doi:10.20944/preprints201704.0007.v1

Article

THE IMPACT OF TOURIST TRAFFIC ON THE CONDITION AND CELL STRUCTURES OF ALPINE SWARDS

Marlena Kycko [1,*], Bogdan Zagajewski [1], Elżbieta Romanowska [2], Magdalena Zwijacz-Kozica [3], Samantha Lavender [4]

[1] University of Warsaw, Faculty of Geography and Regional Studies, Department of Geoinformatics, Cartography and Remote Sensing, Warsaw, Poland; e-mails: marlenakycko@uw.edu.pl; bogdan@uw.edu.pl
[2] University of Warsaw, Faculty of Biology, Department of Molecular Plant Physiology, Warsaw, Poland; e-mail: romanela@biol.uw.edu.pl
[3] The Tatra National Park, Zakopane, Poland; e-mail: mzwijacz@tpn.pl
[4] Pixalytics Ltd, Plymouth, UK, e-mail: slavender@pixalytics.com
* Correspondence: marlenakycko@uw.edu.pl; Tel.: +48225521507

Abstract: This research focuses on the effect of trampling on vegetation in high-mountain ecosystems through the electromagnetic spectrum's interaction with plant pigments, cell structure, water content and other substances that have a direct impact on leaf properties. The most heavily visited part of the High Tatras in Poland was divided into polygons and, after selecting the dominant species within alpine swards, a detailed analysis of trampled and reference patterns was performed. An ASD FieldSpec 3 was used to acquire high-resolution spectral properties of plants, their fluorescence and the leaf chlorophyll content with the ts-ta temperature index and fraction of accumulated radiation in the range of photosynthesis (fAPAR) used as reference data. The results show that, along tourist trails, vegetation adapts to trampling with the impact depending on the species. A lower chlorophyll value was confirmed by a decrease in fluorescence, and the state of cellular structures was degraded in trampled compared to reference species, with a lower leaf reflectance. Also, at the extreme, trampling can eliminate certain species such as *Luzula alpino-pilosa*.

Keywords: High Tatras; trampling; spectroscopy; vegetation indices; fluorescence; *Juncus trifidus; Agrostis rupestris; Luzula alpino-pilosa; Oreochloa disticha; Festuca picta*

---

1. Introduction

Low intensity anthropopression may increase plant resistance and defensive physiological adaptations, and at higher intensities it damages fragile biosphere cover. Mountain plants have developed specific adaptations to survive at the fringe of life (pigment content, plant tissue structure, leaf wax covers, etc.) that have a direct impact on reflectance, which can be quantified using hyperspectral techniques.

Remote sensing methods allow identification of plants and vegetation communities in mountains [1,2] and mapping or monitoring of vegetation can be carried out using various sensors [3-5]. Non-invasive remote sensing methods may be accomplished by assessing the spectral reflectance curve, fluorescence and vegetation indicators [6]. The spectral reflectance curve depends on how the substances, which make up the object, absorb and scatter radiation originating from different parts of the electromagnetic spectrum [7]; the reflectance depends on the state of cell structure, chlorophyll content and the content of other parameters such as quantity of water in the

 doi:10.20944/preprints201704.0007.v1

cell [8]. Exploring the mechanism(s) of stress resistance within a plant's assimilation apparatus will therefore allow the identification of stressed species [9-11].

Mountain vegetation grows under adverse environmental conditions, which leads to the development of specific adaptations [12]. The vulnerability of vegetation to trampling damage is expressed by three indices: resistance (ability of vegetation to resist change when trampled), resilience (ability of vegetation to recover following the cessation of trampling) and tolerance (ability of vegetation to tolerate a cycle of disturbance and recovery) [13]. Reductions in cover or abundance of vascular plants have repeatedly been shown to be a response to trampling, and several studies show decreases in shrub cover [14-19]. Several mechanical traits, including leaf toughness, root strength and stem flexibility have also been observed to play a key role [20-25]. Studies have also showed that even trampling-resistant species that grow well, and some which are very sensitive [26-27], are constrained by the packed earth of trampled land that's a barrier to germination and thus plant regeneration [28]; soil bulk density of trampled and untrampled soils increases with increasing exposure [29].

An application on Cadillac Mountain [30] used single-spectral high-resolution remote sensing datasets captured in 1979, 2001, and 2007, and applied pre-classification change detection analysis techniques in order to measure temporal fractional vegetation cover changes. The overall study suggested a trend in the desired direction for the site, and visitor management strategies were designed to reduce vegetation impact and enhance vegetation recovery. However, the recovery since 2000 was rather minimal and did not reach the level of cover observed during 1979. This may be because summit-wide vegetation changes were detected rather than changes along specific trails, at specific recreation sites or at the plant-growth form level. Therefore, the use of remote sensing techniques and fluorimetry can provide an accurate and precise determination of changes in trampled alpine swards with spectrometric measurements and remote sensing vegetation indices allowing information to be transferred from the ground to satellite level. However, both spectral and spatial resolution are key if the remote monitoring of alpine swards is going to provide a full understanding of the processes.

The Tatra environment is constantly monitored both by the employees of the TPN (Tatra National Park) and by scientists from research centres across Poland [31-34]. Vegetation was investigated around the Kasprowy Wierch mountain, with research focused on changes caused by tourism [35]. Past analyses have shown that processes of erosion and denudation are present on and around the trails, in addition to changes in plant cover – plants in the vicinity of the trail are beaten i.e., they are indicators of anthropopressure. Plants resistant to trampling included *Agrostis rupestris* and *Festuca airoides* [36] that grow in sward habitats near the trails; the synanthropic species *Plantago major* and *Urtica dioica* [35] were also present.

In her study, Jakomulska [37] used laboratory testing methods, based on plant physiology, to mark photosynthetically active pigment content, measure transpiration and estimate the water content. The diversified construction of each of the studied species, and its adaptation, are reflected in the results of spectrometric, bio-radiometric and fluorescence measurements. The highest content of carotenoids (28% of the pigment in the leaves of a species) was found for *Juncus trifidus*, compared with the species *Luzula spadicea* that had an 18% share of carotenoids in its leaves. *Juncus trifidus* is found on the top shelf of rock, and exposed to excessive sunlight, so had high carotenoid values that exceeded the optimum range; for example, the Carotenoid Reflectance Index 2 (CRI2) had a value of more than 12.75 compared to the optimal range of 1–12. The chlorophyll content of *Juncus trifidus* is from 0.5 to 0.9 mg per 1 g dry weight. In the late stage of development, the chlorophyll value decreases relative to carotenoids; the chlorophyll-to-carotenoid ratio in the middle stage of development is 3.9:1, while in the late stage of development it is 2.6:1. The greatest amount of chlorophyll (1.6–2.5 mg per 1 g dry weight) was recorded for *Luzula spadicea*. In contrast, tissue hydration for *Luzula spadicea* (78.9% water in the tissues) is higher than that for *Juncus trifidus* (71.3% water in the tissues) [37].

Measurement of chlorophyll fluorescence provides a useful probe of photosynthetic performance *in vivo* and the extent to which performance is limited by photochemical and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

non-photochemical processes. Coupling fluorescence measurements with other non-invasive techniques such as gas exchange, thermal imaging and absorption spectroscopy can provide insights into stress conditions before visible external symptoms such as wilting, necrosis and chlorosis are present [38-40]. The use of fluorescence determines the actual damage of cellular structures, which is reflected in the amounts of light-related reaction products in the plant [9,41]. The parameters that determine the photosynthetic efficiency and state of photosynthetic apparatus are the maximum quantum efficiency of photosystem II (PSII) photochemistry (Fv/Fm where Fm, maximal fluorescence; Fv, variable fluorescence equals Fm-Fo; Fo, minimal fluorescence) and t½ (half rise time from Fo to Fm, which indicates the size of antenna systems). The excitation energy transfer between the chlorophyll compounds reach about 100%, while between chlorophyll and carotenoid it is much smaller [42]. Fv/Fm describes the photochemical efficiency of PSII, i.e. the structure most sensitive to stress factors. Stress induced decreases in stomatal conductance, carbon metabolism, and transport processes can all decrease PSII efficiency because the quantum yield for PSII fluorescence depends fundamentally on the use and dissipation of excitation energy [43]. Fv/Fm represents the maximum potential quantum efficiency of PSII if all capable reaction centers were opened, and so a value from 0.79 to 0.84 is the optimal value for many plant species, with lower values indicating plant stress [44,45]. In plants adapted to the darkness, the electron transport chain is not active; whereas in the light, all chlorophyll molecules are fully activated and transfers light energy to a reaction center. Therefore both Fv/Fm and t½ may be measured when the vegetation is adapted to darkness or measured without adaptation to darkness, when the names are changed to Fv'/Fm' and t½' [46].

The aim of this study was to find suitable methods for indicating the impact of trampling on alpine swards. We wanted to answer the question, what kind of changes occur in plants subjected to trampling, when species are in natural environment? Which parts of the electromagnetic spectrum show the greatest changes during trampling? Do these changes affect pigment contents and cellular structures or do they occur in the morphology of the plant, and will be also visible in the function of the photosynthetic apparatus? In addition, the selected major species of alpine swards were evaluated for resistance to trampling.

2. Study area and research objects

The Tatra test site is located within the Man & Biosphere Reserve, encompassing alpine and subalpine zones of the Polish and Slovak Tatra National Park (TPN and TANAP). The area extends across the rectangle: 49°10′30′′ to 49°16′00′′N and 19°45′30′′ to 20°07′30′′E, covering approximately 110 km^2. The study area is located at an altitude range of 1500–2300 m above sea level (a.s.l). Due to technical limitations, only the most visited the Czerwone Wierchy mountain (in the Western Tatras) of the Polish Tatra Mountains was investigated.

The core of the massif is made up of sedimentary rocks (limestone and dolomites of the Middle Triassic), and the tops are covered with a mantle of crystalline rocks (granites and gneisses). The Czerwone Wierchy name comes from the red-brown color of the slopes, which comes from a plant called *Juncus trifidus* that fades to red in the autumn; and often as early as mid-summer. The altitude also results in the occurrence of *Oreochloa disticha* and *Festuca picta*. Due to the varied geological substrate very diverse vegetation is present, e.g. both the aforementioned plants that grow on calciphilous and acidophilic soil. Therefore, alpine swards are an excellent example of communities susceptible to change [47]. The research conducted so far [46] has revealed that species react differently to stress factors, depending on their morphology and the type of the cellular structures.

The study was conducted in August 2013, in the Czerwone Wierchy massif. On the basis of experience and previously conducted research, species were selected to provide the greatest percentage of high-altitude communities [48]. After consultation with field scientists of phytosociology, and selection with a map of the main vegetation species in this area, the following species were selected: *Juncus trifidus*, *Agrostis rupestris*, *Luzula alpino-pilosa*, *Oreochloa disticha*, and *Festuca picta*. Trails are frequently visited by tourists, as evidenced by large-scale destruction by the tourist infrastructure (Figure 1). In 2014, TPN began revitalization of the area and reconstruction of routes and their surrounding areas in order to reduce the impact of tourist traffic both in activating erosion processes and in trampling vegetation, and also in order to enable regeneration of damaged vegetation (Project co-financed by the European Union under the European Regional Development Fund nr POIS.05.01.00-00-398/12 Tatra National Park – Reduction of the tourist pressure on habitats and species in the area of NATURA 2000 PLC 120001 Tatra).

Figure 1. Photo of the trail running from the mountain pass under the Kopa Kondracka

3. Materials and Methods

In the study area an ASD FieldSpec 3 spectrometer with ASD Plant Probe was used, operating in the range 350–2500 nm. Measurements were performed for selected species on test sites within 5 of the trails (for species threatened by trampling) and reference measurements were taken (more than 5 m from the trail, where no trampling effects were observed); the distance was dependent on the intensity of hiking and mountain slopes. Each of the 16 polygons covers all investigated species (*Juncus trifidus, Agrostis rupestris, Luzula alpino-pilosa, Oreochloa disticha, Festuca picta*), which were examined 250 times at every training site (Fig. 2). Additionally, bio-radiometric measurements were performed for:

- the fraction of accumulated radiation in the range of photosynthesis – Absorbed Photosynthetically Active Radiation (APAR) [49] that is the total radiation used by the vegetation for photosynthesis, measured using an AccuPAR linear ceptometer;
- the temperature index ts-ta, is the difference between ts – the plant surface temperature, and ta – the air temperature, and defines evapotranspiration and water stress (pyrometer iRtecMiniRay);
- chlorophyll content in leaves – Chlorophyll Content Meter (CCM-200);
- chlorophyll fluorescence;
- all polygons were located by a Trimble GeoXT GPS.

In all polygons fluorescence measurements were performed on all sward species, using a Plant Stress Meter (PSM Mark II) fluorometer for analysis of the actual state of the photosynthetic apparatus; fluorescence measurements were made on the leaves adapted to darkness (Fv/Fm) and in the light period (Fv'/Fm'); in addition, the half-time (t½) was measured.

The results of the spectrometric, bio-radiometric and fluorescence measurements were compiled. The spectral curves for each alpine sward species were analysed using ANOVA, with the spectral characteristics of trampled and reference vegetation used to verify that the spectral range shows the biggest difference in pigments, cellular structures or water content. The result was the electromagnetic spectrum band which, at the assumed significance level of 0.05, represented the impact of trampling on the vegetation and presented the specific characteristics and parameters that are changed in the studied species due to this stress factor.

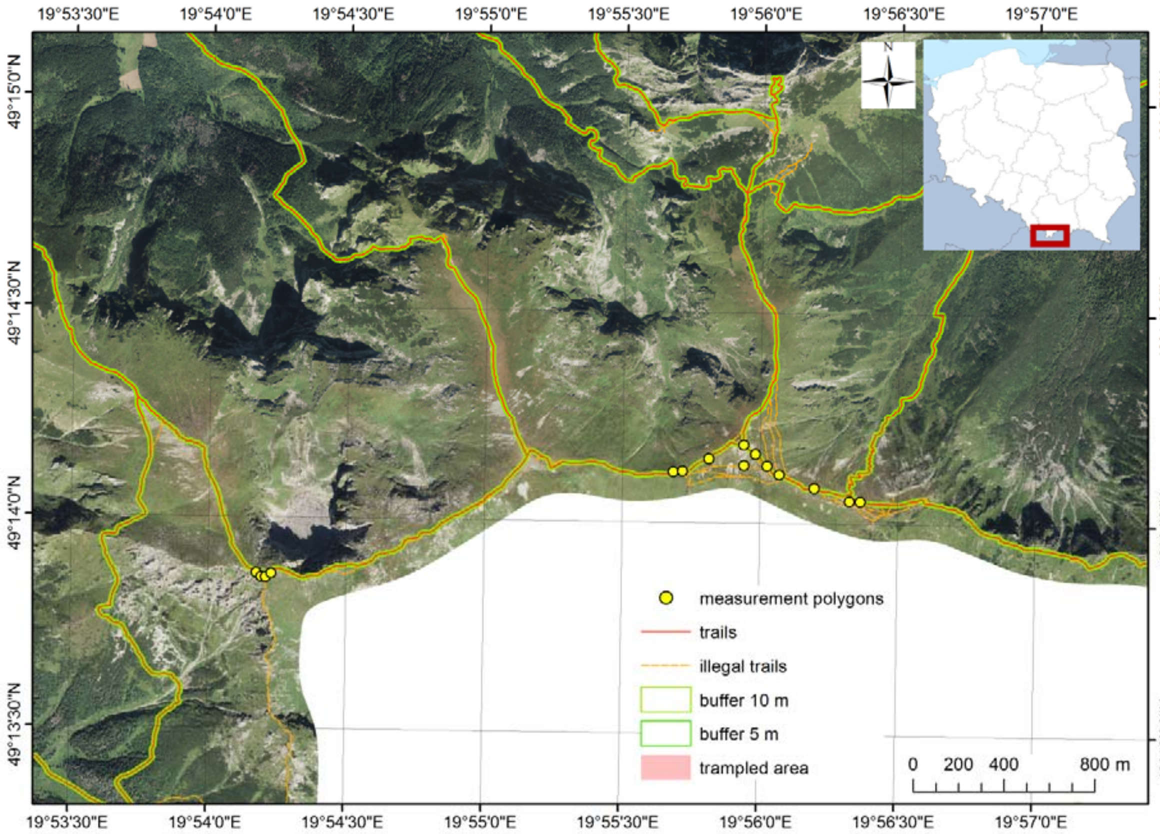


Figure 2 – Test sites in the Czerwone Wierchy research area

On the basis of the spectral characteristics of the tested species', vegetation indices were calculated; mathematical relationships between spectral reflectance values in narrow, well-defined ranges allow more accurate analysis of vegetation parameters. Selected statistical tests were performed to: check if the data distribution was normal, Shapiro–Wilk test; to check equality of variance, Levene's test; identify changes between trampled vegetation and reference vegetation, ANOVA analysis of variance or Kruskal–Wallis one-way analysis of variance; check spectrometric measurements' correlation with bio-radiometry and fluorescence using Pearson or Spearman tests for data correlation. All measurements of vegetation indices had a nonparametric distribution, and therefore, in order to find statistically significant ($p<0.05$ level) indicators showing the differences between the trampled and the reference plant, Kruskal–Wallis one-way analysis of variance was used. Based on previous research [46,48] the choice of indicators was also based on statistically significant ranges of the spectrum for the analyzed species. Then, remote sensing vegetation indices were selected and calculated; designed to provide a measure of the overall quantity and quality of the photosynthetic material in plants, which is necessary for understanding the state of vegetation:

- Wide Dynamic Range Vegetation Index (WDRVI) [50], Soil-Adjusted Vegetation Index (SAVI) [51], Green Normalized Difference Vegetation Index (Green NDVI) [52], Greenness Index (G) [53], Red Edge Position Index (REPI) [54],
- Modified Normalized Difference Vegetation Index 705 (mNDVI705) [55], Transformed Chlorophyll Absorption Reflectance Index (TCARI) [56], Modified Chlorophyll Absorption Ratio Index (MCARI) [57], Normalized Pigment Chlorophyll Index (NPCI) [58], Simple Ratio Pigment Index (SRPI) [59], Normalized Phaeophytinization Index (NPQI) [60],

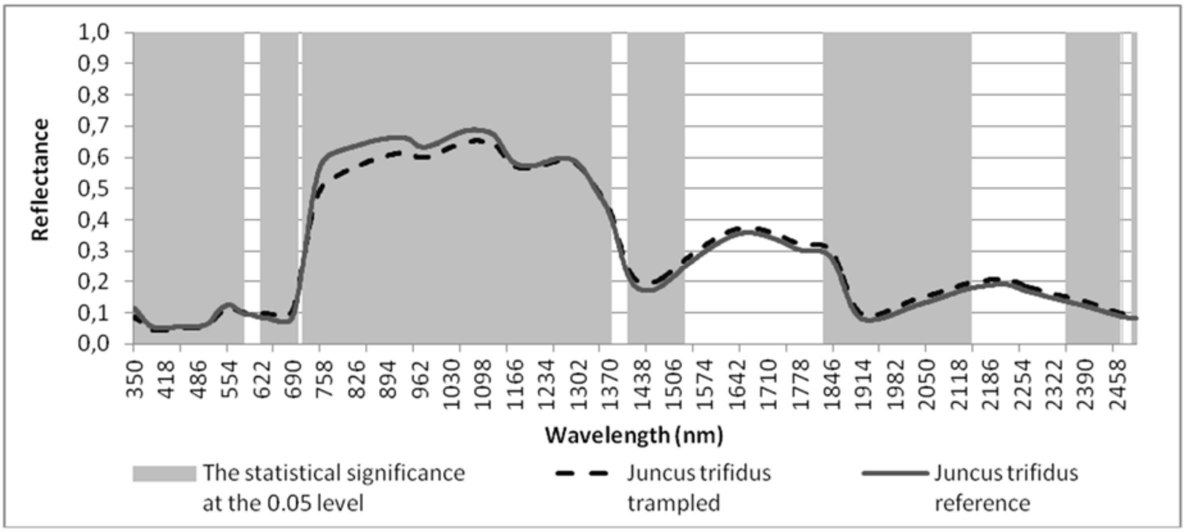
 doi:10.20944/preprints201704.0007.v1

- Photochemical Reflectance Index (PRI) [61], Structure Insensitive Pigment Index (SIPI) [59], Xanthophyll Epoxidation State (XES) [62],
- Normalized Difference Nitrogen Index (NDNI) [63],
- Normalized Difference Lignin Index (NDLI) [63],
- Plant Senescence Reflectance Index (PSRI) [64], Cellulose Absorption Index (CAI) [65]
- Carotenoid Reflectance Index 2 (CRI 2) [66], Carotenoid Reflectance Index 2 (CRI2) [66], Anthocyanin Reflectance Index 1 (ARI 1) [67], Anthocyanin Reflectance Index 2 (ARI 2) [67],
- Moisture Stress Index (MSI) [68], Normalized Difference Infrared Index (NDII) [69], Water Band Index (WBI) [58], Normalized Difference Water Index (NDWI) [70].

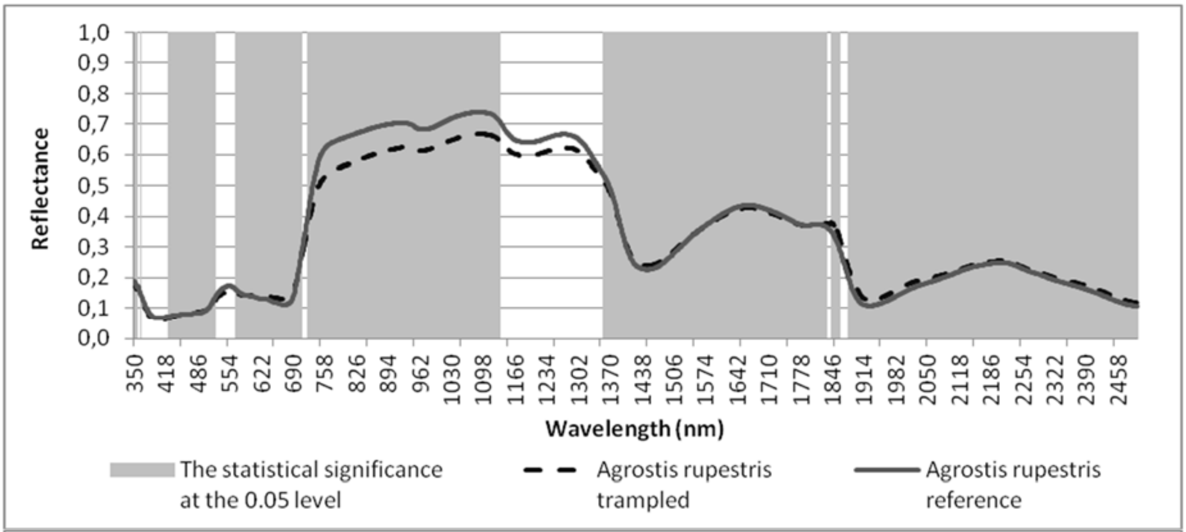
The next step was statistical analysis (Kruskal–Wallis one-way analysis of variance) of these remote sensing vegetation indices, to see which indices show statistically significant changes (level $p<0.05$) in reaction to trampling compared to reference buffer measurements for all investigated species. Then, in cases of statistical significance (at the level $p<0.05$) for changes caused by trampling, parameters were analysed using bio-radiometric measurements and fluorescence. For this purpose the remote sensing vegetation indices (statistically significant at the $p<0.05$ level) were correlated (using Spearman correlation because the data don't have normal distribution) with bio-radiometric indices. This correlation was used to verify the same information obtained from the spectrometer. Meanwhile, the Spearman correlation between remote sensing vegetation indices and measurements of fluorescence (Fv/Fm and t½; Fv'/Fm' and t½') was used to verify that changes are genuinely caused by trampling and relate to cell damage and disorders in the photosynthetic process.

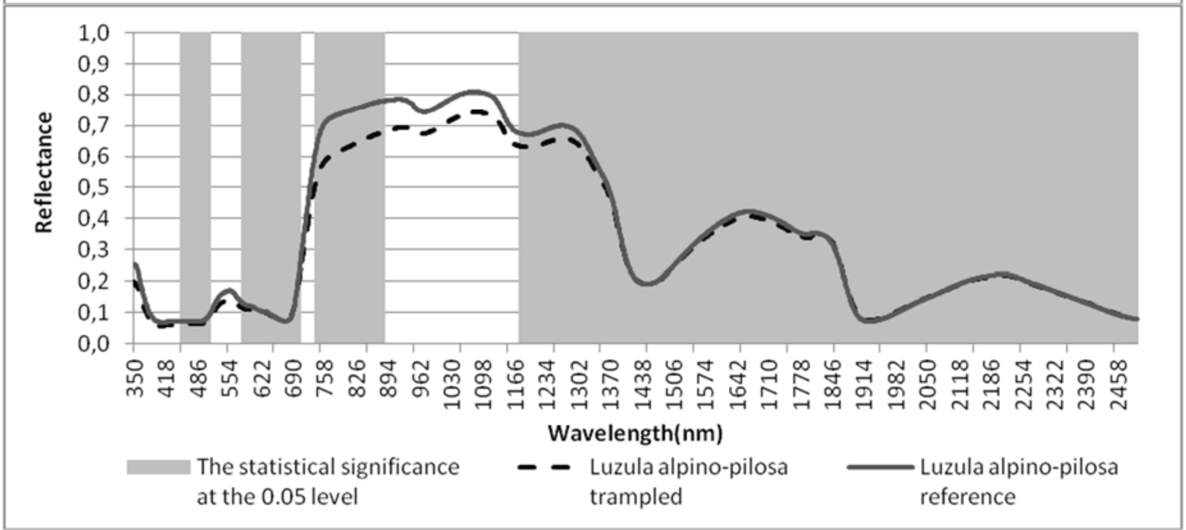
4. Results

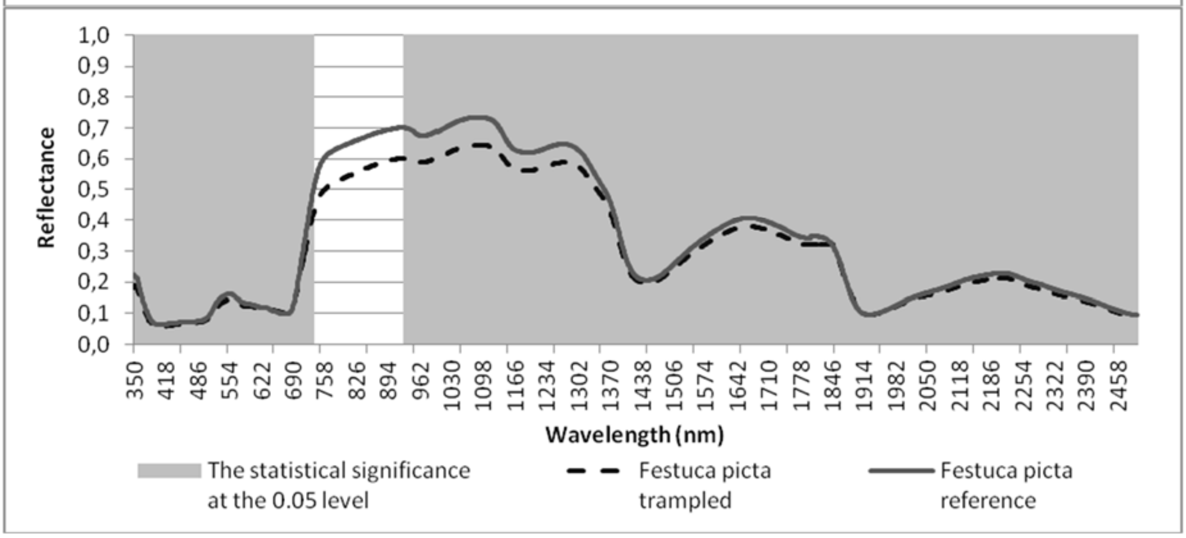
After analyzing statistical tests, all the species studied showed statistically significant changes at the 0.05 level (Fig. 3–4). Confirmation of changes between trampled vegetation and the reference vegetation is visible in both wavelength ranges corresponding to amount of pigments (especially chlorophyll), cellular structures (red edge), and the narrow ranges describing the contents of water (Fig. 3–4). All species showed different ranges of the electromagnetic spectrum as important, when looking for the signature of trampled vegetation, but there is also a common spectral range that is related to the chlorophyll and water content in the vegetation (Fig. 4); additionally, uncovered soil causes increased transpiration and dryness of vegetation around the trail, as evidenced by the decrease in water content for the species in the buffer. Overall, these changes describe the status of vegetation translated across in the remote sensing vegetation indices.



Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1







Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

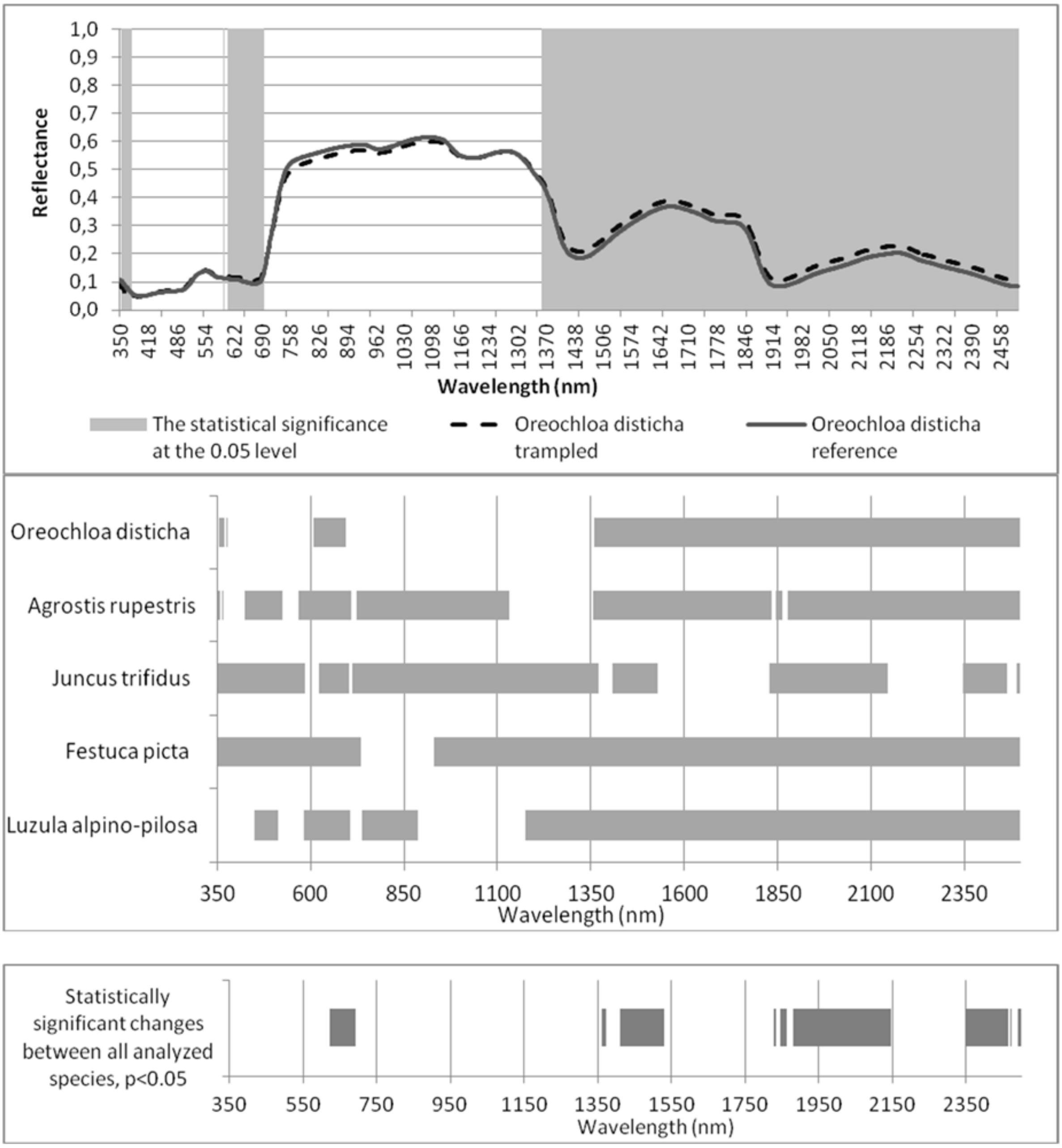


Figure 4 – Effect of the trampling on plant parameters measured in different ranges of the electromagnetic spectrum (350 - 2500 nm). Statistical significance is at a level of 0.05.

To confirm the variation in stress status and contents of individual parameters (e.g., chlorophyll, water) remote sensing indices from the spectral curve were calculated and combined into groups that described specific features, i.e. condition and structure, chlorophyll content, nitrogen content, the amount of light used in the process of photosynthesis, and the water content. For the studied species, calculated values are significantly different (Tab. 1). WDRVI shows a statistically significant decline in the value for trampled plants in the range of 0.03–0.07. There was also a decrease in chlorophyll content, e.g. NPCI decreases by approximately 0.03–0.14. The nitrogen content varies between buffers by approximately 0.01–0.04. The amount of water also decreases, as shown by MSI and NDII, whose value for the trampled species decreases by approximately 0.05–0.11. There are also statistically significant changes that depend on the species; different reactions to trampling.

 doi:10.20944/preprints201704.0007.v1

Table 1 – Remote sensing vegetation indices for the species showing statistically significant changes at the level of 0.05 (AR – *Agrostis rupestris*; LAP – *Luzula alpino-pilosa*; JT – *Juncus trifidus*; FP – *Festuca picta*; OD – *Oreochloa disticha*; T – trampled; R – reference; * – statistical significance at the 0.05 level).

	AR_R	AR_T	LAP_R	LAP_T	JT_R	JT_T	FP_R	FP_T	OD_R	OD_T
mNDVI 705	0.52*	0.47*	0.58*	0.53*	0.61*	0.50*	0.52*	0.47*	0.50	0.49
PRI	-0.03*	-0.04*	-0.02*	-0.03*	-0.03*	-0.08*	-0.03	-0.04	-0.03	-0.04
SIPI	1.08*	1.14*	1.01*	1.04*	1.04*	1.11*	1.07*	1.10*	1.08*	1.11*
NDLI	0.06*	0.05*	0.06	0.06	0.05*	0.05*	0.06*	0.06*	0.05	0.05
PSRI	0.05*	0.08*	0.00*	0.03*	0.03*	0.08*	0.04*	0.06*	0.05*	0.06*
CRI 1	5.25*	3.94*	8.15*	5.80*	7.33*	8.96*	6.43*	4.02*	5.37	4.76
CRI 2	6.20*	5.15*	9.35	7.12	8.26*	12.05*	7.41*	5.20*	6.80	6.07
ARI1	0.95*	1.21*	1.21*	1.32*	0.92*	3.09*	0.97*	1.17*	1.43	1.31
ARI 2	0.50*	0.69*	0.48*	0.74*	0.55*	1.42*	0.52*	0.64*	0.73	0.68
MSI	0.60*	0.69*	0.51*	0.59*	0.51*	0.62*	0.57*	0.63*	0.59*	0.66*
NDII	0.21*	0.15*	0.28*	0.22*	0.28*	0.20*	0.23*	0.18*	0.21*	0.16*
WBI	1.02*	1.00*	1.04*	1.02*	1.04*	1.01*	1.02*	1.01*	1.02*	1.01*
NDWI	0.02*	-0.01*	0.04*	0.01*	0.05*	0.00*	0.02*	0.00*	0.01*	0.00*
SAVI	0.62*	0.55*	0.72*	0.67*	0.68*	0.60*	0.62*	0.58*	0.59*	0.56*
WRDVI	0.69*	0.62*	0.79*	0.74*	0.77*	0.71*	0.70*	0.64*	0.68*	0.65*
Green NDVI	0.59*	0.56*	0.65*	0.63*	0.67	0.66	0.60*	0.56*	0.60	0.58
TCARI	0.21*	0.17*	0.28*	0.25*	0.18*	0.15*	0.21	0.21	0.19*	0.17*
MCARI	0.11*	0.09*	0.11	0.10	0.09*	0.08*	0.10*	0.11*	0.09*	0.09*
NPCI	0.27*	0.30*	0.19*	0.23*	0.22*	0.36*	0.27	0.27	0.28	0.29
SRPI	0.65*	0.55*	0.92*	0.77*	0.71*	0.49*	0.64*	0.62*	0.61*	0.56*
NDNI	0.20*	0.19*	0.22*	0.22*	0.19*	0.18*	0.19*	0.20*	0.19*	0.18*
CAI	-0.01*	0.00*	-0.01	-0.01	-0.01*	-0.01*	-0.01	0.00	-0.01*	-0.01*
NPQI	-0.06*	-0.07*	-0.01*	-0.02*	-0.03*	-0.05*	-0.06	-0.05	-0.07	-0.07
XES	0.14	0.14	0.13	0.13	0.10*	0.09*	0.13*	0.15*	0.12	0.12
G	1.61*	1.28*	2.40*	1.88*	1.77*	1.36*	1.67*	1.40*	1.49*	1.35*
REPI	720.71	720.87	720.48*	719.94*	721.64*	720.85*	720.71*	720.10*	720.04	720.30

To verify the spectrometric measurements and the vegetation indices calculated on their basis, as well as to check conclusions concerning the degraded state of trampled plants, these values were correlated with bio-radiometric measurements. Table 2 shows the results of bio-radiometric correlation for indices such as CCI, ts-ta and fAPAR. The highest correlation was observed for chlorophyll content in the *Agrostis rupestris* and *Luzula alpino-pilosa*. For the trampled species *Agrostis rupestris*, the chlorophyll value correlates with GNDVI to the level of -0.67, with this value indicating that the chlorophyll content has declined due to trampling. However, in the reference buffer we observe a high relationship (0.71) between the values of chlorophyll content and GNDVI, which indicates that the amount of chlorophyll has not changed in the plant (Tab. 2). Overall, species correlation results from the spectrometer and bio-radiometric indices are different depending on the structure of vegetation.

Analyzing the community as a whole, a high correlation is observed between the ts-ta and NDWI or WBI – indices describing water in plants. In both the reference and trampled case, the ts-ta index for *Oreochloa disticha* is highly correlated with NDWI, but for trampled plants it is -0.71, which means that the water content is lower than in the reference plant, where the correlation value is 0.49 meaning that the value of both indices increases. The highest correlation was observed for *Juncus*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

trifidus in the trampled buffer where the correlation between the index of ts-ta and WBI reached a value of -0.85; statistically significant at the 0.05 level (Tab. 2). With an increase in the observed water stress value of the ts-ta index, the value of the WBI decreases, confirming a decreasing water content in the trampled plant.

Table 2 – The Spearman correlation remote sensing vegetation indices and bio-radiometric indices (CCI, ts-ta, fAPAR) for all investigated species marked T – trampled and R – reference; '-' – no correlate; * – statistical significance at the 0.05 level.

	CCI	**ts-ta**	**fAPAR**
Luzula alpino-pilosa _T	SIPI [0.81]*	WBI [0.58]*	NPQI [-0.72]*
Luzula alpino-pilosa _R	XES [0.54]	G [0.62]*	SAVI [0.49]
Festuca picta _T	-	WBI [0.60]	mNDVI 705 [-0.83]*
Festuca picta _R	-	TCARI [-0.78]*	NDII [-0.81]*
Juncus trifidus _T	-	WBI [-0.85]*	PRI [-0.39]
Juncus trifidus _R	-	MCARI [0.42]	CRI1 [0.68]*
Agrostis rupestris _T	GNDVI [-0.67]	NDNI [-0.58]*	NPCI [0.68]*
Agrostis rupestris _R	GNDVI [0.71]	NDWI [-0.75]*	NDWI [-0.69]*
Oreochloa disticha _T	-	NDWI [-0.71]	NPQI [-0.72]*
Oreochloa disticha _R	-	NDWI [0.49]	mNDVI 705 [0.49]

Trampling also influences the use of light within photosynthesis, linking into the functioning of the plant, which has been verified by measurements of the amount of radiation used by photosynthesis. Thus, the light use efficiency coefficient (fAPAR) was calculated. The fAPAR index for *Juncus trifidus* in the trampled buffer zone correlated with the PRI (–0.39), which showed that the amount of light used in photosynthesis decreased through trampling (Tab. 2). NPQI correlated highly with fAPAR for trampled *Luzula alpino-pilosa* and *Oreochloa disticha*, which means that a lower chlorophyll and plant stress (described by NPQI) is accompanied by a decrease in the photosynthetic use of light.

To determine the actual cell status caused by trampling, fluorescence measurements were performed. A very constant value for Fv/Fm of 0.780-0.865 [71], was found for healthy leaves of a very wide variety of species (after darkness or for the night-time period), while stress due to disease or environmental conditions was indicated by lower values. The Fv/Fm ratio was found to fluctuate within the range 0.471–0.627 for dark-adapted leaves for the trampled and buffer plants, which means that the actual state of the cells is good in all cases (Tab. 3). Therefore, it's assumed the growth of the plants and function of photosynthetic apparatus was not damaged, with mechanism(s) involved in the limitation of the production of oxygen species, responsible for oxidative stress, being efficient. The low values of Fv/Fm for leaves adapted to darkness may be also due to the plants' growth in full sunlight slopes, which initiates the process of photoinhibition.

Generally, t½ is smaller when the plastoquinone (PQ) pool is low in shaded compared to sunlit plants, and also under stress conditions; t½ (in ms) is a function of the photochemical reaction rate and pool size of electron acceptors, including the PQ pool. *Juncus trifidus* in the trampled buffer has a value of 145 and 40 ms with and without adaptation to the darkness, respectively (Tab. 3). The half-time after adaptation to darkness is much larger, which means a high PQ pool in the plant. Some species in the reference buffer have a higher value of Fv/Fm than the same species in the trampled buffer. For example, *Luzula alpino-pilosa* has an Fv/Fm value of 0.627 in the reference buffer, while in the buffer where the species was trampled this value is lower, at 0.596, which may indicate lower efficiency of light absorption related to lower chlorophyll (Tab. 3). Furthermore, these values for *Luzula alpino-pilosa* were statistically significant (at the $p<0.05$ level) compared with the difference between the reference and trampled plants as measured using Kruskal-Wallis one-way analysis of variance. This means that the plant cells of this species are most sensitive to changes caused by trampling, and have a limited capacity for photosynthesis. The larger difference between the value

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

of Fm and Fm' shows a greater susceptibility to photoinhibition; larger Fv/Fm ratios may mean that the photosynthetically active pigment concentration is higher. The observed morphological adaptation in previously studied species also confirmed their biochemical condition being due to their growth in the testing mountainous terrain, with leaf thickness and the number of layers of parenchyma contributing to the process of photosynthesis. To confirm this relationship, values obtained from the fluorometer were correlated to the indices illustrating the various vegetation parameters, such as cellular structures, pigments or water content. In the study only those indices which revealed statistically significant differences between both for the buffer zones were used.

Table 3 – The average fluorescence value for the studied species in the two buffers without and after adaptation to darkness. (* – statistical significance at the 0.05 level)

Plant species	Trampled buffer				Reference buffer			
	with adaptation to darkness		without adaptation to darkness		with adaptation to darkness		without adaptation to darkness	
	Fv/Fm	t ½ ms	Fv'/Fm'	t½'ms	Fv/Fm	t ½ ms	Fv'/Fm'	t ½' ms
Juncus trifidus	0.496	145	0.323	40	0.549	173	0.372	34
Luzula alpino-pilosa	0.596 *	116	0.343	27	0.627 *	79	0.407	24
Agrostis rupestris	0.471 *	113	0.285	31	0.597 *	113	0.314	35
Oreochloa disticha	0.520	126	0.391	36	0.598	119	0.381	27
Festuca picta	0.572 *	123	0.302	22	0.496 *	121	0.276	24

Fv/Fm for the species *Luzula alpino-pilosa* in the reference buffer was strictly correlated with WBI (-0.67) that characterizes the water content, while Fv/Fm for the trampled *Luzula alpino-pilosa* correlates with REPI (0.36) (Tab. 4). In addition, a high correlation is observed between Fv/Fm and GNDVI, especially for trampled plants, which means that the plants were stressed; trampled plants have a higher ratio of carotenoids to chlorophyll. The *Juncus trifidus* in the reference buffer shows the highest correlation with fluorescence indices representing the structure and condition of the plants (e.g. CAI or WBI), with values of -0.62 or -0.53. For the trampled species *Juncus trifidus* the correlation value of Fv/Fm with GNDVI is -0.66 and when Fv'/Fm' is correlated with GNDVI it is -0.71. This indicates induction of stress factors and thus decreasing values of Fv/Fm and Fv'/Fm', which demonstrate the damage of photosynthetic apparatus and PSII, thus lowering photosynthetic efficiency (Tab. 4).

Table 4 – The Spearman correlation of remote sensing vegetation indices and fluorometric indices (Fv/Fm, t½ – with adaptation to darkness; Fv/Fm', t½' – without adaptation to darkness) for all species marked T – trampled and R – reference; * – statistical significance at the 0.05 level.

	Fv/Fm	t½	Fv'/Fm '	t½'
Luzula alpino-pilosa _T	REPI [0.36]	REPI [0.46]	PRI [-0.52]	ARI2 [0.49]
Luzula alpino-pilosa _R	WBI [-0.67]*	PRI [-0.52]	WBI [-0.28]	CRI2 [0.59]
Festuca picta _T	GNDVI [0.83]*	SIPI [0.82]*	GNDVI [0.54]	CRI2 [-0.94]*
Festuca picta _R	PRI [0.54]	SIPI [0.49]	PRI [0.86]*	ARI2 [-0.59]
Juncus trifidus _T	GNDVI [-0.66]*	ARI1 [-0.63]*	GNDVI [-0.71]*	PRI [-0.51]
Juncus trifidus _R	CAI [-0.62]	mNDVI705 [0.66]*	WBI [-0.53]	mNDVI705 [-0.64]*
Agrostis rupestris _T	TCARI [0.63]*	mNDVI705 [0.67]*	CAI [-0.70]*	MSI [-0.66]*
Agrostis rupestris _R	mNDVI 705 [0.56]	PRI [0.74]*	CRI2 [0.54]	mNDVI705 [0.49]
Oreochloa disticha _T	CAI [-0.77]	GNDVI [0.37]	PRI [-0.65]	CRI1 [-0.55]
Oreochloa disticha _R	SIPI [0.77]	GNDVI [0.75]	SIPI [0.60]	CAI [0.88]*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

The fluorescence index (Fv/Fm) for the species *Agrostis rupestris* is in most cases positively correlated with remote sensing indices, but there is a noticeable decrease (approx. 0.50) in correlation between Fv'/Fm' and CAI or t½' with MSI. For this species in the reference buffer, the Fv/Fm fluorescence value correlated with mNDVI705 indices (0.56), and the Fv'/Fm' value correlated with CRI2 indices (0.54). Plants in stress contain higher concentrations of carotenoids, which play a prominent role in protection against stress; this index is one measure of stressed vegetation. Higher CRI2 values mean greater carotenoids relative to chlorophyll concentrations. This is also reflected in photosynthesis and other processes that occur in a plant, as shown in Table 4, by the values of the fluorescence correlations between indices depicting chlorophyll (e.g. mNDVI705) or those describing the state of health (e.g. GNDVI) and indices determining the amount of light used in the process of photosynthesis (e.g. PRI), water content (e.g. WBI) or stress-related pigments CRI2 and ARI2 (which determine the condition of trampling vegetation).

Trampling interacts in different ways with the studied species, which is visible via a changing reflectance spectrum and then remote sensing indicators. Changes caused by trampling mostly affect the spectral bands related to pigments and water content. Moreover, the changes are also reflected in the values of Fv/Fm, which reflect the variable adaptation of the photosynthetic apparatus of each species. Differences in the condition of species do not allow us to state clearly that the species are more or less resistant to trampling in this area.

5. Discussion

The study results confirm that the vegetation condition may decline in response to many biotic and abiotic factors. Alpine swards are exposed to full sunlight, insufficient water supply and high temperature. Therefore these high mountain plants evolve adaptations, such as *Crassulaceae*, allowing water retention, storage in the leaves, or wax to reduce evaporation [72]. One of the stress factors is trampling, which leads to changes in some species. Tests were used to determine which remote sensing vegetation indices reflect the currently worsened (damaged structures, lower level of physiological processes) state of swards caused by excessive tourist traffic. The impact of informal trails can be particularly important, because subalpine and alpine plant communities on mountain summits are typically fragile, spatially restricted and rare [73].

Changes caused by trampling are reflected in the health status of vegetation, lower cover, lower plant height, decreased biomass, changes in the typical species composition, relative changes in cover classes and displacement of species [8,14,74-78]. Cole [14] found that plant morphology was more significant than site characteristics (altitude, overstorey canopy cover and total vegetation cover) in determining the response of vegetation to trampling. The same information was compared by Sun and Liddle [79], who showed that plant height and morphological structure appear to be strongly associated with resistance to trampling. As a result, some alpine vegetation types may be significantly more resistant than sub-alpine and low-elevation types, due to the larger proportion of turf-forming graminoids [79,80]. However, Bell and Bliss [81] wrote that the other alpine vegetation types are likely to be less resistant. These studies also showed diverse resistance to trampling among species. All researched species in the Czerwone Wierchy showed a statistically significant ($p<0.05$) change between trampled and reference plants, which was visible in the reflectance spectral curves.

However, vegetation can, of course, rebuild its structure in most cases if trampling is not to a very high degree (devastation sites), but alpine vegetation recovers much more slowly than vegetation in lower altitude areas [78,82]. The conducted research also showed variable species resistance to trampling– a similar relationship to that shown by Dumitrascu [83], who emphasize that habitat characteristics affect the state of trampled plants. Species react differently to stress and this is dependent on their morphology, anatomy and state of development at any given moment. Changes were observed in the chlorophyll concentration within the leaves, revealing, respectively, a decrease or increase in the visible range reflectance around 700 nm [84]. A similar situation can be seen in this study, where the stress caused by excessive trampling is visible in the same wavelength range indicating chlorophyll, as evidenced by the remote sensing of vegetation indicators such as

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

SRPI, that the difference between trampled and reference plants is about 0.10–0.20. Moreover, these changes are statistically significant at the $p<0.05$ level for all species studied [85].

When plants are exposed to abiotic and biotic stress in high light conditions, decreases in Fv/Fm are frequently observed [86]. The optimum value is 0.83, but this varies [41,44,45,87]; a lower value will be seen when the plant has been exposed to stress, indicating photoinhibition [88]. The Fv/Fm parameter appears to be relatively insensitive to severe water limitation, but could be used to differentiate between responses during cold temperatures. High light intensity is observed in the mountains, thus Fv/Fm values obtained in this study ranges between 0.471 and 0.627. Kalaji [9] observed that heat stress significantly prolonged the time to achieve maximum fluorescence (TFM) – 190% compared to controls, and increased the reduction of QA from 0 to TFM (N) by 230% compared to the control plants. In the present study a decrease in half-time ($t½$) between the trampling and the reference plant was not observed [89], which may suggest that, despite the small difference in Fv/Fm values, reaction centers are not disrupted and photochemical processes in the studied plant species work well. We can therefore conclude that temperature is not an important stress factor for alpine swards, where plants are well adapted to adverse environmental conditions [9,90]. Literature data suggest that most of the stresses affecting the photosynthetic apparatus reduce the Fv/Fm ratio, while some authors suggest constant values are measured during drought conditions [91-93].

The high correlation index of Fv/Fm with indicators from the group describing the use of light within the photosynthetic process allows for long-term monitoring of vegetation. One example is the research of Tan et al. [94] where, when determining damage to corn, the SIPI index was used and was highly correlated to Fv/Fm (R=0.88). However, in this study the second of the indicators in this group – PRI – correlated highly with Fv/Fm (R= -0.53 for *Luzula alpino-pilosa*) indicating changes in trampled vegetation that included lower energy consumption. GNDVI – defined in the literature as a modification and improvement of the NDVI index for highlighting the physiological properties of plants [95] – shows a high correlation with Fv/Fm for all the reference species (0.71), which proves the very good development of reference vegetation compared to the disturbed state of trampled vegetation.

Replanting of the vegetation is possible, although very labour intensive [73,96]. Through the analysis of the state of vegetation and information about fluorescence we are able to closely monitor and evaluate species and plants exposed to environmental stress before external signals, such as yellowing or browning of leaves, are visible. Such monitoring of the state of vegetation allows earlier prevention of the destruction of species in such valuable natural areas as, among others, national parks [97].

6. Conclusions

Alpine vegetation is characterised by a set of unique properties that can be quantified by hyperspectral sensors. The most important are plant pigments (including their relative concentrations), protective elements, e.g. the shape and structure of leaves, or waxy cover; allowing the influence of different abiotic and biotic factors to be illustrated. Trampling disintegrates the plant canopy, causing higher transmission of solar radiation and changes in water-temperature relationships (increases in soil temperature and evapotranspiration). Therefore, it is valuable to monitor plant condition, especially in areas often visited by tourists.

The applied methods, which combine spectral characteristics and vegetation indices containing information on fluorescence, allow for a detailed analysis. The hyperspectral measurements confirmed statistically significant differences between trampled and reference plants. The changes were observed in chlorophyll absorption, cell structures and water content; the observations were confirmed by chlorophyll content, fluorescence and the ts-ta temperature index. The fluorescence ratio (Fv/Fm) was used to evaluate the efficiency of chlorophyll in the photosynthetic process. The correlation of both categories of information obtained allowed the impact of heavy tourist traffic on the selected species to be determined, which is important for the protection of valuable natural areas.

The present study of *Juncus trifidus; Agrostis rupestris; Luzula alpino-pilosa; Oreochloa disticha; Festuca picta* has failed to clearly determine which species are resistant or more sensitive. The most resistent to trampling is *Nardus stricta*, which in 59% ($p<0.05$) and 83.3% ($p<0.01$) of analysed targets did not show statistically significantly differentiated spectral properties; similar observations were recorded for *Deschampsia flexuosa*. The most sensitive were *Agrostis rupestris* and *Juncus trifidus* (87% where the trampled plants were different from the reference species at the $p<0.05$ level of statistical significance, and 75% at the $p<0.01$). Fv/Fm had a high correlation with the indices, indicating the amount of chlorophyll and the use of light in photosynthesis. For example, for the reference samples of species *Oreochloa disticha* the Fv/Fm ratio correlates at 0.77 with the SIPI index, while for the same species threatened by trampling, we have the opposite (negative) correlation. The best vegetation indices, which were statistically significant for the analyses, are NDVI, mNDVI, NDVI705, mSR705, ARVI, EVI, WBI, NDWI and NDII.

The proposed methodology allows for remote monitoring of various types of vegetation. The use of hyperspectral data confirms the rich information on the state of vegetation, especially vegetation endangered by trampling, but also confirms fluorescence as an indicator for describing the actual state of the pigments and cell structures.

Acknowledgements: Authors would like to thank the Tatra National Park, Institute of Geography and Spatial Organisation of Polish Academy of Sciences and the Anna Pasek Foundation, which in 2012/2013 funded a scholarship to Marlena Kycko. The method of field data acquisition was developed in the frame of the Polish-Norwegian Research Programme, National Centre for Research and Development project: *Ecosystem stress from the combined effects of winter climate change and air pollution – how do the impacts differ between biomes? (WICLAP)*, No: POL-NOR/198571/83/2013.

Special thanks also go to students participating in the study: Aneta Modzelewska, Olga Kurek, Ignacy Kaźmierczak, as well as the Tatra National Park Volunteers Adam Lubiński and Michał Kozioł.

References

1. Roberts, D.A.; Batista, G.T.; Pereira, J.; Waller, E.K.; Nelson, B.W. Change identification using multitemporal spectral mixture analysis: applications in Eastern Amazonia. In *Remote sensing change detection: environmental monitoring applications and methods*, Elvidge, C., Lunetta, R., Eds.; Ann Arbor Press, Ann Arbor, Chelsea, USA, 1998, pp. 137 – 161.
2. Raczko, E.; Zagajewski, B.; Ochtyra, A.; Jarocińska, A.; Marcinkowska-Ochtyra, A.; Dobrowolski, M. Forest species identification of Mount Chojnik (Karkonoski National Park) using airborne hyperspectral APEX data. *Sylwan* **2015**, *159(7)*, 593-599. Available online: https://sylwan.lasy.gov.pl/apex/apex_util.get_blob?s=701130105435&a=105&c=1170502307818324&p=10&k1=19388&k2=&ck=XLVbaH8GXqwpYjx-2vS8hzMGr5opetjkFCpYchomGRB-cxCS4w8nZj6wU2za5POEVS-y7vgtCspwRluX44hP-A&rt=CR (accessed on 21.01.2017).
3. Raczko, E.; Zagajewski, B. Comparison of Support Vector Machine, Random Forest and Neural Network Classifiers for Tree Species Classification on Airborne Hyperspectral APEX images. *Euro. Jour. of Rem. Sens.* **2017**, *50(1)*, 144-154, DOI: 10.1080/22797254.2017.1299557.
4. Tucker, C.J.; Sellers, P.J. Satellite remote sensing of primary production. *Inter. Jour. of Rem. Sens.* **1986**, *7(11)*, 1395-1416, DOI: 10.1080/01431168608948944.
5. Knipling, E.B. Physical and physiological basis for the reflectance of visible and near-infrared radiation from vegetation. *Rem. Sens. of Env.* **1970**, *1(3)*, 155–159, DOI: 10.1016/S0034-4257(70)80021-9.
6. Gamon, J.A.; Field, C.B.; Roberts, D.A.; Ustin, S.L.; Valentini R. Functional patterns in an annual grassland during an AVIRIS overflight. *Rem. Sen. of Env.* **1993**, *44(2–3)*, 239-253, DOI: 10.1016/0034-4257(93)90019-T.
7. Cierniewski, J.; Kazmierowski, C.; Krolewicz, S.; Piekarczyk, J.; Wrobel, M.; Zagajewski, B. Effects of Different Illumination and Observation Techniques of Cultivated Soils on Their Hyperspectral Bidirectional Measurements Under Field and Laboratory Conditions. *IEEE Jour. of Sel. Topics in Ap. Earth Obser. and Rem. Sen.* **2014**, *7(6)*, 2525-2530, DOI: 10.1109/JSTARS.2014.2298098.

 doi:10.20944/preprints201704.0007.v1

8. Cole, D.N. Experimental trampling of vegetation. I. Relationship between trampling intensity and vegetation response. *Jour. of App. Ecol.* **1995**, *32*, 203-214. Available online: https://www.treesearch.fs.fed.us/pubs/download/24581.pdf (accessed on 21.01.2017).
9. Kalaji, M.H.; Bosa, K.; Kościelniak, J.; Hossain Z. Chlorophyll a fluorescence - A useful tool for the early detection of temperature stress in spring barley (Hordeum vulgare L.). *OMICS: A Jour. of Integr. Biol.* **2011**, *15(12)*, 925-934, DOI: 10.1089/omi.2011.0070.
10. Whinam, J.; Chilcott, N.M. Impact after four years of experimental trampling on alpine/sub-alpine environments in western Tasmania. *Jour. of Env. Manag.* **2003**, *67(4),* 339–351, DOI: 10.1016/S0301-4797(02)00218-9.
11. Whinam, J.; Chilcott, N.M. Impact of trampling on alpine environments in central Tasmania. *Jour. of Env. Manag*. **1999**, *57(3)*, 205–220, DOI: 10.1006/jema.1999.0302.
12. Della Beffa, M.T. Rośliny górskie; Horyzont, Publishing Group Bertelsmann Media, Warsaw, Poland, 2002; pp. 223.
13. Cole, D.N. Experimental trampling of vegetation. II. Prediction of resistance and resilience. *Jour. of App. Ecol*. **1995**, *32*, 215-224. Available online: *https://www.treesearch.fs.fed.us/pubs/download/23582.pdf* (accessed on 21.01.2017).
14. Cole, D.N.; Bayfield, N.G. Recreational trampling of vegetation: standard experimental procedures. *Biol. Conser.* **1993**, *63(3)*, 209-215, DOI: 10.1016/0006-3207(93)90714-C.
15. Gremmen, N.J.M.; Smith, V.R.; van Tongeren, O.F.R. Impact of trampling on the vegetation of subantarctic Marion Island. *Arct. Antarct. and Alp. Research* **2003**, *35(4)*, 442-446, DOI: 10.1657/1523-0430(2003)035[0442:IOTOTV]2.0.CO;2.
16. Jägerbrand, A.K.; Alatalo, J.M. Effects of human trampling on abundance and diversity of vascular plants, bryophytes and lichens in alpine heath vegetation, Northern Sweden. *SpringerPlus* **2015**, *4*, 95, DOI: 10.1186/s40064-015-0876-z.
17. McDougall, K.L.; Wright, G.T. The impact of trampling on feldmark vegetation in Kosciuszko National Park, Australia. *Aust. J. Bot.* **2004**, *52*, 315-320, DOI: 10.1071/BT03145.
18. Barros, A.; Gonnet, J.; Pickering, C. Impacts of informal trails on vegetation and soils in the highest protected area in the Southern Hemisphere. *Jour. of Env. Manag.* **2013**, *127*, 50-60, DOI: http://dx.doi.org/10.1016/j.jenvman.2013.04.030.
19. Ballantyne, M.; Pickering, C.M.; McDougall, K.L.; Wright, G.T. Sustained impacts of a hiking trail on changing windswept feldmark vegetation in the Australian Alps. *Aust. Jour. of Bot*. **2014**, *62(4)*, 263-275, DOI: 10.1071/BT14114.
20. Ballantyne, M.; Pickering, C.M.; McDougall, K.L.; Wright, G.T. Sustained impacts of a hiking trail on changing windswept feldmark vegetation in the Australian Alps. *Aust. Jour. of Bot*. **2014**, *62(4)*, 263-275, DOI: 10.1071/BT14114.
21. Kobayashi, Y.; Kaya, H.; Goto, K.; Iwabuchi, M.; Araki, T. A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **1999**, *286(5446)*, 1960–1962, DOI: 10.1126/science.286.5446.1960.
22. Cole, D.N.; Monz, C.A. Trampling Disturbance of High-Elevation Vegetation, Wind River Mountains, Wyoming, U.S.A. *Arct., Antarct., and Alp. Research* **2002**, *34(4)*, 365-376, DOI: 10.2307/1552194.
23. Sunohara, Y.; Ikeda, H.; Tsukgashi, S.; Murata, Y.; Sakurai, N.; Noma, Y. Effects of trampling on morphology and ethylene production in asiatic plantain. *Weed Scien.* **2002**, *50(4)*, 479-484, DOI: 10.1614/0043-1745(2002)050[0479:EOTOMA]2.0.CO;2.
24. Sunohara, Y.; Ikeda, H. Effects of trampling and ethephon on leaf morphology in trampling-tolerant Plantago asiatica and Eleusine indica. *Weed Res.* **2003**, *43(3)*, 155-162, DOI: 10.1046/j.1365-3180.2003.00329.x.
25. Striker, G.G.; Mollard, F.P.O.; Grimoldi, A.A.; Leon, R.J.C.; Insausti, P. Trampling enhances the dominance of graminoids over forbs in flooded grassland mesocosms. *Ap. Veg. Scien.* **2010**, *14(1)*, 95-106, DOI: 10.1111/j.1654-109X.2010.01093.x.
26. Emanuelsson, U. Recreation impact on mountainous areas in northern Sweden. In: Proceedings: The Ecological Impacts of Outdoor Recreation on Mountain Areas in Europe and North America, Wye, England, 1985; Bayfield, N.G.; Barrow, G.C., Eds.; Recreation Ecology Research Group, pp. 63-73.

27. Grabherr, G. Damage to vegetation by recreation in the Austrian and German Alps. In: *Proceedings: The Ecological Impacts of Outdoor Recreation on Mountain Areas in Europe and North America*, Bayfield, N.G.; Barrow, G.C., Eds.; Recreation Ecology Research Group ; Wye, England, 1985; pp. 74–91.
28. Klug, B.; Scharfetter-Lehrl, G.; Scharfetter, E. Effects of trampling on vegetation above the timberline in the eastern Alps, Austria. *Arctic, Antarctic, and Alpine Res.* **2002**, *34(4)*, 377–388, DOI: 10.2307/1552195.
29. Scott, J.J.; Kirkpatrick, J.B. Effects of human trampling on the sub-Antarctic vegetation of Macquarie Island. *Polar Rec.* **1994**, *30(174)*, 207-220, DOI: 10.1017/S003224740002427X.
30. Kim, M.K.; Daigle, J.J. Detecting vegetation cover change on the summit of Cadillac Mountain using multi-temporal remote sensing datasets: 1979, 2001, and 2007. *Env. Monitor. and Assess*. **2011**, *180(1)*, 63-75, DOI: 10.1007/s10661-010-1772-1.
31. Jakomulska, A.; Zagajewski, B.; Traut, A. Application of field remote sensing techniques for vegetation investigation. Case study of Siwica Glade Reserve. *Miscel. Geograph*. **2002**, *10*, 279-306.
32. Sobczak, M.; Folbrier, A.; Kozłowska, A.; Krówczyńska, M.; Pabjanek, P.; Wrzesień, M.; Zagajewski, B. Assessment of the potential of hyperspectral data and techniques for mountain vegetation analysis. In: Imaging Spectroscopy. New quality in environmental studies; Zagajewski, B.; Sobczak, M. Eds.. EARSeL & Warsaw University, Faculty of Geography and Regional Studies, Warsaw, Poland, 2005, pp. 761-780.
33. Wrzesień, M.; Zagajewski, B.; Sobczak, M.; Zwijacz-Kozica, T. Estimation of leaf area index in dwarf mountaine pine (*Pinus mugo* Turra) using hyperspectral data. In: Imaging Spectroscopy. New quality in environmental studies; Zagajewski, B.; Sobczak, M. Eds.. EARSeL & Warsaw University, Faculty of Geography and Regional Studies, Warsaw, Poland, 2005, pp. 809-816.
34. Ochtyra, A.; Zagajewski, B.; Kozłowska, A.; Marcinkowska-Ochtyra, A.; Jarocińska, A. Assessment of the Tatra National Park forests condition using decision tree method and multispectral Landsat TM satellite images. *Sylwan* **2016**, *160(3)*, 256-264. Available online: https://sylwan.lasy.gov.pl/apex/f?p=105:10:::NO::P10_NAZWA_PLIKU,P10_ARTYKUL:F1737022461%2F2016_03_0256au.pdf,2015039 (accessed on 05.03.2017).
35. Kozłowska, A.; Rączkowska, Z.; Zagajewski, B. Links between Vegetation and Morphodynamics of High-Mountain Slopes in the Tatra Mountain. *Geograph. Polon*. **2006**, *79(1)*, 27-39. Available online: https://www.geographiapolonica.pl/article/item/5350.html (accessed on 05.03.2017).
36. Mirek, Z. Studies in Polish endemic species - vascular plants. 1. Gladiolus felicis Mirek. *Acta Societ. Botanic. Polon.* **1985**, *54(2)*, 157-167. Available online: https://pbsociety.org.pl/journals/index.php/asbp/article/view/asbp.1985.015/2628 (accessed on 05.03.2017).
37. Jakomulska, A. Physiology and Spectral Signatures of the Alpine Species: *Juncus trifidus*, *Luzula spadicea* and *Calamagrostis villosa*. Assessment of Potential for Remote Identification of Vegetation in High-MountainEnvironments. In: *Geoecological research in the Kasprowy Wierch Area*; Kotarba, A.; Kozłowska A. Eds.; Prace Geograficzne, Wrocław, Poland, 1999, Vol. 174, pp. 45-61.
38. Tsimilli-Michael, M.; Strasser, R.J. In vivo assessment of plants' vitality: applications in detecting and evaluating the impact of mycorrhization on host plants. In *Mycorrhiza: State of the Art, Genetics and Molecular Biology, Eco-Function, Biotechnology, Eco-Physiology, Structure and Systematics*, 3rd ed.; Varma, A. Ed.; Springer Berlin Heidelberg, Berlin, Germany, 2008, pp. 679-703, DOI: 10.1007/978-3-540-78826-3.
39. Kuckenberg, J.; Tatrachnyk, I.; Noga, G. Temporal of spatial changes of chlorophyll fluorescence as a basis for early and precise detection of leaf rust and powdery mildew infections in wheat leaves. *Precision Agric.* **2009**, *10*, 34-44, DOI: 10.1007/s11119-008-9082-0.
40. Sharma, D.K.; Andersen, S.B.; Ottosen, C.O.; Rosenqvist, E. Wheat cultivars selected for high Fv /Fm under heat stress maintain high photosynthesis, total chlorophyll, stomatal conductance, transpiration and dry matter. *Physiol. Plant.* **2015**, *153(2)*, 284-298, DOI: 10.1111/ppl.12245.
41. Kalaji, M.H.; Guo, P. Chlorophyll fluorescence: A useful tool in barley plant breeding programs. In *Photochemistry Research Progress*; Sanchez, A.; Gutierrez, S.J., Eds.; Nova Publishers, NY, USA. 2008, pp. 439-463.
42. Hall, D.O.; Rao, K.K. Photosynthesis, 5th ed.; Cambridge University Press, Cambridge, UK, 1994; pp. 211.

43. Schreiber, U. Pulse-Amplitude-Modulation (PAM) fluorometry and saturation pulse method: an overview. In *Chlorophyll a Fluorescence: A Signature of Photosynthesis*; Papageorgiou, G.C., Ed.; Springer, Dordrecht, The Netherlands, 2004, pp. 279–319, DOI: 10.1007/978-1-4020-3218-9.
44. Björkman, O.; Demmig, B. Photon yield of O2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins . *Planta* **1987**, *170(4)*, 489-504, DOI: 10.1007/BF00402983.
45. Johnson, G.N.; Young, A.J.; Scholes, J.D.; Horton P. The dissipation of excess excitation energy in British plant species. *Plant, Cell & Env.* **1993**, *16(6)*, 673–679, DOI: 10.1111/j.1365-3040.1993.tb00485.x.
46. Kycko, M.; Zagajewski, B.; Zwijacz-Kozica, M.; Cierniewski, J.; Romanowska, E.; Orłowska, K.; Ochtyra, A.; Jarocińska, A. Assessment of Hyperspectral Remote Sensing for Analyzing the Impact of Human Trampling on Alpine Swards. *Mount. Res. and Devel.* **2017**, *37(1)*, 66-74, DOI: 10.1659/MRD-JOURNAL-D-15-00050.1.
47. Kycko, M.; Zagajewski, B.; Kozłowska, A.; Oprządek, M. Variability of spectral characteristics of selected high-mountain plant species of Gasienicowa Valey exposed for trampling. *Teledetekcja Środowiska* **2012**, *47*, 75–86. Available online: http://geoinformatics.uw.edu.pl/wp-content/uploads/sites/26/2014/03/TS_v47_Kycko.pdf (accessed on 25.03.2017).
48. Kycko, M.; Zagajewski, B.; Kozłowska, A. Variability in spectral characteristics of trampled high-mountain grasslands. *Miscel. Geograph.* **2014**, 18(2), 10–14, DOI: 10.2478/mgrsd-2014-0003.
49. Pitman, J.I. Absorption of Photosynthetically Active Radiation, Radiation Use Efficiency and Spectral Reflectance of Bracken [*Pteridium aquilinum* (L.) Kuhnl] Canopies. *An. of Botany* **2000**, *85* (Supplement B), 101-111, DOI:10.1006/anbo. 1999.1096.
50. Gitelson, A.A. Wide Dynamic Range Vegetation Index for Remote Quantification of Biophysical Characteristics of Vegetation. *Jour. of Plant Physiol*. **2004**, *161(2)*, 165–173. DOI: 10.1078/0176-1617-01176.
51. Huete, A. A Soil-Adjusted Vegetation Index (SAVI). *Rem. Sen. of Env.* **1988**, *25(3)*, 295-309, DOI: 10.1016/0034-4257(88)90106-X.
52. Gitelson, A.A.; Kaufman, Y.J.; Merzlyak, M.N. Use of a green channel in remote sensing of global vegetation from EOS-MODIS. *Rem. Sen. of Env.* **1996**, *58(3)*, 289-298, DOI: 10.1016/S0034-4257(96)00072-7.
53. Zarco-Tejada, P.J.; Bejron, A.; Miller, J.R. Stress Detection in Crops with Hyperspectral Remote Sensing and Physical Simulation Models. Airborne Imaging Spectroscopy Workshop, 8 October 2004 - Bruges, Belgium, 2004. Available online: http://quantalab.ias.csic.es/pdf/paper_bruhyp_workshop_zarco_tejada.pdf (accessed on 25.03.2017).
54. Dawson, T.P.; Curran, P.J. A new technique for interpolating the reflectance red edge position. Technical note, *Inter. Jour. of Rem. Sen.* **1998**, *11*, 2133 – 2139, DOI: 10.1080/014311698214910.
55. Sims, D.A.; Gamon, J.A. Relationships Between Leaf Pigment Content and Spectral Reflectance Across a Wide Range of Species, Leaf Structures and Developmental Stages. *Rem. Sens. of Env.* **2002**, *81(2-3)*, 337-354, DOI: 10.1016/S0034-4257(02)00010-X.
56. Haboudane, D.; Miller, J.R.; Pattey, E.; Zarco-Tejada, P.J.; Strachan, I.B. Hyperspectral Vegetation Indices and Novel Algorithms for Predicting Green LAI of Crop Canopies: Modeling and Validation in the Context of Precision Agriculture. *Rem. Sen. of Env.* **2004**, *90(3)*, 337-352, DOI: 10.1016/j.rse.2003.12.013.
57. Daughtry, C.; Walthall, C.L.; Kim, M.S.; Brown de Colstoun, E.; McMurtreym, J.E. Estimating Corn Leaf Chlorophyll Concentration from Leaf and Canopy Reflectance. *Rem. Sen. Env.* **2000**, *74(2)*, 229–239, DOI: 10.1016/S0034-4257(00)00113-9.
58. Peñuelas, J.; Gamon, J.A.; Fredeen, A.L.; Merino, J.; Field, C.B. Reflectance Indices Associated with Physiological Changes in Nitrogen and Water Limited Sunflower Leaves. *Rem. Sen. of Env.* **1994**, *48(2)*, 135-146, DOI: 10.1016/0034-4257(94)90136-8.
59. Peñuelas, J.; Baret, F.; Filella, I. Semi-Empirical Indices to Assess Carotenoids/Chlorophyll-a Ratio from Leaf Spectral Reflectance. *Photosynthetica* **1995**, 31, 221-230. Available online: http://www.creaf.uab.es/Global-Ecology/Pdfs_UEG/Photosyn1995.pdf (accessed on 25.03.2017).

60. Barnes, J.D.; Balaguer, L.; Manrique, E.; Elvira, S.; Davison, A.W. 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.* **1992**, *32*, 85–100, DOI: 10.1016/0098-8472(92)90034-Y.
61. Gamon, J.A.; Penuelas, J.; Field, C.B. A Narrow-Waveband Spectral Index That Tracks Diurnal Changes in Photosynthetic Efficiency. *Rem. Sen. of Env.* **1992**, 41, 35–44, DOI: 10.1016/0034-4257(92)90059-S.
62. Gamon, J.A.; Field, C.B.; Bilger, W.; Bjorkman, O.; Fredeen, A.L.; Peñuelas, J. Remote sensing of xanthophyll cycle and chlorophyll fluorescence in sunflower leaves and canopies. *Oecologia* **1990**, *85*, 1–7, DOI: 10.1007/BF00317336.
63. Fourty, T.; Baret, F.; Jacquemoud, S.; Schmuck, G.; Verdebout, J. Leaf Optical Properties with Explicit Description of Its Biochemical Composition. Direct and Inverse Problems. *Rem. Sen. of Env.* **1996**, *56(2)*, 104–117, DOI: 10.1016/0034-4257(95)00234-0.
64. Merzlyak, J.R.; Gitelson, A.A.; Chivkunova, O.B.; Rakitin, V.Y. Non-destructive Optical Detection of Pigment Changes During Leaf Senescence and Fruit Ripening. *Physiol. Plant.* **1999**, *106(1)*, 135–141, DOI: 10.1034/j.1399-3054.1999.106119.x.
65. Nagler, P.L.; Inoue, Y.; Glenn, E.P.; Russ, A.L.; Daughtry, C.S.T. Cellulose absorption index (CAI) to quantify mixed soil–plant litter scenes. *Rem. Sen. of Env.* **2003**, *87(2-3)*, 310-325, DOI: 10.1016/j.rse.2003.06.001.
66. Gitelson, A.A.; Zur, Y.; Chivkunova, O.B.; Merzlyak, M.N. Assessing carotenoid content in plant leaves with reflectance spectroscopy. *Photochem. and Photobiol.* **2002**, *75(3)*, 272–281, DOI: 10.1562/0031-8655(2002)0750272ACCIPL2.0.CO2.
67. Gitelson, A.A.; Merzylak, M.N.; Chivkunowam, O.B. Optical properties and nondestructive estimation of anthocyanin content in plant leaves. *Photochem. and Photobiol.* **2001**, *74(1)*, 38-45, DOI: 10.1562/0031-8655(2001)074<0038:OPANEO>2.0.CO;2.
68. Rock, B.N.; Williams, D.L.; Vogehnann, J.E. Field and airborne spectral characterization of suspected acid deposition damage in red spruce (*Picea rubens*) form Vermont. Proceedings of 11th International Symposium Machine processing of Remotely Sensed Data, Purdue University, Lafayette, IN, USA 1985, pp. 71–81. Available online: https://www.researchgate.net/profile/Jim_Vogelmann/publication/4659490_Field_and_airborne_spectral_characterization_of_suspected_damage_in_red_spruce_picea_rubens_from_Vermont/links/546529ed0cf2052b509f2d37/Field-and-airborne-spectral-characterization-of-suspected-damage-in-red-spruce-picea-rubens-from-Vermont.pdf (accessed on 25.03.2017).
69. Hardisky, M.A.; Klemas, V.; Smart, R.M. The Influences of Soil Salinity, Growth Form, and Leaf Moisture on the Spectral Reflectance of Spartina Alterniflora Canopies. *Photogram. Eng. and Rem. Sen.* **1983**, *49(1)*, 77–83. Available online: https://www.asprs.org/wp-content/uploads/pers/1983journal/jan/1983_jan_77-83.pdf (accessed on 25.03.2017).
70. Gao, B.C. NDWI - A normalized difference water index for remote sensing of vegetation liquid water from space. *Rem. Sen. of Env.* **1996**, *58(3)*, 257-266, DOI: 10.1016/S0034-4257(96)00067-3.
71. Öquist, G.; Wass, R. A portable, microprocessor operated instrument for measuring chlorophyll fluorescence kinetics in stress physiology. *Physiol. Plant.* **1988**, *73*, 211–217, DOI: 10.1111/j.1399-3054.1988.tb00588.x.
72. Paryska, Z.; Paryski, W.H. Wielka Encyklopedia Tatrzańska. Wydawnictwo Górskie, Poronin, Poland, pp. 1995.
73. Ketchledge, E.H.; Leonard, R.E.; Richards, N.A.; Craul, P.F.; Eschner, A.R. Rehabilitation of Alpine Vegetation in the Adirondack Mountains of New York State. Newtown Square, PA: US Department of Agriculture Forest Service, Northeast Research Station, USA; 1985, Research Paper NE–553, pp. 1-10. Available online: http://www.fs.fed.us/ne/newtown_square/publications/research_papers/pdfs/scanned/OCR/ne_rp553.pdf (accessed on 25.03.2017).
74. Liddle, M.J. A theoretical relationship between the primary productivity of vegetation and its ability to tolerate trampling. *Biol. Conserv.* **1975**, *8(4)*, 251-255, DOI: 10.1016/0006-3207(75)90002-6.
75. Liddle, M.J. *Recreation ecology: the ecological impact of outdoor recreation and ecotourism*; Chapman & Hall, London, UK, 1997; pp. 639.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 3 April 2017 doi:10.20944/preprints201704.0007.v1

76. Cole, D.N.; Spildie, D.R. Hiker, horse and llama trampling effects on native vegetation in Montana, USA. *Jour. of Env. Manag.* **1998**, *53(1)*, 61–71, DOI: 10.1006/jema.1998.0192.
77. Littlemore, J.; Barker, S. The ecological response of forest ground flora and soils to experimental trampling in British urban woodlands. *Urban Ecosys.* **2001**, *5*, 257-276, DOI: 10.1023/A:1025639828427.
78. Monz, C.A. The response of two arctic tundra plant communities to human trampling disturbance. *Jour. of Env. Manag.* **2002**, *64(2)*, 207–217, DOI: 10.1006/jema.2001.0524.
79. Sun, D.; Liddle, M.J. Plant morphological characteristics and resistance to simulated trampling. *Env. Manag.* **1993**, *17(4)*, 511-521, DOI: 10.1007/BF02394666.
80. Price, M.F. Impacts of recreational activities on alpine vegetation in western North America. *Mount. Res. and Develop.* **1985**, *5(3)*, 263–277, DOI: 10.2307/3673358.
81. Bell, K.L.; Bliss, L.C. Alpine disturbance studies: Olympic National Park USA. *Biol. Conser.* **1973**, *5(1)*, 25-32, DOI: 10.1016/0006-3207(73)90051-7.
82. Calais, S.S.; Kirkpatrick, J.B. Impact of Trampling on Natural Ecosystems in the Cradle Mountain-Lake St Clair National Park. *Austral. Geograph.* **1986**, *17*, 6-15, DOI: 10.1080/00049188608702894.
83. Dumitrascu, M.; Marin, A.; Preda, E.; Tibirnac, M.; Vadineanu, A. Trampling effects on plant species morphology. *Rom. Jou. Biol. – Plant Biol.* **2010**, *55(2)*, 89–96. Available online: http://www.ibiol.ro/plant/volume%2055/art203.pdf (accessed on 25.03.2017).
84. Carter, G.A.; Knapp, A.K. Leaf optical properties in higher plants: linking spectral characteristics to stress and chlorophyll concentration. *Amer. Jou. of Bot.* **2001**, *88*, 677-684. Available online: http://www.amjbot.org/content/88/4/677.full (accessed on 25.03.2017).
85. Broge, N.; Leblanc, E. Comparing Prediction Power and Stability of Broadband and Hyperspectral Vegetation Indices for Estimation of Green Leaf Area and Canopy Chlorophyll Density. *Rem. Sen. of Env.* **2000**, *76*, 156-172, DOI: 10.1016/S0034-4257(00)00197-8.
86. Baker, N.R. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *An. Rev of Plant Biol*. **2008**, *59*, 89–113. DOI: 10.1146/annurev.arplant.59.032607.092759.
87. Bolhàr-Nordenkampf, H.R.; Öquist, G.O. Chlorophyll fluorescence as a tool in photosynthesis research. In: *Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual*, Hall, D.O.; Scurlock, J.M.O.; Bolhàr-Nordenkampf, H.R.; Leegoood, R.C.; Long, S.P., Eds.; Chapman & Hall, London, UK, 1993, pp. 193-206, DOI: 10.1007/978-94-011-1566-7_12.
88. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—a practical guide. *Jou. of Exp. Bot.* **2000**, *51(345)*, 659-668, DOI: 10.1093/jexbot/51.345.659.
89. Strasser, R.J.; Srivastava, A.; Tsimilli-Michael, M. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In *Probing Photosynthesis: Mechanism, Regulation and Adaptation*, Yunus, M.; Pathre, U.; Mohanty, P., Eds.; Taylor and Francis, London, UK; 2000, pp. 443-480.
90. Gola Golan, T.; Li, X.P.; Müller-Moulé, P.; Niyogi, K.K. Using mutants to understand light stress acclimationin plants. In: *Chlorophyll fluorescence: a signature of photosynthesis*, Papageorgiou G.C., Ed.; Springer, Dordrecht, Netherlands; 2004, pp. 525-554.
91. Kaiser, W.M. Effect of water deficit on photosynthetic capacity. *Physiol. Plant.* **1987**, *71(1)*, 142–149, DOI: 10.1111/j.1399-3054.1987.tb04631.x.
92. Ohashi, Y.; Nakayama, N.; Saneoka, H.; Fujita, K. Effects of drought stress on photosynthetic gas exchange, chlorophyll fluorescence and stem diameter of soybean plants. *Biol. Plant.* **2006**, *50(1)*, 138–141, DOI: 10.1007/s10535-005-0089-3.
93. Guóth, A.; Tari, I.; Gallé, A.; Csiszár, J.; Horváth, F.; Pécsváradi, A.; Cseuz, L.; Erdei, L. Chlorophyll a fluorescence induction parameters of flag leaves characterize genotypes and not the drought tolerance of wheat during grain filling under water deficit. *Acta Biol. Szeged.* **2009**, *53(1)*, 1–7. Available online: https://www2.sci.u-szeged.hu/ABS/2009/Acta%20HP/5301.pdf (accessed on 25.03.2017).
94. Tan, C.W.; Huang, W.J.; Jin, X.L.; Wang, J.C.; Tong, L.; Wang, J.H.; Guo, W.S. Monitoring the chlorophyll fluorescence parameter Fv/Fm in compact corn based on different hyperspectral vegetation indices. *Spectros. and Spectral Anal.* **2012**, *32(5)*, 1287-1291. Available online: http://www.gpxygpfx.com/qikan/public/tjdjl_en.asp?wenjianming=2012-05-1287&houzhui=.pdf&id=20015 (accessed on 25.03.2017).
95. Gitelson, A.A.; Merzlyak, M.N. Spectral Reflectance Changes Associated with Autumn Senescence of *Aesculus hippocastanum* L. and *Acer platanoides* L. Leaves. Spectral Features and Relation to

Chlorophyll Estimation. *Jou. of Plant Physiol*. **1994**, *143(3)*, 286–292, DOI: 10.1016/S0176-1617(11)81633-0.

96. Pickering, C.M.; Growcock, A.J. Impacts of experimental trampling on tall alpine herbfields and subalpine grasslands in the Australian Alps. *Jou. Env. Manage* **2009**, *91(2)*, 532-540, DOI: 10.1016/j.jenvman.2009.09.022.
97. Mason, S.; Newsome, D.; Moore S.; Admiraal, R. Recreational trampling negatively impacts vegetation structure of an Australian biodiversity hotspot. *Biodiver. and Conserv*. **2015**, *24(11)*, 2685-2707, DOI: 10.1007/s10531-015-0957-x.