**Interactive effects of UV radiation and water deficit on production characteristics in upland grassland and their estimation by proximity sensing**

Petr Holub1\*, Karel Klem1,2, Barbora Veselá1, Kateřina Surá1,2 and Otmar Urban1

*1Global Change Research Institute of the Czech Academy of Sciences, Bělidla 4a, CZ-603 00 Brno, Czech Republic*

*2Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic*

*\*author for correspondence; email: holub.p*[*@czechglobe.cz*](mailto:novak.j@czechglobe.cz)

**Abstract**

Since water deficit (WD) and ultraviolet radiation (UV) trigger similar protective mechanisms in plants, we tested the hypothesis that UV modulates grassland acclimation to WD, mainly through changes in the root/shoot (R/S) ratio, enhances the ability of grassland to acquire water from the soil and hence affects its productivity. We also tested the potential of spectral reflectance and thermal imaging for monitoring the impacts of WD and UV on grassland production parameters. The experimental plots were manipulated by lamellar shelters allowing precipitation to pass through or to exclude it. The lamellas were either transmitting or blocking the UV. The results show that WD resulted in a significant decrease in above-gound biomass (AB). In contrast, below-ground biomass (BB), R/S ratio and total biomass (TB) increased significantly in response to WD, especially in UV exclusion treatment. UV exposure had a significant effect on AB and BB, but only in the last year of the experiment. The differences in the effect of WD between years show that the effect of precipitation removal is largely influenced by the potential evapotranspiration (PET) in a given year and hence mainly by air temperatures, while the resulting effect on production parameters is best correlated with the water balance given by the difference between precipitation and PET. Canopy temperature and selected spectral reflectance indices showed a significant response to WD and also significant relationships with morphological (AB, R/S) and biochemical (C/N ratio) parameters. In particular, the vegetation indices NDVI and RDVI provided the best correlations of biomass changes caused by WD and thus the highest potential to remotely sens drought effects on terrestrial vegetation.

**Key words:** drought; grassland; infrared thermography; nitrogen; precipitation; spectral reflectance; UV radiation

**1. Introduction**

It is widely accepted that the frequency and magnitude of extreme weather events will increase as a result of global climate change (Dai, 2011; IPCC, 2021). The effects of extreme weather events on vegetation and ecosystem functioning are likely to be much greater than the effects of long-term changes in mean temperature and precipitation (Easterling et al., 2000; Jentsch & Beierkuhnlein, 2008; Hoover et al., 2014). Such changes will affect numerous soil, plant, and ecosystem properties and functions of grasslands, ultimately affecting their productivity and biodiversity (Knapp et al., 2002; Fay et al., 2008; Kreyling et al., 2008). The relationships between vegetation productivity and climatic components, mainly precipitation and temperature, and their interaction with soil properties have been studied by several authors. For example, above-ground net primary productivity was positively correlated with mean annual precipitation in grasslands (e.g. Yahdjian & Sala, 2006; Fay et al., 2008; Qin et al., 2018). Furthermore, in a synthesis of 83 studies, Wilcox et al. (2017) showed that cooler mountain ecosystems, in particular, are more sensitive to changes in precipitation.

Less attention has been paid to the analysis of below-ground production (BP), although it is a major carbon sink, especially in areas dominated by grassland vegetation. Among others, Brunner et al. (2015) highlighted that below-ground processes are relatively underrepresented in research of climate change impact on terrestrial vegetation. Conflicting data have been published on root growth and BP under drought conditions. Root biomass and total length of fine roots were often significantly higher in dry sites than in humid sites (e.g. Ibrahim et al., 1997; Bakker et al., 2006; Qaderi et al., 2006), while the shift towards increasing values of root to shoot ratio (R/S) is a well-known drought avoidance strategy (e.g. Rodrigues et al., 1995; Li et al., 2021). However, published data also show that water availability may be a limiting factor for BP and alters the share of BP in total production (e.g. Frank, 2007; Xu et al., 2012). Several studies (e.g. Denton et al., 2017) even reported no general effect of reduced water availability on BP.

However, plants grown under field conditions usually simultaneously experience changes in more than one environmental factor. The interactions of more environmental factors may cause a variety of plant responses which can be adaptive (antagonistic) or cumulative (additive or synergistic). Adaptive interactions arise primarily due to the formation of similar protective mechanisms in plants against oxidative damage caused by several abiotic and biotic stresses such as drought and UV-B (Cechin et al., 2008) and due to morphological adjustment of plants (Robson et al., 2015). A highly efficient antioxidant defence system in plants for detoxification of reactive oxygen species includes either the nonenzymatic (as flavonoids, carotenoids, tocopherols) or the enzymatic constituents (Basu et al., 2010). Accumulation of antioxidants is mainly controlled by the light environment, particularly the intensity of UV radiation (Klem et al., 2015). Because these protective mechanisms play an adaptive role in both water stress regulation (Gitz & Liu-Gitz, 2003) and attenuation of UV-B radiation (Ibañez et al., 2008), the interactive effects between exposure to UV radiation and drought stress in plants are assumed. However, present data concerning the interaction between UV radiation and drought as well as the implications on plant metabolic processes are controversial. For example, ameliorating effects of drought for UV-B sensitivity have been reported by Sullivan & Teramura (1990). In addition, it has been suggested that under multiple stress conditions, exposure to UV radiation moderates the effects of drought (Schmidt et al., 2000; Cechin et al., 2008) or that each stress factor seems to bring about some adaptive effect to reduce the damage caused by the other one (Alexieva et al., 2001; Hofmann et al., 2003; Zhang et al., 2020). In contrast, other findings show synergistic (additive) interactions, resulting in enhanced sensitivity to UV radiation under water limitation (Tian & Lei, 2007; Lu et al., 2009).

Due to the complexity of interactions with other factors, which show high temporal and spatial variability, estimation of drought impacts on the ecosystem productivity is challenging. The primary possibility for repeatedly assessing drought impacts on large areas represents remote sensing approaches of which, particularly thermal infrared imaging and spectral reflectance, can detect drought stress effects. Thermal infrared imaging detects particularly the changes in leaf temperature caused by stomatal closure under drought stress (Grant et al., 2006). Reduction of stomatal conductance leads to reduced transpiration and a subsequent increase in leaf temperature. As stomatal conductance can also be influenced, besides other factors such as wind speed, air humidity and intensity of solar radiation (Jones et al. 2009), by UV radiation (Jansen & van den Noort, 2000), the results of detection drought responses by thermal infrared imaging should be considered concerning this factor. Although the results of thermal infrared imaging depend on a number of environmental factors, their main advantage is the possibility of early detection of drought responses.

On the contrary, spectral reflectance is based on biophysical parameters of vegetation such as leaf area, biomass, chlorophylls or other pigments, and water content that are more stable in time and not directly affected by actual environmental conditions. However, the response to drought is detected in much later stages of drought, which already represent a certain degree of damage such as a decrease in biomass, chlorophyll content, accumulation of oxidative damage products. Hence, the estimation of drought responses using spectral reflectance is based on changes in biomass (NDVI, Aparicio et al., 2000), conversion of xanthophyll cycle carotenoids (PRI, Elsheery & Cao, 2008), water content (WI, Peñuelas et al., 1997) or accumulation of oxidised phenolic compounds (BPI, Peñuelas et al., 2004). Spectral reflectance thus allows detection of different types of response compared to the thermal infrared imaging; however, as an indirect type of measurement, the results can be affected by interactions with other factors (e.g. nutrient availability, light conditions, canopy structure; Hatfield et al., 2008).

The main objectives of the present paper were to investigate and compare the effects of water deficit (WD) and UV radiation (UV) and their combination (WD+UV) on the above- and below-ground productivity of the mountain grassland ecosystem and to evaluate the possibility of detection responses to the combined effect of WD and UV using infrared thermal imaging and spectral reflectance. Since we also aimed to evaluate the potential contribution of UV radiation to the plants´ protective mechanisms against drought, treatment with ambient precipitation and UV exclusion was defined as control (C). We formulated the following hypotheses: (1) UV alleviates the negative effects of WD on plants and ecosystems through changes in the R/S ratio and improved ability to acquire water from the soil, (2) thermal infrared imaging results are more related to physiological status (stomatal conductance) and is therefore affected by both WD and UV, whereas spectral reflectance data are mainly related to biomass accumulation and chlorophyll content which is primarily affected by WD.

**2. Materials and methods**

**2.1. Site description**

The experimental plots were established in the mountain *Nardus* grassland (association *Molinio-Arrhenatheretea,* class *Polygono-Trisetion*) in the Moravian-Silesian Beskydy Mts., the Czech Republic (near the Ecological Experimental Study Site Bílý Kříž, altitude 855 m, latitude 49o30´ N, 18o32´ E). The site is characterised by a mean long-term annual temperature of 6.8 oC and precipitation of 1318 mm. The geological bedrock is formed by Spodo-dystric cambisol on Flysch Godulian sandstone. The experimental area was selected to represent a homogeneous segment of vegetation, allowing proper experimental design with randomised replications in the block. Narrow-leaved grasses *Nardus stricta, Festuca rubra*, *Agrostis capillaris, Holcus mollis*, *Avenella flexuosa* and *Carex pilulifera* are the major components of the vegetation in this grassland. The most frequented forbs were *Hypericum maculatum, Rumex obtusifolius,* *Veronica officinalis, Potentilla erecta* and *Hieracium lachenalii.* The grassland is regularly mowed once a year in July.

**2.2. Experimental design**

The field experiment was carried out in a two-factorial design manipulating WD and UV radiation during 2012–2014. The design consisted of twelve plots, each 2 x 1.5-m in size, with all factorial combinations replicated three times. To determine the responsiveness of mountain grassland vegetation to drought and UV radiation, six transparent shelters (3 x 2-m in size) were constructed as a wood frame covered with transparent acrylic lamellas (thickness of 3 mm; Quinn Plastics, Enniskillen, UK) with different UV-A and UV-B transmittance that had a 20o inclination. The first type (UVT Solar) transmitted more than 90% of incident UV-A and UV-B radiation, whereas the second one (Quinn XT) filtered UV-B radiation and a large part of UV-A.

The shelters were installed in all plots every year from April, just after snowmelt, until the mowing in July. The exclusion of rainfall in dry plots was provided by modifying the position of the acrylic lamellas so that they overlapped in the direction from the top. In this case, the water intercepted by the lamellas was channelled using a gutter out of the experimental area. On the contrary, lamellas in wet treatments overlapped in the direction from the bottom and thus allowed complete penetration of rainfall to the experimental plot. Thus four treatments were maintained: control with ambient precipitation and UV exclusion (C), water deficit and UV exclusion (WD), ambient precipitation and UV exposure (UV), and water deficit and UV exposure (WD+UV). Such definition of treatments enables to determine the effect of UV, WD and their combination as well as to interpret how UV radiation enhances drought resistance of plants. Induced drought period (IDP) was manipulated in spring, thus during the maximal growth of vegetation (Table 1). A 0.2 m wide trench was dug and sheathed with plastic foil to separate the soil of the roofed areas from the neighbouring soil. A 0.25-m wide zone beneath the edge of the shelters was excluded from all measurements and samples. The distance between the shelter and soil surface was 1.3 m to maximise air movement and minimise temperature and relative humidity artefacts. The reduction of heating under the shelters was also ensured by the gaps between individual lamellas, through which the heated air rose upwards.

**2.3. Climatic parameters**

Precipitation and air temperature were measured every 30 min at a meteorological station during the whole growing season. During the experiment, volumetric soil moisture was continually monitored every 10 min using soil moisture (ThetaProbe ML2x, Delta-T Devices, Cambridge, UK) sensors installed at a depth of 15 cm. Precipitation was measured by a rain gauge Rain-O-Matic Meteorological (Pronamic, Denmark). During the experiment, volumetric soil moisture had been reduced at the end of the drought period relatively by 56, 61, 42% in WD plots in 2012–2014, respectively, compared to the control treatment (C; 100%; Fig. 1). The lower reduction of soil moisture in WD treatments in 2012 and 2013 was due to a drier summer during these years (Table 1, Fig. S1).

For the characterisation of hydrological conditions of combination WD treatments and a given year, we analyse the Emberger aridity index (AI) (Emberger, 1932). AI depends on the precipitation P (mm), the mean temperature Tc (°C) of the coldest month and the mean temperature Th (°C) of the hottest month within a specific time period (April–end of IDP in the present study). The index is defined as follows: AI = 1000 × P / (Th2 – Tc2). In addition, we calculated water balance (WB) which is defined as the difference between precipitation P (mm) and potential evapotranspiration PET (mm). PET was estimated using the Penman-Monteith method, and it was calculated using 30 min data sets of air temperature, humidity, wind speed, solar (incident global radiation) and net (the balance between incoming and outgoing energy at the top of the canopy) radiations (Allen et al., 1998).

**2.4. Production parameters**

The above-ground biomass (AB) was determined annually by harvesting all above-ground biomass at the end of the IDP when the biomass reached a seasonal maximum of the growing seasons (July 10, 2012, July 3, 2013 and July 3, 2014). The area 0.3 x 0.3 m was harvested per plot. AB was dried to a constant weight (at 60 °C) and weighed. Simultaneously with AB sampling, below-ground plant biomass (BB) was determined in all treatments with the coring method. Soil cores (9.4 cm in diameter, 15 cm depth, n = 3) were collected within each treatment at the end of IDP of the respective year. Collected samples were washed in nylon bags and on sieves of 0.5 mm mesh size and dried to the constant weight. R/S ratios were assessed on the basis of total below- and above-ground biomass (BB/AB).

**2.5. Spectral reflectance and thermal measurements**

Measurement of spectral reflectance (350–2500 nm) at the canopy level was carried out at the end of the IDP (Table 1) using a FieldSpec 4 Hi-Res spectroradiometer (ASD Inc., Boulder, CO, USA). The reflectance measurements were conducted from a distance of ca 0.8 m perpendicular to the canopy surface using a pistol grip twice for each plot and then averaged. Before each new plot, the reference spectrum was measured using white Spectralon reflectance standard (Spectralon; Labsphere, USA). The reflected radiances were directly converted to spectral reflectance within the RS3 Spectral Acquisition Software (ASD Inc). Subsequently, vegetation and chlorophyll indices were computed from spectral reflectance curves (the calculated indices are listed in Table S1).

Thermal imaging measurements were acquired around noon from a distance of ca 1 m using an infrared thermal camera SC 660 (Flir Systems, USA). Approximately 100 points from each image were selected manually to avoid the effect of pixels from the soil background. The canopy temperature difference (Tdiff; oC) was calculated as the difference between a canopy (Tc) and air (Ta) temperatures (Tdiff = Tc – Ta).

**2.6. Statistical analyses**

Before calculating the analysis of variance, the data for individual parameters were tested for normality using the Kolmogorov–Smirnov test. Three-way ANOVA analysis was used to test the effect of WD, UV radiation and year on AB, BB, total biomass, and nitrogen and carbon in AB. The Fisher LSD post-hoc test was used to analyse differences between means. Significance levels are reported in the figures and tables as a significant with \*p <0.05, \*\*p <0.01, and \*\*\*p <0.001. Pearson correlation coefficients (*r*) were calculated to evaluate the power of relationships between climatic and production parameters (Table S2) and between production parameters and thermal and spectral parameters (Table S3). All statistical tests were done using Statistica 12 software (StatSoft, Tulsa, OK, USA).

In order to identify the variables that explained a higher proportion of the total variance, which could provide insight into the relationships among climatic, production, nutrient, thermal and spectral reflectance parameters, a principal component analysis (PCA) was performed using R 3.5.1 ([R Core Team, 201](https://www.sciencedirect.com/science/article/pii/S0034425720302042" \l "bb0245)4). In addition, to evaluate the interactive effect of WD and UV, linear regression was used to relate measured (i.e. observed) values (in the y-axis) vs. calculated (i.e. predicted) values (in the x-axis) for individual variables (Fig. 6; Piñeiro et al., 2008). The predicted effect was the sum of percentage changes in response to WD and UV effects relative to the control. This predicted effect was compared to the measured combined WD+UV effect relative to the control. In these figures, the Root Mean Square Error (RMSE) was calculated for the 1:1 line.

**3. Results**

**3.1. Production characteristics**

The ANOVA analysis confirmed the significant effect of WD on all production parameters and the significant effect of UV on BB, R/S and TB (Table 2). Significant reduction of AB was observed in WD and WD+UV treatment in comparison with UV treatment in 2012 and in all treatments (WD, UV and WD+UV) compared to C in 2014 (Fig. 2). In addition, the significant effect of year on AB was found (Table 2). AB was significantly higher in year 2014 compared to previous years 2012 and partly 2013 (except WD treatments; Fig. 2).

Generally, WD treatment resulted in a higher accumulation of BB in comparison with C and UV treatments, which led to a higher R/S ratio and TB (Figs. 3A–C). Except 2013, the R/S ratio was significantly higher under WD treatment (4.6–5.6) in comparison with C (1.1–3.4). Moreover, also combined WD+UV effect was higher than C, but significantly only in 2012 (Fig. 3B). Generally, no significant differences in TB accumulation were found during the whole experiment, except significant increase in WD treatment in 2014 (Fig. 3C). Concerning interactions, all biomass characteristics were not affected by the interaction of WD, UV and the year (Table 2).

Most of the TB was allocated to BB within soil horizon 0–5 cm, followed by to the AB, and the smallest fraction of biomass was allocated to soil horizon 5–10 cm and deeper (Fig. 4). Significantly higher biomass allocation to BB within soil horizon 0–5 cm was found under WD at the expense of AB in the last year of the experiment (2014). In addition, UV led to significantly higher allocation to BB within horizon 0–5 cm under the simultaneous effect of WD (comparison of WD and WD+UV treatments; Fig. 4). In treatments without WD (C and UV), a higher biomass allocation to AB was found in 2014 (47–49%) in comparison with the previous two years, 2012 and 2013 (24–29%).

**3.2. Nitrogen accumulation and C/N stoichiometry**

The ANOVA analysis of data from differed precipitation and UV radiation treatments confirmed the significant effect of WD on above-ground N concentration, N uptake in AB, and C/N ratio (Table 3). Generally, N concentration in AB was significantly lower when exposed to both WD and WD+UV treatments (1.36–2.02%) in comparison with treatments without WD (C and UV; 1.76–2.55%). However, such effect was significant only under UV exposure in 2012 and 2013 (Fig. S2). Also, N uptake in AB was reduced in response to WD and WD+UV when compared with C, however significantly only in 2014 (Fig. 5A). C/N ratio increased after WD compared to C, but significantly only in 2012 (Fig. 5B).

No significant effect of UV radiation on nitrogen accumulation and C/N stoichiometry was observed (Table 3). Nevertheless, N uptake in AB was significantly affected by UV in 2014. In this last year of the experiment, UV led to a substantial decrease in N uptake compared to C (Fig. 5A). No effect of UV radiation on C/N ratio was observed in all years studied (Fig. 5B).

**3.3. Interactive effects of water deficit and UV radiation**

To evaluate the combined effect of WD and UV, we compared the predicted effect (the sum of relative changes in individual WD and UV treatments compared to C) with the measured combined effect of WD+UV treatment (compared to C) for selected production and nutrient parameters. The comparison of the real response line between the observed combined effect of WD+UV showed in all parameters decline of the slope, which indicate less than additive (i.e. mutually alleviating) effect of both factors. This means that the real effect of combined factors is lower in both directions, negative and positive, than the sum of both effects acting separately (Fig. 6).

**3.4. Associations among environmental, production, and remote sensing parameters**

In order to find potential associations that would explain the observed effects of WD and UV, and also potential remote sensing indicators for their estimation, a principal component analysis (PCA) based on all measured climatic, production, elemental, thermal infrared and spectral reflectance parameters was performed (Fig. 7). PCA revealed that AB, N concentration and N uptake in AB was positively related to precipitation and other climatic parameters (soil moisture, aridity index, and water balance). In contrast, strong antagonistic relationships between R/S ratio or C/N ratio in AB and climatic parameters were observed (Fig. 7).

PCA also revealed a strong positive association between UV radiation and C concentration in AB, and negative association between UV radiation and TB. The best predictors for estimating changes in R/S and C/N ratios are Tdiff (positive relationship) and NDVI, SIPI and ANMB650-725 (negative relationship). AB is best estimated using spectral indices RDVI and MCARI1 (Fig. 7). In addition, a significant positive relationship between C/N and R/S ratios were observed (Fig. S3).

AB significantly increased with higher water regime parameters except for soil moisture. In addition, the significant negative relationships between BB and R/S ratio and water regime parameters were observed (Table S2). Moreover, the highest correlation coefficient (r = -0.67, p ≤ 0.001) for R/S ratio was found in the relationship with water balance (Fig. 8A). The strong negative correlations between R/S ratio and all water regime parameters were also found separately in both UV exposure and UV exclusion treatments except soil moisture, where the significant relationship with R/S ratio was observed only in UV exclusion treatment. Moreover, N concentration and N uptake were significantly positively correlated with water regime parameters, while a negative relationship between C/N ratio and water regime parameters were found. The most significant negative correlation coefficient for C/N ratio was observed with water balance in both UV radiation treatments (Fig. 8B).

**3.5. Thermal imaging and spectral reflectance measurements**

Thermal infrared imaging results showed that Tdiff was significantly influenced by WD, but not by UV radiation (Table S3). Nevertheless, we found a significant increase in canopy temperature (reduction of canopy temperature difference) in response to UV compared to C in 2013 (Fig. S4). The main effects on Tdiff, however, had WD, when Tdiff raised by more than 80% and 69% (averaged over 2012–2014) in the case of WD and combined WD+UV treatments, respectively. The correlation analysis of thermal infrared imaging data showed significant relationships between Tdiff and AB, BB or R/S ratio and also with N uptake or C/N ratio (Table S4). The highest correlation coefficient was observed between Tdiff and R/S ratio (Fig. 9A).

All the calculated vegetation indices except TCARI, TCARI/OSAVI and NPQI showed statistically significant responses to WD, while these responses to UV radiation were negligible and insignificant (Table S3). In addition, the correlation analysis which has been conducted for spectral reflectance indices and production parameters with data for each plot individually, showed a significant correlation of 19 and 20 vegetation indices from a total of 24 calculated with AB and R/S ratio, respectively (Table S4). The highest correlation coefficients were observed between AB and RDVI or between R/S ratio and NDVI (Figs. 9B, C). Only three significant relationships between BB and spectral reflectance indices (NDGI, VOG2 and GM1) and even no significant relationships with TB were observed (Table S4).

Spectral reflectance indices were also correlated with elemental (C and N) parameters in AB, particularly with N. From 24 calculated vegetation indices, 22 were significantly correlated with N concentration in AB, 21 with N uptake in AB and C/N ratio (Table S4). On the other hand, only four significant correlation coefficients were found between C concentration in AB and vegetation indices. The most statistically significant relationship between C/N ratio and NDVI is shown in Figure 9D.

**4. Discussion**

**4.1. Production characteristics**

Extreme drought events affect numerous soil, plant, and ecosystem properties of grasslands and ultimately influence their productivity, biological diversity (Knapp et al., 2002, Fay et al., 2008, Kreyling et al., 2008) and also their ability to take up and utilise the nutrients (Grange et al., 2021, Kubert et al., 2021). In the present study, we found a significant negative effect of WD on the productivity of mountain grassland. In contrast, no marked effects of reduced precipitation by 50% on the above-ground productivity of grasses and forbs were found in the same mountain site during the water availability manipulation experiment in 2006–2008 (Holub et al., 2013). They found that the cover of dominant species such as *Nardus stricta*, *Festuca rubra*, *Avenella flexuosa*, *Potentilla erecta* and *Vaccinium myrtillus* did not change in response to reduced precipitation (Holub et al., 2013). It is evident that only 50% reduction of precipitation did not change the AB of the mountain grassland with high ambient precipitation. It is in accordance with findings from a manipulative experiment with six precipitation treatments (Zhang et al., 2017). They observed only effect of the most extreme drought treatment (1/12 of annual precipitation) on above-ground productivity in an alpine meadow and it was caused mainly by the reduction of forbs. On the other hand, Johnson et al. (2011) reported that repeated drought events reduced grass biomass, increased forb biomass, and led to an overall decrease in AB compared with controls in alpine snowbed community with dominant *Nardus stricta*.

On the contrary, the effect of UV radiation on the AB was not statistically significant in our study, which is consistent with findings of other authors, who summarised that effects of UV-B on plant biomass production are mostly species-specific (e.g. Deckmyn & Impens, 1999). However, it is evident that UV radiation further modulates the effect of WD. While AB was in our experiment reduced by 36, 3 and 39 % in response to WD, it was reduced by 43, 1 and 7 % in response to combined WD+UV effect in 2012, 2013 and 2014, respectively. This marked difference in the last year of the experiment pointed to the positive effect of UV radiation on AB production under reduced water availability. Drought tolerance can be enhanced by the effect of UV radiation due to various reasons. Some authors suggest an interaction between drought stress and UV radiation through flavonoid biosynthesis (Nogués et al., 1998). Flavonoids play an important role as antioxidants and can mitigate the oxidative stress induced by drought stress. Rapantová et al. (2016) observed higher accumulation of flavonoids under combined effect of WD and UV in grass as well as forbs.

Some authors mentioned that WD led to a significant increase in the fine root length density (root length in soil volume) and dry weight (e.g. Rodrigues et al., 1995; Walter et al., 2011). Robson et al. (2015) reviewed that UV radiation can alleviate the WD effect by higher root development and an increase in R/S ratio. In the present study, we found higher accumulation of BB in response to WD; however, we did not observe any significant change of R/S ratio in response to UV. Rapantová et al. (2016) observed species-specific differences in response to UV radiation in the same drought experiment. They found that UV radiation alleviated the negative impact of WD in *A. capillaris*, *H. mollis* and *H. maculatum*, while the additive effect of UV was observed in *R. obtusifolius* in response to WD. These different species-specific effects can probably explain no significant positive effect of UV on biomass allocation to below-ground plant parts in response to WD at the level of whole plant community in the present study. Thus, the first hypothesis that UV radiation alleviates the negative impact of WD through enhanced below-ground biomass was not confirmed in our study.

**4.2. N and C stoichiometry**

In the present paper, N cycling was significantly affected by WD. The annually recurrent WD periods led to increasing C/N ratio in AB and decreasing N concentration and N uptake in AB in comparison with control. Gleeson et al. (2010) indicated that nitrification, both in terms of process rates and microbial populations responsible for nitrification activity, is highly responsive to soil water availability. Thus, water stress resulted in an increase of ammonium in the soil, which can hardly be taken up by plants. Alternating drying and wetting cycles lead to controversial effects. Although the alternated drying is often reported to increase N mineralisation (Lu et al., 2020; Xiang et al., 2008), the cumulative N mineralisation is mostly smaller under alternated drying and wetting compared to optimum soil moisture (Borken & Matzner, 2009). The effect of drying cycles on N mineralisation also strongly depends on soil texture when in fine textured soils is the effect of drying less pronounced due to non-linear response (Austin et al., 2004). Jentsch et al. (2011) suggested that the composition of different microbial groups in soils remained unchanged in response to drought except for arbuscular mycorrhizal fungi. They explained an increase in C/N ratio in plants by a lower microbial activity and reduced soil respiration under drought conditions, which can result in decreased rate of decomposition (Jentsch et al., 2011).

The effect of elevated UV-B on N metabolism is often caused by changes in nitrite or nitrate reductase activity and reduction in leaf C/N ratio under elevated UV-B suggested competition between sucrose synthesis and nitrate reduction (Singh et al., 2015). However, there was found no significant effect of UV radiation on nutrient characteristics in the present study.

**4.3. Thermal imaging and spectral reflectance measurements**

Thermal imaging represents one of the well-established methods for indirect and non-invasive estimation of stomatal conductance and responses to WD (Jones, 1999). However, the final effect of WD on above-ground biomass can be different from stomatal response. Decline in stomatal conductance can be in short-term a sign of higher water use efficiency and thus lower response to drought stress, however, in long-term it means a severe drop in biomass productivity (Blum, 2005). In the present study, thermal infrared imaging proved that canopy temperature increases due to WD. The UV effect was, however, lower than WD effect and statistically insignificant. Under ambient precipitation, higher UV radiation led to increase in the canopy temperature in comparison with control treatment. These results indicate that UV generally stimulated stomatal closure, but this stimulation was higher under sufficient water availability. Tdiff also indicated the changes in C/N and R/S which are mostly caused by WD.

Here, almost all selected vegetation indices showed statistically significant responses to WD. This is probably because the reflectance is strongly influenced by reduction in biomass and therefore changes in pigment composition are multiplied by differences in biomass. Correlation analysis which have been conducted for spectral reflectance indices and production parameters (AB and R/S ratio) with data for each plot individually, showed the highest correlation for AB with RDVI and R/S ratio with NDVI or NDGI. Various authors reported different vegetation indices as most suitable for detection responses to drought stress. These include indices related to biomass or leaf area (NDVI, Aparicio et al., 2000), xanthophyll cycle carotenoids (PRI, Elsheery & Cao, 2008), water content (WI, Peñuelas et al., 1997) or accumulation of soxidised phenolic compounds (BPI, Peñuelas et al., 2004). Such high diversity of indices sensitive to drought stress is probably caused by different stages and severity of drought stress within individual studies. Significant changes in above-ground biomass under severe drought stress can then mask the effect on pigment composition. This is likely the case also of our study where very similar responses were observed for different vegetation indices.

**5. Conclusions**

Water deficit (WD) during the main vegetation period (May–July) changes biomass allocation between above- and below-ground parts of temperate mountain grassland. While above-ground biomass was reduced, below-ground biomass increased, which led to higher R/S ratio in response to WD. However, the hypothesis that UV radiation alleviates the negative effect of WD through enhanced below-ground biomass was not confirmed. Noticeably, WD led to reduced N uptake and increased C/N, but the interaction with UV radiation was also negligible. On contrary, we confirmed the hypothesis that vegetation indices based on thermal imaging and spectral reflectance (particularly NDVI and RDVI) can detect a reduction of above-ground biomass induced by drought conditions and consequently estimate changes in R/S ratio.

**Acknowledgements**

This research was supported by SustES – Adaptation strategies for sustainable ecosystem services and food security under adverse environmental conditions, project no. CZ.02.1.01/0.0/0.0/16\_019/0000797 and by the Ministry of Education, Youth and Sports of CR within the CzeCOS program, grant number LM2018123.

**Author´s contributions**

P.H. and K.K. conceived the ideas and designed the methodology; P.H., K.K., B.V. and K.S. collected and analysed the data; P.H., K.K. and O.U. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

**Conflict of Interest**

The authors declare that they have no conflict of interest.

**Data Availability Statement**

The data that support the findings of this study are openly available in Mendeley DOI: 10.17632/d79khnmhkf.1

**References**

# Alexieva, V., Sergiev, I., Mapelli, S., Karanov, E., 2001. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ., 24, pp. 1337–1344. <https://doi.org/10.1046/j.1365-3040.2001.00778.x>

Allen, D.J., Nogués, S., Baker, N.R., 1998. Ozone depletion and increased UV-B radiation: is there a real threat to photosynthesis. J. Exp. Bot., 49, pp.1775–1788. <https://doi.org/10.1093/jxb/49.328.1775>

Aparicio, N., Villegas, D., Casadesus, J., Araus, J.L., Royo, C., 2000. Spectral vegetation indices as nondestructive tools for determining durum wheat yield. Agron. J., 92, pp.83–91. doi:10.2134/agronj2000.92183x

Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta, D.A., Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia, 141, pp.221–235. DOI 10.1007/s00442-004-1519-1

Bakker, M.R., Augusto, L., Achat, D.L., 2006. Fine root distribution of trees and understory in mature stands of marine pine (*Pinus pinaster*) on dry and humid sites. Plant Soil, 286, pp.37–51. https://doi.org/10.1007/s11104-006-9024-4

Barnes, J.D., Balaguer, L., Manrique, E., Elvira, S., Davison, A.W., 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophylls a and b in lichens and higher plants. Environ. Exp. Bot., 32, pp.85–100. <https://doi.org/10.1016/0098-8472(92)90034-Y>

# Basu, S., Roychoudhury, A., Saha, P.P., Sengupta, D.N., 2010. Differential antioxidative responses of indica rice cultivars to drought stress. Plant Growth Regul., 60, 51. https://doi.org/10.1007/s10725-009-9418-4

## Blum, A., 2005. Osmotic adjustment is a prime drought stress adaptive engine in support of plant production. Plant Cell Environ., 40(1), pp.4–10. <https://doi.org/10.1111/pce.12800>

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Glob. Change Biol., 15(4), pp.808–824.  <https://doi.org/10.1111/j.1365-2486.2008.01681.x>

Broge, N.H., Leblanc, E., 2001. Comparing prediction power and stability of broadband and hyperspectral vegetation indices for estimation of green leaf area index and canopy chlorophyll density. Remote Sens. Environ., 76(2), pp.156–172. doi:10.1016/S0034-4257(00)00197-8.

# Brunner, I., Herzog, C., Dawes, M.A., Arend, M., Sperisen, C., 2015. How tree roots respond to drought. Front. Plant Sci., 6, 547. <https://doi.org/10.3389/fpls.2015.00547>

# Cechin, I., Corniani, N., de Fátima Fumis, T., Cataneo, A.C., 2008. Ultraviolet-B and water stress effects on growth, gas exchange and oxidative stress in sunflower plants. Radiat. Environ. Bioph., 47, pp. 405–413. https://doi.org/10.1007/s00411-008-0167-y

Dai, A., 2011. Drought under global warming: a review. WIREs Climate Change, 2(1), pp.45–65. <https://doi.org/10.1002/wcc.81>

Deckmyn, G., Impens, I., 1999. Seasonal responses of six *Poaceae* to differential levels of solar UV-B radiation. Environ. Exp. Bot., 41(2), pp.177–184. <https://doi.org/10.1016/S0098-8472(98)00061-6>

# Denton, E.M., Dietrich, J.D., Smith, M.D., Knapp, A.K., 2017. Drought timing differentially affects above- and belowground productivity in a mesic grassland. Plant Ecol., 218, 317–328. https://doi.org/10.1007/s11258-016-0690-x

Easterling, D.R., Meehl, G.A., Parmesan, C., Changnon, S.A., Karl, T.R., Mearns, L.O., 2000. Climate Extremes: Observations, Modeling, and Impacts. Science, 289, pp.2068–2074. [DOI: 10.1126/science.289.5487.2068](https://doi.org/10.1126/science.289.5487.2068)

Emberger, L., 1932. Sur une formule climatique et ses applications en botanique. La Météorologie, 92(1932), pp.423–432.

Elsheery, N.I., Cao, K.-F., 2008. Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. Acta Physiol. Plant., 30, pp.769–777. https://doi:10.1007/s11738-008-0179-x.

Fay, P.A., Kaufman, D.M., Nippert, J.B., Carlisle, J.D., Harper, C.W., 2008. Changes in grassland ecosystem function due to extreme rainfall events: implications for responses to climate change. Glob. Change Biol., 14, pp.1600–1608.  <https://doi.org/10.1111/j.1365-2486.2008.01605.x>

Frank D.A., 2007. Drought effect on above- and below-ground production of a grazed temperate grassland ecosystem. Oecologia, 152, pp.131–139. DOI 10.1007/s00442-006-0632-8

Gamon, J.A., Peñuelas, J., Field, C.B., 1992. A narrow-waveband spectral index that tracks diurnal changes in photosynthetic efficiency. Remote Sens. Environ., 41(1), pp.35–44. <https://doi.org/10.1016/0034-4257(92)90059-S>

Gitelson, A.A., Merzlyak, M.N., 1997. Remote estimation of chlorophyll content in higher plant leaves. Inter. J. Remote Sens., 18, pp.2691–2697. h[ttps://doi.org/10.1080/014311697217558](https://doi.org/10.1080/014311697217558)

# Gitz, D.C., Liu-Gitz, L., 2003. How do UV Photomorphogenic Responses Confer Water Stress Tolerance? Photochem. Photobiol., 78, pp. 529–534.  <https://doi.org/10.1562/0031-8655(2003)0780529HDUPRC2.0.CO2>

Gleeson, D.B., Müller, C., Banerjee, S., Ma, W., Siciliano, S.D., Murphy D.V., 2010. Response of ammonia soxidising archaea and bacteria to changing water filled pore space. Soil Biol. Biochem., 42, pp.1888–1891. <https://doi.org/10.1016/j.soilbio.2010.06.020>

## Grange, G., Finn, J.A., Brophy, C., 2021. Plant diversity enhanced yield and mitigated drought impacts in intensively managed grassland communities. J. Appl. Ecol., 58(9), pp.1864–1875. DOI: 10.1111/1365-2664.13894

# Grant, R.F., Black, T.A., Gaumont-Guay, D., Klujn, N., Barrc, A.G., Morgenstern, K., Nesic, Z., 2006. Net ecosystem productivity of boreal aspen forests under drought and climate change: Mathematical modelling with *Ecosys.* Agr. Forest Meteorol., 140(1–4), pp. 152–170. <https://doi.org/10.1016/j.agrformet.2006.01.012>

Haboudane, D., Miller, J.R., Tremblay, N., Zarco-Tejada, P.J., Dextraze, L., 2002. Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. Remote Sens. Environ., 81, pp.416–426. [https://](https://doi.org/10.1016/S0034-4257(02)00018-4)doi:10.1016/S0034-4257(02)00018-4

Haboudane, D., Miller, J.R., Pattey, E., Zarco-Tejada, P.J., Strachan, I.B., 2004. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. Remote Sens. Environ., 90, pp.337–352. [https://](https://doi.org/10.1016/S0034-4257(02)00018-4)doi:10.1016/j.rse.2003.12.013

Hatfield, J.L., Gitelson, A.A., Schepers, J.S., Walthall, C.L., 2008. Application of spectral remote sensing for agronomic decisions. Agron. J., 100, pp.S-117-S-131. DOI: 10.2134/agronj2006.0370c.

# Hofmann, R.W., Campbell, B.D., Bloor, S.J., Swinny, E.E., Markham, K.R., Ryan, K.G., Fountain, D.W., 2003. Responses to UV-B radiation in *Trifolium repens* L. – physiological links to plant productivity and water availability. Plant Cell Environ., 26, pp. 603–612. <https://doi.org/10.1046/j.1365-3040.2003.00996.x>

Holub, P., Fabšičová, M., Tůma, I., Záhora, J., Fiala, K., 2013. Effects of artificially varying amounts of rainfall on two semi-natural grassland types. J. Veg. Sci., 24(3), pp.518–529.  <https://doi.org/10.1111/j.1654-1103.2012.01487.x>

Hoover, D.L., Knapp, A., Smith, M.D., 2014. Resistance and resilience of a grassland ecosystem to climate extremes. Ecology, 95, pp. 2646­­­–2656.  <https://doi.org/10.1890/13-2186.1>

Chen, J.M., 1996. Evaluation of vegetation indices and a modified simple ratio for boreal applications. Can. J. Remote Sens., 22, 229–242. https://doi:10.1080/07038992.1996.10855178

# Ibañez, S., Rosa, M., Hilal, M., González, J.A., Prado, F.E., 2008. Leaves of *Citrus aurantifolia* exhibit a different sensibility to solar UV-B radiation according to development stage in relation to photosynthetic pigments and UV-B absorbing compounds production. J. Photoch. Photobio. B., 90(3), pp. 163–169. <https://doi.org/10.1016/j.jphotobiol.2008.01.002>

Ibrahim, L., Proe, M.F., Cameron, A.D., 1997. Main effects of nitrogen supply and drought stress upon whole-plant carbon allocation in poplar. Can. J. Forest Res., 27(9), pp.1413–1419. <https://doi.org/10.1139/x97-080>

IPCC, 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change, in: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S. L., Péan, C., et al. (Eds.), Cambridge University Press. In Press.

Jansen, M.A.K., Van Den Noort, R.E., 2000. Ultraviolet-B radiation induces complex alterations in stomatal behaviour. Physiol. Plantarum, 110(2), pp.189–194.  <https://doi.org/10.1034/j.1399-3054.2000.110207.x>

## Jentsch, A., Beierkuhnlein, C., 2008. Research frontiers in climate change: Effects of extreme meteorological events on ecosystems. C. R. Geosci., 340, pp. 621­–628. <https://doi.org/10.1016/j.crte.2008.07.002>

Jentsch, A., Kreyling, J., Elmer, M., Gellesh, E., Glaser, B., Grant, K., Hein, R., Lara, M., Mirzae, H., Nadler, S.E., Nagy, L., Otieno, D., …Beierkuhnlein, C., 2011. Climate extremes initiate ecosystem-regulating functions while maintaining productivity. J. Ecol., 99, pp.689–702. <https://doi.org/10.1111/j.1365-2745.2011.01817.x>

## Johnson, D., Vachon, J., Britton, A.J., Helliwell, R.C., 2011. Drought alters carbon fluxes in alpine snowbed ecosystems through contrasting impacts on graminoids and forbs. New Phytol., 190(3), pp.740–749. <https://doi.org/10.1111/j.1469-8137.2010.03613.x>

Jones, H.G., 1999. Use of infrared thermometry for estimation of stomatal conductance as a possible aid to irrigation scheduling. Agr. Forest Meteorol., 95(3), pp.139–149. <https://doi.org/10.1016/S0168-1923(99)00030-1>

Jones, H. G., Serraj, R., Loveys, B.R., Xiong, L., Wheaton, A., Price, A.H., 2009. Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Funct. Plant Biol., 36(11), pp.978–989. https://doi.org/10.1071/FP09123

Klem, K., Holub, P., Štroch, M., Nezval, J., Špunda, V., Tříska, J., Jansen, M.A.K., Robson, T.M., Urban, O., 2015. Ultraviolet and photosynthetically active radiation can both induce photoprotective capacity allowing barley to overcome high radiation stress. Plant Physiol. Biochem., 93, pp.74–83. <https://doi.org/10.1016/j.plaphy.2015.01.001>

Klem, K., Rajsnerová, P., Novotná, K., Mìša, P., Křen, J., 2014. Changes in vertical distribution of spectral reflectance within spring barley canopy as an indicator of nitrogen nutrition, canopy structure and yield parameters. Agriculture (Polnohospodárstvo), 60, pp.50–59. doi:10.2478/agri-2014-0006

Knapp, A.K., Fay, P.A., Blair, J.M., Collins, S.L., Smith, M.D., Carlisle, J.D., Harper, C.W., Danner, B.T., Lett, M.S., McCarron, J.K., 2002. Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. Science, 298, pp.2202–2205. [DOI: 10.1126/science.1076347](https://doi.org/10.1126/science.1076347)

Kreyling, J., Wenigmann, M., Beierkuhnlein, C., Jentsch, A., 2008.Effect of extreme weather events on plant productivity and tissue die-back are modified by community composition. Ecosystems, 11, pp.752–763. https://doi.org/10.1007/s10021-008-9157-9

## Kubert, A., Kuester, E., Gotz, M., Dubbert, D., Eiblmeier, M., Werner, C., Rothfuss, Y., Dubbert, M., 2021. Combined experimental drought and nitrogen loading: the role of species-dependent leaf level control of carbon and water exchange in a temperate grassland. Plant Biol., 23(3), pp.427–437. <https://doi.org/10.1111/plb.13230>

# Li, X., Zuo, X., Yue, P., Zhao, X., Hu, Y., Guo, X., Guo, A., Xu, C., Yu, Q., 2021. Drought of early time in growing season decreases community aboveground biomass, but increases belowground biomass in a desert steppe. [BMC Ecol. Evol](file:///C:\Users\urban.o\AppData\Local\Microsoft\Windows\INetCache\Content.Outlook\NRCWL40B\BMC%20Ecol.%20Evol)., 21, 106. https://doi.org/10.1186/s12862-021-01842-5

# Lu, Y.W., Duan, B.L., Zhang, X.L., Korpelainen, H., Berninger, F., Li, C.Y., 2009. Intraspecific variation in drought response of *Populus cathayana* grown under ambient and enhanced UV-B radiation. Ann. Forest Sci., 66, 613. https://doi.org/10.1051/forest/2009049

Lu, T., Wang, Y., Zhu, H., Wei, X., Shao, M., 2020. Drying-wetting cycles consistently increase net nitrogen mineralization in 25 agricultural soils across intensity and number of drying-wetting cycles. Sci. Total. Environ., 710, 135574. <https://doi.org/10.1016/j.scitotenv.2019.135574>

Malenovský, Z., Ufer, C., Lhotáková, Z., Clevers, J.G.P.W., Schaepman, M.E., Albrechtová, J., Cudlín, P., 2006. A new hyperspectral index for chlorophyll estimation of a forest canopy: Area under curve normalised to maximal band depth between 650-725 nm. EARSeL eProceedings, 5, pp.161–172.

Nogués, S., Allen, D.J., Morison, J.I.L., Baker, N.R., 1998. Ultraviolet-B Radiation Effects on Water Relations, Leaf Development, and Photosynthesis in Droughted Pea Plants. Plant Physiol., 117, pp.173–181. <https://doi.org/10.1104/pp.117.1.173>

Peñuelas, J., Gamon, J.A., Fredeen, A.L., Merino, J., Field, C.B., 1994. Reflectance indices associated with physiological changes in nitrogen- and water-limited sunflower leaves. Remote Sens. Environ., 48, pp.135–146. [https://](https://doi.org/10.1016/0034-4257(94)90136-8) doi:10.1016/0034-4257(94)90136-8.

Peñuelas, J., Baret, F., Filella, I., 1995. Semi-empirical indices to assess carotenoids/chlorophyll a ratio from leaf spectral reflectance. Photosynthetica, 31, pp.221–230.

Peñuelas, J., Pinol, J., Ogaya, R., Filella, I., 1997. Estimation of plant water concentration by the reflectance Water Index WI (R900/R970). Inter. J. Remote Sens., 18, pp. 2869–2875. https://doi:10.1080/014311697217396

Peñuelas, J., Munné‐Bosch, S., Llusià, J., Filella, I., 2004. Leaf reflectance and photo‐ and antioxidant protection in field‐grown summer‐stressed *Phillyrea angustifolia*. Optical signals of oxidative stress? New Phytol., 162, pp.115–124. https://doi:10.1046/j.1469-8137.2004.01007.x

Piñeiro, G., Perelman, S., Guerschman, J.P., Paruelo, J.M., 2008. How to evaluate models: observed vs. predicted or predicted vs. observed? Ecol. Model., 216(3–4), pp.316–322. <https://doi.org/10.1016/j.ecolmodel.2008.05.006>

Piñol, J., Filella, I., Ogaya, R., Peñuelas, J., 1998. Ground-based spectroradiometric estimation of live fine fuel moisture of Mediterranean plants. Agr. Forest Meteorol., 90, pp.173–186. https://doi:10.1016/S0168-1923(98)00053-7

Qaderi M.M., Kurepin L.V., Reid D.M., 2006. Growth and physiological responses of canola (*Brassica napus*) to three components of global climate changes: Temperature, carbon dioxide and drought. Physiol. Plantarum, 128, pp.710–721. <https://doi.org/10.1111/j.1399-3054.2006.00804.x>

Qin, X., Hong, J., Ma, X., Wang, X., 2018. Global patterns in above-ground net primary production and precipitation-use efficiency in grasslands. J. Mt. Sci., 15, pp. 1682–1692. https://doi.org/10.1007/s11629-017-4772-6

Rapantová, B., Klem, K., Holub, P., Novotná, K., Urban, O., 2016. Photosynthetic response of mountain grassland species to drought stress is affected by UV-induced accumulation of epidermal flavonols. Beskydy, 9, pp.31–40. https://doi.org/10.11118/beskyd201609010031

## Robson, T.M., Klem, K., Urban, O., Jansen, M.A.K., 2015. Re-interpreting plant morphological responses to UV-B radiation. Plant Cell Environ., 38(5), pp.856–866. <https://doi.org/10.1111/pce.12374>

Rodrigues, M.L., Pacheco, C.M.A., Chaves, M.M., 1995. Soil-plant water relation, root distribution and biomass partitioning in *Lupinus albus* L. J. Exp. Bot., 48, pp.947–959. <https://doi.org/10.1093/jxb/46.8.947>

Rondeaux, G., Steven, M., Baret, F., 1996. sOptimisation of soil-adjusted vegetation indices. Remote Sens. Environ., 55, pp.95–107. https://doi:10.1016/0034-4257(95)00186-7

Roujean, J.-L., Breon, F.-M., 1995. Estimating PAR absorbed by vegetation from bidirectional reflectance measurements. Remote Sens. Environ., 51, pp.375–384. https://doi:10.1016/0034-4257(94)00114-3

Rouse, J.W., Haas, R.H., Schell, J.A., Deering D.W., 1973. Monitoring vegetation systems in the Great Plains with ERTS, in: Third ERTS Symposium, NASA SP-351, Vol. 1, NASA, Washington, DC, pp. 309–317.

Schmidt, A.M., Ormrod, D.P., Livingston, N.J., Misra, S., 2000. The Interaction of Ultraviolet-B Radiation and Water Deficit in Two *Arabidopsis thaliana* Genotypes. Ann. Bot., 85, pp.571–575. <https://doi.org/10.1006/anbo.1999.1085>

# Singh, S., Agrawal, S.B., Agrawal, M., 2015. Responses of pea plants to elevated UV-B radiation at varying nutrient levels: N-metabolism, carbohydrate pool, total phenolics and yield. Funct. Plant Biol., 42(11), pp. 1045–1056. https://doi.org/10.1071/FP15003

Smith, R.C.G., Adams, J., Stephens, D.J., Hick, P.T., 1995. Forecasting wheat yield in a Mediterranean-type environment from the NOAA satellite. Crop Pasture Sci., 46(1), pp.113–125. https://doi:10.1071/AR9950113.

Sullivan, J.H., Teramura, A.H., 1990. Field Study of the Interaction between Solar Ultraviolet-B Radiation and Drought on Photosynthesis and Growth in Soybean. Plant Physiol., 92, pp.141–146. <https://doi.org/10.1104/pp.92.1.141>

Team, R Core, 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [www.R-project.org/](http://www.r-project.org/)

# Tian, X.R., Lei, Y.B., 2007. Physiological responses of wheat seedlings to drought and UV-B radiation. Effect of exogenous sodium nitroprusside application. Russ. J. Plant Physiol., 54, pp. 676–682. <https://doi.org/10.1134/S1021443707050160>

Walter, J., Nagy, L., Hein, R., Rascher, U., Beierkuhnlein, C., Willner, E., Jentsch, A., 2011. Do plants remember drought? Hints towards a drought-memory in grasses. Environ. Exp. Bot., 71, pp.34–40. <https://doi.org/10.1016/j.envexpbot.2010.10.020>

Wilcox, K.R., Shi, Z., Gherardi, L.A., Lemoine, N.P., Koerner, S.P., Hoover, D.L., Bork, E., Byrne, K.M., …. Luo, Y., 2017. Asymmetric responses of primary productivity to precipitation extremes: A synthesis of grassland precipitation manipulation experiments. Glob. Change Biol., 23(10), pp.4376–4385. <https://doi.org/10.1111/gcb.13706>

Xiang, S.R., Doyle, A., Holden, P.A., Schimel, J.P., 2008. Drying and rewetting effects on C and N mineralization and microbial activity in surface and subsurface California grassland soils. Soil Biol. Biochem., 40(9), pp.2281–2289. <https://doi.org/10.1016/j.soilbio.2008.05.004>

Xu, X., Niu, S., Sherry, R.A., Zhou, X., Zhou, J., 2012. Interannual variability in responses of belowground net primary productivity (NPP) and NPP partitioning to long-term warming and clipping in a tallgrass prairie. Glob. Change Biol., 18, pp.1648–1656.  <https://doi.org/10.1111/j.1365-2486.2012.02651.x>

Yahdjian, L., Sala, O.E., 2006. Vegetation structure constrains primary production response to water availability in the Patagonian steppe. Ecology, 87, pp.952–962. [https://doi.org/10.1890/0012-9658(2006)87[952:VSCPPR]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87%5b952:VSCPPR%5d2.0.CO;2)

Zarco-Tejada, P.J., Miller, J.R., Noland, T.L., Mohammed, G.H., Sampson, P.H., 2001. Scaling-up and model inversion methods with narrowband optical indices for chlorophyll content estimation in closed forest canopies with hyperspectral data. IEEE Transactions on Geoscience and Remote Sensing, 39, pp.1491–1507. doi:10.1109/36.934080

Zhang, C., Chen, M., Liu, G., Huang, G., Wang, Y., Yang, S., Xu, X., 2020. Enhanced UV-B radiation aggravates negative effects more in females than in males of Morus alba saplings under grought stress. Environ. Exp. Bot., 169, 103903. <https://doi.org/10.1016/j.envexpbot.2019.103903>

Zhang, F., Quan, Q., Song, B., Sun, J., Chen, Y., Zhou, Q., Niu, S., 2017. Net primary productivity and its partitioning in response to precipitation gradient in an alpine meadow. Sci. Rep., 7, 15193. <https://doi.org/10.1038/s41598-017-15580-6>

**Figure 1.** Dynamics of daily mean soil moisture in ambient (C; solid line) and water deficit (WD; dash line) treatments over three experimental seasons 2012–2014. Grey background indicates the induced drought period.

**Figure 2.** The effect of water deficit (WD), UV radiation (UV), and combined effect WD+UV on above-ground biomass in comparison with control (C) in mountain grassland during years 2012–2014. Means (bars) and standard deviations (error bars) are presented (n = 3). Different letters denote statistically significant differences between treatments using Fisher LSD post-hoc test (p ≤ 0.05).

**Figure 3.** The effect of water deficit (WD), UV radiation (UV), and combined effect WD+UV on below-ground biomass (A), R/S ratio (B), and total biomass (C) in comparison with control (C) in mountain grassland during years 2012–2014. Means (bars) and standard deviations (error bars) are presented (n = 3). Different letters denote statistically significant differences between treatments using Fisher LSD post-hoc test (p ≤ 0.05).

**Figure 4.** The effect of water deficit (WD), UV radiation (UV), and combined effect WD+UV on relative allocation of biomass in comparison with control (C) in mountain grassland in 2012–2014. Means (bars) and standard deviations (error bars) are presented (n = 3). Different letters denote statistically significant differences between treatments using Fisher LSD post-hoc test (p ≤ 0.05). AB – above-ground biomass, BB – below-ground biomass.

**Figure 5.** The effect of water deficit (WD), UV radiation (UV), and combined effect of WD+UV on nitrogen uptake per area (A) and C:N ratio (B) in above-ground biomass of mountain grassland in comparison with control (C) during years 2012–2014. Means (bars) and standard deviations (error bars) are presented (n = 3). Different letters denote statistically significant differences between treatments using Fisher’s LSD post-hoc test (p ≤ 0.05).

**Figure 6.** Relationship between observed (Measured effect) and calculated (Predicted effect) combined effect of WD and UV radiation for production parameters (AB – above-ground biomass, BB – below-ground biomass, TB – total biomass, root:shoot – R/S ratio) and nutrient characteristics measured in AB. The predicted effect was calculated as the sum of individual WD and UV effects. The data were fitted using linear regression (best linear fit). Coefficients of determination (r) and significance levels (ns - p > 0.05) are shown. Root Mean Square Error (RMSE) was calculated for the 1:1 line (red). Grey areas indicate more than additive or synergistic effects while white areas indicate less than additive or antagonistic effects.

**Figure 7.** Principal component analysis (PCA) results for effects of precipitation (Prec), soil moisture (SM), aridity Index (AI), water balance (WB), and UV radiation (UV-A, UV-B) on biomass parameters (AB – above-ground biomass; BB – below-ground biomass, TB – total biomass; R.S – root:shoot ratio), nutrient parameters (Nconc – nitrogen (N) concentration in AB; Cconc – carbon (C) concentration in AB, Nupt – N uptake in AB per area, Cupt – C uptake in AB per area, C.N – C:N ratio in AB), spectral reflectance parameters (ANMB – ANMB650-725, NDVI, RDVI, MCARI1, OSAVI, SIPI), and thermal characteristics (Tdiff – Tdiff) in mountain grassland during 2012–2014.

**Figure 8.** The relationships between the R/S ratio (A) or C/N ratio (B) and water balance in the mountain grassland during 2012–2014. The linear functions were fitted separately for both UV radiation treatments. Points represent individual replicates. Coefficients of determination (r) and significance levels (\*\*\*p ≤ 0.001; \*\*p ≤ 0.01) are shown.

**Figure 9.** The relationships between selected production (above-ground biomass – AB and R/S ratio) or nutrient (C:N ratio) parameters and infrared thermography (Tdiff) and selected vegetation indices (NDVI and RDVI) in the mountain grassland during 2012–2014. The linear function was fitted together for both UV radiation treatments. Points represent individual replicates. Coefficients of determination (r) and significance levels (\*\*\*p ≤ 0.001; \*\*p ≤ 0.01) are shown.

**Table 1.** Amount of precipitation (P; mm), aridity index (AI), ratio between precipitation and potential evapotranspiration (P/PET) in control and WD treatment, UV-A (kJ m-2) and UV-B (kJ m-2) in control and UV treatment during vegetation season (from April to measurement day - MD). IDP – Induced dry period

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Year | 2012 | | 2013 | | 2014 | |
| Treatment | control | WD | control | WD | control | WD |
| IDP | 10 May–10 July | | 15 May–3 July | | 21 May–3 July | |
| MD | July 10 | July 10 | July 3 | July 3 | July 3 | July 3 |
| P | 279 | 67 | 279 | 135 | 431 | 175 |
| AI | 1520 | 342 | 1645 | 796 | 3340 | 1357 |
| P/PET | 1.04 | 0.23 | 1.50 | 0.73 | 2.03 | 0.82 |
| Treatment | control | UV | control | UV | control | UV |
| UV-A | 93.4 | 402.7 | 93.6 | 320.5 | 81.9 | 340.0 |
| UV-B | 4.5 | 25.9 | 3.3 | 15.6 | 3.8 | 25.7 |

**Table 2.** Effects water deficit (WD), UV radiation (UV), year (Y) and their interactions on production parameters (above-ground biomass - AB, below-ground biomass - BB, root/shoot ratio - R/S, total biomass - TB) in mountain grassland in 2012–2014. F-values of three-way analysis of variance (ANOVA); ns - not significant, \*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Effects | *df* | AB | BB | R/S | TB |
| WD | 1 | 13.3\*\*\* | 10.5\*\* | 15.2\*\*\* | 5.3\* |
| UV | 1 | 0.2 ns | 5.5\* | 4.8\* | 6.0\* |
| Y | 2 | 34.2\*\*\* | 0.1 ns | 6.8\*\* | 2.4 ns |
| WD x UV | 1 | 2.0 ns | 1.1 ns | 1.7 ns | 0.4 ns |
| Y x WD | 2 | 3.3 ns | 1.7 ns | 1.8 ns | 1.1 ns |
| Y x UV | 2 | 1.9 ns | 2.2 ns | 0.5 ns | 3.3 ns |
| Y x WD x UV | 2 | 2.5 ns | 3.0 ns | 1.9 ns | 1.9 ns |

**Table 3.** Effects of water deficit (WD), UV radiation (UV), year (Y) and their interactions on above-ground N concentration (N%), above-ground C concentration (C%), N uptake in above-ground biomass per area, and C:N ratio in mountain grassland in 2012–2014. F-values of three-way analysis of variance (ANOVA); ns - not significant, \*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Effects | *df* | N% | C% | N uptake | C:N |
| WD | 1 | 14.5\*\*\* | 0.0 ns | 21.0\*\*\* | 21.2\*\*\* |
| UV | 1 | 1.3 ns | 0.0 ns | 0.0 ns | 2.8 ns |
| Y | 2 | 34.0\*\*\* | 14.3\*\*\* | 56.9\*\*\* | 46.3\*\*\* |
| WD x UV | 1 | 1.5 ns | 1.9 ns | 0.2 ns | 0.6 ns |
| Y x WD | 2 | 0.0 ns | 0.1 ns | 2.1 ns | 1.7 ns |
| Y x UV | 2 | 0.3 ns | 1.9 ns | 2.8 ns | 0.7 ns |
| Y x WD x UV | 2 | 1.2 ns | 0.0 ns | 2.3 ns | 0.6 ns |