radiation.

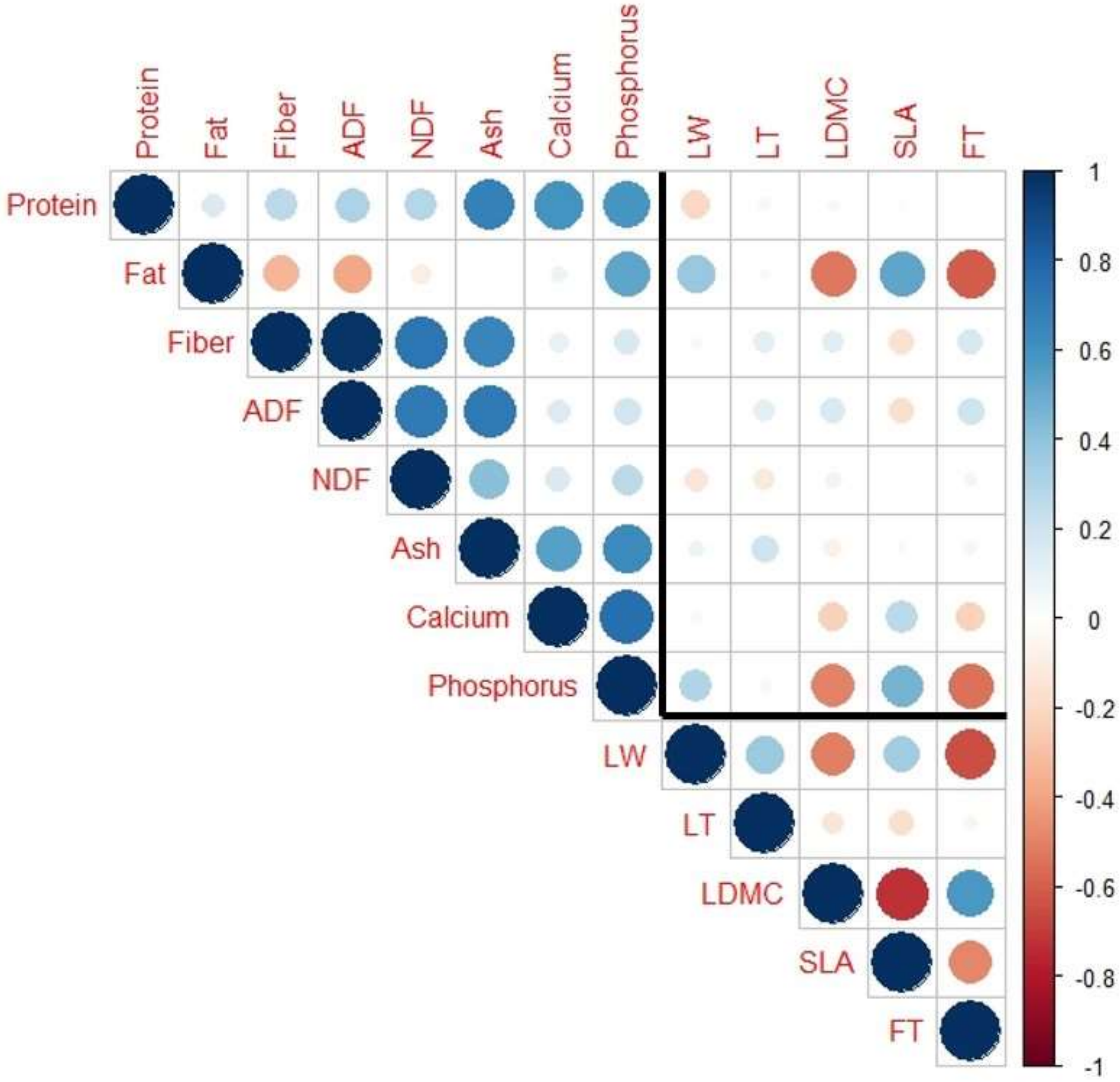


Figure B3 Correlation matrix of all plant functional traits and grazing quality components. ADF = acid detergent fibre, NDF = neutral detergent fibre, LW = leaf width, LT, = leaf thickness, LDMC = leaf dry matter content, SLA = specific leaf area and FT = force to tear.

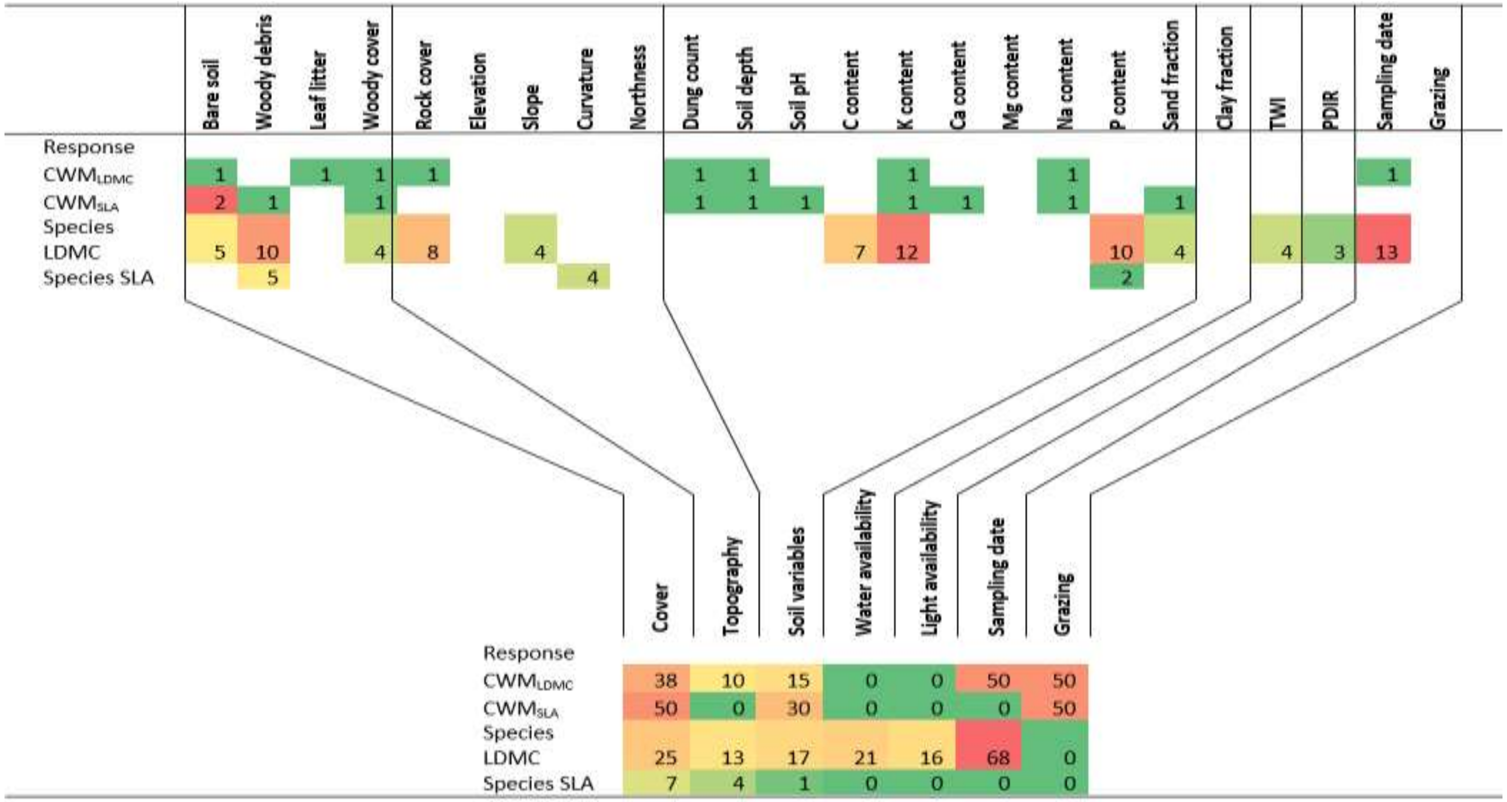
Chapter 4: General conclusion

In the Waterberg region of the South African savanna ecosystem, environmental conditions were related to grass species composition and cover (Chapter 2), and community-level and intra-specific variation in grass functional traits (Chapters 2 and 3). The strength and nature of these relationships varied considerably, and most relationships were idiosyncratic. Grazing quality varied greatly between species and some variation was also observed within species, but intra-specific variation in grazing quality was not strongly related to environmental conditions. The PFTs investigated in this study had no significant relationships with three core grazing quality components: protein content, acid detergent fibre, and neutral detergent fibre. These PFTs are, therefore, unsuitable to act as proxies of grass grazing quality.

Across both study sites, there was only a single environmental variable, bare soil, that had a consistent impact on SLA at the community level (i.e. CWM_{SLA}, Table 4.1, Fig. 4.1). Similarly, at the species-level, no environmental variables were consistently related to SLA in the four species that were dominant in both LWNR and WGR. The lack of consistency in trait-environment relationships between sites suggests that the relationships documented for each reserve are not robust (i.e. are geographically localized), highlighting the challenges of accurately extrapolating trait-environment relationships between sites, even when within the same geographic region and/or the same biome (although see; Kemppinen et al., 2021). In contrast, LDMC was related to six environmental variables in species that are common in both reserves (Table 4.1). Moreover, whenever the same environmental variables impacted the same traits between the reserves, at the species- or community-levels, their relationships with the respective PFTs were always consistent, highlighting that some trait-environmental relationships are comparable, albeit relatively weak, between sites. For example, at both sites the LDMC of *D. eriantha* was negatively related to TWI, suggesting that across both environments this grass species reduced investment in structural plant cell components in wetter environments. In situ measurements of more proximally and ecophysiologically relevant environmental variables (e.g. soil moisture and soil temperature) could potentially provide better tools for comparing trait-environmental relationships between sites compared to several of the more distal variables (e.g. derived from remotely sensed data) used in this study (Lembrechts et al., 2020).

Table 4.1 Trait-environmental relationships at the community- and species-level from the best fit models (combined from Chapters 2 and 3; only showing the species and community traits recorded at both sites). LWNR = Lapalala Wilderness Nature Reserve, WGR = Welgevonden Game Reserve, CWM_{SLA} = community weighted mean specific leaf area, CWM_{LDMC} = community weighted mean leaf dry matter content, Dig eri = *Digitaria eriantha,* Lou sim = *Loudetia simplex,* Sch pap = *Schmidtia pappophoroides,* Set sph = *Setaria sphacelata* U = valley shaped quadratic relationship and ∩ = humped shaped quadratic relationship. - = negative linear relationship and + = positive linear relationship. PDIR = potential direct incidence radiation, TWI = topographic wetness index. Blue = same environmental variable impacting the same trait at the community-level in both LWNR and WGR, Yellow = same environmental variable impacting the same trait at the species-level in both LWNR and WGR.

Site	Response	Rock cover	Bare soil	Woody debris	Leaf litter	Woody cover	Elevation	Slope	PDIR	Curvature	Northness	TWI	Dung count	Grazing	Soil depth	Soil pH	Soil C	Soil K	Soil Ca	Soil Mg	Soil Na	Soil P	Sand fraction	Clay fraction	Sampling date
LWNR	CWM_{LDMC}	U	-			U												∩							+
WGR	CWM_{LDMC}				-								+		U						U				
LWNR	CWM_{SLA}		+	+		+												U					∩		
WGR	CWM_{SLA}		+										-		U	-			+	-					
	Species SLA																								
LWNR	Dig eri											+						+							
WGR	Dig eri								-		U						+								
LWNR	Lou sim											+										-			
WGR	Lou sim															∩					+		∩		
LWNR	Sch pap																								
WGR	Sch pap								∩		-														
LWNR	Set sph			+																					
WGR	Set sph									+														∩	
	Species LDMC																								
LWNR	Dig eri							+	-			-						U				+			+
WGR	Dig eri											-										+			+
LWNR	Lou sim		-					+									+	-				+			+
WGR	Lou sim			-	∩					-							∩	-			+				
LWNR	Sch pap		+					+										∩				U			
WGR	Sch pap			∩					-		+		-											-	
LWNR	Set sph	-		-		+												∩				+			
WGR	Set sph			-																			+		



Response	Bare soil	Woody debris	Leaf litter	Woody cover	Rock cover	Elevation	Slope	Curvature	Northness	Dung count	Soil depth	Soil pH	C content	K content	Ca content	Mg content	Na content	P content	Sand fraction	Clay fraction	TWI	PDIR	Sampling date	Grazing
CWM$_{LDMC}$	1		1	1	1					1	1			1			1						1	
CWM$_{SLA}$	2	1		1						1	1	1		1	1		1		1					
Species LDMC	5	10		4	8		4						7	12				10	4		4	3	13	
Species SLA		5						4										2						

Response	Cover	Topography	Soil variables	Water availability	Light availability	Sampling date	Grazing
CWM$_{LDMC}$	38	10	15	0	0	50	50
CWM$_{SLA}$	50	0	30	0	0	0	50
Species LDMC	25	13	17	21	16	68	0
Species SLA	7	4	1	0	0	0	0

Figure 4.1 A schematic summarizing the number of relationships for each trait-environmental variable combination for each reserve at the species- and community-level. The top section = the total number of trait-environmental relationships excluding reserve or species identity at the community- and species-level. LWNR = Lapalala Wilderness Nature Reserve, WGR = Welgevonden Game Reserve, CWM$_{SLA}$ = community weighted mean specific leaf area, CWM$_{LDMC}$ = community weighted mean leaf dry matter content, PDIR = potential direct incidence radiation, TWI = topographic wetness index. Bottom section = the percentage of possible trait-environmental relationships as trait relationships that were included in top-ranked models for each response variable. The links between the two sections indicate which variables from the top section was classified in which broad environmental group in the bottom section. Green indicates a low proportion of influential relationships out of all potential trait relationships with yellow and orange indicating higher proportions of influential relationships out of all proportions and red ultimately indicating a high proportion of influential relationships out of all potential proportions.

When excluding species that were common in both reserves, a diversity of relationships between traits and environmental conditions were observed (Fig. 4.1). These results help fill the gaps in our understanding of trait-environment relationships for savanna grasses and provide insight into which environmental variables are important drivers of functional traits in this assemblage. Where consistent trait-environmental relationships can be identified, these relationships provide an opportunity for use in ecosystem service monitoring and land management. For example, LDMC, SLA and FT (i.e. resource use- and conversion-related traits) could be used to monitor how grasses, and as a result biomass production would respond to changes in environmental conditions. More broadly, consolidating variables into groups (e.g. soil variables vs topographic variables; Fig. 4.1) may also reveal some general patterns, including that cover- and soil variables were, overall, relatively important drivers of SLA and LDMC at the community level. However, being able to include multiple studies into such a consolidation would provide more reliable results.

Some traits and environmental variables have more consistent relationships than others. At the species level, for example, LDMC was more frequently correlated with environmental variables than SLA. This suggests that LDMC may be a more practical choice when investigating the impact of environmental variables on traits, since this trait appears more sensitive to environmental variation and, therefore, may be useful for monitoring the reaction of individual species (or species assemblages) to changing conditions. Another practical implication of these results stems from the observation that more species showed a relationship between sampling date and LDMC at LWNR where sampling was conducted over a longer time frame compared to the sampling in WGR. This reiterates the importance of sampling within a single season and in as short a time frame as possible when investigating LDMC (and other related traits). A sampling strategy that favours collecting data over as short a period as possible will reduce the potentially confounding impact of plant growth and age on traits. Moreover, irrespective of the duration of a sampling campaign, sampling date should be considered in analyses to control for such temporal effects that could otherwise obscure environment-trait relationships

The large differences in the proportion of variation in grass characteristics at the species- and community-level explained by models in this study (Fig. 4.2) show that some important environmental-trait relationships have been identified, but others may still need to be uncovered. Interestingly the lowest R^2 values at both study sites at the species-level are for SLA in *S. pappophoroides*, the most widespread species in LWNR (but scarcer in WGR). This result may reflect the species' widespread distribution in LWNR, highlighting the species ability to survive in a wide range of conditions without showing clear trends in leaf characteristics. However, the patchy distribution of *S. pappophoroides* in WGR suggests that the species is limited to specific environmental conditions (potentially, environmental conditions similar to those common in LWNR). Using *S. pappophoroides* as an exemplar species, it is clear that much further research is required to understand which environmental variables are influential on the traits, distribution, and grazing quality of the dominant grass species.

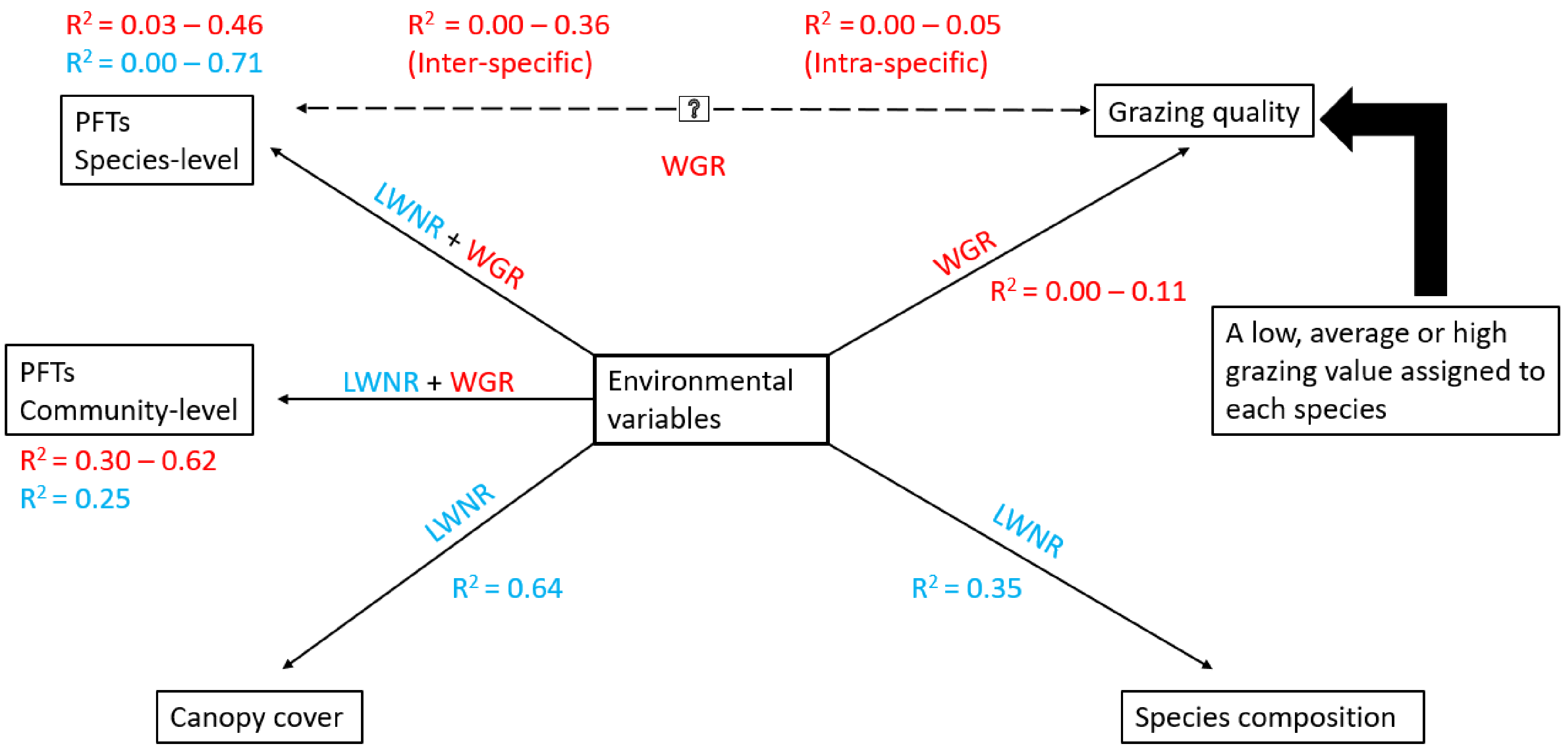


Figure 4.2 Links between the different components of this study. Marginal R^2 values are from the best model for each grass characteristic, at the community-level the range of R^2 values indicate the lowest- to the highest value for the best model for multiple traits and at the species-level for multiple traits and species. LWNR (in blue) = Lapalala Wilderness Nature Reserve, WGR (in red) = Welgevonden Game reserve, PFTs = Plant functional traits.

In this study, I found that the categorization of grass species into two or three grazing quality classes (i.e. high vs low grazing quality) has some merit since the species identity of grasses accounted for a large proportion of the variation in grazing quality. For example, species identity explained up to 91 % of the variation in ADF content. Based on the data reported in Chapter 3, I have calculated a coarse grazing quality index (GQI) for each of the 12 species investigated at WGR, assigning a value for protein content and crude fibre (see Table 4.2). Of the 12 species investigated, four species could clearly be differentiated based on their grazing quality components (Fig. 3.3 in Chapter 3). *Schmidtia pappophoroides* and *E. gummiflua* had high ADF and NDF values and, largely as a result, have the lowest grazing quality index values (GQI; Fig. 4.3). In contrast, *D. eriantha* had the highest protein content, and *C. dactylon* had the lowest ADF and relatively high protein content (giving both species high GQI scores; Fig. 4.3). This agrees with the earlier commercialization of both *D. eriantha* and *C. dactylon*. However, this study also suggests that the general idea of dividing grass species into low, average, and high grazing quality categories is not optimal, with a more continuous grazing quality index allowing for better division of grass species based on their grazing quality (Fig. 4.3). There was only a weak correlation between the GQI determined in this study and the grazing quality category previously assigned in the literature to species (Fig. 4.3), suggesting that these classifications may be of limited value at a national or regional scale. However, the grazing quality categories assigned to grass species also typically take other components, such as palatability, into account and are therefore not necessarily directly comparable with the GQI values calculated here. As a result, there is considerable scope for an improved GQI which takes all the grazing quality and grazing quality components of grasses into account.

Table 4.2 Categories used to calculate the grazing quality index of each species. Categories are assigned based on % crude fibre and crude protein. The two category values are added up per species to obtain a grazing quality index out of 10.

Category value	1	2	3	4	5
Crude fibre (%)	< 55	< 50	< 45	< 40	< 35
Protein content (%)	< 3	< 6	< 9	< 12	< 15

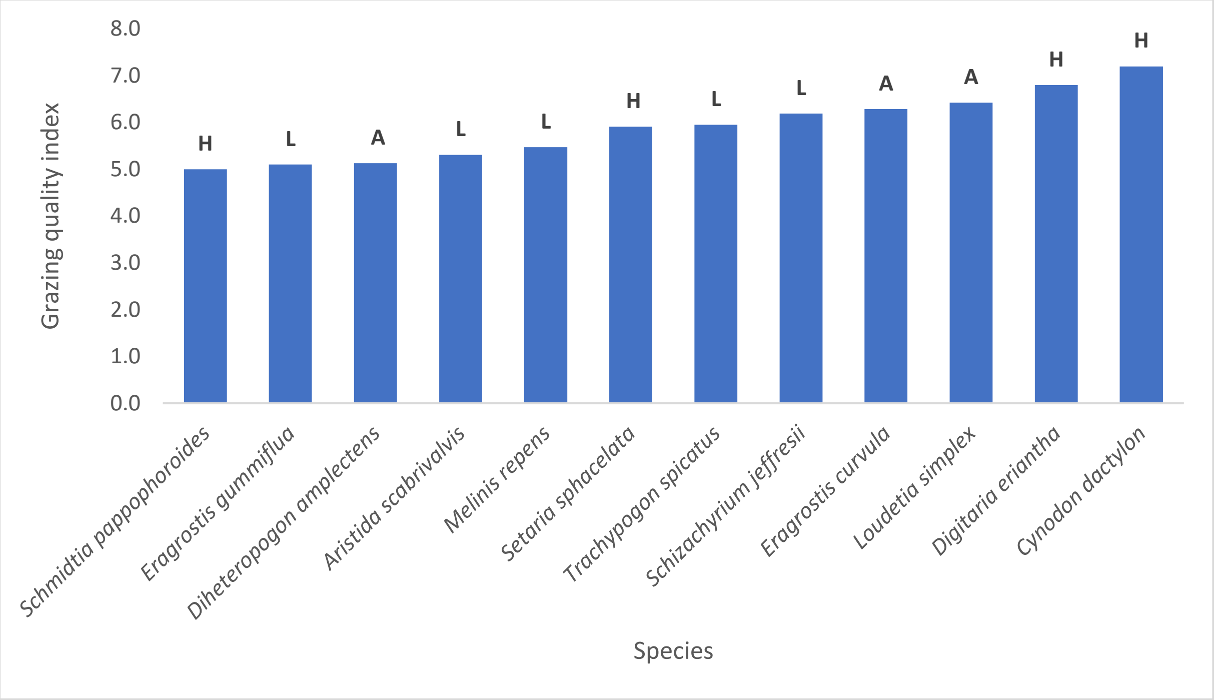


Figure 4.3 Grazing quality index calculated for each dominant grass species at WGR compared to grazing quality categories obtained from literature. The letters above each bar indicate grazing quality categories obtained from literature, L = low grazing quality, A = average grazing quality and H = high grazing quality.

Intra-specific variation in grazing quality was inadequately explained by environmental variables. The variation in ADF and NDF within species could only be weakly related to a single environmental variable (soil Ca), and a maximum of 11% of the variation in protein content could be explained. Therefore, despite the broad range of environmental variables used in this study, strong relationships between grazing quality components and environmental conditions could not be identified. Several other environmental variables could, however, still be considered in these analyses, including climatic conditions (which can potentially vary markedly across the spatial extents examined here; Bennie et al., 2008; Scherrer and Körner, 2011). The intra-specific variation in grazing quality could also potentially stem from genetic differences between populations and individuals in populations, but this is poorly examined in grasses (although see e.g. Valliant et al., 2007; Hagl et al., 2020). Another possible factor that could impact grazing quality is biotic interactions. For example, legume plants that can fix nitrogen, growing in close approximation of grasses that could impact the protein content of the grasses (Schwinning and Parsons, 1996, see also e.g. Paciullo et al., 2017 as an example of shading by trees affecting grass grazing quality).

Plant functional traits could not be significantly related to any of the core grazing quality components. Other PFTs to potentially consider in future studies should include leaf nitrogen content, specifically due to its strong relationship with protein content (and its potential link to soil nitrogen content).

However, since leaf nitrogen content cannot readily be measured in the field and typically requires laboratory analyses post-collection (although see Ecarnot et al., 2013), measuring this trait may not necessarily be pragmatic. Leaf area is another PFT that could potentially be related to protein content and is more easily measured in the field. Other traits with potential relationships with grazing quality are plant size traits (Pérez-Harguindeguy et al., 2013) and growth form (Whitworth-Hulse et al., 2016), and these traits may, therefore, be worth further investigation in this context.

This study provides insight into the relationships between different PFTs, grazing quality components and environmental variables at the species- and community-level. As a result, my findings give some new insights into these relationships and point to the importance of future studies examining how PFTs, environmental variables and grazing quality are interrelated (especially in ecosystems where knowledge on this topic is completely lacking). More practically, this study provides information on which grass species from this system can objectively be considered to be good grazing species. Other wild grass species, as well as other PFTs, should be investigated in future studies to enable us to get a better understanding of the relationships between these different components (and specifically to determine if any functional traits can be used as easily measured proxies for grass grazing quality). Increased knowledge of the relationships between PFTs, grazing quality, environmental conditions at the inter-and intra-specific level may be able to inform decisions on the management of grazing regimes. For example, using this information to determine grazing capacity, stocking rates and the minimum requirements per grazing animal on a particular grazing area. By doing so we will be able to monitor and ensure that one of the most important ecosystem services, grazing provisioning, can be more adequately and sustainably provided in the future.

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