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Changes in the biochemical compounds of *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, and forest litter collected from various forest types

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Abstract: The aim of this study was to evaluate the impact of forest clear-cutting on the phenolic compounds and antiradical activity of *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, and forest litter collected during the vegetation stages in 2 forests types (*Pinetum vacciniosum* and *Pinetum vaccinio-myrtillosum*). The Folin-Ciocalteu method, aluminum trichloride colorimetric assay, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) test were applied to perform the total phenolic content (TPC), total flavonoid content (TFC), and antiradical activity analyses of the extracts of the underground and aboveground parts of the plants and forest litter. The TPC content varied from 35.87 to 229.76 mg/g (expressed as rutin equivalents) and 120.03 to 309.64 mg/g in the aboveground extracts of *V. myrtillus* and *V. vitis-idaea*, respectively. Remarkably lower amounts of phenolic compounds were determined in the underground extracts of the tested shrubs. The TPC content in the forest litter ranged from 3.12 to 11.89 mg/g. The radical scavenging activity (RSA) varied from 3.12 to 434.68 mg/g; the lowest antiradical activity was determined in the underground extracts, while the highest was in the aboveground extracts of *V. myrtillus.* The TFC was dependent on the vegetation phase, forest type, and clear-cutting, and varied from 7.97 to 40.18 mg/g in the aboveground extracts of the tested plants. Flavonoids were not detected in the underground extracts of the samples or in the forest litter. The chemometric analysis revealed statistically significant trends of environmental impact on *V. myrtillus* and *V. vitis-idaea* at the different vegetation stages. Hypotheses testing showed that the TPC, TFC and RSA expressed statistically significant ($\alpha \leq 0.05$) changes in 68%, 60% and 71% of the tested samples after clear-cutting, respectively.

Key words: Bilberry, chemometric methods, lingonberry, total phenolic content, total flavonoid content, vegetation phase

1. Introduction

Vaccinium sp. is a widespread genus of dwarf-shrubs growing in the southern areas of taiga forests to the north areas of tundra forests. The well-known berries of these shrubs, such as cranberry, blueberry, bilberry, and lingonberry, are popular all over the world due to their high source of vitamins, phenolic compounds, organic acids, and minerals (Vendrame et al., 2016).

Arbutin, hydroquinone, pyroside, salidroside, benzoic acid, vanillic acid, and p-hydroxybenzoic acids are the main phenolic compounds detected in *V. vitis-idaea* L. (Saario et al., 2002). Chlorogenic acid is the main acid in *V. myrtillus* L. leaves (Liu et al., 2014; Bujor et al., 2016). In bilberry fruit, high amounts of anthocyanins and hydroxycinnamic acid derivatives, as well as low amounts of flavonols, proanthocyanidins, and coumaroyliridoids, have been identified (Mikulic-Petkovsek et al., 2015).

The berries of *V. myrtillus* and *V. vitis-idaea* are the most popular part of these plant among consumers and scientists, as a research object, due to their high antioxidant potential (Chu et al., 2011; Blumberg et al., 2013; Vendrame et al., 2016). Moreover, the leaves of these bushes are rich in antioxidants and other biologically active compounds that exhibit a wide spectra of biological activities (Vyas et al., 2013; Liu et al., 2014; Ferlemi and Lamari, 2016). Both *V. myrtillus* and *V. vitis-idaea* are forest plants, therefore the growth and production (berries or leaves) of these shrubs might be dependent on forest ecosystem sustainability.

Phenolic compounds are important, not only due to their positive effect on human health, but because they are also important for the survival of plant, since they act as defensive agents against solar UV light, bacterial infections, and herbivores (Haukioja, 2005). Plant phenolics are one of the most widespread groups of secondary metabolites, and

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they play a significant role against competing plants (War et al., 2012). These compounds are found as glycosides in vacuoles as well as in the walls of plant cells. The top of forest litter is also a source of phenolic substances (Kuiters, 1990). These substances are naturally present in forest soil due to the biochemical transformation of organic components. Muscolo et al. (2005) opined that phenolics were released into the soil as root exudates and play a significant role in plant-litter-soil interactions.

Previous studies of natural products have shown that the content of biochemical compounds are highly affected by plant cultivar (Usanmaz et al., 2019), climate (Koç et al., 2018), and production area (Usanmaz et al., 2018). The phenolic composition of forest litter and soil depends on forest plant species and soil types, whereas these compounds act as indicators of litter quality by inhibiting microbial activity (Strobel, 2001; Li et al., 2016). Therefore, understanding the dynamics of phenolic compounds in forest plants and litter can help to identify the litter decomposition mechanisms in forest ecosystems, while these processes influence nutrient cycling by inhibiting organic matter degradation, mineralization rates, and azote availability (Kraus et al., 2003).

The impact of forest clear-cutting on the understory plants and insect populations, as well as the soil or forest litter, is not clear and remains under debate among scientists (Nielsen et al., 2007; Nybakken et al., 2013; Bogdziewicz and Zwolak, 2014; Tariq and Aziz, 2015; Okonogi and Fukuda, 2017). Forest clear-cutting may result in changes in the nutrient availability for plants, such as carbon, nitrogen, phosphorus, potassium, and calcium. It is believed that enhanced solar irradiation may increase the availability of carbon sources due to increased photosynthesis, but it may also enhance the stress level of the plant (Nybakken et al., 2013). The amount of nitrogen, phosphorus, potassium, and calcium in ground vegetation decreases after forest clear-cutting for a few years and after 5 years, it returns to the precutting level (Palviainen et al., 2005). The response of *V. myrtillus* and *V. vitis-idaea* to clear-cutting is not very clear. The aim of this study was to evaluate the impact of clear-cutting on the phenolic compounds, flavonoids, and antiradical activity of the underground and aboveground parts (extracts) of *V. myrtillus* (bilberry) and *V. vitis-idaea* (lingonberry) during their vegetation phases. Furthermore, this study will reveal the total amount of phenolic compounds and antiradical activity of *V. myrtillus* and *V. vitis-idaea* growing in Lithuania, as it is already well-known that the accumulation of biologically active compounds depends on the geographic origin of the plant, climatic and environmental conditions, or its genotype (Kaškonienė et al., 2011; Kaškonienė et al., 2013; Kaškonienė et al., 2015b; Kaškonienė et al., 2016).

2. Materials and methods

2.1. Plant material

Shoots with leaves (aboveground part) and rhizomes (underground part) of *V. myrtillus* and *V. vitis-idaea* were collected at different growing stages in April (the beginning of plant vegetation), May (blooming of *V. myrtillus* and *V. vitis-idaea*), July (development of *V. myrtillus* and *V. vitis-idaea* berries), and November (the end of understory plant vegetation) in 2016. The aboveground and underground parts of *V. myrtillus* and *V. vitis-idaea* were gathered randomly (10 samples) in each plot at a distance of at least 5 m apart from each other. Next, 2 different aggregate samples, comprising 150–200 g of the aboveground and underground parts, were put into plastic bags and stored in a refrigerator at –20 °C until further analysis.

Plant samples were collected in 2 different forest types, i.e. in *Pinetum vacciniosum* (PV) (area between 54°11′62″N to 54°25′61″N and 24°33′13″E to 24°49′16″E) and *Pinetum vaccinio-myrtillosum* (PVM) (area between 54°24′37″N to 54°23′49″N and 25°00′03″E to 24°57′22″E) in Southern Lithuania, in both a mature forest and 1 year after clear-cutting. The investigation plots were selected in mature stands of Scots pine forests with an average age of 110–115 years and the stand volume 330–335 m^3 per hectare. One year prior to sampling, part of each plot was cleared and the tree stems and branches were removed immediately after cutting. This study was implemented on cleared plots and compared with control forest sites. The samples of forest litter were also collected considering the vegetation phases of understory plants of the family Ericaceae, such as *V. myrtillus*, *V. vitis-idaea*, and *Calluna vulgaris*, in the tested forests. Collected samples were stored in a refrigerator at –18 °C until further analysis.

2.2. Extract preparation

Before analysis, each plant material was defrosted and ground into a 1–2 mm particle size. Next, 500 mg of ground material was extracted with 20 mL of 75% aqueous methanol for 24 h in a TiterTek orbital shaker (Titertek-Berthold, Pforzheim, Germany). Extracts were filtered through a filter paper and stored in a fridge at 4 °C until spectrophotometric tests were performed. The moisture content in the samples was evaluated using PMB-53 moisture balance (Adam Equipment, Kingston, UK) according to the recommendations of the manufacturer in order to make the results comparable. All of the results were recalculated using absolutely dry material.

2.3. Biochemical analysis

The total phenolic content (TPC), total flavonoid content (TFC), and radical scavenging activity (RSA) were determined using the Folin-Ciocalteu method, aluminum trichloride colorimetric test, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, respectively, as described previously by Kaškonienė et al. (2015b). Calibration curves with rutin as reference compound were used in all of the tests. The results were expressed as mg of rutin equivalents (RUE) in a gram of absolutely dry plant material. All of the experiments were performed in triplicate and the results were expressed as the mean ± standard deviation (SD).

2.4. Statistical analysis

Statistical data analysis was performed using Matlab v.9.1 (R2016b) software (MathWorks, Natick, MA, USA). Before any data analysis, data set conditioning was performed by subtracting the mean and dividing by the SD. The quantities, processed in such a way, were positioned around zero with normalized scatter, i.e. the variance became equal to unity. Data mining involved hypotheses testing to determine the significance of the observed changes in the measured data set. The testing of hypotheses was performed by applying 2-tailed 2-sample t-tests at $\alpha \leq 0.05$ significance level. The main hypothesis H_0 was formulated, wherein the means of the corresponding variables did not change after clear-cutting, while the alternative hypothesis H_a stated the opposite, where clear-cutting had a strong effect on the shrubs under investigation and the means of the corresponding variables were not equal, i.e. changed significantly. On rejection of the H_0 hypothesis about the equality of the means at the chosen significance level, the 2-tailed hypothesis was converted into a 1-tailed hypothesis, reformulating H_a, in order to obtain information about the incremental or decremental characters of the statistically significant change observed.

Principal component analysis of the aboveground and underground biomass description attributes was performed in order to combine these initial measured quantities into a single output variable that would represent the specified category, i.e. aboveground or underground biomass. The first principal component, which explained the largest part of variance of the initial quantities, was taken as a combined variable for successive correlation analysis. Correlation coefficients between the aboveground and underground biomasses of the shrubs and litter estimation followed that, which exposed the relationship of the changes during different vegetation changes.

Statistical analysis also included evaluation of the Euclidean distance as a cumulative similarity measure, which allowed comparing the integral closeness of the mature forest and clearc-utting areas according to the measured quantities (Kaškonienė et al., 2018). The estimated similarity measure revealed trends of the environmental impact on the different vegetation phases of *V. myrtillus* and *V. vitis-idaea*.

3. Results and discussion

3.1. Variations in the biochemical compounds of *V. myrtillus* and *V. vitis*-idaea

Shoots with leaves and rhizomes of *V. myrtillus* and *V. vitis-idaea* were analyzed for variations in the TPC, TFC, and RSA during different growing stages. The impact of the type of forest and forest clear-cutting was also taken into account. The results are presented in Figures 1 and 2.

The TPC in the aboveground extracts of *V. myrtillus* varied from 35.87 to 229.76 mg/g (expressed as RUE), while remarkably lower TPC was determined in the underground extracts of *V. myrtillus*, as 4.64–72.29 mg/g. The TFC varied from 7.97 to 40.15 mg/g in the aboveground extracts, while flavonoids were not detected in the prepared extracts from the underground extracts. As was expected, the RSA showed higher values in the aboveground extracts of *V. myrtillus* and varied from 43.96 to 434.68 mg/g, than in the underground extracts (16.00–149.74 mg/g). All of the tested parameters varied depending on the growing stage, type of forest (PV or PVM), part of the plant, and, of course, cutting (Figure 1). Variations in the TPC and RSA in the leaves, stems, and fruit of *V. myrtillus* during the different vegetation phases and seasons was also obtained by Bujor et al. (2016). Nybakken et al. (2013) determined the increase of phenolics after forest clear-cutting in the shoots and leaves of *V. myrtillus*, and the are in good agreement with coincided with the data of the current study. Herein, the TPC increased significantly ($a \leq 0.05$) by 1.2–3.5 times in the aboveground extracts of the samples collected in July and November in both forest types and did not show any effect on the samples collected in May. However, the reduction of RSA was observed in many instances in the aboveground and underground extracts of both the PV and PVM forests. No changes or a significant ($a \leq 0.05$) increase, by 1.3–3.1 times, was observed in the TFC. There was no clear evidence in the variations of TPC, TFC, and RSA in the tested samples, as the level of changes was vegetation phase- and forest type-dependent.

Furthermore, the TPC and TFC, together with the RSA, in *V. vitis-idaea*, varied depending on the growing stage, forest type, aboveground or underground parts of the plant, and clear-cutting (Figure 2). The TPC the aboveground and underground extracts varied from 120.03 to 309.64 mg/g and 5.22 to 60.69 mg/g, respectively. The TFC varied from 14.37 to 40.18 mg/g in the aboveground extracts, while flavonoids were not detected in the prepared underground extracts of *V. vitis-idaea*. RSA, again, was higher in the aboveground extracts than in the underground extracts, and it was in the range of 49.58–485.53 and 4.98–99.44 mg/g, respectively. Relatively high RSA values (up to 434.68 mg/g) in the samples were obtained, probably because of the fresh (not dried) material, used during the analysis. Other studies have shown that the drying process of plant material often

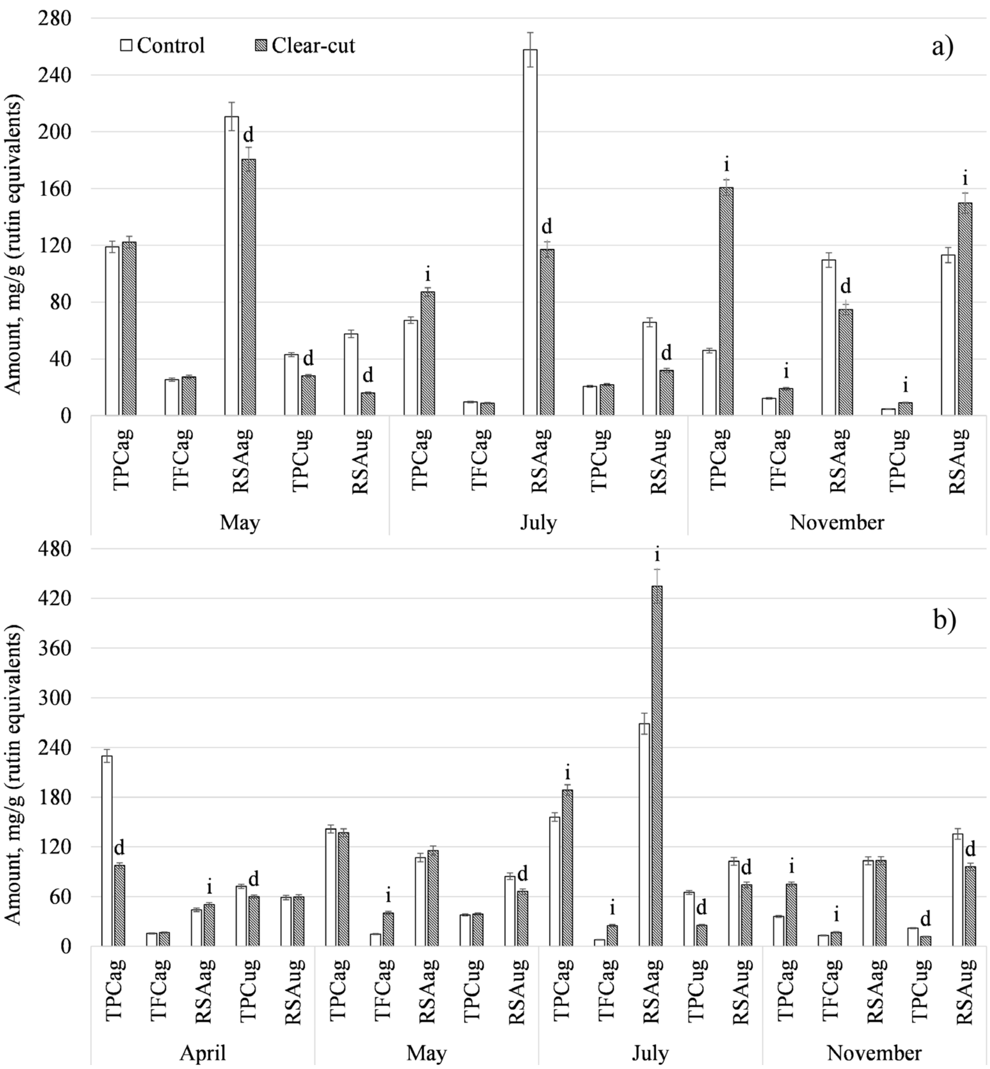

Figure 1. Changes in the TPC, TFC, and RSA in the aboveground (ag) and underground (ug) extracts of *V. myrtillus*: (a) PVM forest type, (b) PV forest type. Each value is expressed as the mean ± SD, n = 3. Statistically significant changes between the control and clear-cutting site were labeled i (increased) or d (decreased) when α ≤ 0.05.

leads to the reduction of TPC and RSA, due to degradation by some enzymatic and nonenzymatic reactions, and the loss of some good antioxidants, such as ascorbic acid and carotenoids (Kaškonienė et al., 2015b).

Bujor et al. (2018) analyzed variations in the phenolic compounds and antiradical DPPH activity in the leaves, stems, and fruit of *V. vitis-idaea*. They found that either no or low variations in the TPC were detected, while the RSA showed different behavior in the stems, leaves, and fruit collected in the different years during 3 different seasons. In the current study, either no or small changes were observed only in the PV forest after clear-cutting (the TPC was 231.24–280.48 mg/g). The TFC was significantly (α ≤ 0.05) reduced in the samples collected in July and November after clear-cutting in the PVM forest, while they were significantly (α ≤ 0.05) increased in 3 out of 4 samples in the PV forest (Figure 2).

Interestingly, all of the TPC values in the *V. vitis-idaea* extracts were higher in the PV forest than in the PVM forest, except for in 1 aboveground sample, collected in November in the mature forest. Similar behavior was found in the *V. myrtillus* extracts, except in 3 out of 14 samples. The tested parameters in the underground extracts of both *V. vitis-idaea* and *V. myrtillus* were similar.

Summarizing the results of the impact of clear-cutting on the TPC in the samples of plants collected in 2 forest types during various vegetation phases, it was observed that in most instances, the TPC exhibited statistically significant (α ≤ 0.05) changes in 19 samples, while it remained constant in the other 9 samples. The TFC,

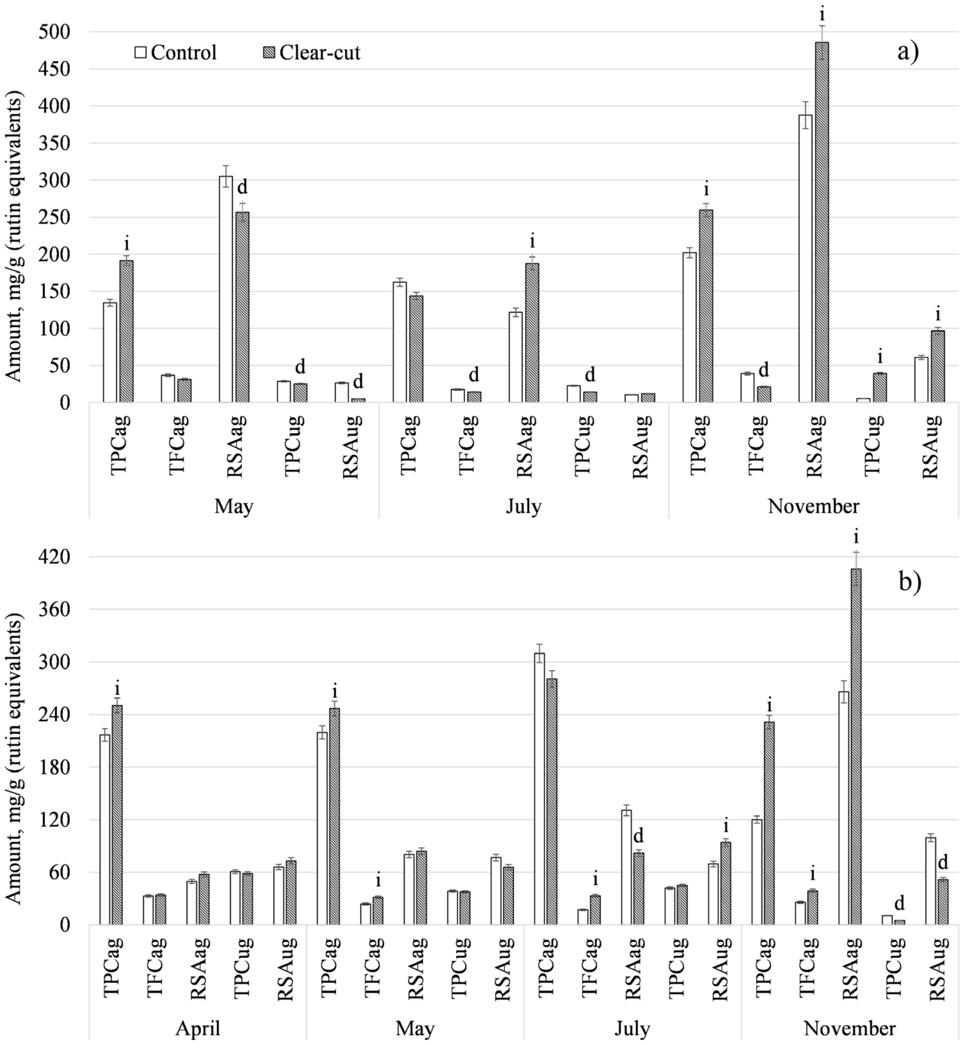

Figure 2. Changes in the TPC, TFC, and RSA in the aboveground (ag) and underground (ug) extracts of *V. vitis-idaea*: (a) PVM forest type, (b) PV forest type. Each value is expressed as the mean ± SD, n = 3. Statistically significant changes between the control and clear-cutting site were labeled i (increased) or d (decreased) when $\alpha \leq 0.05$.

detected in the aboveground extracts only, after clear-cutting revealed statistically significant ($\alpha \leq 0.05$) changes in 9 samples, while it remained unchanged in the other 5 samples. The RSA exhibited a significant ($\alpha \leq 0.05$) change in 20 samples, while it remained constant in the other 8 samples.

To estimate the similarity measure between the mature forest and clear-cutting areas, with expectations to see an overall picture while comparing *V. vitis-idaea* and *V. myrtillus* from these growth sites in different forest types, Euclidean distance calculation was involved. Not only did this cumulative measure reveal the impact of clear-cutting on *V. vitis-idaea* and *V. myrtillus*, but it also allowed trends to be followed during the vegetation stages (see Figure 3a). The results showed that, despite the forest type, *V. vitis-idaea* was affected by clear-cutting and dissimilarity grew with time. The same effect was noted with *V. myrtillus* in the PV forest. However, these trends became opposite in the PVM forest; a high initial impact was observed on *V. myrtillus* in this type of forest, but the gap began to close rapidly during the following months, resulting in *V. myrtillus* being very similar to that of the mature forest in November. These findings differed from those of Palviainen et al. (2005), where slightly faster recovery of *V. vitis-idaea* compared to *V. myrtillus* was determined, due to the different growth and morphology between these 2 species.

The matrices of correlation coefficients between the variables representing the aboveground and underground biomasses of the shrubs and litter are presented in Tables 1 and 2. As the measured attributes (TPC, TFC, and RSA) of the aboveground biomass, as well as underground

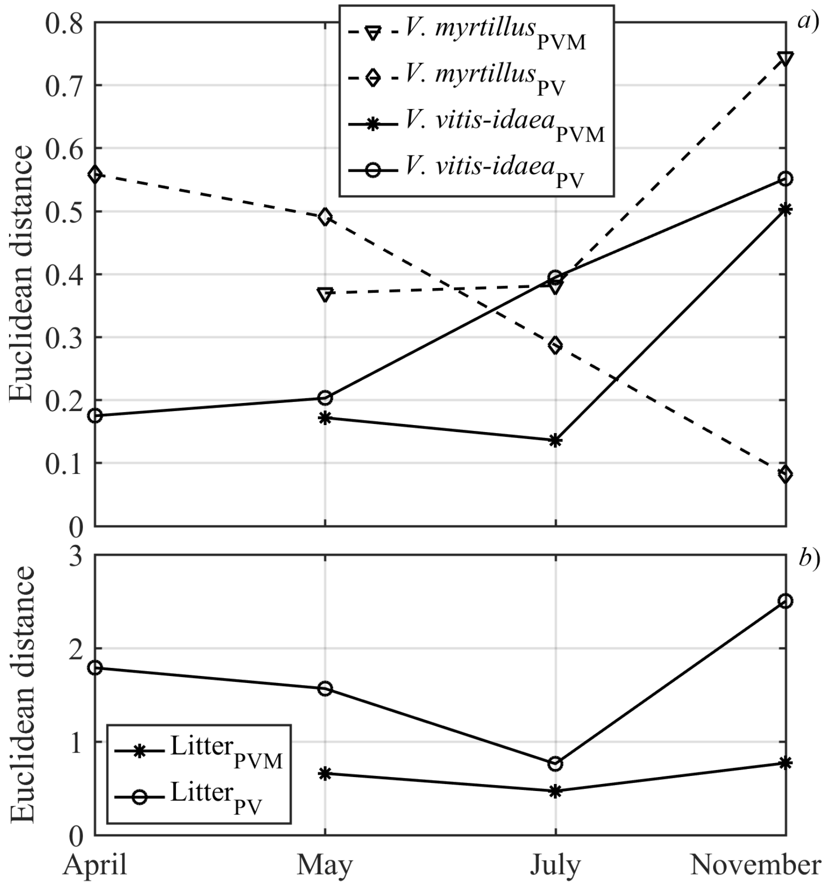

Figure 3. Euclidean distance in the samples of *V. myrtillus* and *V. vitis-idaea*, (a), and forest litter samples, (b), collected in control forest and after clear-cutting.

Table 1. Correlation matrix calculated between the derived (linearly combined) variables representing the aboveground and underground biomasses of the shrubs and litter in the mature forest and clear-cut site of the *Pinetum vaccionio-myrtillosum* forest.

Variables	Control area					Clear-cut area				
	V. vitis-idaea		*V. myrtillus*		Litter	*V. vitis-idaea*		*V. myrtillus*		Litter
	Aboveground	Underground	Aboveground	Underground		Aboveground	Underground	Aboveground	Underground	
1	1.000	0.715	0.213	–0.301	0.806*	0.919*	0.657	0.840*	0.560	0.925*
2		1.000	–0.519	–0.875*	0.970*	0.920*	0.993*	0.248	0.975*	0.519
3			1.000	0.860*	–0.365	–0.159	–0.586	0.689	–0.680	0.411
4				1.000	–0.773	–0.628	–0.910*	0.240	–0.953*	–0.075
5					1.000	0.957*	0.954*	0.400	0.916*	0.638
6						1.000	0.887*	0.592	0.824*	0.782*
7							1.000	0.170	0.989*	0.453
8								1.000	0.048	0.901*
9									1.000	0.346
10										1.000

* Significant correlation ($\alpha \leq 0.05$).

Table 2. Correlation matrix calculated between derived (linearly combined) variables representing aboveground and underground biomasses of shrubs and litter in mature forest and clear-cut site of *Pinetum vaccioniosum* forest.

Variables	Control area					Clear-cut area				
	V. vitis-idaea		*V. myrtillus*		Litter	*V. vitis-idaea*		*V. myrtillus*		Litter
	Aboveground	Underground	Aboveground	Underground		Aboveground	Underground	Aboveground	Underground	
1	1.000	0.617	0.498	0.625	–0.871*	–0.819*	0.745*	0.850*	0.300	0.639*
2		1.000	0.983*	0.979*	–0.242	–0.909*	0.978*	0.130	0.914*	0.917*
3			1.000	0.964*	–0.107	–0.848*	0.942*	–0.015	0.956*	0.897*
4				1.000	–0.225	–0.855*	0.974*	0.160	0.861*	0.848*
5					1.000	0.577	–0.393	–0.934*	0.057	–0.374
6						1.000	–0.937*	–0.430	–0.765*	–0.948*
7							1.000	0.304	0.831*	0.905*
8								1.000	–0.231	0.188
9									1.000	0.878*
10										1.000

* Significant correlation ($\alpha \leq 0.05$).

biomass, changed during vegetation of the shrubs in the mature forest, the correlation analysis helped to track these changes in the clear-cutting area. The correlation coefficient between the aboveground and underground biomasses, describing the quantities of *V. vitis-idaea* and *V. myrtillus* in the mature forest of any tested type, was positive. It showed that both the aboveground and underground parts changed in the same direction during the vegetation of the plant. However, after clear-cutting the shrubs did not behave the same way. While *V. vitis-idaea* in the PVM forest held this tendency (correlation coefficient of 0.887), in the PV forest, the opposite trend was observed (correlation coefficient of –0.937). For *V. myrtillus*, the situation was similar; a correlation between development of the aboveground and underground parts during plant vegetation was not observed in either forest type (correlation coefficients of 0.048 and –0.231, respectively). Summarizing, the correlation analysis revealed that the aboveground and underground biomasses of the shrubs after clear-cutting started to develop differently.

3.2. Biochemical compounds in the forest litter

The samples of forest litter were also tested, considering the vegetation phases of the understory plants, including *V. myrtillus* and *V. vitis-idaea*, in the tested forests. Forest litter has a huge impact on the forest ecosystem, as it is involved in the humification process (Kuiters 1990). It is an important source of nutrients, while phenolic compounds of the litter may participate in microbe inhibition (Li et al., 2016). However, there is lack of analytical data about changes in the TPC and RSA in forest litter during seasonal periods. The TFC in the tested samples varied from 3.12 to 11.89 mg/g, while the RSA range from 59.21 to 137.28 mg/g (Figure 4).

A statistically significant ($\alpha \leq 0.05$) decrease in the TPC after clear-cutting was observed in 7 out of 8 samples of forest litter, the TPC did not change in 1 sample. Changes of RSA in the forest litter after clear-cutting were dependent on the forest type; a significant ($\alpha \leq 0.05$) increase was observed in 2 samples in the PVM forest, and in 1 sample, it did not change significantly. A significant ($\alpha \leq 0.05$) decrease was observed in 3 samples in the PV forest, while in 1 sample, it remained constant.

Euclidean distance calculated to compare the litter similarity of the clear-cut site to the mature forest stand during the time interval from April to November is presented in Figure 3b. The graph shows that trends of the changes in both analyzed forest types were similar; the major dissimilarity of the litter in the clear-cut area and mature forest was observed in November, while during the spring and summer months, this difference had a tendency to diminish.

It was expected that the forest litter had to change after clear-cutting, as the aboveground and underground parts of *V. vitis-idaea* and *V. myrtillus* started to react to this environmental impact. The correlation analysis showed that, despite expectations, the main changes were observed between the underground biomass attributes of the shrubs and the forest litter. There was no correlation between the mentioned categories representing the variables observed in the PV mature forest, but a strong positive correlation

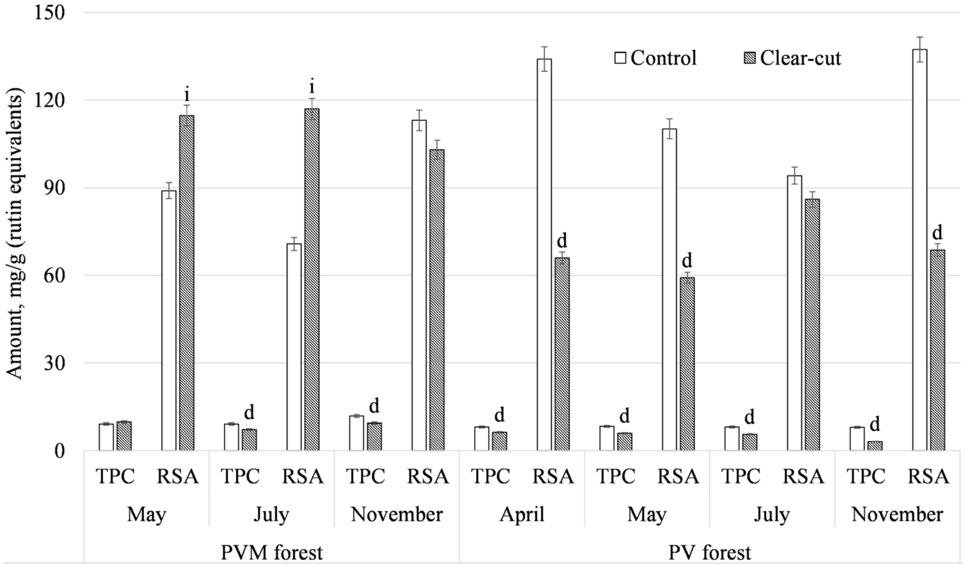

Figure 4. Changes in the TPC and RSA in the forest litter collected in different periods. Each value is expressed as the mean ± SD, n = 3. Statistically significant changes between the control and clear-cutting site were labeled i (increased) or d (decreased) when $\alpha \leq 0.05$.

appeared after clear-cutting (0.905 for *V. vitis-idaea* and 0.878 for *V. myrtillus*), while the observed correlation in the PVM mature forest (0.970 for *V. vitis-idaea* and –0.773 for *V. myrtillus*) disappeared after clear-cutting.

4. Conclusion

The impact of clear-cutting on the biochemical compounds of *V. myrtillus* and *V. vitis-idaea* shrubs and from forest litter in PV and PVM forests was addressed in this paper. The TPC varied from 35.87 to 229.76 mg/g and 120.03 to 309.64 mg/g in the aboveground extracts of *V. myrtillus* and *V. vitis-idaea*, while remarkably lower amounts of the phenolic compounds were determined in the underground extracts of the tested shrubs, at 4.64–72.29 mg/g and 5.22–60.69 mg/g, respectively. The TPC in the forest litter ranged from 3.12 to 11.89 mg/g. The RSA determined in all of the samples scattered from 16.00 to 434.68 mg/g. The lowest antiradical activity was determined in the underground extracts of *V. myrtillus*, while the highest were in aboveground extracts of the same plant. The TFC was dependent on vegetation phase, forest type, and clear-cutting, and varied from 7.97 to 40.18 mg/g in the aboveground extracts of the tested plants. Flavonoids were not detected in the underground extracts of the shrubs or forest litter.

The study revealed the dependency of the impact of clear-cutting on the TPC,TFC, and RSA of plants and forest litter with the vegetation phase and forest type. The The TPC, TFC and RSA exhibited statistically significant ($\alpha \leq 0.05$) changes in 68%, 60% and 71% of the tested samples after clear-cutting, respectively.

The applied hypothesis testing revealed that changes in the TPC, TFC and antiradical activity in the aboveground and underground extracts of the shrubs and litter after clear-cutting were statistically significant at $\alpha \leq 0.05$. The performed chemometric analysis involving the similarity measure evaluation between the mature stand and clear-cutting site during different plant growth phases revealed that the observed changes in the vegetation behavior of *V. myrtillus* and *V. vitis-idaea* could have been attributed to the clear-cutting factor. The correlation analysis proved that the aboveground and underground biomasses of the tested shrubs after clear-cutting started to develop differently when compared to the mature stand forest.

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Contribution of authors

V.K., L.Č., R.D., and A.M. contributed to the design of the study. L.Č. and R.D. were responsible for the sample collection. K.B.S. and N.T. performed the spectrophotometric tests. V.K. wrote the chemical part of the manuscript, performed the literature analysis. P.K. performed the chemometric analysis of the data and wrote the corresponding paper part.

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