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Searching for an ecological indicator based on plant functional diversity along a climatic gradient

Dissertação

Melanie Köbel Batista

Mestrado em Ecologia e Gestão Ambiental

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Abstract

Searching for early indicators of climate change is of utmost importance in drylands, since these regions are particularly sensitive to desertification, due to water scarcity and land-use impacts. Our main objective was to search for a potential ecological indicator of climate change. For that, plant community was assessed along a spatial climatic gradient in a dryland area located in southern Portugal. Plant community was assessed in 15 sites that varied in mean annual precipitation (521-634mm) and mean annual temperature (16-17°C), in Mediterranean grasslands. Plant community was studied both in a classical approach (species diversity and plant cover) and in a functional approach (through the analysis of several *a priori* functional groups and measured traits related to climate) and related to climatic variables. The point-line intercept method was used to assess plant community. *A priori* functional groups were based on life form, life cycle and families. Traits measured were biomass, height and SLA.

The sampled sites were dominated by annual grasses. Species richness and plant cover decreased significantly with increasing aridity. Considering *a priori* functional groups, the cover of hemicryptophytes decreased with increasing aridity, as well as cover of perennial grasses and annual legumes while cover of annual grasses remained unchanged. Along the climatic gradient, a community shift was found based on relative cover (relative % in the plant community): annual grasses and *Plantaginaceae* species increased their relative cover with increasing aridity, while perennial grasses, annual legumes and *Caryophillaceae* species decreased in relative cover. A multivariate analysis grouped species in a manner consistent with the previous result. Among *a priori* functional groups, the most promising groups with potential to be used as ecological indicators are perennial grasses and annual legumes and the previously mentioned community shift.

Biomass and height changed along the climatic gradient, although the response pattern found for dominant species did not always match the response of their respective *a priori* functional groups. For example while height of annual grasses increased with precipitation, height of the dominant annual grass *A. pourretti* did not significantly changed. Specific leaf area, which was analyzed only for the *Compositae* species *Tolpis barbata*, decreased with increasing aridity as well. Considering that the height of this species also decreased, this suggests a change in the physiological performance along the climatic gradient. Moreover it reflects the phenotypic plasticity of this species. In sum, the response of specific

traits (e.g. height or SLA) measured in the same species along the gradient seems to have the potential to be used as an ecological indicator of climate change, especially in species with global distribution.

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Resumo

As alterações climáticas podem ter consequências especialmente graves em zonas áridas, uma vez que a escassez de água aliada a pequenas alterações no clima ou na gestão do uso do solo podem gerar transformações abruptas e dificilmente reversíveis, comprometendo os serviços prestados pelos ecossistemas (MEA 2005). A produtividade nestas zonas já é limitada pela falta de água e, neste contexto, as alterações climáticas podem inclusivé induzir um processo de desertificação iminente (UNCCD 2011). Assim, a procura de indicadores ecológicos, i.e. parâmetros do ecossistema que reflictam a sua resposta a determinado factor ambiental (Turnhout et al. 2007), que permitam antecipar os efeitos das alterações climáticas é de extrema importância (MEA 2005).

As previsões climáticas para Portugal apontam para um decréscimo de precipitação ao longo do próximo século, especialmente acentuado na região sudoeste do país (Costa et al. 2012). Esta região tem atualmente valores de precipitação muito baixos (Rosário 2004) pelo que está classificada como zona árida, de acordo com classificação da Convenção das Nações Unidas para o Combate à Desertificação (MEA 2005), apresentando uma elevada variabilidade interanual (Soares et al. 2012). Estas características tornam-na uma zona susceptível a processos de desertificação que poderão ser acentuados pelas referidas previsões climáticas.

Atributos funcionais são características mensuráveis das plantas, relacionados com o seu funcionamento, modelando a forma como respondem a variáveis ambientais ou influenciam os processos do ecossistema (Lavorel et al. 2007a). A utilização de grupos funcionais – grupos de espécies com atributos semelhantes – é muito vantajosa pois além de fornecer informação sobre os processos dos ecossistemas, inacessível numa abordagem baseada na composição específica, foi também demonstrada a relação entre diversidade funcional e vários factores de perturbação tais como pastoreio, disponibilidade de nutrientes, fogo, etc. (Scherer-Lorenzen 2005, Lavorel et al. 2007a). Acresce que, ao contrário de uma

abordagem clássica baseada apenas na diversidade específica, esta permite comparar diferentes comunidades vegetais sob o ponto de vista funcional.

Neste trabalho, pretende-se encontrar um potencial indicador ecológico dos efeitos das alterações climáticas. Para isso, avaliou-se a comunidade vegetal ao longo de um gradiente climático, localizado em clima mediterrânico, usando quer uma abordagem específica, quer uma baseada na diversidade funcional. A comunidade vegetal foi avaliada ao longo de um gradiente climático espacial, no qual a precipitação média anual variou entre 521 e 634mm. Os 15 locais amostrados localizados em Montado de azinho são homogéneos relativamente a uma série de parâmetros (baixa intensidade de pastoreio, tipo de solo, altitude, pH, litologia e não ocorrência de fogo recente) e foram aleatoriamente selecionados após estratificação baseada na precipitação média anual dos últimos 50 anos.

A comunidade vegetal foi amostrada usando o método dos quadrados pontuais. As espécies encontradas foram classificadas em vários grupos funcionais definidos *a priori*, relacionados com a forma de vida, o ciclo de vida e a família taxonómica (como uma aproximação à classificação por grupos funcionais, uma vez que agrupam espécies que partilham uma série de características). Alguns atributos funcionais foram medidos: biomassa, altura vegetativa e área específica foliar. De uma forma geral, este estudo pretende responder às seguintes questões: i) a diversidade específica e a cobertura de plantas variam ao longo do gradiente?; ii) qual o padrão de resposta dos vários grupos funcionais?; iii) ocorrem mudanças ao nível da comunidade como um todo (em termos de cobertura relativa)?; iv) podem os atributos funcionais ser usados para avaliar gradientes climáticos, ao nível da espécie e ao nível da comunidade?; v) será possível identificar um limiar após o qual ocorram alterações significativas ou abruptas no ecossistema?; vi) quais são os caracteres ou grupos funcionais com maior potencial para se tornarem indicadores ecológicos de alterações climáticas? Uma vez que indicadores ecológicos devem ser parâmetros simples e de medição o

mais expedita possível, de modo a potenciar uma utilização a larga-escala, a estrutura deste trabalho segue uma linha de crescente especificidade nos parâmetros avaliados começando por parâmetros relativamente simples, nomeadamente diversidade específica e cobertura, seguida por uma avaliação ao nível do grupo funcional e terminando numa avaliação ao nível específico.

Considerando os 15 locais amostrados, foram identificadas ao todo 146 espécies, na qual a sua maioria pertence às famílias *Graminae* (37 espécies), *Compositae* (29) e *Leguminosae* (22). A cobertura de plantas foi, em média, ca. de 80%, dominada por gramíneas anuais, que ocupavam, em média, 51.8% da comunidade vegetal. A diversidade específica e cobertura de plantas aumentou significativamente com o aumento da aridez (i.e., ao longo do gradiente climático). No entanto, visto que estes parâmetros estão muito dependentes do clima e uso do solo, sugere-se que um indicador ecológico baseado na diversidade funcional será mais apropriado para uma aplicação a larga-escala. Considerando os grupos funcionais avaliados, os resultados mostraram que a cobertura de espécies hemicriptófitas diminuiu com o aumento da aridez, bem como a cobertura de gramíneas perenes e de leguminosas anuais, enquanto que a cobertura de gramíneas anuais permaneceu inalterada. Esta classificação que tem em conta conjuntamente a família e o ciclo de vida (ex. gramíneas perenes), mostrou ser mais eficaz do que os primeiros grupos funcionais avaliados, que têm em conta apenas um atributo (apenas forma de vida, por exemplo). Portanto, com base nestes resultados, os grupos que parecem ter maior potencial para se tornarem indicadores ecológicos das alterações climáticas são as gramíneas perenes e as leguminosas anuais. Estes resultados estão de acordo com vários estudos que associam gramíneas perenes e leguminosas a sítios mais húmidos.

Ao analisar a cobertura relativa (%) de locais em extremos opostos deste gradiente climático, verificou-se que, além dos grupos acima referidos, também outros taxa variavam a sua cobertura relativa. Verificou-se que 2 grandes grupos variavam inversamente: à medida que os locais são cada vez mais áridos, a

cobertura relativa de um grupo composto pelas gramíneas anuais e espécies Plantagináceas aumenta (de ca. 40% para 60%), enquanto que outro grupo composto pelas gramíneas perenes, leguminosas anuais e espécies Cariofiláceas diminui (de ca. de 30% para 10%). Uma vez que estes resultados são com base em grupos funcionais feitos *a priori*, foi também efetuada uma análise multivariada para verificar como as espécies se associavam entre si. Os resultados são consistentes com os grupos funcionais considerados. No entanto, também permitiu verificar que, dentro dos grupos funcionais, podem existir espécies que não mostram o mesmo padrão de resposta que o grupo funcional em que se esta insere, o que sugere que a comunidade vegetal deve continuar a ser analisada, de modo a refinar os grupos funcionais considerados.

A biomassa e a altura vegetativa são atributos funcionais que mostraram responder ao gradiente climático, embora a resposta varie entre os vários grupos funcionais e as espécies dominantes. A área específica foliar, medida para a espécie *Tolpis barbata*, diminuiu significativamente com o aumento da aridez. Considerando que a altura vegetativa desta espécie também decresceu, a resposta conjunta destes dois atributos funcionais sugere que há uma reposta fisiológica por parte da planta ao gradiente climático. Os resultados sugerem que a resposta destes atributos funcionais medidos na mesma espécie ao longo do gradiente, pode constituir um potencial indicador ecológico. No entanto, para que um indicador ecológico deste tipo seja aplicável em larga-escala, deve ser utilizado numa espécie com distribuição global.

Palavras-chave

atributos funcionais; diversidade funcional; Mediterrâneo; gradiente climático; pastagens

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Figure 12: Bi-plots between measured number of species and A: minimum temperature of the coldest month (mean from Y1950-2000); B: short-term precipitation (mean monthly precipitation from Oct 2011 – Mar 2012). Plant community was sampled in 14 sites along a climatic gradient in Alentejo region.

Figure 13: Plant community composition of the more humid and the driest sites according to Medium-term Precipitation, i.e. mean annual precipitation from years 1998-2011 (MTPrec), using relative cover data of a sampling performed in 14 sites along a climatic gradient in Alentejo region.

Figure 14: Relative cover in the community of functional groups considered in table 12 along sites with increasing long-term precipitation (mean annual precipitation from period 1960-1999). Plant community was sampled in 14 sites along a climatic gradient in Alentejo region.

Figure 15: Non-metric Multivariate Dimensional Scaling (NMDS; first vs. second axes) of cover of the 21 most dominant species and sites. Distance measure used was Bray Curtis and stress was 0.097. Numbers indicate sites sorted in ascending order of long-term precipitation (as in Appendix 1). Simbols were placed manually on the left of the species name to indicate species assignment to functional group or family considered in previous results. Apour= Agrostis pourretii Willd.; Bdyst= Brachypodium distachyon (L.) P.Beauv.; Bhord= Bromus hordeaceus L.; Ccapi= Crepis capillaris (L.) Wallr.; Cdact= Cynodon dactylon (L.) Pers.; Cmixtu= Chamaemelum mixtum (L.) All; Crace= Carlina racemosa L.; Gfragi= Gaudinia fragilis (L.) P.Beauv.; Hglab; Hypochaeris glabra L.; Lgall= Logfia gallica (L.) Coss. & Germ.; Lrigi= Lolium rigidum Gaudin; Ltara= Leontodon taraxacoides (Vill.) Mérat; Ocomp= Ornithopus compressus L.; Pcoro= Plantago coronopus L.; Plago= Plantago lagopus L.; Spurp= Spergularia purpurea (Pers.) G.Don; ; Tbarba= Tolpis barbata (L.) Gaertn; Tgutt= Tuberaria gutatta (L.) Fourr.; Vcili= Vulpa ciliata Dumort.; Vgeni= Vulpia geniculata (L.) Link; Vmyur= Vulpia myuros (L.) C.C.Gmel..

Figure 16: Bi-plots between climatic variables and SLA (m^2kg^{-1}) of annual forb *Tolpis barbata* sampled along a climatic gradient in Alentejo region. This species was present in 12 of the 14 sites sampled. A: relation of SLA with the aridity index (calculated using climatic data from Y1960-1990); B: relation of SLA with long-term precipitation (mean annual precipitation Y1950-2000).

2000). Life cycle: An + Bn = annual and facultative biennial species; Pn= perennial species.

Table 8: Spearman's rank correlation coefficients at P<0.05 for species richness and plant cover of *Compositae* species sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An = annual; Pn= perennial; Tot= total.

Table 9: Spearman's rank correlation coefficients at P<0.05 for species richness and plant cover of *Graminae* species, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An = annual; Pn= perennial; Tot= total.

Table 10: Spearman's rank correlation coefficients at P<0.05 for species richness and plant cover of legume species sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An = annual; Sb = perennial shrubs; Tot = total.

Table 11: Spearman's rank correlation coefficients at P<0.05 for relative cover in the community of family and life-cycle groups, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Groups: An Gram= annual grasses; Pn Gram=

perennial grasses; An Legu= annual legumes; Plantago= *Plantago sp.*; Cary= *Caryophillaceae.*

Table 12: Spearman's rank correlation coefficients at P<0.05 for scores of first and second axes of NMDS presented in figure 11.

Table 13: Signal of Spearman's rank correlation coefficients at P<0.05 for cover, biomass and height of functional groups and dominant species with long-, medium- and short-term precipitation. Simbols: = = non significant correlations; + = positive significant correlations. Dominant species: Cmixt= *Chamaemelum mixtum* (L.) All.; Ltara= *Leontodon taraxacoides* (Vill.) Mérat; Apour= *Agrostis pourretii* Willd.; Gfrag= *Gaudinia fragilis* (L.) P.Beauv.; Ocomp= *Ornithopus compressus* L..

Table 14: Spearman's rank correlation coefficients at P<0.05 for Specific leaf area of *Tolpis barbata*, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000).

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1.Introduction

1.1. Climate change and drylands

Ecosystems and their communities are experiencing changes at a global scale as a result of human activities and climate change, showing global to local effects (MEA, 2005). Global change can significantly modify the structure and functioning of ecosystems in an irreversible way and consequently reduce their biodiversity and provision of goods and services (IGBP 2007). A known example is the abrupt degradation of what is now the Sahara desert, which was a productive verdant landscape during the early Holocene, supporting several animal and human populations (deMenocal et al. 2000). The transformation was highly associated with climatic changes, namely the strengthening of the African monsoon (deMenocal et al. 2000). Learning how to anticipate the effects of these global factors on ecosystems associated to global change is therefore a major need (MEA 2005).

A community existing at a site can be seen as the result of a filtering process, where abiotic conditions (ex.: climate, resource availability) and interactions among organisms (competition, predation, mutualisms) constrain the species that persist from a regionally available pool (Lavorel et al. 2007a). Climate, which acts at the regional scale, is one of the primary filters modelling plant communities. Thus, the current climate change scenario may have major consequences in ecosystems community's spatial patterns.

Global climate projections suggest a generalized warming in the 21st century, increasing precipitation in high-latitude regions and a decrease in subtropical regions (IPCC 2007). In accordance, Luterbacher et al. (2004) concluded that

Europe is currently under climate change and that the 20th century was the warmest in the last 500 years.

Although climate change effects are felt at a global scale, drylands are particularly vulnerable to global environmental change, since in these systems water is one of the main limiting factors for plant productivity and consequently ecosystem services provision (MEA 2005). Drylands occupy 41% of the Earth's surface and include all regions classified as dry sub-humid, semi arid, arid and hyper-arid (MEA 2005). This classification is based on an Aridity Index, which is the ratio of mean annual precipitation to mean annual potential evapotranspiration (MEA 2005). Values range between 0 and 1, and lower values indicate more aridity. Figure 1 shows the aridity index classification for Portugal. A fundamental distinction exists between aridity, which is a long-term climatic phenomenon, and droughts, which are a temporary phenomenon (water deficit). In other words, aridity is a function of both precipitation and the potential evapotranspiration rate (ETp). An additional factor affecting aridity is temperature and the annual timing of precipitation. Rainfall during cold seasons is more effective in areas with sufficiently high temperature for plant growth, because less water is lost to direct evapotranspiration during cold periods than during the hot season (Maliva and Missimer 2012).
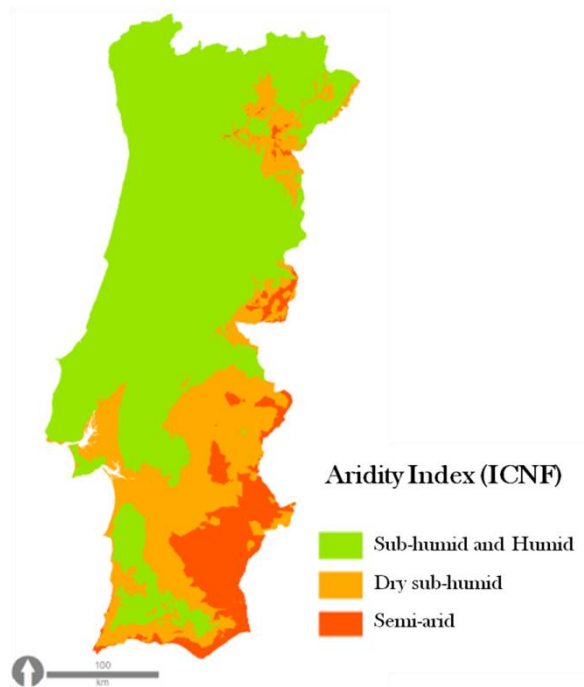
Figure 1: Climate classification of Portugal based on the Aridity Index, using climatic data from years 1961-1999. Source: Rosário (2004).

In Portugal, climate projections made by Costa et al. (2012), for the 2071-2100 period, suggest that total precipitation will decrease in most of the area of the country. The dry period will extend from summer to autumn and spring, amplifying the length of dry spells. On the other hand, extreme precipitation events will increase during winter periods. In Portugal drylands are mainly located in the southern part of the country, where current precipitation levels are low and future precipitation decrease will be more significant (Costa et al. 2012). Moreover this southern region of Portugal has the highest variability of the interannual precipitation (Soares et al. 2012).

One possible consequence of climate change occurring in drylands is the acceleration of the desertification process. Although desertification is the result of various factors, namely chronic droughts and unsustainable land use, climate change may exacerbate desertification through the projected intensification of water scarcity (MEA 2005, UNCCD 2011). Ultimately, arid ecosystems with an ongoing desertification process may shift abruptly to desert, often in an irreversible manner. Several studies focus on measuring only abiotic drivers (e.g.

climatic variables) to evaluate the desertification process (e.g. Costa et al. 2012). However, they do not provide information about its impacts at the ecosystem level. Moreover, the same change in climatic variables might have different impacts in different ecosystems. The changes that occur at the ecosystem level, depend on multiple interactions and on the ecosystem's resistance and resilience. In this work we propose to focus on the ecosystems response along a climatic gradient, in addition to environmental drivers. Thus we want to assess the ecosystems general response pattern to a climatic gradient in the transition towards a more arid environment.

As ecosystem functioning is highly complex, monitoring the effects of environmental drivers in ecosystems on an integrative perspective can be too time and resource consuming. As an alternative, scientists and managers rely on measurable ecological surrogates of the structure, composition, or function of ecological systems, named ecological indicators (Cairns et al. 1993). They can be used to predict ecosystems' changes and help defining ameliorating actions for both anthropogenic and natural disturbances.

1.2. Ecological Indicators

Natural systems are highly complex, i.e. dependent on a multitude of factors and interactions which act at different levels of ecological organization. Thus, there is the need to use "indicator" parameters which are easily measurable and preferably integrate several aspects of ecosystems response to a given factor (or factors). Ecological indicators are measurable parameters that allow us to access nature, based on an observed relationship between environmental factor - biological parameter and the existing knowledge of cause-effect relationships in ecosystems (Turnhout et al. 2007). Ecological indication is broadly used in monitoring programs, either to access the system's quality and/or to evaluate policy performance (Cairns et al. 1993, Turnhout et al. 2007). One kind of

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ecological indicator largely used to access, for example, pollution toxicity (Munn 1988), are biomonitors: a target species or population known to respond to a certain factor. However, the use of target species has several limitations: i) its presence depends on local species assemblages; ii) it gives limited information about ecosystem response as a whole in terms of structure, function and composition (Cairns et al. 1993, Dale and Beyeler 2001). Monitoring at the community or ecosystem level allows a more robust assessment, since it integrates cumulative effects of many stressors (Cairns et al. 1993). Additionally, an integrative approach is more likely to detect early changes (Munn 1988). In this framework, it is possible to apply the ecological indication concept not only for tracking certain substances (as in pollution monitoring programs), but to indicate community changes in response to a given environmental factor.

This work focuses on searching potential ecological indicators of climate change. However, tracking climate change responses would demand long-term datasets (more than 30 years) to cover the usual period for tracking climate changes. This kind of approach is addressed in long-term ecological studies that only started recently in Portugal (SPECO 2012). To expeditiously search for potential ecological indicators we can make a screening using a spatial gradient instead of a temporal one. Thus, in this work we propose to use a spatial gradient that simulates a climate change scenario over time. It is expected that from spatial patterns observed at ecosystem transition (towards more arid environments) associated with climatic gradients, it will be possible to derive a pattern of temporal change, enabling to anticipate ecological changes due to climate change.

1.3. Functional diversity

The functional diversity concept eventually arose from an old discussion between scientists to answer whether biodiversity is important for ecosystem functioning. Scherer-Lorenzen (2005) give a good historical perspective on this

matter. An attempt to answer this main question was done by comparing communities along a biodiversity gradient, while trying to keep extrinsic conditions (ex.: climate) as constant as possible. Species identities were found to be important in biodiversity – ecosystem function relationships. Thus, biodiversity relates to ecosystem processes through functional differences between species (Garnier et al. 2004, Scherer-Lorenzen 2005). In accordance, many studies (see Scherer-Lorenzen (2005) for a review) have shown that species identities within a mixture (i.e. its functional diversity) is more important than the number of species *per se* (Tilman and Knops 1997, Dı́az and Cabido 2001). Traits are related to plant functions, so it is through traits that plants respond to environmental factors and influence ecosystem processes (Garnier et al. 2004, Scherer-Lorenzen 2005).

Functional groups gather species with similar traits (or observed correlations among their various traits), or with similar functions (Cornelissen et al. 2003, Lavorel et al. 2007a). Therefore, there are functional response groups, with species that respond similarly to a particular environmental factor, and functional effect groups, grouping species with a similar effect on one or several ecosystem functions (e.g. nutrient cycling) (Scherer-Lorenzen 2005, Lavorel et al. 2007a).

Functional classification is a very useful tool at various research fields, since it simplifies the floristic complexity of natural communities. It has been widely used in monitoring the effects of global change or of management actions on plant distribution patterns and ecosystem processes (see Lavorel et al. (2007a) for a review). Additionally, since functional classification is not species-specific, it enables the comparison between sites with different floras, belonging to different regions, continents or biomes. Limitations among functional classification have mostly to do with finding/creating an ideal classification, named by Lavorel et al. (2007a) as the holy grail: a single classification that i) would be applicable at the global-scale and ii) can together represent plant responses and effects. This difficulty is largely related to the fact that traits responsible for plant responses

may coincide directly, indirectly, or not at all with traits responsible for plant effects on ecosystem function (Lavorel et al. 2007a).

Functional traits can be morphological, physiological, biochemical, reproductive or demographic characteristics that relate to plant function in ecosystems (Lavorel et al. 2007a). This definition leads to an undefined number of possible measurable traits. Although there may be several methodologies for measuring a certain trait, there are traits that are more laborious than others, independently of the methodology used. For example, traits measured in the roots will probably involve greater labor than traits measured in the leaves. With this in mind, Hodgson et al. (1999) suggested a trait classification where soft traits would be relatively easy to measure, versus hard traits, which involve complex and laborious investigations. Soft traits are therefore favored in functional trait research, and an important effort has been made to standardize its measurement methodology (Cornelissen et al. 2003).

1.4 Measuring functional diversity in inland Alentejo

This study was conducted in Alentejo region, since it is among the more arid regions in Portugal (Rosário 2004) and a decrease in precipitation is predicted during the next century (Costa et al. 2012). Sampled sites are located in *Montado* ecosystem, a semi-natural open woodland, which is the dominant land-use in this region.

In order to analyze functional diversity along the sampled sites it is important that the sampling method enables: i) accuracy in species identification and consequently on traits classification; ii) precision in cover estimation, in order to detect even slight community shifts and iii) a random selection of plant

individuals for trait measurements in the laboratory. Since this study is based on a functional approach and aims to study community composition along a climatic gradient, a thorough registration of species presence *per se* is not the main goal.

Cover estimation through visual estimation methods is common, although an unknown level of observer bias is inherent. Moreover, cover is estimated in classes, so slight alterations in real cover are hardly reported (Elzinga et al. 1998). In order to choose the best sampling method to evaluate shifts in plant community functional diversity, a preliminary essay was performed in two contrasting sites along the study area by Nunes et al. (submitted). Three commonly used methods were compared: two area-based methods (the Modified-Whittaker's method and Dengler's method) and the point-line intercept method (hereafter named PT method)(Stohlgren et al. 1995, Elzinga et al. 1998, Dengler 2009). The PT method displayed higher precision in cover estimates, a similar or higher number of quantified species and a more even cover distribution from more abundant to less abundant species, i.e., a higher evenness. This feature is important in order to: i) not overvalue dominant species in detriment of less abundant ones; ii) have a better picture of the multiple functional traits present in the community; iii) detect even slight shifts in the community whether they depend only on dominant species or also on less abundant ones. The higher precision of the PT method was also verified by Godínez-Alvarez et al. (2009). Additionally, it was highlighted that this method, unlike ocular estimation methods, also enables cover estimates for soil surface.

The gathered knowledge in functional traits enabled the creation of several databases, either regional or international, where average trait values are presented for a growing number of species. Today, several trait data bases are available online (Kattge et al. 2011). This can be very useful since using pre existing trait values, instead of measuring them, can save a lot of resources (Cornelissen et al. 2003, Lavorel et al. 2007b). However, the use of databases information must be used with some caution and has some limitations. First, the

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methodology used for trait measurement must be taken into account for data interpretation and comparison. The use of standardized methods is a way to reduce variability and problems associated with this issue (Cornelissen et al. 2003). Second, functional traits, like any plant feature, reflect intra-specific variability. Therefore, it is expected that trait values vary between species, populations and, to a lower degree, between individuals of the same population. However, general data bases present an average value per species, ignoring the range or level of intra-specific variability within populations (Lavorel et al. 2007b). Another limitation is that data on the species of interest may not be available (de Bello et al. 2006), and this was an important limitation in the present work, although this drawback has the tendency to decrease as more data is added to data bases. Nevertheless, in functional diversity studies, average values are often used (Cianciaruso et al. 2009, de Bello et al. 2011). In these cases, a species comparison approach is used, and intraspecific variability is considered negligible (de Bello et al. 2011). In this work, a mixed approach was used. A number of traits were measured following protocols in Cornelissen et al. (2003). These traits were: plant biomass, a hard trait with large-scale ecological significance; height, which is related to plant competitiveness and may be involved in trade-offs between height and stress tolerance/avoidance; and specific leaf area, a trait related to environmental resources availability (Cornelissen et al. 2003). For another group of traits, mean values from literature were used, namely life form, life cycle and onset of flowering. This approach allows to study plant traits both at the species level (comparing trait values of the same species among sites) and at the community level (via interspecific comparison).

1.5 Objective

The purpose of this study was to search for a potential ecological indicator of early responses to climate change. Plant community was assessed along a spatial climatic gradient composed of a set of 15 sites located in a dryland area in

Alentejo region. The sites were randomly selected after being homogenized for most of the possible confounding variables (soil type, land-use, altitude, inclination) and stratified for precipitation. Plant richness and cover of the understory community of the Quercus ilex (L.) *Montado* were sampled using the point-line intercept method. Plant species found were classified for a series of traits related with response to climate (life form and life cycle) Specific traits such as biomass, height and specific leaf area were measured at functional group and species level of dominant species.

The following specific questions will be addressed in this study:

1) How do species richness and plant cover changes along the climatic gradient?

2) How different plant functional groups respond along a climatic gradient?

3) What are the major shifts at the community level?

4) Can traits at the plant species level or functional group be used to track climatic gradients?

5) Is it possible to identify a critical threshold for significant changes in the ecosystem?

6) What are the most promising ecological indicators of climate change?

The rationale of the structure of this work followed the criteria that indicators should be as simple as possible and as wide applicable as possible. Thus, the quest for an ecological indicator started on the simplest variables that can be obtained in a herbaceous community namely plant diversity and cover. On a following approach a priori functional groups were evaluated in general and within families approach. The work also reaches the species level and their possible associations were tested using multivariate analysis. Finally traits such as biomass, height and specific leaf area were tested from the functional group to the species level.

2.Materials and Methods

2.1.Study area

The study was conducted in the inland Alentejo region, SE Portugal. This region is dominated by semi-natural open woodland called *Montado*. It has a long history of man management, resulting in a mosaic of forest, pastures for extensive grazing and agriculture (Pereira and Fonseca 2003). In the more arid areas, the tree layer is dominated by scattered Holm-oak trees (*Quercus ilex* L.)

Grazing areas are common in the *Montado* ecosystem. Among the sampled sites, pastures with low grazing intensity were the only one land-use type selected, to reduce variability and increase the chance of detecting plant community response to the climatic gradient. In these sites soils are poor and vary between lithosoils and luvisoils.

The study area has a Mediterranean climate, with dry and hot summers and mild to cold and wet winters (Rivas-Martínez et al. 2004). Additionally, the study area is classified as a dryland, based on the Aridity Index (see chapter 1.1). The Aridity Index was calculated for the Portuguese territory by the *Instituto de Conservação da Natureza e Florestas* (ICNF), and the resulting climatic classification is presented in figure 2A. In general terms, the southern part of the country includes the driest regions.
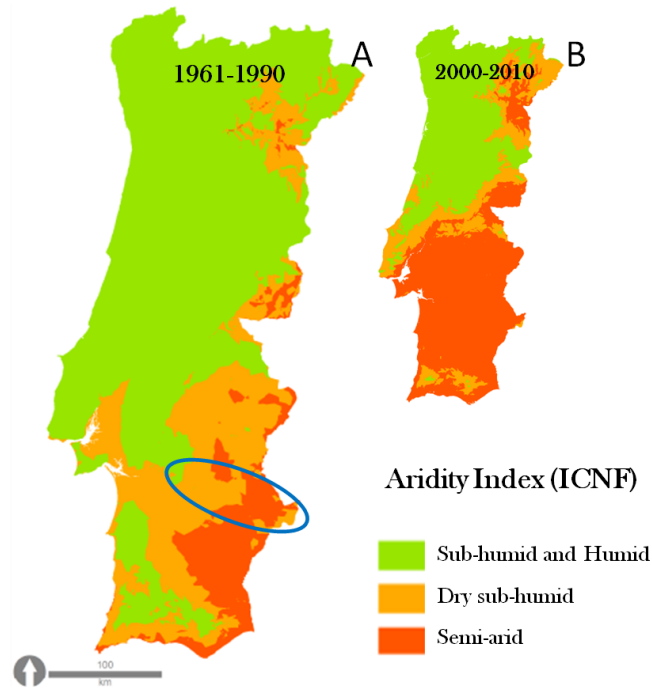
Figure 2: Climate classification of Portugal based on the Aridity Index. A: current official classification, using data from years 1961 to 1990; B: a provisional classification using data from decade 2000 to 2010. The sampling area is marked with a blue circle. Source: do Rosário (2004).

The study area, marked with a blue circle, includes sub-humid, dry sub-humid and semiarid areas which compose the drylands in the region. Figure 2B is a provisional classification (since it uses only data from the last decade, instead of 3 decades) that shows a trend to increasing aridity in the southern part of the country.

2.2. The climatic gradient

Sampling design was made prior to this work, by Pedro Pinho, for the project Modeling Ecosystem Structure and Functional Diversity as early-warning indicators of Desertification and Land-degradation - from regional to local level. The sites sampled in this work are a small part of a large set of sites that are regularly assessed by the ICNF in terms of the tree layer dominance. From this large set of sites, firstly those where *Q. ilex* is the dominant tree species were chosen. Secondly the set of sites was homogenized according to a series of environmental parameters listed in table 1.

Table 1: Parameters used for homogenize the sampling sites.

Parameter	Range admitted
Altitude	158-280m
Soil	Lithosoil ans luvisoil
Soil pH	5.6-6.5 (exceptionally -7.3)
Lithology	Sedimentary and metamorphic
Slope	0.8-4.8
Fire	Non-existence between 1975-2005

Finally a stratified random sampling was performed, with mean annual precipitation (Y1950-2000) as the stratifying parameter. This sampling design ensured a set of sites similar to each other for the parameters listed in table 1, but with different precipitation regimes.

A number of other climatic parameters were also assessed: aridity, evapotranspiration, and temperature. Since these parameters co-vary with precipitation, the sampled sites are actually located along a climatic gradient. In this work 15 sites were sampled. When a randomly selected site was found to be inaccessible or inappropriate, the sampling was performed in the second randomly selected site, which had similar climatic features and was located in the same region. A site could be considered inappropriate if there were marks of recent soil mobilization or severe grazing (plants visibly diminished in height and cover). Appendix 1 shows a detailed characterization of the sampled sites, concerning several temperature and precipitation variables, organic matter content, soil and altitude. Among the 15 sampled sites, located along ca. 115 km (distance between the 2 most distant sites), the mean annual precipitation ranged between 521 and 634 mm and the mean annual temperature varied between 16 and 17 ºC (period Y1950-2000).

Figure 3 shows the location of the sampled sites along the precipitation gradient (total annual precipitation data for the period 1950-2000).
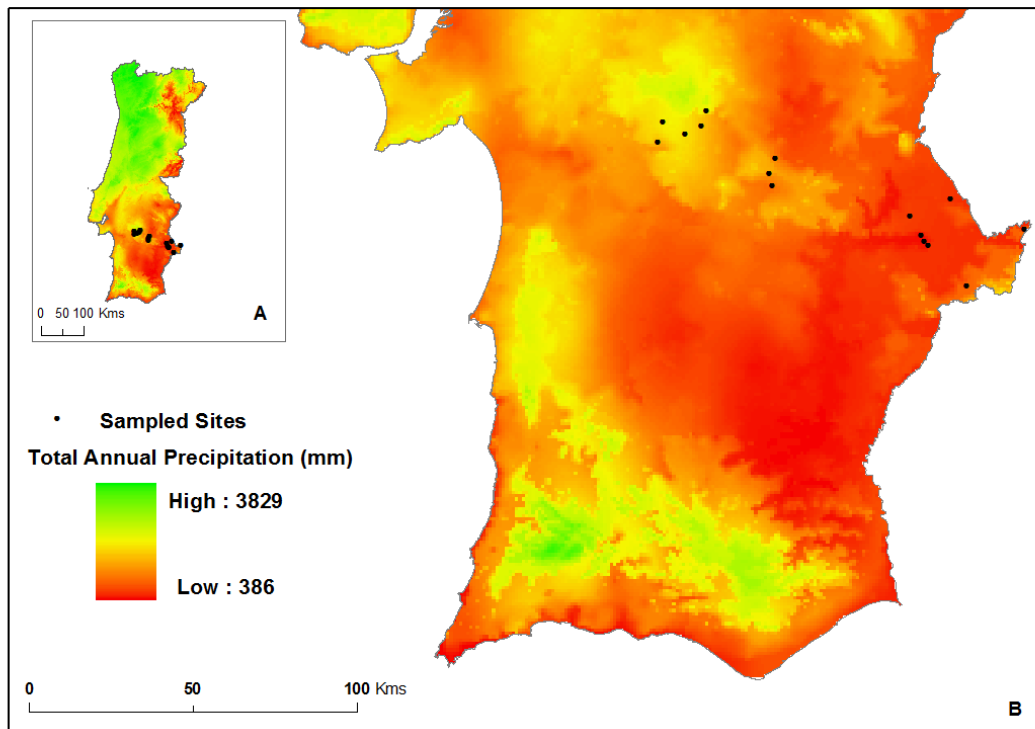
Figure 3: Maps of Portugal showing a precipitation gradient, based on total annual precipitation from years 1950-2010. A: Map of continental Portugal. B: Map of south Portugal, showing the sampled sites marked by dots. Maps constructed using data of Hijmans et al. (2005).

2.2.1. Climatic variables

Table 2 shows the climatic variables considered in this work, including abbreviations used in the Results and Discussion section.

Table 2: Climatic variables considered in this work. For each climatic variable is shown the abbreviation adopted, description, calculation and source.

Abbrev.	Description	Calculation	Source
Arid. Idx	Aridity Index	Mean annual precipitation by Potential evapotranspiration (Y1960-1990)	*Instituto da Conservação da Natureza e Florestas* (ICNF)
LT Prec	Long-term precipitation	Mean annual precipitation (Y1950-2000)	(Hijmans et al. 2005)
MT Prec	Medium-term precipitation	Mean annual precipitation (Y1998-2011)	Calculated using monthly data of *Sistema Nacional de Informação de Recursos Hídricos* (SNIRH)
ST Prec	Short-term precipitation	Mean monthly precipitation (October 2011-March2012)	
LT Temp	Long-term temperature	Mean annual temperature (Y1950-2000)	(Hijmans et al. 2005)
TColdM	Temperature Coldest Month	Mean minimum temperature of the coldest month (Y1950-2000)	(Hijmans et al. 2005)

2.3. Sampling Method

In this study, only the understory vegetation (herbaceous and shrubs species) was sampled, since in the *Montado* ecosystem trees are very often planted and managed, resulting in a tree cover that may reflect not only climate, but management as well.

The herbaceous and shrub vegetation were sampled using the point-line intercept method (PT method) along linear transects. Sampling was performed in late spring, at the end of the growing season. Google Earth and GPS were used to reach each of the sampling sites in the field corresponding to ICNF sites. From these coordinates 6 transects were placed in different directions (Fig.4), so all 6 transects were aligned, with the starting point in the middle. If the site was located in a slope, transects were oriented perpendicularly to the slope, in order to avoid a possible slope-induced gradient. Exceptions to this spatial arrangement were made in order to avoid tree canopy, drainage lines, flooding surfaces and small paths made by livestock transit (fig. 4). This procedure aimed at avoiding heterogeneity among transects.
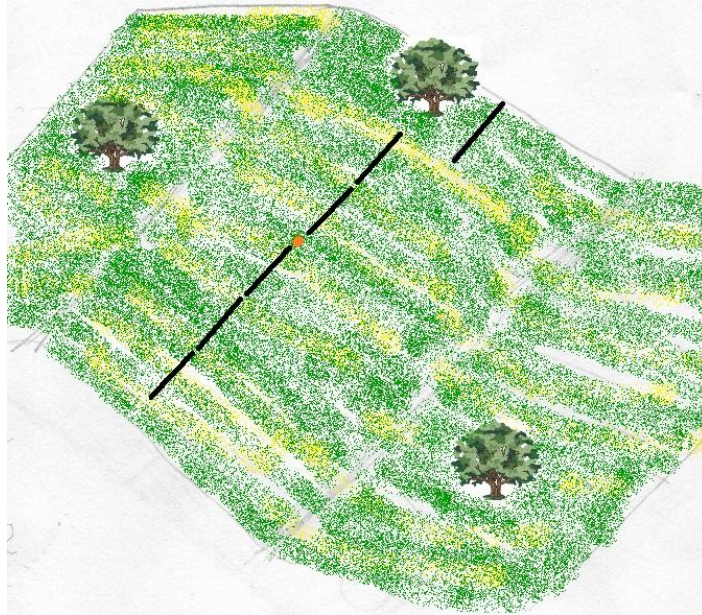
Figure 4: Exemplification of the disposition of the 6 transects in the field. The red point corresponds to the ICNF point, located using a GPS. The 6 transects are arranged perpendicularly to the slope and one transect is deviated to avoid a tree.

Transects were 20m long, and intercept points were spaced every 50cm, summing 41 intercept points per transect and 246 intercept points per site. At each intercept point a metal pin 5 mm thick was placed along the transect perpendicular to the ground.

At each intercept point, the pin was lowered through the vegetation until the ground and all plant individuals touched by the metal pin were collected and putted together in a paper bag. The entire aboveground part was collected, trying to keep individuals as complete as possible for further identification and traits measurement. When no plant was hit, the presence of bryophytes, lichens, litter, dead plant or bare soil were recorded in the absence of plants.
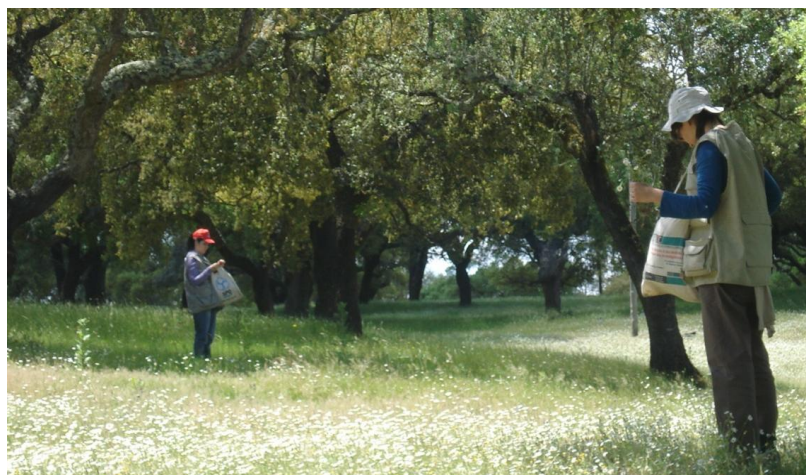
Figure 5: Sampling work. The method used for individuals collection consisted in a packaging system, where all hits of one point were packaged together and these small packs were grouped by transect and then by site for further identification and trait measurement in the lab.

2.4. Data measurements and analysis

2.4.1. Species identification

All collected individuals were identified as close as possible to species level. This identification was conducted in the lab using floras and identification keys of: Flora Iberica (Castroviejo 1986-2012), Nova Flora de Portugal (Franco 1971, 1984, Franco and Afonso 1994, 1998, 2003), Flora Vascular da Andalucía Occidental (Valdés et al. 1987), Catálogo das Plantas Infestantes das searas de trigo (Beliz and Cadete 1982). Species nomenclature was updated using Flora Iberica (Castroviejo 1986-2012), except for species not covered, which follow Nova Flora de Portugal (Franco 1971, 1984, Franco and Afonso 1994, 1998, 2003).

2.4.2. Richness and Cover measurement

Identified species were used to calculate species richness at each site (total species richness and species richness by functional group).

Changes in species cover were calculated by summing up all the points intercepted by plant species at each site (sum of 6 transects). Functional groups cover was calculated by summing the cover of species belonging to the same group. Relative cover in the community (%) was calculated by dividing the total number of hits that touch a specific plant or group by the total number of intercept points measured that hit a plant at each site (sum of 6 transects). This

variable reflects the representativeness of a species/group in the plant community.

2.4.3. Selected traits

A list of traits related to climate was created through a literature review (e.g. Cornelissen et al. 2003, Lavorel et al. 2007a) (Appendix 2). Then, mean values were collected for the species previously identified using databases, floras and papers. Traits for which values were found for all species identified were used for species classification into functional groups. Additionally, some of the listed plant traits were directly measured, namely height, biomass and specific leaf area (SLA).

2.4.3.1. Height

After plant identification height was measured. For each species, height was measured only for the 10% tallest individuals of each transect to evaluate the potential of the community to grow in height. Since the sites had some degree of grazing and the plants were carried out to the lab, the measurement of all plants would bring some error because some were not complete. Furthermore, measuring only the 10% tallest individuals reduces processing time without reducing the accuracy of the measurement. Both vegetative and reproductive height were measured, but in this work only vegetative height is analyzed, since it is the trait described in Cornelissen et al. (2003).

2.4.3.2. Biomass

After height measurement, species were grouped based on family and life cycle. The species were grouped firstly by family: the three most dominant families were kept apart (*Graminae, Compositae* and *Leguminosae*) and the remaining families were measured all together. Then each of these 4 groups was divided by life cycle: i) annuals and species with facultative biennial life cycle and ii) perennial species. Additionally, 11 species were selected to be measured individually. These species were selected by their dominancy in the community in

terms of cover and because they were present in most sampled sites. In resume, 8 a priori functional groups and 11 species were measured. To calculate biomass, plants were dried at 60ºC for a minimum of 72 hours and dry weight was measured with a precision balance (Sartorios, 1mg readability). Biomass of the 11 measured species was added to the biomass of the functional group they belonged.

2.4.3.3. Specific Leaf Area (SLA)

Specific leaf area (SLA) corresponds to a leaf light-intercepting area divided by its dry mass (Garnier et al. 2001). SLA was measured for two dominant species (present with a high cover in most sampled sites sampled): *Agrostis pourretii* Willd. and *Tolpis barbata* (L.) Gaertn. Three fully expanded leaves were collected from the same individuals that were measured for height and biomass following the protocol of Cornelissen et al. (2003).

SLA should be measured within 48h after field collection. This procedure was impossible in this work because field trips would last 3-5 days per week from April to July. To diminish the effects of dry storage, leaves were rehydrated for 6 hours in the dark at ambient temperature (Garnier et al. 2001). After rehydration of Tolpis barbata the area of leaves were measured with a Portable Area Meter (LI-COR, model LI-3000, measurement unit: cm2), while in Agrostis pourretii leaves were scanned using a computer and leaf area was measured using software Adobe Photoshop CS5. Leaves were then dried at 60ºC for 72h and dry mass was measured.

During the scanning process, *Agrostis pourretii* leaves tended to curl or bend, even using an acrylic cover to keep leaves flat. This directly affected SLA measurement. Thus, SLA of *Agrostis pourretii* is not presented in this work.

2.5. Statistical analysis

Research on plant traits responses to environmental variation is mainly based on a correlational approach (Lavorel et al. 2007a), and it is also the approach chosen in this work. Based on the categorical traits compiled from the literature, the community was divided into functional groups made *a priori*. A correlation between the cover of these functional groups with climatic variables was tested.

Correlations between the variation of the measured traits (biomass, height and SLA) and the climatic variables were also tested. All correlations were tested using Spearman rank-order analysis since some relations between variables were not linear. The software used was Statistica 10.0.

Grouping species *a priori* assumes that traits used for that classification may be determinant for the observed species distribution along the climatic gradient. To visually assess if this assumption was confirmed a Non-metric Multidimensional Scaling (NMDS) was performed with species cover data (number of hits per site), independently of their traits. This analysis enables to assess the degree of similarity in plant community composition along the sampling sites as well as the species responsible for it. Species graphically close to each other would have similar traits that led to a common distribution pattern (or different traits that led to the same response). NMDS was performed with only the 21 most abundant species (species with high cover and present in at least 7 sites), because with more species the graphic would be very difficult to read. NMDS was performed with software R, version 2.15.2 using vegan library (R Core Team 2012), and the Bray Curtis dissimilarities as distance measure. The matrix was square root and Wisconsin transformed to minimize outliers. NMDS analysis has advantages in relation to other multivariate methods because it only uses rank information and maps ranks non-linearly onto ordination space, and thus can handle non-linear species responses of any shape and effectively and robustly find the underlying gradients (Oksanen 2011).

3.Results and Discussion

3.1. Plant community in the study area

3.1.1. Pant richness and Plant cover

In this work, 15 sites were sampled for plant richness and cover. At each site 246 intercept points were evaluated making a total of 3690 intercept points evaluated along the climatic gradient. The lack of plant was classified as *no plant cover*. Plant cover was in average 81%. Bugalho et al. (2011) found 83% plant cover in grazed plots under similar climate (mean annual precipitation –MAP- of 587mm) in a study performed in *Montado* at Alentejo.

Figure 6A shows plant cover variation along all 15 sampled sites.
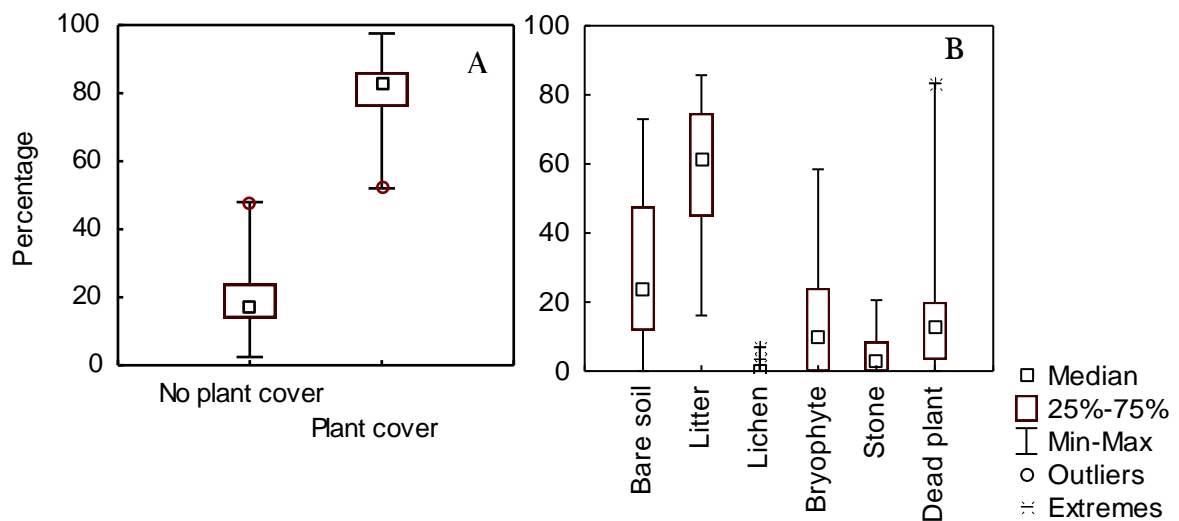
Figure 6: Median, 25th and 75th percentiles, minimum and maximum, outliers and extreme values of A: percentage of plant cover and *no plant cover*; B: percentage of bare soil, litter, lichen and bryophyte within the no-plant cover, in 15 sites sampled along a climatic gradient in Alentejo region.

The variable *no plant cover* was classified for the presence of: bryophytes (15.3%), lichens, litter (56.9%), dead plant, stone/rock, and bare soil (28.4%) (Fig. 6B). The observed low cover of lichens is probably related to the land-use intensity of the *Montado* ecosystem, namely trampling. Lichens were shown to be very sensitive to disturbance, namely to livestock trampling as shown by Zubiri (2012).

A total of 146 plants species were identified and on average there were 36.5 plant species per sampling site, ranging between 26 and 52 (fig.: 7).
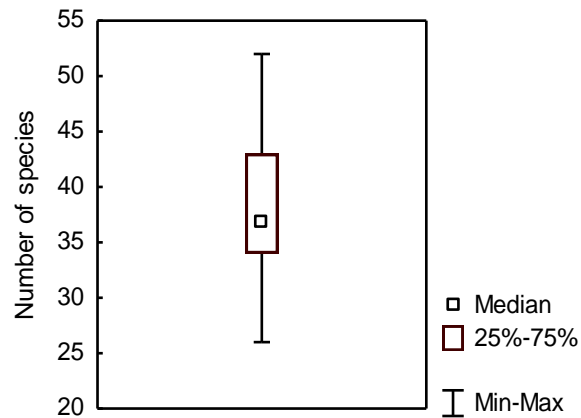
Figure 7: Median, 25th and 75th percentiles, minimum and maximum value of number of species, in all sites sampled (15 sites), along a climatic gradient in Alentejo region.

Castro et al. (2010) found a lower number of plant species (75 species) in plots under extensive grazing and submitted to a climate slightly drier than ours (MAP=438mm and MAT-mean annual temperature=16.8ºC). This could be due to the fact that in the mentioned study only 3 sampling sites were used to measure plant diversity while we measured in 15 different sampling sites. Castro et al. (2010), found a lower median of species per plot (25) ranging from 15 to 38 species based on the observation of 15 plots per site, each with 0.25m^2. Castro et al. (2010) used an area-based method. Nevertheless our driest sites (MAP=521-526mm) showed comparable number of species (26-37) suggesting that climate is an important variable driving biodiversity in this ecosystem.

Under similar climate, Bugalho et al. (2011), found lower plant species (53) than the ones found in this work. The latter author evaluated less plots/sites (5 against 15 sites measured in our study) and had lower total number of intercept points (1440 against 3690) than the ones used in this study. A lower median number of species among the grazed plots (22 species, from 13 to 26) was also found in the study of Bugalho et al. (2011) compared to our results. The authors used the pin-point quadrat method, in which needles are positioned in plots using a frame, with 288 intercept points per plot and a total of 1440 intercept points in grazed plots.

We suspect that distance between intercept points is lower than the used in the present study (0.5m), because frames have 9 needles, positioned 8 times in sub-plots with 2 x 4m. Thus, the lower number of species per plot can probably be due to smaller distance between intercept points, capturing probably lower spatial heterogeneity. One of the main characteristics of drylands is their increasing spatial heterogeneity with increasing aridity. Thus having methods that enable to capture the spatial heterogeneity is of high interest (Kefi et al. 2007).

3.1.2. Diversity of families and of functional groups

Of the 5990 plants collected in 251 hits (4.2%) was not possible to identify plants at the species level. Most of the unidentified individuals were grasses without flowers (88.04% of all unidentified hits), so it was only possible to identify to the family level. The families with higher number of species were *Gramineae*, *Leguminosae* and *Compositae* (Table 3). These three families are also dominant in terms of relative cover in the community (more than 70% except in one site, where it was only 57%).

Table 3: Number of species per family found in 15 sites sampled along a climatic gradient in Alentejo region.

Family	Nr species	Family	Nr species
Gramineae	37	Rubiaceae	2
Leguminosae	29	Boraginaceae	1
Compositae	22	Convolvulaceae	1
Caryophyllaceae	12	Cyperaceae	1
Plantaginaceae	6	Euphorbiaceae	1
Cistaceae	5	Gentianaceae	1
Geraneaceae	4	Guttiferae	1
Scrophulariaceae	4	Isoetaceae	1
Brassicaceae	3	Juncaceae	1
Polygonacea	3	Linaceae	1
Labiatae	2	Primulaceae	1
Campanulaceae	2	Umbelliferae	1
Liliaceae	2		

Of all species identified, 138 are herbaceous and 8 are shrubs. The number of species (fig.: 8A) and the relative cover (fig.: 8B) of herbaceous plants was much higher than that of shrubs. On average, there was only 1 shrub and 3.6% of the shrub cover per site. The herbaceous layer clearly dominates, with an average of 35.6 species and 96.1% of relative cover per site. This low abundance of shrubs is expected in *Montado*, since shrubs are intentionally cleared out for the pastures maintenance (Castro and Freitas 2009). The study of Castro (2008), in the same region and ecosystem, with similar climatic features (MAP of 438mm and MAT of 16.8ºC), found that herbaceous species had a relative cover of more than 90%, similar to what we found in our work.
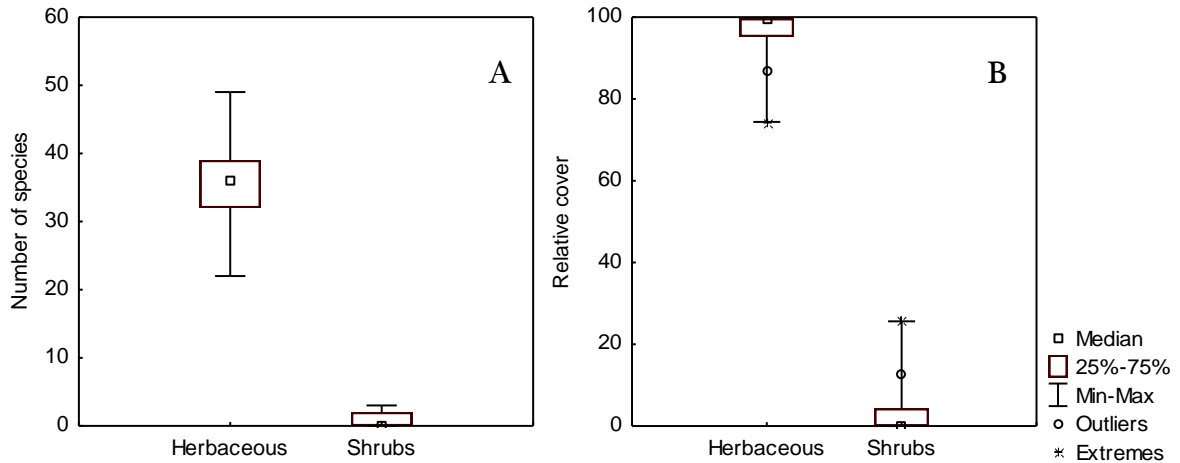
Figure 8: Median, 25th and 75th percentiles, minimum and maximum, outlier and extreme values of A: number of species and B: relative cover percentage of herbaceous and shrub understory, in 15 sites sampled along a climatic gradient in Alentejo region.

Regarding the herbaceous understory, 99 species have annual life cycle, 17 are facultative biennials (i.e. with annual or biennial life cycle) and 22 are perennials. On average, there were 24.1 annual, 5.9 annual or biennial, and 4.9 perennial species per site (fig:.9). In terms of relative cover, annuals represented on average 64.5% of the plant community, annual or biennials 18.8% and the perennials 12.6%.

Many studies in grasslands consider species with annual and facultative biennial life cycles all together (e.g. Kutiel et al. 1998), for this reason the following discussion does not differentiate between annual and facultative biennial species. Our results show that their joint mean relative cover is 83.3%.

The previously mentioned study of Castro (2008) found a slightly higher value of 97.4% of annual species in sites with extensive grazing. In Spain, the study site of Azcarate et al. (2002), located near Madrid with similar ecosystem, land-use and climate (MAP of 450-500mm and MAT of 13ªC), is also dominated by annual species.
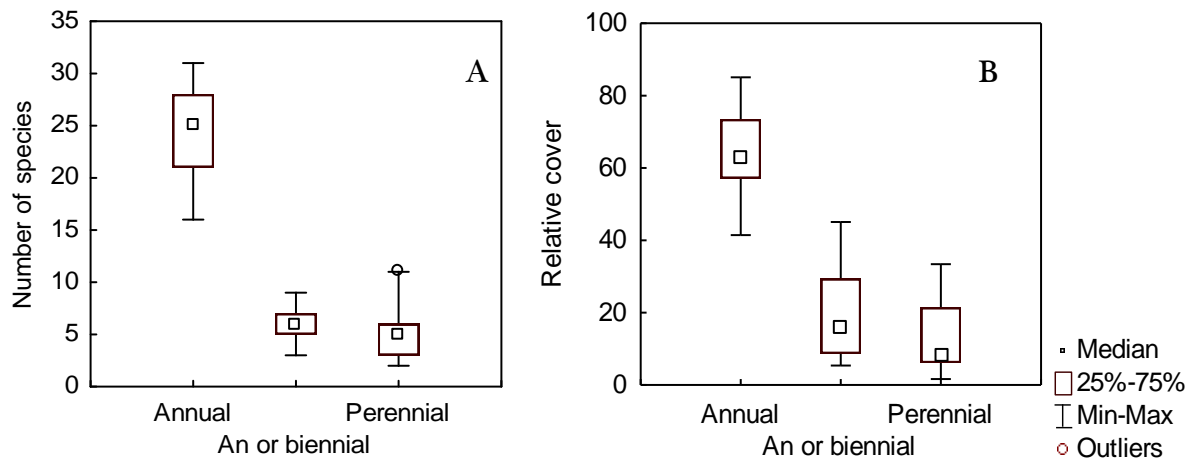
Figure 9: Median, 25th and 75th percentiles, minimum and maximum, and outlier values of annual, facultative biennial and perennial herbaceous species in terms of A: number of species and B: relative cover in the community, in 15 sites sampled along a climatic gradient in Alentejo region.

Sternberg et al. (2000) found a relative cover of 74% in Israel, in a site with Mediterranean climate (MAP of 570mm and MAT of 18ºC) under different treatments of grazing. Since these studies are all located in grasslands with Mediterranean climate, with similar ecosystem and land-use, and are all dominated by annual species, it seems that annual species is a frequently dominating group in grasslands across different regions with Mediterranean climate with extensive grazing land-use. Species with this life cycle have the ecological advantage of growing and reproducing during the favorable conditions of the growing season and spend the dry hot summer that characterizes the Mediterranean climate in a seed form (Cain 1950, Noy-Meir 1973).

Figure 10 shows the number of species (fig. 10A) and relative cover (fig.: 10B) of the main families present in the sampled sites. The *Graminae* family dominates either in number of species (mean 13.3) and relative cover (mean 51.8%), followed by the *Compositae* family (mean number of species 7.1 and mean relative cover 18.6%). The *Leguminosae* family, in comparison with the other families present, has the widest range of variation on the number of species (mean 5.7), but in terms of relative cover varies little (mean 6.9%) (Figure 10).

Caryophillaceae and *Plantaginaceae* are both families with little representation (mean relative cover 3% and 9.2%, respectively). Although mean relative cover of *Plantaginaceae* is higher than legumes, Plantaginaceae species are not present in 3 sites sampled, while legume species are present in all sites. *Cistaceae* family, which in these sites is mainly composed of shrub species (only one herbaceous species), has a low relative cover, something expected in *Montado* land use where shrubs are intentionally cleared out for the pasture maintenance (Castro and Freitas 2009). The remaining families were analyzed together in the category 'Other families'. Their relative cover is very low (mean 5.2%) given the considerable number of species included.
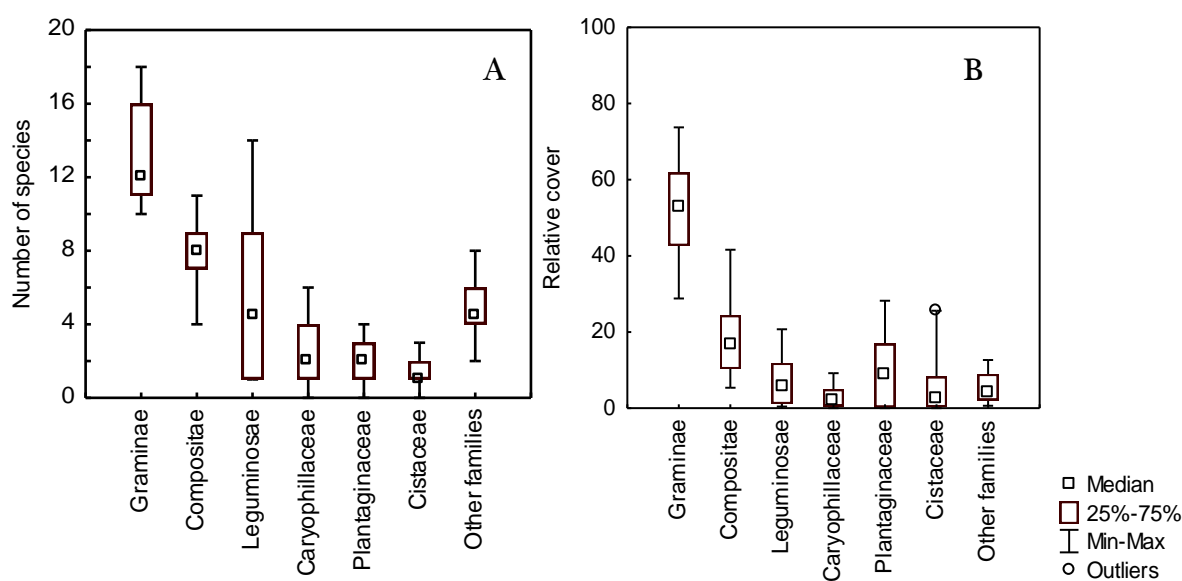
Figure 10: Median, 25th and 75th percentiles, minimum and maximum and outliers values of community composition among the study sites regarding families, in terms of A: number of species and B: relative cover percentage.

The three more abundant families in the present study were *Graminae*, *Compositae* and *Leguminosae*. These three families were also found to be abundant in other studies performed in *Montado* ecosystem in Portugal, with similar climatic features (Castro 2008, Bugalho et al. 2011). In Bugalho et al. (2011) grasses were the dominant family and represented more than 60% of plant

cover. In Castro (2008) the dominant family was *Leguminosae*, followed by *Compositae* and *Graminae*, but the sampling sites included not only sites with grazing land-use but also abandoned sites in a later stage of succession (10 to 20 or more years since abandonment), with higher shrub cover (20 to 75%). *Caryophillaceae* and *Cistaceae* were also present.

3.2. Analysis along the climatic gradient

In this section plant community is analyzed along the climatic gradient, by performing correlations between plant variables and climatic variables. Response patterns of species richness and plant cover (section 3.2.1), functional groups (section 3.2.2) and measured traits (section 3.2.3) are evaluated. The climatic variables considered are described in table 2. One site was excluded from all Spearman analysis (site ID 7, appendix 1) because it was the only site ungrazed.

3.2.1. Species richness and plant cover

The percentage of *No plant cover* significantly decreases with increasing precipitation and increases with long-term temperature. The standard deviation of *No plant cover* among the 6 transects of each site also significantly and negatively correlated to long-term precipitation and aridity index. Plant cover significantly increases with precipitation and negatively with long-term temperature but has stronger correlations (higher coefficient) with aridity index and long-term precipitation (table 4). Figure 11 shows relation of both plant cover and *no plant* percentage with the aridity index.

Table 4: Spearman's rank correlation coefficients at P<0.05 for no plant cover, percentage and standard deviation (SD), plant cover and species richness (Nr sp) of 14 sites sampled along a climatic gradient in Alentejo region. Ns= non-significant. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation

(mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000).

	No plant		Plant	
	Cover (%)	SD	Cover	Nr sp
Arid. Idx	-0.73	-0.56	0.79	0.69
LT Prec	-0.70	-0.60	0.76	0.72
MT Prec	ns	ns	ns	0.72
ST Prec	-0.53	ns	0.66	0.75
LT Temp	0.61	ns	-0.62	ns
TColdM	ns	ns	ns	0.64

Various studies under different conditions found increasing plant cover, or decreasing bare soil cover, with increasing precipitation along spatial climatic gradients. Kutiel et al. (1998) in eastern Mediterranean, along a wider climatic gradient from Mediterranean climate to extreme arid (MAP 620-120mm and MAT 17-23ºC) found decreasing plant cover towards more arid sites. In Spain de Bello et al. (2007) studied different grazing cover along a climatic gradient, (MAP 922-325mm and MAT 7.1-15.2ºC), and concluded that grazing caused a higher bare soil percentage in more arid areas than in more humid areas. In China, Deng et al. (2006) studied a dryland, (MAP 346-192mm and MAT 9.1-8.2ºC), in shrub-dominated communities, where total plant cover decreased from 96 (more humid site) to 37% (drier site).

An example of a temporal precipitation gradient is the work of Collins et al. (2012), who studied grasslands in Kansas, USA, under an experiment simulated scenario of increasing precipitation during a 19-year period from 835 to 1108mm of precipitation, showed that total plant cover increased significantly in comparison to control.
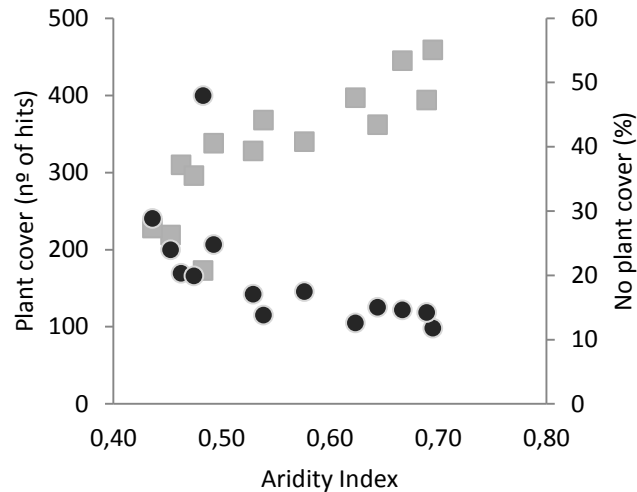
Figure 11: Bi-plot of plant cover (grey quadrats) and no plant cover (black dots) with the aridity index (calculated with data from Y1960-1990).

Species richness significantly increases with several precipitation variables and with minimum temperature of the coldest month (Table 4 and fig:.12).

Other studies showed similar results with species richness increasing with increasing precipitation conditions. Holzapfel et al. (2006), studying herbaceous-shrubs interactions along a climatic gradient in Mediterranean (MAP 780-90mm and MAT 18.1-19.1ºC), found decreasing herbaceous species richness towards more arid sites. In North-American grasslands, Adler and Levine (2007), also found decreasing species richness in a spatial precipitation gradient (MAP 835-321mm).

It is interesting to note that results in the present study for plant cover and species richness are in accordance to the papers discussed, considering the differences between precipitation gradients: the present precipitation gradient has a MAP from 521 to 634mm, a difference of only ca. 110mm, while Adler and Levine (2007), for example, the difference is ca. 510mm. Our results show that these patterns (decreasing plant cover and species richness) may also occur in less pronounced gradients as the one shown in this work. These results show the potential of this type of vegetation to respond to climate and consequently their potential as ecological indicator of climatic gradients.
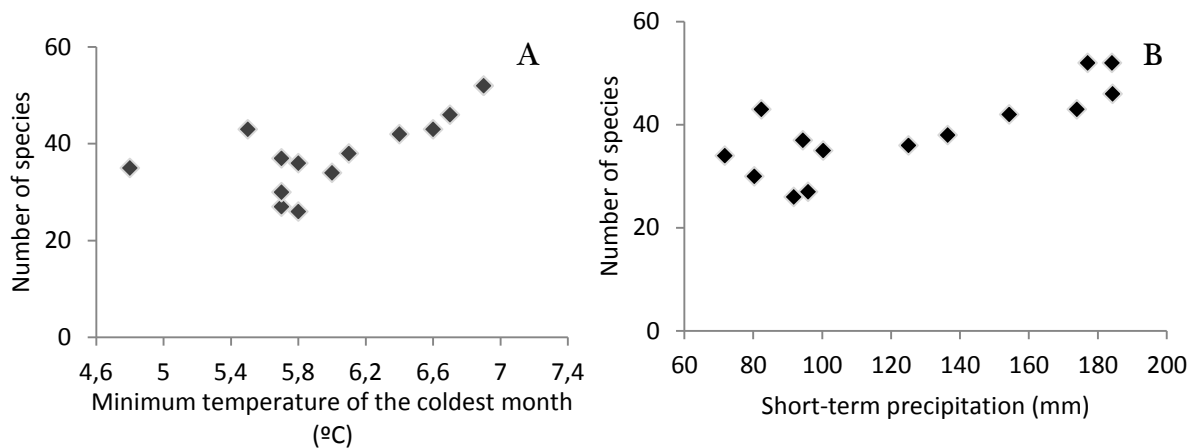
30

Figure 12: Bi-plots between measured number of species and A: minimum temperature of the coldest month (mean from Y1950-2000); B: short-term precipitation (mean monthly precipitation from Oct 2011 – Mar 2012). Plant community was sampled in 14 sites along a climatic gradient in Alentejo region.

Increased plant diversity seems to be favored by higher recent precipitation events and higher lower temperatures of the coldest month (Table 4 and fig:.12). The increase in richness might only depend on germination and emergency and for that, punctual phenomena of optimum conditions, such as, precipitation pulses might be enough to create new niches where new plants can germinate. Whereas the changes in plant cover are more associated to long-term phenomena that occur during all growing season. This seems reasonable if we think that for having a substantial cover of plants it is necessary that the plant not only successfully germinate and emerge but also establish and grow during all the growing season with their optimum conditions of temperature and precipitation.

Both plant cover and species richness seem to have the potential to be used as ecological indicators of climate since they show trends with it. Different habitats in the same climatic region can have completely different values of plant cover and of species richness. Thus this type of indicators can only be used to compare the same type of habitat not having the potential to a widely used ecological

indicator. One of the 11 indicators that was proposed by UNCDD (United Nations for Combating Desertification and Soil Degradation) to be applied all over the world to evaluate the level of desertification at each country was the diversity of plant species (Berry et al. 2009). In this work we suggest that such an indicator does not have the potential to be compared everywhere. We suggest that functional diversity has more potential to be universal since it is not species specific dependent. Thus, the next steps of this work will be to further test functional diversity as an ecological indicator along a climatic gradient.

3.2.2. Functional groups

The following analyses attempt to assess plant community along the climatic gradient, using a functional approach. Plant species were classified in a series of functional groups regarding life form, life cycle. Species richness, plant cover and relative cover in the community (%) of these functional groups were evaluated. Plant cover corresponds to number of intercept points were a functional group was hit, what enables to study functional groups' plant cover fluctuations along the climatic gradient. Relative cover in the community, being a percentage variable, gives a better insight of community composition Additionally, analyzing community in terms of percentage helps to compare with other studies with different methods or in different communities.

Table 5 shows the list of traits for which information was found for all species present in this study but a complete list can be found in Appendix 2. The traits "photosynthetic pathway" and "clonality" showed very little variation among the species present thus were not analyzed. For "height", measured values were chosen to be analyzed instead of the categorical ones. Based on previous references on the effect of climate on plant trait we proposed to analyze as a priority the following functional groups: life form, life cycle, height (de Bello et

al. 2005, Garnier et al. 2007). Analyzes for "growth form", "flower duration" and "dispersal mode" are not shown in this work.

The limitations have to do mostly with: i) a small number of traits and species available in the data bases; ii) data bases are often region specific (Gaucherand and Lavorel 2007, Lavorel et al. 2007b); iii) we need to use the genus for our local flora. Floras and papers concerning regional vegetation were also used to ensures more precise information.

Table 5: Traits compiled from the literature for the species present in the precipitation gradient. Traits analyzed are highlighted.

Abbrev.	Trait's name	Classes	Sources
Gr_form	Growth form	Rosette	(Alday et al. 2011)
		Semi-basal (partial-rosette)	(Ehleringer et al. 1997)
		erect leafy	(Liu et al. 2003)
		tussock	(Liu et al. 2011)
		climber	(Singsaas et al. 2001)
Li_form	Life form	Therophyte	(Still et al. 2003)
		Hemicryptophyte	(Vogel et al. 1986)
		Geophyte	(Waller and Lewis 1979)
		Camephyte	(Wang 2002)
		Phanerophyte	(Wang 2003)
		Variable	(Bonet and Pausas 2004)
Li_cycle	Life cycle	Annual	(Castro 2008)
		Annual or biennial	(Coca and Pausas 2012)
		Perenial	(Luna and Moreno 2009)
		Variable	(Porto et al. 2011)
Height	Height	Short (<40cm)	(Valdés et al. 1987)
		Medium (40-80cm)	(Castroviejo 1986-2012)
		Tall (>80cm)	(Franco 1971, 1984)
Ph_path	Photosynthetic pathway	C3	(Franco and Afonso 1994, 1998, 2003)
		C4	(Kleyer et al. 2008)
		CAM	(Paula et al. 2009)
Clonal	Clonality	Non-clonal	(Clayton et al. 2006 onwards)
		Short-rhyzomes	(Green 2009)
		Stolons	
B_flower	Onset of flowering	January to December	
D_flower	Flowering duration	Nr of months flowering lasts	

Dis_mode	Dispersal mode	Anemochory	
		Barochory	
		Ectozoochory	
		Endozoochory	
		Combinations	

The following analyses aim at assessing plant community along the climatic gradient, using a functional approach. Plant species were classified in a priori functional groups regarding life form and life cycle. Species richness, plant cover and relative cover in the community (%) of these functional groups were evaluated. Plant cover corresponds to the number of intercept points were a functional group was hit, what enables to study functional groups' plant cover fluctuations along the climatic gradient. Relative cover reflects species or groups' relative dominance in the community and thus gives a better insight of changes in community composition as a whole. Additionally, analyzing community in terms of percentage helps to compare with other studies assessed with different methods or in different communities.

3.2.2.1. Life form

The plant community sampled in this study comprised 94 terophytes, 22 hemicryptophytes, 9 phanerophytes, 3 geophytes and 1 chamaephyte. Eleven species were classified as variable. In accordance, Castro (2008) shows that terophyte and hemicryptophyte life forms are highly associated with extensive grazing land-use, in a study performed in *Montado* ecosystem, under similar climatic conditions. Species with annual or biennial life cycle are classified by some sources as terophyte, and by others as hemicryptophyte and we chose to use hemicryptophyte. Because the values for geophytes and chamaephytes were very low, both in number and in cover, analyzes for these groups are not presented. Species richness and plant cover of phanerophytes and variable species showed not significant correlations with all climatic variables (data not shown).

Phanerophytes, as well as chamaephytes, may be influenced by the *Montado* land-use where shrubs are cleared (Castro and Freitas 2009).

Table 6: Spearman's rank correlation coefficients at P<0.05 for species richness, total cover and relative cover of functional groups based on life form. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life forms: TR = terophyte; HM = hemicryptophyte; Other = other life forms present, namely therophytes, phanerophytes and species classified as variable.

	Nr species		Total cover		Relative cover (%)	
	TR	HM	TR	HM	Other	HM
Arid. Idx	0.73	0.72	0.55	0.61	ns	0.54
LT Prec	0.77	0.76	ns	0.62	ns	0.57
MT Prec	0.69	0.61	ns	0.75	-0.69	0.75
ST Prec	0.70	0.78	ns	0.71	-0.62	0.71
LT Temp	ns	-0.69	ns	ns	ns	ns
TColdM	0.62	0.56	ns	0.66	-0.7	0.64

Both terophytes and hemicryptophytes increase their species richness with precipitation and with temperature of the coldest month, but only hemicryptophytes seem to be affected by increasing long-term temperature (mean annual temperature) (table 6). Terophyte cover correlates poorly with the climatic variables, whereas hemicryptophyte cover has, in turn, strong positive correlations with precipitation variables.

Relative cover (%) of terophytes, phanerophytes and variable species was joined (Other in table 6) and shows a community shift between this group and hemicryptophytes. With increasing short-, medium- precipitation, relative cover (%) of hemicryptophytes increases, while joined relative cover (%) of terophytes, phanerophytes and variable species decreases.

3.2.2.2. Life cycle

Although Raunkiaer's life forms are, to some point, related to life cycle, functional groups only based on life cycle were also analyzed. Herbaceous vegetation was classified as annual, facultative biennial (species with annual or biennial life cycle) and perennials; results are shown in table 7. Annual and facultative biennial species were analyzed together since the number of species classified as facultative biennial is low and other studies frequently don't make a distinction between these life cycles (e.g. Castro 2008).

Table 7: Spearman's rank correlation coefficients at P<0.05 for species richness, total cover and relative cover (%) of functional groups based on life cycle. Plant community sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An + Bn = annual and facultative biennial species; Pn= perennial species.

	Nr species		Total cover		Relative cover (%)	
	An+Bn	Pn	An+Bn	Pn	An+Bn	Pn
Arid. Idx	0.62	ns	0.53	ns	ns	ns
LT Prec	0.66	ns	ns	0.52	ns	ns
MT Prec	0.61	ns	ns	0.69	ns	ns
ST Prec	0.59	ns	ns	0.71	ns	ns
LT Temp	ns	ns	ns	ns	ns	ns
TColdM	0.52	ns	ns	0.65	ns	ns

The species richness of annual species and facultative biennial species increases with increasing precipitation and increasing minimum temperature of the coldest month. Perennial species' richness showed no significant correlations with any climatic variables. Cover of annual and facultative biennial species correlates significantly and positively with the aridity index, but this correlation is

weaker and no correlations are observed with other climatic variables. This suggests that cover of annual and facultative biennial species does not vary significantly along the climatic gradient.

Cover of perennial species increases with precipitation and minimum temperature of the coldest month, and showed to have higher correlation coefficient with short-term precipitation. This is interesting since perennial species seem to reflect better the recent climate and thus can act as a responsive ecological indicator of more recent conditions. By analyzing relative cover (%) of these two groups, it is shown that there is no significant community shift along the climatic gradient among annual, biennial and perennial species.

3.2.2.3. Families and life cycle

It is known that traits may have different responses not only among functional groups, but also within groups (Lavorel et al. 1999, de Bello et al. 2005). Additionally, studying several traits together can enhance trait-climate relationships (Barboni et al. 2004). In this section, it was tested if different life cycles would have contrasting responses within families. Although family is not a trait or functional group itself, we considered that families gather species with similar traits or trade-offs among traits. We analyzed *Compositae*, *Graminae* and *Leguminosae*, since those are the families with higher number of species (see Fig. 9A and Table 3). Grasses are frequently analyzed apart from forbs (Lavorel et al. 1999, Bonet 2004, de Bello et al. 2006). Among forbs, it was chosen to separate legumes, because of their N-fixing ability, and the *Compositae* family, because its large numbers enables this family to be analyzed separately from other forbs with lower risk of data deficit.

In the further following analyses facultative biennials were integrated in the annual species group. Thus, the following analyses have three categories: annuals,

perennials and "total". "Total" category joins annuals and perennials, to verify if correlations of both groups together are stronger.

Compositae

In general, the *Compositae* family is poorly related with climatic variables (table 8). Richness and cover of annual species are not significantly correlated with any climatic variable. Perennial species richness shows a trend to increase with long-term precipitation, a pattern that is still verified in the "total" group. Perennial species richness and cover decreases with long-term temperature, but for the total groups these correlations are not significative.

Table 8: Spearman's rank correlation coefficients at P<0.05 for species richness and plant cover of *Compositae* species sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An = annual; Pn= perennial; Tot= total.

	Nr species			Species cover		
	An	Pn	Tot	An	Pn	Tot
Arid. Idx	ns	0.54	ns	0.56	ns	0.56
LT Prec	ns	0.59	0.55	ns	ns	ns
MT Prec	ns	ns	ns	ns	ns	ns
ST Prec	ns	ns	ns	ns	ns	ns
LT Temp	ns	-0.63	ns	ns	-0.64	ns
TColdM	ns	ns	ns	ns	ns	ns

It is not clear if life cycle is an important feature among the *Compositae* species, because of the large number of non significant correlations. However, these results suggest that different life cycles don't have contrasting responses to climate within this family. In resume it seems that *Compositae* family as a group

does not have the potential to work as an ecological indicator along climatic gradients.

Graminae

Annual grasses richness and cover do not vary along the climatic gradient (Table 9). This group has no significant correlations with any climatic variable. On the other hand, perennial species richness and cover increase with increasing precipitation. This result is in accordance to what was found by Clary (2012). This author studied perennial grass cover at grasslands sites across three latitudinal belts, from coastal California inland to the Central Valley, relating perennial presence to environmental variables. Sites with greater May–September precipitation (i.e., less strongly "Mediterranean" seasonality) showed higher perennial cover than the other.

Among the total group the previously observed correlations are no longer significant, suggesting that annual and perennial grasses have contrasting response patterns. A possible reason for this difference could be associated with plants' ability to explore water resources: Harris and Wilson (1970) reported a faster root development among annual grasses, which would effectively compete with slower root growth perennial grasses for water resources.

Perennial grasses, both in number of species and in cover, seem to be a potential ecological indicator of climatic gradients.

Table 9: Spearman's rank correlation coefficients at P<0.05 for species richness and plant cover of *Graminae* species, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An = annual; Pn= perennial; Tot= total.

	Nr species			Species cover		
	An	Pn	Tot	An	Pn	Tot
Arid. Idx	ns	0.62	ns	ns	ns	ns

LT Prec	ns	0.66	ns	ns	0.53	ns
MT Prec	ns	0.70	ns	ns	0.71	ns
ST Prec	ns	0.85	ns	ns	0.74	ns
LT Temp	ns	ns	ns	ns	ns	ns
TColdM	ns	0.80	ns	ns	0.64	ns

Leguminosae

Table 10 shows significant correlations for legumes. Annual legumes have many significant correlations with climatic variables. Annual species richness and cover respond positively to precipitation and negatively to long-term temperature, but stronger correlations are with the aridity index. This may suggest that annual legumes are highly associated not only with water availability, but also with other site conditions, which could be soil nutrients or seed bank diversity, since this association might suggest that legumes are influenced by long-term processes that occur at the ecosystem level.

Perennial herbaceous legumes are not shown because this group was absent among the sampled sites. Legume shrubs were rare among the sampled sites, and are restricted to 3 species. This explains the non significant responses of this group both in species richness and cover.

Table 10: Spearman's rank correlation coefficients at P<0.05 for species richness and plant cover of legume species sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Life cycle: An = annual; Sb = perennial shrubs; Tot = total.

	Nr species			Species cover		
	An	Sb	Tot	An	Sb	Tot
Arid. Idx	0.83	ns	0.82	0.77	ns	0.58
LT Prec	0.81	ns	0.80	0.69	ns	ns
MT Prec	0.73	ns	0.74	0.56	ns	ns
ST Prec	0.71	ns	0.70	0.60	ns	ns
LT Temp	-0.60	ns	-0.57	-0.67	ns	ns

TColdM	0.79	ns	0.80	0.72	ns	0.72

In terms of species diversity, response patterns are similar between annuals and total groups. However, this pattern was not observed for cover. The total group showed much less significant responses than annuals, suggesting that shrub cover does not show the same response trend than annuals. Annual legumes, which show a strong response pattern along the climatic gradient, have the potential to be further analyzed as ecological indicator of climatic gradients.

Considering the overall results of the previous section (3.2.2.4) we hipotyzed that a community shift along the climatic gradient could be observed when plant community was classified by family and life cycle. Additionally to the previously evaluated families (Compositae, grasses and legumes) other relatively abundant (Fig. 10) families were shown to vary considerably, namely *Plantaginaceae* (which in this study are all *Plantago* sp., data not shown) and *Caryophillaceae* species (Fig. 13).

Figure 13 shows plant community composition of the wettest and the driest sites according to long-term precipitation.
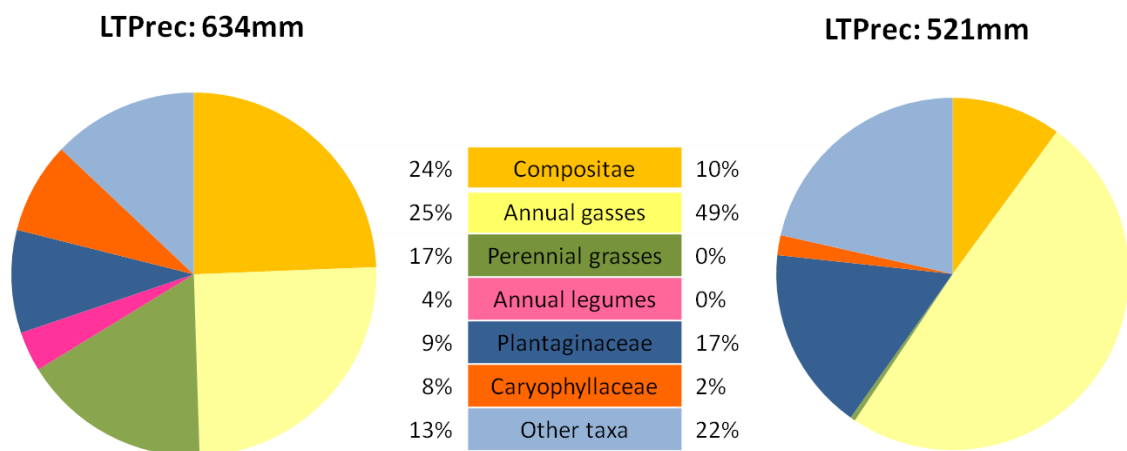
24%	Compositae	10%
25%	Annual gasses	49%
17%	Perennial grasses	0%
4%	Annual legumes	0%
9%	Plantaginaceae	17%
8%	Caryophyllaceae	2%
13%	Other taxa	22%

Figure 13: Plant community composition of the more humid and the driest sites according to Medium-term Precipitation, i.e. mean annual precipitation from years 1998-2011 (MTPrec), using relative cover data of a sampling performed in 14 sites along a climatic gradient in Alentejo region.

Based on all the previous information and other exploratory analysis we proposed an indicator based on community shift between groups of species that were shown to significantly related with all climatic variables (Table 13). In resume, with increasing aridity also increases relative cover in the community (%) of the group composed by annual grasses and *Plantago* species, while relative cover (%) of a group composed of perennial grasses, annual legumes and *Caryophillaceae* species decreases.

Table 11: Spearman's rank correlation coefficients at P<0.05 for relative cover in the community of family and life-cycle groups, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000). Groups: An Gram= annual grasses; Pn Gram= perennial grasses; An Legu= annual legumes; Plantago= *Plantago sp.*; Cary= *Caryophillaceae*.

	Cover in community (%)	
	An Gram + Plantago	Pn Gram + An Legu + Cary
Arid. Idx	-0.71	0.69
LT Prec	-0.72	0.68
MT Prec	-0.74	0.85
ST Prec	-0.61	0.79
LT Temp	0.60	-0.54
TColdM	-0.78	0.77

Figure 14 shows community composition along the climatic gradient. In more humid sites (higher long-term precipitation) relative cover of annual grasses and

Plantago species is approximatly 40% and relative cover of perennial grasses, annual legumes and *Caryophyllaceae* species is around 30%.

In drier sites (lower long-term precipitation) relative cover of these two groups is more dissimilar: annual grasses and *Plantago* sp occupy around 60% of plant community while perennial grasses, annual legumes and *Caryophillaceae* species occupy only around 10% of the plant community. It is interesting to notice that around 600 mm of long-term precipitation the shift in these two groups of plants is most obvious (Fig.14).
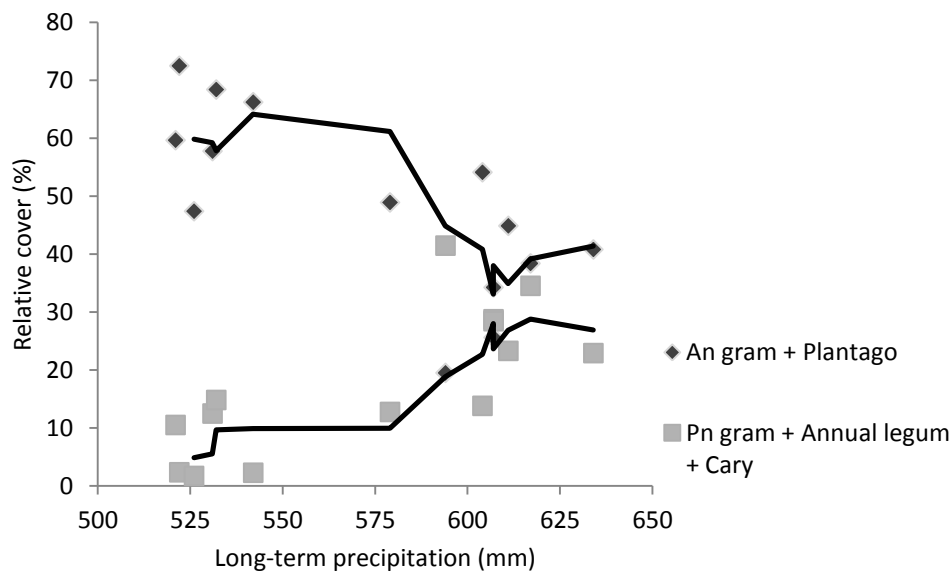
Figure 14: Relative cover in the community of functional groups considered in table 12 along sites with increasing long-term precipitation (mean annual precipitation from period 1960-1999). Plant community was sampled in 14 sites along a climatic gradient in Alentejo region. Line represents a moving average of two measures.

In accordance with results of the present study, Azcarate et al. (2002), in Spanish *Q.* ilex open woodlands with similar climate, found that perennial grasses and *Trifolium* species (legumes) were abundant in more moist sites along an altitudinal-moisture gradient with MAP around 450-500mm and MAT of ca. 13ºC. A recent work of Collins et al. (2012), which studied the effects of increasing precipitation (manipulative approach) over a 19-year long experiment in

American grasslands dominated by perennial grasses (natural MAP of 835mm, experienced MAP of 1108 mm), found increasing cover of perennial grasses, and this increase was more marked in drier upland sites.

Acosta et al. (2008) studied functional diversity in grasslands with extensive grazing land use along an altitudinal gradient of 1100m that leaded to a soil moisture gradient, in Spain. In the mentioned study, perennial species (including the perennial grass *Agrostis castellana* Boiss. & Reut., also present in our study) and horizontal growth species (in which legumes can be included) were associated with high altitude sites, where soil moisture was higher, while annual species (including some annual grasses like *Vulpia* sp, also present in our study) and species with basal rosettes (like *Plantago* sp), were typical of low altitude, drier sites.

Supporting the annual grass and *Plantago* species association made in the present study Ansquer et al. (2009) found that a set of weighted plant traits of grasses and rosette forbs (a growth form shared among *Plantago* species) where closely related. These traits, namely plant height and plant Nitrogen (N) and Carbon (C) content, are related to capture and use of resources and plant competitiveness. The mentioned study was performed in grasslands with livestock farms, but climatic conditions were different from the present study: sites were located in the Pyrennes, with MAP of 1080mm and MAT of 10ºC (5 years' data).

The discussion with other works performed in the *Montado* ecosystem are not useful since most studies are related to land-use change and aimed at studying the effects of abandonment (cessation of ploughing and sowing) (Lavorel et al. 1999, Castro and Freitas 2009, Castro et al. 2010, Bugalho et al. 2011).

The main results concerning functional groups found in this work seem to be in accordance to the ones performed in other countries namely the ones with Meaditerranean climate. This suggests that there is potential that the selected functional groups of species found in the present work, namely perennial grasses, annual legumes and *Plantago* species can be further tested for their universality.

3.2.2.4. Non-metric Multivariate Dimensional Scaling

To test whether plants species associated themselves in relevant functional groups a Non-metric Multivariate Dimensional Scaling (NMDS) was performed. In the graphical representation, in figure 15, drier sites (sites are represented by number sorted in ascending order of long-term precipitation) were placed on the left quadrants, with the three driest sites (lower long-term precipitation) located under -0.5 in the first axis. The more humid sites were placed in the right quadrants, but its location in more scattered.

Figure 15: Non-metric Multivariate Dimensional Scaling (NMDS; first vs. second axes) of cover of the 21 most dominant species and sites. Distance measure used was Bray Curtis and stress was 0.097. Numbers indicate sites sorted in ascending order of long-term precipitation (as in Appendix 1). Simbols were placed manually on the left of the species name to indicate species assignment to functional group or family considered in previous results. Apour= Agrostis pourretii Willd.; Bdyst= Brachypodium distachyon (L.) P.Beauv.; Bhord= Bromus hordeaceus L.; Ccapi= Crepis capillaris (L.) Wallr.; Cdact= Cynodon dactylon (L.) Pers.; Cmixtu= Chamaemelum mixtum (L.) All; Crace= Carlina racemosa L.; Gfragi= Gaudinia fragilis (L.) P.Beauv.; Hglab; Hypochaeris glabra L.;

Lgall= Logfia gallica (L.) Coss. & Germ.; Lrigi= Lolium rigidum Gaudin; Ltara= Leontodon taraxacoides (Vill.) Mérat; Ocomp= Ornithopus compressus L.; Pcoro= Plantago coronopus L.; Plago= Plantago lagopus L.; Spurp= Spergularia purpurea (Pers.) G.Don; ; Tbarba= Tolpis barbata (L.) Gaertn; Tgutt= Tuberaria gutatta (L.) Fourr.; Vcili= Vulpa ciliata Dumort.; Vgeni= Vulpia geniculata (L.) Link; Vmyur= Vulpia myuros (L.) C.C.Gmel.

Note that the NMDS considered only the 21 dominant species (due to graphical/visual issues), while previous results include all species present in 14 sampled sites. *Plantaginaceae* species (namely *Plantago lagopus* L. and *P. coronopus* L.) are located near the dry sites, among several annual grasses (e.g. *Bromus hordeaceus* L., *Brachypodium dystachion* (L.) P.Beauv.). However, other annual grasses (e.g. *Vulpia myurus* (L.) C.C.Gmel. and *Gaudinea fragilis* (L.) P.Beauv.), are scattered along the first axis as well as *Compositae* species (e.g. *Hypochaeris glabra* L. and *Logfia gallica* (L.) Coss. & Germ.), in accordance with the non significant results of cover for these two groups (tables 7 and 8). In the "humid side" we find an annual legume (*Ornithopus compressus* L.), a perennial grass (*Cynodon dactylon* (L.) Pers.) and a *Cistaceae* herb species (*Tuberaria guttata* (L.) Fourr.). However, the *Caryophyllaceae* species (*Spergularia purpurea*) is located in the "dry side", while previous results (table 12) showed that this group cover increases with precipitation. Overall, the results for species are in accordance with previous results, despite the small number of species considered. Shepard plot showed a linear fit of R^2=0.949 and non-metric fit R^2= 0.991. The first axis correlates with all climatic variables considered in this work. Correlations are positive with the aridity index, precipitation variables and minimum temperature of the coldest month and negative with long-term temperature, as shown in table 15. The second axis did not correlate with any climatic variable considered.

Table 12: Spearman's rank correlation coefficients at P<0.05 for scores of first and second axes of NMDS presented in figure 11.

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	NMDS scores	
	First axis	Second axis
Arid. Idx	0.79	ns
LT Prec	0.79	ns
MT Prec	0.78	ns
ST Prec	0,67	ns
LT Temp	-0.69	ns
TColdMonth	0.73	ns

Despite some accordance of species assemblage and previous functional groups that showed a community shift in table 12, the ordination analyses highlighted the fact that species belonging to the same functional group may have different distribution patterns along the climatic gradient, and that some functional groups made a priori (namely annual grasses and *Compositae*) may be too comprehensive, gathering species with different response patterns and traits. In fact, there are annual grasses and annual *Compositae* species in both sides on the first axis. It would be necessary to study more traits to define smaller groups within these broad groups.

These ordination analyses may also enable the opposite approach, with *a posteriori* functional classification based on the assumption that species graphically close to each other have similar response pattern, possibly because these species have similar traits, and could, therefore, constitute a functional group. More time would be needed to disclose the common traits that might associate species together.

3.2.3. Measured Traits

3.2.3.1. Biomass and Height

Biomass is often referred in literature as an ecosystem property at the community level (Roscher et al. 2012) and more seldom, as a trait at the plant level (Cornelissen et al. 2003). This is probably due to the fact that it is considered

as a hard trait because its laborious measurement, although it has proved large-scale ecological significance and can provide important information, especially in combination with other traits (Cornelissen et al. 2003). Therefore, biomass was included in these results both at the specific and functional group level, to enable the comparison of functional group's and dominant species' response patterns. Table 13 summarizes the observed patterns among functional groups and dominant species for cover, biomass and height, based on correlations tested for long-, medium- and short-term precipitation (see table 1 for further information on these climatic variables; see Appendix 3 for Spearman's rank correlation coefficients)

Table 13: Signal of Spearman's rank correlation coefficients at P<0.05 for cover, biomass and height of functional groups and dominant species with long-, medium- and short-term precipitation. Simbols: = = non significant correlations; + = positive significant correlations. Dominant species: Cmixt= *Chamaemelum mixtum* (L.) All.; Ltara= *Leontodon taraxacoides* (Vill.) Mérat; Apour= *Agrostis pourretii* Willd.; Gfrag= *Gaudinia fragilis* (L.) P.Beauv.; Ocomp= *Ornithopus compressus* L..

	Cover	Biomass			Height		
	FG	FG	Dom Sp		FG	Dom Sp	
			Cmixt	Tbarb		Cmixt	Tbarb
Annual Compositae	=	+	=	=	+	+	+
			Apour	Gfrag		Apour	Gfrag
Annual grasses	=	=	=	+	+	=	+
			Ocomp			Ocomp	
Annual legumes	+	+	=		=	=	
Perennial grasses	+	=			+		

Cover of annual Compositae species does not increase with precipitation, but biomass and height do increase. The biomass of dominant species of *Compositae* group (*Chamaemelum mixtum* (L.) All. and *Tolpis barbata* (L.) Gaertn.) do not increase, which suggests that the biomass increase among the functional group is due to other species.

As seen in previous results, cover of annual grasses does not vary along the climatic gradient. Biomass of this group also doesn't vary, while height increases with precipitation. Within the dominant species of annual grasses, *Agrostis*

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pourretii Willd., the results show that its biomass and height do not vary along the climatic gradient, while *Gaudinia fragilis* (L.) P.Beauv increases in both these traits. These results suggest that species within this group may respond differently to precipitation.

Annual legumes increase with precipitation both in cover and biomass, while height doesn't change significantly. The dominant species, *Ornithopus compressus* L., does not increase in biomass or in height. This suggests that the increase in biomass of this functional group with precipitation is due to the presence of other less abundant species, in accordance with the observed increase in legumes species richness with precipitation.

Perennial grasses increase their cover and height, while biomass doesn't change. One possible reason for these results could be that, in more humid sites, perennial grasses tend to grow preferably in height, with less investment on cespitous growth. This is reasonable since in more humid sites plant cover is higher (table 4), what could lead a competition for light among plants. Other possible reason could be perennial grass species turnover along the climatic gradient, and species present in more moist sites would be taller but with less biomass when compared with species present in drier sites . In this functional group, there was no species present in sufficient number of sites to enable an individual species analysis; however, relatively common species within this group were *Poa bulbosa* L. and *Agrostis* castellana Boiss & Reut.

3.2.3.2. Specific leaf area

Specific leaf area (SLA) measurements were made for two species, but results are shown only for *Tolpis barbata* which is an annual *Compositae* species where leafs were able to be measure even some time after field work. A total of 104 leaves were analyzed.

Specific leaf area values range between 12.3 and 37.9 m^2kg^{-1}. This range is within the expected values since herbaceous species in general show SLA higher than 10-15 m^2kg^{-1} (Cornelissen et al. 2003), and are similar to SLA values of *T. barbata* found by Castro (2008) – mean of 27.1 m^2kg^{-1} - in sites located in Alentejo region with similar land-use and climate.

To verify if SLA varied significantly along the climatic gradient, correlations between SLA and climatic variables were performed. Results in table 14 show that SLA of *T. barbata* significantly increases with precipitation and with minimum temperature of the coldest month. Figure 16 shows the biplots between SLA and the aridity index (A) and medium-term precipitation (B).

Table 14: Spearman's rank correlation coefficients at P<0.05 for Specific leaf area of *Tolpis barbata*, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx= Aridity index (using data from Y1960-1990); LT Prec= long-term precipitation (mean annual precipitation Y1950-2000); MT Prec= medium-term precipitation (mean annual precipitation Y1998-2011); ST Prec= short-term precipitation (mean monthly precipitation Oct 2011 to Mar 2012); LT Temp= long-term temperature (mean annual temperature Y1950-2000); TColdM= minimum temperature of the coldest month (mean Y1950-2000).

	T. barbata SLA
Arid. Idx	0,75
LT Prec	0,74
MT Prec	0,75
ST Prec	ns
TColdM	0.71

The study of (Reich et al. 1998) compared SLA and other leaf traits across various functional groups and biomes. This study has a large-scale approach, and thus comparisons with the present study are difficult. However, a comparison between SLA measurements for several forb species between two sites with different MAP shows that the SLA of forbs' increases from the drier to the more humid wet site. The wetter site is a cold temperate forest and prairie with MAP of 820mm whereas, the drier site is composed of desert

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grassland, shrubland and woodland with MAP of 222mm. The only forb species measured in the wettest site showed an SLA of 3.4 m^2kg^{-1}, while the several forb species found in the driest one showed SLA ranging between approximately 10 and 30m^2kg^{-1}. Although reflecting a much lower precipitation gradient, our results ($\approx$500-650mm) showed a comparable and wide range of variation of SLA from 10-40 m^2kg^{-1}.

In previous results, *T. barbata* showed no significant changes in biomass but a significant increase in height with increasing precipitation. It seems that *T. barbata* respond to this gradient of climatic change increasing its physiological performance based on phenotypic plasticity, which means increasing its SLA and height. Another interesting pattern that is observed from the plot that relates SLA with long-term precipitation is that only after $\approx$600mm the SLA considerable increases and reaches values higher than 30 m^2kg^{-1}. It seems that only above the threshold of 600mm of long-term precipitation this species is able to considerably increase its SLA. This threshold is similar to the one found in the shift of communities shown in figure 13.
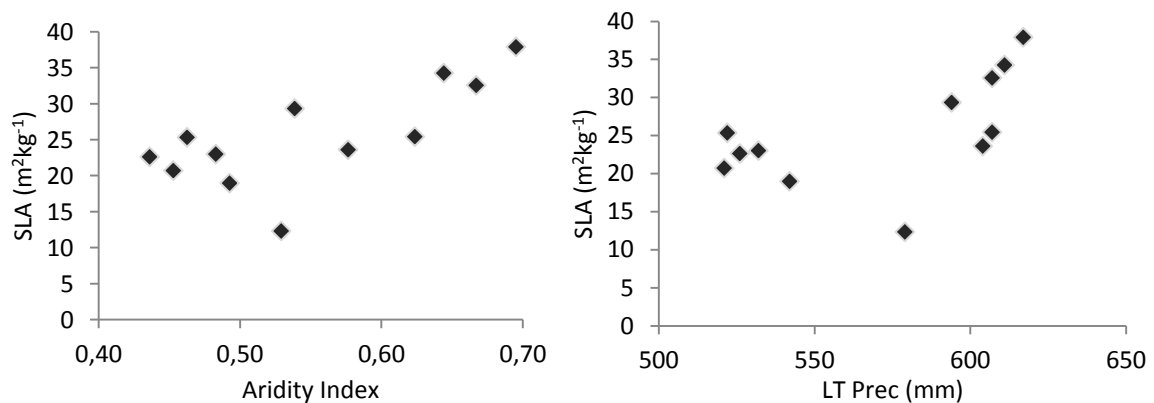
Figure 16: Bi-plots between climatic variables and SLA (m^2kg^{-1}) of annual forb *Tolpis barbata* sampled along a climatic gradient in Alentejo region. This species was present in 12 of the 14 sites sampled. A: relation of SLA with the aridity index (calculated using climatic data from Y1960-1990); B: relation of SLA with long-term precipitation (mean annual precipitation Y1950-2000).

Poorter et al. (2009) reviewed that SLA has a positive relation with water availability across all types of plant species. More specifically along precipitation gradients, Lamont et al. (2002) found decreasing SLA towards drier sites in evergreen shrubs. Interpretation of lower SLA with decreasing water availability relies on the fact that cells are smaller and more tightly packed, leading to a low development of the leaves. Additionally a smaller leaf area leads to a smaller transpiring area, reducing water requirements under dry conditions (Poorter et al. 2009). In addiction, SLA is frequently a good proxy of species potential relative growth rate (Cornelissen et al. 2003).

Specific leaf area is not related only to water resources. In fact, it has been widely discussed whether water or nutrients are the main drivers of SLA (Poorter et al. 2009). In the present work, soil nutrients were not assessed. Moreover, it is expected that soil nutrients co-vary with long-term precipitation and aridity. In general term more precipitation corresponds to more productivity and the existence of more biomass. More biomass is associated with more litter and eventually higher nutrients in the soil. In this work we are not able to disentangle the effect of climate and soil nutrients. Nevertheless in nature they co-vary most of the times. Maybe an indicator that responds more to precipitation than to soil nutrients would be a good choice. Despite this, other authors showed that SLA in shrubs, for example, was more closely related to precipitation than to nutrient availability (namely P and N) (Lamont et al. 2002).

Specific leaf area in *T. barbata* reflected a change of the physiological performance and reflecting phenotypic plasticity along a climatic gradient. This parameter could be a good ecological indicator if detached from other factors such as soil nutrients, something that needs further evaluation. The potential application of this indicator worldwide requires that the chosen species has a universal distribution, such as *Brachypodium distachyon*. This species occurs everywhere and its genome was completed recently (February 2010) which makes

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it a model plant where both phenotypic and genotypic differences could be assessed at the same time.

4.Final Remarks

Among the 15 sampled sites, total plant cover is ca. 80%. A total of 146 plants species were identified. On average there were 36.5 plant species per sampling site, ranging between 26 and 52. These results showed higher values than the

ones recorded for previous works performed in the region. That could be due to differences in the method used or sampling effort and/or in the wider geographical range of the sampled area. Plant community is dominated by herbaceous species, mainly annuals (64.5% relative cover). Most species identified belong to *Graminae* (37 species), *Compositae* (29) and *Leguminosae* (22) families.

Both plant cover and species richness decrease along the climatic gradient, i.e. with increasing aridity. Species richness seems to be highly associated with water availability and minimum temperature of the coldest month, while plant cover seems to be more associated with site long-term aridity (temperature and precipitation), suggesting that it is influenced by more integrated processes. Both plant cover and species richness depend on habitat and land-use type, thus we suggest that functional diversity has more potential to be universal as an ecological indicator of climatic gradients. We are not expecting that long-term precipitation and aridity influence directly the vegetation observed in 2012, although an indirect relation could be occurring. Aridity index and long-term precipitation reflect the long-term effects of climate at the ecosystem level more than simply the amount of water available for plant growth during its vegetative period. These variables reflect the average climatic conditions in the last years (more than 30) "shaping" that place. Thus, they may be associated with other factors such as the amount of soil nutrients and the diversity of the seed bank. The amount of soil nutrients depends on several factors among: plant productivity (litter), litter quality and rate of decomposition (Castro 2008, Fortunel et al. 2009, Dias et al. 2013). Declines in soil structure associated with increasing aridity can adversely impact the water holding capacity andas more water runs off the land surface, less is retained in the soil and available for local recharge and plant establishment and growth. These factors might in turn influence the pattern of plant community which will further affect soil properties. These cascading effects are an evidence of long-term indirect effects of climate in soil properties which in turn model plant community.

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Soil seed bank is the reflection of past diversity patterns. Although most of species present in the sampled sites have an annual life cycle, the percentage of species with persistent banks (i.e. species present in the soil seed bank for more than 1 year) is usually relatively high. Peco et al. (2003) found that 64% of *montado* ecosystem grassland species (with several species common to our study site) had persistent seed banks, as also found in other research (Ortega et al., 1997), including species absent from the vegetation but present in the seed bank (long-term persistent). Some authors have shown that the presence of a reserve of dormant seeds in the soil can stabilize population dynamics diminishing large fluctuations in response to short-term environmental perturbations (Leishman et al. 2000). The presence of these species in the plant community may then vary with interannual fluctuations or short-term climatic conditions. However, long-term perturbations may lead to local extinction of some plants with lower capacity to disperse which might not be present due to the lack of viable seeds storage at a certain site, reflecting long-term climate effects.

Among the sampled sites the more abundant life forms were terophyte and hemicryptophyte. Species richness of both these functional groups decreased with increasing aridity, but only hemicryptophytes' plant cover seems to be affected by decreasing precipitation.

Analyzing both life cycle and family-based functional groups seems to be a step further in finding potential indicators of climate change. Among grasses, different life cycles showed clear contrasting response patterns: while cover of annual grasses remained unchanged, cover of perennial grasses significantly decreased with increasing aridity. Cover and species richness of annual legumes also significantly decreased with increasing aridity. Compositae species correlated poorly with the climatic gradient. Among these *a priori* functional groups perennial grasses and annual legumes seem to be promising ecological indicators of climate change.

Analyzing relative cover in the community (%) enables the perception of a community shift along the climatic gradient. There is a clear community shift between two groups that consider both family and life cycle: a group composed of annual grasses and *Plantaginaceae* species, which increase with aridity, while a group composed of perennial grasses, annual legumes and *Caryophillaceae* species decrease with increasing aridity. Relative cover of the first group varies from ca. 40 to 60%, and relative cover of the second group varies from ca. 30 to 10% from the more humid to drier sites. Functional groups based on life form also showed a community shift: hemicryptophytes increased in relative cover with increasing precipitation, while relative cover of other life forms present (namely terophytes, chamaephytes and one geophyte) decreased with increasing precipitation.

The latter conclusions were based on *a priori* knowledge about plant traits. It is also important to test whether plants associated themselves in groups which have functional relevance. A multivariate analysis of species distribution along the climatic gradient grouped species in a manner consistent with *a priori* functional groups classification, although species included in the same group did not always respond in the same way as functional groups. The results suggest that further analyses of plant community compositional changes along the gradient might be useful to better study potential indicator groups responding to the climatic gradient.

Biomass and height changed along the climatic gradient, but the response pattern is different between *a priori* functional groups and dominant species of the same group. Height decreased with increasing aridity among all functional groups, except for annual legumes, which decreased in biomass. Specific leaf area, which was analyzed only for the *Compositae* species *Tolpis barbata*, decreased with increasing aridity. Considering that height of this species also decreased, this suggests a change in physiological performance along the climatic gradient. Moreover it reflects the phenotypic plasticity of this species. In sum, measuring

specific traits in the same species seems to have a potential to be an ecological indicator of climate change, especially if these traits are measured in a species with global distribution.

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Appendix 1

Table A1: Sampled sites characterization. Coordinates expressed in UTM. Altitude=altitude above sea level (m); LTTemp=long-term temperature (mean annual temperature); TWarmM=maximum temperature of the warmest month; TColdM=mean temperature of the coldest month; T. Annual range=temperature annual range; LTPrec=long-term precipitation; PWarmQ=precipitation of the warmest quarter; PColdQ=precipitation of the coldest quarter; MTPrec=medium-term precipitation (mean annual precipitation Y1998-2011); STPrec=short-term precipitation (mean monthly precipitation October 2011 to March 2012). Temperatures are expressed in Celsius and precipitation in mm. Climatic variables are a mean of the period 1950-2000, except for MTPrec and STPrec

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ID	Coordinates		City	Soil	Altitude	Aridity Index	LTTemp	TWarmM	TColdM	Annual range	LTPrec	PWarmQ	PColdQ	MTPrec	STPrec
1	38°10'52.96"N	7°18'51.09"W	Moura	Litosoil	163	0.45	17.0	32.6	5.8	26.8	521	29	204	472.1	91.7
2	38° 9'15.10"N	7°17'30.04"W	Moura	Litosoil	158	0.46	16.9	32.5	5.7	26.8	522	29	204	474.6	95.9
3	38° 9'47.82"N	7°18'10.74"W	Moura	Litosoil	171	0.44	16.9	32.5	5.7	26.8	526	29	206	473.9	94.4
4	38°16'46.93"N	7°12'36.84"W	Mourão	Litosoil	199	0.47	16.8	32.8	5.5	27.3	531	30	205	473.8	82.3
5	38°14'8.52"N	7°21'12.81"W	Moura	Luvisoil	165	0.48	16.8	32.5	5.7	26.8	532	29	209	469.3	80.3
6	38°11'46.82"N	6°57'15.99"W	Barrancos	Litosoil	262	0.49	16.5	32.8	4.8	28.0	542	30	208	468.9	100.3
7	38° 2'25.94"N	7°9'22.63"W	Moura	Litosoil	276	0.52	16.4	32.0	5.1	26.9	548	30	213	446.8	108.3
8	38°19'8.33"N	7°49'58.54"W	Portel	Luvisoil	209	0.53	16.3	31.6	6.1	25.5	579	30	237	494.0	136.4
9	38°23'43.88"N	7°49'16.19"W	Évora	Luvisoil	235	0.54	16.2	31.4	6.0	25.4	594	31	242	487.9	71.7
10	38°21'18.15"N	7°50'39.19"W	Évora	Luvisoil	280	0.58	16.0	31.2	5.8	25.4	604	31	247	499.7	125.0
11	38°29'9.63"N	8°4'43.15"W	Évora	Luvisoil	194	0.62	16.4	30.8	6.6	24.2	607	31	252	535.8	173.9
12	38°27'48.60"N	8°8'9.45"W	Viana do Alentejo	Luvisoil	185	0.67	16.4	30.7	6.7	24.0	607	31	253	548.3	184.3
13	38°26'27.37"N	8°13'59.94"W	Viana do Alentejo	Luvisoil	163	0.64	16.5	30.6	6.9	23.7	611	30	257	547.6	184.1
14	38°29'42.00"N	8°12'58.31"W	Montemor-o-Novo	Luvisoil	187	0.70	16.5	30.5	6.9	23.6	617	31	258	540.3	177.0

| 15 | 38°31'35.53"N | 8°3'41.12"W | Évora | Luvisoil | 262 | 0.69 | 16.0 | 30.4 | 6.4 | 24.0 | 634 | 34 | 262 | 523.7 | 154.2 |

Appendix 2

Table A2: Traits searched in the literature.

Traits	Description
Whole-plant traits	Habit
	Growth form
	Life form
	Life cycle
	habitat_preferences
	height
	Stress tolerance
	Drough tolerance
	Fire strategy
	Clonality
Leaf traits	Leaf type
	Photosynthetic pathway
	Photosynthetic rate
	Water use efficiency
	Leaf pubescence
	Leaf phenology type
	Leaf size
	Leaf longevity
	Leaf anatomy
	Leaf absorbance
	Specific leaf area (SLA)
	Leaf dry matter content
	Leaf Carbon content
	Leaf Nitrogen content
	Leaf Phosphorus content
	Stomatal conductance
Regenerative traits	Flowering period
	Onset of flowering
	Flower height
	Reproductive maturity
	Pollination mode
	Resprouting capacity
	Dispersal mode
	Seed mass
	Seed size
	Seed longevity
Below-ground traits	Root type
	Root depth
	Root shoot ratio
	Nutrient uptake
Other traits	Soil type
	Litter decomposability

Appendix 3

Table A3: Spearman's rank correlation coefficients at P<0.05 for no plant points percentage and standard deviation (SD), plant cover and number of species (Nr sp) in 14 sites sampled along a climatic gradient, in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (mean from Y1998-2011); ST Prec = short-term precipitation (mean from October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =nminimum temperature of the coldest month; TWarmQ = mean tempretature of the warmest quarter; %OM = organic matter percentage. Long-term variables refer from years 1950-2000.

			No plant		Plant	
Climatic variables			Cover (%)	SD	Cover (%)	Nr sp
Precipitation	Long-term	LT Prec	-0.70	-0.6	0.76	0.72
		Arid. Idx	-0.73	-0.56	0.79	0.69
		Pseasonality	-0.7	ns	0.71	0.60
		PWetM	-0.73	-0.56	0.78	0.75
		PDryQ	ns	ns	ns	ns
	Medium-term	MT Prec	ns	ns	ns	0.72
	Short-term	ST Prec	-0.53	ns	0.66	0.75
Temperature	Long-term	LT Temp	0.61	ns	-0.62	ns
		Diurnal Range	0.74	0.58	-0.71	-0.64
		Isothermality	-0.63	ns	0.55	0.58
		Tseasonality	0.54	ns	-0.52	-0.59
		Annual Range	0.55	ns	-0.53	-0.62
		TColdM	ns	ns	ns	0.64
		TWarmQ	0.71	0.58	-0.72	-0.63
Soil		% OM	0.74	ns	-0.85	-0.67

Table A4: Spearman's rank correlation coefficients at P<0.05 for functional groups based on life form, in 14 sites sampled along a climatic gradient, in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =minimum temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. Life form: TR = terophyte; HM = hemicryptophyte; PN = phanerophyte; vrb = species classified as variable.

| | | | Nr species | | | | Cover | | | | | |
			TR	HM	TR/HM	PN	TR	HM	TR/HM	TR/HM+vrb	PN	TR/HM+PN
Precipitation	Long-term	LT Prec	0.77	0.76	ns	ns	ns	0.62	ns	ns	ns	ns
		Arid. Idx	0.73	0.72	ns	ns	0.55	0.61	ns	ns	ns	ns
		Pseasonality	0.71	0.70	ns	ns	ns	0.58	ns	ns	ns	ns
		PWetM	0.78	0.79	ns	ns	0.53	0.70	-0.55	ns	ns	ns
		PDryQ	ns	0.61	ns	ns	ns	0.53	ns	ns	ns	ns
	Medium-term	MT Prec	0.69	0.61	ns	ns	ns	0.75	-0.73	ns	ns	-0.59
	Short-term	ST Prec	0.70	0.78	-0.53	ns	ns	0.71	-0.55	ns	ns	ns
Temperature	Long-term	LT Temp	ns	-0.69	0.57	ns	ns	ns	ns	ns	ns	ns
		Diurnal Range	-0.73	-0.68	ns	ns	ns	-0.63	0.52	ns	ns	ns
		Isothermality	0.56	0.68	ns	ns	ns	0.68	-0.63	ns	ns	-0.52
		Tseasonality	-0.63	-0.59	ns	ns	ns	-0.65	0.60	ns	ns	ns
		Annual Range	-0.67	-0.57	ns	ns	ns	-0.61	0.58	ns	ns	ns
		TWarmM	-0.73	-0.70	ns	ns	ns	-0.63	0.52	ns	ns	ns
		TColdM	0.62	0.56	ns	ns	ns	0.66	-0.65	ns	ns	-0.62

Table A5: Spearman's rank correlation coefficients at P<0.05 for functional groups based on life cycle in 14 sites sampled along a climatic gradient, in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; PWarmQ MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =minimum temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. Life cycle: An = annual; Bn = biennial; Pn = perennial; Sb = shrub.

			Nr species					Cover (%)			
			An	Bn	An+Bn	Pn	Pn+Sb	An	Bn	An+Bn	Pn
Precipitation	Long-term	LT Prec	0.59	ns	0.66	ns	ns	ns	ns	ns	0.52
		Arid. Idx	0.55	ns	0.62	ns	ns	0.55	ns	0.53	ns
		Pseasonality	0.58	ns	0.56	ns	ns	ns	ns	ns	0.53
		PWetM	0.61	ns	0.69	ns	ns	0.53	ns	ns	0.55
		PDryQ	Ns	ns	ns	ns	ns	ns	ns	ns	ns
	Medium-term	MT Prec	0.61	ns	0.61	ns	ns	ns	-0.54	ns	0.69
	Short-term	ST Prec	0.55	ns	0.59	ns	ns	ns	ns	ns	0.71
Temperature	Long-term	LT Temp	Ns	ns	ns	ns	ns	ns	ns	ns	ns
		Diurnal Range	-0.56	ns	-0.6	ns	ns	ns	ns	ns	-0.56
		Isothermality	Ns	ns	ns	ns	ns	ns	ns	ns	0.58
		Tseasonality	Ns	ns	ns	ns	ns	ns	ns	ns	-0.64
		Annual Range	-0.54	ns	-0.56	ns	ns	ns	ns	ns	-0.59
		TColdM	0.53	ns	0.52	ns	ns	ns	-0.56	ns	0.65
		TWarmM	-0.53	ns	-0.59	ns	ns	ns	ns	ns	-0.58

Table A6: Spearman's rank correlation coefficients at P<0.05 for the Compositae family, sampled in 14 sites along a climatic gradient, in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =minimum temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. Life cycle: An = annual; Pn = perennial; Tot = total.

			Nr species			Cover		
			An	Pn	Tot	An	Pn	Tot
Precipitation	Long-term	LT Prec	ns	0.59	0.55	ns	ns	ns
		Aridity	ns	0.55	ns	0.53	ns	0.53
		Pseasonality	ns	ns	ns	ns	ns	ns
		PWetM	ns	0.6	0.59	ns	0.52	0.51
		PDryQ	ns	0.64	ns	0.58	0.6	0.56
	Medium-term	MT Prec	ns	ns	ns	ns	ns	ns
	Short-term	ST Prec	ns	ns	ns	ns	ns	ns
Temperature	Long-term	LT Temp	ns	-0.63	ns	ns	-0.64	ns
		Diurnal Range	ns	ns	ns	ns	ns	ns
		Isothermality	ns	0.52	ns	ns	0.55	ns
		Tseasonality	ns	ns	ns	ns	ns	ns
		Annual Range	ns	ns	-0.52	ns	ns	ns
		TColdM	ns	ns	ns	ns	ns	ns
		TWarmM	ns	ns	-0.52	ns	ns	ns

Table A7: Spearman's rank correlation coefficients at P<0.05 for the Graminae family sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =minimum temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. Life cycle: An = annual; Pn = perennial; Tot = total.

			Nr species			Cover		
			An	Pn	Tot	An	Pn	Tot
Precipitation	Long-term	LT Prec	ns	0.66	ns	ns	0.53	ns
		Arid. Idx	ns	0.64	ns	ns	ns	ns
		Pseasonality	ns	0.79	ns	ns	0.62	0.6
		PWet	ns	0.69	ns	ns	0.6	ns
		PDryQ	ns	ns	ns	ns	ns	ns
	Medium-term	MT Prec	ns	0.7	ns	ns	0.71	ns
	Short-term	ST Prec	ns	0.85	ns	ns	0.74	ns
Temperature	Long-term	LT Temp	ns	ns	ns	ns	ns	ns
		Diurnal Range	ns	-0.73	ns	ns	-0.60	ns
		Isothermality	ns	0.68	ns	ns	0.59	ns
		Tseasonality	ns	-0.66	ns	ns	-0.61	ns

	An	Sb	Tot	An	Sb	Tot
Annual Range	ns	-0.67	ns	ns	-0.59	ns
TColdM	ns	0.71	ns	ns	0.64	ns
TWarmM	ns	-0.71	ns	ns	-0.60	ns

Table A8: Spearman's rank correlation coefficients at P<0.05 for the Leguminosae family sampled in 14 sites along a climatic gradient, in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM = minimum temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. Life cycle: An = annual; Sb = shrubs; Tot = total.

			Nr species			Cover		
			An	Sb	Tot	An	Sb	Tot
Precipitation	Long-term	LT Prec	0.81	ns	0.80	0.69	ns	ns
		Arid. Idx	0.84	ns	0.83	0.74	ns	0.57
		Pseasonality	0.67	ns	0.65	0.58	ns	0.57
		PWetM	0.82	ns	0.81	0.69	ns	ns
		PDryQ	0.64	ns	0.66	0.55	ns	ns
	Medium-Term	MT Prec	0.73	ns	0.74	0.56	ns	ns

	Short-Term	ST Prec	0.71	ns	0.70	0.60	ns	ns
Temperature	Long-Term	LT Temp	-0.60	ns	-0.57	-0.67	ns	ns
		Diurnal Range	-0.77	ns	-0.76	-0.66	ns	-0.58
		Isothermality	0.78	ns	0.78	0.71	ns	0.61
		Tseasonality	-0.71	ns	-0.71	-0.57	ns	ns
		Annual Range	-0.70	ns	-0.71	-0.56	ns	ns
		TColdM	0.72	ns	0.75	0.54	ns	0.55
		TWarmM	-0.75	ns	-0.74	-0.67	ns	-0.53

Table A9: Spearman's rank correlation coefficients at P<0.05 for relative cover in the community of functional groups based on life cycle, sampled in 14 sites along a climatic gradient, in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =nminimum temperature of the coldest month; TWarmQ = mean tempretature of the warmest quarter; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. Life cycle: An = annual; Bn = facultative biennial; Pn = perennial.

Relative cover (%)		
An	Bn	Pn

Precipitation	Long-term	LT Prec	ns	ns	ns
		Arid. Idx	ns	ns	ns
		Pseasonality	ns	ns	ns
		PDryQ	ns	ns	ns
		PWetQ	ns	ns	ns
	Medium-term	MT Prec	ns	ns	ns
	Short-term	ST Prec	ns	ns	ns
Temperature	Long-term	LT Temp	ns	ns	ns
		Dirnal Range	ns	ns	ns
		Isothermality	ns	ns	ns
		Tseasonality	ns	ns	ns
		Anual Range	ns	ns	ns
		TColdM	ns	ns	ns
		TWarmQ	ns	ns	ns

Table A10: Spearman's rank correlation coefficients at $P<0.05$ for relative cover in the community of functional groups based on life form, sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =nminimum

temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. HM = hemicryptophyte; Other = other life forms present, which includes terophytes, camephytes, nanophanerophytes, geophytes and species classified as variable.

			Relative cover (%)	
			HM	Other
Precipitation	Long-term	LT Prec	0.57	ns
		Arid. Idx	0.54	ns
		Pseasonality	0.64	-0.67
		PWetM	0.65	-0.55
		PDryQ	ns	ns
	Medium-term	MT Prec	0.75	-0.69
	Short-term	ST Prec	0.71	-0.62
Temperature	Long-term	LT Temp	ns	ns
		Diurnal Range	-0.6	0.63
		Isothermality	0.65	-0.59
		Tseasonality	-0.63	0.63
		Anual Range	-0.6	0.61
		TWarmM	-0.61	0.6
		TColdM	0.64	-0.70

Table A11: Spearman's rank correlation coefficients at P<0.05 for relative cover in the community of family and life-cycle groups sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: LT Prec = long-term precipitation; Arid. Idx = aridity index; Pseasonality = precipitation seasonality; PWetM = mean precipitation of the

wettest month; PDryQ = mean precipitation of the driest quarter; MT Prec = medium-term precipitation (Y1998-2011); ST Prec = short-term precipitation (October 2011–March 2012); LT Temp = long-term temperature; Diurnal range = diurnal range; Isothermality = isothermality; Annual range = annual range; TColdM =minimum temperature of the coldest month; TWarmM = maximum tempretature of the warmest month; %OM = organic matter percentage. Long-term variables refer from years 1950-2000. An Gram = annual grasses; Pn Gram = perennial grasses; An Legu = annual legumes; Plantago = Plantago spp.; Cary = Caryophyllaceae.

			Relative cover (%)	
			An Gram + Plantago	Pn Gram + An Legu + Cary
Precipitation	Long-term	LT Prec	-0.72	0.68
		Arid. Idx	-0.71	0.69
		Pseasonality	-0.54	0.70
		PWetM	-0.69	0.74
		PDryQ	-0.70	0.57
	Medium-term	MT Prec	-0.74	0.85
	Short-term	ST Prec	-0.61	0.79
Temperature	Long-term	LT Temp	0.60	-0.54
		Dirnal Range	0.73	-0.72
		Isothermality	-0.81	0.78
		Tseasonality	0.74	-0.74
		Anual Range	0.74	-0.74
		TWarmM	0.74	-0.74
		TColdM	-0.78	0.77

Appendix 4

Table A13: Spearman's rank correlation coefficients at P<0.05 for biomass of a priori functional groups. Plant community was sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx = aridity index (calculated using data from years 1960-1990); LT Prec = long-term precipitation (mean annual precipitation from years 1950-2000); MT Prec = medium-term precipitation (mean annual precipitation from Y1998-2011); ST Prec = short-term precipitation (mean monthly precipitation from October 2011–March 2012).

| | Annual | | | Perennial |
	Compositae	Grasses	Legumes	Grasses
Arid. Idx	0.55	ns	0.69	ns
LTPrec	0.60	ns	0.67	ns
MTPrec	0.52	ns	0.55	ns
STPrec	ns	ns	0.57	ns

Table A14: Spearman's rank correlation coefficients at P<0.05 for biomass of dominant species. Apour = *Agrostis pourretii*; Bdyst = *Brachypodium dystachion*; Cmixt = *Chamaemelum mixtum*; Gfrag = *Gaudinea fragilis*; Lrigi = *Lolium rigidum*; Ltara = *Leontodon taraxacoides*; Ocomp = *Ornithopus compressus;* Tbarb = *Tolpis barbata*; Xgutt = *Xolantha guttata*. Climatic variables: Arid. Idx = aridity index (calculated using data from years 1960-1990); LT Prec = long-term precipitation (mean annual precipitation from years 1950-2000); MT Prec = medium-term precipitation (mean annual precipitation from Y1998-2011); ST Prec = short-term precipitation (mean monthly precipitation from October 2011–March 2012).

	Apour	Bdyst	Cmixt	Gfrag	Lrigi	Ltara	Ocomp	Tbarb	Xgutt
Arid. Idx	ns	ns	ns	0.62	ns	ns	ns	ns	ns
LTPrec	ns	ns	ns	0.64	ns	ns	ns	ns	ns
MTPrec	ns	ns	ns	0.61	ns	ns	ns	ns	ns
STPrec	ns	ns	ns	ns	ns	ns	ns	ns	0.74

Table A14: Spearman's rank correlation coefficients at P<0.05 for vegetative height of a priori functional groups (mean vegetative height of the species composing each functional group). Plant community was sampled in 14 sites along a climatic gradient in Alentejo region. Climatic variables: Arid. Idx = aridity index (calculated using data from years 1960-1990); LT Prec = long-term precipitation (mean annual precipitation from years 1950-2000); MT Prec = medium-term precipitation (mean annual precipitation from Y1998-2011); ST Prec = short-term precipitation (mean monthly precipitation from October 2011–March 2012).

	Annual	Perennial

	Compositae	Grasses	Legumes	Grasses
Arid. Idx	0.65	0.56	ns	ns
LTPrec	0.60	0.54	ns	ns
MTPrec	0.62	0.60	ns	ns
STPrec	ns	ns	ns	0.63

Table 18: Spearman's rank correlation coefficients at P<0.05 for mean vegetative height of dominant species sampled in 14 sites along a climatic gradient in Alentejo region. Apour = *Agrostis pourretii*; Bdyst = *Brachypodium dystachion*; Cmixt = *Chamaemelum mixtum*; Gfrag = *Gaudinea fragilis*; Lrigi = *Lolium rigidum*; Ltara = *Leontodon taraxacoides*; Ocomp = *Ornithopus compressus;* Tbarb = *Tolpis barbata*; Xgutt = *Xolantha guttata*. Climatic variables: Arid. Idx = aridity index (calculated using data from years 1960-1990); LT Prec = long-term precipitation (mean annual precipitation from years 1950-2000); MT Prec = medium-term precipitation (mean annual precipitation from Y1998-2011); ST Prec = short-term precipitation (mean monthly precipitation from October 2011–March 2012).

	Apour	Bdyst	Cmixt	Gfrag	Lrigi	Ltara	Ocomp	Tbarb	Xgutt
Arid. Idx	ns	ns	0.73	0.57	ns	0.62	ns	0.60	ns
LTPrec	ns	ns	ns	ns	ns	0.62	ns	0.59	ns
MTPrec	ns	ns	0.64	0.60	ns	0.67	ns	ns	ns
STPrec	ns	ns	ns	ns	ns	0.88	ns	ns	0.79

Outputs

Oral communication:

Nunes, A., Matos, P., Pinho, P., Batista, M. & C. Branquinho. Functional diversity as ecological indicator of climate change applied to tropical areas. Congresso Internacional Saber Tropical em Moçambique: História, Memória e Ciência, 24 – 26 October, IICT- JBT, Lisboa, Portugal.

Poster:

Nunes, A., Batista, M., Tápia, S., Pinho, P., Correia, O. & Branquinho, C. (2012). Plant functional response to desertification and land degradation in Mediterranean woodlands – contribution to restoration strategies. 4th International Ecosummit – Ecological Sustainability: restoring the planet's ecosystem services. 30 September-5 October, Columbus, Ohio, USA.

Two future outputs of this work are: adding the plant biomass and height measurement data to an existing trait database; and to contribute with plant presence data to the Flora-on project, an interactive website with updated information about the Portuguese flora (Sociedade Portuguesa de Botânica 2012).