CASCADE

Catastrophic shifts in drylands:

How can we prevent ecosystem degradation?

CASCADE REPORT series

Identification of critical changes preceding catastrophic shifts: ecosystems affected by increasing grazing intensity and severe drought

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Identification of critical changes preceding catastrophic shifts: ecosystems affected by increasing grazing intensity and severe drought

Project: CASCADE CAtastrophic Shifts in drylands:
how CAn we prevent ecosystem DEgradation?

Coordinator: Prof. Dr. Coen J Ritsema.
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Preface

Work package 3 (WP3: Identification of critical changes preceding catastrophic shifts: ecosystems affected by increasing grazing intensity and severe drought) focuses on observational and manipulative field experiments to investigate changes in the plant-soil system in response to external stress (i.e. increasing fire frequency and increasing grazing intensity, individually and each of them combined with severe drought). The aim of these experiments was to identify the most sensitive parameters to these changes that then might serve as indicators for sudden catastrophic shifts, i.e. in the form of desertification.

Two WP3 experiments have been conducted in the 6 CASCADE study sites. In the first experiment, named the '*stress- gradient* experiment', measurements of soil quality and plant performance were taken at three levels of stress (low – medium – high) determined by either fire recurrence (in the sites of Várzea in North-Central Portugal and Valencia in Eastern Spain), or grazing intensity (in the sites of Santomera in Eastern Spain, Castelsaraceno in Southern Italy, Messara in Southern Crete and Randi in Southern Cyprus). The second experiment, named the '*drought-stress experiment'*, was a rainfall-exclusion experiment in which the same plant and soil variables were measured during periods of rainfall exclusion. This experiment was carried out under a medium high (grazing or fire frequency) stress level, as we expected that a combination of two stressors would most likely result in a tipping point indicating a regime shift.

We earlier submitted a first Deliverable (D3.1a) that included the results of the stress-gradient experiment in the two sites in which stress was defined by fire frequency, i.e. Várzea in North-Central Portugal and Valencia in Eastern Spain. These results have been published in an international peer-reviewed scientific journal (Mayor et al. 2016).

In the present Deliverable (D3.1b), we present the results of the stress-gradient experiment in the four sites in which stress was defined by grazing intensity, i.e. Santomera in Eastern Spain, Castelsaraceno in Southern Italy, Messara in Southern Crete and Randi in Southern Cyprus, and the results of the *drought-stress experiment* for all six CASCADE study sites.

Contents

1. Introduction

Catastrophic shifts in ecosystems

One of the most pressing environmental issues the world is facing today is the deterioration of ecosystems and the concomitant loss in ecosystem function and services. Human activities and current global climate change adversely affect ecosystems and induce continuous and discontinuous transitions from one ecosystem state to another. Discontinuous transitions are abrupt and relatively irreversible. Such shifts are called 'sudden shifts'. Sudden shifts can take place over different time scales ranging from days to years. One of the most unwanted 'catastrophic' shifts is the transition of a terrestrial ecosystem from a vegetated state to a bare, desert-like state. Such a catastrophic shift implies enormous losses in ecological and economic resources for the local human populations. For this reason, predicting and preventing such unwanted shifts has become a core issue in current ecosystem research.

Ecological mechanisms underlying catastrophic shifts

The key-principle underlying catastrophic shifts in ecosystems, in the present case of dry Mediterranean ecosystems, is the interplay between small scale facilitation (local facilitation) and large scale competition (global competition) between the plants making up the vegetation. Local facilitation occurs when plants that are nearby one another help each other by means of shading, the improvement of local soil quality through the build-up of organic matter, and/or the acquisition of water and nutrients. This facilitation especially works under relatively harsh conditions. The global competition is inevitable given the scarcity of resources, again especially under harsh conditions. The consequence of the combination of local facilitation and global competition is that the vegetation forms patterns, as plants in a patch with other plants will be better able to survive the harsh conditions than alone (facilitation), while the patches compete with each other (competition). Patterns that occur through facilitation and competition can take several forms, for example gaps of bare soil surrounded by vegetation, labyrinths of vegetation and bare soil, and patches of vegetation surrounded by bare soil (Rietkerk et al. 2004). With decreasing vegetation cover the occurrence of a catastrophic shift becomes more likely (Rietkerk et al. 2004).

Another important principle related to catastrophic shifts is hysteresis. Hysteresis implies that the pathway of deterioration is different from the pathway of recovery. For example, in cases of arid ecosystems this means that when the system passes a threshold for a critical environmental factor, for example nutrient availability, the vegetation collapses and a desert emerges. The recovery will require (much) more nutrients than the last passed critical threshold for the vegetation to re-establish and recover. The principle again is local facilitation: passing the critical threshold means that the plants together are not able anymore to survive. Recovery will mostly occur by the establishment of single plants, which will need relatively high levels of resources as there is no facilitation yet, i.e. the resource availability should be enough for single plants to survive and will be higher than the critical level of resource availability at which the patches of plants perished. Also for this reason, the avoidance of catastrophic shifts is very much wanted as recovery can be ecologically difficult and economically expensive.

Predicting catastrophic shifts

Predicting shifts requires the identification of the critical points or thresholds at which the shifts are likely to occur. Several approaches have been developed. A first approach is the analysis of the key-variables in the shift. For example, in shallow lakes, the key-variable, algal biomass, may serve as a signal for shifts, when algal biomass exhibits a strong temporal variation. This phenomenon is known as 'critical slow-down', which means that the system becomes sensitive to a shift to another state because the attraction by the current equilibrium state is relatively weak (Scheffer et al. 2009). Especially relevant to CASCADE is the analysis of desertification shifts in arid terrestrial ecosystems. Here the key variable is vegetation in terms of biomass, land cover or spatial patterning. Especially the spatial patterning of the vegetation seems to be important in this respect. Kéfi et al. (2007) analysed spatial vegetation patterns in terms of the frequency distribution of vegetation patch sizes in three arid Mediterranean ecosystems in Spain, Greece and Morocco. They showed that the patch-size distributions away from tipping points follow a power law, but approaching to the tipping point the patterns deviate from power law distributions. These deviations are a disproportionate high frequency of relatively small patches. The mechanism behind this is that patches shrink to the minimum size for survival through facilitation. In this way, spatial vegetation patterning can serve as an early warning signal for the proximity of tipping points and unwanted sudden catastrophic shifts. Following this approach, Verwijmeren et al. (2014) show that, at the community level, plants under water stress change from negatively associated at no grazing to positively associated at low grazing pressure and randomly associated at higher grazing pressure, while less water-stressed plants the dominant species shifted from excluding each other to co-occurring with increasing grazing pressure. This observational study suggests that environmental pressure increases positive plant-plant interactions, but when the stress level passes certain thresholds, the positive interactions wane. Verwijmeren et al (in review) aimed to prove the above using a manipulative experiment and found that positive plant-plant interactions actually decrease under the combination of high drought stress and grazing pressure, which may undermine ecosystem resilience to increased drought and overgrazing.

A second approach in predicting catastrophic shifts is looking at the occurrence and strength of the environmental drivers that may push the ecosystem to the other unwanted state. Kéfi et al. (2007) show empirically and by modelling that grazing intensity and/or resource availability may force the system towards the shifts and by this that the levels of strengths of these drivers can serve as the point where the shifts are likely to occur. Modelling studies of shallow lakes have successfully adopted this driver approach in assessing critical levels of phosphate loading for ecosystem shifts to occur (Janse et al. 2008; Mooij et al. 2007)

A system approach to predict catastrophic shifts

A third and new way to better understand and predict shifts in ecosystem states is taking a system approach. In such an approach as many ecosystem properties as possible, and the interplay between these properties, are taken into account. Recently, this approach has successfully been applied to the shallow lake example (Kuiper et al. 2015). In this study, a model was used that was parameterized on the basis of observations from a wide array of real shallow lake ecosystems. The model PCLake included abiotic (i.e. nutrients, organic matter) and biotic (food web composition, biomass structure and feeding rates) lake attributes. The combination of all these properties, and the interactions among them, enabled a stability analysis along a gradient of nutrient loading. The results showed that along nutrient loading the ecosystem becomes less stable up to the tipping point where after it shifts from a clear-water to a turbid-water equilibrium state, which is relatively stable again. Going back by reducing the loading, the system stability decreases again up to the critical point, whereafter it shifts back to the original equilibrium state. The advantage of the system approach was that it opened the black box as it revealed that particular key groups of organisms and their interactions, in this case zooplankton, diatoms and detritus, are critical to stability and how trophic structure may serve as early and timely indicator of an ecosystem shift. The application of the ‘system approach’ in predicting and preventing shifts in dryland ecosystems is described in the next section.

The CASCADE-WP3 approach

Characteristics of the CASCADE-WP3 approach are

- It is fully experimentally driven. All observations are carried out in real-world ecosystems under field conditions.

- The ecosystems are selected on the basis of the assumption that they suffer from environmental stress that may lead to catastrophic shifts. Wildfire and grazing are the stressors studied under Mediterranean conditions, with or without extreme drought.
- It is a combination of observations and manipulative experiments. First, the ecosystems are studied in the way they function naturally. Second, the ecosystems are manipulated by enhancing drought, which may push the ecosystem towards tipping point as a precursor to a catastrophic shift.
- The measurements include vegetation and soil variables, as many as achievable, in order to create a completely as possible picture of the plant-soil ecosystem.

The CASCADE-WP3 experiments

In the first experiment we assessed soil quality and plant performance at three levels of stress as they occur in the field. The drivers of these stresses are fire recurrence in the sites of Várzea in North-Central Portugal and Valencia in Eastern Spain, and grazing intensity in the sites of Santomera in Eastern Spain, Castelsaraceno in Southern Italy, Messara in Southern Crete and Randi in Southern Cyprus. In this observational approach we measured soil and vegetation properties at the different stress levels and within plant patches and outside plant patches. This experiment is named the '*stress- gradient experiment'*. In the second experiment we again performed the same set of soil and plant measurements, but now under different manipulations. By means of translucent roofs, we excluded rainfall from the plot to enhance drought. As control we used roofs that let the rain fall through, and sites with no roofs. This experiment was named the '*drought-stress experiment'*.

Aims of the CASCADE-WP3 experiments

The aims of the *stress- gradient experiment* were

- Establish the effects of increased fire- and grazing-induced stress on soil quality and plant performance in order to see whether the plant-soil ecosystem moves into the direction of a catastrophic shift.
- Establish the differences of soil quality within plant patches and outside plant patches to see whether and how facilitation occur in the plant patches.

The aims of the *drought-stress experiment* were

- Establish the effects of artificially-increased drought stress over different extended periods up to 24 months on soil quality and plant performance in order to see whether the plant-soil ecosystem moves into the direction of a catastrophic shift.

- Establish stress levels that seem critical for the plants to survive in order to see how such a critical point is close to the present natural status of the ecosystems.

We earlier submitted a first report (D3.1a) presenting the results of the *stress-gradient experiment* in the two sites in which stress was defined by fire frequency, i.e. Várzea in North-Central Portugal and Valencia in Eastern Spain. These results have been published in an international peer-reviewed scientific journal (Mayor et al. 2016[b]). In the present deliverable (D3.1b) we present the results of the stress-gradient experiment in the four sites in which stress was defined by grazing intensity, i.e. Santomera in Eastern Spain, Castelsaraceno in Southern Italy, Messara in Southern Crete and Randi in Southern Cyprus, and the results of the *drought-stress experiment* for all six CASCADE study sites.

2. Study sites and target species

2.1 Study sites

The WP3 experiments were conducted in the six CASCADE study sites, which are all located in southern Europe. Two are stressed by fire: Várzea in North-Central Portugal and Valencia in Eastern Spain, and four by grazing: Santomera in Eastern Spain, Castelsaraceno in Southern Italy, Messara in Southern Crete and Randi in Southern Cyprus. A general description of the study sites including information on geography, soils, climate, land use, degradation drivers, socioeconomic status and evolution of vegetation is given in CASCADE Deliverables 2.1 and 2.2. For this, we here provide a brief summary in Table 1.

Table 1. Summarized description of the study sites.

Variables	**VARZEA (Portugal)**	**SANTOMERA (Spain)**	**VALENCIA (Spain)**	**CASTELSARA-CENO (Italy)**	**MESSARA (Greece)**	**RANDI (Cyprus)**
Stress factor	Fire	Grazing	Fire	Grazing	Grazing	Grazing
Mean annual T (ºC)	13.4	18	14.6	9.1	17.9	19.5
Mean annual rainfall (mm)	1170	268	385	1290	504	489
Elevation (m.a.s.l)	468 - 530	180 – 270	763 - 1041	1764 - 1861	435	140
Aspect	SSW	NE	NW	E/NE	N	NE/W
Soil type & bedrock	Umbrisols; Cambisols over schists	Calcisols; Cambisols over marls	Regosols over marls/ limestone	Regosols over limestone/ dolomite	Cambisols; Luvisols over marls/limestone	Calcaric Regosols over marls
Plant community	Pine woodland	Open shrubland	Pine woodland	Grassland	Open shrubland	Open shrubland

2.2 Target species

For all six CASCADE sites, we identified the most relevant plant species to be considered as target species for WP3 (Milestone 8). The species selected were either shrubs or perennial grasses (Table 2). The selection was based on the importance of their role (abundance; functional role) in the ecosystems of study. The target species in Várzea, *Pterospartum tridentatum*, is a dominant shrub species in the study site, which quickly resprouts after fire and plays a critical role in the post-fire recovery of the vegetation. *Rosmarinus officinalis*, the target species in Valencia, represents a different post-fire regeneration strategy, a post-fire seeding shrub, which is very common in fire-prone shrublands developed in old agricultural fields in the Mediterranean. *Anthyllis cytisoides* and *Calicotome villosa*, the target species in Santomera and Randi, respectively, represent abundant palatable shrubs and therefore represent the species that are the most likely to be affected by grazing intensity. Similarly, *Brachypodium rupestre* and *Stipa austroitalica* in Castelsaraceno and in *Hyparrhenia hirta* Messara represent the common palatable grasses in these grazed areas.

Table 2. Selected target species

Species	Species description
	Name: *Pterospartum tridentatum* (L.) Family: *Fabaceae* Functional group: Shrub Functional strategy: Resprouter species Site: Várzea (Portugal)
	Name: *Anthyllis cytisoides* (L.) Family: *Fabaceae* Functional group: Subshrub Functional strategy: Drought deciduous species Site: Santomera (Spain)

Name: *Rosmarinus officinialis* (L.)

Family: *Lamiaceae*

Functional group: Shrub

Functional strategy: Seeder species

Site: Ayora-Mariola (Spain)

Name: *Brachypodium rupestre* (Host) Roem. & Schult.

Family: *Poaceae*

Functional group: Herb

Functional strategy: Perennial grass

Site: Castelsaraceno (Italy)

Name: *Stipa austroitalica* (Martinovský)

Family: *Poaceae*

Functional group: Tussock grass

Functional strategy: Perennial grass

Site: Castelsaraceno (Italy)

Name: *Hyparrhenia hirta* (L.)

Family: *Poaceae*

Functional group: Subshrub

Functional strategy: Perennial grass

Site: Messara (Crete)

Name: *Calicotome villosa* (Poir.) Link

Family: *Fabaceae*

Functional group: Shrub

Functional strategy : Droughtdeciduous species

Site: Randi (Cyprus)

3. Stress gradient experiment

3.1 Material and methods

The stress-gradient experiment described in the present deliverable was carried out in the four sites in which grazing was defined as the environmental stressor. The sites included plots with different grazing intensities. Relative grazing intensity at each site was quantified by different methods, including direct measurements of livestock density, tracking herd routes to estimate distances to shelters, and dropping counts along transects or within sampling plots. Counts of livestock dung provide a good index of the amount of time that livestock expend grazing in a particular area (Riginos & Hoffman, 2003), and the frequency of goat visits to a particular area decreases with the distance from the shelter, so both variables can be used as comparable indicators. Based on different indicators, we selected plots that followed a linear gradient of grazing pressure in each site (Figure 1). For this grazing gradient, ungrazed (or barely grazed) plots were considered as control plots (hereafter, Low Stress, LS), plots under medium grazing intensity were considered as Medium Stress plots (hereafter, MS) and plots under high grazing intensity were considered as High Stress plots (hereafter, HS). Although we established a linear grazing gradient in all sites, there were differences between sites regarding both the type of livestock and the overall grazing pressure. Thus, goats were the main livestock in Randi and Santomera, while livestock in Messara combined goats and sheep, and livestock in Castelsaraceno included goats, sheep and cattle. Overall grazing pressure during the study period was very high in Randi and Messara, moderate in Castelsaraceno and relatively low in Santomera.

For the soil variables, in addition to the grazing-stress factor, we also assessed the effect of the soil microsite (i.e. soil underneath plant patches and soil in the bare-soil interpatches). This factor is relevant in dryland sites, where vegetation is arranged in patches interspersed in a matrix of more or less bare soil. Therefore, we assessed this as an experimental factor in the CASCADE dryland sites with patchy vegetation, Santomera, Messara and Randi, but not in the wettest site (Castelsaraceno), where patches and interpatches could not be clearly distinguished. For further information about the study-sites: see Deliverable 2.1.

The experimental design for each site therefore consisted of two factors: stress level (HS, MS and LS) and microsite type (vegetation patch and bare-soil interpatch). In each study site, three plots of 30 x 30 m size, approximately, were set up at each of the three stress levels and, for each of these plots, 5 vegetation-patch microplots (P microsite) and 5 interpatch microplots (IP microsite) were selected for repeated soil and plant measurements.

In each study site, the plots were either interspersed or following a block design, depending on the local conditions. Two main sampling campaigns (spring and autumn) were carried out during approximately one year of study period (mostly in 2013), plus some preliminary exploratory campaigns to define the general soil and plant characteristics of each experimental plot.

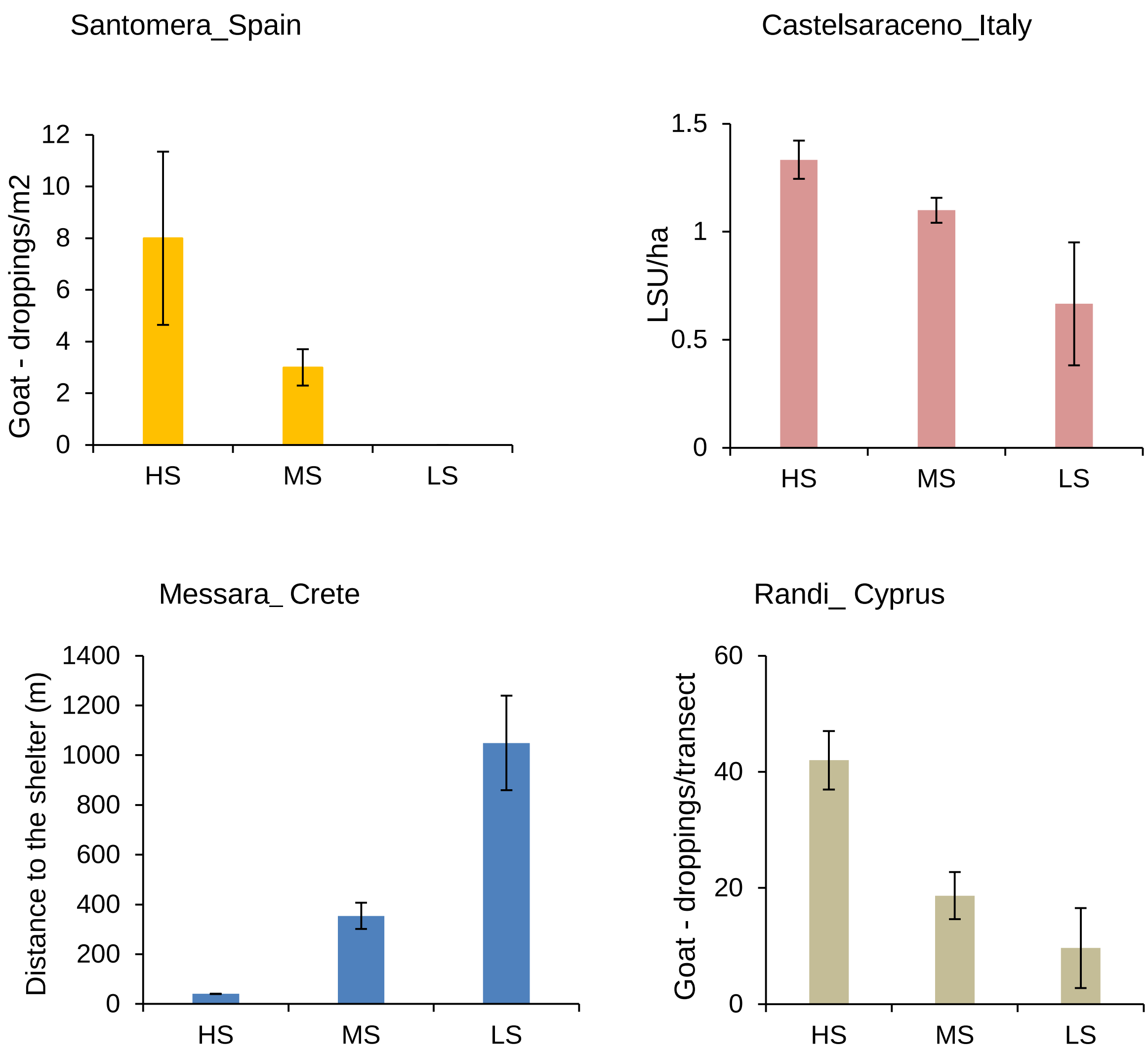


Figure 1. Grazing intensity estimation for each site (different metrics used). LSU/ha means Life Stock Units /ha

Plant and soil measurements

In order to harmonize the sampling and measurement procedures for the assessment of the selected soil-plant system variables (Table 3), an experimental protocol was agreed among the partners. To assess plant performance, we measured Plant Height (PH), Plant Biomass (PB), Plant Cover (PC), Twig Basal Diameter (TBD), Twig length (TL), Branch Basal

Diameter (BBD), Branch length (BL), Specific Leaf Weight (SLW) and Chlorophyll content (SPAD). To assess soil functioning, we measured variables related to soil texture, pH, soil carbon and soil nutrient status. The specific variables measured were Cation-Exchange Capacity (CEC), Soil Organic Carbon (SOC), Potentially Mineralisable Nitrogen (PMN), Available Nutrients (nitrate, ammonium, phosphate), Dissolved Organic Carbon (DOC) and Hot Water Extractable Carbon (HWC). We also assessed soil water content (SWC).

Table 3. Vegetation and soil variables monitored at each site for the grazing stress gradient experiment. Yet, the sets of parameters measured per site could slightly differ, because of plant/soil/ecosystem properties and available expertise/equipment. Acronyms list:

- Vegetation growth: TBD: Twig Basal Diameter; TL: Twig length; BBD: Branch Basal Diameter; BL: Branch length.
- Soft ecophysiological traits: SLW: Specific Leaf Weight; RWC: Relative Water Content; SPAD: Chlorophyll content.
- Soil characterization: CEC: Cation-Exchange Capacity SOC: Soil Organic Carbon.
- Nutrient availability: PMN: Potentially Mineralisable Nitrogen. Available P (Olsen)
- Labile pool of soil C: DOC: Dissolved Organic Carbon; HWC: Hot Water Extractable Carbon.
- SWC: Soil Water Content.

	Variable types	**SANTOMERA (Spain)**	**CASTELSARACENO (Italy)**	**MESSARA (Crete)**	**RANDI (Cyprus)**
VEGETATION	**Vegetation growth**	Height, Canopy, TBD, TL, Biomass	Height, Plant Cover, Biomass	Biomass	Height, Canopy, BBD, BL
	Soft eco-physiological traits	SLW, RWC, Huber index, SPAD	SLW, RWC,		
	Reproductive effort	Flowers, fruits	Spikes		
SOIL	**Nutrient availability**	Available P, PMN, NH_4^+, NO_3^-	Available P, NH_4^+, NO_3^-	Available P, NH_4^+, NO_3^-	Available P, PMN, NH_4^+, NO_3^-
	Labile pool of soil Carbon	DOC			DOC, HWC
	Soil moisture	SWC (Continuous monitoring)	SWC (Continuous monitoring)	SWC (Continuous monitoring)	SWC (Continuous monitoring)

Statistical analyses

Because of the observational character of the stress-gradient experiment, we performed a principal component analysis (PCA) to identify which soil and plant variables were mostly affected by the two factors *stress level (S)* and *microsite (M)*. In addition, the plant and soil variables were analysed using a General Linear Model (GLM) with two fixed factors (Stress level (S), and Microsite (M)) for the soil data and one fixed factor *stress level (S)* for the plant variable. All data met normal distribution of residuals and homoscedasticity assumptions. All analyses were carried out by using Statistical package SPSS version 23.0 (SPSS Inc., Chicago, IL, USA).

3.2 Results

Effect of grazing-stress on soil quality

Figure 2.a summarizes the effect of grazing pressure on the overall soil condition in the four CASCADE study sites for which grazing pressure is the main degradation driver; only results for autumn season are shown, but spring data showed similar patterns (see Annex I for detailed description of effects on individual variables and seasons).

The multivariate (PCA) analysis performed on soil data yielded two main axes in all sites. However, the particular variables that correlated with each axis varied between sites. In Castelsaraceno and Santomera, the wettest and driest site respectively, soil condition did not significantly vary between grazing-stress levels, showing higher variation within each stress level than between levels. However, in Santomera, all soil variables combined in the two PCA axes showed a gradual gradient towards higher nutrient availability and larger amounts of soil organic N and C from high-stress to low-stress plots. Conversely to the previous two sites, soil data from Messara and Randi showed clear variations between stress levels. PC1 in Messara (which positively correlated with total N, SOC, and nitrates) showed a gradual increase from low-stress to high-stress, while PC2 in Randi (which also positively correlated with total N, SOC, and nitrates, and negatively correlated with available P) showed a sharp degradation from low- and moderate-stress to high-stress. In Messara, PC2 correlated with available P and ammonia and showed higher values for moderate-stress than for the other two levels. Similarly, in Randi, PC1, which correlated with ammonia, PMN and labile forms of organic matter, showed higher values for moderate-stress than for the other two levels.

Figure 2.b summarizes the effect of grazing pressure on the overall soil condition of patch versus interpatch microsites (only for the study sites with patchy vegetation: Santomera, Randi, and Messara). Results for Messara were scattered, but in Santomera and Randi patch and interpatch microsites were clearly separated, with patch microsites showing higher values for soil quality variables such as PMN, DOC and Available P (Santomera) and Organic C (SOC), Available P, NO_3 and Total N (Randi). For these two sites, overall differences in soil condition in response to the stress gradient were higher for patch microsites than for interpatch microsites.

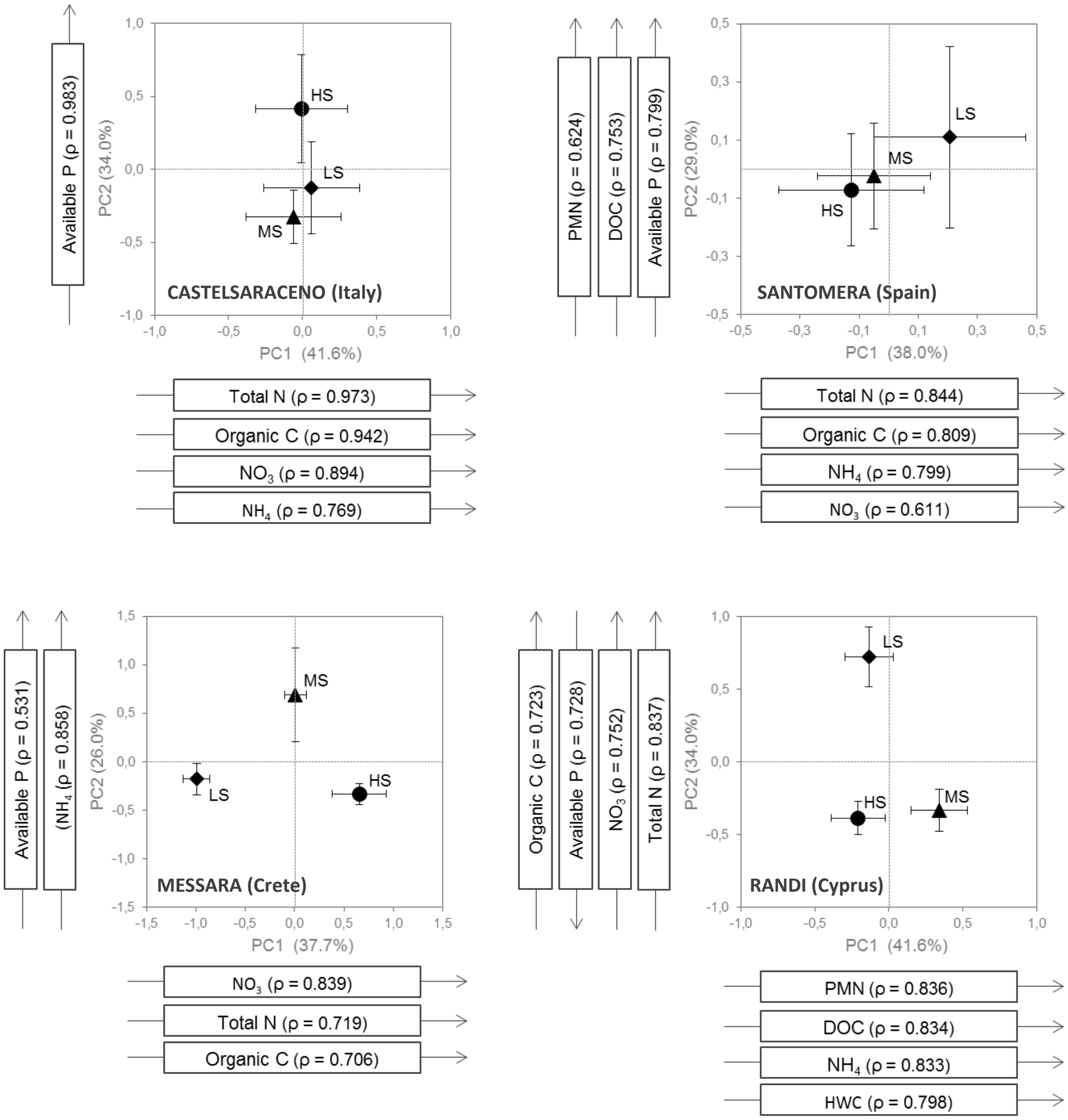


Figure 2.a. Principal component analysis (PCA) of soil variables characterising the different stress levels in the four grazing-pressure CASCADE study sites, considering patch and interpatch microsites pooled. Data represent means ± SE of axis scores for each stress level. Arrows represent soil variables significantly correlated (|ρ| ≥ 0.6) with the first two axes. LS: low stress (diamonds); MS: moderate stress (triangles); HS: high stress (bullets).

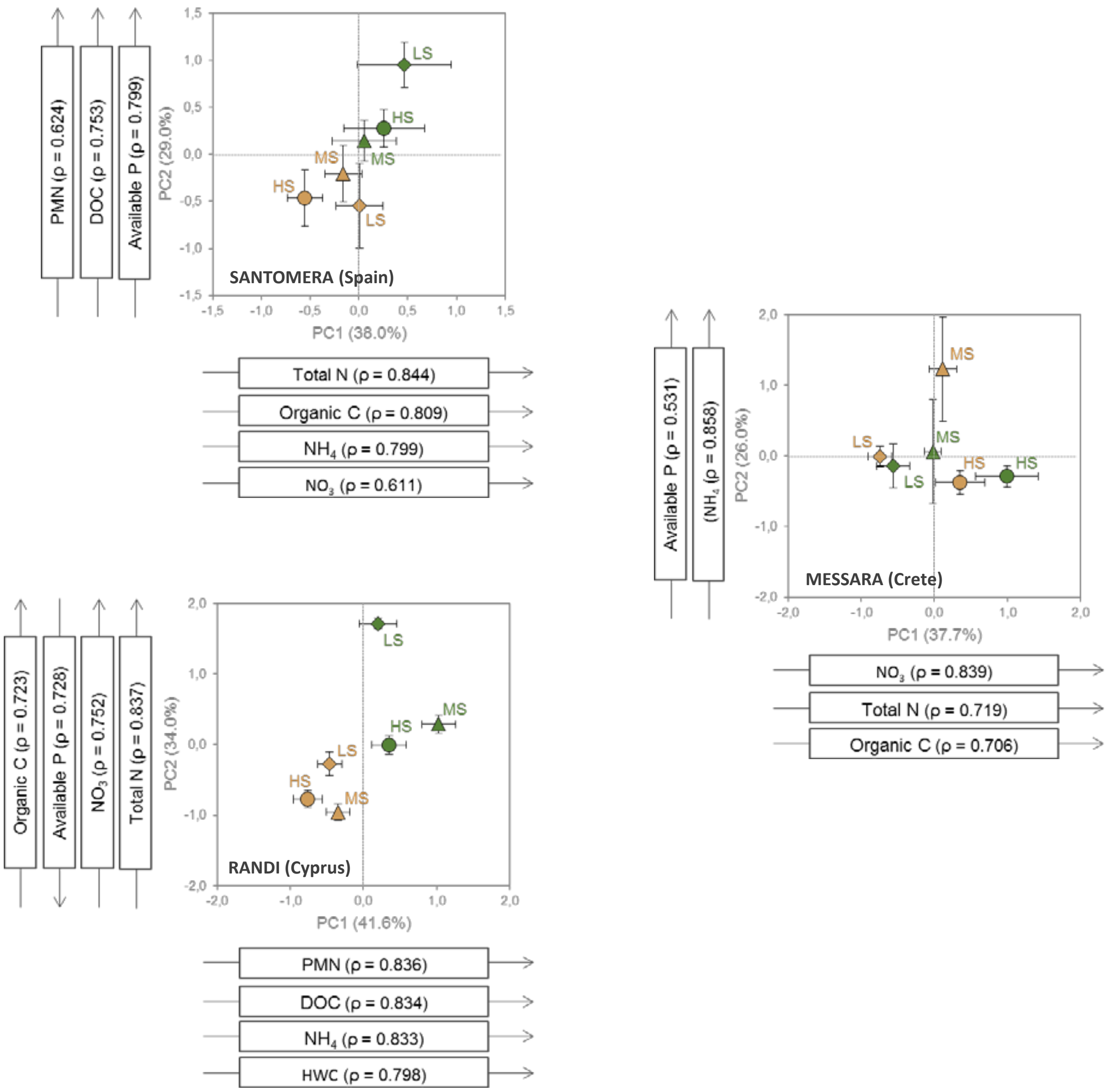


Figure 2.b. Principal component analysis (PCA) of soil variables characterising patch (green symbols) and interpatch (brown symbols) microsites for the different stress levels in the grazing-pressure CASCADE study sites with patchy vegetation. Data represent means ± SE of axis scores for each stress level. Arrows represent soil variables significantly correlated (|ρ| ≥ 0.6) with the first two axes. LS: low stress (diamonds); MS: moderate stress (triangles); HS: high stress (bullets).

For each CASCADE study site with patchy vegetation, Figure 3. shows the variation in the patch/interpatch ratios for soil variables as a function of the grazing stress level. All soil variables showed higher values in soils underneath plant patches than in the interpatches, with the exception of Total N in Messara site and in medium- and low-grazing areas in Santomera site, which showed ratios close to 1. The ratios patch/interpatch were especially high in Randi site. In Randi, patch/interpatch ratios significantly increased from low stress to medium or high stress levels for SOC, ammonium and PMN (Table 4). The same trend (p<0.1) was observed for SOC in Messara and for total N in Santomera. Conversely, DOC ratio in Santomera and available P ratio in Messara were significantly (p<0.1) higher for low grazing-stress than for medium or high stress. The rest of variables did not show any significant effect of the grazing-stress level (Table 4).

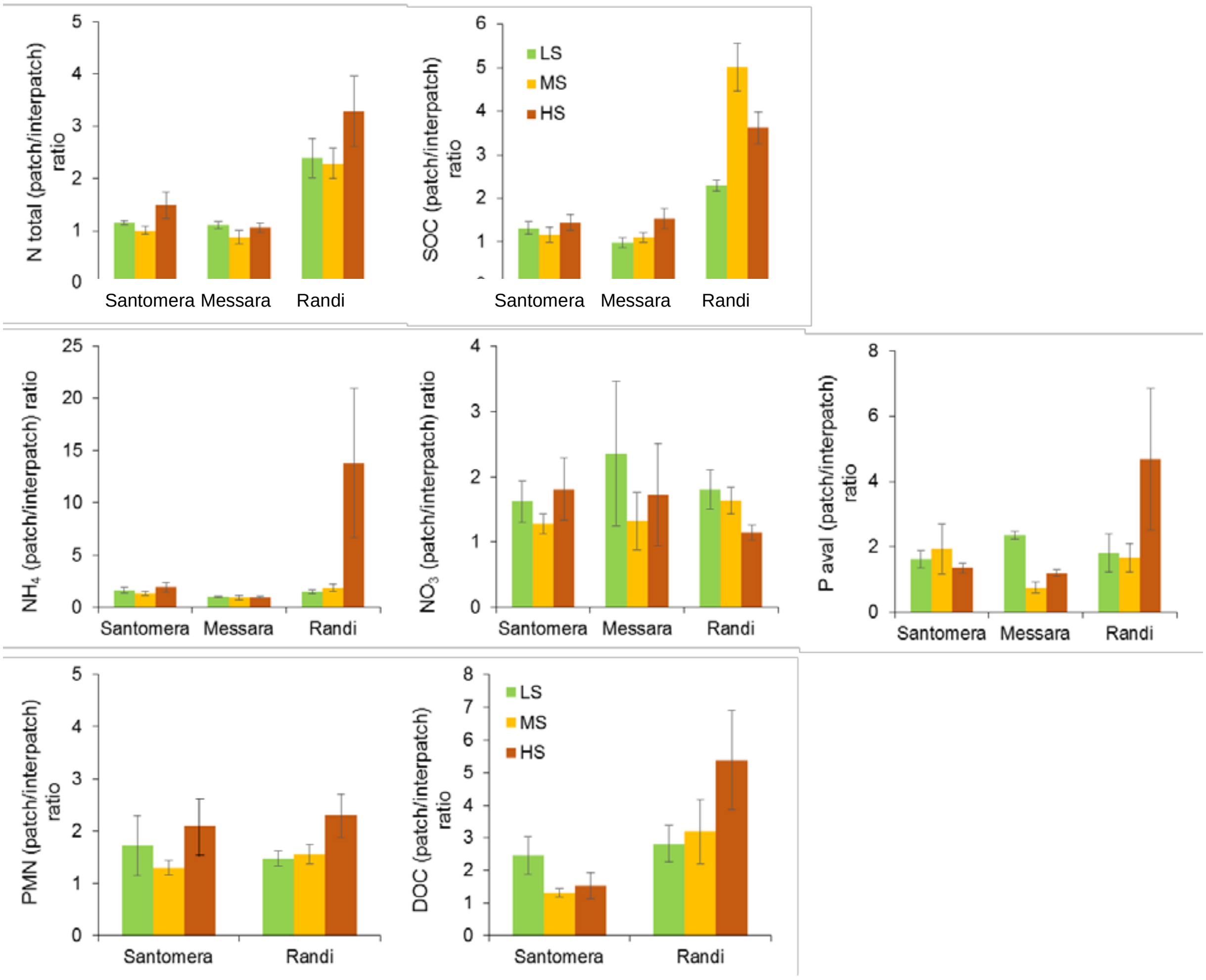


Figure 3. Patch/interpatch ratios for total N and SOC (top), nutrient availability variables (middle) and organic nutrient sources (bottom) for each site and grazing-stress level. The values correspond to the autumn season. LS: low stress level; MS: moderate stress level; HS: high stress level.

Table 4. Statistics results (F and P-values) of the analysis of variance (ANOVA) on the patch/interpatch ratios of the soil variables assessed for Santomera, Messara and Randi sites as a function of the grazing-stress level. SOC: Soil organic carbon; NH_4: ammonium; NO_3: nitrates; Avail-P: available phosphorus; PMN: potentially mineralisable nitrogen; and DOC: dissolved organic carbon. Significant results (p<0.05) are highlighted in bold and marginally significant results (p<0.1) in italics.

	Santomera	Messara	Randi
	F(*P*)	**F(*P*)**	**F(*P*)**
Total N	*2.9(0.072)*	1.4(0.267)	1.3(0.282)
SOC	0.7(0.511)	*2.9(0.079)*	**12.3(<0.001)**
NH_4	0.9(0.411)	0.1(0.891)	*2.9(0.068)*
NO_3	0.8(0.464)	0.3(0.737)	2.4(0.101)
Avail-P	1.1(0.358)	*2.8(0.089)*	1.6(0.207)
PMN	1.6(0.216)		*2.7(0.080)*
DOC	*2.6(0.091)*		1.6(0.213)

Effect of grazing-stress on plant performance

Figure 4 gives the results found for canopy area (CA) and twig basal diameter (TBD) of the shrub target species in Santomera and Randi sites. In Santomera, the canopy of the species *Anthyllis cytisoides* clearly increased in spring time (3 months of monitoring), regardless of the grazing-stress level (Figure 4). After this peak, the canopy area sharply decreased during the summer period. While the variation in canopy area with time was highly significant, this variable did not show any significant effect of the stress level (Table 5). In the case of the shrub species *Calicotome villosa,* the target species in Randi, canopy growth showed significant differences among stress levels (Table 5), with the highest growth in canopy observed for the low-stress level, whereas the lowest values were observed for the high-stress level (Figure 4). In Santomera, *Anthyllis* twigs grew during the first 6 months of monitoring, until the beginning of the summer season. After that, only plants under high stress level continued growing while plants at low and medium stress levels became stagnant. Conversely, for *Calicotome* plants in Randi, the change in the branch basal diameter followed the same pattern as the overall canopy size, growing significantly more for low and moderate grazing stress, and barely growing for high-stress plots (Figure 4; Table 5).

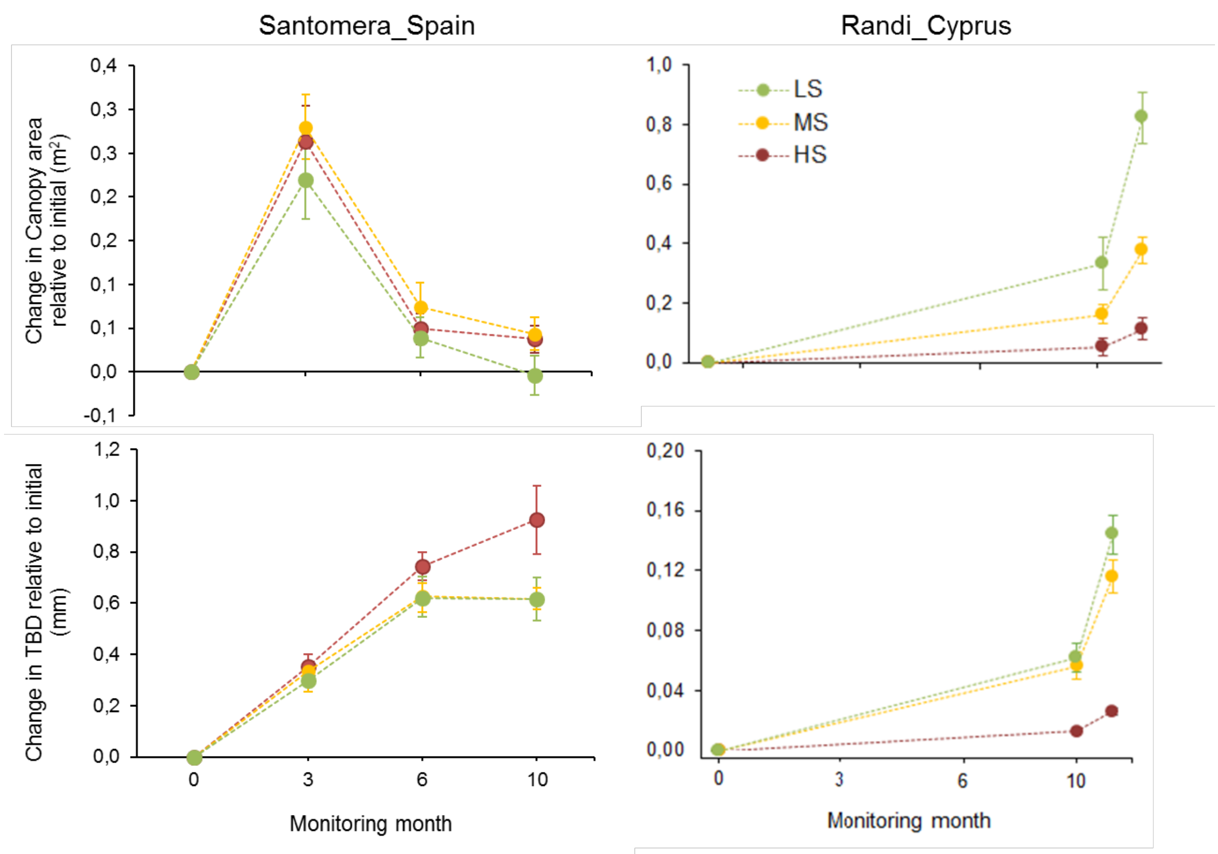


Figure 4. Change in canopy area and twig basal diameter, relative to the initial values at the onset of the experiment, for the two drought-deciduous species, *A. cytisoides* and *C. villosa*, in Santomera and Randi, respectively. LS: low stress level; MS: moderate stress level; HS: high stress level.

Figure 5 shows the changes in plant cover and biomass for the perennial grass species *Brachypodium rupestre* and *Hyparrhenia hirta* in Castelsaraceno and Messara, respectively. The change in plant cover for *B. rupestre*, relative to the initial values, did not show any clear differences among stress levels during the first six months of monitoring (Figure 5). However, the recovery of plant cover after the seasonal decay was significantly higher (Table 5) for the low-stress level than for the other two levels. In Messara, the changes in plant biomass did not show any significant effect of the stress gradient (Figure 5; Table 5)

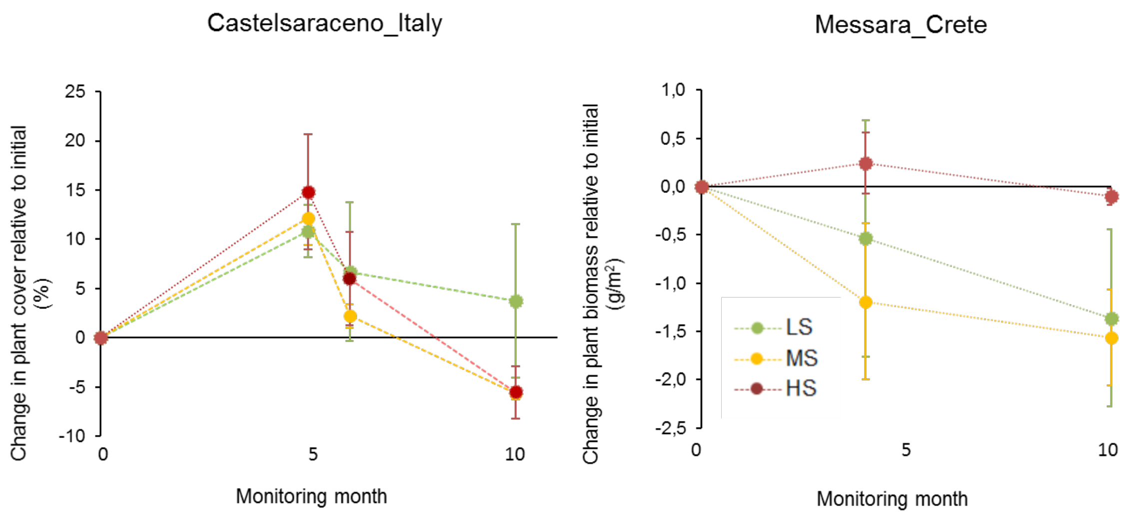


Figure 5. Change in plant cover area and plant biomass relative to the initial plant cover and biomass at the the experiment, for the two perennial grass species, *B. rupestre* and *H. hirta* in Castelsaraceno and Messara, respectively. Green dotted lines show values for low stress, yellow dotted lines show values for medium stress and the red dotted lines show values for high stress level.

Table 5. Statistics results (F and significance, P) of the Repeated Measures Analysis of variance (ANOVA) for the variables "change in canopy relative to the initial", "change in twig basal diameter (TBD) relative to the initial", "change in plant cover relative to the initial" and "change in plant biomass relative to the initial" for Santomera, Castelsaraceno, Messara and Randi respectively. We used Time (T) as a within-subject factor and Stress level (SL) as an intersubject factor. Significant results (p<0.05) and highlighted in bold and marginally significant results (p<0.1) in italics.

		Santomera	**Castelsaraceno**	**Messara**	**Randi**
	Factor	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**
Change in canopy	**T**	**72.4(<0.001)**			**180.3(<0.001)**
	SL	1.1(0.334)			**19.9(<0.001)**
	T*SL	0.2(0.940)			**44.3(<0.001)**
Change in TBD	**T**	**43.7(<0.001)**			**156.2(<0.001)**
	SL	**4.8(0.013)**			**31.1(<0.001)**
	T*SL	**2.5(0.046)**			**23.8(<0.001)**
Change in plant cover	**T**		**48.7(<0.001)**		
	SL		1.0(0.370)		
	T*SL		**3.9(0.008)**		
Change in biomass	**T**			2.7(0.152)	
	SL			0.7(0.523)	
	T*SL			0.4(0.701)	

3.3 Discussion

The aims of the *stress- gradient experiment* were

- Establish the effects of increased stress on the measured soil quality and plant performance parameters in order to see whether the plant-soil ecosystem moves into the direction of a catastrophic shift.
- Establish the differences of soil quality within plant patches and outside plant patches in order to see whether increased potential for facilitation occurs within the plant patches.

Grazing effects on soil quality

It is assumed that the interplay between small scale facilitation (local facilitation) and large scale competition (global competition) between plants underlie catastrophic shifts in drylands (Rietkert et al. 2004). Local facilitation is especially relevant under relatively harsh conditions (He et al. 2013). However, under certain (high) levels of the pressure facilitation may no longer counterbalance the overall competition for scarce resources, leading to a sudden shift towards a degraded state. Facilitation is exerted through multiple mechanisms that, in general, imply improved soil conditions underneath and near plant patches, particularly higher water infiltration capacity (Mayor et al. 2009) and nutrient cycling (Mayor et al. 2016). Within WP3, we assessed changes in the degree of soil improvement as a function of a grazing-stress gradient. In doing so, we aimed to identify functional thresholds (Bestelmeyer, 2006) that might occur after a critical decrease in the soil-plant system took place.

In two of our study sites, Castelsaraceno and Santomera, soil quality parameters did not significantly vary between grazing-stress levels. These two sites represented the wettest and the driest CASCADE sites, respectively, and an overall moderate-low grazing pressure. The relatively low grazing intensity of the ranges considered in both cases could be the most plausible cause for the lack of effect of the stress gradient. Mediterranean rangelands are quite resilient against moderate grazing pressures, which do not significantly hamper ecosystem services provision in these areas (Papanastasis et al. 2015).

An alternative or additional possible explanation for the lack of effect of the grazing level in these two sites might be that the effect of grazing interacts with climatic effects, with the importance of grazing pressure somehow reduced under the most extreme climatic conditions. Castelsaraseno is a relatively wet site with high vegetation cover. These conditions might make the soil ecosystem robust enough to deal with the current range of grazing levels. Santomera is a very dry site, and for this site one might argue that soil conditions are mainly affected by water as the limiting factor for vegetation cover and growth, which in turn determine soil quality parameters.

Conversely, Messara and Randi showed clear effects of grazing-stress levels with some soil quality indicators (PMN, ammonium, available P and labile C forms) peaking under moderate grazing stress and further decreasing under high grazing stress in both sites, and some other indicators (SOC and total N) either decreasing (in Randi) or increasing (in Messara) with increasing grazing pressure. In Randi, we also found the largest differences between the values in the patches compared to the interpatches. For several soil variables, these differences increased with the level of grazing pressure. Only in the case of soil organic carbon, we observed a unimodal response, with the highest contrast between patch and interpatch values at medium level of stress and a further decrease in this contrast at the highest stress level. In the proximity of a critical shift, we expect increased spatial (and temporal) variance (Guttal and Jayaprakash 2009), which will be then reduced after the shift to a degraded state. According to this framework, our results suggest that the range of grazing pressure assessed in the Randi site includes conditions that may drive the system to a sudden shift into a degraded state.

Grazing effects on vegetation

In Santomera, canopy cover and basal twig diameter of *Anthyllis cytisoides* did not vary noticeable with stress level although there was a trend towards higher twig growth for the high-stress plots, probably due to compensatory growth (Oesterheld and McNaughton 1991) under grazing intensities that were not particularly stressful in absolute terms. In Randi, *Calicotome villosa* canopy cover and branch basal diameter showed higher values for low and intermediate stress level and low values for high stress. In Castelsaraceno and Messara, plant cover and biomass of the two target species *Brachypodium rupestre* and *Hyparrhenia hirta* respectively, did not show clear differences among stress levels, except for a slightly higher recovery in plant cover after the seasonal decay for low-stress plots in Castelsaraceno.

An explanation for these results might be found along a similar kind of reasoning as for the grazing effects on soil quality. Santomera is a water controlled ecosystem with, in our study, relatively small effects of grazing. Castelsaraseno is a relatively vegetation rich ecosystem with a high robustness against stress imposed by grazing. Messara showed response in soil quality to stress through grazing, but apparently no response by the vegetation. Only the Randi site show a comprehensive pattern of results with sensitivity for grazing on both the level of soil quality an on vegetation performance.

Conclusions

Regarding the stress-gradient, all statistically significant effects we observed hinted at ecosystems changes in the direction of criticality and ecosystem shifts:

- ✓ The Messara and Randi sites showed increased soil quality under moderate grazing stress and decreased soil quality under high grazing stress. The other two sites, with a low or moderately low overall grazing pressure, appeared to be less sensitive to the grazing level.
- ✓ The Randi site showed reduced plant performance due to increased stress.

Regarding the mechanism of positive feed-backs through local facilitation, we observed that

- ✓ All sites, but especially the Randi site, showed higher soil quality in the patch microsites compared to the interpatch microsites.
- ✓ In Randi, the increasing contrast between patch and interpatch soil conditions with increasing level of grazing points to the proximity of a critical shift into a degraded state.

4. Drought-stress experiment

4.1 Material and methods

The drought-stress manipulative experiment aimed to evaluate the effect of imposed drought on the plant-soil system. It was established for moderate-stress level of either grazing intensity or fire frequency, depending on the experimental site. The experiment was carried out in the six CASCADE study sites, which ranged in natural annual precipitation from 268 mm in Santomera (Spain) to 1290 mm in Castelsaraceno (Italy). Moreover, weather variability between the study sites was particularly high during the experimental period (September 2013- November 2015), with the wettest sites having a very wet period (sites in Portugal and Italy) while the driest sites experienced particularly dry years (sites in Spain and Cyprus).

The drought-stress treatment was applied on plots of around 2 m^2 in size. For each of these plots, we considered two microsites: soils under the target plant, i.e., vegetation-patch microsite (P microsite), and the respective upslope (bare-soil) interpatch microsite (IP).

The drought experiment was set-up using special roofs constructed *ad hoc* that captured rainfall to induce drought below the roof (Figure 6). In order to minimize greenhouse effects on the experimental plots due to the rainfall-exclusion plots, we built a fully translucent cover, supported by metallic poles. To minimize the lateral influx of water from overland flow from the upslope area, metal sheets were inserted into the ground around the plot. The roofs designed for this experiment were made by two layers of V-profile transparent polycarbonate gutters, which conduct the rainfall water to an external gutter connected to a water storage tank, which allow measuring the excluded rain (Figure 6). When placed upside-down, the roof allows the rain to go through and thus works as a roofed control, with similar roof effects (e.g., greenhouse effect) except rainfall exclusion. It is important to highlight that the goal of the treatment was not to completely avoid soil wetting during rainfall but to decrease plant water availability relative to the controls. Indeed, soil wetting during rainfall is possible in the rainfall exclusion roofs when wind is strong and/or there is subsurface runoff.

Figure 6. Pictures of the rainfall exclusion roofs (above left). Below left is a picture of the control roof, i.e. a roof that let rainfall through. Right hand site picture shows a closer view on the roof with the gutter that captures the excluded rainfall.

We established three treatments: (1) Non-roofed controls (hereafter, C_NoR); (2) Rainfall-inclusion roofed control (hereafter, C_R); and Rainfall-exclusion roofs (Drought treatment, hereafter, D). To address the exceptional climatic variation among sites for the study period, special efforts were made to make the drought treatments comparable between sites. The duration of the experiment was established to guarantee that the rainfall received in the roofed plots during the 12 months previous to the end of the experiment were around or below the 1st percentile of the mean annual rainfall for the long-term reference period (30-50 years). Due to the extreme natural drought experienced in some sites the experiment needed a particularly long period to achieve the 1st percentile value. In all sites except Castelsaraceno, the experiments were extended several additional months (the number varying between sites) after achieving the 1st percentile (see Results below), with the longest experiment conducted in the driest sites: Santomera (completed by December 2015) and Messara (completed by November 2015).

Soil nutrient availability and labile C, and plant performance were measured in all plots at the beginning of the experiment and at ~4 months intervals (Table 4). Measurements on plant reproductive effort were taken during the flowering-fruiting seasons. Using TDR probes, soil moisture was measured on a continuous basis for both the soil underneath plant patches and the upslope bare-soil interpatch.

Plant and soil measurements

Table 6. summarizes the soil quality and plant performance parameters measured in the drought experiment.

Table 6. Vegetation and soil variables (0-5 cm)(for repeated measurements at each site for the drought-stress experiment. Yet, the sets of parameters measured per site could slightly differ, because of plant/soil/ecosystem properties and available expertise/equipment. Acronyms list:

- Vegetation growth: TBD: Twig Basal Diameter; TL: Twig length; BBD: Branch Basal Diameter; BL: Branch length.
- Soft eco-physiological traits: SLW: Specific Leaf Weight; RWC: Relative Water Content; SPAD: Chlorophyll content.
- Soil characterization: CEC: Cation-Exchange Capacity SOC: Soil Organic Carbon.
- Nutrient availability: PMN: Potentially Mineralisable Nitrogen. Available P (AvailP)
- Labile pool of soil C: DOC: Dissolved Organic Carbon; HWC: Hot Water Extractable Carbon.
- SWC: Soil Water Content.

	Variables	VARZEA (Portugal)	SANTOMERA (Spain)	VALENCIA (Spain)	CASTELSARACENO (Italy)	MESSARA (Crete)	RANDI (Cyprus)
VEGETATION	**Vegetation growth**	Height, Basal diameter, Canopy, BBD, BL, Biomass	TBD, TL, Biomass	BBD, BL	Canopy, Biomass	Biomass	Height, Canopy, BBD, BL
	Soft ecophysio-logical traits	SLW,RWC	SLA, Huber index	RWC	SLW,RWC	RWC	RWC
	Reproductive effort	Flowers, Fruits	Flowers	Flowers	Flowers, Spikes		Flowers
SOIL	**Available nutrients**	PMN, NH_4^+, NO_3^-	P, PMN, NH_4^+, NO_3^-	P, PMN, NH_4^+, NO_3^-	P, PMN	Available P, PMN NH_4^+, NO_3^-	Available P, PMN, NH_4^+, NO_3^-
	Labile pool of soil Carbon	WSC, HWC	DOC, HWC	DOC	HWC	HWC	DOC, HWC
	Soil moisture	SWC (Continuous monitoring)	SWC (Continuous monitoring)	SWC (Continuous monitoring)	SWC (Continuous monitoring)	SWC (Continuous monitoring)	SWC (Continuous monitoring)

Statistical analysis

We analysed all the variables measured for patch (P) and interpatch (IP) microsites using Repeated Measures ANOVA (GLM) with Treatment (D), with three levels, i.e. C_NoR, C_R and R, as between-subject factor and Time (T) as within-subject factor. All data met the normal distribution of residuals and homoscedasticity assumptions. All analyses were carried out using v.23.0 Statistical package (SPSS Inc., Chicago, IL, USA).

4.2 Results

Effect of drought-stress on soil quality parameters

Figure 7 shows the results for potentially mineralisable nitrogen (PMN) and hot water-extractable carbon (HWC) measured at the Várzea site in Portugal. Both variables showed similar values for patch and interpatch microsites. None of the variables was significantly affected by the drought treatment (Table 7). HWC showed a significant variation in time, peaking in March 2014.

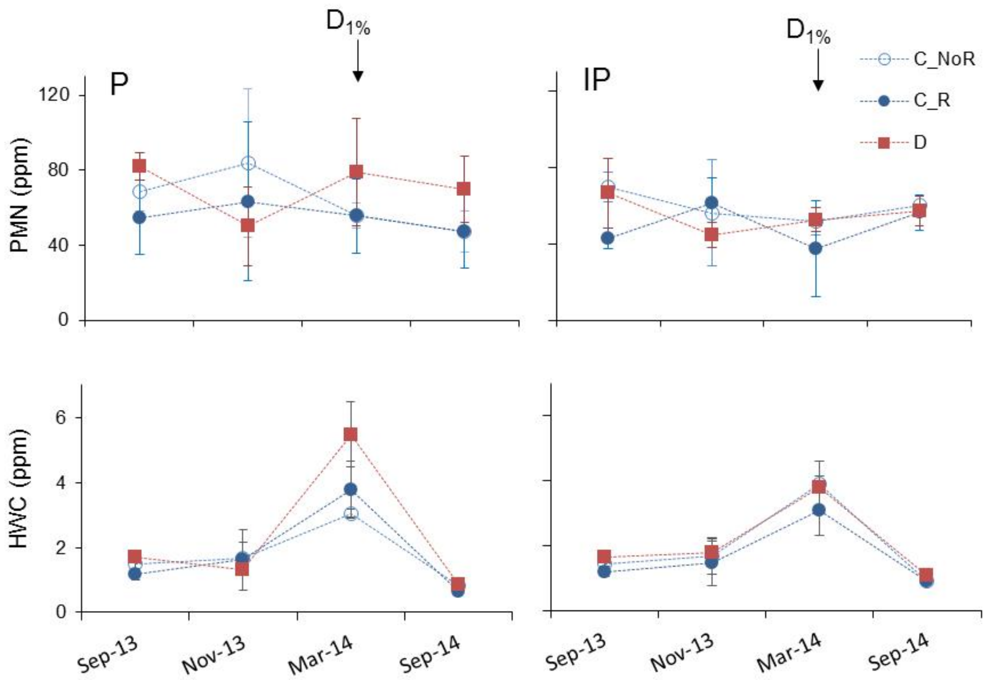


Figure 7. Potentially mineralisable nitrogen (PMN) and hot water-extractable carbon (HWC) measured in Várzea, Portugal, at 0-5 cm soil depth, in both microsites (P and IP). Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). $D_{1\%}$ arrows point to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

In Castelsaraceno, the dynamics of potentially mineralisable nitrogen (PMN) and hot water-extractable carbon (HWC) did not show any significant effect of the drought treatment (Figure 8), yet both variables showed statistical variation in time. Given the homogeneity of the vegetation in Castelsaraceno, we only considered the patch microsite in this site.

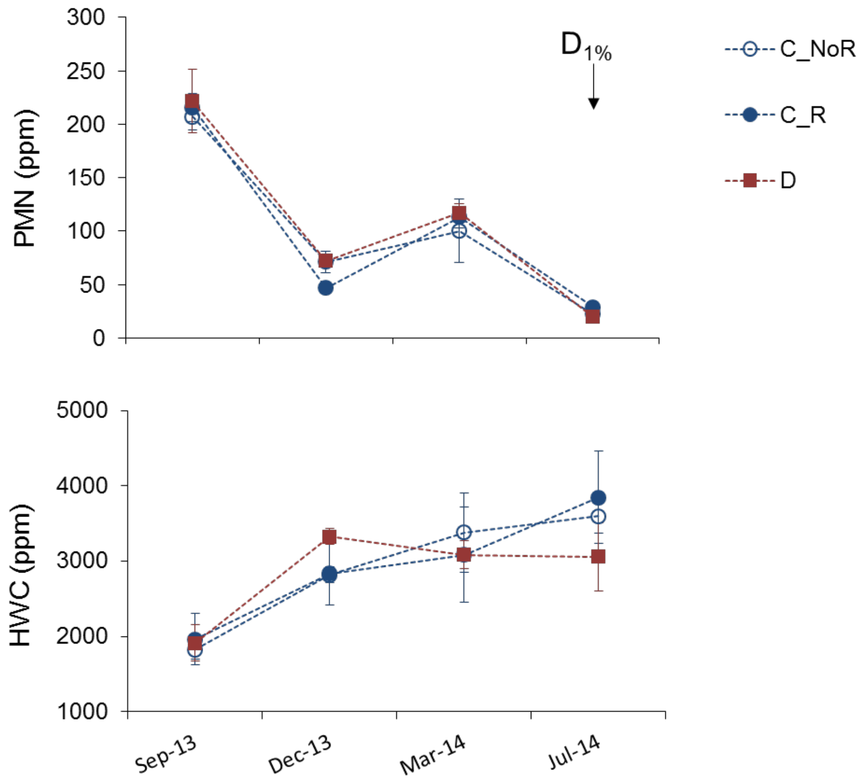


Figure 8. Potentially Mineralisable Nitrogen and Hot water extractable Carbon measured in Castelsaraceno, Italy, at 0-5 cm soil depth, in patch microsite. $D_{1\%}$ arrow points to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

In the Valencia site, patch and interpatch microsites showed similar values (Figure 9) and none of the assessed variables showed any significant effect of the drought treatment (Figure 9¸Table 7), with PMN and DOC showing significant variation in time (Table 7).

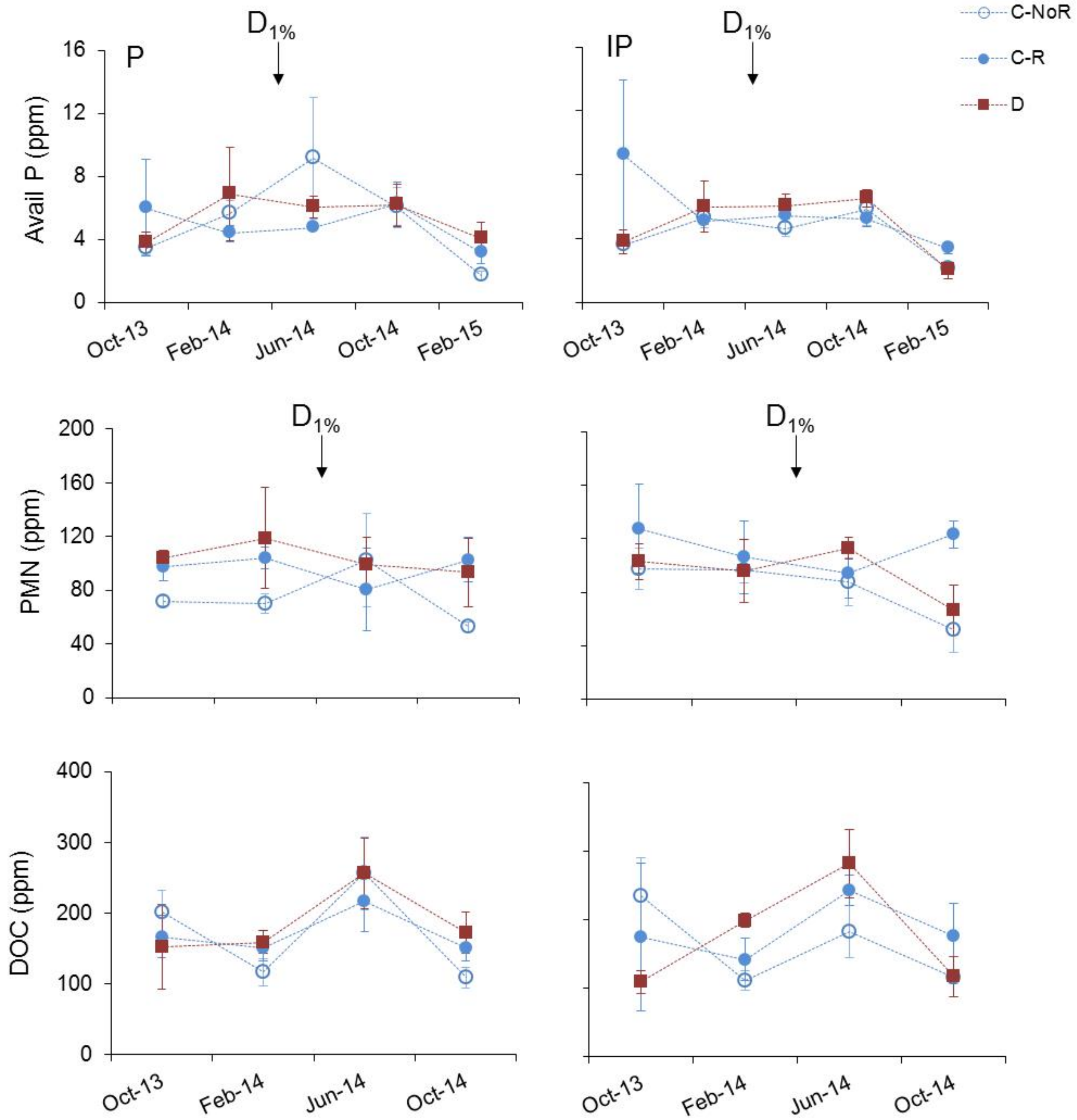


Figure 9. Available phosphorus, potentially mineralisable nitrogen and dissolved organic carbon measured in Valencia, Spain, at 0-5 cm soil depth, in both microsites. $D_{1\%}$ arrows point to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

Figure 10 shows the results from the Messara site in Crete. Likewise the sites reported above, patch and interpatch areas showed similar trends and values. Available phosphorus and potentially mineralisable nitrogen (PMN) did not show any significant effect of the drought treatment (Table 7), while hot water-extractable carbon (HWC) showed significantly different dynamics as a function of the drought treatment, with overall higher values in the open (C_NoR) controls. The three variables showed significant variation in time (Table 7).

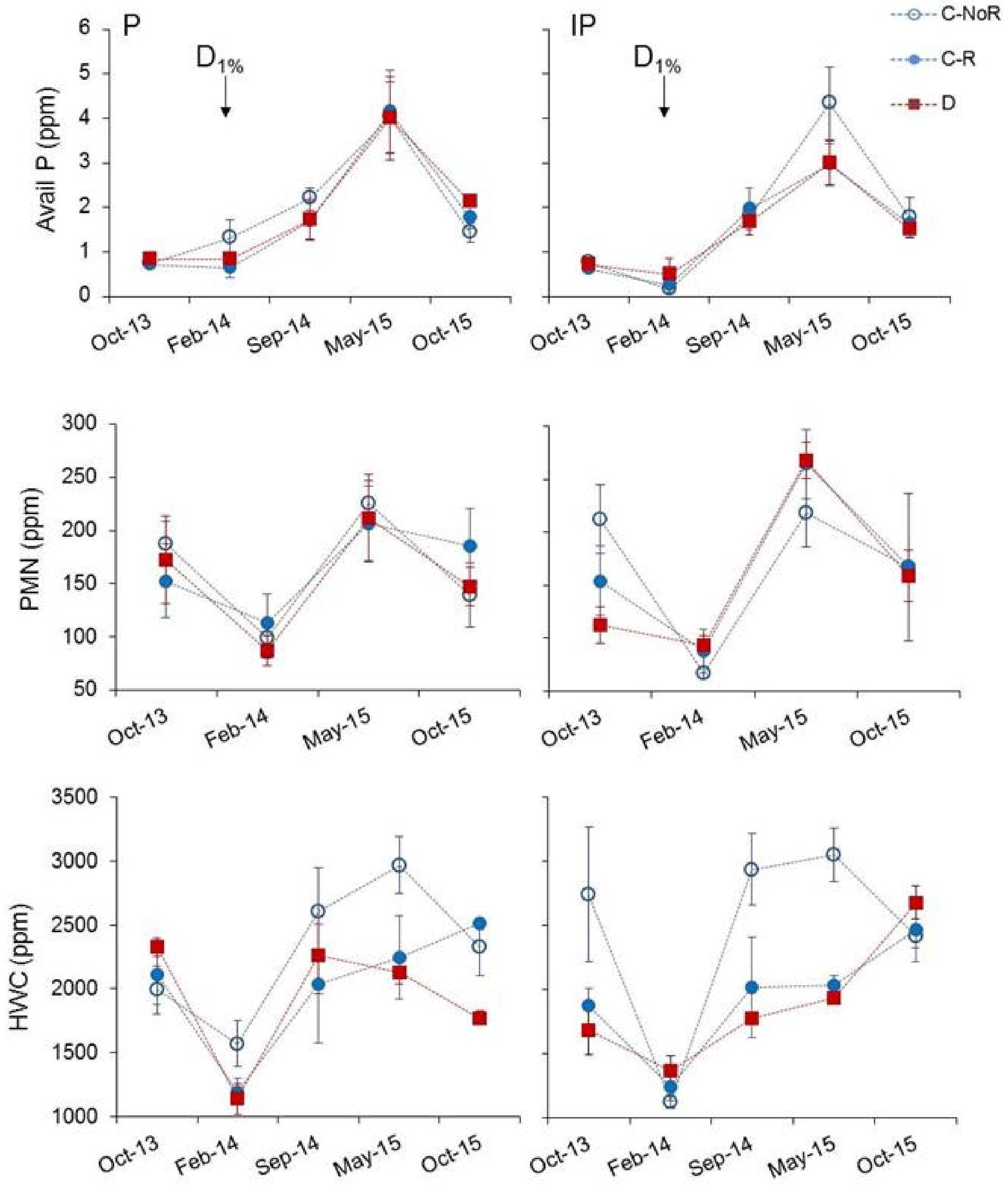


Figure 10. Available phosphorus, potentially mineralisable nitrogen and hot water extractable carbon measured in Messara, Crete, at 0-5 cm soil depth, in both microsites. D1% arrows point to the time when drought treatment achieved the target of the1st percentile of mean annual rainfall.

Figure 11 shows the results from the Santomera site in Spain. Overall, patch soils showed slightly higher values than interpatch soils. Available P did not show a statistically significant effect of the drought treatment, while PMN in interpatches showed significantly different dynamics and higher values for the drought treatment as compared with both controls (Figure 11; Table 7). Similarly HWC showed a marginally significant increase for the drought treatment in interpatches. All the variables showed significant variation along the study period.

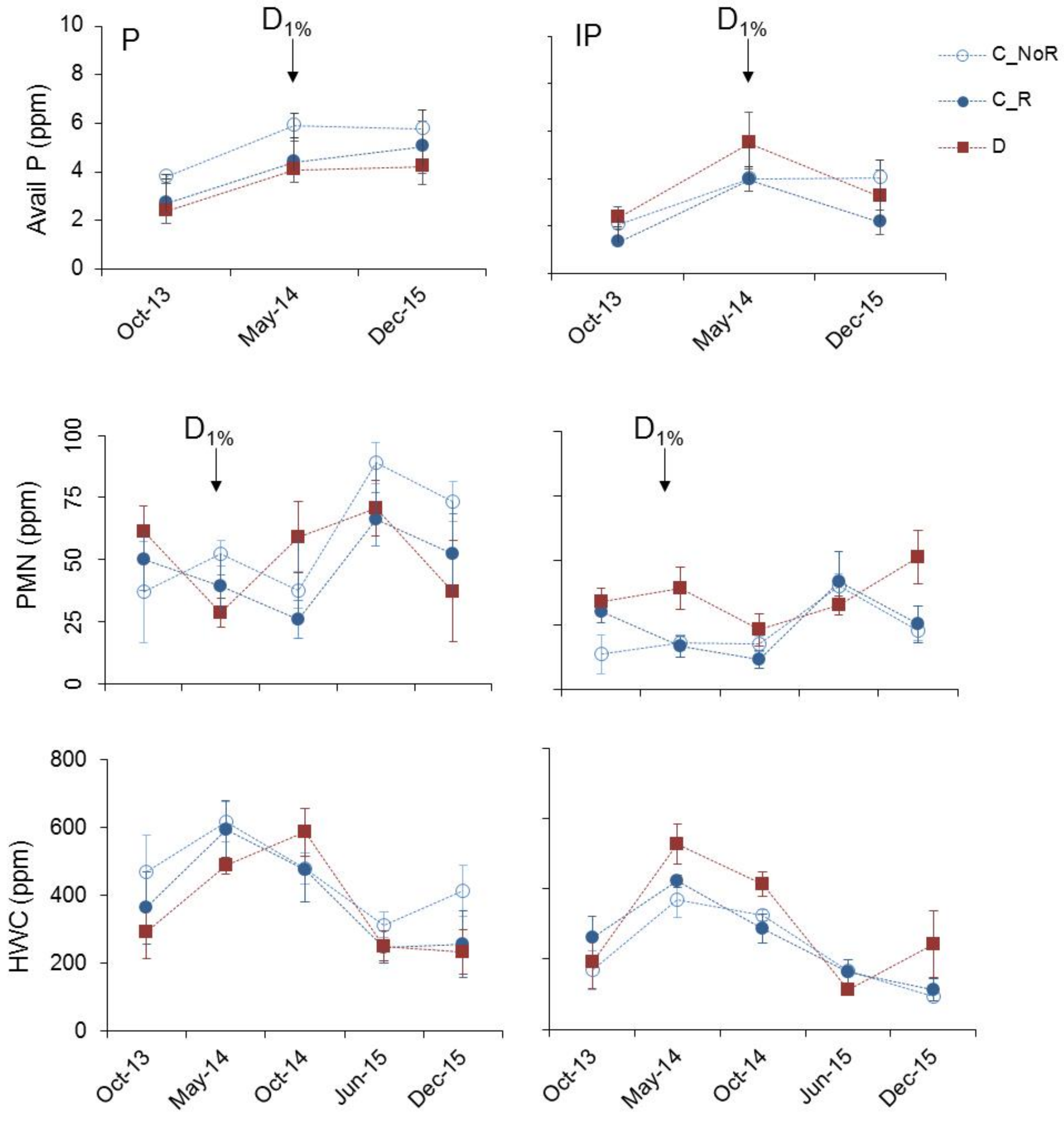


Figure 11. Available phosphorus, potentially mineralisable Nitrogen and hot water extractable carbon measured in Santomera, Spain, at 0-5 cm soil depth, in both microsites. D1% arrows point to the time when drought treatment achieved the target of the1st percentile of mean annual rainfall.

Figure 12 shows the results from the Randi site in Cyprus. Similar to the case in Santomera site, only PMN showed a significant effect of the drought treatment (Table 7), with a higher increase in the patch microsites for the drought plots than for the control plots towards the end of the experiment.

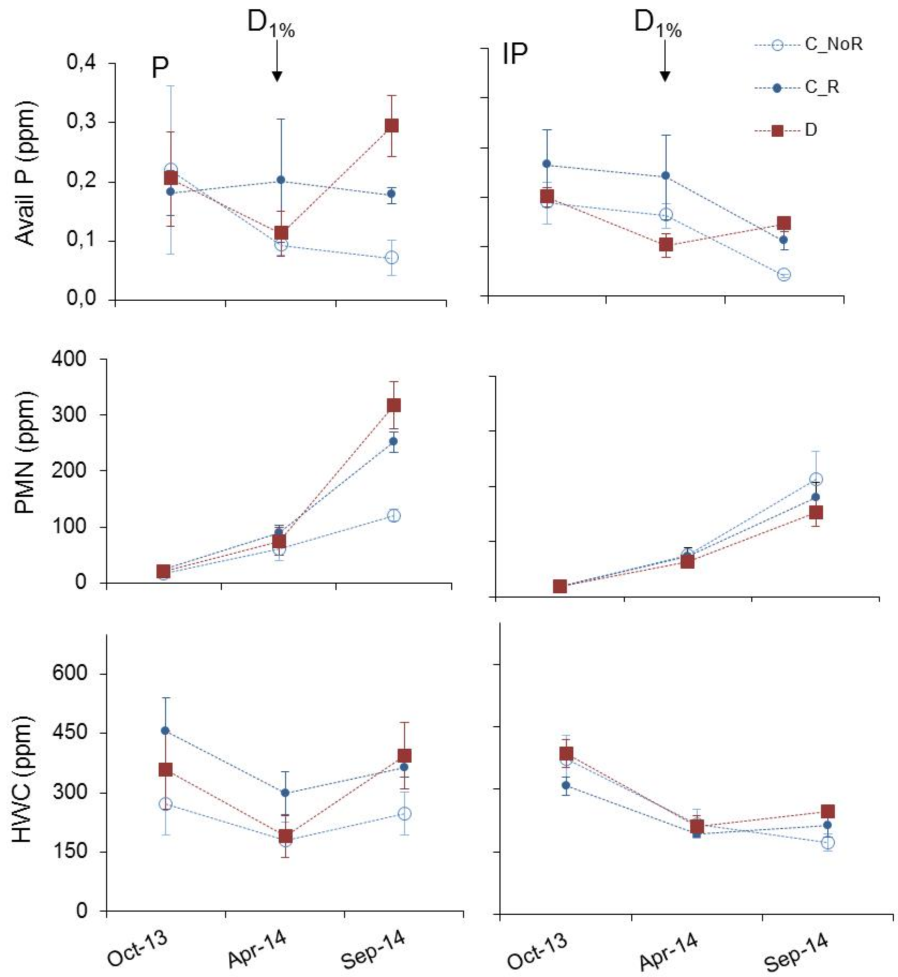


Figure 12. Available phosphorus, potentially mineralisable nitrogen and hot water extractable carbon measured in Randi, Cyprus, at 0-5 cm soil depth, in both microsites. D1% arrows point to the time when drought treatment achieved the target of the1st percentile of mean annual rainfall.

Table 7. Statistics results (F and P-values) of the Repeated Measures Analysis of Variance for the soil variables: Potentially mineralisable nitrogen (PMN), available phosphorus (Avail P), dissolved organic carbon (DOC) and hot water extractable carbon (HWC) for Várzea, Castelsaraceno, Valencia, Messara, Santomera and Randi sites. Time (T) was used as within-subject factor and Drought Stress (DS) as between-subject factor. T * DS represent the interaction between the two factors. Significant results (p<0.05) and highlighted in bold and marginally significant results (p<0.1) in italics.

		Várzea		**Castelsaraceno**	**Valencia**		**Messara**		**Santomera**		**Randi**	
		P	**IP**	**P**	**P**	**IP**	**P**	**IP**	**P**	**IP**	**P**	**IP**
	Factor	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**	**F (*P*)**
PMN	**T**	0.2 (0.896)	0.1 (0.951)	**92.9 (<0.001)**	0.4 (0.759)	1.0 (0.420)	**11.4 (<0.001)**	**19.2 (<0.001)**	**5.4 (0.002)**	**4.6 (0.004)**	**124.0 (<0.001)**	**47.6 (<0.001)**
	DS	0.4 (0.707)	0.1 (0.941)	0.5 (0.651)	1.1 (0.379)	2.0 (0.232)	0.8 (0.924)	0.01 (0.991)	*3.7 (0.068)*	**5.7 (0.025)**	**7.2 (0.025)**	0.7 (0.519)
	T*DS	0.5 (0.82)	0.4 (0.888)	0.4 (0.836)	0.9 (0.509)	0.9 (0.524)	0.8 (0.592)	1.4 (0.251)	1.3 (0.271)	*2.0 (0.082)*	**10.5 (0.001)**	0.6 (0.674)
Avail P	**T**				*2.3 (0.087)*	**3.3 (0.029)**	**26.3 (<0.001)**	**53.9 (<0.001)**	**9.0 (0.002)**	**9.7 (0.001)**	1.0 (0.386)	**7.1 (0.009)**
	DS				0.1 (0.900)	0.9 (0.440)	0.1 (0.893)	0.5 (0.601)	1.2 (0.355)	1.4 (0.303)	0.6 (0.581)	2.0 (0.200)
	T*DS				0.8 (0.569)	1.4 (0.250)	0.4 (0.897)	1.1 (0.362)	0.3 (0.852)	0.8 (0.551)	1.7 (0.214)	1.6 (0.247)
DOC	**T**				**4.2 (0.020)**	**4.2 (0.024)**						
	DS				1.0 (0.409)	0.2 (0.808)						
	T*DS				0.5 (0.790)	1.9 (0.151)						
HWC	**T**	**16.4 (<0.001)**	**7.2 (0.009)**	**15.1 (<0.001)**			**16.5 (<0.001)**	**17.0 (<0.001)**	**16.8 (<0.001)**	**20.1 (<0.001)**	**9.2 (0.004)**	**21.2 (<0.001)**
	DS	0.9 (0.475)	1.2 (0.404)	0.03 (0.974)			2.1 (0.187)	**8.8 (0.012)**	0.9 (0.455)	*3.3 (0.085)*	1.5 (0.305)	2.2 (0.193)
	T*DS	1.4 (0.307)	0.3 (0.923)	0.9 (0.490)			*2.2 (0.060)*	**3.7 (0.005)**	1.1 (0.409)	1.3 (0.264)	1.2 (0.374)	0.9 (0.481)

Effect of drought-stress on plant performance parameters

Figure 13 shows the variation in branch basal diameter (BBD), both as absolute figures and as figures relative to the initial value, for the target plant species as a function of the drought treatment in the Várzea site, Portugal. There was a continuous increase in branch diameter with time, due to the plant regrowth after the wildfire of September 2012, yet there was no statistically significant effect of the drought treatment (Table 8).

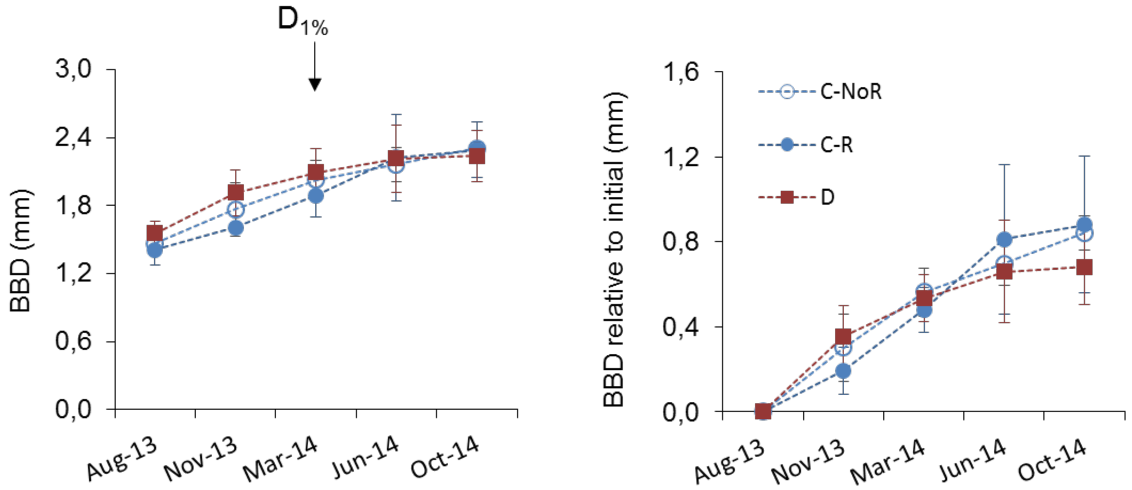


Figure 13. Branch basal diameter in *Pterospartum tridentatum* and its change relative to the initial value in Várzea, Portugal. Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). D1% arrows point to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

Canopy cover (CC) of the target species in Castelsaraseno, Italy, did not show any significant effect of drought, yet there was a significant interaction between Time and Drought factors, with similar variation in canopy for the two controls and drought treatment before the 1st percentile target for the drought treatment was achieved and a higher increase in canopy for the open controls after that moment (Figure 14; Table 8).

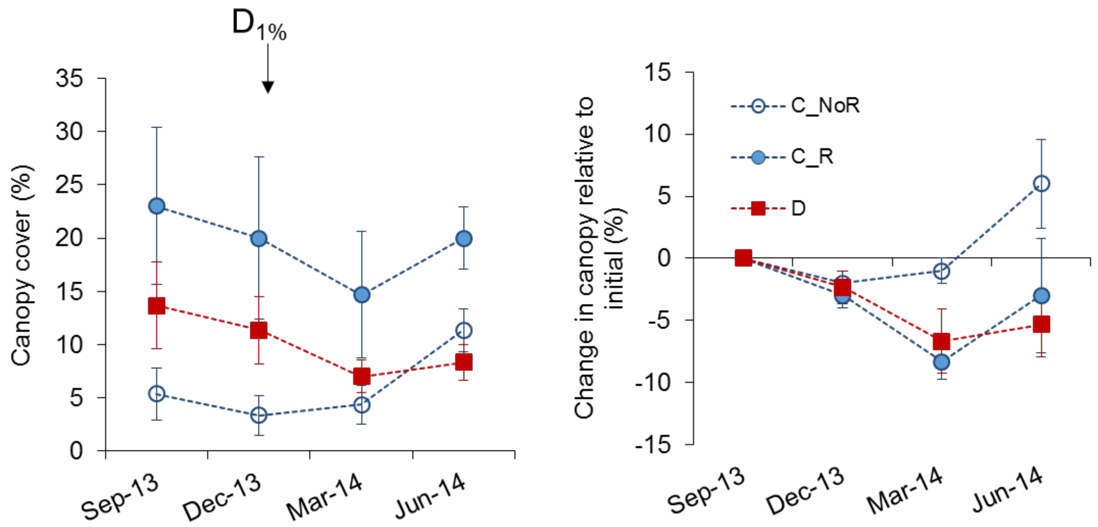


Figure 14. Percentage in canopy cover in *Stipa austroitalica* and its change relative to the initial to the experiment in Castelsaraceno, Italy. Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). D1% arrows point to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

Branch length (BL) of the target species in the Valencia site in Spain showed no significant effect of the drought treatment, yet there was a trend towards a lower increase in BL with time for the drought treatment (Figure 15; Table 8).

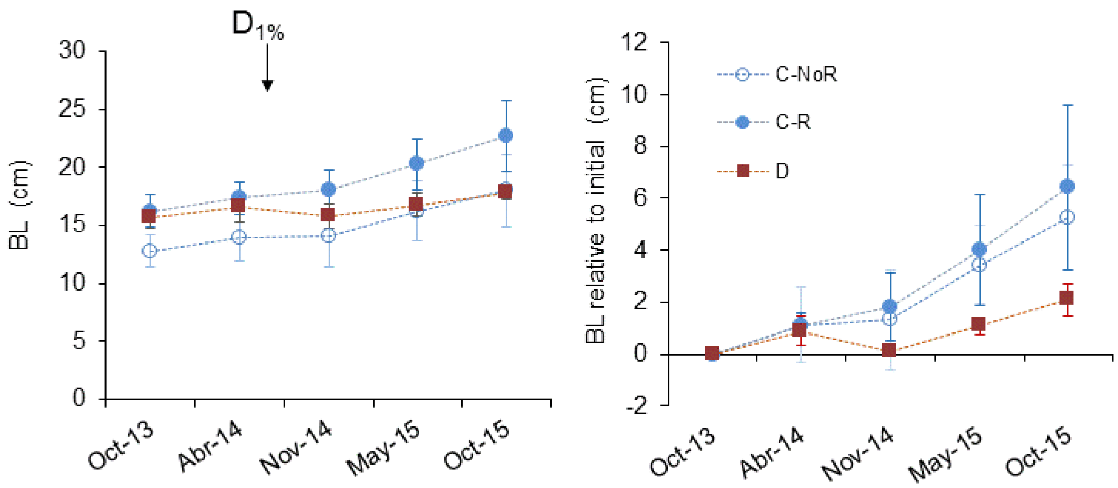


Figure 15. Branch length in *Rosmarinus officinalis* and its change relative to the initial to the experiment in Valencia, Spain. Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). D1% arrows point to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall**.**

For the plant biomass (PB) of the target grass species in Messara, Crete, there was no statistically significant effect of the treatments on the absolute or the relative values (Figure 16; Table 8). Conversely, the change in twig length (TL) of the target shrub species in Santomera, Spain, and the height of the target shrub species in Randi, Cyprus, showed significantly lower values for the drought treatment than for the roofed controls, C_R (Figure 17 and 18, respectively; Table 8).

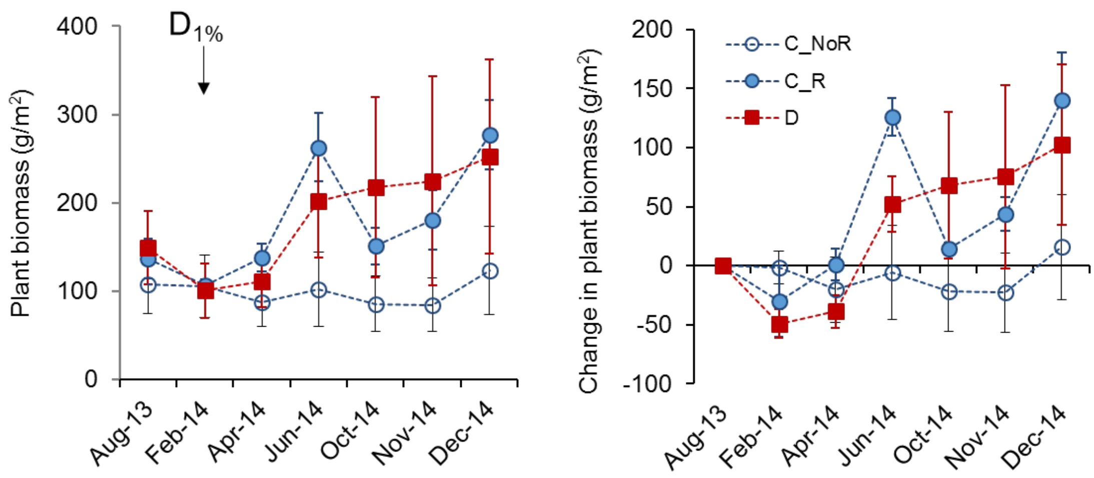


Figure 16. Plant change in *Hyparrhenia hirta* and its change relative to the initial to the experiment in Messara, Crete. Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). D1% arrows point to the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

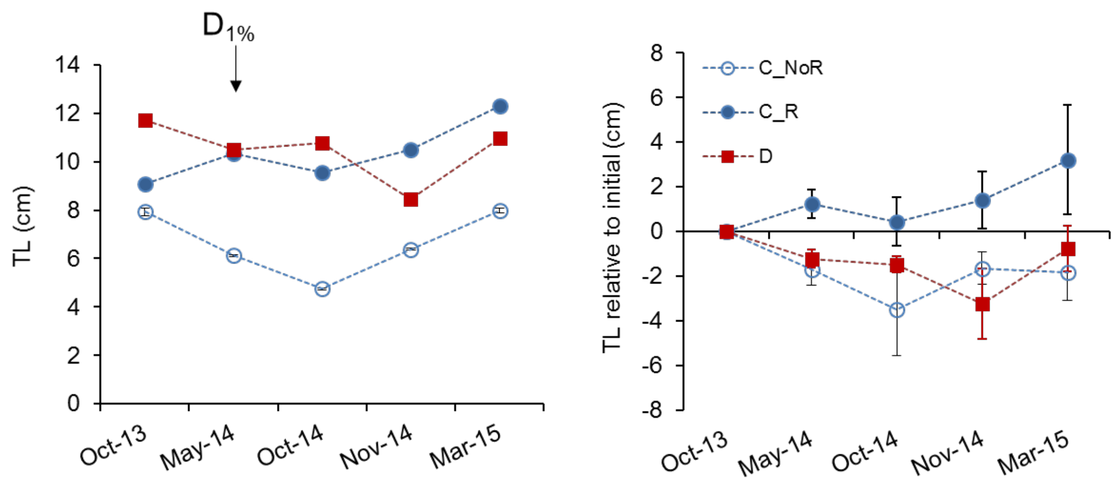


Figure 17. Terminal twig length in *Anthyllis cytisoides* and its change relative to the initial to the experiment in Santomera, Spain. Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). D1% arrows point to show the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

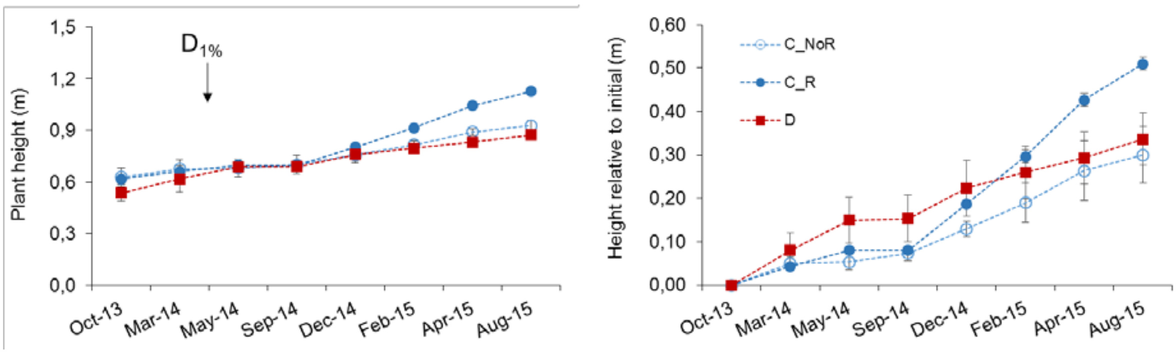


Figure 18. Height *Calicotome villosa* and its change relative to the initial to the experiment in Randi, Cyprus. Blue empty circles show values for unroofed control (C_NoR), blue filled circles represent values for control with roof (C_R), and red squares show values for drought treatment (D). D1% arrows point to show the time when the drought treatment achieved the target of the1st percentile of mean annual rainfall.

Table 8. Statistics results (F. and P-values) of the Repeated Measures Analysis of Variance for plant performance variables: Branch basal diameter (BBD), canopy cover, branch length (BL), plant biomass, twig length (TL) and plant height, and their values relative to the start of the experiment for Várzea, Castelsaraceno, Valencia, Messara, Santomera and Randi. Time (T) was used as within-subject factor and Drought Stress (DS) as between-subject factor. Significant results (p<0.05) and highlighted in bold marginally significant results (p<0.1) in italics.

Site	**Variable**	**T (F(*P*))**	**DS (F(*P*))**	**T*DS (F (*P*))**
Varzea	**BBD**	**13.8(<0.001)**	0.2(0.857)	0.3(0.973)
	Change in BBD	**5.9(0.006)**	0.03(0.966)	0.3(0.932)
Castelsaraceno	**Canopy**	**5.4(0.008)**	3.2(0.115)	**2.8(0.044)**
	Change in canopy	**5.0(0.027)**	2.8(0.135)	*2.7(0.080)*
Valencia	**BL**	**10.8(<0.001)**	1.3(0.338)	1.0(0.463)
	Change in BL	**12.4(<0.001)**	0.7(0.517)	1.2(0.354)
Messara	**Biomass**	**7.4(<0.001)**	1.4(0.310)	*2.0(0.051)*
	Change in biomass	**7.7(<0.001)**	1.8(0.232)	*2.0(0.063)*
Santomera	**TL**	1.8(0.149)	2.2(0.178)	1.4(0.248)
	Change in TL	2.2(0.114)	*3.3(0.099)*	0.5(0.765)
Randi	**Height**	**89.1(<0.001)**	*3.5(0.098)*	**4.5(<0.001)**
	Change in height	**86.6(<0.001)**	1.4(0.306)	**5.5(<0.001)**

Effect of drought-stress on soil moisture

Figure 19 shows the dynamics of monthly rainfall and soil water content (SWC) for the wettest study sites: Várzea (Portugal) and Castelsaraseno (Italy). in both sites, the control plots without roof showed the highest SWC values, yet only for the patch microsite in Várzea site. Castelsaraseno showed particularly high SWC values as compared to the other sites.

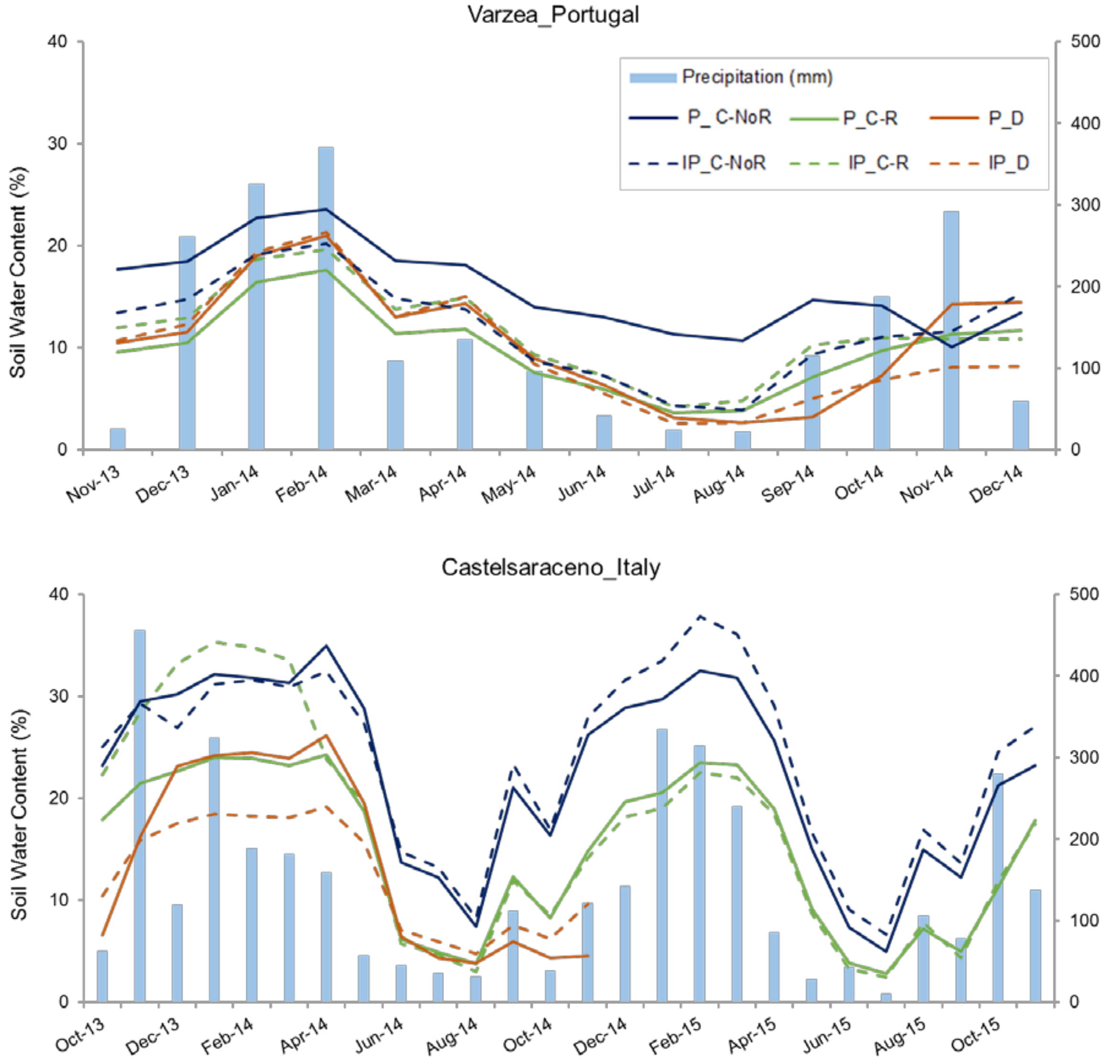
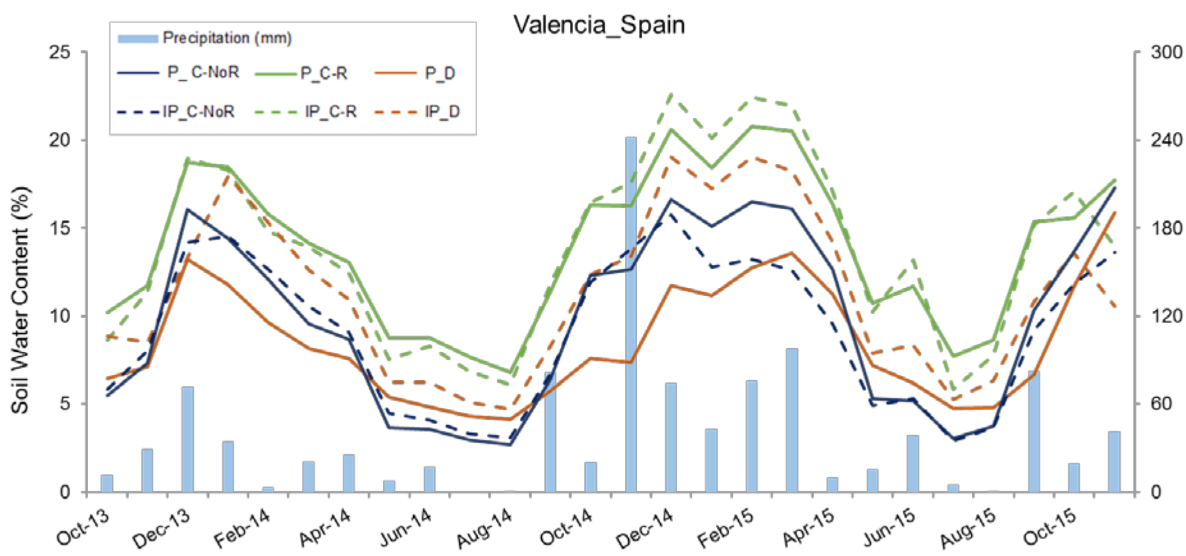


Figure 19. Average soil water content (2.5 cm depth) and monthly precipitation in Varzea and Castelsaraceno.

Figure 20 shows rainfall and SWC dynamics for Valencia (Spain) and the Messara (Crete). In general, SWC showed a trend towards lower values for patches than for interpatches, particularly for the drought (D) treatment and in Messara. In Valencia, the highest SWC values were found for the roofed control treatment (R-C), either for patch or interpatch microsites, and the lowest for the drought treatment (D) in soils under plant patches. In Messara, there were no clear differences between control and drought plots.



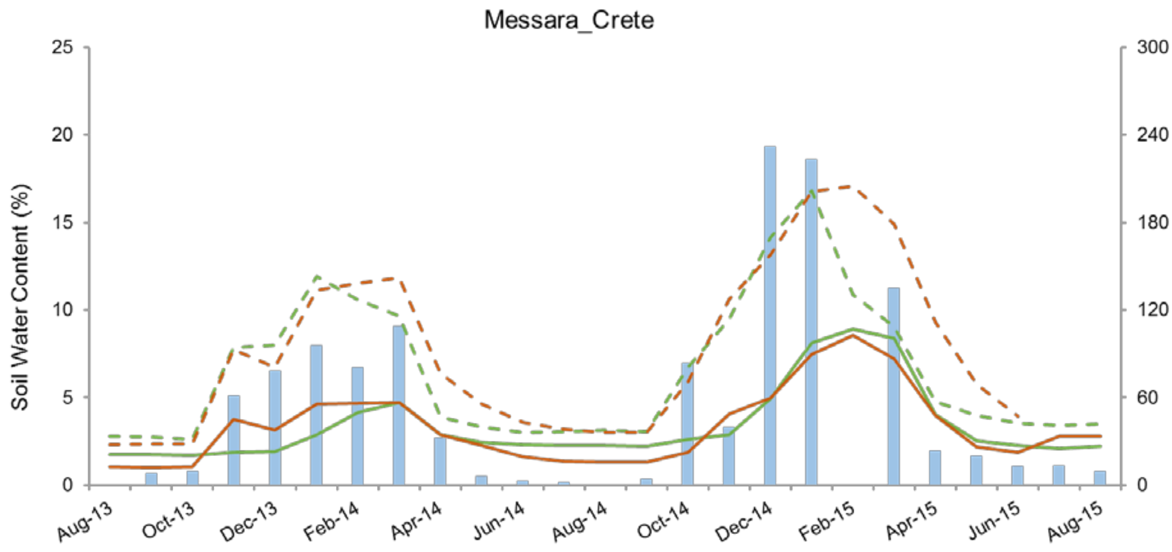


Figure 20. Average soil water content and monthly precipitation in Valencia and Messara.

Soil water content in Randi showed similar dynamics than in Messara, with lower values in patches than in interpatches (Figure 21), while in Santomera, there were no relevant differences between microsites. In Randi, interpatch SWC showed higher values for control than for drought plots, while soils under plant patches did not show any treatment effect. In Santomera, both patches and interpatches showed higher values for control than for drought plots, yet only at the end of the study period, once the natural extreme drought that occurred in the area was over.

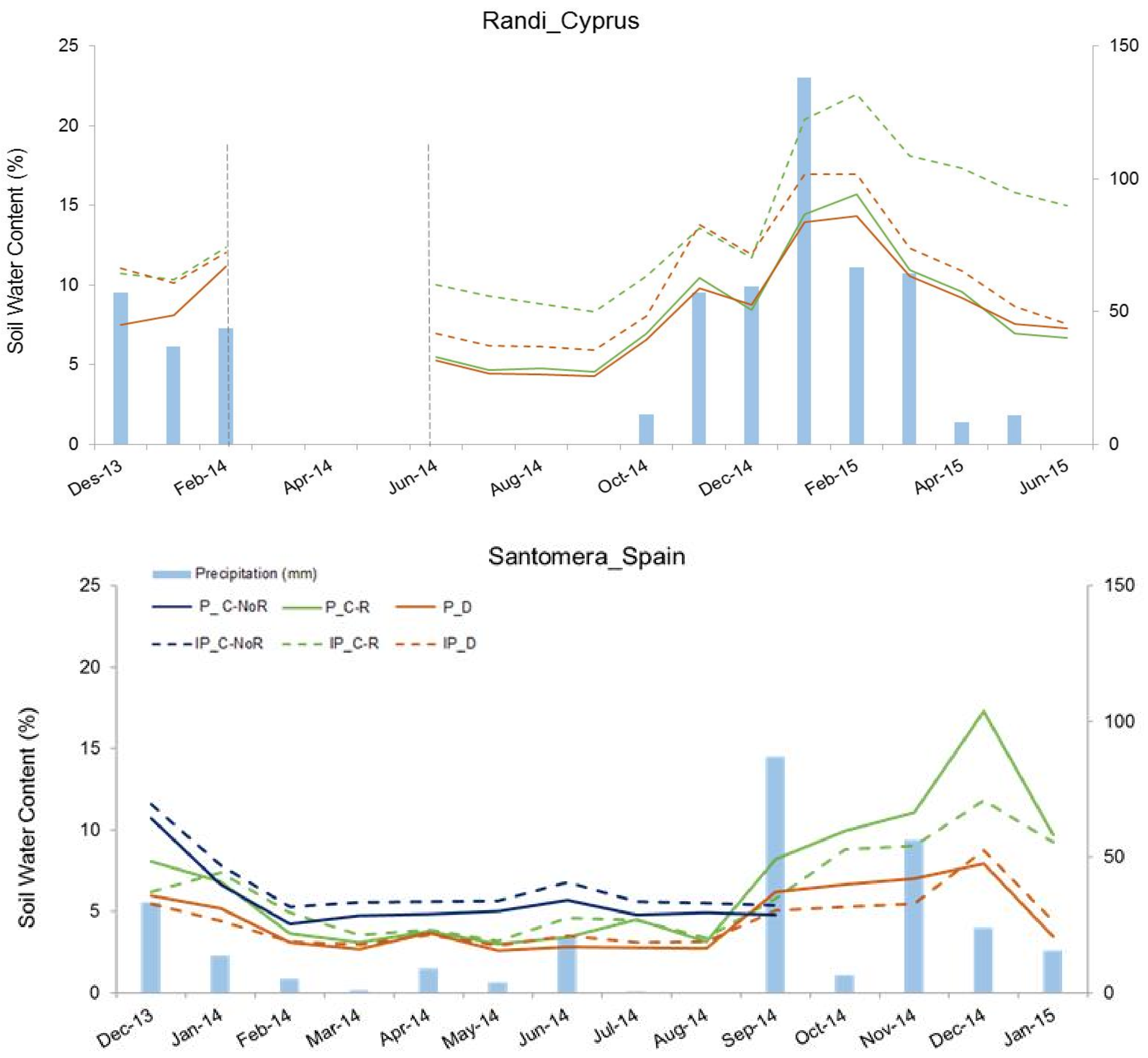


Figure 21. Average soil water content and precipitation in Santomera and Randi..

4.3 Discussion

The aims of the *drought-stress experiment* were

- Establish the effects of increased stress on soil quality and plant performance in order to see whether the plant-soil ecosystem moves into the direction of a catastrophic shift.

- Establish a level a stress that seems critical for the plants to survive in order to see how such critical point is nearby the present natural status of the ecosystems.

We found no consistent effect of increased drought on the selected soil quality and plant performance indicators. For the driest sites, Randi and Santomera, we found increased PMN and, to a lesser extent, labile C forms (HWC) values with increased drought stress towards the end of the experiment, but none of the other four sites showed any relevant effect of increased drought. Similarly, we found no clear treatment effect on plant performance, except for Randi and Santomera, which showed a decreasing trend in plant growth with increasing drought stress. These results indicate an extraordinary capacity of the plant-soil systems of very dry areas to cope with drought, as only the combined effect of a severe natural drought plus the additional experimentally-induced drought finally resulted in decreased plant growth. The increased PMN and HWC values found in the driest sites in response to increased drought could be explained by an increased amount of litter and dead roots being degraded (López-Poma and Bautista, 2014).

The observed soil moisture dynamics confirmed that the roofs captured rainfall, as both controls gave in most cases higher values for soil water content (SWC) than the drought treatments, but also that the impact of the drought treatment was very different between sites. This probably resulted from the occurrence of exceptionally dry conditions during the experimental period in the dry sites, and exceptionally wet conditions in the wet sites, with both cases leading to small differences between roofed and no roofed plots. Hence, in the dry sites insufficient rainfall is captured to obtain differences between treatments, while in the wet sites the soil beneath the roofs stayed wet through influx of water from outside the roofs. For those ecosystems to drive to tipping points for catastrophic shifts to happen requires probably longer and more severe periods of drought.

Also unexpectedly, the results hinted to a positive side effect of the roofs, e.g. in the form shading or keeping air humidity higher, yet this effect would not be consistent across sites. These results highlight the need for using roofed controls to properly assess the effects of rainfall exclusion. Using examples of plant performance data from the various study sites, Figure 22 further explores and illustrates this finding. By comparing each treatment with the appropriate respective control (i.e., drought *versus* roofed control and roofed *versus* non-roofed controls), we found both a negative effect of induced drought and a positive effect of the roof on plant performance, both being higher in drier areas (Figure 22).

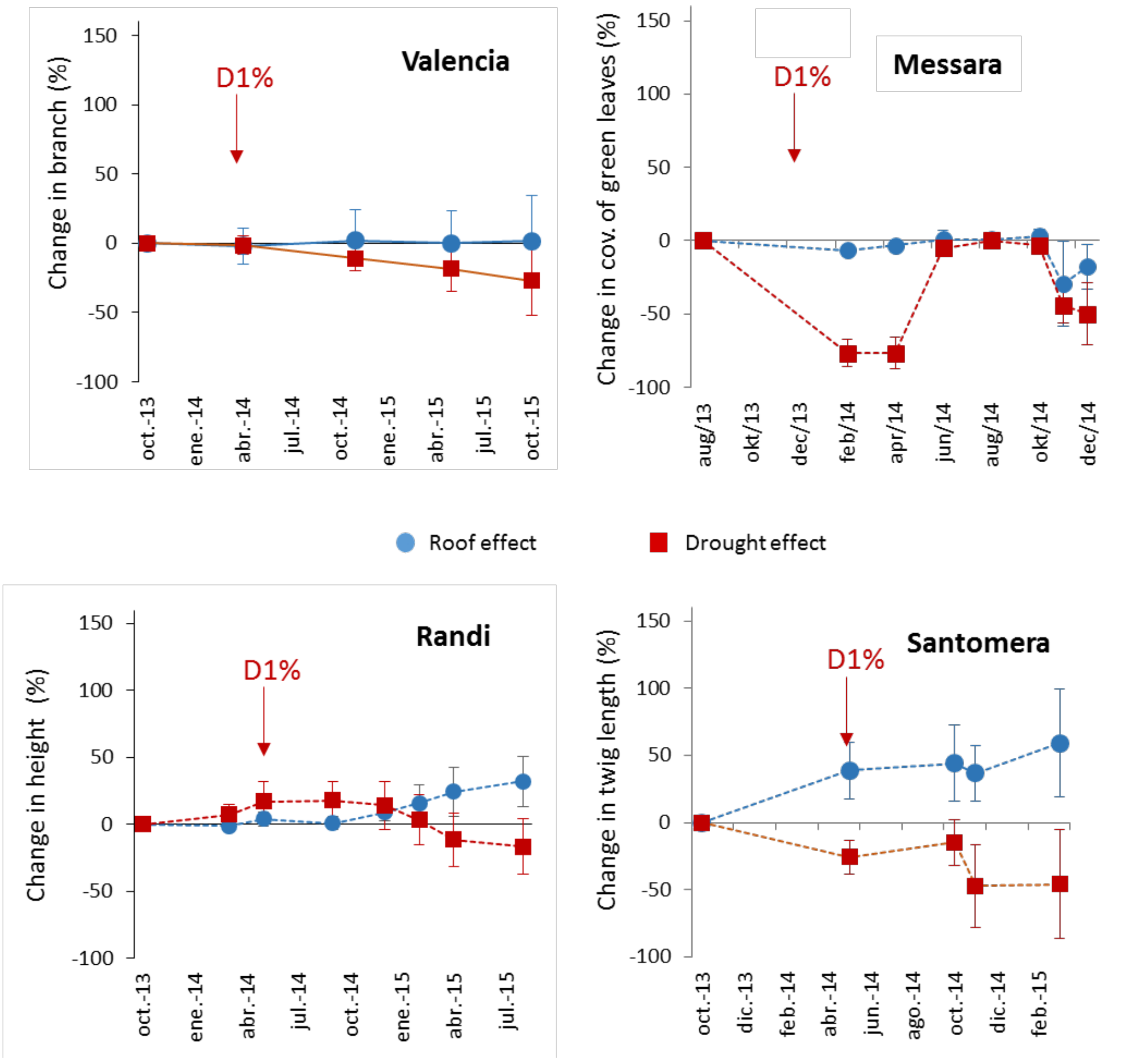


Figure 22. Variation in plant performance attributes as a function of Drought (red squares) and Roof artifact (blue circles) for several CASCADE study sites (the two wettest study sites were not used for this comparison). D1% arrows point to the time when the target reduction in annual rainfall (below the 1st percentile of long-term average) was achieved.

Future experimental work could consider larger roofs to prevent influx of water through infiltration or run-off, or roofs that will move over the plots only during rainfall, although this might be technically and financially difficult to establish under field conditions. Unwanted side-affects could be diminished this way but the costs of the experiments and the logistic difficulties would increase considerably. However, regardless of the type of roof used, our findings support the suggestions made for using roofed controls, combined or not with open ones, to properly assess the effect of rainfall exclusion (Vogel et al., 2013).

Conclusion

The experimentally induced severe drought levels produced some statistically significant effects that hinted at ecosystems changes that might occur in the proximity of criticality and ecosystem shifts but, at the same time:

- ✓ did not produce a consistent response across sites;
- ✓ proved the resistance of dryland plant-soil systems against severe drought
- ✓ proved the existence of significant roof artifacts in rainfall exclusion experiments, which may not be consistent across climatic gradients.

5. Conclusions

Reflecting on the set-up and outcome of the experimental work carried out the CASCADE WP3, we came up with the following conclusions:

Regarding the experimental design

As far as we know the chosen experimental approach is new in its kind. The novelty was:

- ✓ the system-approach characterized by measuring sets of soil quality and plant performance parameters;
- ✓ the comparison of patch and inter-patch microsites;
- ✓ the combination of an observationally established stress-gradient and an experimentally induced stress gradient;
- ✓ In some of the sites (Castelsaraceno, Messara, Randi) it was the first time that research of this kind was carried out;
- ✓ the choice of the experimental roofs used in the drought experiment.

Regarding the results

Regarding the stress-gradient experiment, the sites with higher grazing pressure, Messara and Randi, showed increased soil quality under moderate grazing stress and decreased soil quality and reduced plant performance under high grazing stress. The other two sites, with a low or moderately low overall grazing pressure, did not respond to variations within their respective grazing levels.

Regarding the drought-experiment, increased drought was found to affect soil quality and plant performance only in the driest sites. Results suggested positive side-effects of the roofs on plant performance, particularly for the driest sites.

Regarding the identification of critical changes preceding catastrophic shifts

Most statistically significant results showed that increased stress through grazing (stress-gradient experiment) or drought (drought-experiment) deteriorates the plant-soil ecosystem, probably moving it to critical points for catastrophic shifts to happen. Since we did not encounter an actual shift, we cannot say where these tipping points lie. However, in Randi, the increasing contrast between patch and interpatch soil conditions with increasing level of grazing points to the proximity of a critical shift into a degraded state. Results also indicated positive feed-backs and local facilitation, as most statistically significant results showed higher values for soil quality and plant performance parameters within patches than within inter-patches.

Future research

- ✓ The present choice of sites were relatively different from another in terms of dry-wet conditions (Castelsaraceno and Várzea are relatively wet compared to Valencia, Santomera, Messara and Randi) and drivers of stress (fire recurrence in Várzea and Valencia versus grazing in Castelsaraceno, Santomera, Messara and Randi). This was a correct choice giving the novelty of the research and the need for a relatively broad exploratory approach. Future research directed to more detailed mechanism may benefit from a more restricted focus, e.g. on one type of ecosystem-driver combination. Looking at the effects on patch mortality (results not shown) versus target plant mortality in Messara, one should also reconsider what kind of ecosystem properties should be taken into account.
- ✓ Repeating the drought experiments asks for always using roofed controls or modified version of rainfall capturing roofs, e.g. roofs that are only present during rainfall events diminishing unwanted side effects of the roofs.
- ✓ Even though some sites experienced extreme dry periods (Spain), no ecosystem shifts were encountered here. To be able to learn more from actually occurring tipping points, longer lasting experiments should be performed

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Annex I. Variation in soil variables as a function of grazing pressure and microsite for each site

Santomera site (Spain)

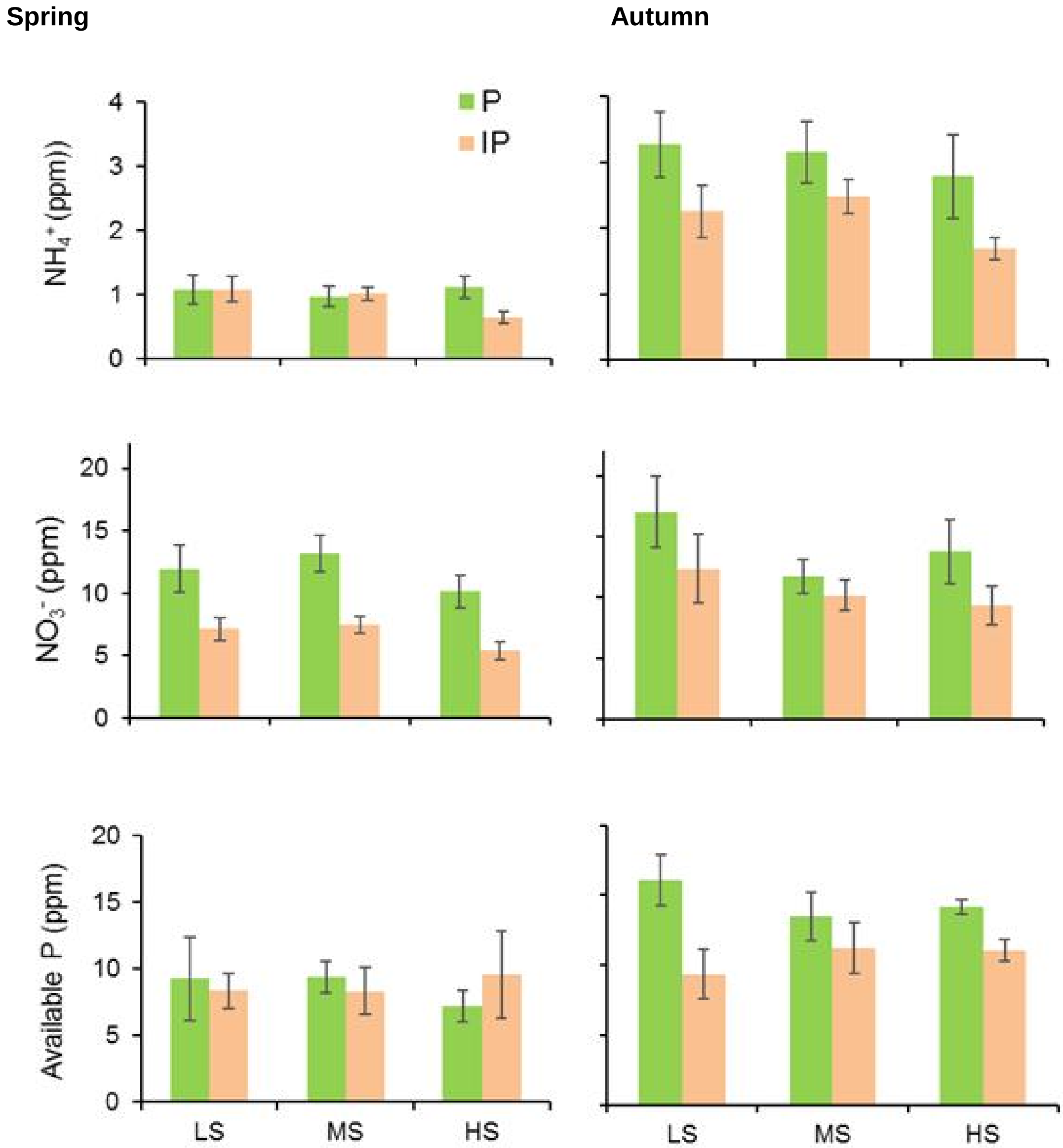

Figure A.1. Variation in dissolved organic carbon (DOC) and potentially mineralisable nitrogen (PMN) at 0-5cm soil depth as a function of grazing pressure, microsite, and season in Santomera (Spain). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.

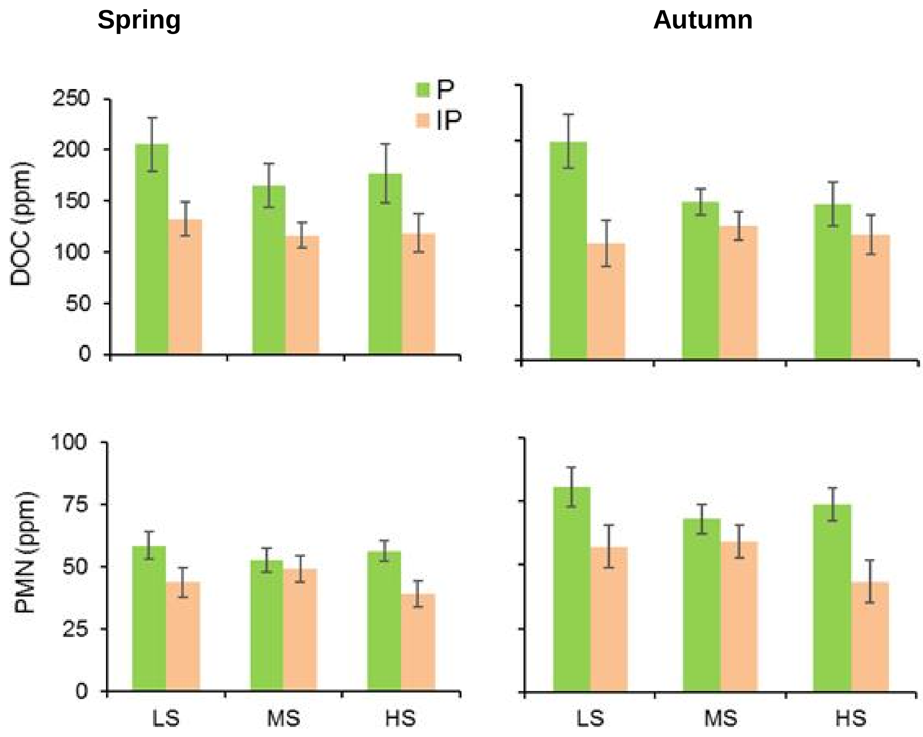

Figure A.2. Variation in nutrient availability: NH_4^+, NO_3^-, and available P, at 0-5cm soil depth as a function of grazing pressure, microsite, and season in Santomera (Spain). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.

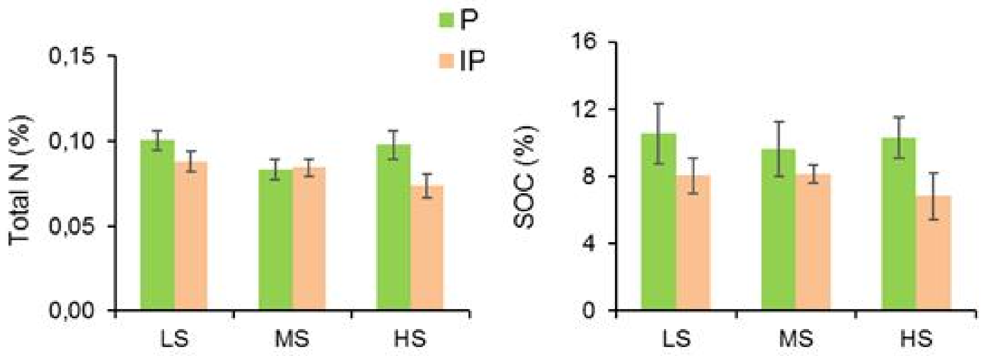

Figure A.3. Variation in one-off measurements of total nitrogen and soil organic carbon (SOC) at 0-5 cm soil depth as a function of grazing pressure and microsite in Santomera (Spain). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.

Castelsaraceno (Italy)[1]

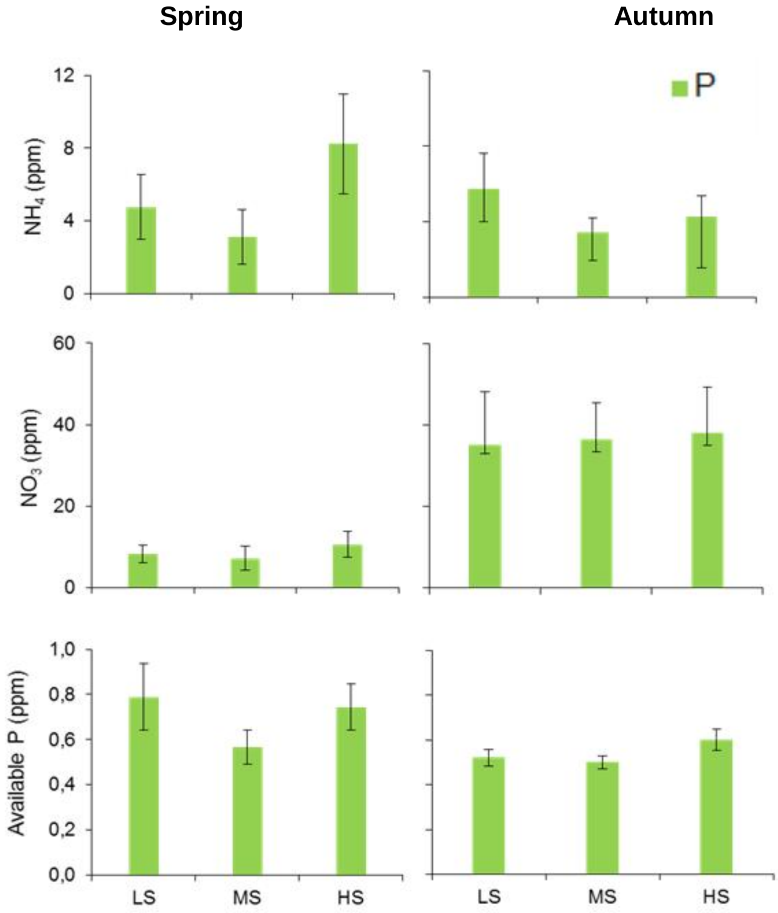

Figure A.4. Variation in nutrient availability: NH_4^+, NO_3^-, and available P, at 0-5cm soil depth as a function of grazing pressure and season in Castelsaraceno (Italy). LS: low stress; MS: moderate stress; HS: high stress.

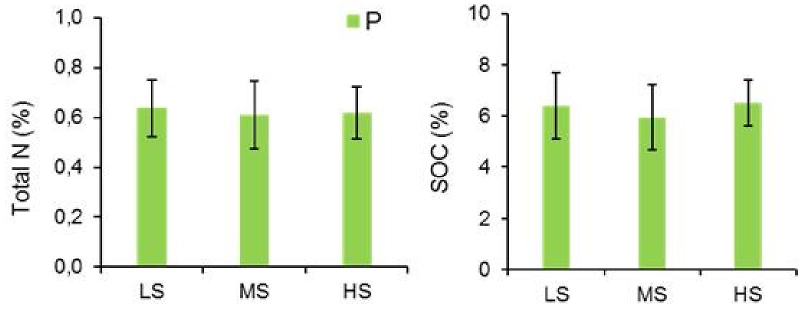

Figure A.5. Variation in one-off measurements of total nitrogen and soil organic carbon (SOC) at 0-5 cm soil depth as a function of grazing pressure in Castelsaraceno (Italy). LS: low stress; MS: moderate stress; HS: high stress.

[1] No patchy vegetation structure in Castelsaraceno site, and therefore the microsite (patch/interpatch) factor is not considered in this site.

Messara (Crete)

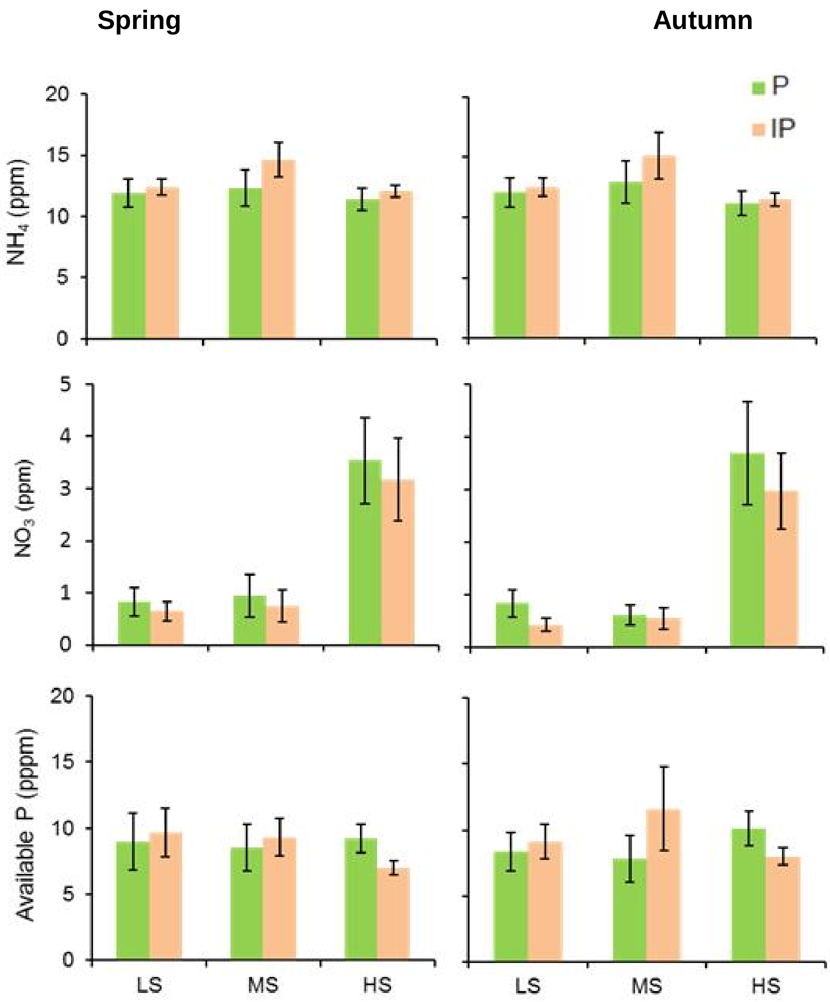

Figure A.7. Variation in nutrient availability: NH_4^+, NO_3^-, and available P, at 0-5cm soil depth as a function of grazing pressure, microsite, and season in Messara (Crete). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.

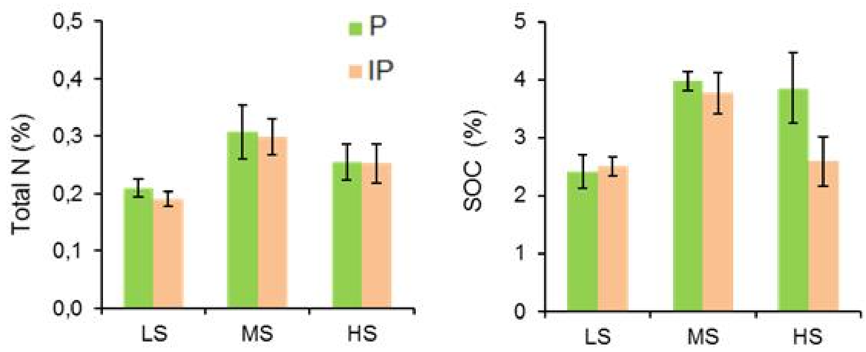

Figure A.6. Variation in one-off measurements of total nitrogen and soil organic carbon (SOC) at 0-5 cm soil depth as a function of grazing pressure and microsite in Messara (Crete). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.

Randi (Cyprus)

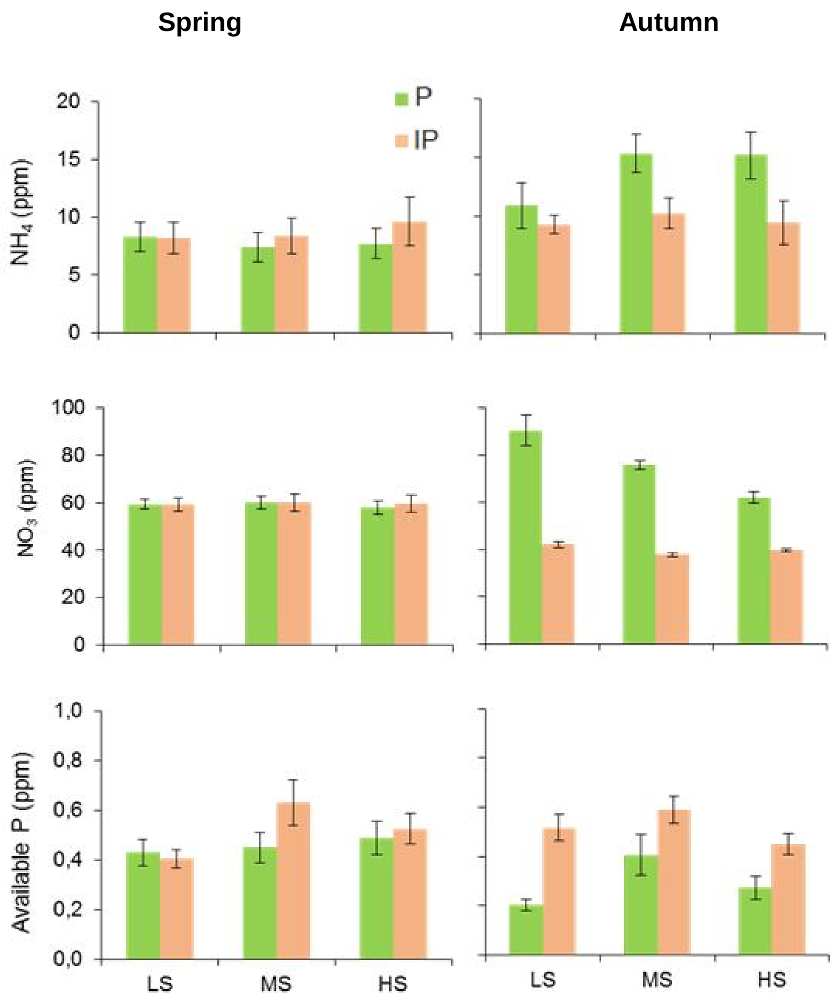

Figure A.8. Variation in nutrient availability: NH_4^+, NO_3^-, and available P, at 0-5cm soil depth as a function of grazing pressure, microsite, and season in Randi (Cyprus). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.

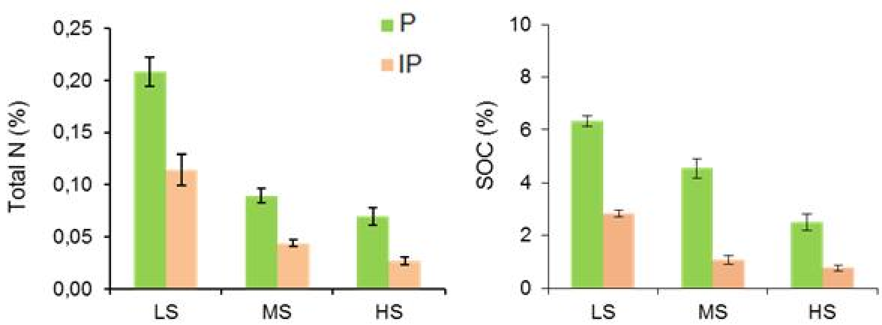

Figure A.9. Variation in one-off measurements of total nitrogen and soil organic carbon (SOC) at 0-5 cm soil depth as a function of grazing pressure and microsite in Randi (Cyprus). LS: low stress; MS: moderate stress; HS: high stress; P: patch microsite; IP: Interpatch microsite.