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Mirsada STARČEVIĆ*[1], Laura SLEBIODA[2]
Dženana KUMALIĆ[3], Azra ČABARAVDIĆ[4]

ENVIRONMENTAL EFFECTS ON PHYSIOLOGICAL INDEX OF BLACK ALDER (*Alnus glutinosa* [L.] Gaertn.*)* DOMINANT TREES IN CENTRAL BOSNIA

SUMMARY

Alnus glutinosa (L.) Gaertn. appears naturally as stands, fragments, patches or river lines mainly around rivers and streams in central Bosnia. Considering spatial frame (mountainous on the headwaters and hilly valley afterward) arises the question of how *A. glutinosa* responds to exposed climate, hydrology and site conditions. We identified twenty tree temporary sample plots on five sites and conducted measurements of tree dominant trees per plot related to tree structural characteristics (diameter et breast height, height, crown projection) and registered the chlorophyll content index (CCI) in leaves of dominant trees monthly in season 2022. To examine structural, climate, hydrology and site effects on CCI quantity and dynamic we used descriptive statistics, analysis of variance and Kruskal – Wallis test. Obtained results indicated that dominant trees of *A. glutinosa* reach similar tree dimensions but physiologically responded differently depending on climate factors (mean annual temperature and maximal precipitation) and water (mean annual water flow and water level) fluctuations pointing out site-dependent responses. We notified increase in CCI on the sites exposed to reduced maximal precipitation and shortage of water at the second half of season. Our results confirmed *A. glutinosa* ability to resist climate challenges and contribute to ecological services on different sites in central Bosnia.

Keywords: black alder, dominant trees, chlorophyll content index, annual variation.

[1]Mirsada Starčević (corresponding author: m.starcevic@sfsa.unsa.ba), Department for Forest Ecology and Urban Greenery, University of Sarajevo, Faculty of Forestry, Zagrebačka 20, Sarajevo, 71000, BOSNIA AND HERZEGOVINA

[2]Laura Slebioda, Department of Mathematical and Statistical Methods, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, POLAND

[3]Dženana Kumalić, University of Sarajevo, Faculty of Forestry, Zagrebačka 20, Sarajevo, 71000, BOSNIA AND HERZEGOVINA

[4]Azra Čabaravdić, Department for Forest Management and Urban Greenery, University of Sarajevo Faculty of Forestry, Zagrebačka 20, Sarajevo, 71000, BOSNIA AND HERZEGOVINA

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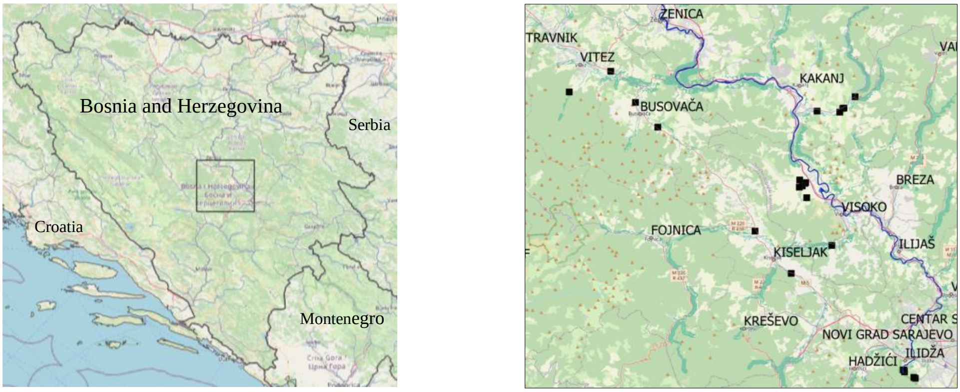
INTRODUCTION

Black alder (*Alnus glutinosa* (L.) Gaertn.) is a tree species situated in different conditions and on different site types across Europe, from central Scandinavia on the north, to the North Africa on the south. Development types of black alder communities range from highly productive closed canopy forest stands (Poland, Croatia) to environmentally important small stands and scattered groups of trees on riversides and planes (Mediterranean region). Similar site conditions for black alder are found in internal Dinarides in the Western Balkans (Vukelić *et al*., 2006; Barudanović, 2007; Laganis, 2007; Rakonjac *et al*., 2009; Spalević *et al*., 2013; Simovski *et al*., 2019). Dominant black alder habitat is situated on plane, riparian areas with available ground water mainly but the species appears also on hilly and lower mountain ranges with changeable environmental impacts. Mandák *et al.* (2016) reported about black alder genetic pools in Europe identifying occurrences of black alder on hilly and lower mountain ranges in internal Dinarides in central Bosnia, at the north of Montenegro and at the east and central Serbia. Although, many studies have been done about black alder interaction with environment in riparian zones on large planes, little literature is available on its interaction on hilly and lower mountain range zones. Recently, black alder situated in hilly and lowland valleys is exposed to rapid urbanization (land-use change and human pressure) and climate changes (temperature increase and water shortage). Like all native plants, black alder is exposed to different environmental conditions influencing its functional responses. Main environmental influences are related to orography, edaphic and climatic characteristics as well as tree and stand structural characteristics. Recently, chlorophyll content index (CCI) non-destructively registered in tree leaves has been promoted as a physiological index (a functional treat) reflecting the interaction between trees and the environment (Hendry *et al*., 1987; Terzi *et al*., 2010; Zhang *et al*., 2011; Talebzadah and Valeo 2022). Several studies confirmed that environmental pressures reflect on CCI index differently between different tree species within ecotypes of single species (Tenkanen *et al*. 2019). Changes of CCI were observed for several tree species during the vegetation season indicating environment x tree species interaction (Demarez *et al*., 1999; Gond *et al*. 1999; Uvalle Sauceda *et al*., 2007; Mõttus *et al*., 2014; Şevik *et al*., 2015; Croft *et al*., 2017; Tenkanen *et al*., 2019; Atar *et al*., 2020). Previous study about black alder reported about high CCI with increasing trend achieving the highest value before leaves falling at the end of vegetation season (Laganis, 2007).

The main aim of this study was to examine CCI annual variation related to main climate factors (temperature and water regime) and different site conditions in central Bosnia. We formulated the following research questions: (i) is average CCI affected by tree dimensions (tree height, DBH, crown area), orography (altitude, slope), climate factors (mean temperature, mean precipitation) and water regime (water level, water flow rate), (ii) is CCI intra-annual variation affected by climate and water regime variables and (iii) is intra-annual CCI variation site dependent. This study may lead to a better understanding of black alder resilience on hilly and lower mountain range areas what is relevant for sustainable forest and environmental management.

MATERIAL AND METHODS

Study area. The selected sites are all located at the subsection of the Bosna River basin examined in this study stretches from Sarajevo and flows north toward the city of Zenica where the Lašva River joins it in central Bosnia and Herzegovina within a smaller area of 1,512 km^2 (41.2 km by 36.7 km) from 43°81' to 44°14' N and 17°77' to 18°29' E (Fig. 1a). (Fig. 1b).



a) b)

Figure 1. Bosnia and Herzegovina with study area (a), Central Bosnia – sampling plan (black square - sample plots) (b)

Here are noticed changeable site conditions: (a) mountainous vs. plane and riverside positions, (b) waterlogged vs. low water level and low water flow rate sites, (c) protected landscape vs. sites under human pressures with sporadic industrial air and water pollution incidents and (d) natural site condition vs. sites surrounded with agricultural land near settlements.

The dominant forest types in the study area with complex petrographic composition (especially tertiary flyschoid complexes) are beech forests, oak forests and oak and hornbeam forests with mosaic spatial distribution (Stefanović *et al*., 1983). The soil types in the study area, stretching from the spring of the Bosna River downstream, are combinations of fluvisols, dystric and eutric cambisols, calcomelanosols and calcocambisols (Ćesir, 2022).

The climate for this region is humid continental tempered by the Adriatic Sea, with an average annual temperature of 10 °C, while minimal average temperatures occur in January (-0.5 to -4.3 °C) in Sarajevo and Zenica, respectively, and maximum temperatures in July/August (20 to 26 °C). Precipitation differs significantly between Sarajevo and Zenica, where in Sarajevo it ranges from around 64 mm in February to 91 mm in September, while in Zenica it ranges from 34 mm in September to 81 mm in May (Federal Hydro-meteorological Institute). The mean, maximal and minimal temperatures and precipitation were recorded monthly at two metrological stations: at the headwaters on the south (central Bosnia) and at the station near the Bosna River 100 km far away on the north.

Data acquisition. Temporary 23 sample plots varying in size from 60 to 300 m^2 with an average area of 190 m^2 were selected within black alder dominant sites. The plots are situated along a longitudinal gradient and are exposed to various environmental and anthropogenic conditions. The elevation of the sample plots ranged from 385 to 500 meters above sea level (m.a.s.l), while the slope ranged from 0 to 5.6°.

The plant communities examined in this study were all black alder dominant, appearing in two general forms: at the Bosna River headwaters as the forest stand (Fig. 2a), and the other sites as isolated stands or groups of trees along waterways (Fig. 2b).

(a)

(b)

Figure 2. Black alder sites: Closed forest stand (a), River line tree formation (b)

Sample plots are clustered on five sites following a longitudinal gradient. Site 1 is located at the Bosna headwaters, below Igman mountain with a shaded position and colder climate (Ilidza). Sites 2 and 3 occupy sunny exposed planes near fertile agricultural fields around Bosna River (Visoko) and near the industrial area (Kakanj). Sites 4 and 5 are situated in uphill positions, but Site 4 is closer to the river (Kiseljak) while Site 5 occupies drier positions (Busovaca). The dominant and neighboring trees were selected at the beginning of spring (March and April 2022) during flowering. The diameter at breast height (DBH), height and crown area of the three largest trees were measured in April 2022. Descriptive statistics of tree, orography and climatic variables are presented in Table 1.

In-situ measurements related to the chlorophyll content in leaves were determined using a CCM-200 hand-held chlorophyll meter (Opti-Sciences, Massachusetts, USA) and registered as CCI between April and October 2022.

Methods. The data analysis began by conducting descriptive statistics to examine the mean, standard deviation, maximum and minimum values for various variables, including tree structural variables, orography variables, climate variables and a physiological variable: Chlorophyll Content Index (CCI). Descriptive statistics were also calculated for chlorophyll content per month. Next, correlation analyses were conducted using both Pearson's and Spearman's correlation coefficients. The strength of correlations was examined between CCI and the variables as well as between CCI, climate variables and water variables during the season. To assess the normality of distribution, the Shapiro – Wilk test was performed. Furthermore, the differences in structural, functional, and environmental variables, as well as CCI values between sites, were examined using both parametric ANOVA and non – parametric Kruskal – Wallis test. The intra–annual CCI variations across different sites were analyzed using repeated – measurement ANOVA, considering Mauchly's sphericity test with Huynh – Feldt correction applied. All statistical analyses were performed using the "dplyr" package (R Statistical Software version 4.2.2; R Core Team 2022).

RESULTS

Correlations between trees' structural and environmental variables with chlorophyll content index in leaves of dominant trees

Table 1. Descriptive statistics (n = 23).

Variable	Mean	Standard deviation	Minimum	Maximum
Tree structural variables				
Tree height (m)	18.43	4.42	9.90	25.50
Diameter at breast height (cm)	38.93	11.84	20.00	72.20
Crown projection area (m^2)	53.21	33.69	5.11	142.08
Orography variables				
Altitude (m.a.s.l)	449.96	35.85	384.40	500.00
Slope (^{o})	1.95	1.70	0.50	5.64
Climate variables				
Mean annual temperature (^{o}C)	14.04	0.70	12.68	15.02
Mean maximal precipitation (mm)	57.73	0.93	56.63	60.28
Mean annual water level (cm)	99.77	60.19	9.30	230.90
Mean annual water flow rate (m^3/sec)	22.03	11.88	3.60	36.10
Chlorophyll content index	27.53	7.31	14.4	40.6

Table 1 provides important findings regarding the descriptive statistics of the variables examined. These descriptive statistics provide an overview of the central tendency, variability, and range of values for each variable. High standard deviation can be seen for crown projection area (33.69), altitude (35.85) and mean annual water level (60.19), whereas for mean annual temperature and mean maximal precipitation standard deviation is quite low (respectively 0.70, 0.93).

Table 2 presents the correlations between the chlorophyll content index (CCI) and the variables examined. What is worth noting, there is a significant moderately positive correlation between mean annual temperature and CCI, with Pearson's correlation coefficient of 0.42 and Spearman's correlation coefficient of 0.45. There is also a significant correlation between mean annual water flow rate and CCI that is moderately positive, with Pearson's correlation coefficient of 0.41. Spearman's correlation coefficient was 0.38.

The findings show that tree height, altitude, mean annual temperature, and mean annual water flow rate may have more notable associations with chlorophyll content, while the other variables show weaker correlations.

Table 2. Correlations between chlorophyll content index and variables

Variable	Pearson's	Spearman's
Tree height (m)	-0.20	-0.19
Diameter at breast height (cm)	0.12	0.13
Crown projection area (m^2)	0.01	-0.03
Altitude (m.a.s.l)	-0.26	-0.23
Slope (^{o})	0.18	0.10
Mean annual temperature (^oC)	0.42*	0.45*
Mean maximal precipitation (mm)	-0.12	-0.17
Mean annual water level (cm)	0.03	-0.02
Mean annual water flow rate (m^3/sec)	0.41*	0.38

Chlorophyll content index intra-annual variation. Table 3 provides the dates of data acquisitions and summary statistics for chlorophyll content per month. The chlorophyll content tends to increase from May to August, reaching its peak in August (maximum = 59.9, minimum = 16.2), and then gradually decreases in September and October. The range of chlorophyll content values also tends to widen from May to August, indicating greater variability in chlorophyll levels during this period. On average, the highest chlorophyll content is observed in September (32.88).

Table 4 presents the correlation matrix, which explores the relationships between the chlorophyll content index (CCI) and various climate and water variables during the season. Notably, significant correlations are observed between CCI and specific variables. First, there is a moderate positive correlation between CCI and mean maximal precipitation (0.47), indicating that higher levels of precipitation are associated with higher chlorophyll content. Secondly, a very strong negative correlation is observed between CCI and mean annual water level (-0.83). This finding suggests that higher water levels are linked to lower chlorophyll content. Furthermore, a very strong negative correlation is observed between CCI and mean annual water flow rate (-0.82). This implies that higher rates of water flow are associated with lower chlorophyll content.

Table 3. List of dates of data acquisitions and summary statistics for chlorophyll content per month

Month	Day of the Year	Mean	St. dev.	Min.	Max.
May	139	22.89	7.90	9.7	32.7
Jun	154	24.65	7.05	10.7	36.3
July	200	27.24	8.99	12.7	43.3
August	229	31.20	11.00	16.2	59.9
September	249	32.88	10.35	11.9	47.9
October	289	27.44	7.55	15.6	41.1

Table 4. Correlation matrix for chlorophyll content index, climate and water variables during the season

Variable	Temp.	Precip.	Water level	Water flow rate
Mean annual temperature (^oC)	1			
Mean maximal precipitation (mm)	-0.12	1		
Mean annual water level (cm)	-0.03	0.04	1	
Mean annual water flow rate (m^3/sec)	0.06	-0.03	0.99	1
Chlorophyll content index	-0.09	0.47*	-0.83*	-0.82*

Abbreviation: Precip. - precipitation.

Figure 3a shows that there are no significant differences in mean air temperature in the southern and northern regions of the study area.

In Figure 3b differences in mean precipitation can be noticed. In May, June, July and September there was more precipitation in the north. Much dissimilarity is seen especially in May. On the other hand, in January, April and August there was more precipitation in the south. In Figure 3c substantial disparity can be observed. The water level was much higher in northern regions. Moreover, in Figure 3d higher water flow rate in the north can also be seen. From February to June this rate is the highest.

Spatial and annual variability of CCI. Table 5 presents the descriptive statistics for various variables measured at different study sites. The table provides a summary of the measurements taken at each site, allowing for comparisons and identification of any differences or patterns among the variables across the study sites.

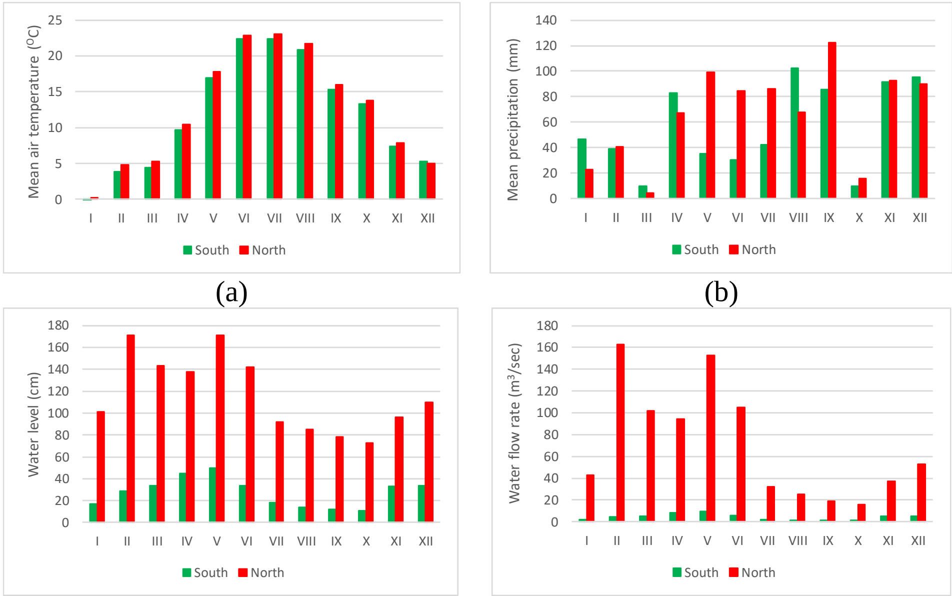


Figure 3. Climate and water intra-annual dynamics on the south and the north of the study area (a) mean air temperature, (b) mean precipitation, (c) water level, (d) water flow rate.

A significant variation in tree height, diameter at breast height, and crown projection area among the different sites can be seen. Site 2 has the tallest trees with an average height of 20.8±2.4 meters, while Site 3 has the shortest trees with an average height of 13.2±4.5 meters. Site 1 has the largest crown projection area with an average of 63.5±24.2 square meters.

The altitude and slope exhibit notable differences across the sites. Site 1 has the highest altitude at 496±2.6 meters above sea level, Site 5 has the lowest altitude at 419±37.2 meters above sea level. Site 4 has the steepest slope with an average of 3.4±1.9 degrees.

Mean annual temperature and mean maximal precipitation show slight variations among the sites. Site 4 has the highest mean annual temperature of 15.0±0.1 degrees Celsius, while Site 1 has the lowest mean annual temperature of 13.4±0.1 degrees Celsius. The mean maximal precipitation ranges from 57.0±0.3 to 58.5±1.3 millimeters, with no significant differences observed. There are significant differences in mean annual water level and mean annual water flow rate among the sites. Site 5 has the highest mean annual water level at 185.7±31.6 centimeters, while Site 1 has the lowest mean annual water level at 9.9±0.4 centimeters. Site 2 has the highest mean annual water flow rate at 32.4±2.1 cubic meters per second, while Site 1 has the lowest mean annual water flow rate at 3.9±0.3 cubic meters per second.

The chlorophyll content index shows higher values at Site 1, whereas the lowest values are at Site 4.

Overall, the findings from Table 5 highlight the significant differences in tree structural variables, orography, water variables and chlorophyll content index among the study sites, while climate variables and show relatively consistent values across the sites.

Table 5. Descriptive statistics of tree structural variables, orography, and annual climate and CCI mean values per site

Variable	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Number of plots/trees	5	6	3	5	4
Tree structural variables					
Tree height (m)	18.9±6.2	20.8±2.4	13.2±4.5	16.8±1.4	20.3±4.5
Diameter at breast height(cm)	36.5±5.3	42.1±11.5	25.9±7.9	41.8±18.5	43.4±5.9
Crown projection area (m^2)	63.5±24.2	62.3±31.9	21.7±15.3	58.3±48.7	51.0±32.7
Orography variables					
Altitude (m.a.s.l)	496±2.6	422±10.6	468±24.7	449±37.2	419±37.2
Slope (^{o})	1.0±0.8	2.1±2.2	0.9±0.4	3.4±1.9	1.9±1.2
Climate variables					
Mean annual temperature (^{o}C)	13.4±0.1	14.4±0.1	13.6±0.5	15.0±0.1	13.5±0.5
Mean maximal precipitation (mm)	57.2±0.1	57.0±0.3	57.7±0.9	58.5±0.7	58.5±1.3
Mean annual water level (cm)	9.9±0.4	97.0±6.5	110.8±44.3	117.7±12.0	185.7±31.6
Mean annual water flow rate (m^3/sec)	3.9±0.3	32.4±2.1	13.9±2.2	32.0±3.1	22.7±4.0
Physiological variable					
Chlorophyll content index	496±2.6	422±10.6	468±24.7	419±37.2	449±37.2

Table 6 provides a summary of the significant values based on the chosen tests for different variables. If the data was found to follow a normal distribution based on the Shapiro – Wilk test, the analysis of variance (ANOVA) test was performed in addition to the Kruskal – Wallis test. However, if the data did not follow a normal distribution, only the Kruskal – Wallis test was conducted.

Table 6. Summary of significant values based on chosen test

Variable	Shapiro-Wilk test	Normal Distribution	Test	*p*-value
Tree structural variables				
Tree height (m)	$p = .586$	Yes	ANOVA	.110
			Kruskal – Wallis	.091
Diameter at breast height (cm)	$p = .088$	Yes	ANOVA	.282
			Kruskal – Wallis	.158
Crown projection area (m^2)	$p = .089$	Yes	ANOVA	.483
			Kruskal – Wallis	.225
Orography variables				
Altitude (m.a.s.l)	$p = .055$	Yes	ANOVA	<.001*
			Kruskal – Wallis	.003*
Slope (^{o})	$p < .001$	No	Kruskal – Wallis	.473
Climate variables				
Mean annual temperature (^{o}C)	$p = .077$	Yes	ANOVA	<.001*
			Kruskal – Wallis	<.001*
Mean maximal precipitation (mm)	$p = .006$	No	Kruskal – Wallis	.009*
Mean annual water level (cm)	$p = .089$	Yes	ANOVA	<.001*
			Kruskal – Wallis	<.001*
Mean annual water flow rate (m^3/sec)	$p = .004$	No	Kruskal – Wallis	<.001*
Physiological variable				
Chlorophyll content index	$p = .774$	Yes	ANOVA	.063
			Kruskal – Wallis	.050·

The ANOVA and Kruskal – Wallis tests done on tree structural variables, both yield non-significant *p*–values, suggesting no significant differences among the groups. Considering orography variables, for altitude the ANOVA and Kruskal – Wallis tests show highly significant *p*–values (respectively, $p < .001$, $p = .003$), showing significant differences in altitude among the groups. For slope, Kruskal – Wallis test yields a non–significant *p*–value ($p = .473$). On the other hand, all climate variables have highly significant *p*–values indicating significant differences among the groups. For chlorophyll content index, ANOVA test yields a non–significant *p*–value ($p = .063$), while Kruskal – Wallis test shows a *p*–value of .050, indicating a borderline significant difference in chlorophyll content index among the groups.

These findings indicate that there are significant differences among the groups in terms of altitude, mean annual temperature, mean maximal precipitation, mean annual water level, and mean annual water flow rate. However, there are no significant differences observed in tree height, diameter at breast height, crown projection area, slope, and chlorophyll content index among the groups.

Figure 4 illustrates the temporal variation of the chlorophyll content index (CCI) across different sites. It visually represents the fluctuations in CCI values over time at each site.

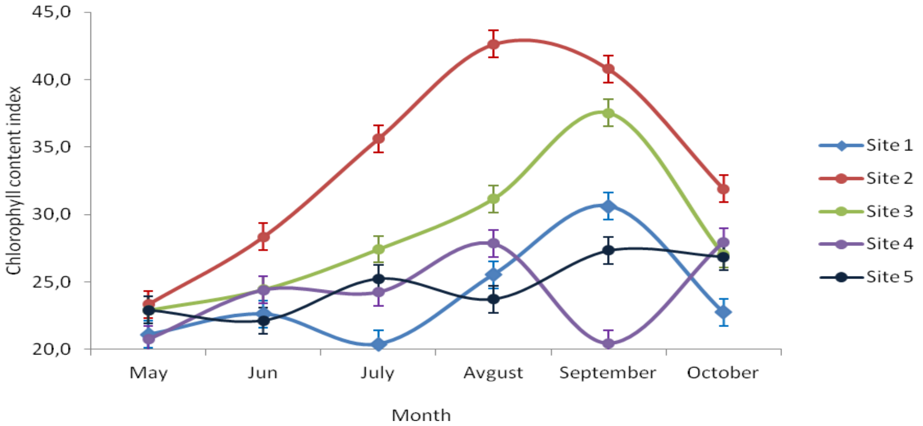


Figure 4. Profile plot month x site with LS confidence intervals

In Table 7, the results of the repeated measures analysis of variance (RM ANOVA) considering Mauchly's sphericity test with applied Huynh–Feldt correction are presented.

The variation between sites was assessed, and it yielded a non–significant F–value ($p = .063$; $F = 2.71$), indicating that there were no significant differences in the dependent variable among the different sites.

The within–subjects error variation (within the tree factor) was calculated. The sum of squares was 4395.57, with 18 degrees of freedom, and the mean square was 244.199. The obtained F–value was statistically significant ($p < .001$; $F = 10.30$), indicating that there were significant differences in the dependent variable within the tree factor.

Table 7. RM ANOVA considering Mauchly's sphericity test (W=0.16, p=0.01) with applied Huynh–Feldt (0.96) correction

Source of variation	Sum of squares	d.f.	Mean square	F	*p*-value
Site	2650.18	4	662.545	2.71	.063
ERROR(Tree)	4395.57	18	244.199	10.30	<.001*
Month	1924.20	4.8	401.24	16.23	<.001*
Month*Site	921.645	19.2	48.05	1.94	.020*
ERROR(Month)	2133.39	90	23.70		

The variation between months was examined, and it showed a highly significant F–value ($p < .001$; F = 16.23), which means that there were significant differences in the dependent variable across different months.

The interaction effect between month and site was evaluated. The F–value was statistically significant ($p = .020$; F = 1.94), showing that there was a significant interaction effect between the month and site factors on the dependent variable.

Notably, Site 2 exhibits the highest CCI values throughout the observed period. In contrast, Sites 1, 4, and 5 demonstrate relatively lower CCI values. Specifically, Site 1 exhibits the lowest CCI values in July, Site 4 in September, and Site 5 in June (Figure 4).

Trend analysis showed significant linear and quadratic trends for CCI changes (Table 8). Trends were significant for sites 1, 2, 3 and 5.

Table 8. Trends of chlorophyll content index on different sites

Site	Trend	t	*p*-value
Site 1	linear	2.46	.016*
	quadratic	-1.37	.175
Site 2	linear	3.52	<.001*
	quadratic	-4.04	<.001*
Site 3	linear	3.35	.001*
	quadratic	-2.29	.024*
Site 4	linear	1.59	.116
	quadratic	-0.64	.523
Site 5	linear	2.40	.018*
	quadratic	-0.51	.612

Sites 1 and 5 exhibited a linear increasing trend present on non-disturbed sites (Atar, 2020) while trends were non-linear with extreme values in the second period of the growing season on sites 2 and 3 (Fig. 4).

DISCUSSION

Chlorophyll content in forest tree leaves. Changeable environmental and climatic conditions influence chlorophyll content and its seasonal dynamics in forest trees (Croft *et al*. 2017; Atar *et al*. 2020). Mainly, chlorophyll content was investigated for deciduous tree species (Bassow, 1997; Demarez *et al*., 1999, Richardson *et al*., 2002) using different instruments. Brown *et al*. (2022) found that CCM-200, among other instruments, represents a suitable choice for CCI determination of some deciduous tree species (ash, beech, silver beech, hawthorn, red maple and sycamore). In the present study, CCI achieved mean of 28.4 units ranging from 10 to 60 in fully developed leaves of black alder during the growing season. In the greenhouse experiment, Sever (2018) reported CCI means in two-year-old oak leaves in control and drought treatment with 13.1 and 21.3 values respectively. Brown *et al*. (2022) reported CCM-200 recordings ranged from 5 to 65 differing between ash, beech, silver beech, hawthorn, red maple and sycamore. Also, similar CCI ranges are presented for hawthorn, hazel and beech in Brown *et al*. (2022) study. Related to the reported results measured by CCM-200 instrument, black alder CCI mean of dominant trees is higher than in silver birch (Tenkanen *et al*. 2019). Assuming similarity between chlorophyll content registered with CCM-200 and other instruments (SPAD-502) mean CCI value of black alder is lower than in oaks (*Quercus petrea* Liebl. and *Q. robur* L.), beech (*Fagus sylvatica* L.) and hornbeam *(Carpinus betulus* L.) leaves (Demarez et al. 1999) and twelve deciduous tree species (*Quercus hartwissiana* Steven, *Ginkgo biloba* L., *Fagus orientalis* Lipsky, *Quercus castaneifolia* C. A. Mey., *Ulmus minor* Mill., *Cinnamomum camphora* (L.) Sieb., *Liquidambar orientalis Mill.*, *Acer negundo* L., *Quercus pubescens* Willd., *Quercus rubra* L. *and Aesculus hippocastanum* L.) in Turkey (Atar *et al*., 2020).

Intra-annual variation of chlorophyll content index of black alder trees. Related to chlorophyll content annual profile, it is known that deciduous trees begin leaf development with strongly increasing chlorophyll content from April to May. Then, between June and August chlorophyll content reaches a stable level and starts to decline from September to October/November (Demarez *et al*., 1999). Annual profiles of chlorophyll content differ between tree species. Demarez *et al*. (1999) studied an annual variation of leaf chlorophyll content of a temperate forest (oak, beech and hornbeam). Croft *et al*. (2017) investigated trembling aspen (*Populus tremuloides* Michx.), red maple (*Acer rubrum* L.), bigtooth aspen (*Populus grandidentata* Michx.) and white ash (*Fraxinus americana* L.) describing slow increase at the start of the season until values stabilize in the middle of the growing season and declining during leaf senescence. The highest CCI values in the middle of the growing season achieved bigtooth and trembling aspen while ash and maple reached lower CCI values. Taulavuori (2006) reported that *Alnus glutinosa* maintains higher chlorophyll concentration and higher ratios of chlorophyll to other pigments which result in prolonged photosynthetic activity in the growing season.

We observed almost full leaf development in the first half of May starting from the beginning of May which was not so early, but we noticed intensive leaf development till the end of August as well as active leaves on the crown base in September too. High CCI values were stable during the long period and a sharp decrease appeared at the end of overall vegetation activities (the end of September to the middle of October). Our CCI results are consistent with other authors' findings related to long and stabile black alder leaf functionality.

Also, we determined a significant annual change of CCI values from the beginning (15 May) and the end (29 October) of the growing season with higher CCI (difference of 17 CCI units). Atar *et al*. (2020) compared chlorophyll contents between the beginning and the end of the growing season for twelve tree species in Turkey and reported significant changes and higher CCI at the end of the growing season for all tree species except *Quercus hartwissiana* Steven, *Cinnamomum camphora* (L.) Sieb., and *Ginkgo biloba* L.

Intra-annual variation of chlorophyll content index of black alder trees on different sites. Considering chlorophyll content on different sites, the black alder appears on various site types such as waterlogged, plateau and riverside sites (Claessens *et al*., 2010) affecting physiological processes and tree responses (Miller, 2012). Talebzadeh and Valeo (2022) stated that chlorophyll content is a species-specific feature that could be upgraded with the assumption that any change in chlorophyll content in a plant is a reflection of weather or environmental impacts on specific sites. Zielewich *et al*. (2020) found that evaluation and monitoring of chlorophyll content in tree leaves reflected site-dependent environmental stressors, weather, climatic and anthropogenic conditions.

In our study, sites differ greatly although CCI mean differences were not significant ($p = 0.06$).

Sites 1 and 2 differ in environmental conditions. Site 2 having the highest CCI values is characterized by plain, sunny positions surrounded by fertile agricultural land while Site 1 is situated at headwaters with a colder climate and hilly positions. Also, sites differ in hydrological conditions. The lowest mean values for water level and water flow rate are registered on Site 1 while higher water levels and water flow rates were present on other sites. Many studies related differences in physiological processes and tree responses to hydrological conditions of sites in interaction with the period of growing season (time).

Related to annual changes in chlorophyll content, environmental variation of different conditions occurs as a complex interaction and species characteristics vary accordingly (Miller, 2012). Annual changes of tree physiological activity depend on the species identity, climate, and site conditions (Hooper *et al*., 2005; Jucker *et al*., 2016). Tenkanen *et al*. (2019) obtained a significant effect of the interaction of dates and sites on CCI means for silver birch in northern Europe.

In the present study, significant differences are obtained for CCI means registered at different sites at different dates. Those differences appeared in July, August and September. In each month, Site 2 reached the highest CCI mean while Site 1 achieved the lowest value or belonged to the group with statistically lower CCI means. In addition, analyzing climate factors, we noticed that Site 1 had lower mean air temperature and maximal precipitation in the first part of the growing season which could contribute to lower CCI. Analyzing water supply conditions, we observed lower water levels and flow rates at Site 1. Hydrological conditions on other sites had two characteristic periods: in the first part of the growing season water level and water flow rate were very high and then in July declined sharply by more than 50%. Recent studies connected CCI increase in forest trees with environmental stress, more specifically with low water level (drought effect) (Arend *et al*., 2016; Hagedorn *et al*., 2016; Sever *et al*., 2018). According to Hagedorn *et al*. (2016), additional photosynthetic activity appears to overcome drought stress after a drought period. In our study, on Site 2 sharp CCI maximal increase appeared shortly after the water decline achieving maximal value and then decreasing progressively.

CONCLUSIONS

Black alder is addressed as an important tree species adaptable to different site conditions in riparian, hilly and lower mountain areas. This study examined CCI annual variation related to main climate (temperature, precipitation) and ground water (water level and flow rate) condition variations on different sites in central Bosnia. Annual variation of climate and water conditions affected annual CCI in interaction month vs. site resulting in CCI significant increase on sites with reduced water quantities. Although climate and water conditions differ on sites, dominant trees reach similar tree dimensions (DBH, height and CPA) as well as mean CCI. It seems that black alder dominant trees resist changeable annual environmental effects what confirm their functionality in similar conditions. Forest management should support sustainable functioning of black alder providing regular water supply and stabile water regime reducing water stress and maintaining conditions for regular black alder growth. Primary strategic and planning objectives should be complemented with the protection of the valuable black alder native habitats and those at highest risk.

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