UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM ECOLOGIA E BIODIVERSIDADE

# PHENOTYPIC AND OPTICAL PLANT TRAIT VARIATION ACROSS SPACE AND TIME IN THE SEASONAL TROPICS: PATTERNS, DRIVERS AND CONSEQUENCES

ANNIA SUSIN STREHER

Dezembro - 2018

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Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutora em Ecologia e Biodiversidade.

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## CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: Phenotypic and optical plant trait variation across space and time in the seasonal tropics: patterns, drivers and consequences

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This work is dedicated to all women in science. You are a true inspiration.

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A learning experience is one of those things that says,
'You know that thing you just did? Don't do that'

-Douglas Adams-

## DECLARAÇÃO

Declaro que as opiniões, hipóteses e conclusões ou recomendações expressas neste material são de responsabilidade do(s) autor(es) e não necessariamente refletem a visão da CAPES e FAPESP.

## RESUMO

O objetivo geral dessa tese foi explorar os conceitos da ecologia baseada em atributos a fim de entender como o ambiente modula a variação de atributos funcionais em diferentes escalas espaciais e temporais, combinando sensoriamento remoto e ecologia vegetal. No segundo capítulo da tese, avaliou-se os padrões de fenologia remota do mosaico de vegetação na montanha do Espinhaço, investigando quais os agentes ambientais são responsáveis pelos padrões observados. O algoritmo TIMESAT foi utilizado para extrair os indicadores fenológicos de uma série temporal de 14 anos de imagens de satélite MODIS/NDVI. A disponibilidade de água e luz, modulada pela topografia, foram os principais responsáveis pelas respostas da fenologia remota na região, determinando o início, fim e comprimento da estação de crescimento. A temperatura teve um papel importante na determinação das taxas de desenvolvimento das folhas e na força da sazonalidade da vegetação. No capítulo três, testou-se a generalidade dos padrões globais de estrutura e função da vegetação na escala da paisagem, na porção sul do Espinhaço, conhecida como Serra do Cipó em Minas Gerais. Também buscou-se determinar a relação dos atributos funcionais que representam as dimensões de estrutura e função da vegetação com os gradientes de elevação e topo-edáficos encontrados na região. Foram coletadas características funcionais (LMA, LDMC – relacionados à economia foliar, área foliar e altura da planta – relacionados com a estrutura da vegetação) de 1650 indivíduos, compreendendo todas as formas de vida, em cinco locais ao longo de um gradiente de elevação, abarcando os diferentes tipos de vegetação encontrados em cada elevação. A organização fenotípica na estrutura e função das plantas encontrada em escalas globais foi análoga à encontrada entre as espécies que co-ocorrem localmente nos campos rupestres. A dimensão relacionada a estrutura da vegetação apresentou variação ao longo dos gradientes ambientais, porém as relações alométricas foram igualmente importantes para explicar as variações encontradas nessa dimensão funcional. A dimensão fenotípica relacionada a função foliar não apresentou variação relacionada a nenhum dos gradientes ambientais avaliados, indicando que os atributos funcionais, LMA e LDMC, não estão relacionados com as estratégias de aquisição e uso dos recursos em ambientes sazonalmente secos. No capítulo quatro, utilizou-se os dados funcionais de folhas coletados no capítulo três, para testar a capacidade da espectroscopia de solo em estimar as características funcionais foliares e diferenciar plantas com diferentes formas de crescimento. Os espectros de reflectância foliar foram capazes de predizer com precisão as características funcionais de folhas da vegetação independente da forma de crescimento, porém os modelos apresentaram imprecisão em torno dos valores maiores de LMA. Este resultado aponta para uma limitação ou do método da espectroscopia e/ou do método de modelagem utilizado neste estudo. Gramíneas e plantas lenhosas apresentaram respostas espectrais mais dissimilares, enquanto as herbáceas representam um tipo espectral intermediário, parcialmente semelhantes as gramíneas (no visível) e

parcialmente semelhantes às plantas lenhosas no infravermelho médio. No capítulo cinco foi investigada a influência dos níveis taxonômicos na relação entre a diversidade espectral e funcional em um subconjunto de plantas coletadas no capítulo três. A variação interespecífica foi maior que a variação intraespecífica para todas as características funcionais e espectrais da vegetação, mas o tamanho da influência intraespecífica foi uma resposta específica de cada espécie. Os resultados indicaram também que a idade foliar pode estar contribuindo mais que o esperado na variabilidade espectral intraespecífica e assim, dificultando o delineamento de um paralelo com os processos reconhecidos pela ecologia baseada em atributos que geram a variação intraespecífica (i.e., plasticidade). A partição da variância mostrou que tanto os atributos funcionais quanto os atributos espectrais variaram principalmente no nível de família, indicando que ambos são conservados evolutivamente. Este estudo contribui para a construção de teorias ligando a diversidade espectral com a diversidade funcional e taxonômica, os quais são muitas vezes difíceis de quantificar nos trópicos, auxiliando a impulsionar um sistema de monitoramento da biodiversidade baseado em sensoriamento remoto hiperespectral.

**Palavras-chave**: ecologia baseada em atributos, sensoriamento remoto, fenologia, atributos funcionais, LMA, LDMC, campo rupestre, espectroscopia.

## ABSTRACT

Here, I explore trait-based ecology to understand how the environment shapes plant trait variation at multiple scales, combining remote sensing technologies and plant ecology. In the second chapter, the patterns and drivers of land surface phenology were assessed for the Meridional Espinhaço Range in Brazil. The TIMESAT algorithm was used to extract the phenological indicators from a14-year time series of MODIS/NDVI satellite images. Water and light availability, modulated by topography, are the most likely drivers of land surface phenology in the region, determining the start, end, and length of the growing season, while temperature had an important role in determining the rates of leaf development and the strength of vegetation seasonality. In chapter three, I tested if the generalities of global patterns of plant form and function dimension held in finer scales in the seasonally dry tropics, and its relation with the an elevational and topo-edaphic environmental gradient. Leaf functional data (LMA, LDMC and leaf area) and plant height from 1650 individual comprising all life-forms of locally co-occurring plants was gathered, at five sites along an elevational gradient, sampling all vegetation types found within each elevation, at the southern portion of the Espinhaço range, known as Serra do Cipó, in Minas Gerais. The phenotypic organization of plant form and function found at global scales was similar to the one found among locally co-occurring species in *campo rupestre*. The whole-plant size dimension varied along the elevational gradient, however, the leaf economics dimension (LES) behaved differently than plant size, and no variation of key LES traits along environmental gradients was found. In chapter four, the ability of spectroscopy to estimate leaf functional traits and to differentiate plants comprising different growth forms was investigated. Leaf reflectance spectra was able to accurately predict leaf functional traits from different growth forms, but the models lost precision towards higher LMA values, pointing out a saturation point from spectroscopy and/or a limitation from the modelling approach adopted in this study. Grasses and woody plants were the most spectrally dissimilar, while forbs represented an intermediary spectral group. In chapter five the influence of taxonomic levels on the relationship between spectral and functional diversity was investigated. Interspecific variation was greater than intraspecific variation for functional and spectral traits, but the amount of intraspecific variation was a specific response of each species. Leaf age may be contributing more than expected to intraspecific spectral variability, hampering the delineation of a full parallel with trait-based ecology. The variance partition showed that both, functional and spectral traits varied mainly at the family level, indicating that both are evolutionary conserved. This study contributes to the construction of theories linking spectral to functional and taxonomic diversity, helping to build a biodiversity monitoring system based on remote sensing.



**Keywords:** trait based ecology, remote sensing, phenology, functional traits, LMA, LDMC, spectroscopy.

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# 1. GENERAL INTRODUCTION

This thesis is a tale of two stories. The first story is about a tropical mountain of recognized ecological importance as an ecotone among three tropical biomes, and how the environment shapes functional traits and ecosystem function across scales. The second story is about a less known biodiversity component, the spectral component, and its links with functional and taxonomic diversity, providing a fresh perspective on how we understand key plant structural and physiological features, emerging as an important piece of the puzzle that is ecology.

## 1.1 The appeal of trait based ecology

Predicting species abundances is one of the most fundamental pursuits in ecology, and despite over a century of scrutiny, understanding the drivers of species diversity across space and time remains a crucial research area (Whittaker, 1967; McGill *et al*., 2006; Kraft *et al*., 2011), given the accelerated biodiversity losses during the Anthropocene era (Schimel *et al*., 2013). Although the early plant geographers had perceived that species phenotypic variability influenced their abundance and distribution (Schimper, 1903; Grime, 1974), the species-centric approach, in which the numbers and types of specimens were compared within and between environments (Whittaker, 1967), was adopted in ecology's early phases as a way of understanding diversity. Recently, it has been argued that the focus on species identities has led to a loss of ecological generality and predictability (McGill *et al*., 2006), especially in highly diverse systems, where using species as the working unit is often overwhelming (Messier *et al*., 2010).

The shift from a species-centric view to a trait-based approach was the conceptual framework proposed to reconcile long-standing hypotheses within ecology (Lavorel & Garnier, 2002; McGill *et al*., 2006) and enhance predictability (Houlahan *et al*., 2017). The appeal of the so called "trait based ecology" resides in its promise of generality, by providing a common basis for the comparison of individuals, populations and species from different phylogenetic histories and environments (Shipley *et al*., 2016). This new type of ecology seems curiously similar to the older functional ecology and comparative ecology, and the boundaries between such disciplines are not clear (Shipley *et al*., 2016). For the sake of simplicity, I use functional ecology and trait-based ecology as synonyms. I use the definition of trait-based ecology as a description of organisms that emphasizes the values of their phenotypic traits over their taxonomic or phylogenetic affiliations (thus "trait-based" ecology), allowing for the explicit comparison of trait values between many species, environments and ecological scales in order to elucidate general trends (McGill *et al*., 2006; Garnier *et al*., 2015; Shipley *et al*., 2016).

The advantages of the trait-based approach are threefold. First, a summarized list of plant traits that are good indicators of ecological strategies, easily available to plant ecologists (Westoby *et al*., 2002). Second, traits are usually straightforwardly measured by following standardized protocols, allowing the comparison of multiple trait values using a common basis, across multiple scales, even in highly diverse communities (i.e. Kraft et al. 2008). This approach thus provides a link between population, community, and ecosystem processes (e.g., growth, metabolism, reproduction; McGill et al. 2006). Third, since traits are closely coupled to the environment and to interactions, their use improve our understanding of the selective pressures constraining species distributions, and provides a mechanistic plant-environment relationship (McGill *et al*., 2006).

By defining trait-based ecology, a second question emerges: what is a *trait*? Many definitions can be found in the current literature, causing some confusion not only in the use of the term ‘‘trait’’ itself, but also in the underlying concepts it refers to (Violle *et al*., 2007). Originally, traits were defined by Darwin as proxies for organismal performance (Violle *et al*., 2007; Garnier *et al*., 2015). Over the last three decades, the “trait bandwagon” in ecology (McGill, 2015) has pushed the concept of traits beyond these original boundaries, and trait-based approaches are now used in studies ranging from organismal to ecosystem levels. Unarguably, the two key aspects defining a trait are: 1) something measured from a single individual, and 2) this measure can be conceivably linked to function or performance (e.g fitness or a component of it such as growth rate). In this thesis, I use the definition of *trait* as any morphological, physiological or phenological feature measurable at the individual level, without need to reference the environment or any other level of organization (Violle *et al*., 2007; Garnier *et al*., 2015), which can be understood as a “performance currency” derived from the processes of acquiring, allocating and/or spending energy and resources (McGill *et al*., 2006).

Our understanding of the functional component of biodiversity has been advanced by the concept of strategy dimensions (Grime, 1974), and a core assumption of trait-based ecology is that trade-offs and constraints have shaped phenotypic variation in different trait dimensions (Westoby *et al*., 2002). *Trait dimensions* are sets of correlated phenotypic traits that reflect distinct aspects of a plant’s ecological strategy (Westoby *et al*., 2002), representing the continuous functional variation among plants, relevant to growth, survival and reproduction. Across broad spatial and taxonomic scales and/or large environmental gradients, trait dimensions describe global trends in phenotypic diversity (Wright *et al*., 2004; Reich, 2014; Díaz *et al*., 2016). Our understanding of several plant traits has taken substantial advances in the last two decades, in the light of

empirical data, and ecologists have used these resources to make remarkable progress in understanding plant ecological strategies at large scales (Moles, 2018). However, it is widely understood that patterns and processes change with spatial, temporal and biological scales (Wiens, 1989; McGill, 2010). In fact, recent studies have suggested that relationships among traits detected in large scale studies cannot be interpreted in the context of the predicted tradeoffs at local scales (Messier *et al*., 2010; Funk & Cornwell, 2013; Messier *et al*., 2017b; Anderegg *et al*., 2018), and it remains unclear whether trait dimensions are also present at the smaller spatial and biological scales that are relevant for community assembly processes.

The knowledge gap in the scaling of trait relationships exists not only because we do not know the nature of trait-trait relationships across scales, but also because spatially and temporally explicit information on plant traits is still lacking in ecology (Asner & Martin, 2009; Schimel *et al*., 2015; Jetz *et al*., 2016). Although trait databases keep continuously expanding, available global data on plant functional biodiversity are grossly incomplete and non-representative taxonomically, geographically, environmentally, and temporally (Jetz *et al*., 2016). Most of our theoretical and empirical understanding of trait-trait and trait-environment relationships comes from broad species-level studies. We do not know what to expect in terms of local and regional diversity of traits. According to Jetz et al. (2016), only 2% of currently known vascular plant species have any trait measurements available at the regional scale. This gap is even larger across the tropics. Existing trait databases often overlook tropical regions, and trait sampling is still biased towards the northern hemisphere, yielding a gap of local observations of plant functional traits exactly where available trait data is relatively small when compared to the high level of taxonomic diversity (Schimel *et al*., 2015; Jetz *et al*., 2016). This limits our ability to generalize how variation in trait values changes across environmental gradients and ecological scales.

### 1.2 Trait based ecology in a remote sensing era

The term remote sensing usually refers to the measurement of reflected, emitted, or backscattered electromagnetic radiation from the Earth's surface, using instruments stationed at a distance (far or close range) from the object of interest (Jensen, 2007). The remote sensing definition sounds like a theoretician's dream: a way to obtain data without leaving their chair (Roughgarden *et al*., 1991). Unfortunately, while many remote sensing activities can be done in a chair, remote sensing analysis is far from simple and straightforward, and substantial legwork is still needed to ensure the accurate interpretation of remotely sensed signals (Roughgarden *et al*., 1991). Also, many ecologists seem to ignore the physical principles behind remote sensing, which allow reliable retrieval of biophysical properties from the Earth's surface, generating products that go beyond "pretty study area pictures" or land cover maps (Milton *et al*., 2006; Jensen, 2007). To put it another way, plants can be understood essentially as solar energy factories with leaves and canopies structured to optimize sunlight capture within existing resource constraints, shaping phenotypic variability (Ustin & Gamon, 2010). Consequently, by inverting the viewpoint and looking down from above, remote sensing can assesses key plant structural and physiological features related to its interaction with electromagnetic radiation, giving a new perspective to plant ecology studies (Ustin & Gamon, 2010).

Generally, the link between remote sensing and plant biodiversity is twofold (Turner *et al*., 2003). First, we can obtain direct remote sensing measurements of optical properties (reflectance, absorbance and transmittance) from individual organisms, species assemblages, or ecological communities from hand-held, airborne, or space-borne sensors (Turner *et al*., 2003). Data on ecosystem structure (such as ecosystem extent and fragmentation, or land cover types),

ecosystem function (net primary productivity, land surface phenology or disturbance regimes) and community composition can be derived from satellites and are key to understand large-scale processes, currently comprising the so-called Remotely Sensed Essential Biodiversity Variables (RS-EVB) (Paganini *et al*., 2016). The second link is the indirect remote sensing of biodiversity by deriving environmental variables, such as topography, cloud cover, climate and others, and using them as proxies for estimating species distribution and abundances (Turner *et al*., 2003).

Another important approach is to retrieve temporal information from "historical remote sensing" (Reed *et al*., 2009a). For example, one of the most challenging aspects of understanding vegetation phenology in the tropics is the lack of long-term monitoring datasets (Morellato *et al*., 2013, 2016). Through the use of remotely sensed information of vegetation dynamics, known as *land surface phenology* (LSP), ecologists can have a glimpse of otherwise unknown patterns. For instance, by retrieving information from the *Moderate Resolution Imaging Spectrometer* (MODIS), we can access LSP information every eight days from approximately 20 years back, or even more than 30 years back with more sparse images available from the Landsat series sensors or coarser spatial resolution from the AVHRR series. LSP is defined as the seasonal pattern of variation in vegetation "greenness" of vegetated land surfaces (de Beurs & Henebry, 2004), and although not a trait *per se*, (because is not measured at the individual scale - while ground-based phenology is), it allows us to infer landscape-scale patterns about the timing of phenological events and duration of growing seasons, which are important features of the temporal niche. Nevertheless, our ability to apply remote sensing to map and retrieve plant traits is still a challenge to be conquered, and has been limited by the spatial, temporal and spectral scales of the available technology, combined with the fact that correlative models are also limited by the *in-situ* gaps in trait data (Jetz *et al*., 2016; Cavender-Bares *et al*., 2017).

Spectroscopy can be one solution to this challenge.

### 1.3 New avenues for remote sensing in a trait-based ecological era

With the goal of advancing trait-based ecology, spectrometers are a very promising avenue. Spectroscopy is the acquisition of reflected radiation measurements at contiguous narrow adjacent spectral bands (i.e. "full spectra"), providing a continuous representation of radiation reflectance across the full optical electromagnetic spectrum (Milton *et al*., 2006; Goetz, 2009). Spectroscopy has already proven to be a 'sharper tool', making novel contributions to our understanding of functional traits (Milton *et al*., 2006; Ustin & Gamon, 2010; Asner & Martin, 2016). The integration between remote sensing spectroscopy and plant ecology occurs mainly at the leaf level. Spatial and temporal variations in resource utilization by plants result in chemical, metabolic, structural, and phenological differences that ultimately influence leaf optical properties, namely reflectance, absorbance and transmittance (Ustin & Gamon, 2010; Cavender-Bares *et al*., 2017). Leaf spectroscopy has shown the potential to link leaf optical properties to a wide group of foliar traits (Fig 1.1), including many of the traits used in leaf trait dimensions.

However, a small number of selected leaf traits are unlikely to fully capture the variation contributing to a particular phenotype. In this sense, leaf spectra has an advantage over commonly measured traits: it incorporates more of the total variation in function associated with leaf chemistry, anatomy and morphology, including variation that is difficult to measure as a single trait or may be of unrecognized importance (Cavender-Bares *et al*., 2017; Schweiger *et al*., 2018). Consequently, the leaf reflectance spectra are able to reproduce plant life-history strategy, and can be understood as an overall expression of a "leaf phenotype".

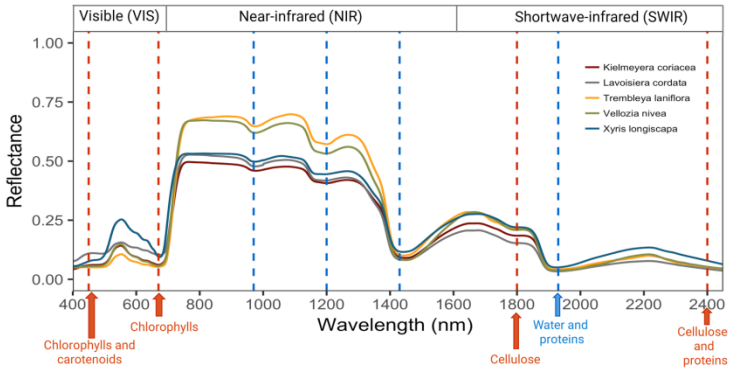


***Figure 1.1:*** Full spectra of different *campo rupestre* plant species, corroborating the potential of spectroscopy for species discrimination. The visible (400–700 nm) region of the spectrum is dominated by leaf pigments absorption; the near-IR (700–1500 nm) region expresses leaf water content and leaf internal structural scattering; and the shortwave-IR (1500–2500 nm) regions are influenced by leaf water, nitrogen and carbon (e.g. lignin and cellulose) constituents. The blue vertical lines indicate water absorption bands.

Besides the potential to retrieve the functional component of biodiversity, the spectral domain is also tied to the taxonomic component of plant diversity (Asner & Martin, 2009; Asner *et al*., 2014a; Schweiger *et al*., 2018) and niche theory. According to the spectral or optical diversity hypothesis (Palmer *et al*., 2002; Rocchini *et al*., 2015; Schweiger *et al*., 2018), the larger the spectral heterogeneity, the higher will be niche availability for different organisms, suggesting that species within a plant community occupy unique spectral spaces delineated by their chemical, anatomical and morphological characteristics. The precept for the optical diversity hypothesis is the same underlying classical niche theory, and more recently, trait-based ecology: interspecific variability in spectral reflectance exceeds intraspecific variability. Hence, the higher the spectral variability of an environment, the higher would be species diversity. Such a hypothesis has been tested with taxonomic data (Rocchini *et al*., 2015, 2018; Schweiger *et al*., 2018) and often resulted in a positive statistical relationship, although this link does not always hold (Schmidtlein & Fassnacht, 2017).

Plant spectra can be sampled at different spatial scales, providing the means to link an array of biological disciplines at different ecological scales (Asner *et al*., 2015, 2016b; Cavender-Bares *et al*., 2016). The comparisons between field sampling of functional traits and leaf-level reflectance measured using imaging spectrometers, be it airborne (including UAV-based) or spaceborne, are still lacking. There is a major knowledge gap between small-scale field studies (≤1 ha) of plant functional traits and broad-scale, remotely sensed estimates of vegetation properties (Asner *et al*., 2016b). We still do not know how traits vary across scales (Messier *et al*., 2010), and we do not know how pixel grain size (spatial resolution) will shape our perception of trait distributions (Asner *et al*., 2015), but the few results already found in the literature are encouraging (Asner *et al*., 2016b; Schweiger *et al*., 2018). Assessing all components of biodiversity in a concise and scalable manner is increasingly urgent. The only low-Earth orbit spectroscopic satellite available is the low-fidelity Hyperion-1, which has proven difficult for estimate functional traits and species composition (Townsend & Foster, 2002; Asner *et al*., 2016b). Recently, researchers have envisioned a global biodiversity observatory by integrating remotely sensed information on functional traits (through an imaging spectroscopy mission) with other remotely sensed information and *in situ* observations of phylogenetic relationships, functional traits and species distributions (Jetz *et al*., 2016; Cavender-Bares *et al*., 2017).

Trait-based ecological studies can be greatly improved by multiscale spectroscopic studies, but to fully realize the potential of spectroscopy, data must be combined with sound ecological theory to pave the way for a more integrated global assessment of plant functional biodiversity (Ustin & Gamon, 2010; Jetz *et al*., 2016; Cavender-Bares *et al*., 2017). While I do not directly address the multiscale relationship of trait based ecology and spectroscopy in this work, my thesis touches on both worlds: ecology and remote sensing. By combining trait-based ecology

with the physical basis of remote sensing, I was able to assess trait-trait, trait-environment and trait-spectra relationships of individual plants. I contribute not only with ecophysiological trait sampling at the individual level, helping to fill the existing gap of local trait observations in tropical regions, but also with standard spectroscopy measurements. Together, these results allowed me to draw some first-order inferences about the shape of the environment-phenotype-spectral relationships for tropical seasonal environments across scales.

### 1.4 Study scales

My thesis is built around a specific study area (Fig 1.2). To "move" research across scales is not something straightforward (Wiens, 1989). The nomenclature of scales is ambiguous and not well defined across the literature. The term "ecological scales" refers here to biological, spatial, and temporal scales on which ecological processes act (Chave, 2013). For this thesis, I will use the term "spatial scales" as the spatial extent defining the range over which a pattern or process occurs. I thus define my spatial scales of interest in three levels: **a)** *regional*: representing patterns often spanning large geographical areas and multiple biomes (Fig. 1.2a); **b)** *landscape*: representing the different elevational bands along a small geographical area, with high environmental heterogeneity, where the main ecological mechanisms (environmental filtering) is expected to occur at the species level; and **c)** *local*: representing local interaction at the individual level (e.g. within a transect), where the main ecological mechanisms are expected to act at the individual level (Fig 1.2c). In addition, ecological scales also include the nested hierarchical nature of "biological scales", and in this thesis, they will be referred to as the levels of individual, species, genus and family.

The Espinhaço Range (*Cadeia do Espinhaço)* is one of the most important biogeographic regions

of South America, so much that in 2005 it was designated as a Biosphere Reserve by UNESCO (UNESCO 2005) (Fig 1.2a). The unique location of the Espinhaço Range places this mountain within an ecotone among three biomes: Atlantic Rainforest to the East and South, Cerrado to the West, Caatinga to the North and, on the highlands, the *campos rupestres*, a montane vegetation mosaic comprising mainly non-forest formations (Silveira *et al*., 2016; Streher *et al*., 2017). Mean elevation is over 1000m above sea level, with peaks reaching 1800 to 2100m, and the rugged topography produces a wide diversity of soils and micro-environmental conditions (Giulietti & Pirani, 1988; Schaefer *et al*., 2016a,b).

The southern portion of the Espinhaço Mountain Range, known as "Serra do Cipó", was chosen for this study as it comprises a large range of environmental heterogeneity within a small geographical area, hosting a megadiverse flora with more than 1800 species recorded within 200 $km^2$ (Alves *et al*., 2014). Variations on vegetation structure and growth form turnover occur naturally across two nested scales associated with distinct environmental gradients: (i) a *landscape* scale, along a short elevational gradient (~600m, from 800m to 1400m) (Fig 1.2b), where subtle changes from forest-*cerrado* (savanna) formations towards grassy-shrubby *campo rupestre* vegetation can be observed Fig. 1.2b); and (ii) a *local* scale within each elevational band, where vegetation mosaics characterized by a high number of endemic plant species and lineages can be associated with soil conditions mostly determined by local topography and microenvironmental aspects (Fig 1.2c). The *campo rupestre* is considered, alongside the *fynbos* (Cape Floristic Region) and *kwongkan* (south-western Australia), as an example of the old climatically-buffered infertile landscapes (OCBILS) theory on which infertile soils and climatic stability are the core explanations for the high floristic richness and levels of endemism (Silveira *et al*., 2016).

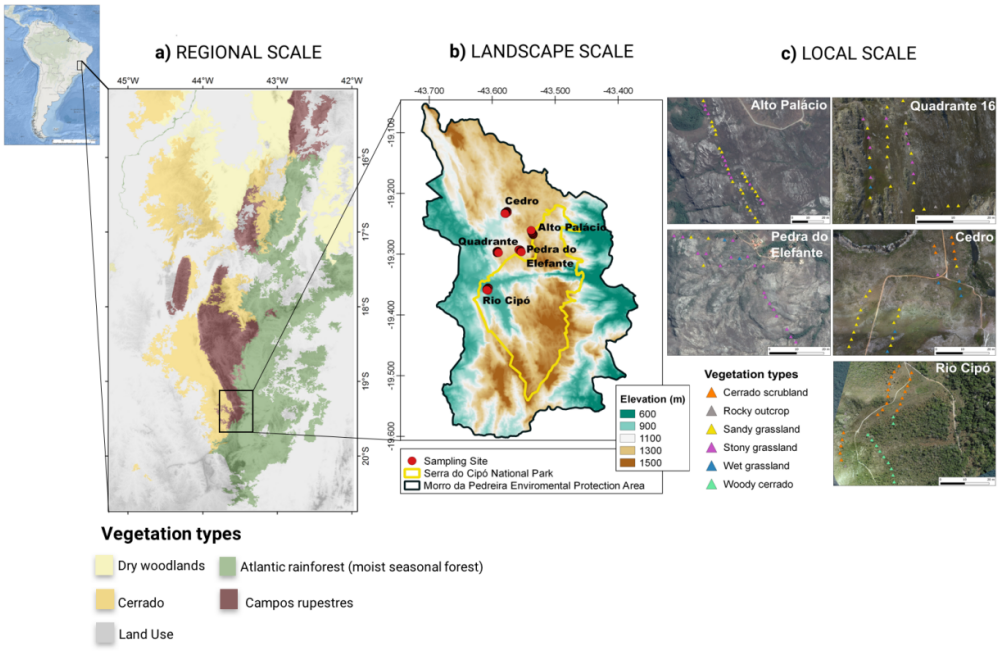


***Figure 1.2:*** Upper left the location and extension of the Espinhaço Mountain Range in South America. The regional scale (a) assessed in this study, comprise the meridional portion of the Espinhaço Mountain Range in the state of Minas Gerais, and its unique location as an ecotone of vegetation types: Atlantic rainforest (moist seasonal forest) to the east and south, cerrado vegetation types to the west, dry woodlands in the north and, on the highlands, *campos rupestres*. The landscape scale (b) shows an overview of the extension and the topography of Serra do Cipó. The red dots mark the locations of the sampling sites along the elevational gradient. Within each sampling site, the local scale (c) comprise a mosaic of microenvironment conditions and vegetation types found across the elevational gradient, and the transects established at each sampling site, with the corresponding vegetation types found at each plot. The UAV images used on (c) are a courtesy of Dr. Patricia Morellato.

## 1.5 Overview of thesis goals, assumptions and general outline

My overarching thesis goal was to explore trait-based ecology to understand how the environment shapes trait variation at multiple scales, from leaves to ecosystems, and from space to species, by combining remote sensing and plant ecology. Together, the work presented here explores three assumptions that are largely untested along the Neotropics, resulting in four chapters. This thesis was written under a format where each chapter comprises a journal manuscript, so the reader may find some redundancy among chapters, especially within the methods section. First, I present the assumption that drove my research question, followed by a

small description of each chapter.

### *1.4.1 The temporal dimension*

Time represents an important resource axis, that may be partitioned by an assemblage of competing species, thus reflecting different selective forces in assemblages of plants (Gotelli & Graves, 1996a). Leaf phenology represents a major temporal component of ecosystem functioning, regulating processes such as carbon, water and energy exchange, forage availability, competition, and coexistence (Cleland *et al*., 2007; Polgar & Primack, 2011). The degree of partial or full loss of leaves is an indicator of shifts in the intensity and timing of species activities (Camargo *et al*., 2018) and changes in leaf deployment and leaf lifespan are therefore likely to have far reaching implications at a wide range of spatial scales. At large scales, vegetation units are better defined by structural and functional attributes rather than by species composition (Higgins *et al*., 2016), consequently phenology is a key component of biomes, and has even been considered as way of delineating and classifying biomes, the so-called *phenomes*, which are based on the link between physiological niche space and leaf habit classes (Givnish, 2002; Buitenwerf & Higgins, 2016).

However, our understanding of where and why different leaf habits are favored is incomplete. In tropical ecosystems, phenology might be less sensitive to temperature and photoperiod, and more tuned to seasonal shifts in precipitation, but we still have a poor understanding of the climatic drivers of tropical phenology (Cleland et al. 2007, Chapter 2), which can be linked to the overall paucity of long-term ecological monitoring. Along the Neotropics, few studies have addressed leaf exchange patterns at the community (Camargo *et al*., 2018) and biome levels - with the exception of the Amazon - (van Leeuwen *et al*., 2013). This knowledge gap is more acute for

tropical mountains, since patterns from temperate mountainous regions are not readily transferrable, as the dominant topography-induced controls on phenology in the tropics are not related to snow cover. Identifying patterns and constraints regulating leafing phenology, and how these mechanisms differ among tropical vegetation types, is therefore critical for understanding vegetation dynamics and for accurately forecasting future changes on tropical mountain ecosystems (Polgar & Primack, 2011).

**Chapter 2:**

The second chapter is based on assumption 1.4.1. We used LSP as a proxy for large-scale phenology to evaluate how a mountainous environment, the Espinhaço range (Fig 1.2a), shapes the timing of leaf phenological events across multiple tropical vegetation types. This chapter consists of a remote sensing analysis of vegetation phenology for the meridional portion of Espinhaço mountain range. I produced a regular time series of vegetation activity based on 15 years of NDVI/MODIS satellite images, acquired from the University of Natural Resources and Life Sciences, Vienna, (http://ivfl-info.boku.ac.at/index.php/eo-data-processing/dataprocess-global/master-global). Leaf phenology metrics, such as start and end of the growing season, peak growing date, season length and amplitude, and rates of growing and senescence were assessed using the TIMESAT 3.2 algorithm (Jönsson and Eklundh 2004). Also based on remote sensing, I produced environmental datasets of clear sky radiation budget, cloud cover, precipitation, topographical soil moisture and temperature, and tested the association between these variables and the satellite-derived metrics of leaf phenology.

*1.4.2. Functional trait dimensions*

Díaz et al. (2016) showed that most variation in plant form and function could be capture by only

two trait dimensions: the first reflecting variations in whole-plant size, broadly understood as architectural constraints (Niklas, 2004; Price *et al*., 2014), and the second representing the construction costs for photosynthetic leaf area, known as the leaf economics spectrum (Wright *et al*., 2004). Although the database used in Díaz et al. (2016) study represents one of the largest compilations of global trait data, it is still lacking in measurements from the tropics (Brian J. McGill personal communication). Furthermore, it is unclear whether global-scale interspecific trait correlations will hold at smaller spatial scales. Indeed, recent studies have questioned if the LES dimension is expressed within communities (Messier *et al*., 2017b; Anderegg *et al*., 2018). While the suite of traits used to describe the two-dimensional global spectrum of plant form and function have been extensively used, the processes of interest at smaller scales might not affect those specific traits and /or affect other traits more strongly. We still lack theoretical and empirical understanding of trait-trait and trait-environment relationships at smaller spatial scales.

**Chapter 3:**

The third chapter of my thesis is based on assumption 1.4.2. I tested if the LES and the plant-size trait dimensions (Díaz *et al*., 2016) are present at the landscape and local scales (Fig 2b and 2c), and how the key traits from both dimensions are related to the environment. I sampled traits from 1650 individual plants, two representing the LES - leaf mass per area (LMA), leaf dry matter content (LDMC), and three representing the plant size dimension- leaf dry mass, leaf area, and plant vegetative height. I expected the patterns of both dimensions to emerge, due to the high phenotypic diversity; I also had expectations regarding the structure of variance of traits in relation to the environmental gradients: (1) if the elevational gradient (landscape scale) is responsible for filtering trait values, then I would expect large differences in modal trait values

and narrow variances among elevations, specifically showing an increasing trend for leaf traits values associated with greater conservation of nutrients, and a decrease trend for plant height, due to a decline in temperature and differences in precipitation with the increase of elevation (Read *et al*., 2014; Pescador *et al*., 2015); and (2) if plant-soil associations (vegetation types) are the major source of variation in trait distributions, I expect modal trait values to vary by vegetation type, and larger variances within elevations largely independent of elevation. I expect that leaf traits and plant height will vary accordingly with the dominant growth form of each vegetation type: higher mean values for vegetation types dominated by woody growth forms, while lower values would be associated with the different grasslands types independent of elevation (Rossatto & Franco, 2017).

### *1.4.3. The spectral dimension*

The assumption that plants can be positioned along an axis of "light" is based on the fact that leaf optical properties are a function of leaf structure, water content, and concentration of biochemical elements (Curran, 1989; Curran *et al*., 2001; Asner *et al*., 2011a; Asner & Martin, 2016). Along an environmental gradient, changes in the use of sunlight and other resources by plants will likely change trait ecophysiological values and so the full-spectrum reflectance profile. This means that, analogous to the functional trait space, spectral space is conceptually an *n*-dimensional hypervolume populated by spectra of individual plants measured at the leaf level or through remote sensing (Schweiger *et al*., 2018). There is sufficient theoretical basis linking the spectral, chemical, and taxonomic diversity of tree species, but our general knowledge is hindered by too few and spatially clustered measurements and also by the lack of measurements of other growth forms and deciduousness strategies. Major advances are still required for a deeper understanding and wider application of the spectral domain as a biodiversity component.

**Chapter 4:**

The fourth chapter is based on assumption 1.4.3. Here I tested if leaf level spectra is able to predict the two key LES traits (LMA and LDMC) in a seasonally dry tropical vegetation and if the spectra profile is able to reflect the expected functional changes among growth forms. For the same set of samples from Chapter 3, I measured high-fidelity leaf reflectance spectra and used a partial-least square modelling approach to predict leaf functional traits from spectra. I used the Bhattacharya distance to measure the dissimilarities among growth forms and draw further insights in leaf ecophysiological differences between them.

**Chapter 5:**

The fifth chapter is also based on assumption 1.4.3. Here I explore the hypothesis of the optical diversity, testing (1) if interspecific variations surpass intraspecific in spectral variability, and 2) if the trait x spectral relationship holds across biological scales. From the 1650 individuals sampled for chapter 3, I selected 231 individuals from 31 different species. I used ideas from the remote sensing community and methods widely used in ecology. I describe the spectral diversity based on the distances among individuals, species, genus and families in a spectral space and tested the convergence with the functional dissimilarities for each biological scale. I expect interspecific variation to exceed intraspecific variation, as predicted by classical niche theory. I also expect that convergence of spectral x trait dissimilarities are stronger at the species and genus level (Asner & Martin, 2009).

**6. Concluding remarks:**

The final chapter synthesizes the findings and limitations of this research and outlines current shortcomings for future research.

## 2. Land surface phenology in the tropics: the role of climate and topography in a snow-free mountain[1]

[1] Streher, A.S., Sobreiro, J.F.F., Morellato, L.P.C. et al. Ecosystems (2017) 20: 1436. https://doi.org/10.1007/s10021-017-0123-2

**Abstract**

Leaf phenology represents a major temporal component of ecosystem functioning, and understanding the drivers of seasonal variation in phenology is essential to understand plant responses to climate change. We assessed the patterns and drivers of land surface phenology, a proxy for leafing phenology, for the Meridional Espinhaço Range, a South American tropical mountain comprising a mosaic of savannas, dry woodlands, montane vegetation and moist forests. We used a 14-year time series of MODIS/NDVI satellite images, acquired between 2001 and 2015, and extracted phenological indicators using the TIMESAT algorithm. We obtained precipitation data from the Tropical Rainfall Monitoring Mission (TRMM), land surface temperature from the MODIS MOD11A2 product, and cloud cover frequency from the MODIS MOD09GA product. We also calculated the Topographic Wetness Index and simulated clear-sky radiation budgets based on the SRTM elevation model. The relationship between phenology and environmental drivers was assessed using general linear models. Temporal displacement in the start date of the annual growth season was more evident than variations in season length among vegetation types, indicating a possible temporal separation in the use of resources. Season length was inversely proportional to elevation, decreasing 1.58 days per 100m. Green-up and senescence rates were faster where annual temperature amplitude was higher. We found that water and light availability, modulated by topography, are the most likely drivers of land surface phenology in the region, determining the start, end, and length of the growing season. Temperature had an important role in determining the rates of leaf development and the strength of vegetation seasonality, suggesting that tropical vegetation is also sensitive to latitudinal temperature changes, regardless of the elevational gradient. Our work improves the current understanding of phenological strategies in the seasonal tropics, and emphasizes the importance of topography in shaping light and water availability for leaf development in snow-free mountains.

## Introduction

Plants are finely tuned to the seasonality of their environment, and phenology has been considered a key element to track vegetation responses to climate change (Polgar & Primack, 2011; Morellato *et al.*, 2016). Leaf phenology represents a major temporal component of ecosystem functioning, regulating processes such as carbon, water and energy exchange, forage availability, competition, and coexistence (Gotelli & Graves, 1996b; Cleland *et al.*, 2007). Identifying patterns and constraints regulating leaf phenology, and how these mechanisms differ among vegetation types, is therefore critical for understanding vegetation dynamics and for accurately forecasting future changes on ecosystems (Peñuelas *et al.*, 2009; Polgar & Primack, 2011).

Phenological events are considered highly plastic traits, as they reflect flexible responses to environmental triggers (Davies *et al.*, 2013). Leaf phenology triggers are well understood in temperate systems (Richardson *et al.*, 2006; Polgar & Primack, 2011; Hwang *et al.*, 2014), but drivers are still under debate for tropical environments (Morellato *et al.*, 2013; Borchert *et al.*, 2015). In seasonal tropical environments, water and light availability have been identified as the main external factors directly or indirectly controlling vegetation seasonal rhythms, placing physiological and evolutionary constraints on the timing of phenological events (Wright & van Schaik, 1994; Morellato *et al.*, 2000; Rivera *et al.*, 2002; Jolly *et al.*, 2005; Archibald & Scholes, 2007; Pau *et al.*, 2013; Jones *et al.*, 2014; Borchert *et al.*, 2015; Guan *et al.*, 2015; Bi *et al.*, 2015). The seasonality in annual rainfall and radiation budgets often has different timings; when water is available, light is likely to limit the production of new leaves, and vice-versa (Rivera *et al.*, 2002). According to Wright & van Schaik (1994), seasonal forests often receive 25 to 50%

more photosynthetically active radiation (PAR) during the dry season, when water is potentially limiting, and 20 to 30% less PAR during the rainy season.

Plant strategies and adaptations to water and light limitations are directly related to the distinct life forms. Tree species have a better ability to access and store groundwater, and are less dependent on the timing of rainfall (Borchert *et al.*, 2002; Rivera *et al.*, 2002; Archibald & Scholes, 2007), thus light-limited trees are predicted to produce new leaves during the season of maximal irradiance, regardless of moisture levels (Wright & van Schaik, 1994; Borchert *et al.*, 2005; Calle *et al.*, 2010). Conversely, at a regional scale grasses are expected to have their maximum leaf area limited by hydraulic and architectural constraints, being more dependent on rainfall seasonality and soil water-holding capacity (Archibald & Scholes, 2007), particularly in upland sites (Schimel *et al.*, 1991). Spatial-temporal variability in grasslands biomass accumulation is also driven by a combination of topography and fire history (Briggs & Knapp, 1995; Reich *et al.*, 2001; Nippert *et al.*, 2006), and light limitation is usually more significant in unburned sites where considerable PAR is intercepted by detritus accumulation (Knapp & Seastedt, 1986; Schimel *et al.*, 1991).

As elevational changes and topographic variability modulate the availability of abiotic resources such as light and water (Inouye & Wielgolaski, 2013), influencing species distribution, biomass production and the timing of leaf production. Although there is a growing number of studies seeking to understand phenological changes across elevational ranges (Richardson *et al.*, 2006; Hudson Dunn & de Beurs, 2011), direct investigation of tropical mountains has seldom been undertaken (Rocha *et al.*, 2016) and little is known about the relationship between topoclimatic conditions and leaf phenology in seasonal, snow-free tropical mountain environments (van Leeuwen *et al.*, 2013; Krishnaswamy *et al.*, 2014).

One of the most challenging aspects of understanding phenology in the tropics is the lack of long-term monitoring datasets (Morellato *et al.*, 2013, 2016; Chambers *et al.*, 2013). In this sense, historical remote sensing allows us to retrieve temporal information and a synoptic view of vegetation dynamics (Reed *et al.*, 2009b). Known as *land surface phenology* (hereafter, LSP) (de Beurs & Henebry, 2004), these remote sensing analyses are defined as the seasonal pattern of variation in vegetated land surfaces, being usually based on the calculation of spectral vegetation indices, derived from orbital sensors with high temporal resolution and moderate spatial resolution. The Normalized Difference Vegetation Index (NDVI) has been widely used to assess LSP, as it is capable of measuring photosynthetic activity and seasonal to interannual changes in leaf cover and canopy structure as a proxy for vegetation leaf phenology (Myneni *et al.*, 2007). While the observed patterns are related to biological phenomena, LSP is distinct from traditional definitions of vegetation phenology, since it describes the seasonality of reflectance characteristics that are associated with stages of vegetation development (Henebry & de Beurs, 2013), instead of specific events in a plant's life history.

The competing effects of light and water are also difficult to assess at regional ecosystem scales, and remote sensing can provide spatially explicit measurements of these factors that are fundamental to understand the effects of abiotic conditions on vegetation dynamics (Myneni *et al.*, 2007; Reed *et al.*, 2009b). Here, we use a 14-year time series of MODIS/NDVI satellite imagery to characterize and understand the patterns of land surface phenology for the mosaic of highly diverse and endangered tropical seasonal forests and woodlands, grasslands and cerrado savannas distributed across the latitudinal and elevational gradients of the meridional portion of the Espinhaço Mountain range, in southeastern Brazil. We also combine satellite-based phenological, climatic and topographic information to evaluate how climate and topography

interact to shape water and light availability in the Espinhaço Range, thus driving the LSP of its several vegetation types.

**Methods**

We studied the meridional portion of the Espinhaço Range, including the "Iron Quadrangle" mountain formation, in the state of Minas Gerais, Southeastern Brazil (Figure 2.1). The Espinhaço Range (*Cadeia do Espinhaço)* is one of the most ancient landscapes on Earth, where different and highly diverse biomes converge, resulting in a complex biogeographic history and high levels of diversity and endemism (Fernandes, 2016; Silveira *et al.*, 2016). This environment thus offers a unique opportunity to understand the effects of topoclimatic variability on LSP.

The Espinhaço Range spans ca. 1200 km in the N-S direction, varying between 50 and 100 km in width along its length (Giulietti & Pirani, 1988). The range emerged during Gondwana formation, nearly 640 Mya, evolving under prolonged tectonic stability and extreme weathering, with sedimentary rocks dating back to 1.8–1.75 Ga (Schaefer *et al.*, 2016b) . The Iron Quadrangle is a major mineral province in Brazil, located south of the Espinhaço Range and covering an area of approximately 7000 km², with rocks dating back to 2.5 Ga). Mean elevation in the studied area is over 1000 m above sea level, with peaks reaching 1800 to 2100 m. The relief has rugged and sharp features, producing a wide diversity of soils and micro-environmental conditions (Giulietti & Pirani, 1988; Schaefer *et al.*, 2016b).

Moist forest vegetation occurs at the eastern and southern portions of the meridional Espinhaço range, forming a transitional belt between the coastal humid Atlantic rainforest and the dryland vegetation of interior Brazil (de Lima *et al.*, 2015). The *cerrado* is a highly diverse Neotropical

savanna, including a wide range of vegetation types from open grasslands to woodlands with varying tree densities (Simon *et al.*, 2009), which can be found mainly at the western side of the Espinhaço Range. The northern portions of the Espinhaço range are characterized by an ecotone of savannas, dry forests and *caatinga*, which are unique to NE Brazil (Portillo-Quintero & Sánchez-Azofeifa, 2010), and broadly named here as Dry Woodlands. Finally, the Espinhaço highlands mountain vegetation are dominated by *campos rupestres*, a montane vegetation mosaic comprised of fire-prone grasses and shrubs growing on quartzite, sandstone or ironstone rocky outcrops, grasslands growing on sandy, stony or waterlogged soils, and patches of transitional vegetation such as cerrado, gallery forests, and relictual hilltop forest patches occurring along the drainage network (Fernandes, 2016; Silveira *et al.*, 2016).

Human disturbances in the region are associated with urbanization, deforestation, and land appropriation for agriculture and *Eucalyptus* plantations, especially on the lowland regions (Silveira *et al.*, 2016). On the highlands, the major current threats are annual anthropogenic burnings to support the cattle industry, wood extraction, invasive species and, mainly in the Iron Quadrangle, opencast mining of gold, precious stones, and iron and manganese ore (Silveira *et al.*, 2016).

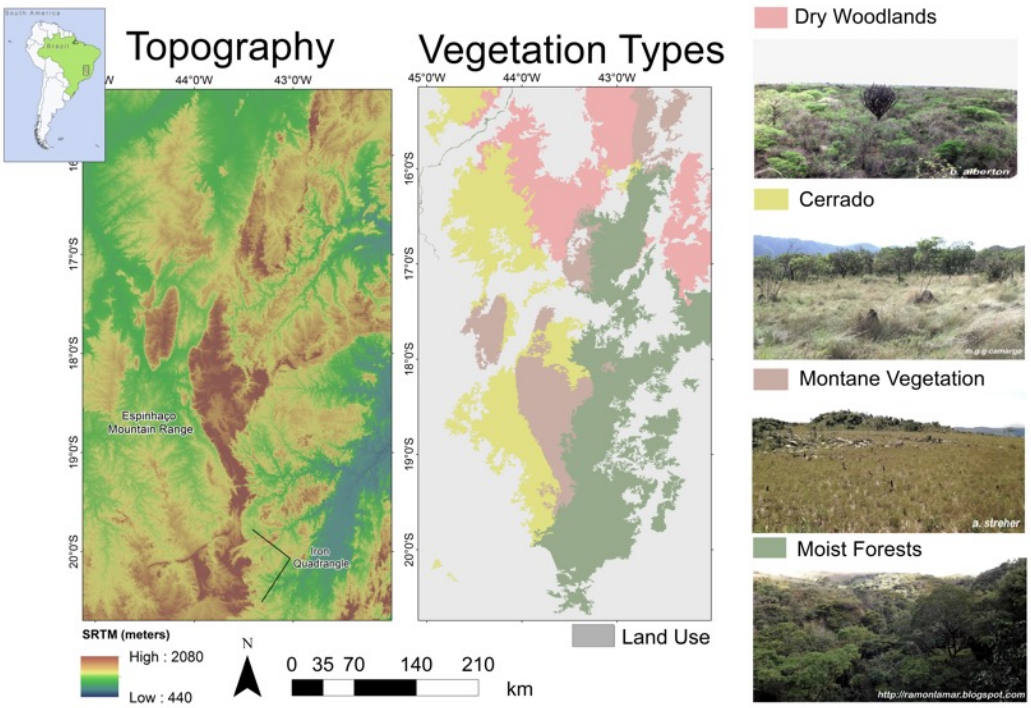


***Figure 2.1:*** Overview of the topography and extent of the meridional portion of the Espinhaço Mountain Range, and the Iron Quadrangle, state of Minas Gerais, Brazil (left). On the right, the unique position of the Espinhaço Range as an ecotone of vegetation types: Atlantic Rainforest (moist seasonal forest) to the East and South, Cerrado (savanna) vegetation types to the West, Dry Woodlands in the North and, on the highlands, campos rupestres, a montane vegetation mosaic comprising mainly non-forest formations growing over sandy/rocky soils and rocky outcrops. Photos: courtesy from Bruna Alberton (Dry Woodlands); Maria Gabriela Gutierrez Camargo (Cerrado); Annia Streher (Montane Vegetation); Moist Forest photo taken from http://ramonlamar.blogspot.com.

*Land surface phenology*

This study was based on a time series of 884 Normalized Difference Vegetation Index (NDVI) images derived from the Moderate Resolution Imaging Spectroradiometer (MODIS) sensor, acquired between 2002 and 2015, at 7-day intervals. We used the global NDVI dataset provided by the Institute of Surveying, Remote Sensing and Land Information (IVFL) of the University of Natural Resources and Applied Life Sciences (BOKU). This improved NDVI dataset uses as input the standard MOD13Q1 and MYD13Q1 NDVI 16-day maximum value composite products, from

MODIS Aqua and Terra satellites (collection 5 products from NASA) (Huete *et al.*, 1999). MOD13Q1 and MYD13Q1 NDVI are derived from the level-2G daily surface reflectance gridded data (MOD09 and MYD09 series) using the constrained view angle-maximum value composite (CV-MVC) compositing method. The BOKU dataset improves the standard NDVI products by filtering images for data noise and interpolating original observations to daily NDVI values using a Whittaker smoothing filter, which takes into account the quality of the observations according to the MODIS VI Quality Assessment Science Data Set (QA SDS) and the compositing day for each pixel (Vuolo *et al.*, 2012; Klisch & Atzberger, 2016). The user can then choose the desired temporal aggregation step, such as our choice of 7-day intervals. This dataset has a spatial resolution of 250m (Vuolo *et al.*, 2012; Klisch & Atzberger, 2016).

NDVI is related to the photosynthetic capacity of vegetation and to biophysical variables such as the fraction of photosynthetically active radiation absorbed (fPAR) and fractional green cover (Tucker, 1979), and is a measurement of the difference in light reflectance between the visible and near-infrared spectral regions:

$$NDVI = \frac{\rho NIR - \rho RED}{\rho NIR + \rho RED}$$

where ρ is surface spectral reflectance. Higher NDVI values indicate increasing amounts of photosynthetically active vegetation cover. Roughly, NDVI values can be interpreted as: a) negative values, approaching -1, represent water bodies; b) Very low values of NDVI (-0.1 to 0.1) usually corresponding to barren areas of rock or sand; c) moderate values in the range of 0.1 to 0.3, associated with shrubs and grasslands; d) high values, usually between 0.6 and 0.9, attributed to temperate or tropical forests. Although several vegetation indices exist in the specialized

literature, NDVI was chosen for its lesser susceptibility to terrain illumination effects when compared to other indices (Teles *et al.*, 2015; Galvão *et al.*, 2016).

The TIMESAT 3.2 software suite was used to extract phenological metrics from the MODIS/NDVI time series (Jonsson & Eklundh, 2002; Jönsson & Eklundh, 2004). To further smooth noisy variations in the time series, the seasonal curves were fitted using a double logistic model (Jönsson & Eklundh, 2004). A user-defined threshold of 20% of the seasonal amplitude, above the left minimum of the seasonal curve was defined as the start of the growing season (SOS), and a similar threshold (20%) was applied to the right minimum of the seasonal curve to define the end of the growing season (EOS). Amplitude is determined separately for each side of the curve, thus accounting for possible hysteresis in the seasonal response curves. This threshold was chosen by visual inspection of the fitted function for several random pixels representing each vegetation type, using TIMESAT's graphical interface, as the best compromise between capturing the maximum extent of the growing season without being overly influenced by minimum seasonal values, which can be strongly affected by noise (Jönsson and Eklundh 2004). The timing of the mid-season peak (MID) was then computed as the mean value of the curve values for which the left edge had increased to the 80 % level and the right edge had decreased to the 80 % level, as pre-defined by the TIMESAT algorithm. Season length (LOS) was defined as the time from the start (SOS) to the end (EOS) of the season. Green-up (GR) and senescence (SR) rates were calculated as the ratio of the difference between the left/right 20% and 80% of total curve amplitude levels and the corresponding time difference, also as pre-defined by the TIMESAT algorithm. Season amplitude (SA) was obtained as the difference between the peak value and the average of the left and right minimum values. All phenological metrics were extracted following Jönsson and Eklundh (2004) (Table 1 and Figure 2.3).

***Table 2-1:*** Land surface phenology metrics (Jönsson and Eklundh 2004) used to describe phenological patterns along the Espinhaço Range, SE Brazil.

| Phenological metrics | Description | Unit |
|---|---|---|
| Start of the growing season (SOS) | Represents the date of the start of the growing season. It is measured as an increase of 20% of the total seasonal amplitude, measured in relation to the left side of the curve. | Days |
| End of the growing season (EOS) | Represents the end date of the growing season. It is measured as a decrease to 20% of the total seasonal amplitude, measured in relation to the right side of the curve. | Days |
| Length of the growing season (LOS) | Length of time from SOS to EOS. | Days |
| Season Amplitude (SA) | Represents the strength of seasonality. It is measured as a difference between the highest and the average of the left and right minimum fitted values. Vegetation with stronger seasonality will have higher SA values. | Unitless |
| Timing for mid-season (MID) | Represents the proximate date of maximum photosynthetic activity. It is measured as the mean value of dates for which left edge increased to 80% and right edge decreased to 80%. | Days |
| Green-up Rates (GR) | Represents the rate of increase of leaf flush events. It is measured as the ratio of the difference between the left 20 % and 80 % levels and the corresponding time difference. It is a ratio of NDVI values per Days. | Unitless |
| Senescence rates (SR) | Represents the rate of increase of senescence events. It is measured as the ratio of the difference between the right 20 % and 80 % levels and the corresponding time difference. It is a ratio of NDVI values per Days. | Unitless |

*Topoclimatic and environmental variables*

To characterize the geographical distribution of environmental drivers of plant phenology, we analysed remote-sensing derived temperature, precipitation, and cloud cover frequency, and modelled topographical water availability and incoming solar radiation, based on terrain elevation data.

For temperature, we used the MODIS Terra Land Surface Temperature (LST) product (MOD11A2, version 5), which provides estimates of surface temperature at 1 km spatial resolution and 8-day time intervals. We used the MODISTools package in R (Tuck *et al.*, 2014), combined with the Modis Reprojection Tool by the NASA Land Process DAAC (https://lpdaac.usgs.gov/tools/modis_reprojection_tool) to download, mosaic and reproject the MODIS LST time series from 2001 to 2015. We converted LST values to degree Celsius (°C), and calculated the per-pixel daytime $90^{th}$ percentile and nighttime $10^{th}$ percentile of the time series to characterize the geographic and topographic distribution of temperature extremes. We then computed thermal amplitude as the difference between maximum and minimum temperature percentiles.

We characterized annual precipitation using data from the Tropical Rainfall Measuring Mission (TRMM), product 3B43 version 7, at 0.25º (~27 km) spatial resolution, covering the same period of the NDVI observations. The 3B43 product comprises estimated values of average monthly rainfall (mm $hr^{-1}$) inferred by an assimilation algorithm that ingests data from multiple sensors of the TRMM mission, as well as rain gauge data provided by the Global Precipitation Climatological Center (GPCC) and the Climate Assessment and Monitoring System (CAMS), and is produced by the National Oceanic Atmospheric Administration (NOAA). Image processing was carried out in

ENVI 5.1 (Exelis Visual Information Solutions 2013) and precipitation values were converted from mm $hr^{-1}$ to mm $month^{-1}$. The agreement between monthly TRMM estimates and monthly ground observations from 112 weather stations distributed throughout the study area, covering the entire studied period, was of $R^2 = 0.96$ (Sobreiro *et al.*, 2015), and did not indicate any bias.

Elevation was derived from the Shuttle Radar Topography Mission (SRTM) 3-arcsec (90 m) dataset (±15 m vertical accuracy), obtained from the Earth Explorer server of the United States Geological Survey (http://earthexplorer.usgs.gov/). Based on elevation, we derived the Topographical Wetness Index (TWI), which represents a relative measure of potential long-term soil water storage capacity at a given site in the landscape. TWI was calculated using the SAGA GIS software, according to Beven and Kirkby (1979):

$$TWI = ln\frac{a}{tanB}$$

where $a$ is the specific catchment (the cumulative upslope area draining through the cell, divided by its contour width) and $B$ is the local cell slope (Beven & Kirbk, 1979). The specific catchment area describes the tendency of the site to receive water from upslope areas, and the local slope describes the tendency of the site to lose water through surface and subsurface runoff (Beven & Kirbk, 1979).

We simulated the solar insolation budget using the Solar Radiation Tool of the ArcGIS Surface Analysis Toolset (Rich *et al.*, 1994). Insolation maps were obtained from an insolation model that accounts for clear-sky atmospheric conditions, elevation, surface orientation, and occlusion from surrounding topography (Fu & Rich, 2002). Our resulting variable was the total annual budget of incoming insolation (*TII*), corresponding to the sum of direct and diffuse radiation (Fu

& Rich, 2002). The atmospheric parameters of transmissivity and diffuse to direct radiation proportion were set as 0.4 and 0.6, respectively. Sky size was set to 200 cells, and we used the *standard overcast sky* diffuse model type (Fu & Rich, 2002).

Since the algorithm calculates the radiation budget under continuous clear sky conditions, we also derived a cloud cover frequency map using the Quality Assessment (QA) flags indicating cloud occurrence from the Terra MODIS MOD09GA daily surface reflectance product, at 1 km spatial resolution. Cloud cover was expressed as the percentage of observed cloudy days for the entire period between 2001-01-01 and 2015-12-31. Data processing was done using the Google Earth Engine cloud computing platform, using a custom JavaScript script that interfaces with the Engine API (https://developers.google.com/earth-engine/playground).

*Data extraction and analysis*

Five broad vegetation classes, chosen based on their relevance and abundance across our study region, were delineated to aid the characterization of phenological patterns (Figure 2.1). We classified each vegetation type based on elevation (SRTM), and the mean and variance of each pixel in the entire MODIS/NDVI series. Class interpretation was complemented by Google Earth imagery, the official vegetation map of Brazil (IBGE, 2002), and a land use map produced by the SIMBRASIL/Otimizagro Project for 2013 (http://csr.ufmg.br/simbrasil). The following classes were mapped:

- **Moist Forests:** areas covered by tall trees, including gallery forests and cloud forests. This vegetation type is composed mostly by evergreen plants or partially by plants that lose part of their foliage for a very short period, when old leaves are shed and new foliage growth starts (Morellato *et al.*, 2000; de Lima *et al.*, 2015).

- **Dry Woodlands:** an ecotonal zone of forests or woodlands dominated by sparse trees, the majority losing their foliage at the end of the growing season (Borchert *et al.*, 2002; Portillo-Quintero & Sánchez-Azofeifa, 2010; Lima & Rodal, 2010).
- **Montane Vegetation**: a heterogeneous mosaic of vegetation known in Brazil as *campo rupestre sensu lato,* occurring over elevations above 800 m and comprising mainly non-forest vegetation types dominated by rocky and sandy grasslands surrounding vegetation growing on rock outcrops (Fernandes, 2016; Silveira *et al.*, 2016).
- **Cerrado**: a vegetation dominated by woody and shrubby Neotropical savannas, also including a vegetation gradient spanning from C4 grasslands to closed canopy seasonal forests (Simon *et al.*, 2009).
- **Land Uses**: agriculture, pasture, *Eucalyptus* plantations, urban areas and other land uses that are not natural vegetation.

We characterized the general spatial patterns of land surface phenology along the Espinhaço Range by averaging the per-pixel value of each phenological metric across the 14 growing seasons of the time series. All images were resampled to the MODIS spatial resolution (250 m) and phenological metrics and environmental variables were stacked in a multilayer image. We then sampled the stack to retrieve only pure pixels from each vegetation class, to avoid the inclusion of signals from mixed vegetation and from human modified surfaces. Pure vegetation samples (polygons containing pixels with unmixed vegetation cover for each class) were manually delineated using visual interpretation of high resolution Google Earth™ and Bing Maps™ imagery, using the OpenLayers plugin of QGIS 2.4.0 software (www.qgis.org). We took 25 samples for each vegetation class, comprising a total of 26122 pixels (Dry Woodlands = 4945 pixels; Cerrado= 6611 pixels; Montane Vegetation = 7512 pixels; Moist Forests = 7054 pixels). We also crosschecked

our sample classification with the TreeCO bibliographical database for forest descriptions (http://labtrop.ib.usp.br/doku.php?id=projetos:treeco:start) (de Lima *et al.*, 2015), to ensure they correctly represented each of the described vegetation types.

Using the pure vegetation samples extracted, we then fitted several general linear models using each phenology metric as the dependent variable (Y), and different combinations of environmental drivers as predictors (X), each model formulation representing different competing hypothesis on the expected relationship between phenology and environment. The models were fitted for all samples (global model), and then separately for each vegetation type (specific models). For each phenology metric, we specified two full models, representing our main environmental hypotheses, the first one using all single variables as independent explanatory terms, and the second including interaction terms between *TWI* and rainfall and between solar radiation and cloud cover frequency:

$$Y = \beta_0 + \beta_1(TII) + \beta_2(CCF) + \beta_3(TA) + \beta_4(AAP) + \beta_5(TWI) + \varepsilon_i$$

$$Y = \beta_0 + \beta_1(TII) + \beta_2(CCF) + \beta_3(TII * CCF) + \beta_4(TA) + \beta_5(AAP) + \beta_6(TWI) + \beta_7(AAP * TWI) + \varepsilon_i$$

where $Y$ is the phenological metric, $TII$ is the total incoming insolation (Wh $m^{-2}$), $CCF$ is the cloud cover frequency (%), $TA$ is the thermal amplitude (ºC), $AAP$ is the average annual precipitation (mm $year^{-1}$), $TWI$ is the topographic wetness index (unitless) and $\varepsilon_i$ is the error term.

The term combining $AAP$ and $TWI$ describes the interaction between the amount of water (precipitation) entering the system and the soil capacity for water accumulation, which therefore determines *potential water availability.* The combination between annual insolation and cloud cover frequency ($TII * CCF$) was used to describe how clouds can alter the balance between direct and diffuse radiation, as well as the total incoming PAR, therefore representing *potential light*

*availability*. From the two global models, we assessed the contribution and significance of each environmental variable, using standardized model coefficients, and manually derived competing nested parsimonious models using Akaike's information criterion (AIC) as a model selection method (Burnham & Anderson, 2004). We assessed multicollinearity among covariates by calculating the variance inflation factor (VIF) for each model predictor, and no evidence for collinearity was found. The highest VIF value was found for the TWI variable (VIF = 2.69; for the models relating SA, GR and SR), still below the recommended threshold of 3 (Zuur *et al.*, 2010). All models were also checked for normality and independence of the residuals, with no issues found for any model.

**Results**

*Land surface phenology patterns*

The spatial distribution of the 14-year averages for all phenological metrics revealed a well-defined geographical pattern for vegetation phenology along the meridional Espinhaço Range (Figures 2.2, 2.5 and 2.6), following both latitudinal and elevational gradients. The maps show how phenology tracks the topography of the region, emphasizing the distinctive phenological patterns of higher elevation vegetation.

The start of the growing season (SOS) happened earlier at lower elevations and in the western portions of the study area, and was only later followed by the highest elevations and eastern portions of the Espinhaço range. Timing for the mid-season (MID) and end of season (EOS) dates tracked SOS, indicating that season displacement was more important than season length in separating each vegetation (Figures 2.3 and 2.4). Dry Woodlands started their growing season first, by late August (doy 238 ± 14d, ±1sd), followed by Cerrado in the beginning of September

(doy 250 ± 21d) and Montane Vegetation by late September (doy 267 ± 27d), while Moist Forests had the latest start dates, in early October (doy 284 ± 34d) (Figures 2.3 and 2.4). MID dates were consistently reached 140 to 145 days after the SOS, occurring towards the end of January for Dry Woodlands and Cerrado, and in mid-late February, for Montane Vegetation and Moist Forests (Figures 2.3 and 2.4). Dry Woodlands were the first to shed their leaves, at the end of May (doy 145 ± 15d), followed by Cerrado in the beginning of June (doy 159 ± 18d), Montane Vegetation in late June (doy 170 ± 20d), and Moist Forests at the beginning of July (doy 187 ± 31d). Leaf senescence occurred during the dry season, approximately nine months after leaf flush, and the duration of the leaf-off period was between two and three months.

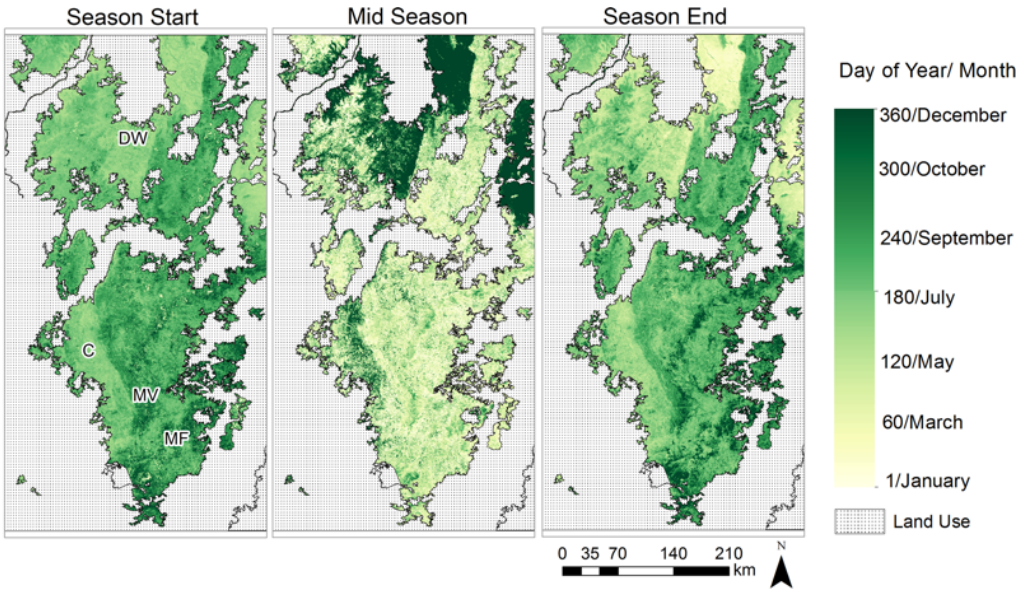


***Figure 2.2:*** Mean Day of Year values for LSP metrics derived from a 14 year MODIS/NDVI time series in the meridional Espinhaço Mountain range, south-eastern Brazil. Start of season (SOS), Time of the mid of season (MID) and End of season (EOS) show a clear geographic pattern with similar trends in dates, with start, peak and end of growing season occurring later for higher elevation vegetation. Also, the sharp discontinuity seen at lower

latitudes is due to an abrupt change in topography leading to a transition between vegetation types. DW: Dry Woodlands; C: Cerrado; MV: Montane Vegetation; and MF: Moist Forest.

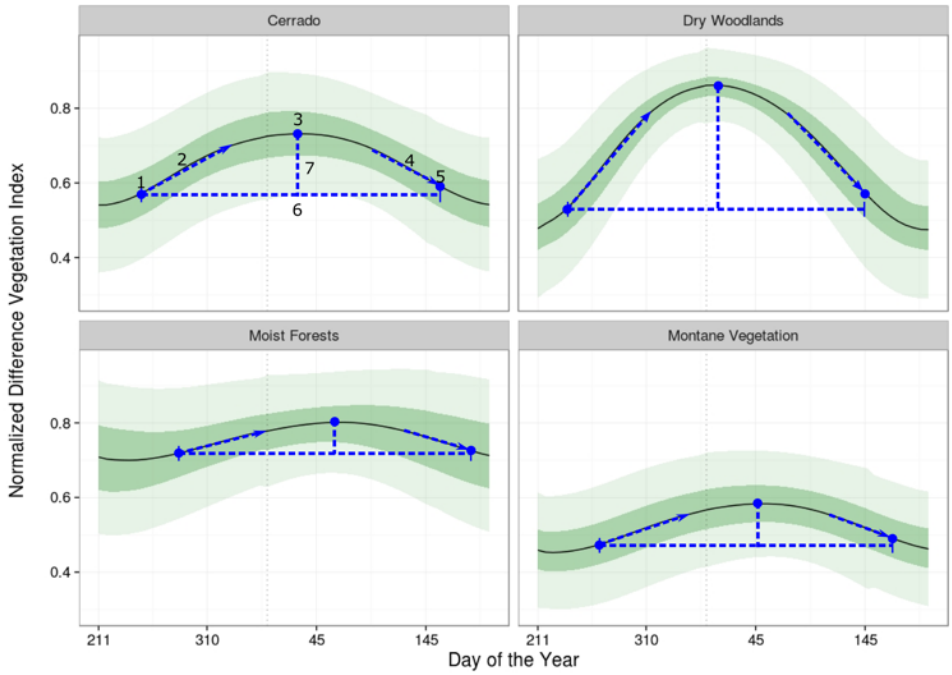


***Figure 2.3:*** Phenological metrics extracted on a per-pixel basis from the NDVI/MODIS time series, through the TIMESAT program (Jönsson and Eklundh 2004). The grey line represents the fitted time series. The start of the season (SOS) is given in Day of Year, and is the time in which the left edge increased to a user-defined level (20%), while the end of season (EOS), also given in Day of Year, is the time in which the right edge decreased to a user-defined level (20%). Season length (LOS) is the number of days between SOS and EOS. The mid-season position (MID) is the mean date value between the points where the left edge of the curve increases to 80%, and right edge decreases to 80%. Season amplitude (SA) is the difference between the maximum value and the mean of the lowest fitted value for both SOS and EOS. Green-up (GR) and Senescence (SR) rates are the ratio of increase for values between the 20% and 80% level of the right and left edges, respectively. The numbers indicate respectively: 1) SOS; 2) GR; 3) MID; 4) SR; 5) EOS, 6) LOS; and 7) SA.

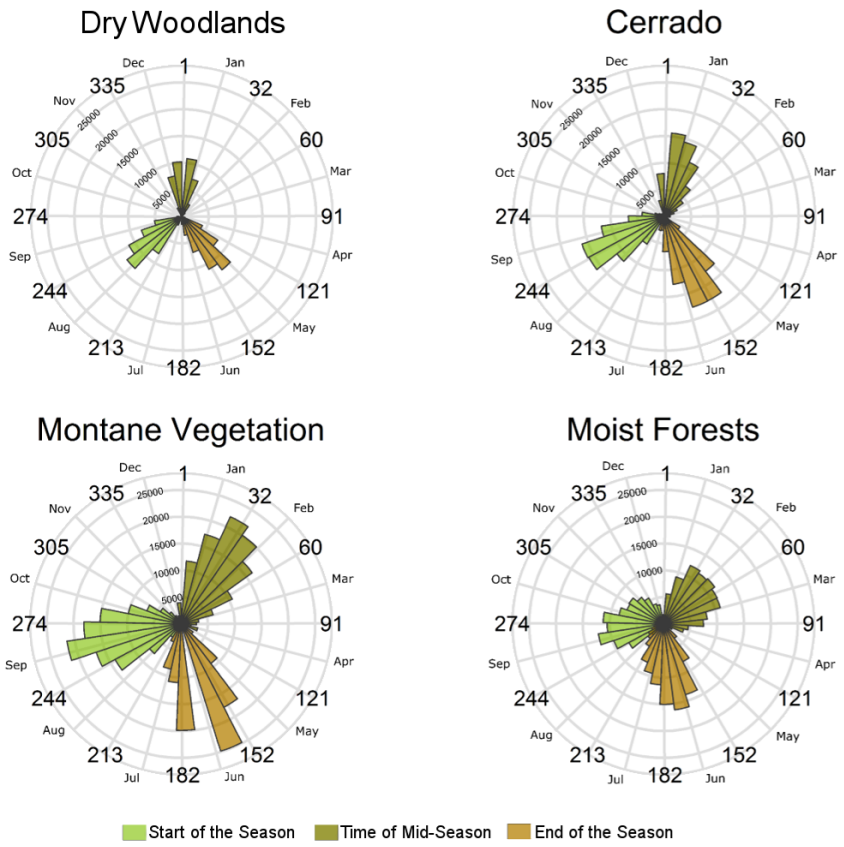


***Figure 2.4:*** Circular histograms of pixels' frequencies for three phenological metrics derived from the average of the 14 year MODIS/NDVI time series, acquired from 25 samples of different vegetation types along the Espinhaço Mountain range in south-eastern Brazil. Light green bars indicate the variation of the Start of the Growing Season (SOS) dates; Olive bars represent the Time of the mid of season (MID dates; and Brown bars variation on End of the Growing Season (EOS) dates. Each bar corresponds to a bin width of 10 days, and bar length indicates the frequency (number of pixels) within each bin range.

The mean length of the season (LOS) was larger than 250 days for almost the entire region, and season length was inversely proportional to elevation, decreasing 1.58 days per 100m (Figure 2.5). Montane Vegetation along the Espinhaço Range and higher elevation Moist Forests in the Iron Quadrangle (above 900m) had the shortest LOS, averaging 266 days (±27d and ±34d, respectively -). Moist Forests at lower elevations had mean LOS similar to Dry Woodlands and Cerrado, of 273 days (±16d and ±23d respectively). We also observed distinct latitudinal and

elevational patterns for season amplitude (SA), emphasizing the strong seasonality of Dry Woodlands in the northern portion of the Espinhaço Range (SA = 0.39 ±0.08, unitless) (Figure 2.5). Cerrado also had a marked seasonality (0.19 ± 0.09), more similar to Montane Vegetation and Moist Forests that presents the lowest variability on season amplitude (0.14 ± 0.04 and 0.12 ± 0.05, respectively).

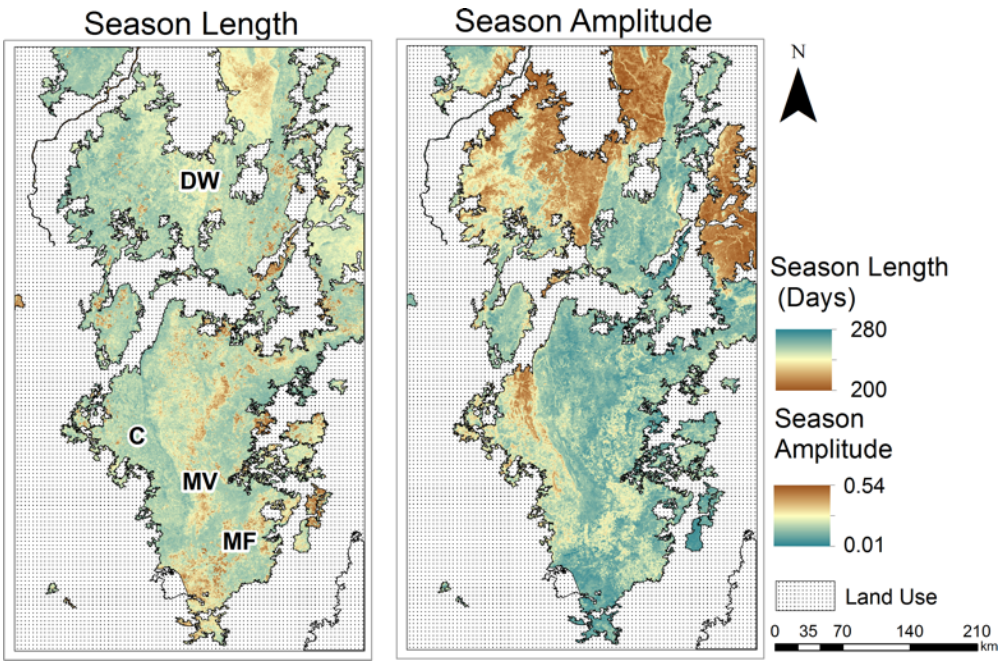


***Figure 2.5:*** Mean length of the growing season (LOS) in number of days, and mean season amplitude (SA), unitless, estimated from a 14 year MODIS/NDVI time series of the Espinhaço Mountain range, in south-eastern Brazil. DW: Dry Woodlands; C: Cerrado; MV: Montane Vegetation; and MF: Moist Forest.

Mean green-up (GR) and senescence (SR) rates were similar for the entire area, having well-defined patterns of variation across the latitudinal and elevational gradients (Figure 2.6), with each vegetation type having comparable GR and SR rates. Dry Woodlands at the northern Espinhaço Range had the fastest rates of growing (GR = 0.036 ± 0.009, unitless) and senescence (GR = 0.031 ± 0.009), followed by Cerrado (GR = 0.016 ± 0.009 and SR=0.016 ± 0.01).

Montane Vegetation had slower rates (GR=0.011 ± 0.004 and SR= 0.012 ± 0.007), and Moist Forests had the slowest rates (GR=0.010 ± 0.005 and SR= 0.009 ± 0.007).

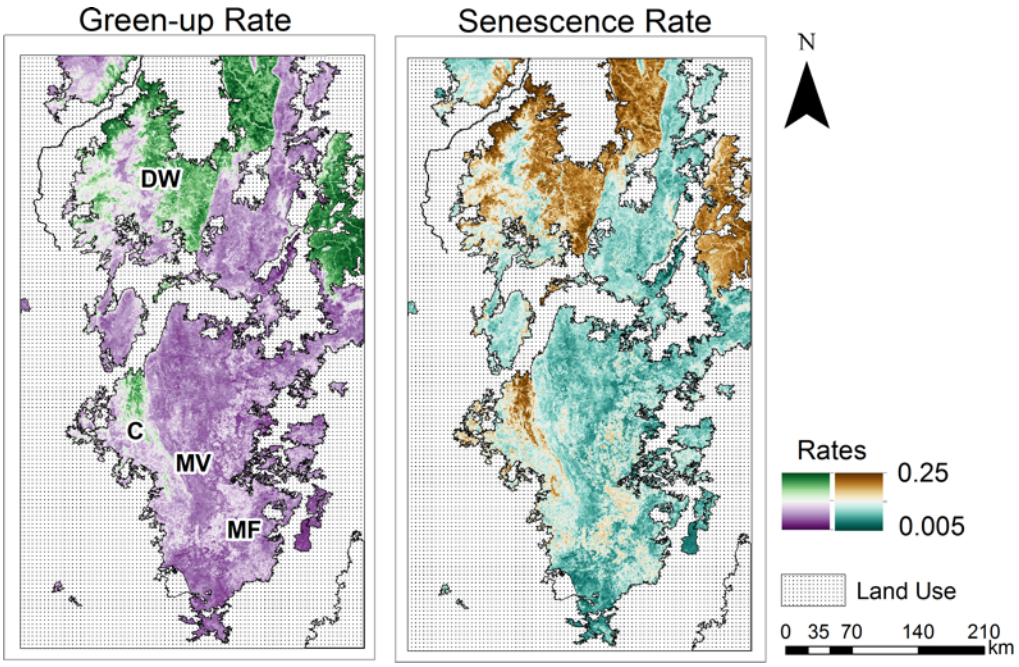


***Figure 2.6:*** Mean rates of green-up and senescence for a 14 years MODIS/NDVI time across the Espinhaço Mountain range in south-eastern Brazil. DW: Dry Woodlands; C: Cerrado; MV: Montane Vegetation; and MF: Moist Forest.

*Environmental drivers*

The temperature gradient along the Espinhaço Range had a well-defined pattern, showing increasing temperatures from east to west and south to north and lower temperatures at higher elevations. Thermal amplitude varied approximately 7°C in average along the Espinhaço Range, with a latitudinal pattern of lower amplitudes from north to south (Figure 2.7). At the northern portion of the Espinhaço Range, higher temperatures were observed at lower elevations, but this relationship did not hold for the southern portion of the range, where temperatures were more homogenous. Average annual precipitation (AAP) had a marked latitudinal pattern: higher precipitation rates were found at the southern parts of the Espinhaço Range, decreasing towards

the northeastern areas (Figure 2.7), with precipitation ranging from 852 to 1569 mm y-1. Rainfall patterns in the state of Minas Gerais are closely linked to the effects of the South Atlantic Convergence Zone (SACZ), which operates in a stationary way, especially in the 19°S and 20°S latitude, explaining the larger precipitation volume in these latitudes (Carvalho *et al.*, 2004). TWI had a gradient from east to west, with lower values at the eastern side of the Espinhaço Range due to the steeper terrain and small contributing drainage areas. Terrain-estimated insolation had higher values at the highest elevations, and several shaded areas occurred due to the Espinhaço rugged topography (Figure 2.7). Cloud cover frequency (CCF) had a strong pattern of variation from east to west, being more frequent in the eastern and northern regions of the Espinhaço Range, where Moist Forests and high elevation grasslands can be found (Figure 2.7).

*Environmental Drivers of Land Surface Phenology*

Water and radiation variables were the best predictors of LSP across the Espinhaço Range, being always included in the most parsimonious models (Table S1 – Supplementary Data). For season metrics (SOS, EOS and LOS), potential water availability and potential light availability (interaction terms) were the strongest predictors. We found strong influence of both potential light availability and potential water availability in SOS dates ($R^2$= 0.78, $p < 0.0001$), while EOS dates were better explained by potential water availability and cloud cover frequency ($R^2$ = 0.77, $p < 0.0001$). LOS was also strongly influenced by potential light availability and potential water availability ($R^2$=0.67, $p < 0.0001$). For phenological metrics related to productivity (GR, SR and SA), temperature amplitude also had a strong influence, being positively correlated and explaining a significant portion of variation for GR and SR ($R^2$= 0.77, $p < 0.05$ and $R^2$= 0.76, $p < 0.0001$, respectively) as well as for SA ($R^2$=0.77, $p < 0.0001$).

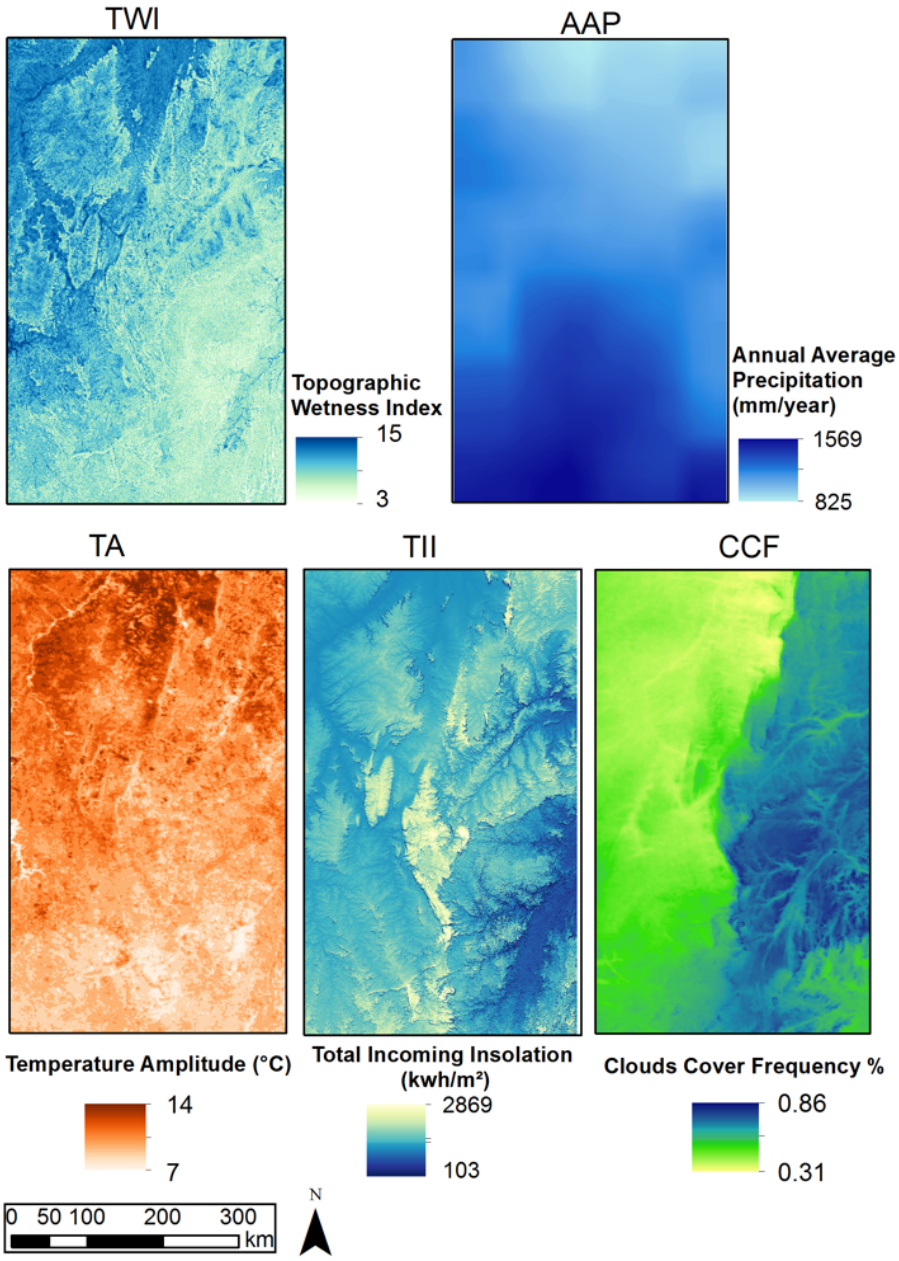


***Figure 2.7:*** Distribution of environmental drivers across the Espinhaço Mountain range, in south-eastern Brazil. TWI is the Topographic Wetness Index (TWI, unitless), representing soil water accumulation capability - higher values indicate higher potential soil moisture. AAP represents the average annual of precipitation from 1998 to 2015, obtained from TRMM. TA is the temperature amplitude, derived from a MODIS time series (MOD11A2 product) spanning 2001 to 2015, and is the difference between the 90$^{th}$ percentile of day temperatures and the 10$^{th}$ percentile of night temperatures for the entire series. TII is the total incoming insolation from all sky directions, modelled based on topography in ArcGIS 10.3. CCF is the cloud cover frequency, obtained from MODIS data, during the period from 2013 to 2015.

Looking at the vegetation specific-models of phenological metrics, we observed the relative contribution of water, light and temperature related drivers (Table S2 – Supplementary Data). A combination of potential light availability, potential water availability and *TA* influenced most of the phenological metrics described for Cerrado, explaining more than 69% of variation, with the exception of SOS dates, which had 78% of variation explained only by potential light and water availability (Supplementary Table S2). Potential light availability and potential water availability also had a strong influence on Montane vegetation phenology, explaining more than 68% of SOS, EOS and LOS dates, but less than 40% for GR, SR and SA. No evidence of TA influence was found for Montane vegetation. Moist Forests were also influenced by potential water availability and potential light availability, which explained more than 50% of the variance found for SOS, EOS and LOS dates. The combination of potential water availability, potential light availability and *TA* had a strong influence on GR, SR and SA, explaining more than 80% of the variance for Moist Forests. Dry forests were influenced by *TA* for all phenological metrics assessed, except for LOS, where no significant relation was found. *TA* explained just about 20% of the variance on the SOS and EOS dates and about 28% for GR, SR and SA for Dry Woodlands.

**Discussion**

While climate can be considered as the main abiotic factor controlling phenological dynamics, topography had a key role in modulating the environmental processes influencing LSP in the Espinhaço Range. In temperate mountains, topography is pointed out as a strong predictor of snowmelt, snow accumulation and temperature change across elevations, influencing leaf onset and leaf development (Hudson Dunn & de Beurs, 2011; Hwang *et al.*, 2014). However, we show that in the absence of snow and/or freezing temperatures, complex topography still results in

high variability of LSP patterns in the Espinhaço mountains, which appear to be a consequence of interactions between topography-mediated water and light availability, compounded by temperature amplitude. This complex topographic matrix and resulting topoclimatic variability allows the co-existence of highly diverse vegetation types (Fernandes, 2016; Silveira *et al.*, 2016).

The importance of rainfall and its interaction with topography for LSP is shown by the consistent selection of *potential water availability* (interaction term between *AAP* and *TWI*) in the statistical models. In terrestrial plants, root water uptake is the most common water-acquisition mechanism (Oliveira *et al.*, 2016) and it has been used to explain species co-existence in the Cerrado savannas (Rossatto *et al.*, 2014). Herbaceous plants, as found in cerrado and montane grasslands, with their dense and shallow root systems, tend to colonize shallow soils, were water is more superficial (Tannus *et al.*, 2006; Archibald & Scholes, 2007; Rossatto *et al.*, 2014). Woody plants, on the other hand, are highly plastic with respect to preferential water uptake, varying from shallow to deep soil sources according to small changes in topography (Rossatto *et al.*, 2014). According to Rossato and others (2014), competition for water between woody and herbaceous species is maximized at shallow slopes with deeper soils, while woody plants are outcompeted by herbaceous species along shallow soil conditions

Our results also highlight the importance of combined total incoming insolation and cloud cover *(potential light availability)* on plant phenology. Insolation is widely known to drive the timing of leaf phenology in the tropics (Wright & van Schaik, 1994; Borchert *et al.*, 2005, 2015; Mittelbach *et al.*, 2007; Calle *et al.*, 2010; Jones *et al.*, 2014; Bi *et al.*, 2015). Seasonal and spatial variations in cloud coverage can result in substantial seasonality in light availability to plants. Cloud cover had a strongly defined spatial pattern, due to the influence of the Atlantic

tropical mass, forming an area of stationary nebulosity along the eastern faces and higher elevational areas of the ER mountains (Coelho *et al.*, 2016). Clouds can reduce direct beam radiation and increase diffuse radiation, which penetrate canopies more efficiently and can increase whole canopy carbon uptake even when total irradiance is reduced (Graham *et al.*, 2003).

Our results also show that temperature has a less strong, but still fundamental role in determining vegetation dynamics in snow-free tropical mountains. Temperature has long been recognized as a fundamental constraint on many biological processes, which interacts with radiation and water to impose complex and varying limitations on vegetation activity (Myneni *et al.*, 2007; Calle *et al.*, 2010; Pau *et al.*, 2013; Borchert *et al.*, 2015). We found that temperature amplitude influenced the rates of plant development, i.e. the speed of green-up and senescence, and also the strength of seasonality, across all vegetation types. According to Körner (2006), temperature does not necessarily control critical ontogenetic phase changes such as bud-break induction, flowering or leaf senescence, but the speed at which plants and their organs pass through developmental phases does depend on temperature. Furthermore, temperature is one of the key environmental factors driving evapotranspiration, thus contributing to water availability and plant-water relations.

Plant species can adapt and/or acclimatize to a variety of environmental conditions (Colwell *et al.*, 2008), often displaying different strategies, as we observed for LSP across the Espinhaço Range mountains. With the exception of Moist Forests, leaf flush occurred mostly before the start of the rainy season, indicating that vegetation types occurring across the Espinhaço range are predominantly of the *drought-tolerant* type (Reich & Borchert, 1984). This means that most plants are capable of storing or accessing water throughout the season, rehydrating after leaf

shedding regardless of the length of the dry season (Borchet *et al.*, 2002). Since the timing of leaf flush requires predictable temporal cues in a limiting environment, it is possible that leaf flush is triggered by light-related environmental cues that precede the wet season, such as changes in irradiance (Borchert *et al.*, 2005, 2015; Zimmerman *et al.*, 2007; Davies *et al.*, 2013; Jones *et al.*, 2014). We suggest that the timing of leaf flush for vegetation in the Espinhaço Range has evolved in response to seasonal variations in both irradiance and water availability, since total incoming radiation, cloud frequency and potential water availability were found to be the best predictors of leaf flush (start of the season). Our results (Figure 2.4) are consistent with previous findings showing that tropical tree phenology has evolved to produce new leaves coinciding with peaks in solar irradiance (Wright & van Schaik, 1994; Myneni *et al.*, 2007; Jones *et al.*, 2014; Borchert *et al.*, 2015; Bi *et al.*, 2015). By flushing leaves before the wet season, plants may keep leaves for longer periods, having more opportunities for photosynthetic assimilation and growth (van Schaik *et al.*, 1993; Calle *et al.*, 2010; Jones *et al.*, 2014; Bi *et al.*, 2015) while minimizing damage by herbivory, which is more intense during the rainy season (Coley, 1988). This strategy may also apply to several perennial grass species at the mountaintop of Espinhaço Range, but this has not been studied to date.

Leaf fall in seasonal forests has been directly or indirectly related to water stress and the occurrence of a more or less severe dry season (Reich & Borchert, 1984). Since leaf fall had different timings for each vegetation type but always occurred during the dry season, vegetation types in the ER must have different strategies to deal with water shortage, and shedding their leaves may be a response to the first signs of water deficit (Reich & Borchert, 1984; Morellato *et al.*, 2000; Lima & Rodal, 2010). Our results are consistent with water deficit influencing leaf fall events, but residual variance in the models may be explained by endogenous processes rather

than environmental cues. Lers (2007) states that leaf senescence is highly regulated by physiological processes promoting plant survival, growth, and reproduction.

Season length is usually determined by the presence of snow in cold mountain environments (Inouye & Wielgolaski, 2013) and no patterns and associated drivers have been reported for snow-free mountains. We propose that water and light availability act simultaneously to define the length of the growing season for the snow-free Espinhaço Mountain Range vegetation.

LSP patterns of green-up and senescence rates, as well as season amplitude are connected with the dominance of phenological functional types of each vegetation. Usually, the mix of evergreen and deciduous species gives tropical dry forests a phenological complexity that is not found in other tropical forest formations (Reich & Borchert, 1984; Portillo-Quintero & Sánchez-Azofeifa, 2010). The faster green-up and senescence rates of Dry Woodlands observed in this study is explained by the predominance of strongly seasonal, deciduous species in this vegetation (Pezzini *et al.*, 2014). Moist Forest vegetation, on the other hand, encompasses semideciduous and cloud forests that are dominated by a different proportion of deciduous, semideciduous and evergreen species (Lopes *et al.*, 2012). Semideciduous forests may have up to 50% of deciduous to semideciduous trees (Morellato *et al.*, 1989, 2013; Morellato & Leitão-Filho, 1992), maintaining reduced leaf cover during the dry season and increasing coverage during the wet season, with short deciduous stages (Singh & Kushwaha, 2005). On cloud forests, however, leaf flush and leaf fall may be observed year-round (Morellato *et al.*, 2000), and leaf cover is not reduced, leading to slower green-up and senescence rates and lower seasonality.

**Concluding Remarks**

Our study unravels land surface phenology patterns along elevational and latitudinal gradients, highlighting the distinctive phenology of higher-elevational tropical vegetation and describes for the first time the large-scale patterns of growing season length in seasonally dry neotropical vegetation. Although we did not find evidence of temperature-related periodicity, such as observed for temperate mountain vegetation, we did observe effects of temperature amplitude over the rates of green-up and senescence events, indicating that tropical seasonally dry vegetation could be sensitive to the increases in global temperatures. That is especially true for Dry Woodlands and Cerrado vegetation, which had a more pronounced seasonality and were more susceptible to the effects of temperature.

We demonstrated that different vegetation types have diverse leaf phenology strategies in seasonally dry tropical environments, and seem to maximize the use of water and light, whose availability is strongly influenced by topography. Our spatially explicit analysis of the processes driving phenological patterns in a snow-free tropical mountain show the importance of environmental factors acting simultaneously as proximate cues for tropical plant phenology, from the highest elevations at different latitudes, to the "*less seasonal"* lower elevation Moist Forests, as has been broadly shown for the lowland tropics (Morellato *et al.*, 2000). Our results highlight the need for further and combined ecological studies from local to regional scales, to disentangle the relative importance of direct and indirect effects of water, light, temperature and especially cloud cover seasonality on tropical land surface phenology dynamics.

**Supplementary Tables**

Table S1. General models of leaf phenology

Table S2. Best-fitting *specific models* of leaf phenology

Table S1. General models of leaf phenology, based on a time series of MODIS/NDVI imagery. The general models were fitted using data acquired from all vegetation types. Clouds and potential water availability were always present in the best-fit models. These models explained the *fundamental drivers* of leaf phenology at Espinhaço Range Mountain. *Phenological metrics assessed:* SOS= Start of Growing Season; EOS = End of Growing Season, LOS = Length of Growing Season, SA = Season Amplitude, GR= Green-up Rate, SR= Senescence Rate. Environmental drivers: TII = Total incoming radiation; CCF = Cloud frequency coverage; TA = Thermal amplitude; AAP = Annual average precipitation, TWI = Topographical wetness index; TII*CCF= potential light availability, AAP*TWI = potential water availability.

| **SOS** | **AIC** | **R²** |
|---|---|---|
| 1) 257.48 -0.363(TII) + 11.13(CCF) -0.37(TA) + 5.65(AAP) + 0.58(TWI) | 734.330 | 0.74 |
| 2) 257.66 + 2.48(TII)+ 9.91(CCF) -3.94 (TII*CCF) + 2.55(TA) + 5.59(AAP) -2.26(TWI) -3.95(AAP*TWI) | 718.66 | 0.79 |
| **3) 257.68 + 2.02(TII)+ 9.73(CCF) -3.24 (TII*CCF) + 4.67(AAP) -1.35(TWI) -3.13(AAP*TWI)** | **716.66** | **0.78** |
| 4) 256.36 + 10.96(CCF) + 5.349(AAP) -1.30(TWI) -2.64(AAP*TWI) | 723.97 | 0.76 |
| 5) 258.81 + 0.72(TII)+ 10.05(CCF) -2.83 (TII*CCF) + 5.38(AAP) | 726.61 | 0.75 |
| 6) 256.25 + 11.05(AAP) -4.35(TWI) -2.90(AAP*TWI) | 785.26 | 0.55 |
| **EOS** | **AIC** | **R²** |
| 1) 167.41 -2.39(TII) + 12.60(CCF) -3.76(TA) + 5.21(AAP) + 1.24(TWI) | 762.22 | 0.73 |
| 2) 165.4 -0.14(TII) + 11.99(CCF) -0.04(TII*CCF) -1.54(TA) + 4.70(AAP) -2.90(TWI) -4.76(AAP*TWI) | 750.68 | 00.77 |
| 3) 165.40 + 0.12(TII)+ 12.20(CCF) -0.46(TII*CCF) + 5.26(AAP) -3.45(TWI) -5.26(AAP*TWI) | 749.74 | 0.77 |
| **4) 165.19 + 12.21(CCF) + 5.28(AAP) -3.53(TWI) -5.24(AAP*TWI)** | **745.87** | **0.77** |
| 5) 165.07 + 11.64(AAP) -6.92(TWI) -5.52(AAP*TWI) | 807.03 | 0.57 |

Table S1 continues...

Table S1 continuation....

| **LOS** | **AIC** | **R²** |
|---|---|---|
| 1) 268.56 -0.01(TII) -5.46(CCF) + 0.23(TA) - 0.21(AAP) + 1.05(TWI) | 637.54 | 0.56 |
| 2) 270.50 -0.99(TII) -5.35(CCF) -1.46(TII*CCF) -0.71(TA) + 0.23(AAP) + 2.90(TWI) + 2.94 (AAP*TWI) | 614.48 | 0.68 |
| **3) 270.49 -0.86 (TII) – 5.30(CCF) -1.66 (TII*CCF) + 0.49(AAP) + 3.58 (TWI) + 2.72 (AAP*TWI)** | **610.82** | **0.67** |
| 4) 269.55 – 5.59(CCF) -1.87(TII*CCF) + 3.16 (TWI) + 2.42 (AAP*TWI) | 617.26 | 0.41 |
| **SA** | **AIC** | **R²** |
| 1) 0.205 -0.01(TII) -0.06(CCF) + 0.03(TA) - 0.02 (AAP) - 0.02(TWI) | -257.26 | 0.72 |
| 2) **0.191 -0.02(TII) -0.05(CCF) + 0.03 (TII*CCF) + 0.02 (TA) – 0.02 (AAP) -0.02 (TWI) + 0.008 (AAP*TWI)** | **-274.42** | **0.77** |
| 3) 0.191 -0.02 (TII) – 0.05 (CCF) + 0.04 (TII*CCF) – 0.03 (AAP) – 0.01 (TWI) + 0.01 (AAP*TWI) | -271.27 | 0.76 |
| 4) 0.193 -0.03 (TII) – 0.04 (CCF) + 0.03 (TII*CCF) + 0.01 (TA) – 0.02 (AAP) + 0.01 (AAP*TWI) | -271.04 | 0.74 |
| **GR** | **AIC** | **R²** |
| 1) 0.09 -0.007(TII) -0.02(CCF) + 0.01(TA) - 0.01 (AAP) - 0.01(TWI) | -405.20 | 0.71 |
| 2) **0.082 -0.01(TII) -0.02(CCF) + 0.01 (TII*CCF) + 0.008 (TA) – 0.01 (AAP) -0.01 (TWI) + 0.001 (AAP*TWI)** | **-425.05** | **0.77** |
| 3) 0.082 -0.01 (TII) – 0.02 (CCF) + 0.04 (TII*CCF) – 0.01 (AAP) – 0.009 (TWI) + 0.004 (AAP*TWI) | -422.72 | 0.76 |
| 4) 0.08 -0.01 (TII) – 0.02 (CCF) + 0.02 (TII*CCF) – 0.02 (AAP) – 0.01 (TWI) | -420.35 | 0.75 |
| 5) 0.089 -0.02 (CCF) + 0.01 (TA) – 0.01 (AAP) – 0.01 (TWI) + 0.003 (AAP*TWI) | -402.00 | 0.70 |
| **SR** | **AIC** | **R²** |
| 1) 0.088-0.004(TII) -0.02(CCF) + 0.01(TA) - 0.006 (AAP) - 0.008(TWI) | -439.87 | 0.71 |
| 2) **0.083 -0.009(TII) -0.02(CCF) + 0.01 (TII*CCF) + 0.009 (TA) – 0.007 (AAP) -0.007 (TWI) + 0.003 (AAP*TWI)** | **-452.74** | **0.76** |
| 3) 0.083 -0.01 (TII) – 0.02 (CCF) + 0.01 (TII*CCF) – 0.01 (AAP) – 0.004 (TWI) + 0.006 (AAP*TWI) | -447.92 | 0.74 |
| 4) 0.08 -0.007 (TII) – 0.02 (CCF) + 0.01 (TII*CCF) – 0.01 (AAP) – 0.009 (TWI) | -443.89 | 0.72 |
| 5) 0.088 -0.02 (CCF) + 0.01 (TA) – 0.008 (AAP) – 0.009 (TWI) | -440.25 | 0.71 |

Table S2. Best-fitting *specific models* of leaf phenology, accordingly to the vegetation type. Slope values are given outside the brackets. *Phenological metrics assessed:* SOS= Start of Growing Season; EOS = End of Growing Season, LOS = Length of Growing Season, SA = Season Amplitude, GR= Green-up Rate, SR= Senescence Rate. Environmental drivers* TII = Total incoming radiation; CCF = Cloud frequency coverage; TA = Thermal amplitude; AAP = Annual average precipitation, TWI = Topographical wetness index; TII*CCF= potential light availability, AAP*TWI = potential water availability.

| **Vegetation Type** | ***Phenological event*** | **$R^2$** | **p-value** |
|---|---|---|---|
| *Dry Woodlands* | **SOS** = 236.45 - 2.390(TA) | 0.24 | 0.02 |
| | **EOS =** 144.25 - 3.027(TA) | 0.20 | 0.04 |
| | **GR** = 0.181 + 0.017(TA) | 0.27 | 0.04 |
| | **SR** = 0.162 + 0.016(TA) | 0.28 | 0.01 |
| | **SA** = 0.39 + 0.037(TA) | 0.29 | 0.01 |
| | **LOS** = 272.78 - 0.709(TA) | 0.04 | 0.36 |
| *Cerrado* | **SOS** = 251.96 + 3.071(TII) + 6.832(CCF) - 3.419(TII*CCF) – 3.291(AAP) + 0.670(TWI) + 0.697(AAP*TWI) | 0.78 | < 0.05 |
| | **EOS =** 162.14 + 2.799(TII) + 3.169(CCF) – 3.437(TII*CCF) – 2.510(TA) – 4.646 (AAP) + 0.138(TWI) + 0.295(AAP*TWI) | 0.69 | < 0.05 |
| | **GR** = 0.081 - 0.009(TII) – 0.016 (CCF) + 0.013(TII*CCF) + 0.031(TA) – 0.032(AAP) – 0.008(TWI) + 0.001(AAP*TWI) | 0.77 | < 0.05 |
| | **SR** = 0.086 - 0.005(TII) – 0.014 (CCF) + 0.002(TII*CCF) + 0.026(TA) + 0.029(AAP) – 0.007(TWI) + 0.002(AAP*TWI) | 0.81 | < 0.05 |
| | **SA** = 0.19 - 0.020(TII) – 0.035(CCF) + 0.026(TII*CCF) + 0.064(TA) + 0.029(AAP) – 0.016(TWI) + 0.001(AAP*TWI) | 0.79 | < 0.05 |
| | **LOS =** 275.31 - 0.557(TII) – 4.521(CCF) – 1.286(TII*CCF) – 1.676(TA) -0.93 (AAP) + 0.113(TWI) + 0.133 (AAP*TWI) | 0.85 | < 0.05 |

Table S2. continue….

Table S2. continuation

| Vegetation Type | *Phenological event* | $R^2$ | p-value |
|---|---|---|---|
| *Montane Vegetation* | **SOS** = 265.26 -2.879 (TII) + 3.20(CCF) – 0.395(TII*CCF) + 10.559(AAP) + 1.471(TWI) - 1.81(AAP*TWI) | 0.86 | < 0.05 |
| | **EOS** = 170.31 + 3.02(CCF) + 5.298(AAP) - 1.024(TWI) - 0.829(AAP*TWI) | 0.72 | < 0.05 |
| | **GR** = 0.059 - 0.005(AAP) – 0.0004 (TWI) + 0.003 (AAP*TWI) | 0.42 | < 0.05 |
| | **SR** = 0.067 - 0.004(AAP) – 0.0003 (TWI) + 0.005 (AAP*TWI) | 0.37 | < 0.05 |
| | **SA** = 0.146 - 0.011(AAP) – 0.0001 (TWI) + 0.009 (AAP*TWI) | 0.38 | < 0.05 |
| | **LOS** = 267.85 - 2.922(CCF) – 3.00(AAP) + 1.385(TWI) + 1.253(AAP*TWI) | 0.65 | < 0.05 |
| *Moist Forests* | **SOS** = 261.64 - 4.297(TII) + 9.037(AAP) – 2.238(TWI) - 4.364(AAP*TWI) | 0.53 | < 0.05 |
| | **EOS** = 185.66 + 2.288(TII) + 6.512(CCF) + 7.353(TII*CCF) + 9.350(AAP) – 4.554(TWI) - 5.772(AAP*TWI) | 0.72 | < 0.05 |
| | **GR** = 0.055 - 0.001(TII) – 0.003 (CCF) + 0.004(TII*CCF) + 0.011(TA) - 0.003(AAP) – 0.003(TWI) + 0.004(AAP*TWI) | 0.84 | < 0.05 |
| | **SR** = 0.052 - 0.003(TII) – 0.005 (CCF) + 0.003(TII*CCF) + 0.012(TA) + 0.0002(AAP) + 0.001(TWI) + 0.005(AAP*TWI) | 0.85 | < 0.05 |
| | **SA** = 0.012 - 0.006(TII) – 0.012 (CCF) + 0.012(TII*CCF) + 0.027(TA) - 0.003(AAP) - 0.001(TWI) + 0.013(AAP*TWI) | 0.85 | < 0.05 |
| | **LOS** = – 3.847 (CCF) - 3.073(AAP) + 2.310(TWI) + 2.083(AAP*TWI) | 0.62 | < 0.05 |

## 3. Testing the generality of plant functional trait trade-offs in the seasonally dry tropics [2]

[2] Streher, A.S; McGill, B.J; Morellato, L.P.C.; Silva , TSF. To be submitted to the jornal Functional Ecology.

**Abstract**

1. The sparsity of tropical trait sampling and the distinct nature of tropical climates raises the question of whether the two global trade-off dimensions, whole-plant size and leaf economics spectrum (LES) extend to the tropics (Díaz *et al*., 2016). *Campo rupestre*, a tropical mountain vegetation mosaic dominated by grasslands, comprises one of the highest levels of plant biodiversity on earth, representing a model landscape to understand if the broader scale patterns of trait-trait and trait-environment relationships holds at finer scales. Here, we investigated if global whole-plant size and LES trade-offs occurs in a Neotropical, seasonally dry *campo rupestre* mountain vegetation, and how the key traits from those dimensions are filtered by environmental gradients.
2. We gathered data from 1650 individuals comprising all life-forms of locally co-occurring plants, at five sites along an elevational gradient, sampling all vegetation types found within each elevation. We examined bivariate correlations between the pairs of traits, and used ordination method to check if those traits are organized along the two-known independent trait dimensions found in global scales. We partitioned the variance of four key functional traits (plant height, leaf area, leaf mass per area, and leaf dry matter content) into three nested ecological scales: individual within edaphic gradients, within elevation band).
3. We verified that the orthogonality of trait dimensions found in global scales do hold among locally co-occurring species, in tropical landscapes. Our results suggest that the whole-plant size dimension is a trade-off that varies along environmental gradients, with the allometric patterns of plant size playing a major role, as found worldwide.
4. However, the LES behaved differently than plant size, and we did not find variation of key LES traits along edaphic condition and elevational gradients at Serra do Cipó. We propose that the dry season experienced regionally may be a stronger environmental filter than elevational and edaphic gradient in the dry tropics, and that the resulting drought-tolerant leaf strategies are not necessarily tightly coupled with resource-use strategies at landscape scales.

**Key-words**: trait-based ecology, trait dimensions, LES, whole plant size dimension, Serra do Cipó, climatic constrains

## Introduction

Since ecology's earliest days, plant geographers have noted that phenotypic traits influence species distribution and abundances across landscapes (Schimper, 1903). Recently, the quest for linking species to environmental gradients has shifted from a taxonomic-centered perspective towards a more mechanistic trait-based approach (Lavorel & Garnier, 2002; McGill *et al.*, 2006). Trait based ecology offers the promise to improve ecological generality and predictability by focusing on generalizable properties shared by all plants, providing a basis for comparisons between different taxa and growth forms, revealing trends that are not limited to taxonomic groups or geographical locations (Shipley *et al.*, 2016).

The majority of vascular plants has a relatively short list of organs (leaves, stems, and roots), with ecological differences arising mostly from acquiring and allocating the same major resources (light, water, $CO_2$, and mineral nutrients) in different ways, which leads to variation in construction costs and lifespan between individuals (Westoby *et al.*, 2002). Thus, each plant organ potentially yields unique information about how a plant functions within its environment (Laughlin, 2014). Given the shared and limited resources plants have access to, individuals and species will differentiate by making distinct allocation decisions, leading to the idea of phenotypic trade-offs, or "trait dimensions" in the trait literature. These trait dimensions capture the continuous functional variation among plants, relevant to growth, survival and reproduction (Westoby *et al.*, 2002; Wright *et al.*, 2007b; Laughlin, 2014; Díaz *et al.*, 2016; Messier *et al.*, 2017a).

A recent global search using more than 45,000 vascular plant species from around the world revealed that the trait space actually occupied by plants is strongly restricted when compared to

the range of possible combinations if traits could vary independently, and major variations in plant phenotype are characterized by only two independent trait dimensions (Díaz *et al.*, 2016). These same two trade-offs have been speculated to be important axes of variation in plant phenotype from the beginning (Westoby *et al.*, 2002) and had been shown in studies of regional flora (e.g. Lavergne *et al.*, 2003; Golodets *et al.*, 2009; Laughlin *et al.*, 2010; Abe *et al.*, 2018). The first key dimension expresses the size of the whole plant, usually associated with tradeoffs in water balance. Plant height is an important trait from the first dimension, influencing the ability of individuals to compete for light and disperse (Westoby, 1998; Díaz *et al.*, 2016). Leaf area and seed mass also co-vary with plant height on the first trade-off dimension; leaf area (LA) (Wright *et al.*, 2004; Díaz *et al.*, 2016) is relevant for light interception and related to leaf energy and water balances (Wright *et al.*, 2017). The second trait axis represents the construction costs for photosynthetic leaf tissue, and has been referred to as the leaf economics spectrum (LES), expressing important tradeoffs in resource acquisition and storage strategies in plants (Wright et al., 2004). From the second dimension, two of the most commonly evaluated traits are leaf mass per area (LMA) and leaf dry matter content (LDMC); LMA reflects a tradeoff between carbon gain and leaf lifespan (Poorter *et al.*, 2009), while LDMC is a proxy for the proportion of investment in structural versus liquid-phase processes (Hodgson *et al.*, 2011). LDMC has been suggested to be the key variable underlying the correlations among the LES traits (Shipley *et al.*, 2006).

Although the dataset analyzed by Díaz *et al.* (2016) represents one of the largest trait compilations in the literature, and open trait databases have been continuously expanding, available global data on plant functional biodiversity are still grossly incomplete and non-representative taxonomically, geographically, environmentally, and temporally (Jetz *et al.*,

2016). Most of our theoretical and empirical understanding of trait-trait and trait-environment relationships comes from broad species-level studies, and according to Jetz et al. (2016), only 2% of currently known vascular plant species have any trait measurements available at the regional scale, with the largest data gap in the tropics. This limits our understanding and ability to generalize how variation in trait values changes across environmental gradients and ecological scales.

Like every other aspect of ecology, traits have been most heavily sampled in the temperate zones (Martin *et al.*, 2012). While there is a large number of samples, they are under representative of the 36% of the Earth's land that lies within the tropics. The GLOPNET database (Wright *et al.* 2004) lists 488 tropical observations, comprising 22% of the data. The TRY database seems to have a fair representation of the tropics, with 50.7% of the data, but they comprise only 65/1419 (4.6%) of the uniquely sampled locations. The BIEN trait database (Maitner *et al.*, 2018) has only 6.8% of its recorded observations in the tropics. Tropical habitats are not all equally sampled either. The tropics differ from temperate zones in fundamental ways. While diurnal temperature ranges are often greater than seasonal temperature variation, rainfall seasonality can be quite high in the tropics, with only wet tropical forests having near-constant precipitation rates.

Additionally, recent studies have suggested that relationships among traits detected in large scale studies cannot be interpreted in the context of the predicted tradeoffs at local scales (Messier *et al.*, 2010, 2017b; Anderegg *et al.*, 2018), but none of these studies have occurred in the tropics and none have tested the first trade-off of plant size. The sparsity of tropical trait sampling and the distinct nature of tropical climates raises the question of whether the two global trade-off dimensions identified by Díaz *et al.* (2016) extend in to the tropics. This is important because if

they do not, it may be suggestive of processes (i.e. those that differ between temperate and tropical regions) that are important in overall trait trade-off dimensions.

To test this theory, we investigated if whole-plant size and LES trade-offs do occur across a Neotropical, seasonally dry mountain vegetation mosaic, the *campo rupestre.* We also examined how variation in five ecological important traits are correlated with two nested environmental gradients: along a short elevational gradient (~600m), and across an edaphic gradient within each elevational band. *Campo rupestre* is dominated by grasslands covering the higher elevations of the Espinhaço Mountain range, and variations on vegetation structure and growth forms turnover occur naturally across environmental gradients. This region supports one of the highest levels of plant biodiversity on earth, with more than 1800 species catalogued in 200 $km^2$, most endemic and narrowly distributed (Alves *et al.*, 2014; Silveira *et al.*, 2016), but little is known about the mechanisms that generate and maintain such diversity. Considering the preeminent biodiversity and the large environmental heterogeneity within small geographical region, *campo rupestre* plant communities represent an excellent model to answer our/the question whether the proposed global scale patterns of trait-trait and trait-environment relationships holds for tropical environments and also at finer scales, where the known process structuring ecological communities' acts. The answer will help to determine the ecological relevance of trait variation for tropical community ecology.

## Methods

### *Study site*

The Espinhaço range is the largest interior mountain range in Brazil, bordering three tropical biomes: Atlantic forest at east, cerrado in the west, and caatinga drylands in the north (Silveira *et*

*al.*, 2016; Morellato & Silveira, 2018). The study was developed in the Serra do Cipó region, located in the southern portion of the Espinhaço range (Giulietti *et al.*, 1987). Ranging from 600 m to 1400 m above sea level, the Serra do Cipó comprises a seasonally dry tropical elevation gradient, with a mosaic of topo-edaphic conditions occurring within each elevational band, supporting patches of vegetation types differing from each other in composition and structure (Fig. 3.1).

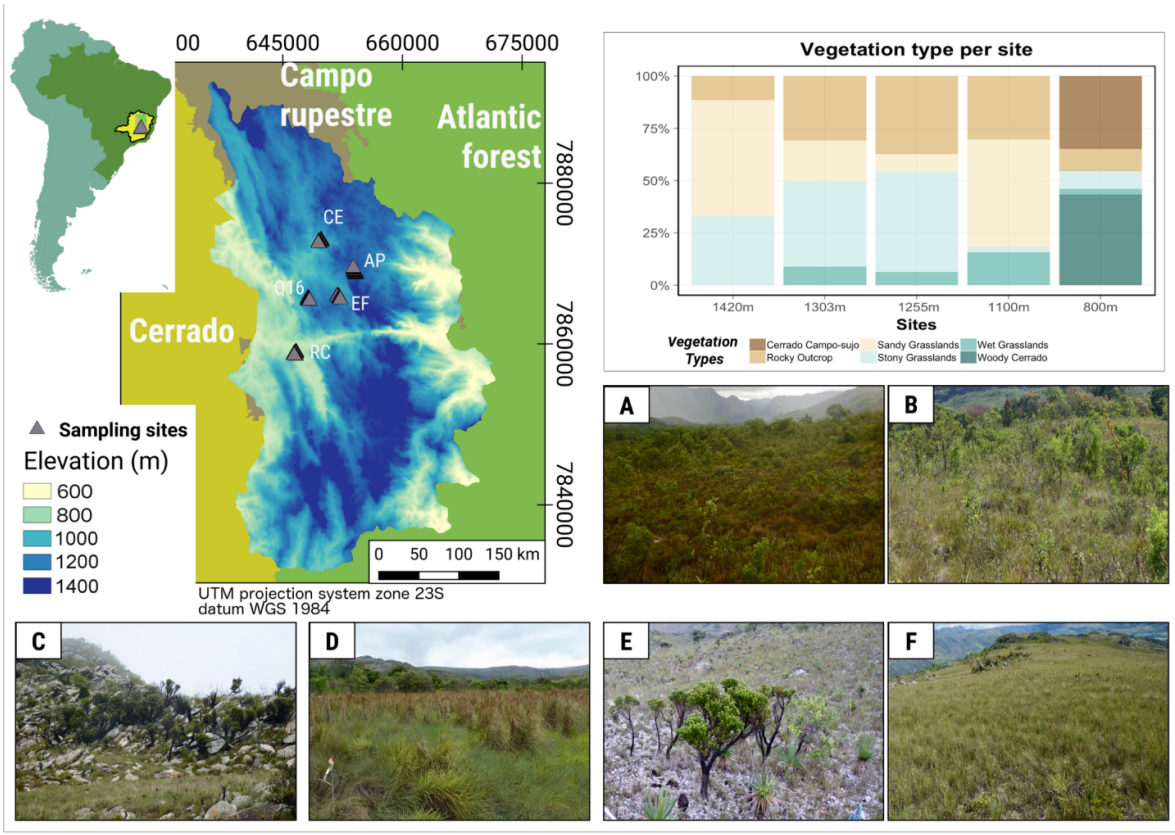


***Figure 3.1:*** Upper left - overview of the topography and extent of Serra do Cipó in the state of Minas Gerais, Brazil, and its unique location within an ecotone of vegetation types: Cerrado to the west; Atlantic Forest to the east, and Campo Rupestre (sensu lato) formations along the highlands. The grey triangles mark the locations sampling site and climatological stations (data courtesy of L.P.C. Morellato and G.W. Fernandes). Upper right – overview of the sampling effort, showing the proportion of vegetation types sampled within each elevation. The photographs illustrate vegetation types found across the elevational gradient, within each site: **A** – woody cerrado; **B** – cerrado campo sujo; **C** rocky outcrops; **D** – wet grasslands; **E** – stony grasslands; and **F** – sandy grasslands.

Vegetation structure in the Serra do Cipó changes along the elevation gradient (Fernandes et al., 2016; Mota et al., 2018): cerrado (savanna) formations, dominated by woody plants, are found at lower altitudes, transitioning to natural areas of *campo rupestre sensu stricto* above 900 m (Silveira *et al.*, 2016). *Campo rupestre* is characterized as a fire-prone montane vegetation, dominated by a matrix of grasslands growing on shallow sandstone soils, with interspersed quartzite rocky outcrops where woody vegetation is dominant (Giulietti *et al.*, 1987; Silveira *et al.*, 2016). The *campo rupestre* is considered an example of the old climatically-buffered infertile landscapes (OCBILS) theory (Hopper, 2009), on which infertile soils and climatic stability are the core explanation for its high floristic richness and endemism (Silveira *et al.*, 2016). Water is the major limiting factor for plant gas exchange at Serra do Cipó, and strategies to cope with seasonal water shortage vary from drought avoidance to desiccation tolerance (Negreiros *et al.*, 2014; Alcantara *et al.*, 2015; Oliveira *et al.*, 2016; Brum *et al.*, 2017; Dayrell *et al.*, 2018). Most plants from this region adopt drought-tolerant strategies with a perennial-leaf phenology (Le Stradic *et al.*, 2018), while drought-avoiding with deep or dimorphic root system species (Oliveira *et al.*, 2015; Brum *et al.*, 2017) and different degrees of deciduousness are also found (Streher *et al.*, 2017). Along the Espinhaço mountaintops, patches of Atlantic forest can also be found scattered within the grassland matrix (Coelho *et al.*, 2018). Although these forest patches co-occur with *campo rupestre*, their tree species differ substantially from the cerrado/*campo rupestre* species in their growth rates, ecology, and structure (Rossatto *et al.*, 2009).

The regional climate is mesothermal, marked by strong seasonality with two distinct seasons: a warmer rainy season from November to April and a colder dry season from May to October ((Streher *et al.*, 2017). The region has a mean annual precipitation of ca.1400 mm (INMET 1911-2015). Short-term climate data from weather stations at each elevational band (Fernandes

*et al.*, 2016) shows a trend of higher precipitation at mid-altitudes (between 1100 to 1200 m), where annual average precipitation reaches c.a. 860 mm, with monthly average precipitation reaching c.a. 300 mm at the peak of the rainy. The lowest amount of precipitation is found at the lowest elevation site (800 m), with annual total precipitation reaching 3000 mm (Fernandes et al., 2016). Minimum daily temperatures range between 13 and 17°C, and maxima between 23 and 28°C (INMET 1911-2015). Average annual temperature decreases linearly with elevation, with a 5°C difference between the lowest and highest elevations (Fernandes et al., 2016), but annual temperature amplitudes are highest at the lowest sites. Relative air humidity is higher at the highest elevations (90% at 1400 m), and lowest at the lower elevations (70% at 800 m), with mid-elevation sites having similar median humidity values (80% at 1100, 1200 and 1300 m). This distribution is due to an area of stationary nebulosity caused by the influence of the Atlantic tropical mass, which increases cloud coverage at higher elevations (Streher *et al.*, 2017; Coelho *et al.*, 2018). Photosynthetically active radiation (PAR) shows a reverse pattern, with the lower median values at the highest site (400 u/E at 1400 m), increasing towards lower elevations (500 u/E at 800 m).

Within each elevational band, a diversity of soil environments and associated vegetation mosaics can be found, largely determined by local topography and microenvironmental aspects. Variations of grasslands vegetation types are related to soil depth and drainage (edaphic factors) (Le Stradic *et al.*, 2015; Schaefer *et al.*, 2016a; Abrahão *et al.*, 2018), and rocky outcrops are widespread (Benites *et al.*, 2007). Soils are generally acidic with high aluminum saturation and nutrient impoverishment (Le Stradic *et al.*, 2015; Oliveira *et al.*, 2015; Abrahão *et al.*, 2018). For our study, we defined six vegetation types based on expert knowledge and current literature, namely: *Woody cerrado*; C*errado campo-sujo*; *Rocky outcrops; Wet grasslands; Stony*

*grasslands;* and *Sandy grasslands* (Silveira *et al.*, 2016; Morellato & Silveira, 2018) (Table 3.1 and Fig. 3.1).

***Table 3-1:*** Main characteristics of the vegetation types found along the elevational gradient at Serra do Cipó, Brazil

| **Vegetation type** | **Principal characteristics** |
|---|---|
| *Woody Cerrado* | Formed by a dense woodland of fire-adapted trees, with different degrees of deciduousness, and presence of a restricted herbaceous layer. This vegetation type occurs in lowlands and is constrained to the lowest site sampled (800 m), occurring over deeper sandy and nutrient poor soils, with low cation exchange capacities and high levels of aluminum saturation and acidity. |
| *Cerrado campo-sujo* | Found on shallower soils with similar properties to *Woody Cerrado*, and dominated by an herbaceous-grassy layer with scattered small shrubs and trees. Can be found up to 1000 m of elevation. |
| *Rocky outcrops* | Dominated by scattered evergreen sclerophyllous shrubs growing on organic soil patches formed on depressions among the bare rock surfaces (Benites *et al.*, 2007) |
| *Wet grasslands* | Dominated by a tall herbaceous layer with scattered small shrubs, occurring in low lying areas where soils are waterlogged during the summer months, retaining some of that moisture during the dry season (de Carvalho *et al.*, 2012) |
| *Stony grasslands* | Dominated by grasses and sub-shrubs, found along smoother topography, with stony lag deposits and soil surfaces covered by gravel and quartz concretions (Le Stradic *et al.*, 2015) |
| *Sandy grasslands* | Formed by a continuous herbaceous layer, dominated by graminoids along shallow coarse soils with low water-holding capacity. Soils may be flooded during the rainy season, but have low water holding capacity and impose water deficit conditions for plants during part of the year (Le Stradic *et al.*, 2015; Oliveira *et al.*, 2016) |

*Sampling design*

Our study design was based on environmental stratification by elevation and vegetation type (representing variations in soil conditions). We set five sampling sites along the elevation gradient (Fig 1.), chosen to coincide with previous studies in the region (Rocha *et al.*, 2016) and with ongoing floristic and phylogenetic surveys within a long-term ecological research program. Four permanent transects of 250 m, distant 50 m from each other, were established based on interpretation of high-resolution satellite and aerial images, to ensure the inclusion of all vegetation types occurring at each elevational band (Table 1). Sampling points were established at seven meter intervals along each transect, and a 3.5 m search radius was established around each point. Within each radius, we searched outwards from the center of the radius for up to three individuals belonging to morphotypes not sampled before in the same transect, identified by their phenotypic characteristics and, when possible, taxonomic designation. If less than three individuals from unsampled morphotypes were found within the search radius, then plant individuals closest to the center point, regardless of species, ensuring three individuals were sampled every seven meters.

Our sampling design sought to balance trait variability and abundance along the transects without considering species taxonomy, to ensure maximal sampling of phenotypical variation and thus, presumably, traits. Our choice of a species-free sampling strategy was based on the endemic and hyperdiverse nature of Serra do Cipó (Silveira *et al.*, 2016; Zappi *et al.*, 2017), with relatively low abundances from a very large number of contrasting species, and an even smaller number of species shared among vegetation types (personal observation) with more than 70% of species turnover between elevations (Mota et al., 2018), hindering intraspecific replication. Ongoing taxonomic work by a group of specialists working on the same transects used in this

work, has identified 482 species to date, from 58 families, with around 100 morphospecies still remaining to be identified.

Our sampling design yielded 330 individual plants per site, for a total of 1650 individual plants encompassing all encountered life-forms, and representing the majority of plant phenotypes found at Serra do Cipó. Although we sampled all vegetation types present at each site, the number of sampled individuals per vegetation type was uneven, given the naturally patchy distribution of vegetation types at each elevation (Fig 1).

*Trait sampling*

We performed trait sampling during the October 2016 to March 2017 growing season (Streher et al., 2017). The height of every individual plant was measured in the field to the nearest centimeter using a measuring tape. We then harvested leaf samples from tree and shrub branches with fully expanded and illuminated mature leaves, avoiding serious herbivore or pathogen damage, and sampled the whole plant for grasses, keeping the roots attached when possible (Pérez-Harguindeguy *et al.*, 2013). Partial rehydration protocols were followed by storing the samples in moistened sealed plastic bags, under elevated $CO_2$ concentration and saturated air humidity (Garnier *et al.*, 2001; Vaieretti *et al.*, 2007). We removed three healthy leaves from each sampled branch/individual after partial rehydration, including petioles, blotted them dry to remove surface water, and immediately weighed them to determine saturated fresh mass (LFM - Garnier, Shipley, Roumet, & Laurent, 2001). Samples were kept at ± 4 ºC in the dark, and measured between six to eight hours after harvesting the plants. Their projected area (LA - one-sided leaf area, Pérez-Harguindeguy et al., 2013) was determined by photographing each leaf with a digital camera under a straight overhead (nadir) view. Leaves were gently pressed

between a glass plate and a sheet of paper including a printed distance scale over a table, to ensure accurate area measurements and allow posterior photo scale calibration. We then calculated leaf areas using the ImageJ2 software (Schindelin *et al.*, 2015). After photographing, we oven-dried leaf samples at 80 °C for at least 2 days to determine leaf dry mass (LM) to the nearest 0.01 g. Finally, we calculated leaf mass per area (LMA) as LM/LA, and leaf dry matter content (LDMC) as LM/LFM (Pérez-Harguindeguy et al., 2013).

*Data analysis*

Leaf measurements were used as replicates for each individual plant, so we first averaged the triplicate measurements of all leaf-traits to the individual level. Second, we assessed the degree of covariance between all measured traits by computing the Pearson correlation coefficient for each pair of traits. We then performed a principal component analyses (PCA) on observed trait data, after z-transforming variables, to evaluate if trait dimensions emerged at the landscape scale.

We used variance decomposition to determine the influence of environmental gradients on trait variation (Messier *et al.*, 2010, 2017b). We applied logarithmic transformations to height, LA and LMA to reduce distribution skewness prior to further analysis. LDMC had a Gaussian distribution and was not transformed. We then decomposed the variance of each trait into three nested scales: *site*, representing changes of elevation (regional scale); *vegetation type,* representing changes in vegetation type mostly related to different edaphic environments (local scale); and *individual* – representing residual trait variance at the individual plant level (*i.e*. residual variance). The contribution of each hierarchical level to total trait variation was estimated by using linear mixed models accounting for the nested structure of the sampling

design. As observations were naturally unbalanced across the ecological scales analyzed (*i.e.* not the same number of vegetation types per elevation) we fitted a nested ANOVA using restricted maximum-likelihood with random effects, following Messier et al. (2010). The analysis was implemented in the R statistical language version 3.4.0 (R Development Core Team 2007), using the "ape" (Paradis *et al.*, 2004) and "nlme" (Pinheiro *et al.*, 2017) packages.

## Results

### *Trait variation*

Trait variation differed across all traits sampled. LDMC was the least variable trait, with a five-fold range of variation, followed by LMA with a 20-fold variation, plant height with 145-fold, and LA as the most variable trait, varying about 990-fold (Table 3.2). Trait variations also had different elevational trends (Figs. 3.2 and 3.3), with taller plants at lower elevations and shorter plants towards higher elevations (Fig. 3.2). Variance in leaf area was similar between elevations, but means were different across vegetation types (Fig. 3.2). The distributions of LMA and LDMC were surprisingly uniform, with relatively constant variance and similar means among different elevations and vegetation types (Fig. 3.3).

***Table 3-2*:** Summary statistics of trait values sampled across the whole data set. Min = minimum observed trait value; Median = median trait value; Mean = average trait value; Max = maximum trait value. Traits were plant height (Height), leaf dry mass (LM), leaf area (LA), leaf mass per area (LMA), and leaf dry matter content (LDMC).

| **Traits** | **Summary statistics** | | | | |
|---|---|---|---|---|---|
| | *Min* | *Median* | *Mean* | *Max* | *Global range* |
| **Height (cm)** | 6 | 61 | 98 | 875 | 0.1 - 9000 † |
| **LM (g)** | 0.060 | 0.110 | 0.334 | 21.923 | $7.9*10^{-7}$ – 2092♠ |
| **LA ($cm^2$)** | 0.564 | 8.120 | 19.435 | 554.94 | $0.79*10^{-4}$ - $2.79*10^{6}$† |
| **LMA ($g\ m^{-2}$)** | 31.8 | 142.8 | 157.6 | 620.8 | 4.9 - 1507† |
| **LDMC ($g\ g^{-1}$)** | 0.120 | 0.386 | 0.385 | 0.670 | $2*10^{-4}$ - 3.54 ♠ |

† Global range data for LMA and Height comes from Díaz et al. (2016), in the same units as our study.

♠ Global range data for LDMC and LM from the Botanical Information and Ecology Network Database BIEN, Maitner et al. (2018), in the same units as our study.

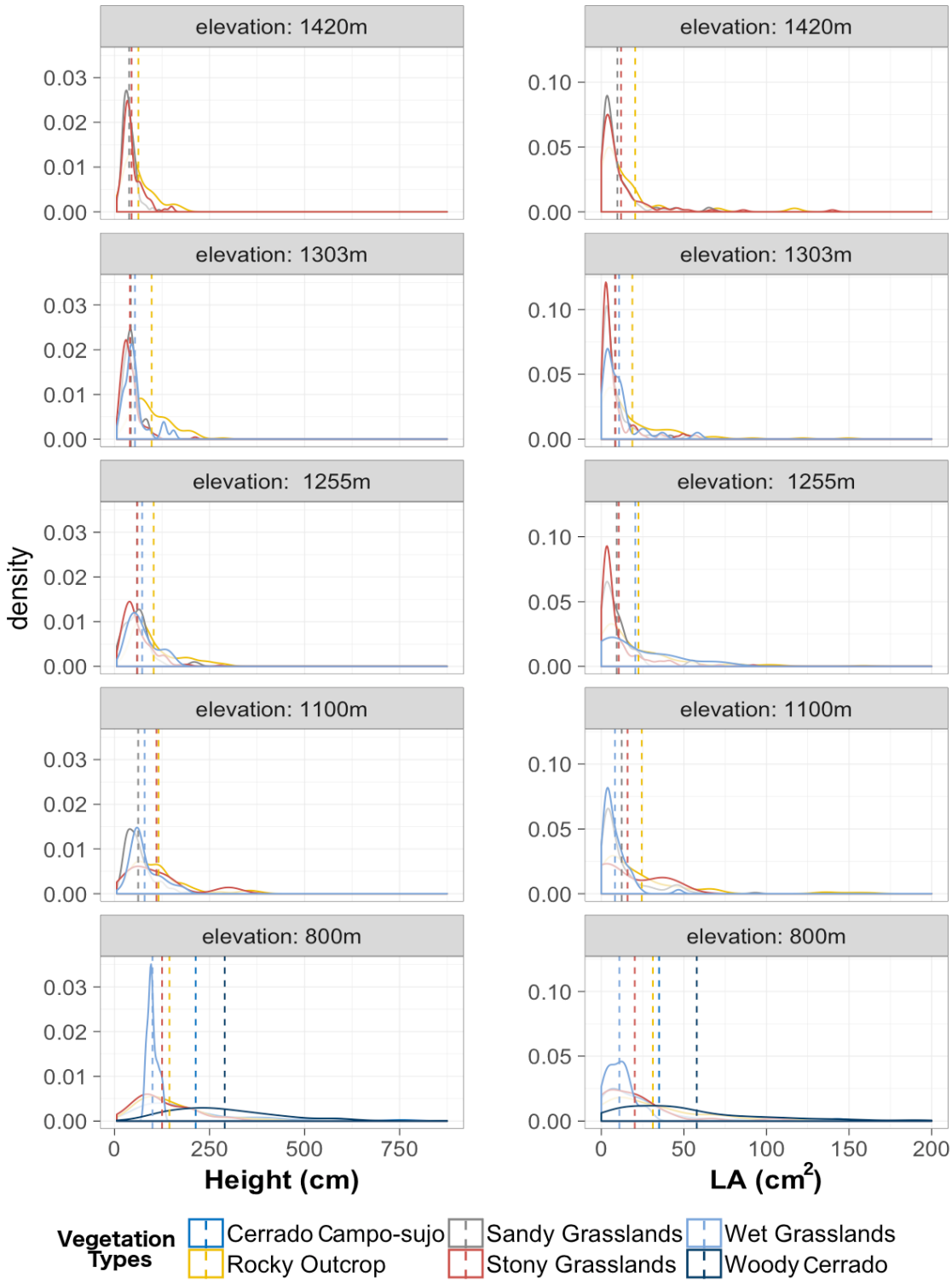


***Figure 3.2:*** Frequency distributions of plant height (Height) and leaf area (LA) of individual plants sampled at each elevation. Colored lines represent observed trait distributions for different vegetation types/edaphic conditions, and dashed vertical lines indicate mean trait value per vegetation type at each elevation.

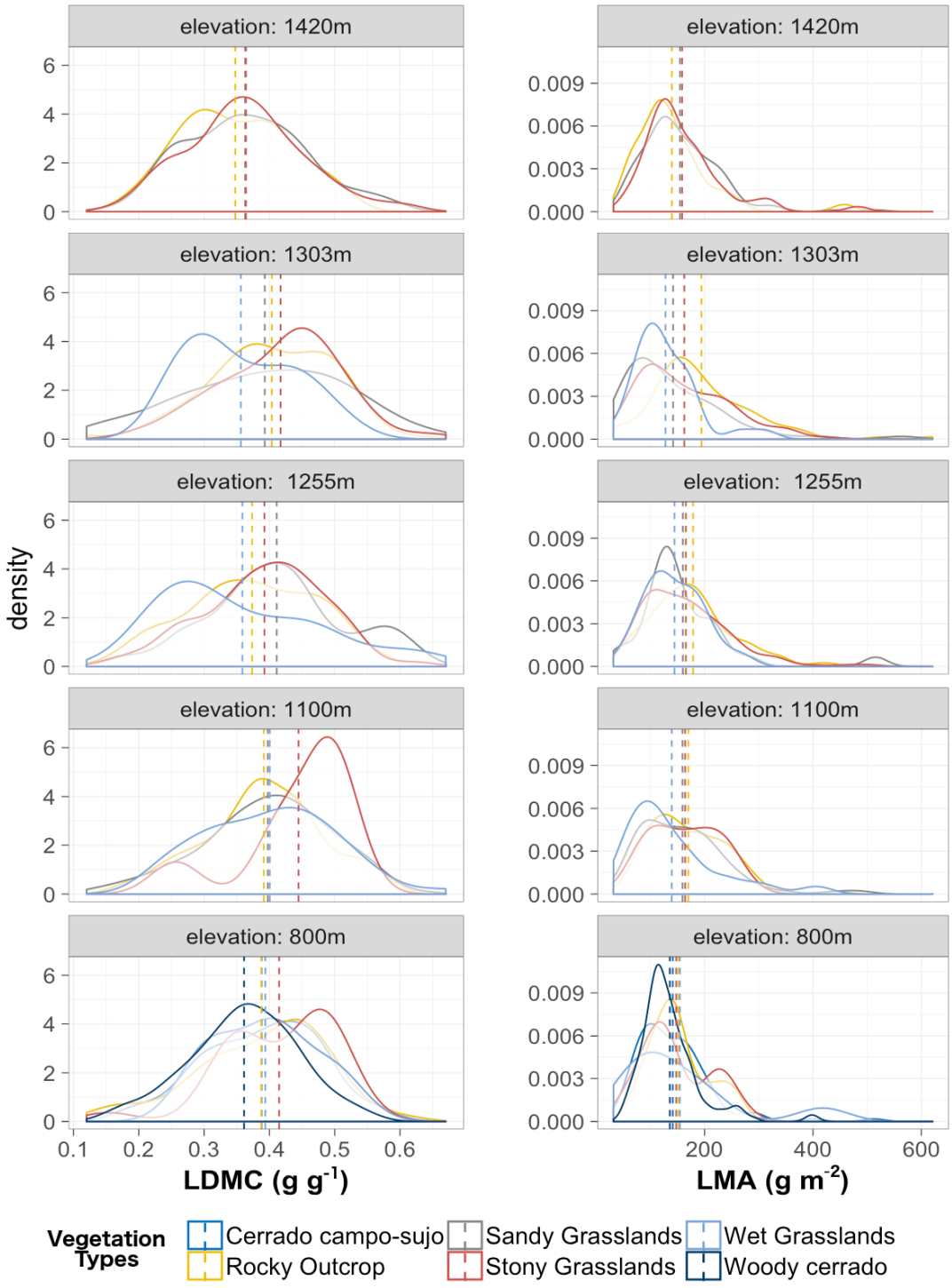


***Figure 3.3:*** Frequency distributions of leaf dry matter content (LDMC) and leaf mass per area (LMA) of individual plants sampled at each elevation. Colored lines represent observed trait distributions for different vegetation types/edaphic conditions, and vertical dashed lines indicate mean trait value per vegetation type at each elevation.

*Trait-trait relationships*

Bivariate trait correlations ranged from non-correlated ($r \approx 0$) to $r=0.95$ and showed notable covariation patterns (Table 3.3). LMA and LDMC were moderately correlated with each other, but weakly correlated with all remaining traits, and a positive moderate correlation was also found between plant height and LA. Plant height and LA were not correlated with LDMC and LMA. LA and LM were strongly and positively correlated, which combined with the lack of correlation between LA and LMA suggest that most of the variation in leaf mass was driven by leaf area and not by leaf thickness or density.

***Table 3-3***: Pearson correlation coefficients between log-scaled traits (except LDMC, which was not normalized). Correlations had $p < 0.001$ except when indicated. Traits were plant height (Height), leaf dry mass (LM), leaf area (LA), leaf mass per area (LMA), and leaf dry matter content (LDMC).

| | **LM** | **LA** | **LMA** | **LDMC** |
|---|---|---|---|---|
| **Height** | 0.45 | 0.47 | 0.11 | 0.01 (p = 0.581) |
| **LM** | | 0.94 | 0.47 | 0.03 (p = 0.276) |
| **LA** | | | 0.15 | -0.11 |
| **LMA** | | | | 0.37 |

Principal component analysis of these data largely confirmed the results from the pairwise analyses. We identified the main independent dimensions of trait variation, with 69% of the variation in the trait-space accounted by the first two principal components (Fig. 3.4a). The first principal axis explained about 43% of the total trait variation and was most strongly correlated with LA and LM (Fig. 3.4a and 3.4b). Variation in plant height was also associated with this axis, which can be interpreted as "whole-plant size dimension", in agreement with Díaz *et al.* (2016). The second axis explained a further 26% of variation, reflecting the positive, but weaker

relationship between LMA and LDMC (Fig. 3.4a and Fig. 3.4c), echoing the LES expected trait-variation. Weak correlations between the two-axes indicated that covariation between traits from leaf structure/function, and whole-plant size traits were largely independent (orthogonal). Therefore, trait variation across the first axis (whole-plant size) was much larger than the trait variation from the second dimension (LES).

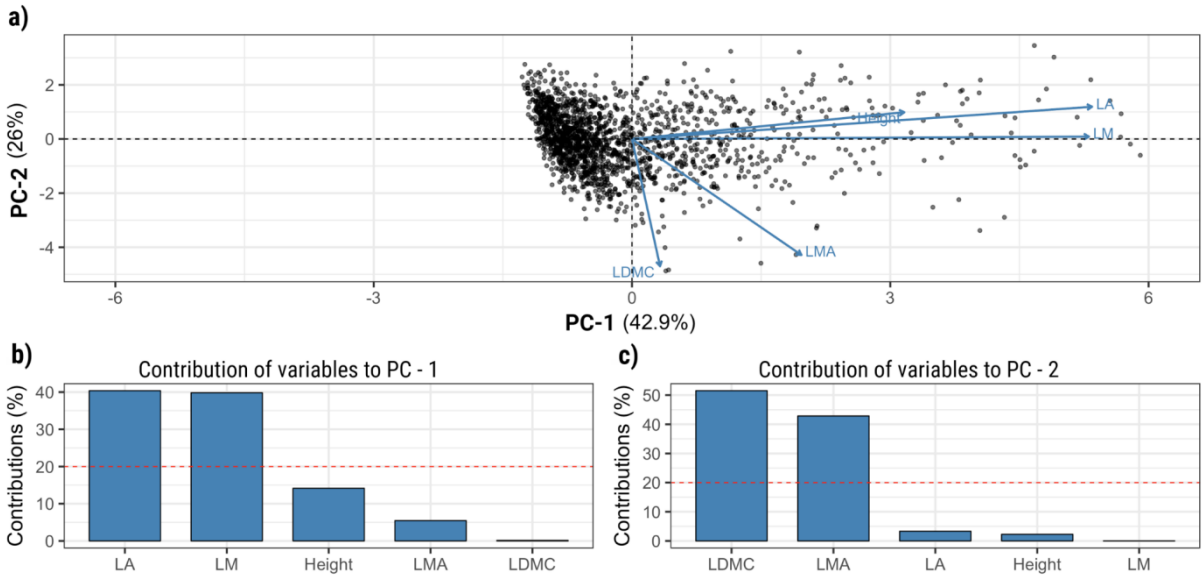


***Figure 3.4 :*** The upper panel (a) shows the principal component analysis (PCA) highlighting independent axes of leaf functional traits among 1650 woody and herbaceous angiosperms in a species-rich neotropical savanna and campo rupestre transitional area. Solid blue arrows indicate the direction and weighing of vectors representing the five traits considered: plant height (Height), leaf dry mass (LM), leaf area (LA), leaf mass per area (LMA), and leaf dry matter content (LDMC). Bar plots at the lower panels represents the percentage contribution from traits to (b) the first principal component (PC-1) describing whole-plant size dimension, and (c) the second principal component (PC-2) representing the leaf economics spectrum dimension. The red dashed line on the bar graphs indicates the expected average value if trait contributions were uniform.

*Trait-environment variance decomposition*

Trait variance partitioning showed uneven results across the scales assessed (Fig. 3.5). For all traits, variance at the individual level was higher than any other ecological scale. After recognizing intraspecific variation as the largest source of variation, as expected, elevation accounted for much of the explained variation for plant height (33%), reflecting the tree-to-grass life-form turnover observed between elevations. Leaf traits had a remarkable amount of variation at the individual level, with almost all of the variation found between individuals within a transect and almost no variation in mean trait values explained by elevation or vegetation types (edaphic gradients). LMA and LDMC stood out with more than 90% of the variance of either leaf trait not explained by any environmental gradient (Fig. 3.5). While larger than LMA and LDMC, the amount of variance in LA explained by elevation was still only 9%. Similarly, the different vegetation types found within each elevation were responsible for c.a. 11% of total LA variance.

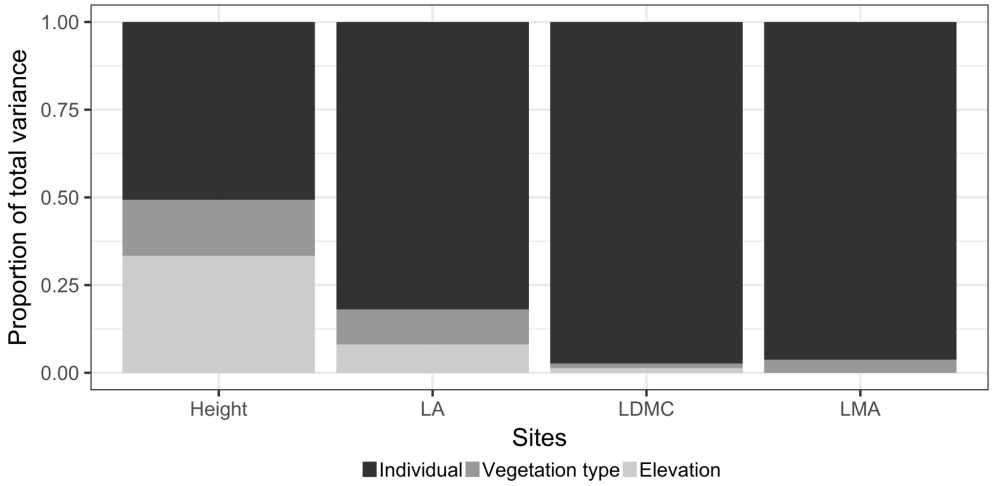


***Figure 3.5:*** Partition of variance between structural (Height: plant height) and leaf functional traits (LA: Leaf area; LDMC: leaf dry matter content; LMA: Leaf mass per area) across three ecological scales, showing traits varying differently from each other and the low variance explained by environmental scales for LDMC and LMA.

**Discussion**

Although our dataset is confined to a small geographic area, our study system has substantial environmental heterogeneity across two distinct scales: elevation and vegetation type/ edaphic gradients, and covers a wide range of plant phenotypes, drawn from one of the most biologically diverse ecosystems in the world (Silveira *et al.*, 2016). Within our dataset, three main patterns of trait variation emerged. First, we verified the existence of the two main axes of variation in plant form and function, confirming the two trait-tradeoff dimensions of Díaz *et al.* (2016) for the first time in a tropical setting at a local scale, and also in a dry tropical setting. Second, both PCA and variance portioning suggest that plant size is a trade-off that varies along environmental gradients, nevertheless the allometric consequences of plant size and architecture seems to play an important role in our system, as found worldwide. Third, the LES behaves differently than plant size. Although a strong trade-off independent of plant size was identified in the PCA, variation was almost entirely across individuals within a single community, with essentially no variation in LES attributable to elevation gradient or topo-edaphic conditions. This contradicts most existing literature that finds LES variation along climatic gradients (Ackerly, 2004; Wright *et al.*, 2005; Messier *et al.*, 2010).

Although plant ecological strategies have been studied in tropical forests (Wright *et al.*, 2005, 2007a; Messier *et al.*, 2017b; Poorter *et al.*, 2018), there are few, limited studies in Neotropical savannas and grasslands (Abe *et al.*, 2018; Dayrell *et al.*, 2018). Cerrado plants are known for allocating more biomass below-ground, and have greater phenotypic plasticity than forest trees (Hoffmann & Franco, 2003), which could result in distinct trait dimensions patterns. In a worldwide context, our results contribute to a better understanding of ecological trait variation among seasonally dry tropical plants at community scales, and reinforces that these two

dimensions capture important generalities about how plants survive natural selection, physiological challenges and competitive exclusion.

The plant size dimension had the strongest correlations among its associated traits, and even though our range of variation in LA, LM and plant height values is quite small when compared to Díaz *et al.* (2016), this axis accounted for the same amount of variation in our dataset as in the Díaz dataset. This dimension runs from short species tending to have small leaves, to tall species tending to have larger leaves. The relative effect size of elevation gradient and edaphic conditions over LA and plant height were considerably different. Leaf area varied more across the diversity of life forms adapted to different topo-edaphic conditions, following the known constrains imposed by small scale edaphic and microclimatic conditions (Wright *et al.*, 2017), while the observed trends for plant height can be attributed to the elevational gradient, reflected in the gradual decrease of woody individuals as they are replaced by grasslands at altitudes above 900m (Fernandes *et al.*, 2016; Silveira *et al.*, 2016). The tendency of LA to increase with plant stature suggests that LA variation can be also partially explained by the allometric consequences of plant size than by environmental constraints. Generally, the LA-plant height allocation pattern can be related to water availability, with taller plants as found at lower elevations at Serra do Cipó demanding structural and anatomical adaptations that minimize hydraulic limitations, thus being able to afford larger leaves (Givnish, 1988, 1995).

Our results indicate an orthogonal axis representing leaf structure and function, but the moderate bivariate relationship between the LES traits prevented LMA and LDMC from clustering tightly together into the PCA space. More strikingly, LMA and LDMC did not show any systematic variation across environmental gradients, restricting the interpretation of the LES patterns as the expected tradeoffs in the resource acquisition-conservation spectrum. In the LES literature,

drought tolerant plants are usually expected to be associated with a resource-conservative strategy (high LMA and LDMC values), while drought avoidance species are expected to be associated with a resource-acquisitive strategy (low LMA and LDMC) (Reich, 2014). In our dataset, LDMC and LMA span 15% and 39% of the range covered by global datasets (Díaz *et al.*, 2016; Maitner *et al.*, 2018), and despite including different growth forms and leaf habits, our results clearly reflect only the 'slow' part of the spectrum. Most strikingly, variation in leaf traits occurs only between individuals in the same transect. This suggests that processes such as competition are much more likely to be driving the LES spectrum in our study area than climatic or soil gradients.

One reason the expected LES tradeoffs break down across environmental gradients in our study is the heavy dependence of LES relationships on variations in leaf-lifespan (LLS) (Funk & Cornwell, 2013). Generally, Cerrado woody species can be categorized into deciduous, semi-deciduous and evergreen species (Streher *et al.*, 2017; Camargo *et al.*, 2018), but LLS differences between them are quite small when compared to other plant communities under the same regional climate, and do not result in distinct leaf functional trait values (Cianciaruso *et al.*, 2013). Moreover, the herbaceous-grassy layer has perennial leaf strategies, with several species showing tolerance to desiccation and high longevity (Negreiros *et al.*, 2014; Alcantara *et al.*, 2015; Le Stradic *et al.*, 2018), reducing even more the overall differences in LLS within the community. This pattern suggests that despite the wide growth form variation and species diversity along the environmental gradients, the "payback time" (the time that a leaf needs to compensate its cost in terms of carbon investment) may be similar even if plants have different phenological strategies.

The common denominator overriding both environmental gradients at our study site is the marked water seasonality. Seasonal water shortage might act as a strong environmental filter on community assembly (Negreiros *et al.*, 2014; Oliveira *et al.*, 2016; Brum *et al.*, 2017; Dayrell *et al.*, 2018). Temperature seasonality has also been repeatedly reported as a constraint over herbaceous and woody deciduous LLS (Givnish, 2002), although the exact role of high LMA and LDMC in communities where LLS is climatically constrained is uncertain. Moisture seasonality may cause LLS to play a role as an important strategy to increase water-stress tolerance (Dayrell et al., 2018; Negreiros et al., 2014; Poorter et al., 2009; Wright, Westoby, & Reich, 2002). Water stress may reduce turgor pressure and hence cell expansion, resulting in approximately the same dry mass being contained within a smaller leaf area, thus raising density and increasing LMA values (Witkowski & Lamont, 1991). In fact, it has been shown that herbaceous species from Serra do Cipó have stress-tolerance syndromes (Negreiros *et al.*, 2014; Dayrell *et al.*, 2018), with higher LMA and LDMC values than plants from other grassland types (Silveira *et al.*, 2016). Even elsewhere within Cerrado environments, the ground-layer vegetation has lower LMA values (Rossatto *et al.*, 2015) than woody plants, as this stratum is completely destroyed during the passage of fire, implying that these plants need to grow faster (Rossatto & Franco, 2017). Although *campo rupestre* is considered part of the Cerrado biome, this predicted fast-return strategy does not seem to hold, given the conservative LMA and LDMC distributions across vegetation types and elevation. Our results reassert the extremely nutrient-conservative nature of *campo rupestre* vegetation.

The idea of regional climate as an explanation for the lack of variability in leaf physiological traits could be the result of the lasting influence (legacy effects) from past climatic and environmental conditions, in agreement with the core of the OCBIL theory. Serra do Cipó is an

old landscape which evolved under climatic stability, favoring the persistence of old lineages that continue to diversify (Silveira *et al.*, 2016), but apparently maintaining the tendency for mature individuals to conserve the traits and environmental tolerances of their ancestors (at least at the leaf level). We believe that this lack of variability in leaf trait variation are due to evolutionary adaptations, possible associated with past climatic stability, and have important implications for the conservation status of *campo rupestre*, since overall, this vegetation may not have enough plasticity to keep up with the fast-anthropogenic climatic changes.

In summary, our study is part of a broader push to identify and understand the major dimensions of trait variation within vegetation communities, and suggests that in a seasonal tropical landscape, the overall patterns of phenotypic variation in plant form and function are similar to the global scale patterns in one way. However, there is no variation of key LES traits along edaphic and elevational gradients, suggesting that the LES may not be tightly coupled with nutrient-use strategies at the landscape level (Abrahão *et al.*, 2018). We suggest that the seasonal drought strongly filters for a narrow range of high LMA an LDMC values, regardless of other environmental gradients. The little variation in LMA and LDMC existing in our study is among individuals within a single transect, and it is possible that this variation is due solely to competition among individuals. We still know little about the phenotypic traits that lead some species to dominate ecological functions, and we emphasize that plant ecologists who wish to use phenotypic leaf traits as indicators of plant function should first confirm that they do in fact correlate with resource-use tradeoff strategies within their communities. For example, in our study system, below-ground structure variations such allocation patterns in roots, rhizomes and fleshy swellings may be more informative than leaf trait variation to the understanding of vegetation resource-use strategies.

## 4. More than a proxy: leaf spectroscopy reveals dissimilarities in growth form even in the absence of leaf trait variation[3]

[3] Streher, A.S.; Torres, R.S.; Morellato, L.P.C.; Silva, TSF. To be submitted to the New Phytologist jornal.

**Abstract**

1. We tested the ability of reflectance spectroscopy to estimate leaf functional traits and differentiate among plant growth forms, to fill current gaps in surveys of leaf optical properties, and to fully understand the integrative nature of leaf reflectance as a leaf phenotype.
2. We measured leaf reflectance spectra of 1648 individual plants comprising all growth forms within a tropical, snow-free seasonally dry mountain vegetation type, the *campo rupestre* and associated woody *cerrado* (savanna) plants. We developed quantitative models linking two leaf functional traits (LMA and LDMC) to leaf spectra (400–2500 nm). We explored the wavelengths with the greatest discriminatory power between growth forms using the Bhattacharyya distance.
3. We were able to accurately predict leaf functional traits from different growth forms. Our general model was not biased towards any growth form, and growth-form specific models showed that leaf traits from woody plants were better estimated by spectroscopy, independently of the trait measured. Grasses and woody plants were the most spectrally dissimilar.
4. Our findings show that leaf reflectance spectra provided important information on leaf ecophysiology, and indicated that an overall *leaf spectra phenotype* can be derived from leaf reflectance spectra. More importantly, leaf reflectance added more information than the LES central traits in the characterization of plant diversity in a seasonally dry, tropical area

**Keywords**: *leaf spectroscopy; phenotype; LMA; LDMC; growth form, prediction, cerrado; campos rupestres; partial least square regression (PLSR).*

## Introduction

In trait-based ecology, the trade-offs in allocation of finite resources to plant functions related to growth, survival, and reproduction yield a variety of plant strategies. Changes in leaf biochemical and structural properties are often interpreted as expressions of differences in how plants acquire and store resources, establishing an important functional dimension, known as the "leaf economics spectrum" (LES) (Wright *et al*., 2004). The LES is a one-dimensional set of correlated functional traits that are associated with a singular trade-off in function, representing a continuum of carbon and nutrient investment strategies and leaf persistence. Within this set of leaf traits, two particularly stand out: leaf mass per area (LMA), a key trait related to plant growth that responds to the trade-off between energetic cost of leaf construction and achieved light intercepting area (Poorter *et al*., 2009); and leaf dry matter content (LDMC), which captures the investment trade-off between structural versus liquid-phase processes (Kikuzawa & Lechowicz, 2011; Hodgson *et al*., 2011), a pivotal trait of leaf structural properties (Kazakou *et al*., 2006). In the LES context, LMA and LDMC values reflect two fundamental plant functioning strategies, whit low values suggesting rapid production of biomass, lower physical strength, and shorter leaf lifespan, and high values suggesting efficient conservation of nutrients, slow growth rates, and long-lived leaves (Garnier *et al*., 2001). The success of the LES theory has encouraged ecologists to conceptually advance trait ecology over an increasing number of publications, even though the relationships between LES traits and abiotic factors are either weak or not consistent across ecological scales (Messier *et al*., 2010, 2017b; Anderegg *et al*., 2018).

Remotely-sensed optical spectroscopy provides a window into the functional composition of vegetation. A wide group of leaf traits, including many of the LES traits, can be detected and accurately predicted using leaf reflectance data (Serbin *et al*., 2014; Asner *et al*., 2016a). The

main biochemical constituents of LMA (proteins, cellulose, lignin, starch, and sugar) have overlapping absorption properties at nearby wavelengths in the visible (VIS), near (NIR), and shortwave-infrared (SWIR), and consequently more than one spectral region can be empirically correlated to this trait (Curran, 1989; Sánchez-Azofeifa *et al*., 2009; Asner *et al*., 2011a,b; Cheng *et al*., 2014; Serbin *et al*., 2014; Feilhauer *et al*., 2015; Cavender-Bares *et al*., 2016; Doughty *et al*., 2017). To date, few studies have investigated the optical properties of LDMC, but results indicate that it is the structural trait that best correlates with reflectance for trees (Asner *et al*., 2011a; Ali *et al*., 2016) and herbaceous plants (Roelofsen *et al*., 2014). LDMC shows strong spectral features related to leaf water content, and is usually correlated with the spectral region dominated by water absorbance, around 1400-1500 nm (Roelofsen *et al*., 2014).

Despite its widespread applications, spectroscopic predictions of leaf morphological traits are particularly focused on forested systems. Trees reaching the top of the forest canopy have been successful in competing for light, and have consequently developed trait combinations that maximize growth rates in these environments (Falster & Westoby, 2005), and sun-exposed leaves are expected to be more similar with respect to growth strategy and nutrient stoichiometry (Niinemets, 2010). This is not generalizable to other vegetation types, such as savannas, due to differences in biomass allocation; savanna plants tend to allocate less biomass to leaves and stems than forest individuals (Hoffmann & Franco, 2003). These allocation differences arise as light competition shifts towards competition for water and other limiting resources, as well as adaptations to fire, resulting in a greater plasticity of leaf morphological traits (Hoffmann & Franco, 2003). Diversification of leaf functional strategies is also conditioned by the integration of multiple traits at plant level, underlined by the overall growth form of the plant. The larger phenotypic plasticity of leaves and growth forms in savannas may affect the consistency of leaf-

trait-reflectance relationships, and potentially hamper the empirical trait–spectra relations usually applied in forested systems.

Furthermore, studies focusing on the LES are usually centered on a few traits, typically LMA and LDMC, which are unlikely to fully capture the ecophysiological and plastic variation of a particular leaf phenotype. Although measuring multiple traits require additional effort to ensure proper species or site replications (which is one requirement for the appealing generality of trait ecology), recent studies have shown that integrating multiple phenotypic traits greatly improves predictions of performance and fitness (Laughlin 2014, Kraft et al. 2015). In this regard, while the leaf reflectance spectrum can be used to predict functional traits, it also captures more of the total ecophysiological variation of leaf function that cannot be or is usually not measured (Schweiger *et al.,* 2018). As spatial and temporal variations in resource utilization by plants result in chemical, metabolic, structural, and phenological differences, leaf reflectance spectra will carry the full signal of plant life-history strategies, and understood as the overall expression of a "leaf phenotype" (Ustin & Gamon, 2010; Cavender-Bares *et al*., 2017).

Still, despite our growing understanding of how leaf morphological traits relate to reflectance spectra, we do not know what to expect in terms of local and regional diversity of leaf traits (Jetz *et al*., 2016) and thus cannot determine how sensitive are spectroscopy-based methods to trait variations. This shortfall sets a fundamental limit to our knowledge regarding the generality of correlations between optical and morphological traits from plants with different growth forms, life histories, and deciduousness strategies.

The cerrado (Brazilian savanna) and *campo rupestre* (mountain grasslands, Silveira *et al*., 2016) biomes are well suited to address this knowledge gap, since a variety of growth forms with

different evolutionary trajectories occur side by side in these biomes. Here, we focused on measuring functional leaf trait values and leaf reflectance among dominant growth forms found in cerrado and *campo rupestre,* to assess how well spectroscopy can predict leaf-level trait variations, and if leaf spectra indeed reflect the expected dissimilarities among growth forms. More specifically we asked: (i) Can leaf spectra accurately estimate LMA and LDMC from plants with different growth forms? (ii) Are leaf functional traits better predicted by different spectral regions between growth forms? (iii) How do leaf spectra quantitatively differ across growth forms?

## Methods

### *Study area and sampling design*

The Espinhaço Mountain Range, in Southeastern Brazil, is among the most ancient landscapes on Earth, having high levels of diversity and endemism with more than 5000 plant species (Fernandes, 2016; Silveira *et al*., 2016; Fernandes *et al*., 2018). Located at the southern portion of the Espinhaço Range, the Serra do Cipó subregion (19°23'29.8" S, 43°32'00.7" W) is also known for its megadiverse vegetation, with more than 1800 species recorded within a 200 $km^2$ area (Giulietti *et al*., 1987; Alves *et al*., 2014). The climate of Serra do Cipó is marked by strong seasonality with two distinguishable seasons: a warm rainy season from November to April and a cold dry season from May to October (Fernandes *et al*., 2016).

The rugged topography of Serra do Cipó provides a complex combination of topographic and edaphic conditions, which can lead to frequent and abrupt changes in vegetation structure and composition, hosting a large variety of plant growth forms and phenotypes (Schaefer *et al*., 2016b; Silveira *et al*., 2016). At lower elevations, a gradient of cerrado vegetation types differing

from each other in structure, composition, and deciduousness can be found, ranging from *campo sujo* (grasslands with scattered trees) to *cerrado sensu stricto* (woody savanna), while above 900 m, natural areas of *campo rupestre sensu stricto* (Silveira *et al*., 2016) dominate the landscape, growing on shallow soils. C*ampo rupestre* has been described as a montane, fire-prone grassland vegetation growing on sandy, stony, or waterlogged soils, interspersed with rock outcrops dominated by evergreen shrubs, forbs and a few herbs.

We sampled leaf traits and leaf reflectance spectra during the October/2016 – March/2017 growing season (Streher *et al*. 2017). Our study design included five sampling sites distributed along the elevation gradient, from 820 m to 1500 m, based on the natural environmental stratification of elevation and edaphic conditions. Within each elevation, four transects of 250m, distant at least 50m from each other, were established based on expert knowledge and interpretation of high-resolution aerial images, ensuring the inclusion of all vegetation types (a proxy for edaphic conditions) found within each site. The vegetation sampled encompasses all types of cerrado and *campo rupestre,* and hereafter are referred only as *campo rupestre*.

Sampling points were established at 7 m intervals along each transect, with a 3.5 m search radius delimited around each point. Within each search radius, we identified and sampled three individual plants, applying the following selection criteria: 1) we identified the three individuals closest to the center of the search radius belonging to morphotypes not sampled before in the same transect; 2) if less than three individuals from new morphotypes were found, we sampled the closest individuals to the center of the search radius, regardless of species, to reach three samples per sampling point. This sampling strategy was designed to ensure maximal sampling of morphotypic variation, maximizing trait variability, while also reflecting relative abundances.

For each plant individual, three fully-expanded sun leaves were sampled. In total, we sampled 4944 leaves from 1648 individual plants encompassing all encountered growth forms, representing the majority of plant phenotypes found at Serra do Cipó.

*Plant growth form definitions*

We followed the 'growth form' classification system proposed by Dansereau (1951), which relies on the forms (morphological aspects and height) shown by plants in their aboveground structure, and has already been applied to cerrado plants by Rossatto & Franco (2017). The plants at Serra do Cipó encompass an array of woody and herbaceous growth forms, comprising trees, shrubs, sub-shrubs, herbs, and grasses (Zappi *et al*., 2014). Based on the proposed classification system and field observations, we classified all the growth forms encountered into three dominant classes found in cerrado (Warming, 1908):

- "*Woody*": taller plants with secondary vascular growth, such as trees (woody plants with a defined stem, taller than 2m) and shrubs (height between 2 and 3 m, without a dominant stem and having lignified branches and stems);

- "*Forb*": plants with herbaceous and/or partially lignified stems, but with herbaceous branches, such as herbs (small eudicots from 0.1– 0.6m height, with herbaceous stems and branches) and sub-shrubs (plants with 0.5 – 1m height, generally with a thickened, partially lignified stem, and with aerial parts growing annually from an underground woody xylopodium);

- "*Grass*": monocot plants, including grasses and sedges from the *Poaceae, Xyridaceae,* and *Cyperaceae* family.

From the 1648 sampled individuals, 369 (22%) were classified as "*Forbs"*, 564 (34%) as "*Grasses"* and 715 (54%) as "*Woody"*.

*Leaf trait sampling protocol*

For trees and shrubs, we harvested branches of individual canopies containing sunlit and mature leaves, while for grasses we sampled the whole plant, keeping roots when possible (Pérez-Harguindeguy *et al*., 2013). We followed partial rehydration protocols by immediately storing the samples in moistened sealed plastic bags, under elevated $CO_2$ concentrations and saturated air humidity, stored in lightproof containers filled with ice (Garnier *et al*., 2001; Pérez-Harguindeguy *et al*., 2013). We kept the samples at ± 4 ºC in the dark, and measurements were taken between six to eight hours after harvesting. From each branch/individual sampled, we removed three healthy leaves with no serious herbivore or pathogen damage, including petioles, blotted them dry to remove surface water, immediately weighed them to determine saturated fresh mass (Garnier *et al*., 2001) and then measured reflectance spectra (see below). We then determined one-sided leaf area (Pérez-Harguindeguy *et al*., 2013) by photographing each leaf under a straight overhead (nadir) view, while gently pressing individual leaves between a glass plate and a sheet of paper including a printed distance scale, ensuring photo scale calibration and thus accurate area measurements. We then calculated leaf area using the ImageJ2 software (Schindelin *et al*., 2015). After photographing, we oven-dried leaf samples at 80 °C for 72 hours to determine leaf dry mass to the nearest 0.01 g. Finally, we calculated LMA as the ratio between dry mass and leaf area, and LDMC as the ratio between leaf fresh mass and dry mass (Pérez-Harguindeguy *et al*., 2013).

*Leaf spectral measurements*

We acquired leaf spectra using a full-range (350–2500 nm) ASD FieldSpec 4 Standard spectroradiometer (Analytical Spectra Devices, ASD, Boulder, CO, USA), with spectral resolution of 3 nm in the VNIR and 10 nm in the SWIR, and wavelength accuracy of 0.5 nm. We used the ASD leaf probe accessory, which measures the spectral reflectance at close range from the leaf. The probe contains its own calibrated light source and the measuring end of a bare fiber-optic cable (25° field-of-view (FOV)) mounted at 42º perpendicular to the contact surface (Serbin *et al*., 2014), minimizing measurement errors produced by variations in illumination geometry.

Bi-directional reflectance measurements were taken for the same three replicate leaves from which LMA and LDMC were estimated, immediately after obtaining saturated fresh mass. Leaves were arranged over a large black non-reflective surface, covering the whole diameter of the contact probe (10 mm) and ensuring that no light escaped the measurement. Plants with small leaves or leaflets were arranged so that the probe FOV was fully covered, without any gaps or excessive overlap, using more than a single leaf or leaflet when necessary. For each leaf, ten measurements were taken at one to six different parts of the leaf adaxial surface (depending on leaf size), following the protocols and standards by Asner & Martin (2009), available at the Carnegie Spectranomics website (http:// spectranomics.ciw.edu). For compound leaves, we took up to 10 measurements of different leaflets. The final leaf spectrum of each leaf was then given as the average of the 10 scans.

To ensure measurement quality and improve signal-to-noise ratio (SNR), we re-calibrated the spectrometer for dark current and stray light between each set of leaf replicates measured, using

a white reflectance reference (Spectralon; Labsphere Inc., Durham, NH, USA). Recorded spectra were read using the "FieldSpectra" package (Serbin *et al*., 2014) of the R statistical language, version 3.4.0 (R Development Core Team 2007), and underwent quality assurance by visual assessment. Finally, we averaged the triplicate measurements of all leaf traits and leaf reflectance to the individual level, and trimmed the full-range leaf spectra at the far edges (450 to 2400 nm), to remove data with low SNR.

*Leaf trait predictive modelling*

We used partial least squares regression (PLSR) modelling (Geladi & Kowalski, 1986; Wold *et al*., 2001), adapting the approach from Serbin *et al*. (2014), to predict LMA and LDMC from leaf spectral properties. PLSR is the current state-of-the-art method for linking leaf spectroscopy and leaf traits, due to its capacity to compensate for multicollinearity and reduce a large predictor matrix down to a relatively low number of predictors, the non-correlated latent components (Serbin *et al*., 2014; Feilhauer *et al*., 2015; Wu *et al*., 2017).

We fit four models to predict each of the two leaf traits: a model based on all observations ("All"), and three models restricted by plant growth form ("Woody", "Forb", and "Grass"), for a total of eight PLSR models. For each model, we split our data into training (70%, hereafter train set) and validation (30%, hereafter test set), using the "createDataPartition()" function from the "caret" package (Kuhn, 2008) in R, to ensure that both sets spanned the entire range of measured values for each trait. To reduce overfitting, we optimized the number of PLSR latent variables in the final models by minimizing the root mean square error (RMSE) of the prediction residual sum of squares (PRESS statistic, Chen *et al*., 2004). For the larger datasets ("All", "Woody", and "Grass") we calculated the PRESS statistic of successive model components using a 10-fold

cross-validation scheme, while for the "Forb" dataset we used a standard leave-one-out cross validation (LOOCV) analysis as recommended for datasets with fewer observations (Serbin *et al*., 2014). We assessed the final accuracy of each model by calculating the RMSE value between predicted and observed trait values in the test set, expressing it in the original variable units (RMSE), as percentage of the sample data range (%RMSE), and as the ratio of each model RMSE to the mean value of the trait dataset (mRMSE). We also report RMSECV, the RMSE obtained from the cross-validation procedure using the 10-fold or LOOCV methods, as discrepancies between RMSECV and RMSE can indicate model overfitting (Kuhn & Johnson, 2013).

Lastly, we computed the variable importance of projections (VIP, Wold (1994)) metric for each model, to identify the spectral regions that contributed the most to the prediction of each leaf trait. VIP is the weighted sum of squares of the PLSR-weights, with the weights calculated from the amount of variance from the response variable explained by each PLS component (Wold 1994).

*Spectral dissimilarities among plant growth forms*

We evaluated spectral dissimilarity between plant growth forms using the Bhattacharyya distance (Bhattacharyya, 1943; Kailath, 1967) (Eqn. 1). This metric quantifies the integrated difference between two individuals of different growth forms over the full spectral range, identifying the wavelengths with the greatest discriminatory power. This metric has been successfully applied for the recognition of differences between species (Baldeck & Asner, 2014), and plants with different growing habits (Sánchez-Azofeifa *et al*., 2009) .

$$B = \frac{1}{8}(\mu_i - \mu_j)^T \sum^{-1} (\mu_i - \mu_j) + \frac{1}{2} ln(\frac{|\Sigma|}{\sqrt{|\Sigma_i| - |\Sigma_j|}}) \quad \textbf{Eqn 1}$$

where $\mu_i$ and $\mu_j$ are the mean values across all spectral bands for species i and j, Σi and Σj are the covariance matrices for each individual, and Σ is the pooled covariance matrix. $B$ is the Bhattacharyya distance.

We used a randomized approach to estimate the distribution of $B$ by randomly sampling 1000 pairs of spectra for each combination of growth forms ("Woody" x "Grass"; "Woody" x "Forb" and "Forb" x "Grass"), and then computing the average and spread (standard deviation) of the 1000 calculated pairwise distances for each combination.

**Results**

*Leaf trait variability*

Differences in LMA and LDMC were subtle among growth forms (Fig. 4.1 and Table 4.1). Overall LDMC values varied between 0.12 and 0.67 g/g, with a similar range of variation between growth forms (Fig. 4.1 and Table 4.1), with the largest LDMC range observed for "*Grass"* (0.12 – 0.67 g/g) and the smallest for "*Forb"* (0.12 – 0.61 g/g). Average LDMC values per growth form were lowest for "*Forb"* (mean = 0.34; sd =± 0.09 g/g), followed by "*Woody"* (0.38 ± 0.09 g/g) and "*Grass"* (0.41 ± 0.09 g/g) (Fig. 4.1). The total measured range of LMA values was 31.8 to 621 g/m$^2$. Average LMA values by growth form were lowest for "*Grass"* (137.9 ± 78.7 g/m$^2$), and similar for the other two growth forms (168.7 ± 77.8 g/m$^2$ for "*Forb"*, 167.9± 73.9 g/m$^2$ for "*Woody"*) (Fig. 4.1). Grasses had the smallest LMA range (32.8 – 529 g/m$^2$), and woody plants the largest LMA range (41.9 – 621 g/m$^2$).

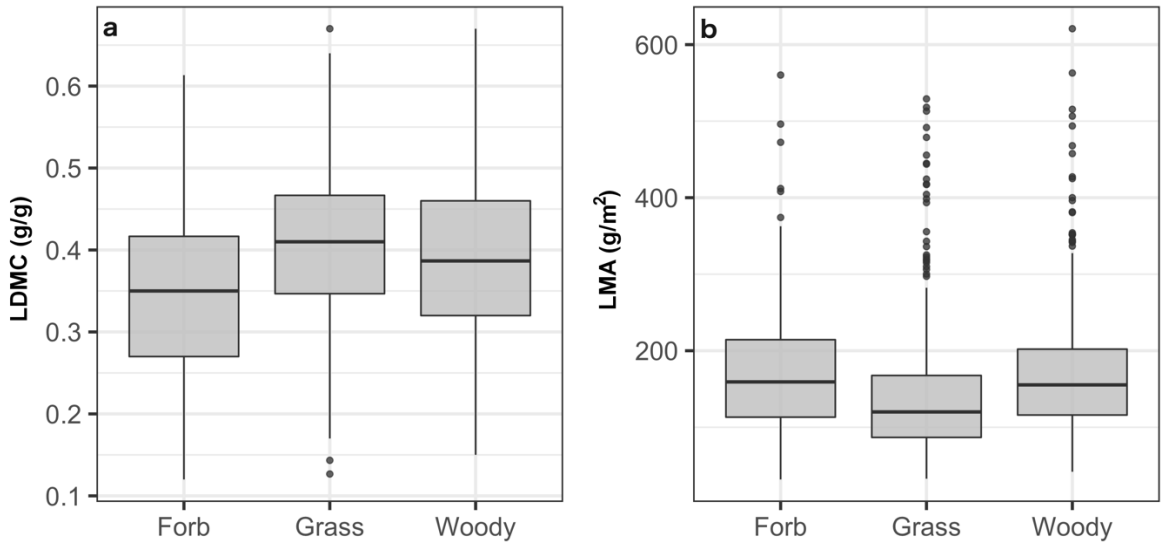


***Figure 4.1:*** Variability of leaf functional traits measured for 1648 individuals of *campo rupestre* vegetation at Serra do Cipó, Southeastern Espinhaço range, Brazil, including 369 individuals of the "*Forb*" class, 564 individuals of the "*Grass*" class, and 715 individuals of the "*Woody*" class. **(a)** Leaf dry matter content (LDMC); **(b)** leaf mass per area (LMA).

*PLSR modeling*

Both leaf traits were predicted with high accuracy from reflectance measurements of fresh leaf material, and no models showed signs of overfitting (Table 4.1). Overall, LDMC was estimated from leaf reflectance with higher accuracy than LMA.

***Table 4-1:*** Results of the partial least-squares regression (PLSR) modeling and cross-validation for each leaf trait, showing the number of samples and range of trait variation for the global data set (all) and per growth form. RMSECV is the root mean square error (RMSE) of the cross-validation procedure with train data set; RMSE is the measured error using the test data; mRMSE is the percentage error of each model in relation to the mean values (RMSE/mean); and the RMSE percentage (%RMSE) shows the error of each model as a percentage of the observed data range. All results are presented for the entire range of LMA and LDMC values ("All" class) and per growth form. "LMA < 300" represents the data set containing only LMA values bellow 300 g/m$^2$.

| *Growth form* | *Number of samples* | *Range of variation (min - max)* | *RMSECV* | *Final number of latent variables* | *RMSE* | *mRMSE (unitless)* | *%RMSE* |
|---|---|---|---|---|---|---|---|
| ***LDMC*** | | | | | | | |
| *ALL* | 1648 | 0.12-0.67 (g/g) | 0.052 (g/g) | 20 | 0.054 (g/g) | 0.14 | 9.83 % |
| *Grass* | 564 | 0.12-0.67 (g/g) | 0.063 (g/g) | 18 | 0.059 (g/g) | 0.14 | 10.92 % |
| *Forb* | 369 | 0.12-0.61 (g/g) | 0.046 (g/g) | 18 | 0.054 (g/g) | 0.16 | 11.08 |
| *Woody* | 715 | 0.15-0.67 (g/g) | 0.047 (g/g) | 17 | 0.044 (g/g) | 0.11 | 8.63 |
| ***LMA*** | | | | | | | |
| *ALL* | 1648 | 31.80 - 620.81 (g/m$^2$) | 44.72 (g/m$^2$) | 17 | 50.58 (g/m$^2$) | 0.32 | 8.58 % |
| *Grass* | 564 | 32.77 - 529.12 (g/m$^2$) | 45.61 (g/m$^2$) | 18 | 43.31 (g/m$^2$) | 0.31 | 8.72 % |
| *Forb* | 369 | 31.80 - 560.29 (g/m$^2$) | 47.75 (g/m$^2$) | 14 | 51.83 (g/m$^2$) | 0.31 | 10.46 % |
| *Woody* | 715 | 41.89 - 620.81 (g/m$^2$) | 45.40 (g/m$^2$) | 20 | 37.13 (g/m$^2$) | 0.22 | 6.41 % |
| ***LMA < 300*** | | | | | | | |
| *ALL* | 1571 | 31.80 – 298.94 (g/m$^2$) | 31.77 (g/m$^2$) | 20 | 31.215 (g/m$^2$) | 0.21 | 11.68 % |
| *Grass* | 539 | 32.77 - 297.23 (g/m$^2$) | 33.69 (g/m$^2$) | 20 | 36.42 (g/m$^2$) | 0.28 | 14.24 % |
| *Forb* | 337 | 31.80 - 298.94 (g/m$^2$) | 31.28 (g/m$^2$) | 20 | 34.83 (g/m$^2$) | 0.21 | 13.98 % |
| *Woody* | 695 | 41.89 - 298.52 (g/m$^2$) | 27.87 (g/m$^2$) | 17 | 26.14 (g/m$^2$) | 0.16 | 10.26 % |

Our PLSR spectral model had an overall error (RMSE) of 0.054 g/g, c.a. 9 % of the range of LDMC values of the entire dataset (Table 4.1 and Fig. 4.2). Among growth-form restricted models, accuracy was higher for *Woody* plants, with %RMSE of c.a. 8% (RMSE = 0.044 g/g). The "*Grass*" and "*Forb"* models yielded similar error rates; although "*Grass*" models had higher overall error (RMSE = 0.059 g/g) than "*Forb"* (RMSE = 0.054 g/g), these errors represented

lower percentages of the mean class value ("*Grass*" mRMSE = 0.14; "*Forb*" mRMSE = 0.16) and %RMSE considering the full range of values ("*Grass*" %RMSE = 10.92%; "*Forb*" %RMSE = 11.08%).

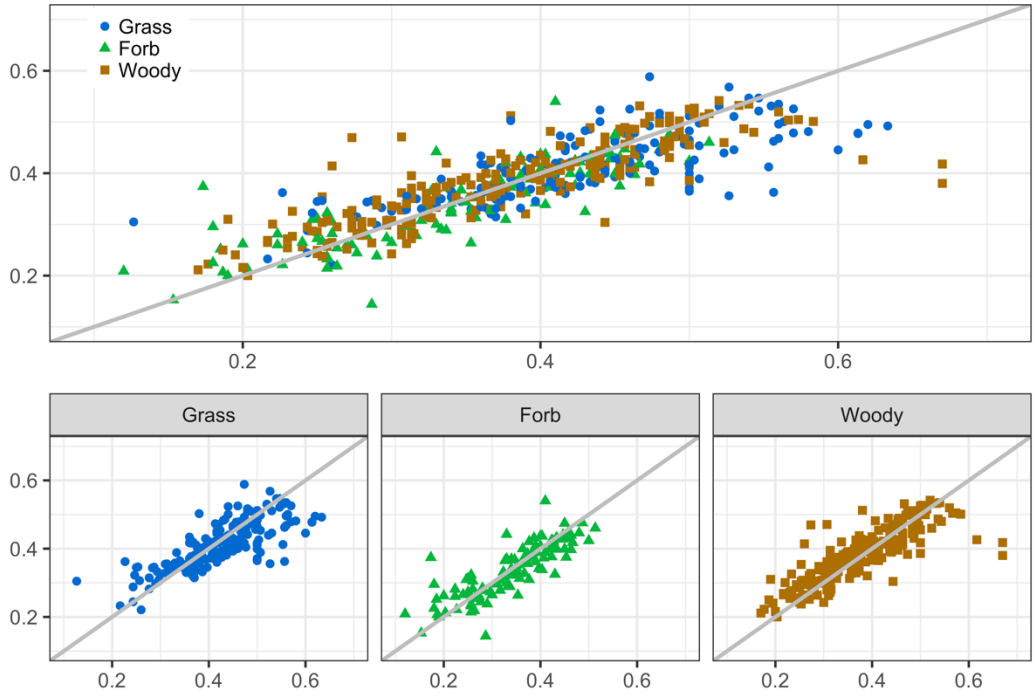


***Figure 4.2:*** Leaf dry matter content (LDMC) as observed and predicted from leaf level reflectance using partial least-squares regression (PLSR) models. The upper panel shows the prediction for the total range of LDMC values ("*All*" class). The lower panels show the relationship between observed and predicted LDMC values for each growth form. Symbols and colors indicate the growth form of each individual plant: blue dots as "*Grass*"; green triangles as "*Forb*", and brown squares as "*Woody*".

The PLSR model for LMA had the highest overall accuracy with a RMSE of 50.58 g/m$^2$, representing an error percentage around 8 % of the range of LMA values of the entire dataset (Table 4.1 and Fig. 4.3). Following the same pattern as LDMC, the restricted model with highest accuracy corresponded to the "*Woody*" data set, with a RMSE of 37.13 g/m$^2$ and error percentage

of c.a. 6 % of the range of values within the class. The lowest accuracy was yielded by the "*Forb*" restricted model, with RMSE of 51.83 g/m², ca. 10 % of the "*Forb*" LMA value range.

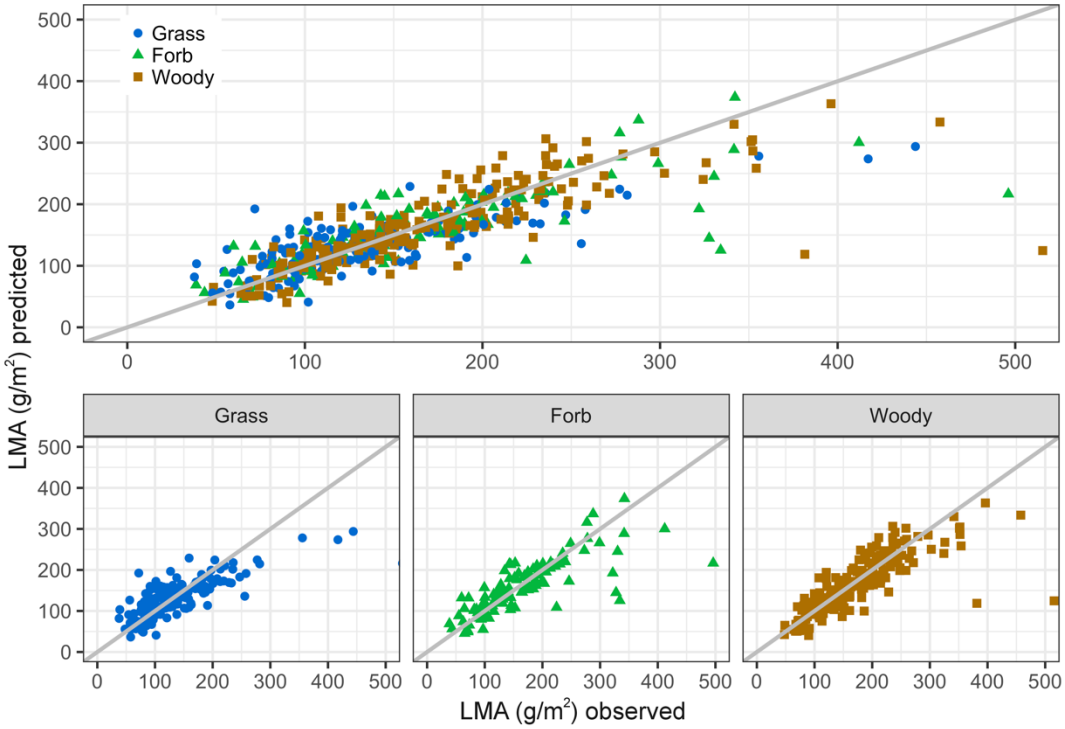


***Figure 4.3:*** Partial least-squares regression (PLSR) results for observed vs. predicted leaf mass per area (LMA). The upper panel shows the prediction for the total range of LMA values ("*All*" class). The lower panels show the relationship between observed and predicted LMA values for each growth form. Symbols and colors indicate the growth form of each individual plant: blue dots as "*Grass*"; green triangles as "*Forb*", and brown squares as "*Woody*".

Overall, VIP values had consistent patterns across the spectrum, with notable variations corresponding to the contribution of particular wavelengths for leaf traits (Fig. 4.4). For LDMC, the wavelength region centered in 1400 nm corresponded the highest VIP value, but the wavelengths in the VIS (550 to 650 nm), red-edge (700-750 nm), and SWIR (around 1700 and 1900 nm) were also important (Fig. 4.4a). The most important spectral region for LMA was the red-edge (700-750 nm), followed by the VIS region at the wavelength centered in 550 nm (Fig.

4.4b). The VIP metric also varied in the position of peak importance among growth forms for both traits, but specially for LMA, where a SWIR spectral region from 1900 to 2100 nm stood out for "*Grass*" form (Fig. 4.4b). The red-edge (700-750 nm) was the spectral region with the closest agreement of VIP values among growth forms for both leaf traits.

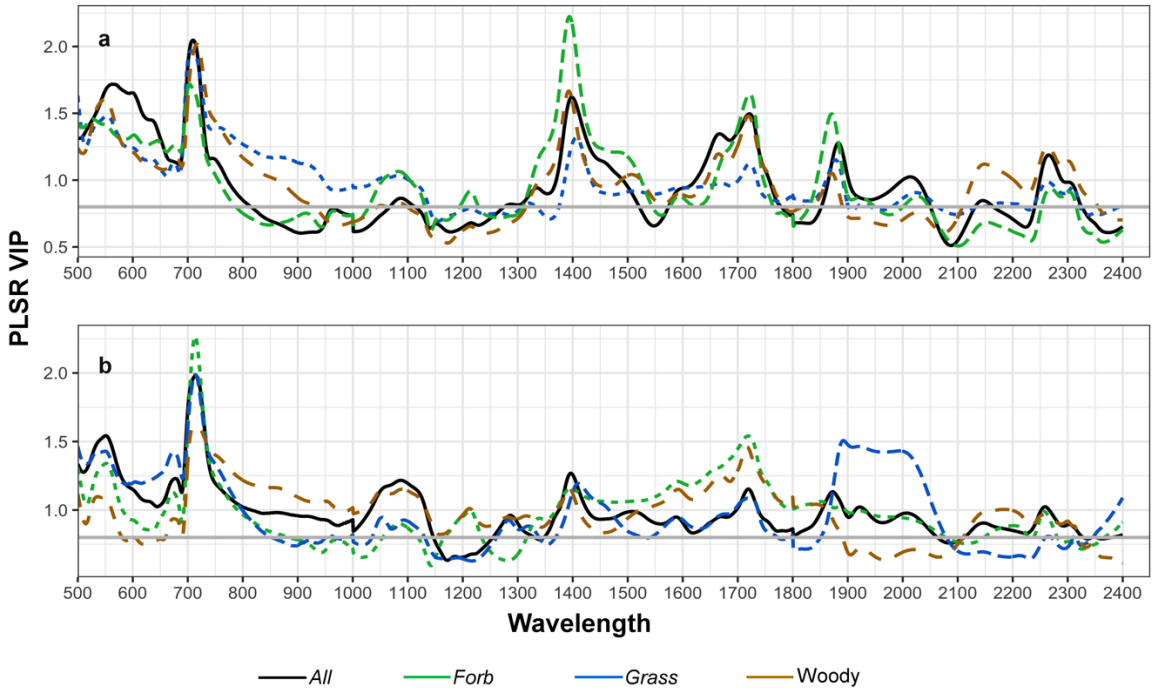


***Figure 4.4:*** Partial Least Squares Regression (PLSR) variable importance of prediction (VIP) plotted by wavelength for **(a)** leaf dry matter content (LDMC), and **(b)** leaf mass per area (LMA), measured for *campo rupestre* plants at Serra do Cipó, Southern Espinhaço Range, Brazil. Colored lines represent the three growth forms investigated in this study with the green dashed line representing "*Forb*", the blue dashed line representing "*Grass*" and the brown dashed line representing "*Woody*". The black solid line represents "*All*" growth forms combined.

We observed a loss of predictive power for all PLSR models for high LMA values, *i.e.* above 300 g/m$^2$ (Fig. 4.3). To quantify the influence of this loss, we refitted the PLSR models using only LMA values between 0 and 300 g/m$^2$ (Table 4.1), matching the range of LMA values

usually observed for tropical (Asner *et al*., 2011a,b) and temperate (Serbin *et al*., 2014) forested systems, which are also typically used in radiative transfer models (Féret & Asner, 2011) and thus most frequently reported in the literature of leaf trait spectroscopy. These restricted-range PLSR models had higher accuracies (Fig. 4.5), yielding a 1.5-fold decrease in overall error (RMSE = 31.21 g/m$^2$) and overall mRMSE of 0.21 in LMA values (Table 1 – LMA < 300 g/m$^2$). This decrease was also uniformly observed for models of each growth form. The highest improvement was found for the "*Forb"* class, with a restricted range mRMSE of 0.21, down from mRMSE= 0.31 from the full range model (Table 4.1 and Fig. 4.5). The lowest performance of the restricted model was found for "*Grass"* (mRMSE= 0.28), with 1-fold change improvement.

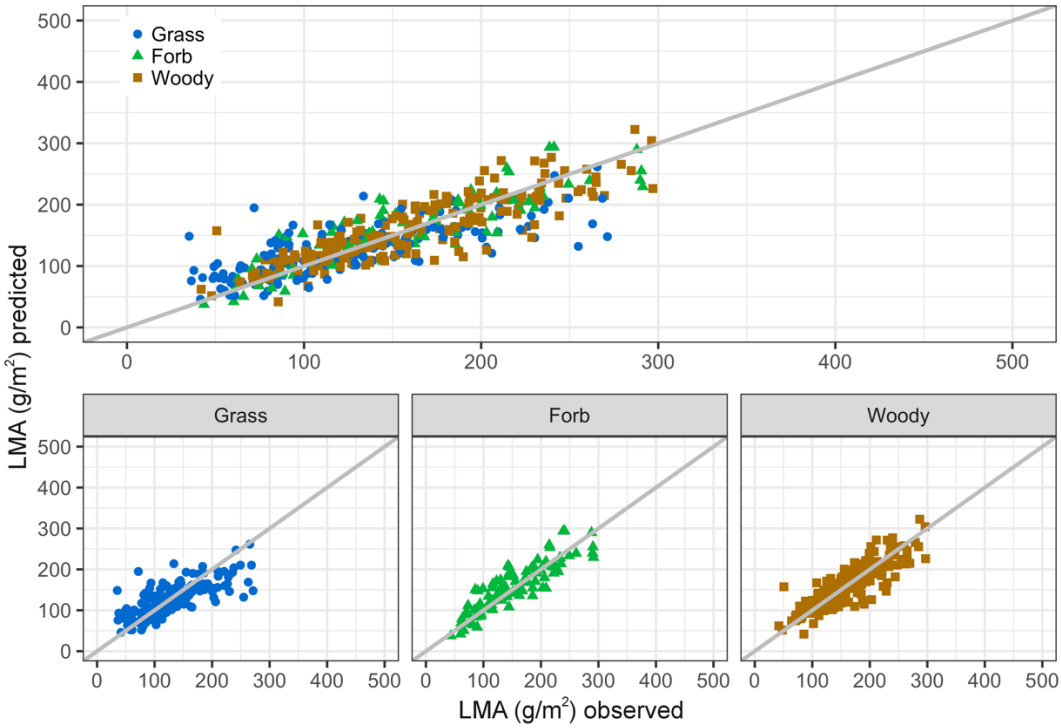


***Figure 4.5:*** Partial least-squares regression (PLSR) results for observed vs. predicted leaf mass per area (LMA), with values restricted to 0 - 300 g/m$^2$. The upper panel shows the prediction for the total range of LMA values ("*All*" class). The lower panels show the relationship between observed and predicted LMA values for each growth form class. Symbols and colors indicate the growth form.

*Leaf reflectance spectra dissimilarity among growth forms*

Overall, full leaf reflectance spectra were able to track the expected ecophysiological changes in leaves from different growth forms (Fig. 4.6a). Reflectance measurements showed a reduction in reflectance along VIS wavelengths and a steep red-edge transition around 700 nm, where variance in reflectance of all plants was very low. Minor water absorption features were visible around 1000 and 1200 nm, while major absorption features stood out around 1400 and 1900 nm for all the three growth forms. Comparisons among growth forms showed that "*Woody"* plants had the lowest reflectance on the VIS range and the highest reflectance on the NIR region, while the average reflectance spectra of "*Grass*" plants had the opposite pattern, with the highest reflectance in the VIS and lowest in the NIR regions, while also having the highest reflectance on the SWIR. "*Forbs*" had intermediate reflectance values, with a spectral profile closer to "*Grass*" in the VIS region, while more similar to "*Woody"* in the SWIR.

Bhattacharrya distances (*B*) indicated a greater degree of dissimilarity between the leaf reflectance spectra of "*Woody"* and "*Grass"* plants at the VIS (400 – 700 nm), around 1500 nm, and highest at the edge of the SWIR (>= 1900 nm) (Fig. 4.6c), in comparison to other pairwise interactions (Fig. 4.6b; 4.6d). As "*Forb*" is an intermediate group between "*Grass"* and "*Woody"* plants, the dissimilarity between these pairs of interactions was subtler. The 1450 nm wavelength feature and the SWIR region yielded the highest degree of separability between "*Forb*" and "*Grass"* (Fig. 4.6b), while "*Forb*" and "*Woody"* were the most spectrally similar growth forms, as indicated by the smallest values of *B*, with the VIS region having the highest degree of separability (Fig. 4.6d).

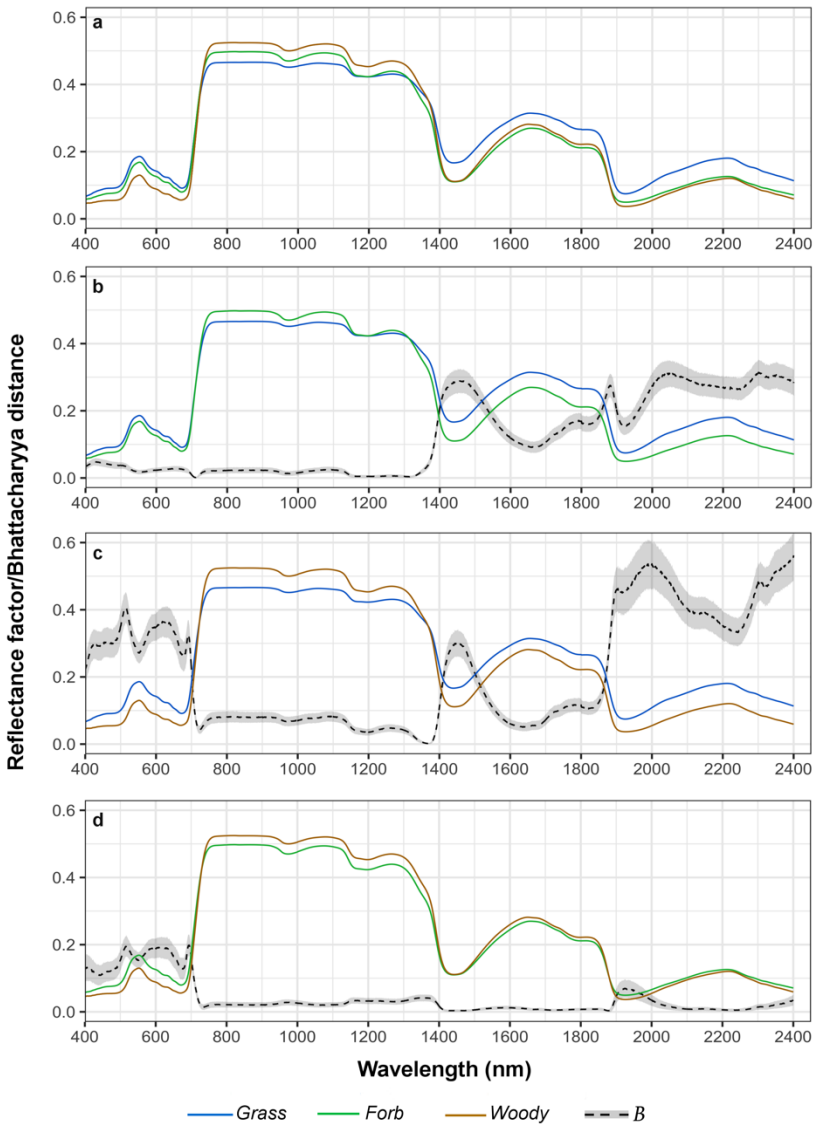


***Figure 4.6:*** Comparison of leaf reflectance spectral averages per growth form **(a)**, and the spectral dissimilarity (Bhattacharyya distance) among the different growth forms across the full wavelength range (400 – 2400 nm): **(b)** "*Forb*" and "*Grass*", **(c)** "*Woody*" and "*Grass*" and **(d)** "*Woody*" and "*Forb*". The peaks observed on the Bhattacharyya index (B, dashed line and the gray shaded area represents ± 1 standard deviation) indicate the spectral bands with highest dissimilarities among growth forms.

## Discussion

Overall, our results indicate that leaf reflectance spectra quantitatively captured leaf morphological traits in seasonal tropical vegetation, and that main growth forms can be distinguished solely based on leaf reflectance. Our findings support the suggested ability of spectroscopy to predict leaf traits, here explored among growth forms. However, more efforts are needed to address possible methodological shortcomings when predicting high LMA values, usually not observed in forest data sets. The power of spectroscopy to capture the ecophysiological correspondence of the LES traits with the environment is highlighted by the wavelengths selected in our modelling approach. Overall, spectroscopy was able to discriminate woody, herbaceous, and grass plants, and the full- spectrum profile allowed us to draw insights on leaf anatomy variation, adding more ecological information than the LES traits *per se*. We thus endorse a shift from the view of spectroscopy as a "tool" to measure traits indirectly, towards a "*spectral phenotype*" characterization based on the complex set of ecophysiological properties that can be summarized by the full leaf reflectance spectrum.

### *Ecophysiological mechanisms linking leaf traits and leaf reflectance*

Leaf functional traits did not vary substantially among growth forms at Serra do Cipó. Plant growth form and leaf structure are expected to be related, and groups with distinct leaf anatomical syndromes can be identified. Usually, plants from the cerrado ground-layer are described with thin, mesomorphic leaves (*i.e*, low LMA and LDMC), since this stratum is completely destroyed during the passage of fire (Rossatto & Franco, 2017). Instead, woody plants have thick and rigid, sclerophyllous leaves, with large amounts of mechanical tissue, palisade parenchyma, and a well-developed vascular system (Rossatto et al., 2015; Rossatto &

Franco, 2017), normally related to seasonal water deficit, nutrient-poor soils, high irradiance and herbivory rates (Givnish, 1988; Coley, 1988; Rossatto *et al*., 2015). Contrary to expectations, at Serra do Cipó, LMA values found for grasses, eudicots herbs, and sub-shrubs are comparable to those found for woody plants. This similarity can be linked to leaf persistence during drought conditions (Negreiros *et al*., 2014; Brum *et al*., 2017), with plants from abundant families (e.g., *Velloziaceae,* here classified as Forb, and *Cyperaceae,* here classified as grass, having species with desiccation-tolerant strategies and dormancy during the dry season (resurrection plants) (Oliveira *et al*., 2005; Alcantara *et al*., 2015).

Considering the lack of variation in leaf traits, our results reinforce the potential of spectroscopic methods to quantitatively describe structural foliar properties (see Kokaly *et al*., 2009 for a review). Our general models were able to successfully explain variations related to leaf strategy without bias towards any growth form, going one step further towards the development of generalized global models. Still, the restricted PLSR models had overall better performances for woody plants than other growth forms for both measured traits. To put our modeling results in perspective, our error rates for woody species (%RMSE 7% - 15%) are comparable to rates observed for tropical (Asner *et al*., 2011a,b ; %RMSE = 5.9%) and temperate forests (Serbin *et al*., 2014; %RMSE= 10.1%). To the best of our knowledge, there is only one study addressing spectroscopy of LMA and LDMC from "herbaceous" plants, with a focus on grasses (Roelofsen *et al*., 2014).

Unlike vegetation indexes commonly used, the PLSR approach captures the simultaneous contribution of several important absorption and scattering properties of leaves across the spectrum, as different wavelengths are sensitive to specific leaf structure and/or pigmentation

characteristics. The spectral regions selected for predicting LDMC were conservative among growth forms, and can be associated with a strong water absorption feature found at 1400 nm (Curran, 1989; Kokaly *et al*., 2009) and the red-edge inflection position (Horler *et al*., 1983) centered at 740 nm. Surprisingly, the red-edge was also the most important spectral region to predict LMA for seasonally dry vegetation, despite the SWIR being usually reported as the most important region of the spectrum for this trait (Asner *et al*., 2011b). Roelofsen *et al*. (2014) have also found the VIS region to be the most important for SLA ($LMA^{-1}$) of grasses. Correlations between reflectance and chemical concentration in some wavebands may not be fully explicable in terms of elemental bonds, and it is usually the result of a strong interaction among several leaf components (Curran, 1989). The red-edge region is known for being strongly related to chlorophyll content, but nitrogen, lignin, and cellulose concentrations can also be correlated with reflectance at the ~650 nm range (Curran *et al*., 2001). This is also consistent with the link between LMA and plant investment in chemical compounds distributed throughout the leaf mesophyll, which strongly affect leaf thickness and mass, and is also broadly correlated with nitrogen content (Poorter *et al*., 2009; Asner *et al*., 2011b). Therefore, although unexpected, we do not consider the importance of the red-edge to predict LMA a spurious correlation, and this interrelation can indicate structural limitations to photosynthesis, as a result of increased LMA (Niinemets, 1999).

Although our empirical models provided good estimates of both leaf traits, it underestimated LMA values above 300 $g/m^2$. Trees usually have LMA values up to 350 $g/m^2$, and most of the literature on empirical and radiative transfer models has tested the ability of spectroscopy to quantify LMA up to this value (Asner *et al*., 2009, 2011b; Cheng *et al*., 2014; Serbin *et al*., 2014; Asner *et al*., 2014a; Feilhauer *et al*., 2015; Doughty *et al*., 2017). The global range of LMA

variation is about two orders of magnitude (14 -1515 g/m$^2$; Glopnet data – Wright *et al*., 2004), and most studies of forest systems capture only c.a. 20% of this range, while our dataset covers c.a. 39% of the LMA worldwide variance. When we refitted our PLSR models constraining LMA values up to 300 g/m$^2$, our predictive power improved considerably for all models (Table 1 and Fig. 5), and specially for eudicot herbs and sub-shrubs. Two key implications emerge from this result: 1) spectroscopy may not be sensitive to variations of high LMA values (*i.e*., it has a saturation point); and/or 2) the PLSR method may not be able to predict large LMA variations. We highlight this result as an important direction for future studies, to increase efforts in sampling leaf spectra for seasonally dry and dry vegetation sites, high light environments (*i.e*., high LMA), and plants with contrasting resource use strategies, to better characterize the sensitivity of leaf spectroscopy and the feasibility of developing general, globally applicable methods for spectral LMA quantification.

*Further insights from full reflectance spectra on leaf phenotype characterization*

Despite sharing very similar morphological trait values, *campo rupestre* growth forms could be distinguished based solely on leaf reflectance spectra. Our findings indicate that there are significant differences in pigment levels, structural tissue, and consequently optical properties between growth forms that the key LES traits did not capture. Leaf carbon gain is one element of the larger life-history of a plant and not all plants adopt a strategy favoring net carbon gain over other fitness components (Messier *et al*., 2017b). Over commonly measured traits, leaf spectra have the advantage of incorporating more of the total variation associated with leaf chemistry, anatomy and morphology, including variations that are difficult to measure or may be of unrecognized importance (Schweiger *et al*., 2018).

The potential of using leaf reflectance to discriminate growth forms has been previously shown (Knapp & Carter, 1998; Sánchez-Azofeifa *et al*., 2009; Asner *et al*., 2011a; Ball *et al*., 2015), but ours is the first study in the seasonally dry tropics showing the use of full range spectroscopy as an integrative representation of the leaf phenotype among growth forms. This allow us to draw insights on leaf anatomy and growth/allocation strategies, as follows. All growth forms had a substantial amount of mesophyll tissue, indicated by the high reflectance values along the NIR, but the mesophyll of trees and shrubs were generally thicker in comparison to other growth forms. This can be grasped from the fact that reflectance will increase when the amount of scattering structures per unit thickness increases (Knapp & Carter, 1998; Ustin & Gamon, 2010). The fact that NIR reflectance values from grasses were consistently lower than other growth forms indicate that the lack of LMA variation is not a consequence of leaf thickness, which is highly correlated with NIR wavelengths (Knapp & Carter, 1998), but most likely related to variations in leaf area (Streher et al, unpublished results from the same dataset). Woody plants and grasses showed reflectance profiles with the largest differences in magnitude, and spectroscopy was able to capture the expected patterns: grasses had the highest VIS and lowest NIR reflectance, while woody plants had the opposite profile. The predominance of C4 grasses in savannas suggests that grasses should have higher photosynthetic rates per unit of leaf area in comparison with other growth forms (Rossatto *et al*., 2015). The SWIR was the most important region to discriminate woody plants from grasses, suggesting differences in structural components, water content and water-use strategies (Curran, 1989) between these two growth forms, not captured by LMA and LDMC.

Eudicot herbs and sub-shrubs represented an intermediate growth form between woody plants and grasses. On one hand, they were differentiated from grasses by the amount of leaf water and

structural properties absorbing along the SWIR, in contrast to the subtle differences found in the VIS from woody plants. The lack of proper spectral discrimination can be due to our inclusion of herbs and sub-shrubs within the same growth form, to improve sample sizes. Sub-shrubs are unique since they have leaf anatomies similar to herbs (Rossatto *et al*., 2015), but are functionally clustered with trees and shrubs (Rossatto & Franco, 2017). This implies that even though they are on an evolutionary trajectory of ecological convergence with herbaceous plants, they are not phylogenetically independent of the tree lineages from which they evolved (Simon *et al*., 2009; Rossatto & Franco, 2017). Although we lack the data to properly test the phylogenetic structure of foliar traits, the strong spectral similarity in the SWIR of woody and sub-shrubs (and also herbs) may suggest an evolutionary signal, as the SWIR tends to be phylogenetically conserved (McManus *et al*., 2016).

*Concluding remarks and emerging opportunities*

Our work expands previous studies of spectral-leaf traits correlations by considering the full-electromagnetic spectra as representations of leaf phenotypes, instead of merely a proxy used to predict leaf functional traits at canopy scales (Asner & Martin, 2016). The scientific advantage of using the full spectra profile rests in a standardized unique measurement that adds more information about life-history strategies, by integrating functional, phylogenetic and taxonomic diversity at scales on which natural selection occurs (Cavender-Bares *et al*., 2017; Schweiger *et al*., 2018). Despite its high plant diversity, vegetation at Serra do Cipó is generally sclerophyllous with leaf traits associated to drought survival, but the shape and magnitude of the full reflectance spectrum provided important information on leaf ecophysiology, indicating that an overall *leaf spectral phenotype* can be derived from leaf reflectance measurements.

We also accurately predicted LMA and LDMC for seasonally dry tropical plants from spectroscopy, even though these traits had little variation among growth forms, reinforcing the ability of leaf spectroscopy to predict morphological leaf traits reliably. However, we also found an important limitation in using PLSR methods to predict high LMA values (> 300 $g/m^2$), resulting in underestimated values over ranges that have not been observed for forests to date. More work is needed to determine if these are methodological shortcomings of PLSR and/or biophysical limitations of spectroscopy. Furthermore, our work contributes not only to increase ecophysiological trait sampling effort at the individual level, helping to fill the gap in local trait observations for tropical regions (Schimel *et al*., 2015; Jetz *et al*., 2016), but also with a standard for spectroscopy measurements of non-forested, highly diversity tropical ecosystem.

Ideally, we should seek to scale our individual spectra-phenotype and trait predictions to the community level, using next-generation airborne (especially UAV-based) and orbital hyperspectral data. Scaling up processes from local studies to larger regions remains an urgent challenge for all environmental sciences (Schimel *et al*., 2015; Asner & Martin, 2016; Asner *et al*., 2016b). Spectroscopy offers a powerful way to conduct spatially explicit assessments of canopy trait distribution from leaves to ecosystems, as spectral data can be easily sampled at multiple scales (Asner & Martin, 2016; Cavender-Bares *et al*., 2017). An important next step for spectral ecology is to fully explore the differences in full-spectra among different growth forms and across biomes, to better understand the ecophysiological differences between individuals, species and higher taxonomic levels, especially at scales where the LES might not be tightly coupled with resource-use strategies (Messier *et al*., 2010; Anderegg *et al*., 2018).

We thus highlight three directions for further work on plant spectroscopic modeling. First, spectroscopy offers a powerful tool for acquiring trait data across scales, and ecological linkages between LMA or LDMC and life-history strategies encourages these traits as starting points for modeling plant communities using spectroscopy data. Beyond that, to fully understand the sensitivity and potential of leaf reflectance for plant ecology, researchers should focus on sampling vegetations with contrasting life-history strategies and leaf longevities, from forests to grasslands and across larger seasonality gradients, seeking reliable and standardized data and methods that can support global models relating foliar traits to leaf spectroscopy. Third, studies assessing the effects of scale-dependence on spectroscopy analysis remains a priority, and we suggest approaches that expand beyond the usual functional trait predictions towards the integrative analyses of “leaf spectral phenotypes”.

# 5. Exploring leaf spectra and functional association in tropical communities: implications for remote sensing at the intersection of ecology and evolution

**Abstract**

Spectral diversity is an emerging component of plant biodiversity that integrates functional variation within and across species, but the spectral - functional diversity linkage across the tree of life remains poorly tested. Here we examine the extent of intraspecific variation in functional and optical traits and its influence on the correlation strength and variation structure of leaf functional traits and leaf reflectance in tropical plants, from the seasonally dry and hyperdiverse *campo rupestre* system. We analyzed LMA, LDMC and leaf reflectance spectra in 231 plants, from 31 species comprising different growth forms, and quantified intraspecific variation as the coefficient of variation of each trait measured. We also evaluated the coupling between functional and spectral diversity with Procrustes analyses and their dissimilarities in variance structure across biological scales. We find that interspecific variation exceeded intraspecific variation for all traits, but intraspecific variation was species-specific, and leaf-age may be playing an important role as a process generating intraspecific variability in spectral diversity. With moderate positive correlation, the relationship between spectral and functional traits was not constant across scales and the variance was not equally structured, but all traits varied primarily at the family level, indicating that both traits and spectra are evolutionarily conserved. We provide evidence that spectral diversity is linked to functional and phylogenetic diversity in a tropical *campo rupestre* vegetation.

Keywords: spectral diversity, campo rupestre, intraspecific variation, interspecific variation, variance structure

## Introduction

Trait-based ecology has gained an increasingly important role in explaining the rules by which species assemble into communities. The trait-based approach rests over the basic assumption that traits should vary more among (interspecific) than within species (intraspecific), and consequently existing intraspecific trait variation would not obscure the broader trends and patterns (McGill *et al.*, 2006). One of the reasons for the widespread focus on interspecific variation are the practical constraints of time and space: sampling a smaller number of individuals allows researchers to cover a wider range of species in their studies, theoretically achieving a greater degree of generality (Shipley *et al.*, 2016). However, there is a growing body of evidence showing that intraspecific trait variability can be an ecologically important source of trait variation in plant communities, renewing the interest in individual variability (Messier et al. 2010, Jung et al. 2010, Albert et al. 2010, Violle et al. 2012, Siefert 2012, Auger and Shipley 2013, Siefert et al. 2015, Funk et al. 2017). Individuals are the "working unit" on which natural selection acts, and not species *per se* (Clark *et al.*, 2011), and individual-level variation is important for understanding the mechanisms behind species coexistence (Chesson, 2018). Theoretically, phenotypic trait variation can lead to higher fitness for one individual, whereas another individual from the same species with different trait values may be unable to survive. Therefore, by ignoring intraspecific trait variation we disregard more than a century of research in evolutionary biology (Laughlin *et al.*, 2012; Violle *et al.*, 2012).

Leaf traits have been extensively used in trait based ecology, particularly leaf mass per area (LMA) and leaf dry matter content (LDMC), because they are easy to measure and have known links to ecosystems functioning, plant life-history strategy and demography (Wright *et al.*, 2004; Reich, 2014). Differences in leaf traits are the basis for differences in leaf optical properties.

Recently, leaf spectral reflectance - the reflected radiation along the electromagnetic spectrum – has emerged as an important component of biodiversity, not only as a proxy for leaf traits, but also adding unprecedented information about plant ecology by integrating chemical, phenological and morphological adaptations to environmental conditions, biotic interactions and phylogenetic signal (Cavender-Bares *et al.*, 2017; Schweiger *et al.*, 2018). Therefore, spectral diversity provides an integrated measure of the variability of leaf phenotypes within plant communities.

We can use "optical diversity", sometimes called "spectral diversity" (Palmer *et al.*, 2002; Ustin & Gamon, 2010) to describe the variation in spectral reflectance detected by optical remote sensing. The optical diversity hypothesis suggests that species within a plant community have distinct spectral spaces due to their unique chemical, anatomical and morphological characteristics (Palmer *et al.*, 2002; Wang *et al.*, 2018b; Schweiger *et al.*, 2018). Still, the main assumption at the base of the optical diversity hypothesis is the same as for classical niche theory, and more recently, trait-based ecology: interspecific variability in spectral reflectance exceeds intraspecific variability.

Biological diversity can be observed at many organizational levels: within individuals, among individuals, within populations, among populations, within species, among species, within lineages (clades), and among lineages, at increasing hierarchical levels (Cavender-Bares *et al.*, 2016). The degree of trait divergence between biological taxa is expected to be proportional to the amount of time they have diverged from a common ancestor, and as a consequence, distantly related taxa are expected to be phenotypically more dissimilar (Cavender-Bares, 2018). Spectral information can also be considered another form of trait, becoming increasingly dissimilar among groups with greater evolutionary distance (Cavender-Bares *et al.*, 2016), and may be

hypothesized to reveal evolutionary relatedness among organisms both within and across lineages (McManus *et al.*, 2016).

The relationship between phylogeny and leaf spectra is only now starting to be explored (Asner & Martin, 2011; Asner *et al.*, 2014a; Cavender-Bares *et al.*, 2016; McManus *et al.*, 2016; Schweiger *et al.*, 2018). Recent studies have shown that leaf spectral data is able to differentiate populations within a species (Cavender-Bares *et al.*, 2016) and that spectral similarity is indeed influenced by the phylogenetic relatedness of species (McManus *et al.*, 2016). A strong taxonomic organization of leaf traits derived from leaf reflectance spectra has also been found in humid tropical forests (Asner & Martin, 2011; Asner *et al.*, 2014b,a), with plant genera, and often species, as unique contributors to the chemical and spectral diversity of any given rainforest (Asner & Martin, 2016). Nonetheless, the role of intraspecific spectral variation generating the overall observed spectral diversity remains uncertain. Usually, interspecific spectral variability is greater than intraspecific spectral variability (Clark et al. 2005, Asner et al. 2011, 2014, Ferreira et al. 2013, 2016, Schweiger et al. 2018, Wang et al. 2018), but within-taxa variation in leaf-level properties still poses a key challenge to differentiate taxa at any level using spectral information, as a small number of independent variables are responsible for characterizing the spectral profile (Price, 1994).

The term spectral variability is usually applied when attempting to understand the potential and caveats of spectroscopic (hyperspectral) sensors to discriminate species at a given spatial scale (Wang *et al.*, 2018b). Here, we apply the concept to leaf level reflectance differences found across the tree-of life, aiming to determine the degree to which spectra and traits are phylogenetically organized. For that, we assessed the role of taxonomy as well as within- (intraspecific) and among-species (interspecific) variation influencing the spectral and functional

diversity. We are motivated by existing parallels with trait-based ecological patterns observed across biological scales. If traits and/or spectra are plastic, then biological diversity may be decoupled from functional and/or spectral diversity. Alternatively, if a phylogenetic organization of spectra exists, then spectral diversity may express functional and taxonomic diversity and vice versa. We thus address the following questions: (i) how variable are functional and optical traits within species (intraspecific)? (ii) To what extent are dissimilarities in leaf spectra correlated with dissimilarities in functional traits across biological scales? and (iii) Is the hierarchical variance structure of optical and functional traits similar across taxonomic scales? In combination, these questions address the degree to which spectral diversity is indeed part of biological diversity.

**Methods**

We sampled 231 individual plants from 31 species, belonging to 26 genera, and 14 different families (Supplementary information S1), at Serra do Cipó, in Southeastern Brazil. Serra do Cipó is a seasonally dry mountainous landscape hosting a megadiverse plant assemblage with more than 1800 plant species recorded within 200 $km^2$ (Alves *et al.*, 2014). Variation and turnover in vegetation structure and growth form occur naturally across the elevation gradient, where subtle changes from forest-*cerrado* (savanna) formations towards grassy-shrubby *campo rupestre* vegetation can be observed. Within each elevational band, there are also complex mosaics of vegetation types, associated with soil conditions mostly determined by local topography and microenvironmental aspects (Silveira *et al.*, 2016), that are often characterized by a high number of endemic plant species and lineages. The *campo rupestre* is considered, alongside the *fynbos* (Cape Floristic Region) and *kwongkan* (south-western Australia), as an example of the old climatically-buffered infertile landscapes (OCBILS) theory on which infertile soils and climatic

stability are the core explanations to the high floristic richness and endemism (Silveira *et al.*, 2016).

We selected mature plant individuals, and we sampled three sunlit and fully expanded leaves per individual, measuring leaf dry mass per area (LMA), leaf dry matter content (LDMC), and leaf-level reflectance spectra. LMA and LDMC were measured using the partial-rehydration method following protocols from Pérez-Harguindeguy et al. (2013). This method has been shown to not produce significant biases relative to the more labour-intensive full-rehydration method (Garnier *et al.*, 2001; Vaieretti *et al.*, 2007). We kept samples at ± 4 ºC in the dark, and measurements were taken between six to eight hours after harvesting the plants. Leaf reflectance spectra was acquired for the same leaves used to quantify LMA and LDMC, immediately after the determination of fresh mass, using the leaf probe accessory of the ASD FieldSpec 4 Standard spectroradiometer (Analytical Spectra Devices, ASD, Boulder, CO, USA). This instrument measures bidirectional reflectance along the optical spectrum, from 350 to 2500 nm. Spectra were calibrated for dark current and stray light, and referenced to a Spectralon white calibration disc approximately every 10 min. We followed the protocols and standards by Asner & Martin (2009), available at the Carnegie Spectranomics website (http:// spectranomics.ciw.edu).

*Data analyses*

Taxonomic patterns in spectral and trait data were examined with respect to the nested biological scales of family, genus, species, and individuals. To assess the overall importance of intraspecific variability on species spectral diversity (*question i*), we computed the coefficient of variation (CV) on a per band basis for each species spectra (Asner *et al.*, 2011b, 2014a; Wang *et al.*, 2018b). We also compared similarities among the shape and amplitude of the spectral signal

(Price, 1994) within each species and between species pairs (Castro-Esau *et al.*, 2006; Ferreira *et al.*, 2013, 2016). The two-spectral metrics described by Price (1994) enabled comparisons between pairs of spectra, as follows:

$$D = \left\{\frac{1}{\lambda_a - \lambda_b} \int_{\lambda_b}^{\lambda_a} [S_1(\lambda) - S_2(\lambda)] d^2\lambda \right. \quad \text{(Eq 1)}$$

$$\theta = \cos^{-1}\left[\frac{\int S_1(\lambda) - S_2(\lambda)\, d\lambda}{[\int S_1(\lambda)^2 d\lambda]^{1/2}\, [\int S_2(\lambda)^2 d\lambda]^{1/2}}\right] \quad \text{(Eq 2)}$$

*D* represents the difference in amplitude between two spectra and is computed as the root mean square difference between a pair of spectra (S1 and S2) averaged over the spectral range of interest ( $\lambda_a$ to $\lambda_b$). The metric $\theta$ determines the angle (in degrees) between two spectra and may be interpreted as the difference in shape between the pair of spectra, with amplitude dependence from brightness variations removed (given by the denominator in Eq. (2)). These are both distance metrics describing different aspects of the dissimilarity between a pair of spectra.

We tested the association between species spectral and functional spaces across biological scales using dissimilarity matrices, ordination methods and Procrustes analyses (*question ii*). The spectral dissimilarity matrix was based on the spectral distance metrics (*D* and $\theta$) described above, while the functional dissimilarity matrix was based on Euclidean distances between positions in multivariate trait space using *z*-score standardized traits (zero mean, unit variance). All intraspecific distances were calculated between individuals, and for higher taxonomic levels dissimilarities were calculated between the mean trait and mean per band spectral values of each level (e.g. species means, genus means, and families means) and each trait. Four dissimilarity

matrices were created for each morphological trait (LMA and LDMC), and for each spectral descriptor ($D$ and $\theta$), yielding four comparisons in each biological scale.

We assessed the overall degree of association between the dissimilarity matrices with Procrustes tests as implemented in the *vegan* R package (Oksanen *et al.*, 2018). In Procrustes analysis, a rotational-fit algorithm is used to minimize the sum of squared residuals between the pair of matrices under comparison (Gower, 1975). Prior to Procrustes analyses, we computed for each dataset a Principal Coordinates Analyses (PCoA) (Peres-Neto & Jackson, 2001). PCoA is identical to principal component analysis, except it uses a distance matrix as input. For each PCoA, we retained the same number of axes for each matrix combination such that the cumulative variance explained was greater than 50% for all datasets. The results were used as input for the Procrustes analysis. We then calculated the Procrustes associative value of the $r$ statistic ($r = 1 - m_{12}$, where $m_{12}$ is the sum of the squares of the residual differences) which indicates the strength of the match between the two ordinations, and can be interpreted similarly to the $r$ coefficient from the Pearson correlation (Peres-Neto & Jackson, 2001). The significance of $r$ was estimated using a permutation test with 9999 samples (PROTEST) (Peres-Neto & Jackson, 2001).

We compared the dissimilarity in variance structure across biological scales for the two leaf traits and the two-spectral metrics ($D$ and $\theta$), by conducting a nested permutational multivariate analysis of variance (PERMANOVA). PERMANOVA is best described as a geometric partitioning of multivariate variance in the space of a chosen dissimilarity measure, according to a given design, with $p$-values obtained using appropriate distribution-free permutation techniques (Anderson, 2017). The rationale behind it is that the variance structure reflects the amount of cumulative variation on each taxonomic scale, and if functional traits and spectra are actually

accounting for the same ecophysiological processes, they should vary similarly across biological levels. In the absence of a well-supported phylogenetic hypothesis, an approach based on taxon rank using nested ANOVA can provide a good approximation (Ricklefs, 2007; McGill, 2008). By apportioning the variance of trait dissimilarities between species within genera, between genera within families, and so on, one can place a trait on a spectrum between evolutionary conservatism and evolutionary lability (Ricklefs, 2007; McGill, 2008). We used the *adonis* function as implemented in the *vegan* R package (Oksanen *et al.*, 2018), with a nested design to partition dissimilarities for LMA, LDMC, $D$ and $\theta$, with 9999 permutations, and a blocking nested design (same as above) to account for the non-independence of observations among hierarchical taxonomic levels.

**Results**

*Intraspecific variation*

Our dataset includes 31 species for which there are four or more replicates, allowing their use in a comparison between inter- and intraspecific variation. We did not find a general trend for intraspecific variation on functional traits (Figure 5.1). The minimum LMA value was 47.97 $g/m^2$ and the maximum was 345.13 $g/m^2$, with a mean value of 166.82 $g/m^2$. The minimum LDMC value was 0.14 g/g and the maximum was 0.67 g/g, with a mean value of 0.37 g/g. The coefficients of variation (CV) of functional traits within species varied between 7 and 37% for LMA, and from 4 to 31% for LDMC, averaging ca. 18% for LMA and 12% for LDMC, with *Vochysia pygmea* (Vochysiaceae - subshrub), and *Kielmeyera coriacea* (Calophyllaceae - tree) having the lowest LMA (7.4%) and LDMC (4.9%) intraspecific variation, respectively. The highest LMA variability within-species was 37.7%, observed for *Chamaecrista ochnacea* –

(Fabaceae - subshrub), while *Homolepsis longispicula* (Poaceae – grass) had 31.9% of LDMC intraspecific variation (Figure 5.1).

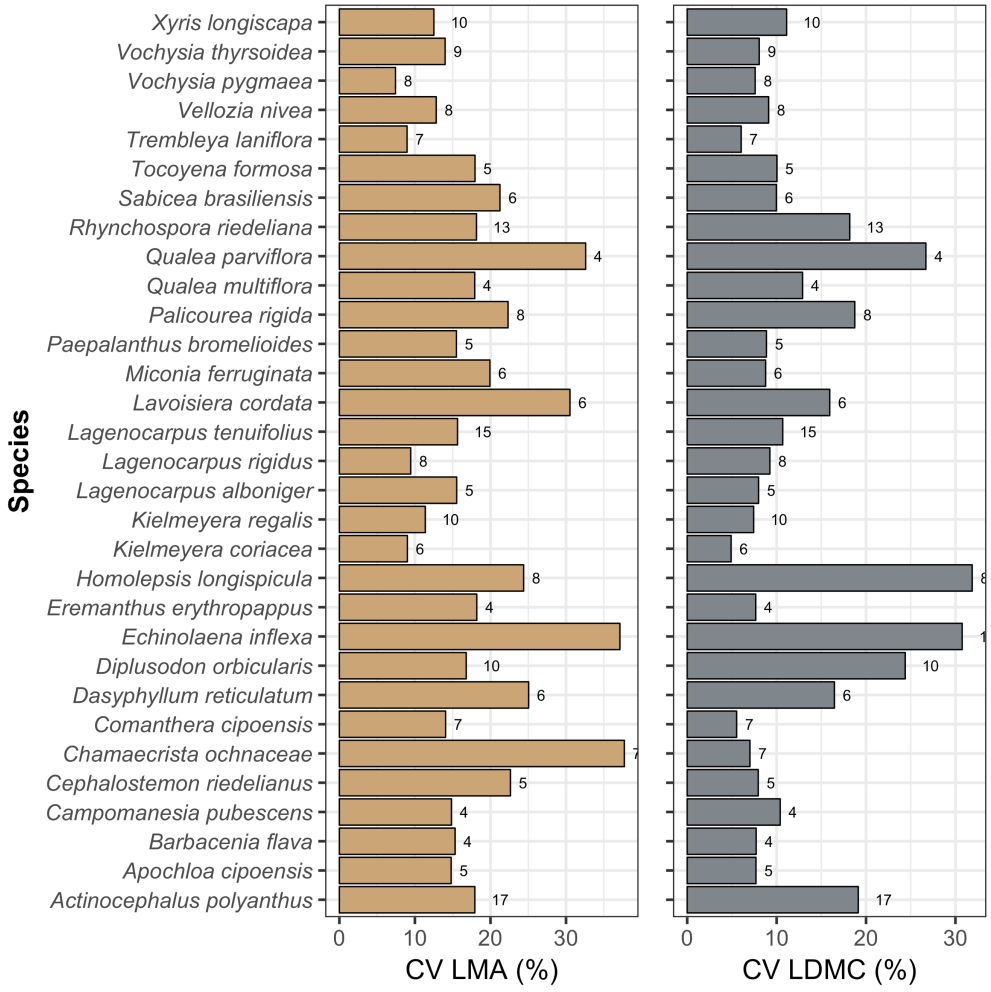


***Figure 5.1:*** Within-species variation calculated as the coefficient of variation for LMA (left panel) and LDMC (right panel). Numbers after the bars in both panels correspond to the number of samples for each species.

Overall, leaf spectra showed the typical patterns for vegetation (Figure 5.2): low reflectance at the visible (VIS) caused by absorption by chlorophyll and other pigments, high near-infrared (NIR) reflectance due to multiple-scattering within the leaf structure, weak NIR water absorption

features at 970 and 1200 nm, and moderate reflectance in shortwave infrared (SWIR) with peaks at 1650 and 2200 nm caused by dominant water absorption features at 1400, and 1900 nm. Intraspecific spectral variability (measured as CV) was dependent on wavelength (Fig 5.2a). Reflectance variability was highest in the SWIR region (CV = 5 to 55%) and generally lowest in the NIR (5% to 30%), except for the region between the red edge (~700 nm) and 1400 nm, where we observed CVs of up to 40%. Similar to functional traits, patterns of reflectance variability were different for each species. For example, *Homolepsis longispicula* (Poaceae – grass) had the largest overall within-species variation across the full spectrum, with the highest variation in the NIR, while *Sabicea brasiliensis* (Rubiaceae - shrub), had its highest variability on the VIS and SWIR regions. The species with the lowest overall variability across the full spectra was *Cephalostemon riedelianus* (Rapataceae - herb). Interspecific variation in leaf reflectance was wavelength dependent (Figure 5.2b), with all species showing an average spectral profile visually different from others, at least in one spectral region.

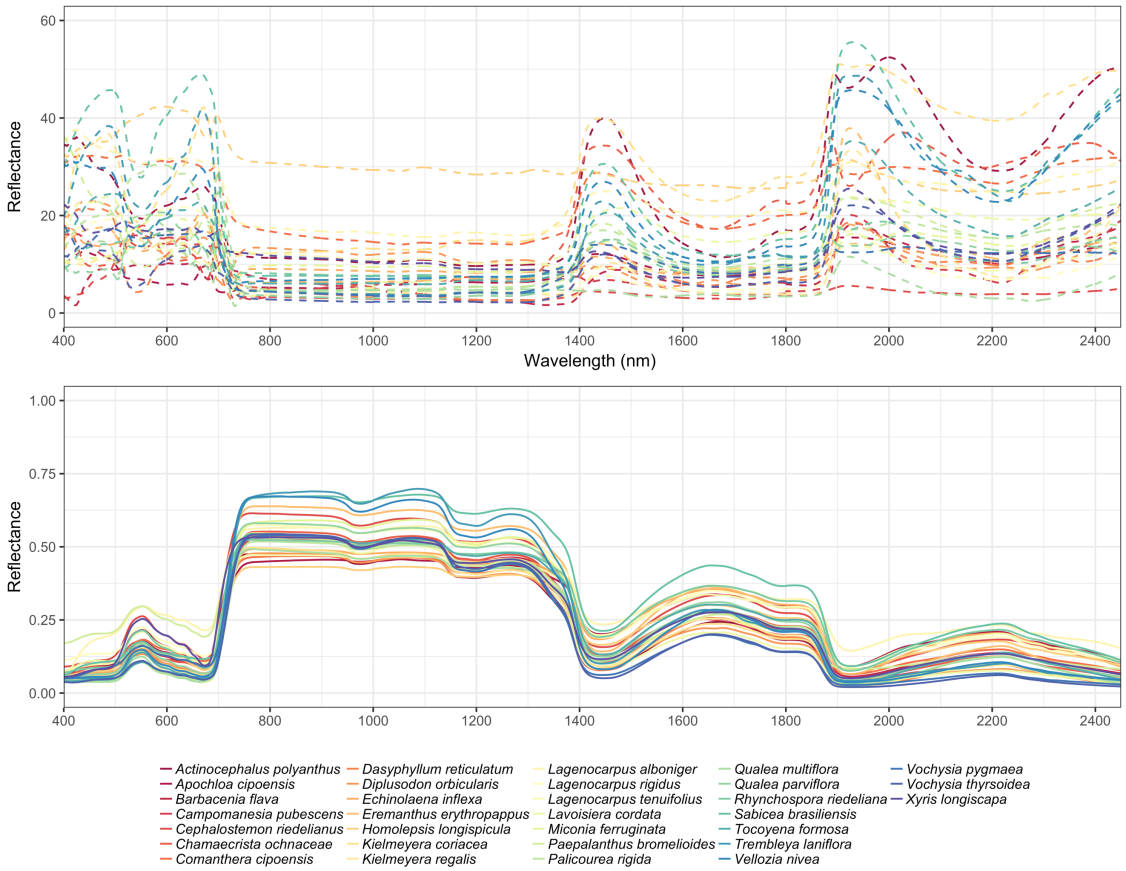


***Figure 5.2:*** Intraspecific spectral variation **(a)**, measured as the coefficient of variation in leaf reflectance spectra of each species, while interspecific variation **(b)** is the average spectral response of each species.

The spectral distance (for both $D$ and $\theta$) was typically greater between a given species and all other species (i.e. interspecific) than between individuals within a species (i.e. interspecific) (Figure 5.3). But interspecific distances were noticeably larger relative to intraspecific distances for the shape measure ($\theta$). Among-species $D$ varied from 0.01 to 0.162, with an average value of 0.066 (0.03 sd). *Qualea parviflora* (Vochysiaceae - tree, $D_{max}$ - $D_{min}$ = 0.18) was the species with the lowest intraspecific variation in $D$, while *Homolepsis longispicula* ($D_{max}$ - $D_{min}$ = 0.20) was the species with the highest intraspecific variability in amplitude. Spectral shape ($\theta$) varied between 0.409° and 19.916° within species and from 1.135° to 20.661° among species. The

average value of within species was 3.587° (2.68 sd), while 8.35° (4.27 sd) was the average value between species. *Eremanthus erythropappus* (Asteraceae - Tree, $_{max}$ - $_{min}$ = 15.3°) was the species with the lowest intraspecific variation in shape, while *Actinocephalus polyanthus* (Ericaulaceae – herb, $_{max}$ - $_{min}$ = 19.3°) was the species with the highest intraspecific variability

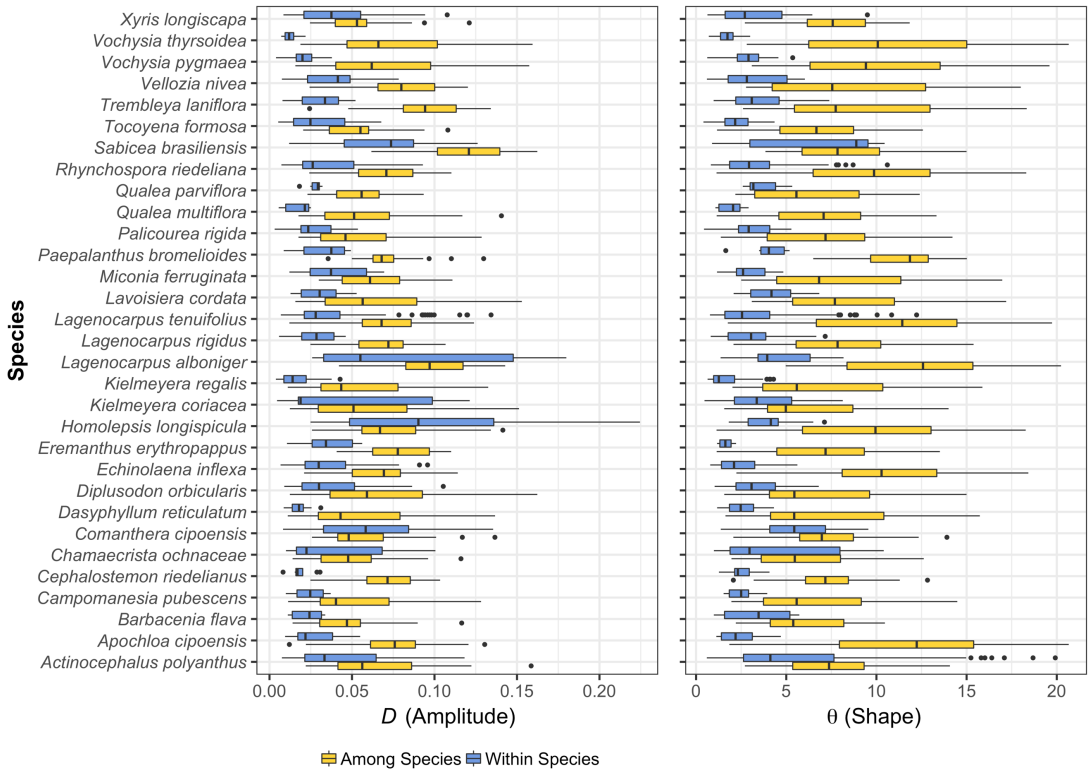


***Figure 5.3:*** Comparison of the amplitude (*D* - left panel) and shape ($\theta$ - right panel) spectral features within (blue boxplots) and among species (yellow boxplots), for 31 *campo rupestre* species from the seasonally dry tropics in Brazil.

The Procrustes analysis showed that the structure of any pair of trait and spectral distance matrices between individuals within a species and between species means were moderately correlated (r=0.35 to 0.39) for both amplitude (*D*) and shape ($\theta$) (Table 5.1). The strongest correlation between trait and spectra dissimilarity matrices was found for LMA vs. *D* or $\theta$, at the

genus and family levels (r=0.48 to 0.52), while the weakest similarity in distance structure between traits and spectra were between LDMC and *D* or $\theta$ ($r \sim 0.2$, $p > 0.05$). Thus, the structure of pairwise distances between LMA and *D* or $\theta$ converged as we moved up on the taxonomic hierarchy, while the correlation between LDMC and *D* or $\theta$ diverged within increasing taxonomic levels.

***Table 5-1:*** Functional x Spectral relationships across biological scales measured as r from a Procrustes analyses. LMA is leaf mass per area, LDMC is leaf dry matter content, amplitude is calculated as *D* (eq.1) and shape as $\theta$ (eq.2)

| | **LMA** | | | | **LDMC** | | | |
|---|---|---|---|---|---|---|---|---|
| | ***D* (amplitude)** | | **(shape)** | | ***D* (amplitude)** | | **(shape)** | |
| **Taxonomic Scale** | *r* | *p-val* | *r* | *p-val* | *r* | *p-val* | *r* | *p-val* |
| *Individual* | 0.368 | 0.001 | 0.421 | 0.0001 | 0.348 | 0.000 | 0.346 | 0.0001 |
| *Species* | 0.389 | 0.015 | 0.383 | 0.0235 | 0.389 | 0.017 | 0.383 | 0.020 |
| *Genus* | 0.476 | 0.004 | 0.519 | 0.0015 | 0.17 | 0.689 | 0.227 | 0.420 |
| *Family* | *0.49* | *0.064* | *0.501* | *0.0503* | *0.201* | *0.821* | *0.42* | *0.159* |

Examining the variance portioning based on distance matrices for each of LMA, LDMC, *D* and $\theta$ showed some similarities (Figure 5.4). In all cases, the contrast between species accounted for the least amount of total variation and was often very small. Variation between genera and families accounted for most (50-80%) of the variation, and the amount of variation between individuals was intermediate. LDMC and *D* showed more intraspecific variation than LMA and $\theta$.

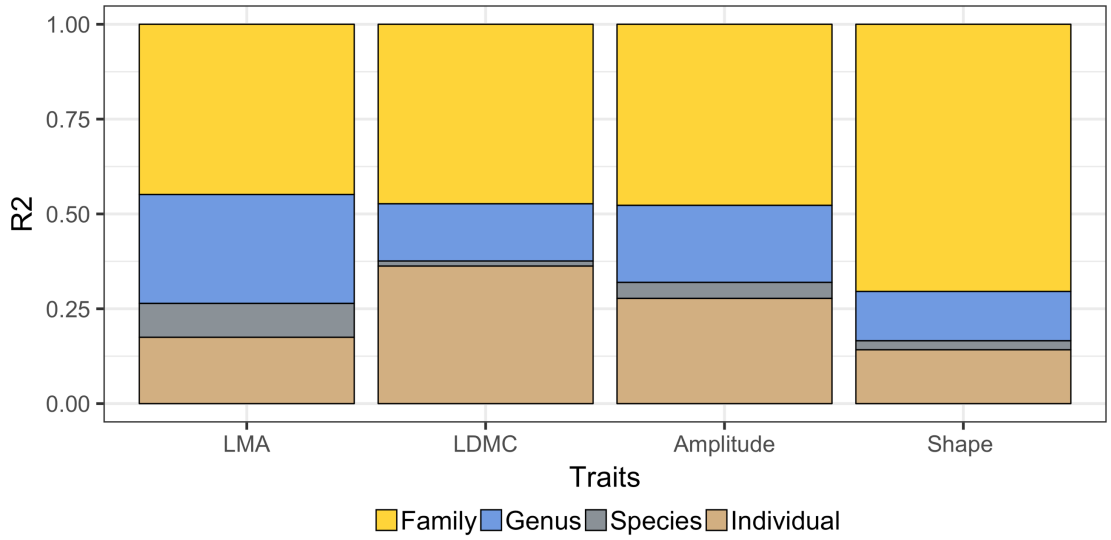


***Figure 5.4:*** Variance structure of two leaf functional traits, and two spectral components across four biological scales. LMA: leaf mass per area; LDMC: leaf dry matter content; Amplitude (*D*): represents differences in the magnitude of the spectral data; Shape ($\theta$): represents differences in the shape (given by angle distances) of the spectral data.

## Discussion

In this study, we examined the degree to which spectral and functional trait variations are structured similarly across the tree of life, using tropical plants of different growth forms from the highly diverse *campo rupestre*. We found that (*i*) interspecific variation exceed intraspecific variation, for trait values and leaf spectra, but both were highly variable within species, and this variability depended on trait and species; (*ii*) LMA was more correlated with the shape of the spectra ($\theta$), the spectral metric that showed the largest interspecific variation; (*iii*) there was almost no shared structuring in dissimilarity between spectra and traits at the species level, with most of the shared structure of dissimilarity found at the higher levels of genus and family.

*Intraspecific variability*

For all traits (including spectral metrics), interspecific variability exceeded intraspecific variability, confirming the general assertion that trait values vary more among than within species (Jung *et al.*, 2010; Albert *et al.*, 2010). In our study, the amount of intraspecific variability was species-specific and we did not detect any pattern in terms of general variation, suggesting that species are affected by the environment and biotic interactions in idiosyncratic ways. Nonetheless, non-trivial amounts of intraspecific variation in spectra occurred, similarly to what has been already documented for leaf traits, challenging the use of average values to describe species, as commonly used in trait-based and spectral diversity studies. This is especially true for single-species studies, since intraspecific variability can obscure the response of a species to environmental changes or to biotic interactions by not accounting for niche differentiation within the functional space (Jung *et al.*, 2010; Albert *et al.*, 2010; Clark *et al.*, 2011; Violle *et al.*, 2012; Siefert, 2012; Siefert *et al.*, 2015).

We found the same pattern and magnitude of intraspecific spectral variability in the seasonally dry *campo rupestre* as previously found for tropical humid, semi-deciduous, and dry forests (Castro-Esau *et al.*, 2006; Ferreira *et al.*, 2013; Asner *et al.*, 2014a; Chavana-Bryant *et al.*, 2016; Ferreira *et al.*, 2016; Wang *et al.*, 2018b). Within-species spectral variability (given by the spectral CV) was especially high at the VIS, at the red-edge and on spectral regions along the SWIR linked to water absorption. Usually, intraspecific variation is associated with differences associated to developmental instability, plant age, sexual genetic mixing, but mainly to plastic and filtering responses to micro-environmental gradients (Messier *et al.*, 2017b). However, leaf-level spectral properties can show strong age dependence (Roberts *et al.*, 1998), with the reported most age-sensitive spectral regions having the highest variability as in our data

(Chavana-Bryant *et al.*, 2016; Wu *et al.*, 2017), indicating that intraspecific spectral variability may be strongly related to leaf-age. This is also in agreement with previous studies by Horler et al. (1983) and Roberts et al. (1998), which also observed red edge shifts attributable to leaf age. Thus, spectral shape ($\theta$) showed more interspecific variation relative to intraspecific variation, when compared to spectral amplitude ($D$). This is consistent with the observation that the magnitude of the spectral signal is overall more susceptible to leaf senescence than spectral shape (Roberts *et al.*, 1998; Chavana-Bryant *et al.*, 2016). Although we restricted our sampling to the same growing season and selected sunlit, fully expanded leaves of mature individuals, we did not control our sampling strategy for leaf age. It goes beyond the scope of our study to fully understand the mechanisms of intraspecific spectral variation, but if our hypothesis that leaf age differences account for a significant fraction of leaf spectral variation is correct, then the interpretation of how we currently understand intra- and interspecific leaf variation in trait-based ecology might not hold for spectral diversity.

*Phylogenetic organization of spectral and functional traits*

The taxonomically nested variance portioning showed that the structuring of dissimilarity of both leaf functional traits and leaf reflectance were evolutionarily conservative, with most of the variance concentrated deep in the phylogeny. The proportion of explained variation decreased successively to lower taxonomic scales, with interspecific variation capturing a very small proportion of variation. Our results are consistent with previous findings showing that LMA and LDMC are less sensitive to interspecific differences, and broadly sensitive to environmental filtering and intraspecific variation (Messier *et al.*, 2010, 2017b; Anderegg *et al.*, 2018). More impressive, we found that spectral metrics captured much of the same filtering patterns detected by traits, following the same taxonomical organization that functional diversity.

Despite broadly sharing a rather similar taxonomical organization, LMA and LDMC do not show an identical dissimilarity structure. Both traits are strongly filtered at the family level but the moderate level of correlation between the two traits (r= 0.36, Chapter 2) allows for considerable independence in the variance structure of each trait. LMA is highly conserved with strong ties to life-history strategies, while LDMC is more labile within the genetic constrains. Thus, LMA had a more similar variance structure to the shape of the spectral, while LDMC had a more comparable variance structure with the spectral amplitude. This pattern is corroborated by our Procrustes analyses, which shows that correlation in structured dissimilarity between functional and spectral traits (Table 1) increased with taxonomic level for LMA, while it decreased with taxonomic level for LDMC. Intraspecific variability (residual) was considerably high for LDMC and the amplitude of the spectral data, indicating that both traits are well suited to detect plastic and filtering responses to environmental variation (Albert *et al.*, 2010; Messier *et al.*, 2017b), as predicted by trait-based approaches, and/or to leaf-age differences (Chavana-Bryant *et al.*, 2016; Wu *et al.*, 2017), as suggested by the analysis of spectral data.

Ca*mpo rupestre* is a low-resource tropical vegetation, where individual plants have to cope with seasonal water shortage, and species that allocate limited resources towards longer-lived, better-defended foliage are favored (Chapter 2; Negreiros *et al.*, 2014; Silveira *et al.*, 2016; Dayrell *et al.*, 2018). Across a diverse phylogeny, such strong environmental filtering may be expected to promote the convergence of distantly related species towards similar trait values (Kraft & Ackerly, 2010). The taxonomical organization and evolutionary conservatism of functional and spectral traits may thus be an outcome of the lasting influence from past climatic and environmental stability on which the Serra do Cipó landscape evolved (Silveira *et al.*, 2016).

*Comparison with other studies*

We expand on previous studies (Asner *et al.*, 2014a; Cavender-Bares *et al.*, 2016; Schweiger *et al.*, 2018) and demonstrate the potential contributions of spectral diversity as an evolutionary and ecologically sound component of biodiversity. Our study is focused on tropical species from a seasonally dry region, with nutrient-conservative strategies, and further studies are needed to determine whether our results are generalizable to other biomes, while also considering other leaf functional traits. Of course, we would expect different spectral-functional relationships for contrasting biomes and communities, since we do expect differences in the overall resource use strategies (Reich, 2014). For example, when comparing our results with tropical humid forest trees, we find contrasting patterns, as in these forested systems the species level accounted for more LMA variation (derived from spectroscopy) than any other biological level (Asner & Martin, 2011; Asner *et al.*, 2014b,a). Since different processes operate to maintain *campo rupestre* and humid forests communities, and each biome represents more or less a different end of the leaf economics spectrum (Wright *et al.*, 2004), it is expected that functional and spectral taxonomical organization would be different between each other.

*Consequences for the future use of spectral diversity measurements*

The taxonomical organization of leaf functional traits and leaf spectra has important implications for the scaling-up of spectral diversity. The linkages between phylogeny and spectroscopy remain a new scientific area of inquiry, but we provide evidence that spectral features such as the magnitude and shape of the spectral signal are consistent metrics linking taxonomy and function in seasonally dry tropical vegetation. Specifically, spectral shape offers a potential approach to quantify spectral diversity using airborne spectroscopic data, and also to estimate and map LMA

across different biological scales. The fact that LDMC is more related to plant plastic responses puts into question the ability of remote spectroscopic data to map this trait in highly diverse systems, or at least in the seasonally dry tropics. Moreover, the scaling-up of leaf reflectance to remote sensing pixels is influenced by other factors like leaf area index and angle distribution, shading, background signals, plant geometry, and sun-sensor illumination angles, which all combined can enhance even more intraspecific variability effects (Clark et al. 2005, Asner and Martin 2016, Cavender-Bares et al. 2016, Ferreira et al. 2016, Jetz et al. 2016, Wang et al. 2018). Nevertheless, we show that spectral data can be used to explore functional diversity in an evolutionary context, providing a link between remote sensing, trait-based ecology and eco-evolutionary theory.

We thus highlight two key messages. First, intraspecific variability is an important source of variation in spectral diversity, and understanding the underlying mechanisms behind within-species spectral variation is fundamental if we want to draw a complete parallel between spectral diversity and trait-based ecology. We raise two hypotheses for intraspecific spectral variability: (*i*) plastic and filtering responses to micro-environmental gradients, usually acknowledged by trait-based ecology; and/or (*ii*) variability in leaf age. Leaf age is a difficult property to measure, and even restricting the sample to "mature and fully expanded leaves", intrinsic leaf age differences could have had more influence in the full spectrum variability than in soft traits like LMA and LDMC, thus hindering our understanding of intra-to-interspecific relationships as expected from trait based ecology. This clearly calls for more multitemporal studies to disentangle ontogenetic effects from plasticity in intraspecific spectral diversity.

Second, our results emphasize the strong taxonomical organization of spectral and trait variation, reflecting the overall evolutionary conservatism of leaf traits in *campo rupestre* vegetation. Still,

we found a strong environmental component influencing traits like LDMC, and to some extent the amplitude of the spectra, which are free to drift at lower taxonomic levels. Such plasticity may be important to prevent selective pressures (Sultan, 2000). In summary, despite major taxonomical differences, there is a striking similarity in leaf function between *campo rupestre* plants, which was also closely followed by their spectral properties. Combined, our results corroborate the link between spectral, functional and taxonomical diversity (Asner & Martin, 2011; Cavender-Bares *et al.*, 2016; Schweiger *et al.*, 2018), and reinforce spectral diversity as a component of biodiversity.

**Supplementary Tables**

Table S1. Species list

Table S2. PCoA summary results

Table S1: Species and number of individual number sampled.

| Family | Spec | Life_Form | Number of individual sampled |
|---|---|---|---|
| Xyridaceae | Xyris longiscapa | Grass | 10 |
| Vochysiaceae | Vochysia thyrsoidea | Trees | 9 |
| Vochysiaceae | Vochysia pygmaea | Sub-Shrubs | 8 |
| Velloziaceae | Vellozia nivea | Sub-Shrubs | 8 |
| Melastomataceae | Trembleya laniflora | Shrubs | 7 |
| Rubiaceae | Tocoyena formosa | Trees | 5 |
| Rubiaceae | Sabicea brasiliensis | Shrubs | 6 |
| Cyperaceae | Rhynchospora riedeliana | Grass | 13 |
| Vochysiaceae | Qualea parviflora | Trees | 4 |
| Vochysiaceae | Qualea multiflora | Trees | 4 |
| Rubiaceae | Palicourea rigida | Shrubs | 8 |
| Eriocaulaceae | Paepalanthus bromelioides | Herbs | 5 |
| Melastomataceae | Miconia ferruginata | Trees | 6 |
| Melastomataceae | Lavoisiera cordata | Shrubs | 6 |
| Cyperaceae | Lagenocarpus tenuifolius | Grass | 15 |
| Cyperaceae | Lagenocarpus rigidus | Grass | 9 |
| Cyperaceae | Lagenocarpus alboniger | Grass | 5 |
| Calophyllaceae | Kielmeyera regalis | Shrubs | 10 |
| Calophyllaceae | Kielmeyera coriacea | Trees | 6 |
| Poaceae | Homolepsis longispicula | Grass | 8 |
| Asteraceae | Eremanthus erythropappus | Trees | 4 |
| Poaceae | Echinolaena inflexa | Grass | 11 |
| Lythraceae | Diplusodon orbicularis | Sub-Shrubs | 10 |
| Asteraceae | Dasyphyllum reticulatum | Shrubs | 6 |
| Eriocaulaceae | Comanthera cipoensis | Herbs | 7 |
| Fabaceae | Chamaecrista ochnaceae | Sub-Shrubs | 7 |
| Rapataceae | Cephalostemon riedelianus | Herbs | 5 |
| Myrtaceae | Campomanesia pubescens | Shrubs | 4 |
| Velloziaceae | Barbacenia flava | Sub-Shrubs | 4 |
| Poaceae | Apochloa cipoensis | Grass | 5 |
| Eriocaulaceae | Actinocephalus polyanthus | Herbs | 17 |

Table S2: PCoA results. GOF is a measure of the goodness of fit, it represents the amount of variance captured by the PCoA.

| **Taxonomic Scale** | **LMA** | **LDMC** | **Amplitude (D)** | **Shape (θ)** |
|---|---|---|---|---|
| | *GOF* | *GOF* | *GOF* | *GOF* |
| Individual | 0.71 | 0.71 | 0.473 | 0.547 |
| Species | 0.75 | 0.75 | 0.498 | 0.56 |
| Genus | 0.736 | 0.75 | 0.50 | 0.56 |
| Family | 0.779 | 0.739 | 0.55 | 0.645 |

## 6. Concluding Remarks

This study has established an important link between trait based ecology and remote sensing, combining sound ecological theory to the physical basis of remote sensing. By addressing the multidimensional and integrated nature of the plant phenotype, and examining similarities and differences between trait-trait, trait-environment, and trait-spectra relationships across ecological scales, this work contributes to a more integrated assessment of tropical plant biodiversity.

Four main messages emerge from this body of work: i) defining vegetation units at regional scale by their phenological traits rather than by floristic composition enables the depiction of mechanisms determining the duration of leaf deployment and its geographical distribution, which are closely linked to large scale environmental gradients; ii) the global pattern of plant form and function from Díaz et al (2016) can be detected at the landscape scale in the seasonally dry tropics, but there is no simple relationship between traits, performance, and environment; iii) embracing the multidimensionality of the leaf full-reflectance spectra as a "leaf phenotype" provides different insights into plant ecology that are not accessible by a handful of individual leaf traits; iv) relationships among functional and spectral diversity reflects a rather similar taxonomic organization and evolutionary constraints, and endorse the spectral diversity concept as an important component of biodiversity.

Commonly, phenological patterns and its environmental relations are inferred from generalized climatic variables, such as total amount of precipitation and temperature means (Waldock *et al*., 2018). However, at a snow-free tropical mountain, topography played a key role in shaping light and water availability for leaf development, allowing the co-occurrence of highly diverse vegetation types that partition the resources across space and time (Chapter 2). The interaction between precipitation and topographic wetness index (a proxy for soil moisture availability), and

light availability, given by the combination of incoming insolation and spatial and seasonal cloud coverage patterns, were the most likely drivers of land surface phenology in the Espinhaço range, determining the start, end, and length of the growing season. Although temperature is not usually acknowledged as an important cue for tropical phenology (Cleland *et al*., 2007), it had an important role in determining the rates of leaf development and the strength of vegetation seasonality, suggesting that tropical vegetation is also sensitive to latitudinal temperature changes. Temporal displacement in the start date of the annual growth season was more evident than variations in season length among vegetation types, indicating a temporal separation in the use of resources (Chapter 2).

A closer look into the emergent patterns of leaf traits (chapters 3, 4 and 5) resulted in the following reasoning: yes, the LES is observable at local scales, but the two key traits from this dimension, LMA and LDMC, do not map the resource-use strategy as predicted by the fast-slow continuum theory at the studied site. This may be a result from the climatic stability under which the Serra do Cipó flora has evolved, favoring the persistence of old lineages that continue to diversify but conserve the traits and environmental tolerances of their ancestors (Chapter 3). These same traits, which are usually measured in ecological studies, also did not show ecologically significant variations (average and range) among plant growth forms. Leaf-reflectance spectra, however, was able not only to accurately predict functional traits, but also to discriminate major *campo rupestre* growth forms, despite the lack of LMA and LDMC variability. This indicates that neither trait fully captures the ecophysiological variation contributing to a particular leaf phenotype (Chapter 4), and that the full leaf spectra is integrating more information about chemical and structural leaf properties than isolated traits.

By placing functional and spectral diversity in an evolutionary context, we confirmed that not only functional traits are evolutionarily conserved in *campo rupestre* vegetation, but also the leaf reflectance spectra, as most of the variance for both biodiversity components were found deep in the phylogeny (*i.e.* at the family level, Chapter 5), thus corroborating findings from Chapter 3. Moreover, the relationship between functional and spectral diversity was confirmed in the seasonally dry tropics, but intrinsic leaf age differences may impact intraspecific spectral variability more strongly, and hamper the understanding of intra-to-interspecific relationships as we know it from trait based ecology.

As the world enters the Anthropocene, ecology faces the challenge of transitioning from a descriptive science into a predictive one, in time to provide relevant understanding of how ecosystem structure and function will be altered by global environmental change (Schimel *et al*., 2013; Higgins *et al*., 2016; Jetz *et al*., 2016; Houlahan *et al*., 2017). Taken together, the findings from this thesis imply that changes in the length and intensity of the dry season may act as a severe threat to all vegetation types found along the Espinhaço range, but particularly for the seasonally dry vegetation at Serra do Cipó, which comprises one of the richest flora of the world (Silveira *et al*., 2016). The strongly conservative nature of leaf traits, combined with effects of temperature amplitude over the rates of green-up and senescence indicates that climatic stability is an important element in this region. These observations provide one more piece of evidence corroborating the OCBIL theory (Hopper, 2009), and highlights the peculiarities of the Espinhaço Mountain Range, but also show that this vegetation may not be plastic enough to keep up with the fast pace of anthropogenic climate change. Fernandes *et al*. (2018) show that 82% of *campo rupestre* areas along the Espinhaço range may be lost within the next 50 years due to climate change only. No specific information has been provided by the Brazilian panel of climate

change to the Espinhaço Mountain Range (Fernandes *et al*., 2018), but it should be an important goal on the future research agenda of ecologists to address the impacts that climatic changes will have on vegetation structure and function in this unique system.

Predicting the future of ecosystems will increasingly require species- and trait-level data. This study works towards predictive ecology by empirically testing the generalities of ecological theory at the undersampled dry tropics and examining some of the foundational assumptions of trait-based plant ecology. A deeper understanding and wider application of leaf reflectance spectra can help integrate components of biodiversity that are difficult to assess, and help promote a global biodiversity monitoring system.

The only universal rule in ecology seems to be that nothing is universal, and the most common ecological result by far is "it depends on the scale". While our current ecological understanding is still far from a "Newtonian unifying theory", remote-sensing approaches are likely the best option to address the scale dependence of ecological process (Kerr & Ostrovsky, 2003; McGill, 2010; Schimel *et al*., 2013; Jetz *et al*., 2016). Currently, we lack a clear understanding of scale dependences in the spectral–biodiversity relationship (but see Asner *et al*., 2016; Wang *et al*., 2018), and this is likely to vary for different ecosystems. Nonetheless, this thesis has yielded encouraging findings by linking spectra to plant function and diversity at the leaf level in a tropical system, combining traditional field approaches with innovative remote observation techniques. In doing so, it attempts to contribute to the theory that guides future trade-offs for the scalability of biodiversity through remote sensing, and provides a positive encouragement for future studies to focus in comparisons between field sampling and reflectance measured across spatial and temporal scales using imaging spectrometers, be it airborne (including UAV-based) or (future) spaceborne, to greatly enhance our understanding in our rapidly changing planet.

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