Effects of Clear-Cuts in Scots Pine-Dominated Forests on *Vaccinium myrtillus* and *Vaccinium vitis-idaea* Vegetative Characteristics, and Accumulation of Phenolic Compounds

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Abstract

The clear-cuts cause considerable changes to forest ecosystems, including physical and chemical changes to understory vegetation. Forest restoration processes strongly depend on the degree of disturbance caused by this activity. Our aim was to assess short-term changes to *Vaccinium myrtillus* and *V. vitis-idaea* populations in *Pinus sylvestris*-dominated forests following the clear-cuts. Vegetative performance of *V. myrtillus* and *V. vitis-idaea* populations was determined for mature forest stands and for clear-cuts. Spectrophotometric tests of plant samples were used to measure the total phenolic compound content, as well as flavonoid content, together with free radical scavenging activity in both aboveground and underground parts of *V. myrtillus* and *V. vitis-idaea.* Statistical data mining using MATLAB v9.1 software was performed on vegetative characters of *V. myrtillus* and *V. vitis-idaea*, as well as on data relating to the antioxidant properties of these samples. Investigation of *V. myrtillus* and *V. vitis-idaea* revealed different responses following the clear-cut. We detected immediate changes in aboveground and underground parts, as well as in the number of individual shrubs of *V. vitis-idaea* and *V. myrtillus* after clear-cuttings. The both species showed higher vitality in *Pinetum vaccinio-myrtillosum* following the clear-cut. An increase in total phenolic and free radical scavenging activity was observed in *V. vitis-idaea* populations after clear-cutting in both types of forest.

Keywords: biomass, clear-cuts; phenolic compounds; *Vaccinium*

Introduction

Scots pine (*Pinus sylvestris* L.) is the dominant tree species in forest stands of Southern Lithuania. Two pine forest types, namely *Pinetum vacciniosum* and *Pinetum vaccinio-myrtillosum* comprise 35-37% of the total forest area (Navasaitis et al. 2003). Since pine forests are an important source of wood, they are commonly affected by intensive commercial activity that causes significant changes to the ecosystem. Tree felling, generally performed during the clear-cuts in extensive forested areas of plantation forests, is the main driver of changes to the microclimate of the forest floor, affecting for example, the regeneration of tree seedlings (Roberts and Zhu 2002, Godefroid et al. 2005). Changes to the microclimate and soil properties following the clear-cuts also affect nutrient uptake and promote the appearance of other species in forest ecosystems (Heinrichs and Schmidt 2009), including alien plants, provided that an abundant local seed source is present (Davis and Puettmann 2009). The effects of clear-cuts can be observed not only in aboveground, but also in underground parts of plants (Palviainen et al. 2005).

Since the diversity of vascular plant species associated with mature Scots Pine forests is poor, good focal plants are members of *Ericaceae*, including *Vaccinium myrtillus* L. and *V. vitis-idaea* L., both dominant pine forest understory species of significant economic value. Both species are important berry plants, and their leaves are also medicinally valuable because they contain high concentrations of biologically active substances. These include various phenolic compounds with characteristic antioxidant, anticarcinogenic and antimicrobial properties. Biologically, these compounds are carbon-based substances that play a role in plant resistance and defence against herbivores and parasites (Haukioja 2005, Witzell et al. 2013).

Many authors (Frego 2007, Heinrichs and Schmidt 2009, Widenfalk and Weslien 2009, Rodríguez and Kouki 2015) have reported how the various activities associated with the clear-cuts destroy the rhizomes of forest floor plants and thereby reduce shrub cover and the competitive capacity of ericaceous plants in favour of nitrophilous species. If the connections between shrubs are cut (especially if the cutting of clearings is followed by soil preparation), subterranean transport of nutrients in bilberry (*V. myrtillus*) is impeded (Nybakken et al. 2013). Furthermore, tree extraction may also result in enhanced environmental stress in response to increased solar radiation and drought. Even so, the effects of clear-cuts on the growth of *V. myrtillus* appear to be contradictory. Some authors have reported that *V. myrtillus* cover was greater in uncut forests (Atlegrim and Sjöberg 1996), whereas other studies have shown that clear-cuts have a positive effect (Nielsen et al. 2007, Nybakken et al. 2013). Other common ericaceous species, such as cowberry (*Vaccinium vitis-idaea*), have become adapted to a wide range of coniferous forests and occur on infertile soils (Palviainen et al. 2005). However, there is less information available on the impact of forest management on populations of this species. Jalonen and Vanha-Majamaa (2001) reported that increased forest density had adverse effects on the abundance of *V. vitis-idaea*, whereas Rodríguez and Kouki (2015) emphasized the greater resistance of this species to increased light and drier soil conditions. Such responses may be mediated via changes in the chemical composition of plants subjected to drastic habitat alteration. As Li et al. (2010) have stated, particular phenolic compounds behave like common plant allelochemicals within ecosystems. These compounds are found both in the aboveground and underground parts of understory plants, as well as in plant decomposition products. The ericaceous shrubs *V. myrtillus*, *V. vitis-idaea* and *Calluna vulgaris* (L.) Hull are considered a source of phenolic compounds that constitute a significant proportion of total biomass carbon (Kraus et al. 2003, Witzell et al. 2013). A number of studies have demonstrated that different phenolic compounds impact on the decomposition of forest litter, the decline in nitrogen mineralisation and the germination of conifer seeds (Northup et al. 1998, Bradley et al. 2000). Consequently, estimation of the concentration and range of phenolic compounds within dominant understory plants would help explain changes of forest ecosystems. We assessed the initial changes to the vigour of dominated understory vascular plant species *V. myrtillus* and *V. vitis-idaea,* as well as the variability in phenolic compounds immediately after clear-cuts in *P. sylvestris*-dominated forests.

Materials and Methods

Study sites

Suitable study plots were located in Scots pine forests in Southern Lithuania. The dominant types of forest occurring in this region are *Pinetum vacciniosum* (hereafter *PV*) and *Pinetum vaccinio-myrtillosum* (hereafter *PVM*). Eight *PVM* and six *PV* plots, measuring 2.4 to 7.1 ha, were selected from mature forest stands dominated by Scots pine (*P. sylvestris*) of average age 115-130 years at 54°24’37”N - 54°23’49”N and 25°00’03”E - 24°57’22”E, respectively. The stand volume range was 330-335 m^3 per hectare. All sites occurred on oligotrophic soils of *Vaccinium* type (Karazija 2003). The forest type was determined in accordance with prevailing tree species, understory vegetation, and humus fraction (Navasaitis et al. 2003). Common understory vascular plant species in *PV* included *V. vitis-idaea*, *V. myrtillus*, *Calluna vulgaris*, *Arctostaphylos uva-ursi* (L.) Spreng. and in *PVM: V. vitis idaea, V. myrtillus, Festuca ovina* L. Mosses were dominant in both forest types, covering, on average, 70 to 85% of the forest floor. The most abundant moss species were *Pleurozium schreberi* (Wild. ex Brid.) Mitt. and *Dicranum polysetum* Sw. The lichens *Cladonia rangiferina* (L.) Weber ex F.H. Wigg. and *C. arbuscula* (Wallr.) Rabenh. were also present in *PV*. The total cover of vascular plants in the latter was lower (to 14%) compared with *PVM* (20% to 40%). The depth of rather crude forest floor was, on average, 2-4 cm. The mineral horizon of the soil varied in depth from 0 to 20 cm. *PV* is distinctive in its possession of very infertile soil (the humus fraction composing ca. 1.5-2%), whereas the humus fraction of *PVM* reached ca. 3% (Navasaitis et al. 2003). One year prior to sampling, part of each plot was cleared and tree stems and branches removed immediately after cuttings in 2015. Our sampling was therefore conducted on cleared subplots and compared with control samples obtained from intact (mature) forest sites in 2016.

Assessment of Vaccinium populations

In order to estimate the impact of clear-cuts, the vegetative characters of *V. myrtillus* and *V. vitis-idaea* populations were determined before reforestation. Aboveground and underground parts of *V. myrtillus* were sampled in July meanwhile plant material of *V. vitis-idaea* was collected in October when the growth of shrubs finished. The total number of shrubs, the dry weight of aboveground and underground parts, the number of shrubs per 1 metre length of rhizome, the total length of rhizomes, and the dry weight of a single shrub were determined for ten replicates from each subplot measuring 1 m^2. The subplots were ranged randomly to cover mature stands or cleared areas. The shrubs and rhizomes were left to dry at room temperature. The average height of shrubs was determined by measuring of 50 shrubs.

Evaluation of phenolic compounds, flavonoids, and radical scavenging activity

In order to study changes to the phenolic compounds present in plant material, aboveground shrubs and underground rhizomes of *V. myrtillus* and *V. vitis-idaea* were gathered randomly for ten replicates, then aggregate samples from each plot were made and stored in a freezer (at -18 °C) prior to investigation.

Each thawed sample was ground to 1-2 mm particle size. Then, 500 mg of ground material was extracted with 20 ml 75% aqueous methanol for 24 hours at room temperature in a dark place in a TiterTek orbital shaker (Germany). Extracts were filtered through filter paper and stored at +4 °C until spectrophotometric tests could be undertaken. Spectrophotometric tests were performed within one week after extract preparation. The moisture content of samples was determined using a PMB-53 Moisture balance (Adam Equipment, Kingston, UK) according to the recommendations of the manufacturer. The moisture content in the tested samples varied from 39 to 55%. All results were expressed per gram of absolutely dry plant material.

Total phenolic and flavonoid content, and free radical scavenging activity were determined by means of the Folin-Ciocalteu method, aluminium trichloride colorimetric test and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay, respectively, as described by Kaškonienė et al. (2015). Rutin was used as a reference compound for all tests. The results were expressed as mg of rutin equivalents (RUE) per gram of absolutely dry plant material.

All spectrophotometric measurements were performed in triplicate. Results were presented as the average of three measurements.

Statistical data analysis

Statistical data analysis using *MATLAB v9.1* software package was performed on vegetative data of *V. myrtillus* and *V. vitis-idaea* from mature forest stands and cut clearings, and on the antioxidant data of these samples. Statistical data mining included descriptive statistical analysis, hypothesis-testing, principal component analysis and *k*-means clustering technique.

The analysis commenced by estimating the significance of the dependence of vegetative characteristics and antioxidant properties on forest type and growth site by applying two-tailed hypothesis tests using *t* statistics (Student's distribution). Further data processing by means of the Monte Carlo method was undertaken, that allowed generation of a large data set having data distribution probability density function with known or estimated parameters (mean, standard deviation, etc.).

After this, principal component analysis (*PCA*) and clustering analysis was carried out. *PCA* allowed not only to reduce the number of variables and to obtain orthogonal principal components representing variance within the initial data in diminishing order, but also to have the capacity to represent data scatter plots following clustering analysis in the principal components space. The number of significant principal components was chosen according to Cattell's scree plot test. *K*-means clustering analysis (*KMCA*) was then applied to cluster the data into groups. Since *KMCA* is an iterative clustering technique that minimizes the sum, over all clusters, of the within-cluster sums of point-to-cluster-centroid distances, the analysis was repeated 200 times so as to find the best clustering outcome distinguished by the minimum sum of the distances. This was done in order to estimate and eliminate the effect of random initial initialization of the *KMCA* algorithm on clustering results. The significance of clustering results relative to the specific number of clusters was tested by calculating the Davies-Bouldin index, Calinski-Harabasz criterion and distortion function, as described by Kaškonas et. al. (2016).

Results

Vegetative characters of V. myrtillus and V. vitis-idaea

Comparison of aboveground and underground parts of *V. myrtillus* in *PVM* forests showed that the weight of rhizomes exceeded the weight of shrubs in mature forests (283.7±35.0 g m^{-2} and 247.7±17.6 g m^{-2}, respectively) and clear-cuts (519.7±42.3 g m^{-2} and 343.7±24.1 g m^{-2}, respectively), i.e. by 1.2- and 1.5-fold. The weight of aboveground and underground parts of *V. myrtillus* did not differ significantly for mature plots of *PV* forest (215.8±18.1 g m^{-2} and 210.5±18.2 g m^{-2}, $p = 0.95$, respectively), whereas the weight of underground rhizomes (171.2±16.4 g m^{-2}) was 0.8-fold less than the weight of shrubs (233.8 ±20.1 g m^{-2}) in clear-cuts of *PV* forest. Similar trends were seen for *V. vitis-idaea*, where the weight of rhizomes was 1.7- to1.8-fold greater in mature stands of *PVM* and *PV* forests (173.3±19.1 g m^{-2} and 104.8±11.6 g m^{-2}; 132.9±15.7 g m^{-2} and 73.5±7.4 g m^{-2}, respectively). However, the weight of aboveground part of *V. vitis-idaea* (177.6±21.6 g m^{-2}) exceeded the weight of underground part (104.9±8.7 g m^{-2}) in clear-cuts of *PV*.

Rhizomes are important in the development of both *Vaccinium* species in this ecosystem. It was found that the total length of rhizome per m^2 did not differ significantly for *V. myrtillus* in mature stands and clear-cuts (109.6 ±7.8 m m^{-2} and 115.7± 5.7 m m^{-2}, p =0.95) in *PV* forest. A statistically reliable increase in rhizome length following clear-cuts was seen in *PVM* forest (153.1±8.6 m m^{-2} in mature stands and 228.8±12.7 m m^{-2} in cut clearings). A significant increase in rhizome length in clear-cut compared to mature stands was observed for *V. vitis-idaea* in *PVM* forest (111.6±3.8 m m^{-2} in mature forest and 188.7±9.8 m m^{-2} in clear-cuts, p =0.95) as well as in *PV* forest (99.0±5.6 m m^{-2} and 120.3±8.5 m m^{-2}, p =0.95).

Two-tailed hypothesis-testing revealed that vegetative characters of *V. myrtillus* remained unchanged following the clear-cuts, except for the length of rhizomes and the average height of shrubs in *PV*. For *V. vitis-idaea*, growing in the same type of forest, we recorded a significant reduction in shrub height and an increase in all other characteristics studied, except for the weight of underground biomass (Table 1).

Table 1. Two-tailed hypothesis-testing of results. Corresponding characteristics of *V. myrtillus* and *V. vitis-idaea* populations in clear-cuts were compared with ones in mature forest stands

Forest type	Species	WAG	WUG	RL	SNR	SW	NS	SH
Pinetum vaccinio-myrtillosum	*V. vitis-idaea*	I	I	I	D	I	C	I
	V. myrtillus	I	I	I	C	C	I	I
Pinetum vacciniosum	*V. vitis-idaea*	I	C	I	I	I	I	D
	V. myrtillus	C	C	C	C	C	C	D

I – increase, D – decrease, C – unchanged. Statistical significance p = 0.95. WAG – weight of aboveground biomass, g m^{-2}; WUG – weight of underground biomass, g m^{-2}; RL – length of rhizomes, m m^{-2}; SNR – number of shrubs per m length of rhizomes; SW – weight of one shrub, g; NS – number of shrubs per m^2; SH – height of shrub, cm

In *PVM* sites, there was a general increase in the most characteristics of both studied *Vaccinium* species, except for a reduction in the number of shrubs per 1 metre length of rhizome of *V. vitis-idaea*, but no such change was seen in *V. myrtillus*.

The further statistical mining included four variables: weight of aboveground biomass, weight of underground biomass, length of rhizomes and number of shrubs per 1-meter length of rhizomes. Initial descriptive statistical analysis showed a large variance in data (relative standard deviation (*RSD*) had increased to 40%). Since the data demonstrated large scatter, the Monte Carlo technique was applied in order to generate data points artificially based on statistical parameters, like mean, standard deviation and probability density function, as described by Kaškonas et. al. (2016). It was generated n'=10000 data points in each case. Thus, the data sets became [80000×4], where 4 was the number of selected variables, 80000 represents the generated data corresponding to *V. myrtillus* growing in *PVM* and *PV* forests (mature forest stand and clear-cuts) and *V. vitis-idaea* growing in *PVM* and *PV* forest (mature forest stand and clear-cuts), respectively.

After the Monte Carlo procedure, principal component analysis was carried out. The decision regarding what numbers of principal components should be retained was taken based on Cattell's scree plot test. The test results showed that all four principal components should be retained as significant analysing both *V. myrtillus* and *V. vitis-idaea*, growing in *PVM* and *PV* types of forest, respectively. The *PCA* was followed by clustering analysis, and this resulted in data scatter plots for *V. myrtillus* and *V. vitis-idaea* shown in Figures 1 and 2, which are given in the principal components *PC1 – PC2 – PC3* space.

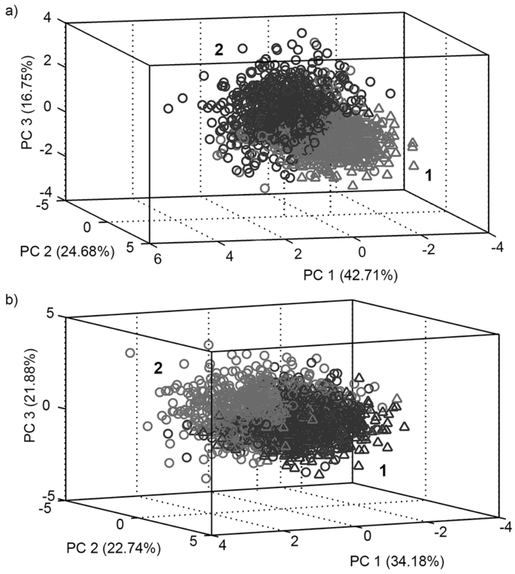

Figure 1. Data scatter plots of clustering analysis of vegetative characters of *V. myrtillus; 1* (triangles) denote mature forest stands, *2* (circles) denote clear-cuts. *a* and *b* represent clustering results of *V. myrtillus* growing in *Pinetum vaccinio-myrtillosum* and *Pinetum vacciniosum* forests, respectively. For clarity purposes, only 2000 points are shown

It is clear from the presented figures that owing to the large amount of data, an overlap of the clusters was exposed. Nevertheless, it could be concluded that populations of *V. myrtillus* and *V. vitis-idaea* are affected by the growth site in both types of investigated forest, since all the selected tests to determine the number of

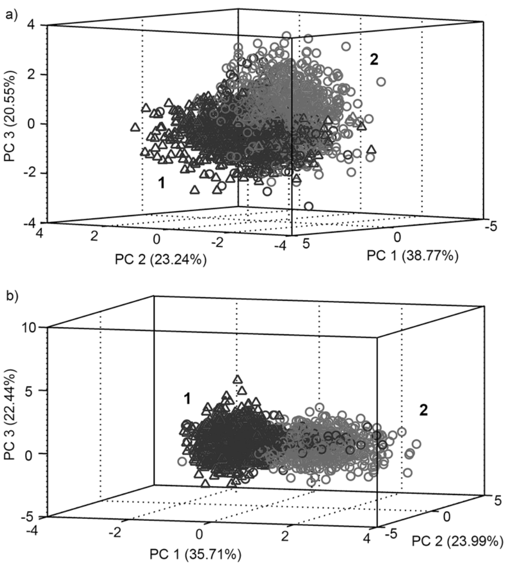

Figure 2. Data scatter plots of clustering analysis of vegetative characters of *V. vitis-idaea; 1* (triangles) denote mature forest stands, *2* (circles) denote clear-cuts. *a* and *b* represent clustering results of *V. vitis-idaea* growing in *Pinetum vaccinio-myrtillosum* and *Pinetum vacciniosum* forests, respectively. For clarity purposes, only 2000 points are shown

significant clusters, namely, the Davies-Bouldin index, Calinski-Harabasz criterion and distortion function, revealed two distinct clusters. Further analysis showed that calculating the Euclidean distances between the centroids of the clusters corresponding to mature stand and clear-cut could help reveal their closeness. The result values obtained, 2.3 and 1.8 for *V. myrtillus*, and 2.0 and 2.1 for *V. vitis-idaea* in *PVM* and *PV*, respectively, indicated that while there is an impact on *V. myrtillus* and *V. vitis-idaea* populations after clear-cutting, this effect is not extremely strong after one year after cutting. The large data scatter (variance) affected the percentage of clusters overlap (data misclustering rate). The overlap percentage observed was as follows: *V. myrtillus* – 11.5% and 25%, *V. vitis-idaea* – 15.9% and 15.3%, in *PVM* and *PV* forests, respectively.

The analysis showed that there are significant changes in these vegetative characters for *V. vitis-idaea* and *V. myrtillus* in clear-cuts, as compared with mature forest stands. *Vaccinium myrtillus* showed stronger dependency in *PVM* forest and weaker dependency in *PV* forest, while the dependency of *V. vitis-idaea* remained constant regardless of the type of forest.

Changes in phenolic compounds

Total phenolic content (TPC) varied between 120.0 and 259.5 mg g^{-1} and between 5.2 and 39.2 mg g^{-1} (RUE) in the extracts obtained from the aboveground and underground parts of *V. vitis-idaea,* respectively (Figure 3a). *Vaccinium myrtillus* contained lower concentrations of TPC in the aboveground parts (110.4 and 218.3 mg g^{-1}, RUE), but elevated concentrations in the underground parts (25.4 and 86.1 mg g^{-1}, RUE) (Figure 3b). Flavonoids were determined only for the aboveground parts of both plants. *Vaccinium myrtillus* accumulated 8.9-25.8 mg g^{-1} (RUE) of flavonoids and *V. vitis-idaea,* 21.1-39.1 mg g^{-1} (RUE) (Figure 3). Total flavonoid content (TFC) varied more between forest types than between mature stands and clear-cuts. Greater amounts of TFC were found in both species collected from *PV* forests.

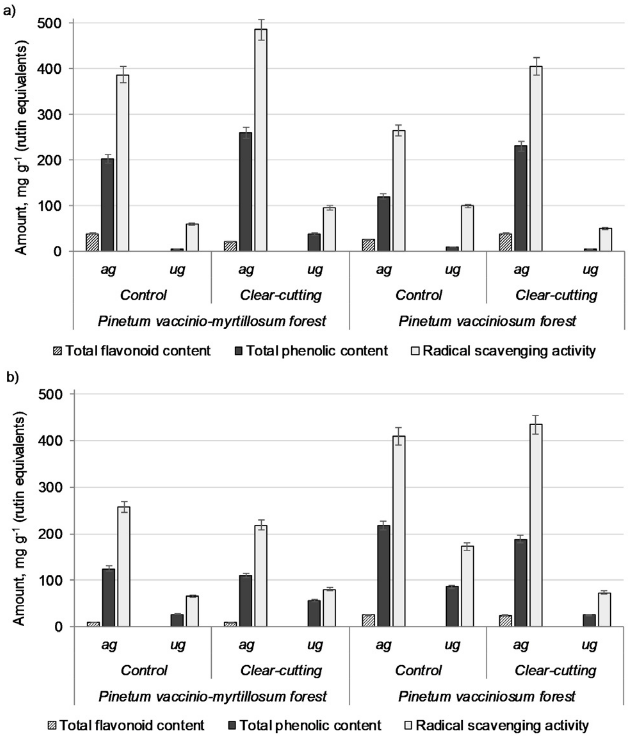

Figure 3. Variation in total phenolic content, total flavonoid content and radical scavenging activity in *Vaccinium vitis-idaea* (*a*) and *Vaccinium myrtillus* (*b*) aboveground (*au*) and underground (*ug*) parts extracts collected in different forests before and after the clear-cut

Radical scavenging activity (RSA) varied between plant species, aboveground and underground parts and forest types (Figure 3). Totals of 265.8-485.5 and 51.7-99.4 mg g^{-1} (RUE) were determined for aboveground and underground parts of *V. vitis-idaea,* respectively. By comparison, lower RSA was present in the aboveground parts of *V. myrtillus*, i.e. 218.7-434.7 mg g^{-1} (RUE), but higher values in the underground parts of this species, 65.8-172.8 mg g^{-1} (RUE). TPC showed very strong correlation with RSA in the tested samples (correlation coefficient 0.98). Correlation coefficients of between 0.87 and 0.97 for TFC and RSA were obtained for *V. vitis-idaea* and *V. myrtillus,* respectively.

Chemometric analysis of the antioxidant properties of *V. myrtillus* and *V. vitis-idaea*, as represented by the total flavonoid content, the total phenolic content and radical scavenging activity of aboveground biomass, as well as the total phenolic content and radical scavenging activity of underground biomass was undertaken. This included two-tailed hypothesis-testing, *PCA* and *KMCA*. Two-tailed hypotheses were tested using *t* statistics in order to check the significance of previously mentioned changes to variables. This was done for forest type and growth site, and the properties of populations growing in clear-cuts and in mature forest stands (control) compared.

Overall, for *V. vitis-idaea* in *PVM*, we found an increase in all assayed compounds except for the total flavonoid content of aboveground biomass, whereas in *PV*, an increase was observed only in aboveground parts, and a reduction in underground parts. For *V. myrtillus* in *PVM,* there was a reduction in these compounds in aboveground parts, and an increase in underground parts. All parameters, except for the radical scavenging activity of aboveground biomass diminished in this species in *PV* (Table 2).

Table 2. Two-tailed hypothesis-testing of results. Corresponding properties of *V. myrtillus* and *V. vitis-idaea* populations in clear-cuts were compared with mature forest stands

Forest type	Species	TFCag	TPCag	RSAag	TPCug	RSAug
Pinetum vaccinio-myrtillosum	*V. vitis-idaea*	D	I	I	I	I
	V. myrtillus	D	D	D	I	I
Pinetum vacciniosum	*V. vitis-idaea*	I	I	I	D	D
	V. myrtillus	D	D	I	D	D

I – increase, D – decrease. Statistical significance p=0.95.
TFCag – total flavonoid content of aboveground biomass; TPCag – total phenolic content of aboveground biomass; RSAag – radical scavenging activity of aboveground biomass; TPCug – total phenolic content of underground biomass; RSAug – radical scavenging activity of underground biomass

The Monte Carlo method was adopted again to inflate the data set based on the *RSD* obtained recently. Here, similar quantities were used, as well as the same apparatus and measurement method, and the number of measurements was relatively large. The procedure for generating data was that described by Kaškonas et al. (2016), when *RSD* was 5% and the number of generated data points was *n*'=10,000. A data matrix of [80000□5] was built for further analysis, where 5 is the number of variables (as given in Table 2) and 80000 represents the generated data corresponding to *V. myrtillus* occurring in *PVM* and *PV* forests (mature forest stand and clear-cut), and *V. vitis-idaea* growing in *PVM* and *PV* forest (mature forest stand and clear-cut), respectively.

Principal component analysis was performed on formed data matrix and by applying Cattell's scree plot test, the number of significant principal components was chosen. The first two principal components were left as significant when analysing *V. myrtillus* and *V. vitis-idaea* growing in *PVM* and *PV* forests. These principal components explained 89.11% and 81.76% of the total initial data variance when analysing *V. myrtillus* growing in *PVM* and *PV* forests, respectively, and 96.73% and 98.05% of the initial data variance when analysing *V. vitis-idaea*, growing in the same types of forest, respectively. Small variations in amount of the explained variance (about 0.1%) were observed because of the data set being randomly generated applying the Monte Carlo method.

After calculating orthogonal principal components clustering analysis followed. The *k*-means analysis results concerning the number of possible clusters underlying the data structure were again tested calculating the previously mentioned indices, the Davies-Bouldin index, Calinski-Harabasz criterion and distortion function, namely, which exposed the existence of two significant clusters. Data scatter plots following *k*-means clustering analysis for *V. myrtillus* and *V. vitis-idaea* represented in principal components *PC1 – PC2* space are shown in Figures 4 and 5.

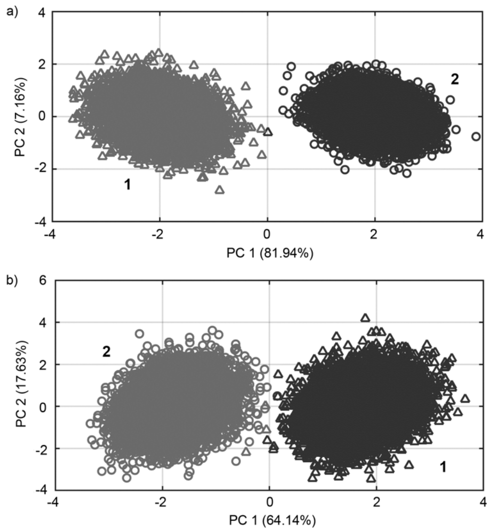

Figure 4. Scatter plots for the clustering analysis of the antioxidant properties of *V. myrtillus; 1* (triangles) denote mature forest stands, *2* (circles) denote clear-cut. *a* and *b* represent clustering results for *V. myrtillus* growing in *Pinetum vaccinio-myrtillosum* and *Pinetum vacciniosum* forests, respectively

Regardless of the type of forest, the antioxidant properties of both species changed significantly between mature forest stands and clear-cut. Clustering analysis showed very small overlap (misclustering) percentage (up to 0.05%) compared to the analysis results of the vegetative characteristics. Nevertheless, the impact of

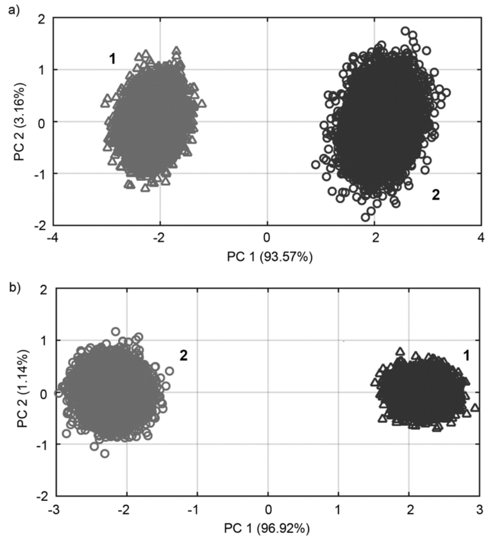

Figure 5. Scatter plots for the clustering analysis of the antioxidant properties of *V. vitis-idaea*.
1 (triangles) denote mature forest stands, *2* (circles) denote clear-cuts. *a* and *b* represent clustering results for *V. vitis-idaea* growing in *Pinetum vaccinio-myrtillosum* and *Pinetum vacciniosum* forests, respectively

clear-cuts was greater on *V. vitis-idaea* than for *V. myrtillus* populations. This conclusion can be drawn looking at estimated Euclidean distances between centroids of the built clusters representing mature stand and clear-cut. These distances were as follows: 4.3 and 4.4 clustering in *V. vitis-idaea* and 3.9 and 3.4 clustering in *V. myrtillus* data for *PVM* and *PV*, respectively.

Discussion

Anthropogenic disturbances of the boreal forest ecosystems are defined as substantial ecological processes that have important long-term impact on biogeochemical cycles and vegetation. Our focal plants, *V. myrtillus* and *V. vitis-idaea,* demonstrated different vegetative responses following the clear-cuts. An increased vegetative response (underground and aboveground biomass, length of rhizomes per 1 m^2, average height of shrubs) was observed for *V. vitis-idaea* in *PVM* forests (Table 1). The opposite, however, was demonstrated by Palviainen et al. (2005), who recorded a reduction in the biomass of *V. myrtillus* and *V. vitis-idaea* during the first seven years following the clear-cut. Miina et al. (2009) noted that the coverage of *V. myrtillus* diminished as a result of clear-cuts. Other authors reported an increase in *V. myrtillus* parameters, including percentage cover, shrub height, biomass, etc. following the clear-cut (Nielsen et al. 2007, Nybakken et al. 2013).

Both studied species are rhizomatous plants that spread vegetatively by means of underground rhizomes. These dwarf shrubs accumulate carbon and nutrient reserves in the old shrubs and underground parts, and this may increase their resistance to physical damage (Pakonen et al. 1991, Salemaa et al. 1999). Although both *V. vitis-idaea* and *V. myrtillus* are members of *Ericaceae*, their biological characteristics differ. *V. vitis-idaea* is an evergreen plant, whereas *V. myrtillus* is deciduous. Evergreen plants are usually better adapted to dry habitats and drought than deciduous species, owing to anatomical differences and a better tolerance of osmotic stress (Sobrado 1986). Results obtained for other studies indicate that mature leaves of *V. myrtillus* are very sensitive to drought (Taulavouri et al. 2010). Other authors reported that *V. vitis-idaea*, showed more efficient repair and a lower turgor loss point than *V. myrtillus* (Ganthaler and Mayr 2015). These features could perhaps contribute to the different tolerance levels of both species to environmental change after clear-cuts.

Phenolic compounds that have been detected in *V. myrtillus* and *V. vitis-idaea* may protect these plants from high levels of solar radiation. Changes in TPC, TFC, and RSA levels were observed in the tested samples. TPC varied according to type of forest and between mature forest stands and clear-cuts. Changes of TPC in the above- and underground parts presented different response of *V. myrtillus* and *V. vitis-idaea* to drastic changes after clear-cuttings. It is evident that the cutting a greater impact on TPC in extracts obtained from the aboveground parts of *V. vitis-idaea*, since here, TPC increased by 1.3- to 1.9-fold more than in *V. myrtillus* (where TPC levels diminished by 1.1 to 1.2-fold). Changes to TPC levels in underground parts depended on the type of forest (Figure 4). These findings are important in the ecological point of view. As other authors have supposed, phenolics release into the soil as root exudates and play significant role in plant-litter-soil interactions (Kraus et al. 2003, Muscolo et al. 2006). Haukioja (2005) noted the importance of phenolic compounds because of influence on survival of plant, since they act as defensive agents against solar UV light, bacterial infections and herbivores.

Hofland-Zijlstra and Berendse (2009) also observed changes in the TPC levels of *V. vitis-idaea* and *V. myrtillus* growing in shaded and non-shaded sites, however in the mentioned study, *V. myrtillus* showed remarkably higher changes than *V. vitis-idaea*. It is difficult to compare obtained data with other studies due to different plant part used for the analysis, different plant collection time, different extract preparation procedure and different reference compounds used for the calibration curve in colorimetric tests. The use of different standards for evaluation of phenolics provides a possibility to compare date obtained considering their different stoichiometry in the colorimetric reaction. In previous study by Kaškonienė et al. (2015), three different calibration standards were used for the determination of phenolic compounds, i.e. gallic acid, rutin and quercetin. Values obtained with rutin were 1.7 and 1.8 times higher than those with gallic acid and quercetin, respectively. Values obtained with gallic acid were 1.1 times higher than with quercetin (Kaškonienė et al. 2015). For example, Hofland-Zijlstra and Berendse (2009), who reported greater changes in *V. myrtillus* than in *V. vitis-idaea,* detected 430 mg g^{-1} and 230 mg g^{-1} TPC in the leaves of these two species, respectively, expressed as tannic acid equivalents, which is also roughly the order of magnitude found in our study. Ehala et al. (2005), while studying fresh berries of *V. vitis-idaea* and *V. myrtillus* found only 0.36 mg g^{-1} and 0.43 mg g^{-1} (expressed as tannic acid equivalents)*,* respectively, whereas Katsube et al. (2003), who also studied fresh berries in these two species, found 35.4 mg g^{-1} and 55.1 mg g^{-1}, respectively, expressed as catechin equivalents. Perhaps this reflects natural variation in wild populations, since Jovančević et al. (2011), who analysed fresh berries in different *V. myrtillus* populations, found variation in TPC ranging from 3.9 mg g^{-1} to 5.2 mg g^{-1}, expressed as gallic acid equivalents (GAE). Katsube et al. (2003) reported greater RSA concentrations in berries of *V. myrtillus*, than in those of *V. vitis-idaea* (287.9 µmol Trolox g^{-1} and 196.9 µmol Trolox g^{-1}, respectively).

Conclusions

This study demonstrates the initial effect of forest disturbance on the understory species *V. myrtillus* and *V. vitis-idaea* caused by the clear-cuts. Our focal plants, *V. vitis-idaea* and *V. myrtillus,* are able to survive following damage caused by the clear-cuts owing to their well-developed rhizomes. Statistical analysis revealed that significant vegetative changes occur in *V. vitis-idaea* and *V. myrtillus* within a year of clear-cuts, as compared with mature forest stands. *Vaccinium myrtillus* showed a greater response in *Pinetum vaccinio-myrtillosum* and weaker dependency in *Pinetum vacciniosum* forests, whereas the growth response of *V. vitis-idaea* remained constant, regardless of the type of forest.

The capacity to produce increased amounts of total phenolic compounds, as well as radical scavenging activity, might explain the greater resistance of *V. vitis-idaea.* The analysis of antioxidant data revealed that the antioxidant properties of both species changed significantly on comparing mature forest stands and clear-cuts, regardless of forest type. Two-tailed hypothesis-testing revealed completely converse trends in the reactions of *V. vitis-idaea* and *V. myrtillus* populations growing in *Pinetum vaccinio-myrtillosum* and *Pinetum vacciniosum* forests. With regard to the growth site, the impact on *V. vitis-idaea* populations was greater than on *V. myrtillus* populations. Trends relating to early changes in populations of dominant understory plants are important for predicting the development and natural restoration of damaged habitats and in proposing methods of reforestation.

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