



Mediterranean Forests in Transition (MEDIT): Deliverable No7

Title: Report of functional trait inter-relationships

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Summary

This report presents the statistical techniques and methods applied to the MEDIT dataset, aiming at revealing important trait relationships and thus identify potential conversion of life history strategies, as expressed via functional traits. A functional description of the key forest species found in Greece is presented, which can be used as a first proxy to describe important ecological processes. In addition the functional relationships identified here were used to parameterise and constrain key algorithms of the vegetation dynamics models used in deliverables D6.1 and D6.2. For the small scale vegetation dynamics model (GREFOS) species specific values of the traits of interest were used to parameterise the allometric relationships and the mortality function. For the large scale JULES models a set of plant functional types was defined and used to simulate gross primary productivity and net primary productivity across the Mediterranean basin. Finally for the hybrid TFS model the identified functional relationships were directly plugged into the model in order to simulate vegetation dynamics under both current and climate change conditions. All analyses were made with the R programming language (R Development Core Team, 2015).

1. Introduction

Understanding plant functional strategies and how these strategies reveal the way plants interact and respond to changes in environmental conditions is a central aim in Ecology (Lavorel and Garnier 2002, Westoby and Wright 2006). Plant functional traits are measurable plant properties that strongly influence plants performance across environmental gradients. Functional traits are usually measured at the individual level and are comparatively considered across species (McGill et al. 2006). Great effort has been made during the last decade for the development of global dataset of plant functional traits (Wright et al. 2004, Kattge et al. 2011). In such datasets it is important not only to cover the wealth of plant species found at different places around the world, but also to "link" measures of their performance with the environmental (climatic, edaphic, disturbance) conditions that they experience. Recent research has highlighted that trait variation even within the same species has an important phylogenetic and environmental component, usually referred to as ecotypic vs environmental

plasticity (Fyllas et al. 2009, Niinemets 2015). Estimating and understanding these sources of variation in key functional characters is a "hot" issue in current ecological research.

The functional traits measured during the MEDIT field campaigns and laboratory work include:

- Leaf Area (L_A [m^2]) defined as the projected area of an individual leaf and related to the climatic and edaphic conditions plants are experiencing. Smaller leaves are generally favoured in environments of increased heat, cold, drought and nutrient stress,
- Leaf thickness (L_{th} [mm]) is frequently used as an indicator of plant water status and plays an important role in determining the physical strength of leaves.
- Leaf Mass per Area (LMA [gm^{-2}]) is estimated as the dry mass to leaf area ratio. LMA is considered one of the key components of the leaf economic spectrum, with higher LMA leaves showing a higher leaf life span and lower nutrient concentrations and photosynthetic rates. Low LMA leaves work better in resource-poor environments Wilson et al. (1999).
- Leaf dry matter content (DMC [gg^{-1}]) is the dry to water-saturated mass ratio of a leaf. LDMC is usually negatively correlated with the growth rate of a plant and positively correlated with leaf longevity. High LDMC species are assumed to be more tolerant to hazards such as herbivory and frequently illustrate lower decomposition rates.
- Leaf C concentration, i.e. the per dry mass leaf C content is relatively stable between plant species (45%). In general higher C content is found in species with higher LMA and leaf longevity.
- Leaf N and P concentration per unit dry leaf mass. N and P concentrations are usually related to LMA, while N concentration is also tightly associated to photosynthetic rate. Frequently the leaf N/P ratio is used as an index to assess whether plant growth is limited more by N or P with transition values around 14-16 mass basis.
- Leaf Ca, K, Mg concentration. The concentration of the so called macronutrients are related to plant growth but the physiological mechanisms that regulate them have not yet been clearly identified.
- Wood density ($g\ cm^{-3}$) is considered a key functional trait and it has been suggested as the central element of the wood economic spectrum (Chave et al. 2009). WD is measured as the dry weight of wood per wood volume and it is important for the stability, the defence the hydraulics and the growth potential of plants. It expresses the growth survival trade-off with low wood density species presenting higher growth rates and high wood density species having higher survival.
- Light saturated Photosynthetic Rate (A_{sat} [$\mu mol\ m^{-2}\ s^{-1}$]) is used as a metric of metabolic capacity and average C assimilation. A_{sat} scales with LMA, N and P concentration
- Leaf dark Respiration Rate (R_{dark}) is a metabolic index that correlates with the average night-time respiration. It scales with other traits such as N and A_{sat} . However comparing R_{dark} between species and among sites and time of year is challenging due to its sensitivity and acclimation to temperature.
- In addition to the above commonly used functional traits we have additionally quantified leaf carboxylation rate (V_{max}) leaf electron transport rate (J_{max}) and leaf stomatal conductance.

Systematic research along this lines is missing for forest species found in Greece and the northeastern part of the Mediterranean Basin. One of the aims of the MEDIT project was to cover this research gap with an extensive network of plots covering the dominant forest species across a range of environmental conditions. Within MEDIT, plant traits for more than forty species have been systematically measured from the one of the southernmost mountains (Taygetos) in the country to one of the northernmost parts of the country (Rodopi). Across our study sites mean annual temperature ranged from 5.6°C to 16°C and total annual precipitation from 488 to 1197mm per year. All major forest soil types have been covered with soil properties like pH ranging from 4.6 to 8.5 and leaf N:P ratio ranging from 11.16 in *Abies cephalonica* stands to 22.75 in Mediterranean mixed woodlands.

In the analysis presented here we firstly explore the key interrelationships between the studied functional traits and then explore to what extent trait variation is explained by the environmental gradients identified within the MEDIT study sites network.

2. Materials and Methods

Within the MEDIT study plots at least ten trees of the dominant species were selected for leaf and branch sample collection. Sunlit leaf samples were used for estimating the leaf structural and chemical traits (L_A , L_T , M_A , DMC, C, N, P, Ca, Mg, K and S). The water saturated leaf mass and area were measured within 48 hours. All leaves were scanned with a portable scanner and then analysed in the Imaje-J software. Leaf samples were then stored and transported to the laboratory for measurements of dry mass (48h at 60°C) and micro and macro nutrient concentrations. Leaf C, N, and S content were measured using an elemental analyser (LECO CNS200), leaf P was measured spectrophotometrically after acid digestion, leaf Ca and Mg content using atomic absorption spectroscopy and leaf K with a flame photometer. Branch wood density (WD) was also measured, using a sample from the branch oven dried for 48 h.





In the field, leaves from the same branches were used for conducting photosynthesis and respiration measurements using the LICOR-6400 gas analyzer. Three types of gas exchange measurements were performed. Instantaneous measurements of leaf photosynthesis where maximum stomatal conductance was estimated (g_{max}), light response curves (A-I) where A_{max} was estimated, and CO_2 response curves (A-Ci) where maximum carboxylation efficiency (V_{max}) and electron transport rates (J_{max}) were estimated.

In the first case the leaf was placed inside the chamber and was left to reach a stable state. Then the photosynthetic rate along with the respective stomatal conductance was recorded. Maximum stomatal conductance was extracted from all the instantaneous measurements taken.

To estimate A_{max} through the A-I measurements we programmed the LICOR to change the amount of Light in the chamber between 0 and 2000 PAR. The leaf was left to stabilise for 5 minutes and then three measurements were taken at each light level. We then used these data to fit the non rectangular hyperbolic equation (de Pury and Farquhar 1997) where the photosynthetic rate (A) at any given light level (I) is estimated by:

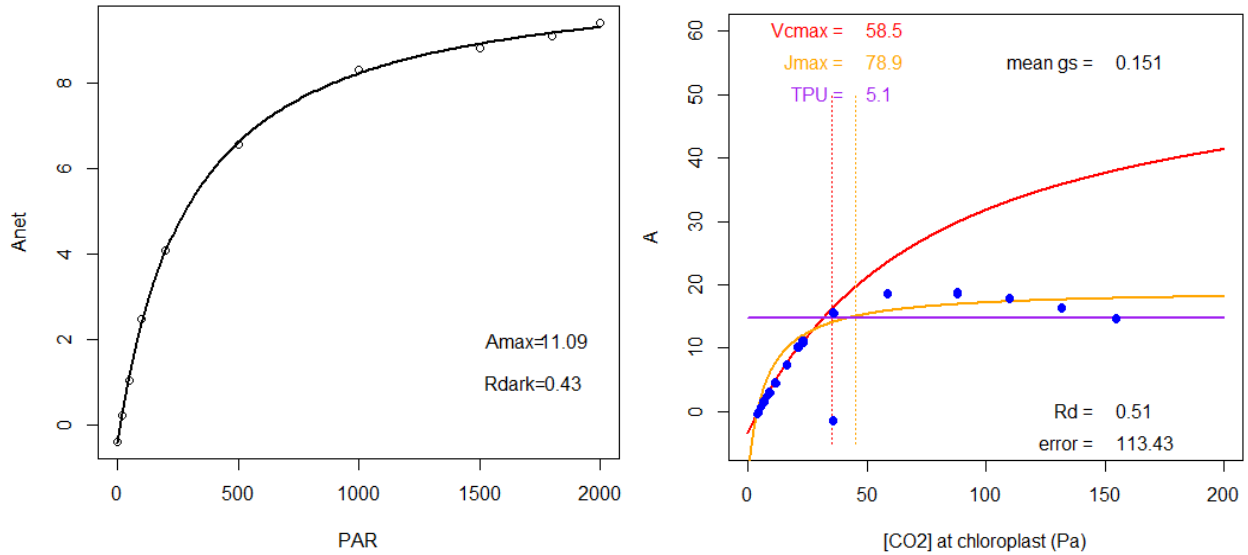
$$A = \frac{a \cdot I + A_{max} - \sqrt{(a \cdot I + A_{max})^2 - 4 \cdot \theta \cdot a \cdot I \cdot A_{max}}}{2 \cdot \theta} - R_{dark}$$

with α the initial slope of the light response curve and ϑ determining sharpness of the curve. We used an optimization function in R to estimate α , A_{max} , ϑ and R_{dark} .

In order to calculate V_{max} and J_{max} we programmed the gas exchange analyser to change CO_2 concentration in the chamber at a saturating light level (identified from the A-I curve for each species). CO_2 concentration was set to range between 0 and 2000 ppm. We then fitted the full A-Ci curve as reported in Domingues et al. (2010). The original R code to do that was provided from the first author of this paper and modified to the MEDIT dataset needs. In general this model assumes that photosynthetic rate is limited by either J or V_c based on CO_2 availability. This model was fit to each one of our A-Ci curves and the traits of interest were extracted.

At the end of the light and CO₂ response curves, the light source was turned off and the leaf was left in the dark inside the chamber for 5 minutes. Then respiration censuses were taken. The mean value of these measurements was used to estimate R_{dark} for each one of the studied trees. Typical measurements of the A-I and A-Ci curves are illustrated in the following figures.

Figure 1: Typical light (A-I) response curve on the left panel and CO₂ (A-Ci) response curve on the right panel



The following table (Table 1) provides a list of the traits measured and abbreviations used throughout this document.

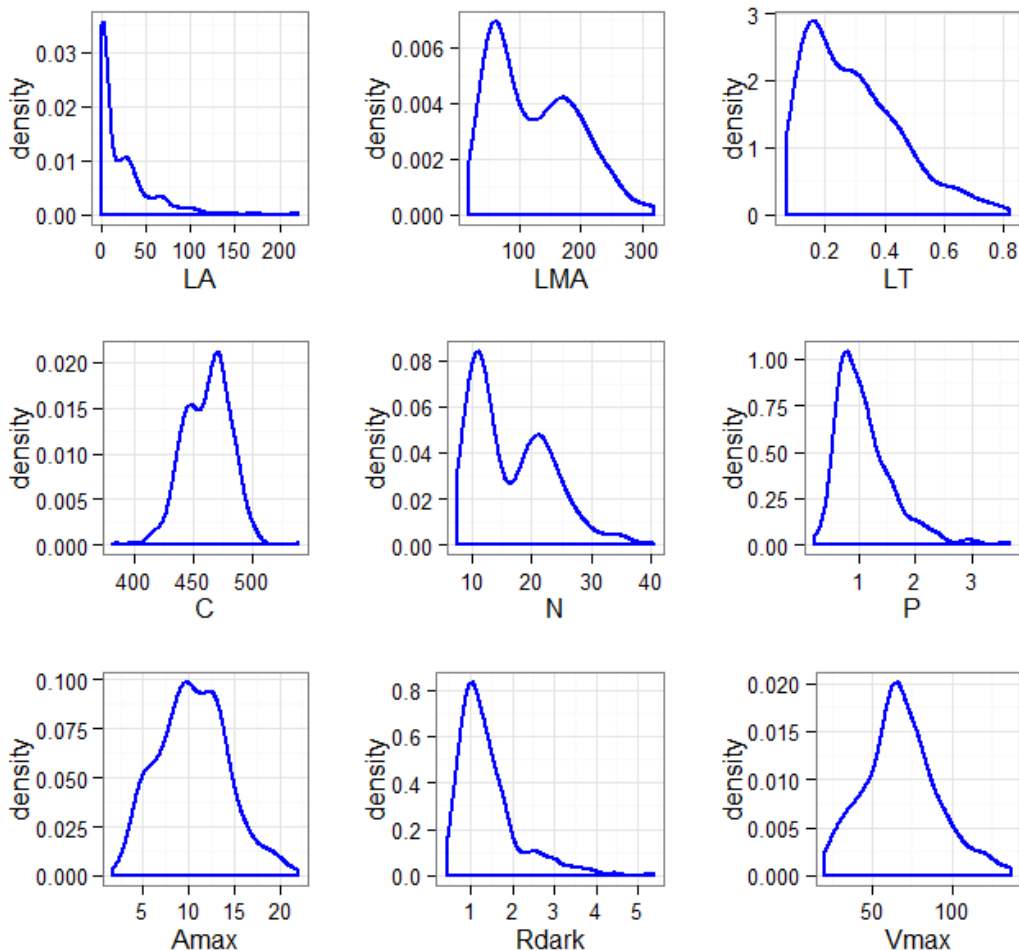
Table 1: Description of the functional traits measured during the MEDIT project

Functional Trait	Symbol	Units
Leaf Area	L _A	cm ²
Leaf Thickness	L _T	mm
Leaf dry Mass per Area	LMA	g m ⁻²
Leaf dry matter content	DMC	g g ⁻¹
Branch wood density	WD	g cm ⁻³
Leaf Nitrogen content	N	mg g ⁻¹
Leaf Phosphorous content	P	mg g ⁻¹
Leaf Calcium content	Ca	mg g ⁻¹
Leaf Magnesium content	Mg	mg g ⁻¹
Leaf Potassium content	K	mg g ⁻¹
Leaf Photosynthetic rate at light saturation	A _{max}	μmol m ⁻² s ⁻¹
Leaf dark respiration	R _{dark}	μmol m ⁻² s ⁻¹
Maximum carboxylation capacity	V _{max}	μmol m ⁻² s ⁻¹
Maximum electron transport rate	J _{max}	μmol m ⁻² s ⁻¹
Maximum stomatal conductance	g _{max}	m s ⁻¹

3. Results

All these functional characters were then assembled in a database and linked through the tree id tag. The structure of the MEDIT dataset is described in Deliverable 6 (D4.1). We initially explored the distribution of each functional character and tested for normality using a Shapiro-Wilk test. Most of them did not follow a normal distribution and were thus \log_{10} transformed for further analysis. The following figure presents the distributions of some key characters related to leaf structure (upper panel), leaf chemistry (mid panel) and fluxes (lower panel). Significant trait variation was observed for most characters. For example leaf area (L_A) ranged between 0.01 and 200 cm^2 , leaf mass per area (M_A) from 40 to 300 g m^{-2} and leaf thickness from 0.01 to 0.8 mm. In terms of leaf chemistry leaf C content ranged between 40 to 50 mg g^{-1} , leaf N from 5 to 40 mg g^{-1} and leaf P from 0.1 to 4 mg g^{-1} . Leaf light saturated photosynthesis (A_{max}) ranged between 3 and 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, dark respiration (R_{dark}) from 0.5 to 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ while the carboxylation rate (V_{max}) between 25 and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This range is considered to have two main source of variation: the genetic (ecotypic) and environmental (plastic) component. The analysis applied to account for these components is described later on (Niinemets 2014).

Figure 2: Variation in some key structural, chemical and carbon exchange functional characters.



3.1 Functional Traits and Plant Functional Types

We explored for differences in the functional traits of interest between plant functional types (PFTs). Three PFTs were *a priori* defined namely, the conifers group (CO), the evergreen broadleaved group (EB) and the deciduous broadleaved group (DB). A repeated measures ANOVA was applied for each log10 transformed functional character with results summarised in Table 2 and the following figures.

Table 2. Analysis of variance for the key functional traits studied in MEDIT. A significant difference ($p < 0.001$) in the mean PFT trait value was observed for all traits apart from Rd.

Trait	F	p
L _A	130.6	<0.001
L _{th}	111.7	<0.001
LMA	124.1	<0.001
DMC	4.6	0.016
N	299.9	<0.001
P	76.4	<0.001
Ca	76.4	<0.001
Mg	24.2	<0.001
K	9.2	0.001
Asat	3.8	0.031
Rd	2.3	0.112
g _{max}	14.3	<0.001
WD	49.9	<0.001

Figure 3: Mean values of key leaf structural traits for the three PFTs.

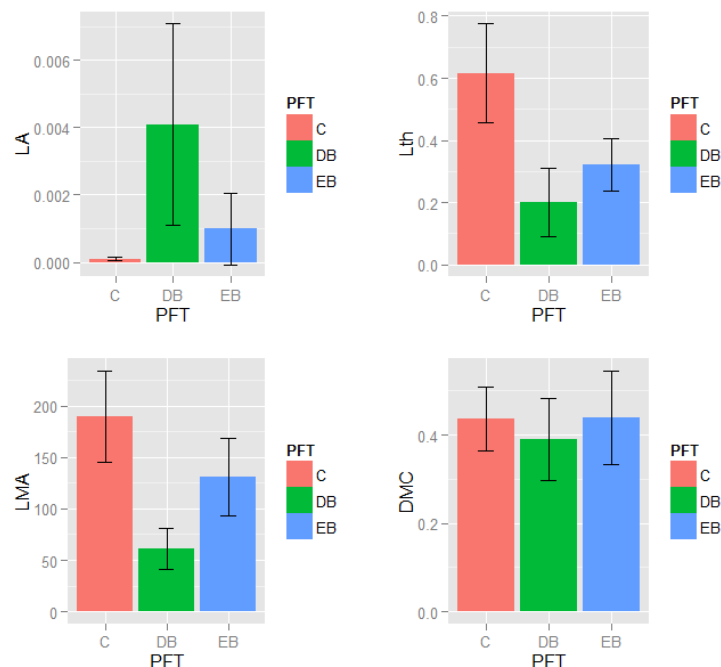


Figure 4: Mean values of key leaf chemical traits for the three PFTs.

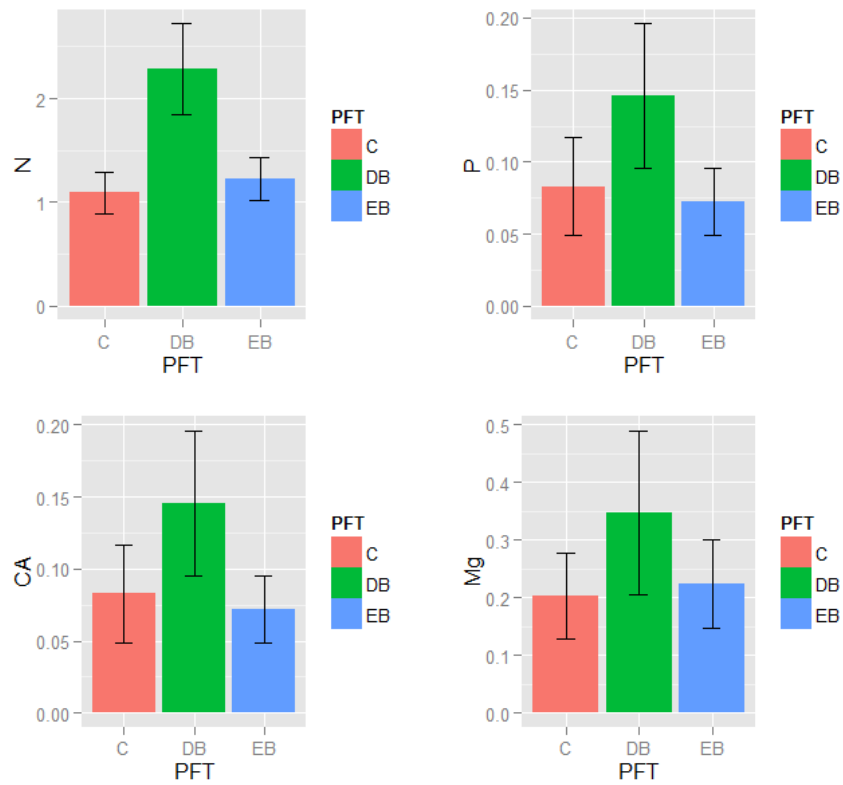
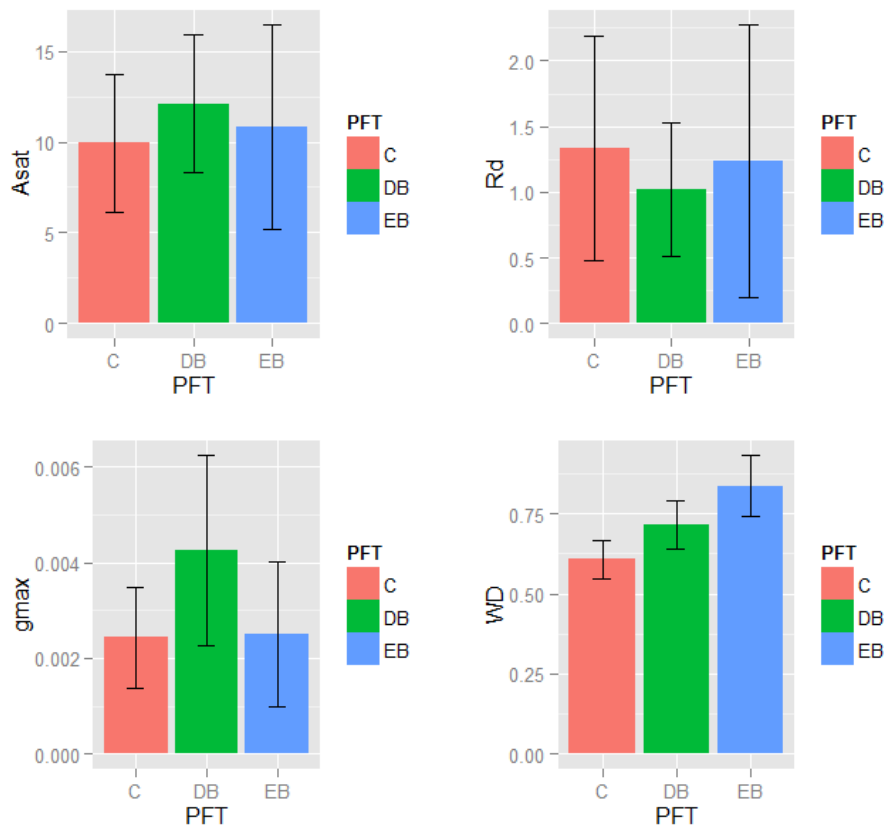


Figure 5: Mean values of key leaf gas exchange traits and WD for the three PFTs.



All functional characters indicated a significant difference between PFTs apart from Rd. Leaf area, nutrients concentration and Asat was higher in DB species. Lth and LMA was higher in the CO type while DMC and WD was higher in the EB type. These differences are in agreement with what was expected from the general description of plant strategies for the PFTs of interest. DB are presenting high photosynthetic rates and nutrient concentrations as they need to photosynthetically accumulate C in a shorter time period due to their deciduous leaf habit. CO are known to have a longer leaf longevity and thus are expected to have a higher LMA. Finally EB are experiencing harsh drought conditions and thus have evolved traits related to a tolerant life strategy expressed by increased WD and DMC. DMC is also related to the leaf sclerophylly.

3.2 Species Functional Traits

As a second step to our analysis we estimated the mean trait values per species. The values for the most important functional characters are summarised in table 3. Note that in this table functional variation between sites is not taken into account, rather a general description of the expected values per species is given. This table can be used as a first proxy to functionally describe the most important forest tree species found in Greece. *Pinus nigra* presented the highest LMA followed by *Pinus brutia*. The highest nutrient concentrations were observed in broadleaved species such as *Juglans regia*, *Corylus avellana* and *Castanea sativa*. The lowest WD indicating fast growth and mortality rates was observed in species like *Salix alba*, *Cercis siliquastrum* and *Pinus sylvestris* while typical Mediterranean sclerophylls like *Phyllirea latifolia* and *Quercus coccifera* illustrated the highest WD. The highest light saturated photosynthetic rates were observed in *Pinus brutia*, *Cotinus coggygria* and *Fraxinus excelsior* and the lowest ones in the two fir species i.e. *Abies borisii* and *Abies cephalonica*. Respiration was also high in *Abies cephalonica* and *Picea abies* with the lowest rates observed in *Carpinus orientalis* and *Pinus sylvestris*.

The species mean traits table was then used to identify relationships between the functional traits of interest. We applied Standardised Major Axis (SMA) regression techniques to identify potential traits intercorrelations. We used the *smatr* package in the R statistical language. Some key associations are presented in the following tables. A positive relationship between leaf thickness and LMA was observed indicating that higher LMA leaves are in general thicker. Such leaves also have a higher C concentration but relatively lower N and P concentrations. N and P scale positively and the same hold true for Asat and maximum stomatal conductance.

Table 3: Mean Trait values per species. NA indicate non available measurements. These values can be used to give a general functional description of the dominant forest species in Greece.

<i>Species</i>	LA	Lth	DMC	LMA	C	N	P	Ca	Mg	K	WD	Asat	Rd	gmax
<i>Abies borisii</i>	0.00005	0.510	0.408	158.569	46.791	1.083	0.089	0.969	0.180	1.264	0.617	5.973	1.580	0.0015
<i>Abies cephalonica</i>	0.00005	0.615	0.461	187.437	48.059	1.057	0.092	0.885	0.225	1.094	0.609	7.569	1.870	0.0019
<i>Acer campestre</i>	0.00149	0.130	0.381	47.814	43.933	2.140	0.134	1.363	0.350	1.317	0.736	8.913	0.852	0.0021
<i>Acer obtusatum</i>	0.01513	0.105	0.336	41.094	43.685	2.755	0.203	1.275	0.255	2.530	0.660	8.056	0.904	0.0019
<i>Acer platanoides</i>	0.01285	0.105	0.340	39.578	48.430	2.065	0.184	2.190	0.495	2.460	0.663	14.635	NA	0.0009
<i>Arbutus andrachne</i>	0.00300	0.377	0.380	131.350	46.702	1.383	0.083	1.090	0.269	1.688	0.808	14.365	1.859	0.0030
<i>Arbutus unedo</i>	0.00196	0.339	0.353	102.881	48.162	1.041	0.060	1.104	0.262	1.131	0.735	10.479	1.013	0.0021
<i>Betula pendula</i>	0.00174	0.169	0.347	50.838	NA	NA	NA	NA	NA	NA	0.650	14.497	0.902	0.0035
<i>Carpinus orientalis</i>	0.00127	0.107	0.377	44.325	43.307	2.063	0.116	1.797	0.320	1.127	0.736	7.518	0.603	0.0023
<i>Castanea sativa</i>	0.00777	0.311	0.352	71.748	45.050	2.818	0.175	0.724	0.529	1.368	0.655	12.905	1.004	0.0070
<i>Cercis siliquastrum</i>	0.00704	0.210	0.281	64.706	44.875	2.470	0.192	0.705	0.200	2.295	0.530	13.161	1.483	0.0029
<i>Cornus mas</i>	0.00170	0.127	0.264	37.755	41.180	2.345	0.188	1.630	0.540	3.695	0.762	8.945	1.013	0.0023
<i>Corylus avellana</i>	0.00611	0.233	0.315	35.159	47.430	2.880	0.135	0.640	0.270	0.710	0.559	7.765	1.423	0.0027
<i>Cotinus coggygria</i>	0.00340	0.247	0.349	67.895	44.242	2.090	0.124	1.574	0.266	1.774	0.577	17.992	1.258	0.0061
<i>Fagus sylvatica</i>	0.00311	0.161	0.374	49.697	44.879	2.198	0.126	0.877	0.313	1.379	0.708	11.570	0.836	0.0042
<i>Fraxinus excelsior</i>	0.00200	0.240	0.337	68.838	NA	NA	NA	NA	NA	NA	0.688	NA	NA	NA
<i>Fraxinus ornus</i>	0.00210	0.170	0.322	59.851	44.360	1.833	0.107	1.373	0.443	1.290	0.732	14.309	1.126	0.0050
<i>Ilex aquifolium</i>	0.00292	0.470	0.364	145.807	47.330	1.050	0.091	0.940	0.320	1.520	0.703	4.998	0.192	0.0010
<i>Juglans regia</i>	0.01059	0.100	0.677	66.701	44.950	2.900	0.309	2.050	0.880	2.710	0.630	13.938	1.196	0.0070
<i>Malus communis</i>	0.00073	0.170	0.153	34.413	43.380	2.790	0.209	1.240	0.780	4.660	0.772	11.285	0.947	0.0048
<i>Ostrya carpinifolia</i>	0.00239	0.161	0.407	53.767	44.260	2.236	0.119	1.556	0.307	1.494	0.719	11.828	0.961	0.0040
<i>Phyllirea latifolia</i>	0.00031	0.278	0.473	134.740	48.450	1.324	0.073	1.251	0.205	1.165	0.837	12.320	1.701	0.0030
<i>Picea abies</i>	0.00003	0.681	0.424	160.706	NA	NA	NA	NA	NA	NA	0.583	7.623	2.317	0.0012
<i>Pinus brutia</i>	0.00017	0.694	0.419	194.039	NA	NA	NA	NA	NA	NA	0.593	17.638	0.823	0.0034
<i>Pinus halepensis</i>	0.00010	0.482	0.468	157.485	47.633	1.261	0.083	0.703	0.260	1.117	0.658	12.275	1.154	0.0024
<i>Pinus nigra</i>	0.00017	0.695	0.427	227.426	47.247	1.067	0.073	0.514	0.175	0.965	0.602	10.591	1.140	0.0028
<i>Pinus sylvestris</i>	0.00008	0.597	0.394	166.522	NA	NA	NA	NA	NA	NA	0.553	13.573	0.695	0.0032
<i>Pistacia terebinthus</i>	0.00122	0.173	0.413	84.538	45.234	2.236	0.129	1.194	0.282	1.390	0.794	11.844	1.175	0.0039
<i>Populus tremula</i>	0.00224	0.223	0.440	67.311	NA	NA	NA	NA	NA	NA	0.642	15.410	1.172	0.0039
<i>Prunus spp</i>	0.00353	0.073	0.497	57.625	44.180	1.967	0.150	1.860	0.460	2.633	0.766	11.311	0.807	0.0038
<i>Quercus cerris</i>	0.00393	0.196	0.395	67.197	45.021	2.277	0.151	0.781	0.246	1.402	0.759	12.491	1.648	0.0034

<i>Quercus coccifera</i>	0.00036	0.321	0.509	155.218	46.836	1.175	0.073	0.979	0.214	1.014	0.894	8.992	1.184	0.0020
<i>Quercus frainetto</i>	0.00717	0.283	0.442	75.424	45.285	2.313	0.164	0.831	0.339	1.528	0.749	12.493	1.119	0.0049
<i>Quercus ilex</i>	0.00123	0.255	0.465	130.590	46.733	1.335	0.066	0.902	0.202	0.885	0.817	10.806	0.722	0.0030
<i>Quercus pubescens</i>	0.00246	0.223	0.445	84.218	44.913	2.138	0.126	1.097	0.331	1.008	0.794	14.717	1.049	0.0043
<i>Salix alba</i>	0.00105	0.210	0.206	63.949	41.390	2.440	0.353	0.244	0.660	2.590	0.490	16.888	1.869	0.0051
<i>Salix caprea</i>	0.00243	0.243	0.297	48.156	NA	NA	NA	NA	NA	NA	0.588	14.082	0.987	0.0039
<i>Sorbus domestica</i>	0.00153	0.130	0.226	35.950	43.270	2.580	0.233	1.610	0.230	3.630	0.785	13.414	0.873	0.0064
<i>Tillia platyphyllos</i>	0.00880	0.230	0.312	53.225	42.460	2.630	0.197	2.500	0.810	2.180	0.561	13.909	1.012	0.0034

Table 4: Summary of the Standardised Major Axis regression applied at the mean species values for some key functional characters.

Y	X	n	elevation	slope	r	p
Lth	LMA	42	-2.72	1.11	0.67	<0.001
C	LMA	32	1.50	0.08	0.42	<0.001
N	LMA	32	1.48	-0.64	0.75	<0.001
P	LMA	32	0.64	-0.81	0.51	<0.001
P	N	32	-1.24	1.27	0.73	<0.001
Asat	gmax	39	2.64	0.63	0.52	<0.001

3.3 Functional Variation and Traits Interrelationships

The following analysis is applied on the full dataset without grouping individual trees into neither species nor PFTs, in order to explore for common axis of variation that could highlight the key functional dimensions (Wright et al. 2004). We used a PCA on the correlation matrix in two cases. In the first case we only included plant chemical and structural properties. Results of this first PCA are summarised in table 5. The three first axes explained 66% of the variation. The first axis expresses the classic leaf economic spectrum and it is highly correlated with the area of the leaf (L_A), the leaf dry mass per area (M_A), the thickness (L_T) and the C, N and P concentrations of the leaf. It agrees with global wide analyses where this dimension represents a trade-off that "contrasts inexpensive short-lived leaves with rapid return on C and nutrient (N, P) investment with costly long-lived leaves with slow returns on investment" (Reich 2014). The second dimension identified in our structural/chemical PCA is related to leaf dry matter content (DMC) and wood density (WD). This can be considered a drought tolerance dimension that coordinates at both the leaf and the wood level. Species that are tolerant to drought and usually exhibit a high DMC and WD value. Finally the third axis (accounting only for 0.08 of the variation) is strongly related only to leaf Ca concentration. The role of Ca in leaf structure and function is important but only lately has started to gain the attention of ecological research.

Table 5: Principal Components Analysis on structural and chemical traits.

eigenvalues	0.385	1.576	0.943
variance	0.449	0.131	0.079
LA	0.737	-0.098	-0.399
LMA	-0.893	-0.212	0.066
DMC	-0.400	0.608	-0.298
LT	-0.799	-0.441	0.177
WD	0.214	0.776	-0.064
C	-0.736	0.001	-0.301
N	0.880	-0.067	-0.184
S	0.586	-0.226	0.127
P	0.797	-0.295	-0.143
Ca	0.409	0.390	0.696
Mg	0.652	0.022	0.078
K	0.561	-0.247	0.061

Subsequently in a second PCA, along with the structural/chemical traits we included the leaf carbon exchange related ones. Here all nutrients are expressed on an area basis, after being multiplied with M_A . This PCA is summarised in Table 6 and the first three axes explained 63% of the variation. In this case the leaf economic spectrum dimension is retained on the first axis, while the second axis is strongly related to the gas exchange traits. PCA axes are by definition considered independent (orthogonal) so this results is interesting because it suggests that photosynthetic

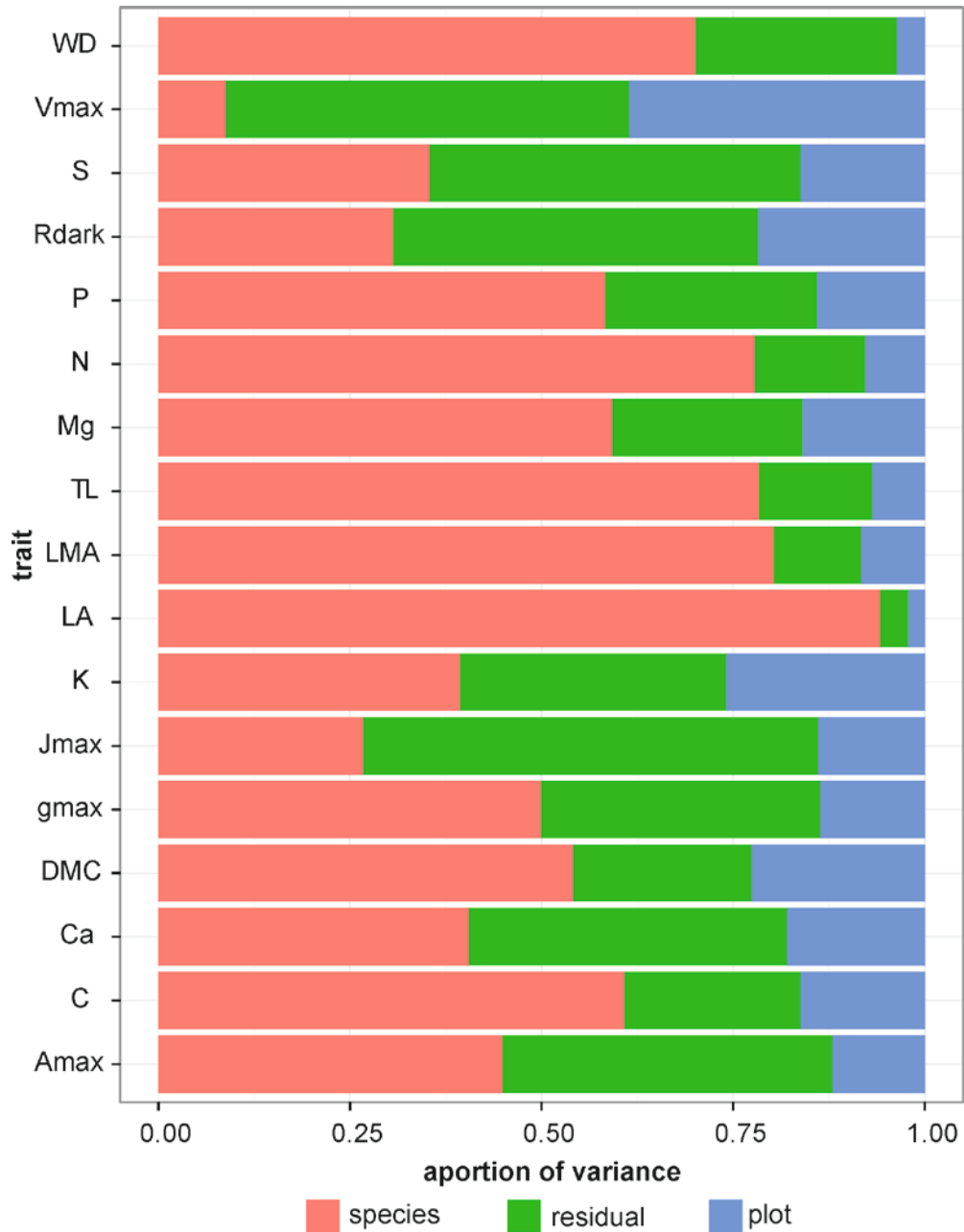
properties could to some extent function independently of leaf structure. The third axis now is mainly associated to leaf dark respiration.

Table 6: Principal Components Analysis on structural, chemical and gas exchange traits.

eigenvalues	7.135	2.257	1.392
variance	0.420	0.133	0.082
LA	0.653	-0.350	0.335
LMA	-0.962	0.014	0.033
DMC	-0.258	-0.179	-0.249
LT	-0.912	0.030	0.141
WD	0.547	-0.164	-0.248
C	-0.963	0.026	0.024
N	-0.733	-0.335	0.295
S	-0.549	-0.125	0.161
P	-0.768	-0.148	0.297
Ca	-0.671	0.041	-0.227
Mg	-0.604	-0.236	0.273
K	-0.716	-0.024	0.231
Amax	0.318	-0.865	0.074
Rdark	-0.492	-0.119	-0.517
Vmax	-0.224	-0.668	-0.445
Jmax	-0.464	-0.449	-0.424
gmax	0.540	-0.675	0.322

After identifying some important trait dimensions, we further investigate our dataset by applying a mixed effect multilevel linear model, where taxonomic information (i.e. species identity) was set as a fixed factor and plot as random (Fyllas et al. 2009). Results from the mixed effect model (Figure 6) indicate that for most traits the major source of variation is associated with its taxonomic (ecotypic). The most strongly genetically constrained characters were L_A , LMA, N and WD. On the other hand the photosynthetic parameters were highly plastic, i.e. showing a great variation with site and thus suggesting that the growing conditions a plant is experiencing could have a large control on these properties. These results are in agreement with the independent second dimension of leaf gas exchange data, identified in the PCA analysis.

Figure 6: Variance Partitioning of the functional characters



We then applied Standardised Major Axis (SMA) regression to identify potential traits intercorrelations. We used the `smatr` package in the R statistical language. Some of the most important associations are presented in the following figure (7) and table (7). We present results only for the most important traits that are extensively reported in the literature and used in vegetation dynamics models.

Figure 7: Interrelationships between the raw trait values. Straight lines are significant SMA regression fits. Colors represent the three discrete leaf types, C for conifers, DB for Deciduous Broadleaves and EB for Evergreen Broadleaves. C and EB were considered together in this analysis as evergreens. All trait values are \log_{10} transformed. Solid lines indicate a significant SMA fit.

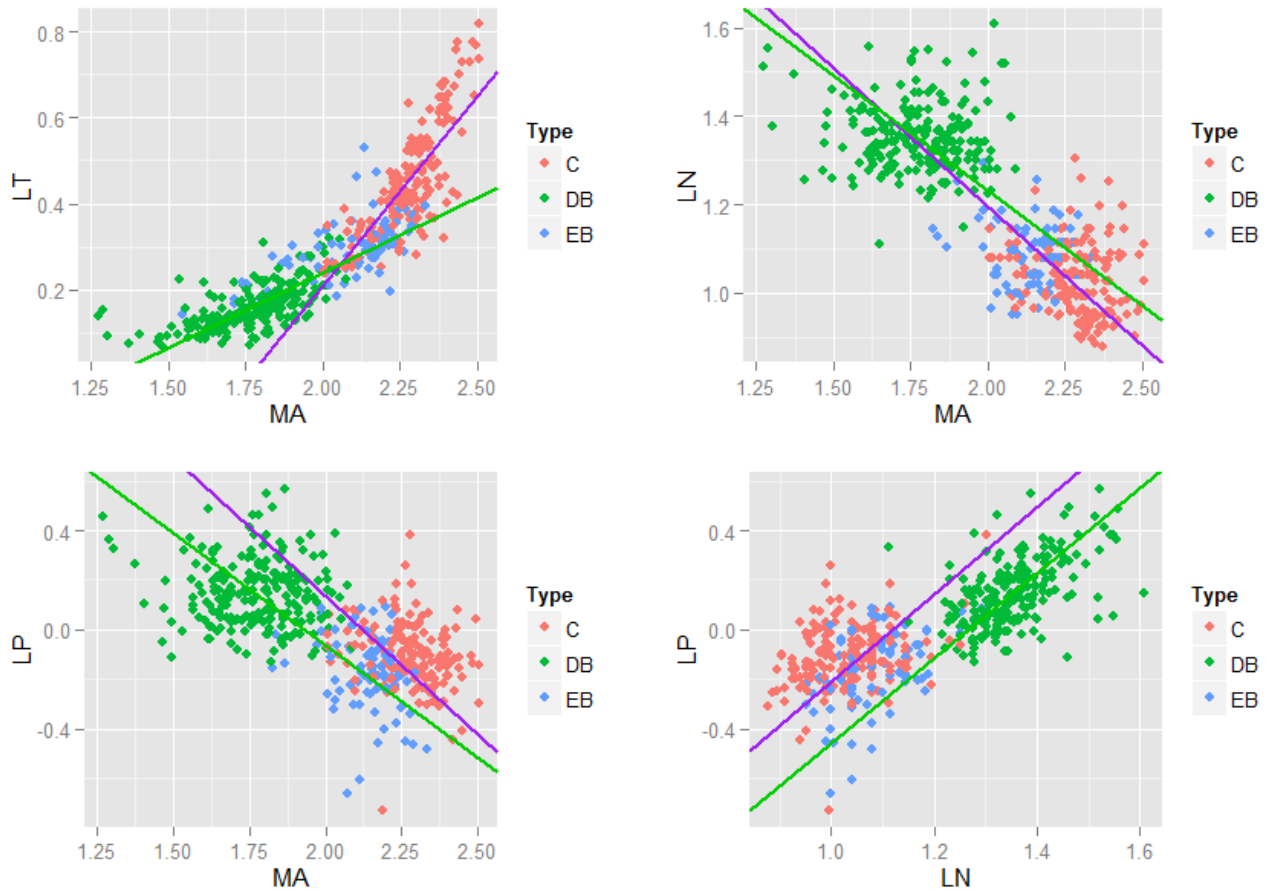


Table 7: Summary of the Standardised Major Axis regression applied at the raw values of some key functional characters.

Y	X	evergreens				deciduous			
		elevation	slope	r	p	elevation	slope	r	p
LT	LMA	-1.549	0.880	0.779	0.000	-0.455	0.349	0.669	0.000
LN	LMA	2.453	-0.629	-0.311	0.000	2.278	-0.523	-0.113	0.105
LP	LMA	2.364	-1.114	-0.081	0.200	1.752	-0.906	-0.104	0.136
LP	LN	-1.964	1.755	0.259	0.000	-2.168	1.711	0.519	0.000

In order to look into the genetic component of trait intercorrelations, we extracted the species effects terms from the mixed effect model and then explored for associations between them. These values represent the "best" estimate of the value a trait would have in a "neutral" environment, i.e.

after removing the environmental plasticity effects. A full correlation table of the traits genetic component is presented below (Table 8), with the upper diagonal reporting the *Pearson's* ρ and the lower the p value of the correlation. In addition we applied again the SMA regression technique to the genetic component of key traits. This analysis revealed important patterns of trait associations. Figure 8 summarises some interrelationships between the structural and the chemical characters. For example leaf DMC and L_T scaled with leaf mass per area, suggesting that thicker leaves have a higher mass per area ratio. As predicted from the leaf economic spectrum leaf N and P content is lower at more expensive leaves (higher LMA). This was also confirmed with our dataset. Leaf N and P content are also strongly linked. Finally WD positively scaled with DMC suggesting a species conservative strategy is illustrated at different plant organs, in our case both leaves and branchwood.

Figure 8: Interrelationships between the genetic component of key functional traits revealing convergence between leaf structural and chemical properties. Solid lines are significant SMA fit (see text and table for details). All trait values are \log_{10} transformed, apart from WD.

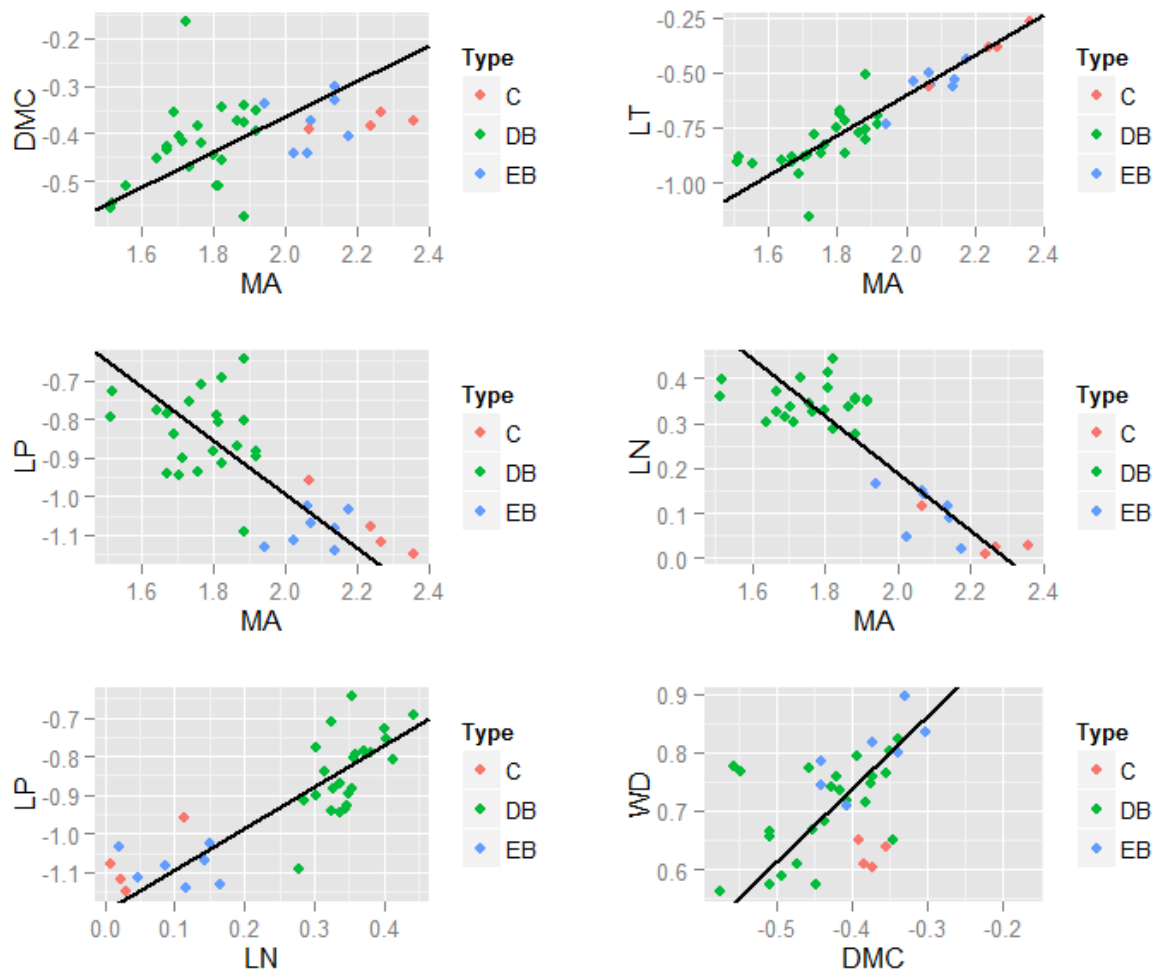


Table 8: Intercorrelations between the functional traits of the MEDIT dataset. Upper diagonal indicates Pearson's ρ and lower diagonal the respective p value.

	LA	LMA	DMC	LT	C	N	S	P	Ca	Mg	K	WD	Amax	Rdark	Vmax	Jmax	gmax
LA		-0.496	-0.159	-0.480	-0.285	0.666	0.538	0.603	0.277	0.486	0.520	-0.120	0.578	-0.533	0.164	-0.185	0.462
LMA	0.001		0.474	0.858	0.670	-0.856	-0.748	-0.700	-0.604	-0.687	-0.721	-0.031	-0.242	0.640	0.210	0.124	-0.318
DMC	0.334	0.002		-0.043	0.412	-0.477	-0.425	-0.558	-0.312	-0.562	-0.607	0.214	-0.389	0.278	-0.011	-0.110	-0.399
LT	0.002	0.000	0.797		0.564	-0.785	-0.671	-0.549	-0.550	-0.538	-0.509	-0.141	-0.056	0.649	0.266	0.228	-0.148
C	0.096	0.000	0.012	0.000		-0.670	-0.568	-0.594	-0.642	-0.692	-0.576	-0.054	-0.126	0.651	0.353	0.173	-0.356
N	0.000	0.000	0.003	0.000	0.000		0.749	0.825	0.500	0.724	0.557	-0.102	0.502	-0.605	-0.015	-0.182	0.597
S	0.001	0.000	0.010	0.000	0.000	0.000		0.702	0.571	0.547	0.558	-0.084	0.468	-0.486	0.055	0.043	0.403
P	0.000	0.000	0.000	0.001	0.000	0.000	0.000		0.556	0.749	0.619	-0.284	0.430	-0.234	0.216	-0.067	0.373
Ca	0.108	0.000	0.064	0.001	0.000	0.002	0.000	0.000		0.657	0.652	0.028	0.077	-0.289	-0.387	0.086	0.107
Mg	0.003	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000		0.643	-0.134	0.264	-0.402	-0.047	-0.119	0.300
K	0.001	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000		-0.078	0.267	-0.323	-0.102	-0.013	0.212
WD	0.472	0.851	0.191	0.398	0.756	0.553	0.628	0.094	0.869	0.436	0.651		-0.242	-0.201	-0.370	-0.213	-0.170
Amax	0.000	0.150	0.016	0.741	0.463	0.002	0.004	0.009	0.657	0.119	0.115	0.149		-0.359	0.539	0.071	0.810
Rdark	0.006	0.001	0.179	0.000	0.000	0.001	0.014	0.260	0.161	0.046	0.115	0.336	0.078		0.381	0.319	-0.567
Vmax	0.435	0.313	0.957	0.199	0.083	0.943	0.795	0.299	0.056	0.822	0.627	0.069	0.005	0.060		0.439	0.206
Jmax	0.376	0.555	0.599	0.273	0.410	0.383	0.840	0.749	0.681	0.572	0.949	0.306	0.738	0.121	0.028		-0.082
gmax	0.004	0.055	0.013	0.384	0.033	0.000	0.015	0.025	0.534	0.076	0.214	0.314	0.000	0.003	0.324	0.698	

The SMAs approach was also implemented to look for associations between leaf chemical and structural traits and characters related to leaf gas exchange. Strong interrelationships have already been revealed from the correlation matrix. For example A_{\max} is strongly related to L_A and leaf N and P content. R_{dark} on the other hand is negatively related to L_A and N but positively to LMA and L_T and C content. Some key scaling relationships are presented in the following figure (Fig. 9) with the respective estimates of the SMA fit given at Table 9.

Table 9: Summary of the Standardised Major Axis regression analysis on genetic trait values. All trait values are \log_{10} transformed, apart from WD.

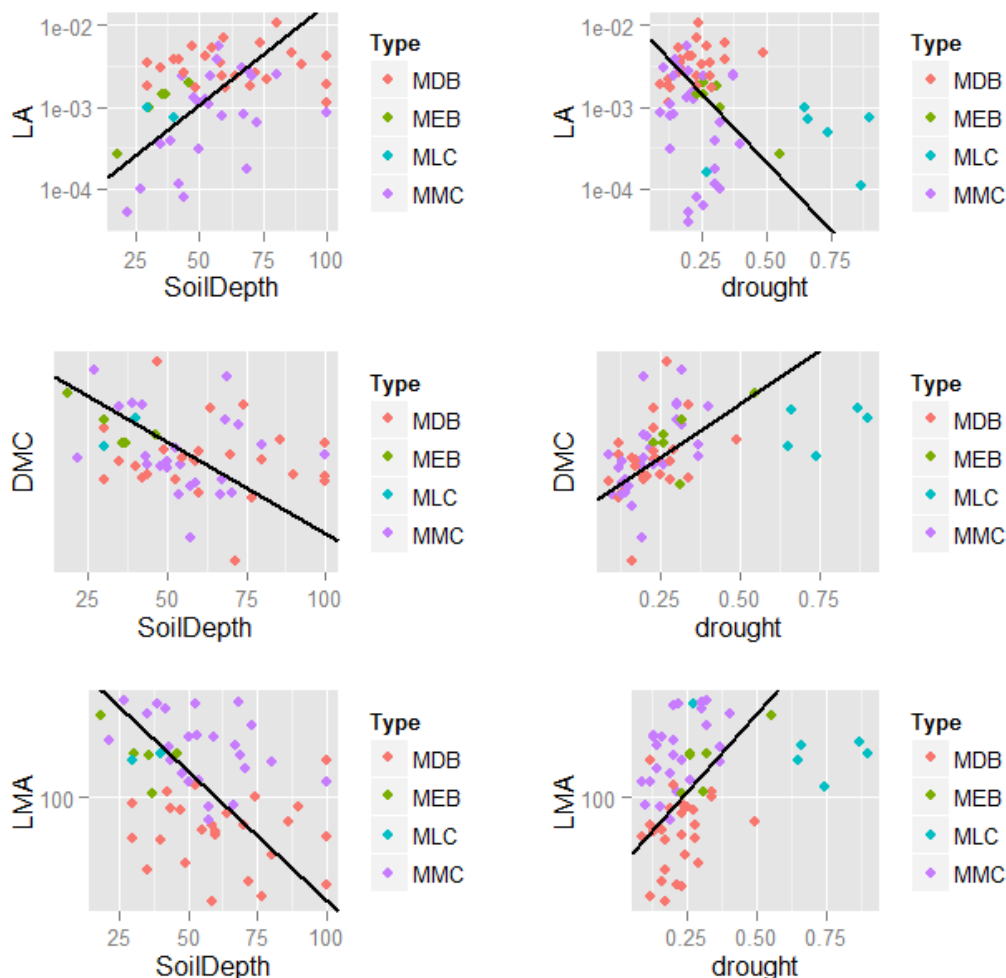
Y	X	n	intercept	slope	R^2	p
DMC	LMA	35	-1.104	0.371	0.158	0.018
L_T	LMA	35	-2.425	0.911	0.805	<0.001
P	LMA	33	0.392	-0.694	0.542	<0.001
N	LMA	33	1.466	-0.640	0.727	<0.001
P	N	34	-1.201	1.079	0.703	<0.001
WD	DMC	35	1.238	1.251	0.208	0.006
A_{\max}	LMA	35	1.966	-0.522	0.053	0.185
R_{dark}	LMA	23	-0.657	0.401	0.406	0.001
V_{\max}	LMA	23	1.542	0.137	0.033	0.410
g_{\max}	LMA	35	-0.954	-0.855	0.088	0.084
A_{\max}	N	34	0.779	0.813	0.259	0.002
R_{dark}	N	23	0.272	-0.622	0.362	0.002
V_{\max}	N	23	1.746	0.213	0.000	0.972
g_{\max}	N	34	-2.896	1.320	0.348	<0.001
A_{\max}	P	34	1.684	0.754	0.213	0.006
R_{dark}	P	23	-0.447	-0.598	0.063	0.248
V_{\max}	P	23	1.992	0.205	0.049	0.309
g_{\max}	P	34	-1.426	1.224	0.144	0.027

3.4 Trait - Environment Relationships

The final part of our analysis focused on the identification Trait-Environment relationships. This was achieved by looking for associations between 1) the mean plot level trait value as estimated from the raw data and the respective set of environmental parameters characterizing a site (climatic and edaphic) and 2) the environmental effects vs environmental parameters as extracted from the mixed effect model. The first analysis integrated the effects of environment and biogeography while the second one uses only the estimated environmental plasticity component.

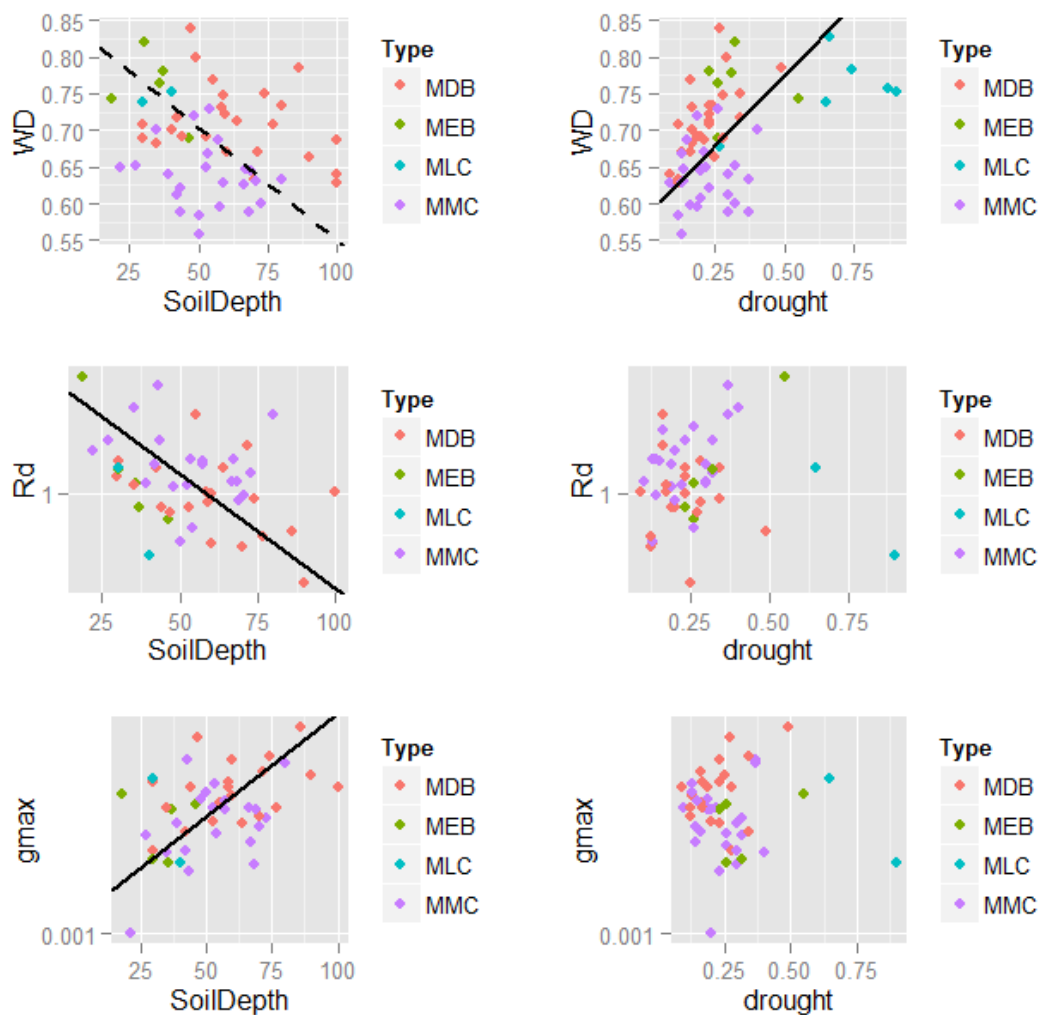
When the mean trait value per plot was used strong associations between LA, LMA and DMC with soil depth and climatic drought were identified. An illustration of these associations is provided in figure 9. LA increased with soil depth and decreased with drought with DMC and LMA decreasing with soil depth and decreasing with drought. This indicates that more conservative leaf strategies are found at drier sites.

Figure 9: Mean plot level trait value for leaf area (LA), leaf dry matter content (DMC) and leaf mass per area (LMA) against soil depth and climatic drought. Y-axis is \log_{10} transformed. Solid lines indicate a statistically significant association (at $p < 0.05$) and broken lines a significant association (at $p < 0.1$).



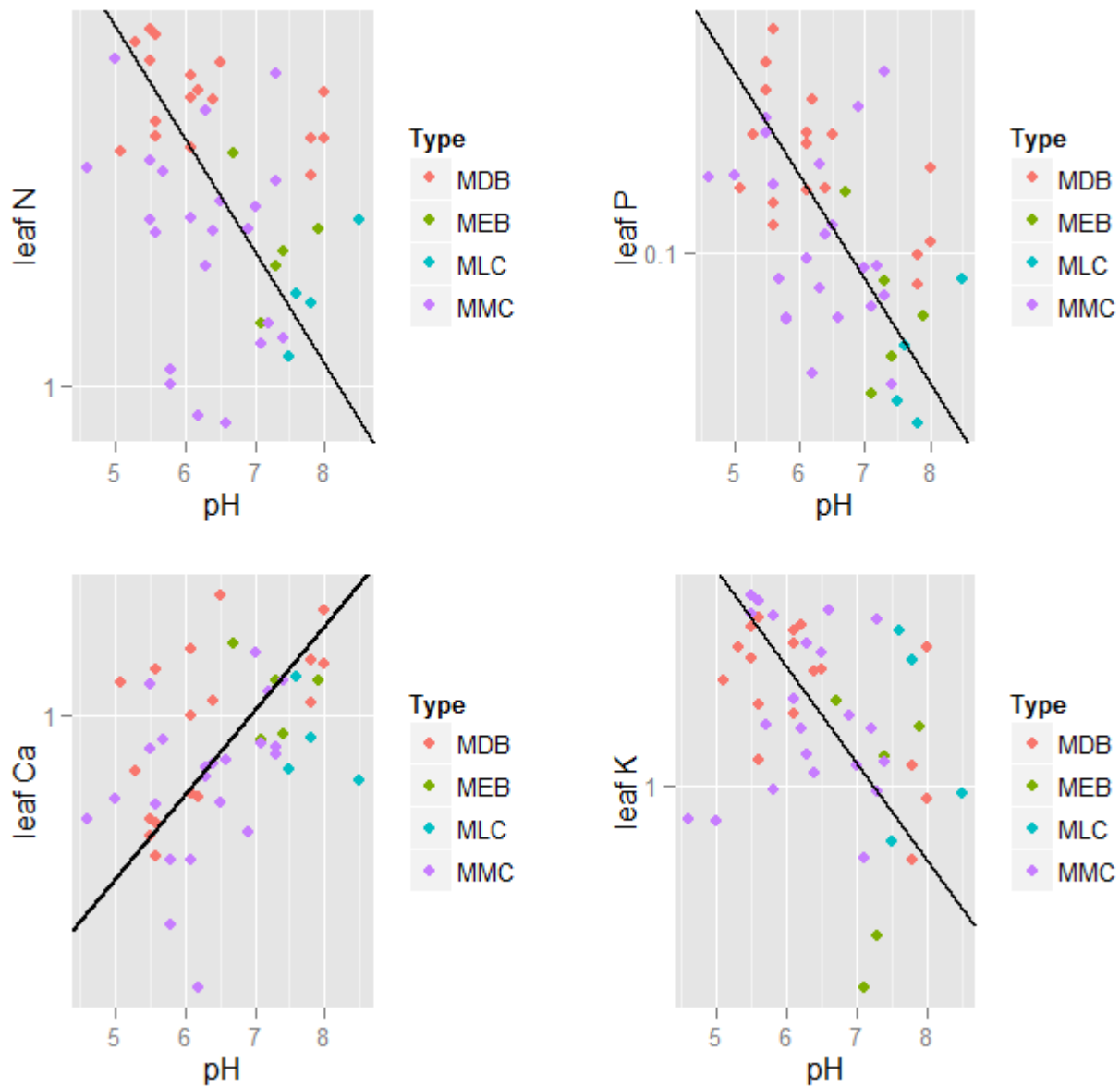
Wood density seems to co-vary with leaf structural properties, leading to more conservative strategies (higher WD) at lower soil depth and higher drought. This is an important finding, indicating functional convergence through different plant components (both leaf and wood) leading to an overall coordinated strategy. In terms of leaf traits related to biochemical fluxes, Asat did not presented any significant association with soil depth or drought, but stomatal conductance did vary in accordance with leaf and wood structural variation. Higher g_{max} was observed in deeper soils, i.e. where water limitation is less frequent. R_d on the other hand seems to operate independently of the other traits, showing higher values at shallower soils. It would have been expected that trees found there (shallower) soils wood respire less if R_d was directly related to stomatal conductance. However our data suggest that R_d seems to follow the LMA and WD trend probably expressing that more expensive leaf and woody components need a higher maintenance respiration.

Figure 10: Mean plot level trait value for wood density (WD), \log_{10} leaf dark respiration (Rd) and \log_{10} maximum stomatal conductance (g_{max}) against soil depth and climatic drought. Solid lines indicate a statistically significant association (at $p < 0.05$) and broken lines a significant association (at $p < 0.1$).



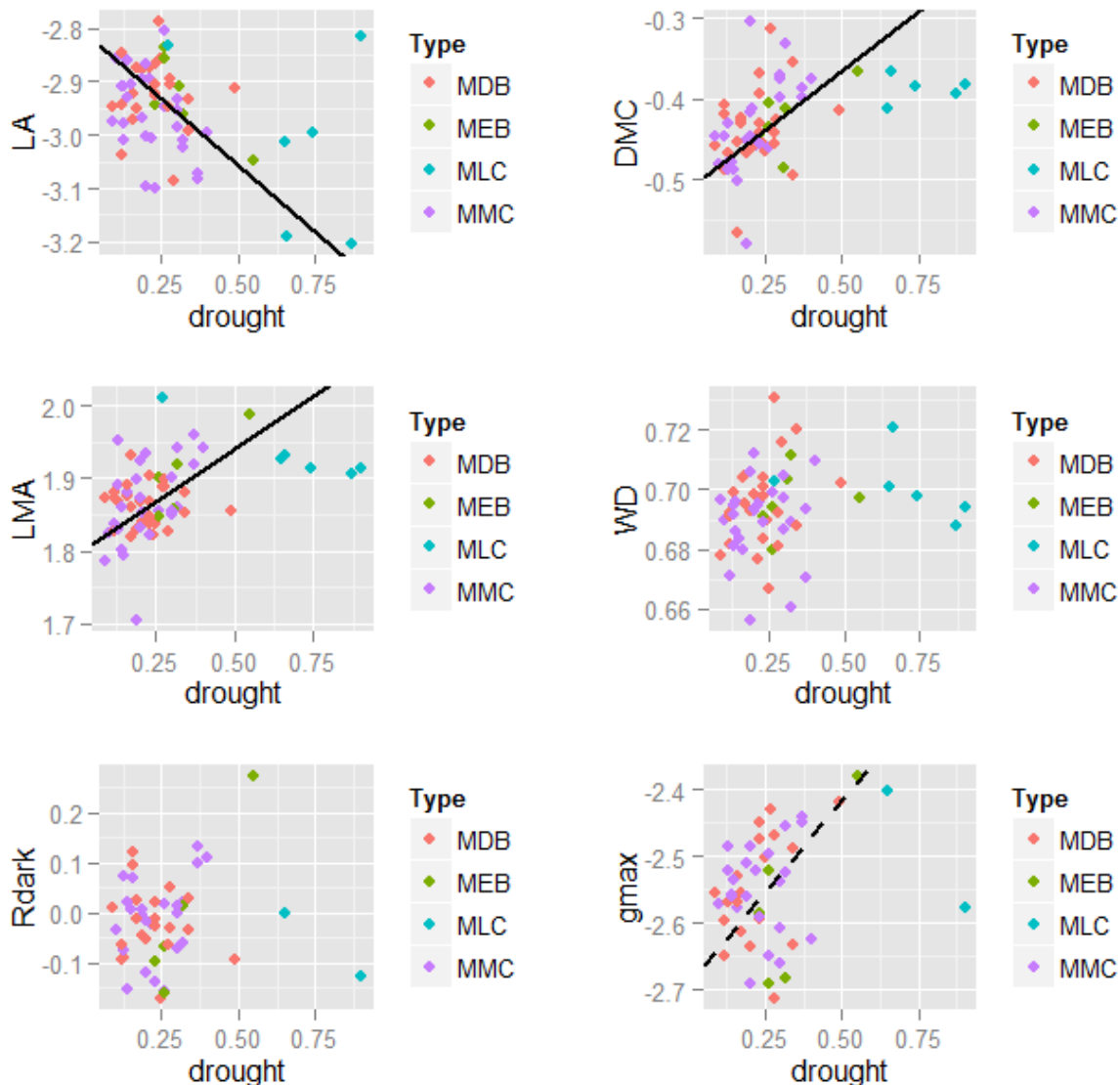
In terms of the leaf and soil chemical associations pH was the stronger predictor of leaf N, P, Ca and K (Figure 11). Apart from Ca all leaf level nutrient concentrations decreased with pH, i.e mean stand level N, P and K leaf concentrations are higher at infertile soils in contrast to Ca. These findings seems to suggest that for N, P and K the biotic control i.e. the species synthesis is a stronger regulator of leaf chemistry. Interestingly this agrees with strong variance component estimated particularly for N and P through the mixed effect model. Ca on the other hand seems to be highly driven by soil chemistry.

Figure 11: Mean plot level trait value for leaf N, P, Ca and K concentrations against soil pH. All leaf traits are \log_{10} transformed. Solid lines indicate a statistically significant association (at $p < 0.05$) and broken lines a significant association (at $p < 0.1$).



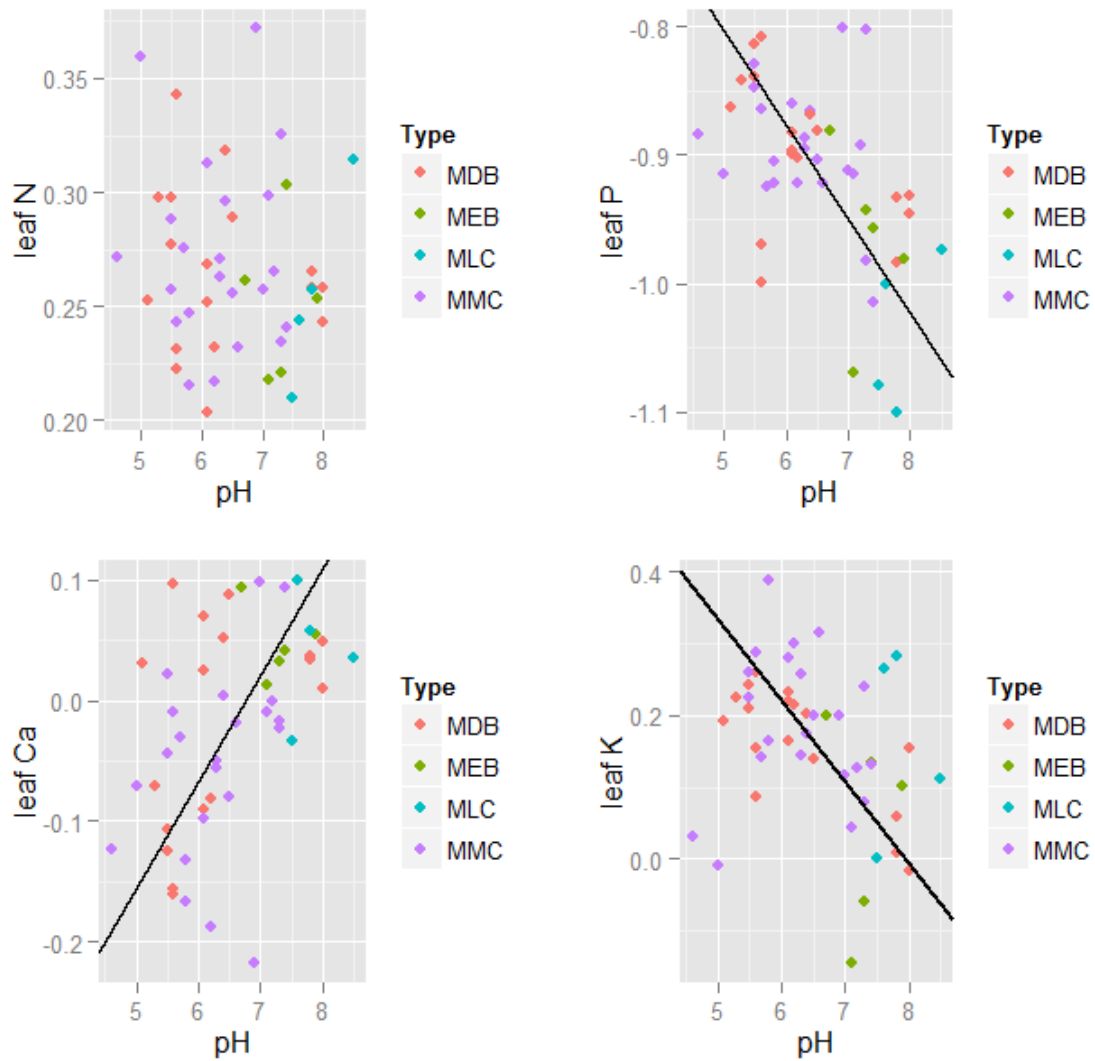
We then moved on to try to explain the environmental component of the trait variation, as estimated through the plot effect term of the mixed effect model. In this case climatic drought was a stronger predictor of environmental component of trait variation (Figure 12). Similar to the plot means approach, LA decreased with drought while DMC and LMA increased. WD did not showed any statistically significant trend suggesting that there a potential biotic (through species syntehsis) effect that leads to an increased WD at drier places (Figure 10). Interestingly the environmental component of gmax increased with drought, an effect not found in the mean stand level approach, suggesting that when the taxonomic effect is removed, there seems to be an increased gmax with drought in most forest types apart from MLC.

Figure 12: Plot effect trait terms for leaf area (LA), leaf dry matter content (DMC), leaf mass per area (LMA), wood density (WD), leaf dark respiration and maximum stomatal conductance (gmax) against climatic drought. All leaf traits are log₁₀ transformed apart from WD. Solid lines indicate a statistically significant association (at p<0.05) and broken lines a significant association (at p<0.1).



Finally in terms of leaf - soil chemistry associations the environmental term for P and K were similar to the mean stand level approach with a negative association. Leaf Ca increased with pH showing again a strong association with soil fertility. A similar trend was found with soil ECEC (not shown)

Figure 13: Plot effect trait terms for leaf N, P, Ca and K concentrations against soil pH. All leaf traits are \log_{10} transformed. Solid lines indicate a statistically significant association (at $p < 0.05$) and broken lines a significant association (at $p < 0.1$).



4. Discussion - Conclusions

Our analysis identified a wide variation in the functional traits studied between species and among study sites, as well as significant associations between many trait pairs. For most traits the greater portion of the observed variation can be found within individuals of the same species underlying the importance of interspecific variation and phenotypic plasticity (Sultan 2000). However for some functional characters such as the carboxylation, the electron transport and the dark respiration rates, the between plots variation was of the same magnitude as the taxonomic variation, suggesting that growing conditions could be also important (Fyllas et al. 2009). This seems to agree with studies that suggest that particularly dark respiration is able to adapt to the prevailing climatic conditions a phenomenon known as acclimation (Atkin and Tjoelker 2003, Zaragoza-Castells et al. 2008). The above suggest that modelling studies that use a range of functional traits should take into account these sources of variation by either using site specific trait values and distributions or by shifting these parameters along environmental gradients in a systematic way.

Many trait pairs presented statistically significant associations. Three important axes of trait variation have been identified, with the first one expressing the leaf economic spectrum (Reich et al. 1997) and the second and third one referring to the photosynthetic and respiratory carbon fluxes. Interestingly deciduous and evergreen species were located in distinct areas along the axis of the leaf economic spectrum, presenting in some cases different scaling coefficients (ex L_T vs LMA). Such finding suggest that different scaling equations should be used for ecologically distinct species groups, such as evergreens and deciduous trees (Reich 1995). When only the genetic-taxonomic component of trait variation was considered, and leaf habit differentiation was not taken into account trait associations remained significant. For example LMA was highly negatively associated with dry mass N and P leaf concentrations while A_{max} showed a strong positive relationship with the concentrations of those two nutrients. These findings are in agreement with the leaf economic theory (Wright et al. 2004, Osnas et al. 2013), with the scaling coefficient estimates being however different for the species measured in MEDIT. N scaled with LMA with a less steep slope (-0.694 vs -0.782) and the same was true for the P - LMA relationship. A_{max} scaled positively with leaf N and P concentrations with however a steeper slope (0.813 vs 0.63) and less (0.754 vs 0.984) respectively, suggesting that for the species studied within MEDIT leaf N concentrations might strongly limit photosynthesis. Finally R_{dark} scaled positively with LMA and negatively with N concentration. Taking into account the strong association of WD with DMC, i.e a positive relationship that shows that plants that follow a conservative leaf strategy also follow a conservative stem strategy, our findings support the expansion of the fast to slow plant economic spectrum across various plant organs (Reich 2014).

Although for most traits the environmental component of trait variation was not very strong, a systematic prediction of environmental effects on this variation at either the individual or the community level could be achieved. Soil depth, climatic drought and soil pH were the stronger predictors of this variation. In general drier conditions (lower soil depth and higher temperature to precipitation ratio) led to a decrease in LA and an increase in LMA, DMC and WD. This is in accordance

with an expectation of more conservative strategies under higher stress environments. On the other hand lower soil pH (infertile conditions) was associated with higher leaf nutrient concentrations, suggesting the biotic component of the nutrient cycles is probably more important than the sole impact of soil chemistry.

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