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ORIGINAL RESEARCH PAPER

Evaluation of species inter-relations and soil conditions in *Arnica montana* L. habitats: a step towards active protection of endangered and high-valued medicinal plant species in NE Poland

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Abstract
Arnica montana L. is a critically endangered and highly valued medicinal plant species in Europe. We show the inter-relationships between arnica and accompanying plant species, as well as soil factors, that promote the persistence of the studied forest arnica populations in terms of active protection of this species in the northeast region of Europe. The population characteristics and plant species composition were assessed during a field study. Additionally, soil samples were taken and analyzed to assess variation in soil conditions in the habitats of arnica populations. Correlations between population characteristics and soil properties were highlighted. The forest habitats of arnica presented in this study differ from those described in other European mountain and submountain areas. The sandy and very poor soils are characterized by a very low content of macro- and microelements, and a strong acid reaction. The positive correlation between population characteristics and Ca and K indicates an important role of these macroelements in flower head production. Acidity, K, Ca, the sum of exchangeable bases, and base saturation play crucial roles in the persistence of arnica populations in pine forests. The level of acidity and its consequences result from soil-forming processes and climatic conditions rather than air pollution. When planning active protection scenarios, special attention should be paid to the frequency and cover of *Vaccinium myrtillus*, which can act as a competitor in forest habitats. Assessment of soil conditions that favor the persistence of the studied arnica populations and species relationships is important for improving knowledge of the ecology of the species and for the active protection of endangered plant species.

Keywords
mountain arnica; rapid decline; pine forest populations; population characteristics; abiotic conditions

Introduction

Mountain arnica (*Arnica montana* L.) is an herbaceous perennial herb and a medicinal plant mainly found in grasslands and shrublands of mountain environments [1–5]. It also grows in meadows on siliceous soils, marginal parts of spruce forests, open forest edges, and dry pine forests [6–8]. The species is typically found in nutrient-poor habitat types, some of which are included in the Habitats Directive of the European Union, especially seminatural dry grasslands with *Nardus stricta*, heaths, and *Juniperus communis* scrub (Commission of the European Communities 2009) [1,9]. *Arnica montana* is a typical European plant species growing in a large gradient of altitudes from nutrient-poor dry grasslands and heathlands in the Netherlands [3,10–12] to 2,830 m a.s.l. [13] in the mountain grasslands of the Alps, and in a relatively large gradient of geographical latitudes from the Scandinavian Peninsula and Lithuania southwards to the South Carpathians and Apennines [14].

In recent decades, changes in land use, habitat fragmentation, and nutrient enrichment through fertilization or cessation of traditional agricultural practices, aerial deposition of nitrogen [4,10,15,16], and collection for medicinal purposes have led to a rapid decline in this species across Europe. An increase in the frequency and coverage of different species of graminoids, which are important competitors, is a particular problem [4,17,18]. The inflow of mineral nitrogen from the atmosphere is the main cause of expansion and domination of grasses [10,11,16]. In some parts of the Eastern Carpathians, arnica populations are still relatively large, but the destruction or transformation of habitats endanger its resources. Grazing pressure in arnica habitats and land-conversion [19,20], as well as uncontrolled harvesting of inflorescences and rhizomes have a negative effect on the populations [21,22]. Therefore, arnica is regarded as a critically endangered plant species in most European countries [5,8,9,23]. Natural populations constitute resources of important genetic diversity endemic to Europe. Additionally, arnica introduces a pool of genes that are very valuable to humans [24], as well as various valuable chemotypes. This plant, which is rich in secondary metabolites [25–33], is commonly used in pharmacy, homeopathy, and cosmetics [34,35]. Moreover, arnica is a source of research material. Differences in the chemical composition of *A. montana* flowers obtained from different accessions are analyzed along the geographical gradient [31,32,36] and altitude [37,38]. A result of breeding work based on natural populations is the Arbo variety [39], which is successfully cultivated in Europe and New Zealand [40] and has been used in experimental studies [41]. In the last decade, arnica genotypes taken from natural sites and from gardens and collections have been the subject of various studies on agricultural factors that determine and modify the yield and composition of secondary metabolites [33,38,42–44]. For the protection of plant species, characterization of soil conditions is necessary but is a frequently omitted element. This plant is sensitive to air pollution and, consequently, to the eutrophication and acidification of soils [10–12]. Information on the soil conditions preferred by arnica can help to achieve success during the introduction or reintroduction of this plant species. In the literature, the effect of air pollution on arnica habitats in Western Europe has been described [10]. However, little is known about the properties of soils in arnica sites located in the lowlands of Central and Eastern Europe, and in the Bohemian Massif and the Eastern Carpathians. In studies of arnica populations, soil characteristics are very often presented as simple data and are very often based on ecological indicators [4,17,18] as substitutes [45,46] for the level of soil moisture, pH, and nitrogen. Godefroid et al. [47] analyzed the reintroduction of approximately 250 plant species worldwide by assessing the methods used and the results obtained from these reintroduction experiments. The analyses indicate that an incorrect habitat (changing habitat) is the main reason accounting for the failure of the reintroduction, as opposed to the biology of the introduced plants. Therefore, the elucidation of factors determining arnica persistence should form the first stage of research in terms of plant species conservation.

Studies of arnica populations and vegetation including this species in the floristic composition conducted in different regions of Poland during the past 2 decades have confirmed the extinction of this plant species in the mountains [16,48] and in lowland sites [5,7,8,18]. The most important area of the species occurrence in Poland is still the northeastern part of the country, in particular, the Augustów and Knyszyn forests, where

at least 50 populations are now known. This has been confirmed by current monitoring [5]. Presently, arnica occurs almost exclusively in forests, while it is noted sporadically in open habitats. There are no current data on the threat from graminoids or invasive plant species. Similarly, the effect of soil conditions on arnica populations, especially in pine forests in this region of Europe, remain unknown. Therefore, the aims of this study were (***i***) to determine the inter-relationships between arnica and accompanying plant species, (***ii***) to characterize the soil conditions of *A. montana* habitats in northeast (NE) Poland, (***iii***) to identify biotic/abiotic and natural/anthropogenic factors favoring the persistence of the studied arnica populations from the perspective of an active protection and conservation strategy.

Material and methods

Field study

The climate of NE Poland is temperate with a mean air temperature of 7.0°C (the monthly average temperature ranges from −3.9°C in January to 17.8°C in July). The average rainfall is 585 mm year^{-1} (1951–2015) and permanent snow cover persists over 70–80 days on average between December and March. The growing season begins in early April and lasts 180–200 days [49].

In June 2016, populations of *A. montana* characterized by different sizes (from 11 to 1,480 rosettes) were selected in NE Poland (Fig. 1): seven from Knyszyn Forest (near Białystok) and five from Augustów Forest (near Augustów). These forest complexes are the only areas in Central Europe similar to the southwestern taiga. The vegetation is characterized by the presence of subboreal plant communities [e.g., *Serratulo-Pinetum* (W. Mat. 1981) J. Mat. 1988, *Sphagno girgensohnii-Piceetum* Polak. 1962, *Thelypterido-Betuletum pubescentis* Czerw. 1972) with a share of boreal plant species (e.g., *Carex limosa*, *Diphasiastrum complanatum*, *Goodyera repens*, and *Linnaea borealis*) [50,51].

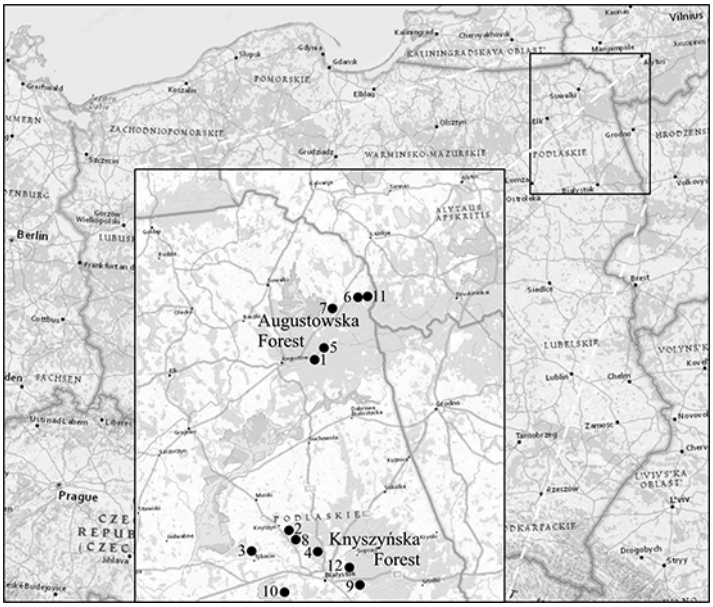

Fig. 1 Distribution map of the studied *Arnica montana* populations in northeast Poland.

They represent a large part of all extant populations in these regions. Only 12 populations were studied, as this species faces high threat as well as the high rate of species extinction in Poland. In NE Poland, no *A. montana* (AM) populations have been reported from strictly protected areas. Most populations, including those included in this study research, are located in Natura 2000 sites covered by various forms of partial protection and the economic use of forests.

In each site, one plot of 25 m^2 with *A. montana* and one plot (25 m^2) without this species were selected. In the richest populations, two plots in the patches with arnica and two plots in the patches without this plant were established; therefore, 30 plots were analyzed. Plots with arnica were chosen in sites representing the species composition and the cover of the analyzed species. In turn, the plots without arnica were randomly established 10 m from the boundary of the areas with arnica in eight directions. In each stand, the percent cover of the shrub layer, herb layer, and bryophyte layer were estimated visually. In the case of the tree layer, color photographs were taken 20 cm from ground level in the central point of every plot. We evaluated the coverage using histogram analysis tools in the GIMP program for the processing of raster graphics [52]. All vascular plant species were recorded, and the percent cover of each plant species was evaluated visually with an accuracy of up to 10%. The abundance of individual plant species was assessed using a 10-degree scale (1 – for cover 1–10%, 2 – for 11–20%,

…, 10 – for 91–100%). Based on the prepared list of vascular plant species, the plant growth form, including trees, shrubs, herbs, monocots, and dicots, was distinguished [53]. Additionally, to assess variation in the soil conditions of arnica populations, 10 soil samples were taken using a sampler from a depth of 0–15 cm at each studied site, pooled, and averaged for each site.

Population characteristics

During the field study, the population characteristics were measured. The population size was assessed by counting the total number of rosettes (number of ramets, TNR). Direct counting of rosettes or flowering stems is used to determine the population size [3,4,6]. Arnica genets can produce several compacted flowerings or nonflowering rosettes [3,44], and identification of individuals without the use of invasive methods (checking the rosette connection through rhizomes) or genetic studies is impossible. In addition, the total number of flowering rosettes (NFR) was measured in the studied populations, and the percent of flowering rosettes in relation to the total number of rosettes (PSFR) was calculated.

Analysis of soil samples

The soil samples obtained for laboratory analysis were air-dried and sieved through a polyethylene sieve (2-mm mesh). Soil texture was analyzed using the Malvern Mastersizer analyzer with the HydroG dispersion unit (Mastersizer MS-2000; Malvern, UK). The samples were prepared according to the procedure proposed by Agrawal et al. [54]. The pH was potentiometrically measured in water and 1 M KCl. The $CaCO_3$ content was assessed using Scheibler's volumetric method. The sum of exchangeable bases (TEB) (Ca, Mg, K, and Na) was determined using atomic absorption spectrometry (AAS) after extraction from the soil with 1 M CH_3COONH_4. Exchangeable acidity was determined as the sum of hydrolytic acidity (Hh) and exchangeable aluminum (Al exch.), which denotes acidity (H+Al) released upon exchange by an unbuffered 1 M KCl solution according to van Reeuwijk [55]. Hh was measured using the Kappen method [56]. Based on the results, cation exchange capacity (CEC) and base saturation (BS) were calculated. The content of organic carbon (TOC) and total nitrogen (Ntot) were determined using a LECO CNS elementary analyzer (LECO Truspec CN; St. Joseph, Michigan, USA). The accuracy of the calculations was tested against certified reference material (calibration soil sample ref. No. 502-062; LECO Corporation is A2LA accredited in accordance with the International Standards Organization ISO/IEC 17025:2005, certificate No. 3285.01). To determine the pseudo total (hereafter referred to as the total) content of heavy metals, the soil samples were dissolved with aqua regia (ISO 11466). Potentially bioavailable forms of Cu (Cu-B), Cd (Cd-B), Cr (Cr-B), Ni (Ni-B), Pb (Pb-B), and Zn (Zn-B) were extracted using 0.01 M $CaCl_2$ as previously described [57]. Trace elements in soil extracts were determined using the F-AAS technique (Agilent 240 FS F-AAS; Santa Clara, California, USA). Geochemical analyses were carried out based on reference samples SO-2 and SO-4 from the Canada Center for Mineral and Energy Technology. The precision of the analyses was within the range of approximately 1.9% to approximately 8.0% (e.g., 1.92% for Cu, 2.02% for Cd, 2.43% for Pb, 3.04% for Zn, 7.4% for Cr, and 8.1% for Ni). The detection limits for F-AAS were 1.5 µg/L for Cd, 3 µg/L for Cu, 6 µg/L for Cr, 10 µg/L for Ni and Pb, 1 µg/L for Zn.

Statistical analysis

Prior to the analysis, all data were tested for normality with the Shapiro–Wilk test. Variance heterogeneity was checked using Levene's test. As most data were not normally distributed or the variance was not homogeneous, the nonparametric Spearman rank correlation coefficient was used to analyze the correlations between population characteristics (NR, NFR, PSFR) and soil properties, as well as the correlations between chemical data. The Kruskal–Wallis test was used to compare more than two samples

that were independent or unrelated, and the Mann–Whitney *U* test was used to analyze significant differences between specific sample pairs. The coverage of plant species, the coverage of particular forest layers, and the number of particular growth forms were expressed as means and standard deviations (*SD*), and the differences were considered significant at $p < 0.05$. All statistical analyses were carried out using the Statistica 6.0 software [58]. Variation in the plant species composition of the studied plots was explored using principal component analysis (PCA), as the detrended correspondence analysis (DCA) results (the length of the first DCA axis was 2.8 *SD*) detected a modal structure of the vegetation data [59]. Sporadic plant species, such as *Betula pendula*, *Larix decidua*, and *Pteridium pinetorum* (frequency lower than 10%), were excluded from the analyses. The data were centered, standardized, and log-transformed. Ordination analyses were conducted using the multivariate statistical package (MVSP) [60].

Results

Population characteristics

In the studied populations of AM, individual ramets were commonly identified in small clusters as well as in single isolated rosettes. The total number of rosettes of AM in the studied habitats was in the range of 11–1,480 (Tab. 1). In the largest populations, the number of flowering rosettes was in the range of 154–298, and the percent share of flowering rosettes was in the range of 12.4–20.1%. The NFR in the other populations was lower, with a maximum value of 32 and the PSFR was in the range of 0–17.1%.

Tab. 1 Characteristics of the *Arnica montana* population.

	Sites											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
TNR	11	74	76	77	86	88	159	214	372	1,246	1,479	1,480
NFR	0	2	13	3	0	6	17	9	32	154	243	298
PSFR	0.0	1.4	17.1	3.9	0.0	6.8	10.7	4.2	8.6	12.4	16.4	20.1

TNR – the total number of rosettes; NFR – the number of flowering rosettes; PSFR – the number of flowering rosettes in relation to the total number of rosettes (%).

Vertical structure of forest plant communities and interspecies relationship

The results of the PCA based on the cover of particular forest layers, the number of particular growth forms, and the cover of most of the studied plant species are presented in Fig. 2. The studied forest populations of AM were found in open stands near forest block lines or pathways, and in sites where the average cover of the tree layer was 48% (Fig. 3). The three PCA axes explained 54.25% of the total variance (Tab. 2). Axis 1 was positively correlated with *P. sylvestris* and *Vaccinium myrtillus*, and negatively correlated with *L. pilosa*, *M. pratense*, *A. montana*, *A. capillaris*, *Calamagrostis arundinacea*, and *Calluna vulgaris*. Axis 2 of the PCA clearly separated the studied plots, with the presence of AM on the right side and those without AM on the left side. The plots with and without AM in the study area differed in their community composition (Fig. 2), indicating that *A. montana* is restricted to areas characterized by the high frequency of *C. arundinacea*, *C. vulgaris*, *Convallaria majalis*, and *Festuca ovina*. In turn, the plots without AM were characterized by the absence of *B. pubescens*, *J. communis*, *L. pilosa*, *M. pratense*, and *A. capillaris*, and a higher frequency of *P. sylvestris* and *V. myrtillus*.

The cover of the tree layer, shrub layer, and bryophyte layer in the studied plots with and without AM was similar. However, statistically significant differences were demonstrated in the case of the herb layer; the mean coverage was 34.7% in the plots with

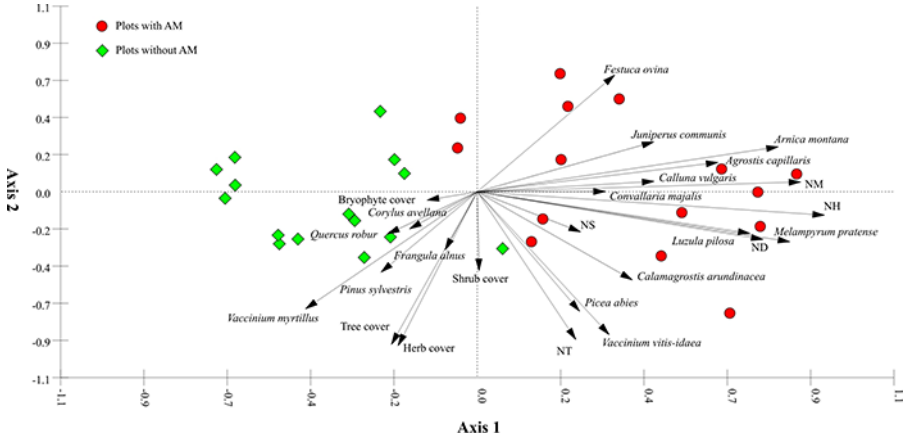

Fig. 2 Comparison of the coverage of particular forest layers (mean ±*SD*) in the two plot groups with and without *A. montana* (AM); different letters indicate significant differences according to the Mann–Whitney test ($p < 0.05$).

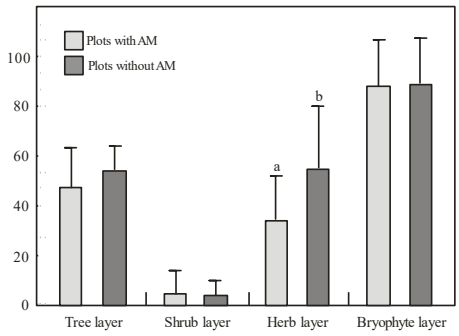

Fig. 3 Comparison of the coverage of particular forest layers (mean ±*SD*) in the two plot groups with and without *A. montana* (AM); different letters indicate significant differences according to the Mann–Whitney test ($p < 0.05$).

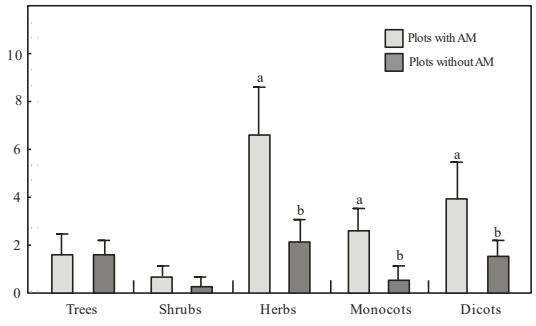

Fig. 4 Comparison of the number of specific growth forms (mean ±*SD*) in the two plot groups with and without *A. montana* (AM); different letters indicate significant differences according to the Mann–Whitney test ($p < 0.05$).

Tab. 2 Results of principal component analysis (PCA) based on the 15 randomly chosen plots with a share of *A. montana* (AM) and 15 randomly chosen plots without AM.

Plant species	Axis 1	Axis 2	Axis 3
Eigenvalues and variance (%) explained by the first three PCA axes			
Eigenvalues	3.662	2.019	1.914
Percentage	26.16	14.425	13.67
Cumulative percentage	26.16	40.585	54.255
Loading components for each variable associated with the three axes			
Juniperus communis	−0.291	0.133	−0.376
Picea abies	−0.057	0.226	0.035
Pinus sylvestris	0.215	0.312	−0.307
Quercus robur	0.074	−0.420	0.179
Agrostis capillaris	−0.318	−0.303	0.203
Arnica montana	−0.375	−0.093	−0.016
Calamagrostis arundinacea	−0.270	−0.224	0.391
Calluna vulgaris	−0.279	0.346	0.193
Convallaria majalis	−0.041	0.384	0.393
Festuca ovina	−0.161	0.091	−0.380
Luzula pilosa	−0.459	−0.029	−0.136
Melampyrum pratense	−0.438	0.081	−0.153
Vaccinium myrtillus	0.188	−0.081	−0.002
Vaccinium vitis-idaea	−0.026	0.466	0.398

AM and 55.3% in the plots without AM (Fig. 3). The studied plant communities were very poor in vascular plant species, especially for trees and shrubs. The mean number of herbs, monocots, and dicots in the plots with AM and in the plots without AM was 6.7, 2.7, and 4.0 and 2.2, 0.6, and 1.6, respectively (Fig. 4). Among the analyzed growth forms, both the number of herbs and the number of monocots and dicots was significantly higher in the plots with AM than in the plots without AM. However, direct comparison of cover by particular plant species in the studied plots revealed significantly higher values for *V. myrtillus* (40%) in the plots without AM than in the plots with AM (16%) (Fig. 5). The mean cover of *P. sylvestris* in the studied plots

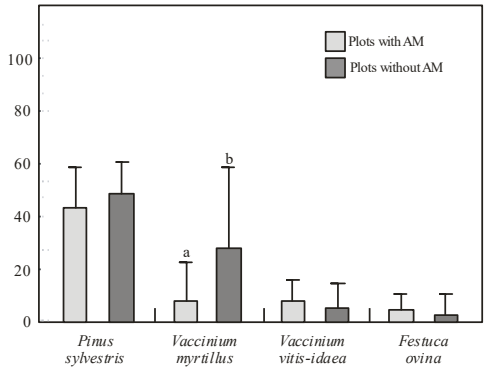

Fig. 5 Comparison of the coverage of chosen plant species (mean ±*SD*) in the two plot groups with and without *A. montana* (AM); different letters indicate significant differences according to the Mann–Whitney test ($p < 0.05$).

of the two compared groups was over 40%, and that of *V. vitis-idaea* and *F. ovina* did not exceed 11%. However, no statistically significant differences were found between these groups for these species.

Soil properties

The very poor sandy soils of forest arnica habitats are characterized by very low contents of macro- and microelements and a strong acid reaction. In the present study, the soil reaction was acidic and strongly acidic; the values of soil pH(H_2O) ranged from 4.32 to 4.87 and pH(KCl) ranged from 3.51 to 3.99 (Tab. 3). Higher values of active acidity (pH in H_2O 4.72–4.87) were recorded in the population characterized by the presence of more than 1,200 rosettes. The soil samples did not contain $CaCO_3$. Soil was characterized by a moderate content of C and a low content of N. The C:N ratio was in the range of 13–32, indicating the low biogenic activity of soils.

The studied soils exhibited a low content of bioavailable forms of potassium and phosphorus (Tab. 3). In acidic soils, the decline in Ca reduces the amount of K displaced into the solution and, hence, reduces the availability to roots. At a soil pH lower than 5.5, the ion Al most commonly reacts, as well as Fe and Ca. This gradually results in the formation of insoluble P compounds, which are generally not available to plants [61]. Similarly, a very low abundance of calcium, magnesium, sodium, and potassium cations were detected (Tab. 4). The largest amount of exchangeable cations were determined in sites characterized by a high number of rosettes, and the lowest levels were detected in sites with a low number of rosettes. The soils within the AM sites were extremely sandy (from 91.62% to 96.23% of the 2–0.05 mm fraction) and the upper layer (0–15 cm) was formed by the granulometric group of sands, according to USDA [62], with a very small proportion of the silt fraction (Tab. 5). No clay fraction was found in the 10 soil samples.

The total concentrations of heavy metals in the upper 0–15 cm soil layer collected from the arnica stands are presented in Tab. 3. The mean total metal concentration

Tab. 3 Chemical properties of soils of *A. montana* habitats.

	Sites											
Properties	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
pH(H_2O)	4.42	4.61	4.6	4.32	4.55	4.74	4.71	4.63	4.63	4.72	4.87	4.78
pH(KCl)	3.64	3.9	3.9	3.51	3.87	3.98	3.93	3.95	3.99	3.93	3.97	3.95
Ntot (%)	0.16	0.14	0.10	0.10	0.10	0.11	0.14	0.10	0.14	0.14	0.12	0.15
TOC (%)	3.15	3.06	2.45	1.97	1.69	2.63	2.64	1.88	3.08	2.87	2.97	2.88
P (mg kg^{-1})	7.79	10.15	7.43	5.44	5.08	10.33	6.53	8.70	7.43	5.98	6.89	5.26
K (mg kg^{-1})	18.5	19.4	13.2	11.8	13	15.1	20.3	18.9	22	22.3	36.8	26.6
Fe-T (mg kg^{-1})	3,664	3,867	3,989	1,700	3,461	3,185	3,340	3,767	5,190	4,257	4,895	4,081
Mn-T (mg kg^{-1})	161.2	160.1	196.8	33.9	86.6	71.6	197.7	124.8	113.4	169.3	33.3	100.8
Zn-T (mg kg^{-1})	77.50	40.40	48.50	28.20	42.90	25.20	56.30	32.50	34.60	59.40	38.30	44.10
Pb-T (mg kg^{-1})	12.10	10.80	11.90	12.30	9.52	0.10	11.30	10.90	13.50	0.10	12.80	12.90
Cr-T (mg kg^{-1})	8.19	7.69	6.82	5.28	4.35	8.23	8.16	8.35	9.14	9.63	11.30	9.37
Cu-T (mg kg^{-1})	3.13	0.09	0.04	0.03	0.04	0.08	3.37	0.09	0.12	4.91	8.82	0.04
Fe-B (mg kg^{-1})	13.01	6.15	11.20	14.40	3.05	6.68	1.50	7.47	11.60	3.50	3.18	2.99
Mn-B (mg kg^{-1})	15.90	3.75	3.87	4.02	7.80	1.50	14.60	11.50	4.31	10.10	1.70	19.10
Zn-B (mg kg^{-1})	1.63	0.44	0.71	0.67	0.61	0.75	2.44	2.07	0.65	2.55	0.76	3.67

Ntot – total nitrogen; TOC – total organic carbon; T – total forms; B – bioavailable forms.

Tab. 4 Exchangeable cations and sorptive characteristics of the soils in *A. montana* sites.

Properties	Sites											
	1	2	3	4	5	6	7	8	9	10	11	12
Na^+ [cmol(+) kg^{-1}]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.01	0.01
K^+ [cmol(+) kg^{-1}]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
Mg^{2+} [cmol(+) kg^{-1}]	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02	0.01	0.03	0.02	0.05
Ca^{2+} [cmol(+) kg^{-1}]	0.10	0.06	0.12	0.05	0.05	0.12	0.10	0.05	0.13	0.16	0.12	0.16
Hh [cmol(+) kg^{-1}]	15.30	10.12	11.17	10.87	7.35	8.92	8.10	9.22	12.52	12.52	12.67	11.70
Al^{3+} [cmol(+) kg^{-1}]	3.01	2.47	1.83	1.78	1.09	1.82	1.45	1.87	2.69	2.72	2.73	2.22
Al^{3+}/Ca^{2+}	27.36	41.17	15.28	13.72	21.80	15.17	14.50	37.40	29.89	17.00	22.75	13.88
TEB [cmol(+) kg^{-1}]	0.14	0.10	0.17	0.10	0.09	0.16	0.14	0.09	0.17	0.22	0.17	0.24
CEC [cmol(+) kg^{-1}]	15.44	10.22	11.34	10.97	7.44	9.08	8.24	9.31	12.69	12.74	12.84	11.94
BS (%)	0.91	0.98	1.50	0.91	1.21	1.76	1.70	0.97	1.34	1.73	1.32	2.01

Hh – hydrolytic acidity; TEB – total exchangeable bases; CEC – cation exchange capacity; BS – base saturation.

Tab. 5 Percent share of particular fractions of soils in *A. montana* sites.

Fraction (mm)	Sites											
	1	2	3	4	5	6	7	8	9	10	11	12
<0.002	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.02
0.002–0.02	1.60	2.13	1.89	1.63	1.24	2.33	1.62	2.46	1.81	2.41	2.22	3.29
0.02–0.05	2.76	2.96	2.95	3.05	2.53	3.46	3.55	3.01	3.92	4.19	4.27	5.06
0.05–0.1	3.09	7.60	3.17	5.20	2.62	8.06	5.34	5.28	8.10	13.73	5.11	9.83
0.1–0.25	36.92	39.02	33.98	47.27	31.50	38.51	28.92	28.44	17.84	50.68	21.67	37.87
0.25–0.5	43.83	33.54	44.32	37.31	43.98	33.65	38.31	35.55	20.10	21.71	37.37	28.23
0.5–1.0	11.76	13.41	13.68	5.54	17.29	12.39	19.56	20.91	32.99	5.05	24.68	12.26
1.0–2.0	0.04	1.34	0.01	0.00	0.84	1.60	2.70	4.35	15.17	2.23	4.68	3.44

sequence in the studied soils was as follows: Fe > Mn > Zn > Cr > Pb > Cu. The Fe and Mn concentrations were in the range of 1,700–5,190 mg kg^{-1} and 33.9–197.7 mg kg^{-1}, respectively. The concentration of other metals was as follows: Pb 0.10–13.50 mg kg^{-1}, Cr 4.35–11.30 mg kg^{-1}, and Cu 0.04–8.82 mg kg^{-1}. The concentration of exchangeable forms was in the following range: Fe-B 1.50–14.40 mg kg^{-1}, Mn-B 1.50–19.10 mg kg^{-1}, Zn-B 0.44–3.67 mg kg^{-1}. The concentration of the total forms of Ni and Cd was very low and did not exceed 0.02 mg kg^{-1}, similar to the potentially bioavailable forms of Pb, Cr, Cu, Ni, and Cd; therefore, these data are not presented.

Soil properties vs. population characteristics

The correlations between the population characteristics and soil conditions are presented in Tab. 6. All population characteristics (TNR, NFR, PSFR) were positively correlated with the pH(H_2O), K, K^+, Ca^{2+}, TEB, and BS soil parameters. Additionally, TNR and NFR were positively correlated with pH(KCl) and Cr-T. Moreover, the correlation results indicated a dependence between the population characteristics and the soil fractions; there was a positive correlation with the silt fraction and a negative correlation with the sand fraction.

Tab. 6 Spearman rank correlation coefficients (soil properties vs. population characteristics; $n = 12$).

Soil properties	Population characteristics		
	TNR	NFR	PSFR
pH(H^2O)	0.82**	0.79**	0.69*
pH(KCl)	0.71**	0.65*	0.51
Ntot	0.10	0.13	−0.05
TOC	0.30	0.47	0.35
P	−0.36	−0.26	−0.21
K	0.76*	0.80**	0.59*
Fe-T	0.55	0.66*	0.54
Mn-T	−0.27	0.00	0.12
Zn-T	−0.11	0.07	0.14
Pb-T	0.26	0.40	0.32
Cr-T	0.79**	0.77*	0.56
Cu-T	0.32	0.35	0.15
Fe-B	−0.51	−0.41	−0.36
Mn-B	0.17	0.11	0.01
Zn-B	0.56	0.59*	0.54
Na^+	0.39	0.39	0.19
K^+	0.65*	0.65*	0.58*
Mg^{2+}	0.13	0.28	0.46
Ca^{2+}	0.58*	0.76**	0.75**
Al^{3+}	0.17	0.29	0.14
Al^{3+}/Ca^{2+}	−0.17	−0.25	−0.40
Hh	0.19	0.37	0.28
TEB	0.58*	0.80**	0.80**
CEC	0.19	0.37	0.29
BS	0.59*	0.67*	0.74**
Silt	0.85***	0.87***	0.73**
Sand	−0.87***	−0.89***	−0.74**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. TNR – the total number of rosettes; NFR – the number of flowering rosettes; PSFR – the number of flowering rosettes in relation to the total number of rosettes (%); Ntot – total nitrogen; TOC – total organic carbon; Hh – hydrolytic acidity; TEB – total exchangeable bases; CEC – cation exchange capacity; BS – base saturation; T – total forms; B – bioavailable forms.

Discussion

The distribution pattern of arnica individuals was similar to that in nonforest [3,63] and forest plant communities in Europe [6]. This results from vegetative (multiramet genets) and generative (single ramets) propagation [3]. Most of the studied forest stands have been characterized by their direct vicinity to open habitats near forest separating lines and pathways. This localization is typical for pine forest populations [6–8].

The studied arnica populations occur in the Scots pine forest belonging to the *Dicrano-Pinion sylvestris* alliance [64]. The forest habitats of arnica are different from those described in other European mountain and submountain areas [2,4,21,22,63] and in heatlands and grasslands of lowlands areas [2–4]. In turn, their floristic composition is similar to phytocoenosis described from other parts of NE Poland [7,8] as well as southeastern and eastern Lithuania [6]. The studied arnica pine forest habitats are located on nutrient-poor, often acidic, podzolic soils, which are common in NE Poland [65,66].

Generally, the dominance of grasses, which are regarded as very serious competitors in plant communities with arnica, negatively influence the vitality of this species [4,11,16,18]. During arnica reintroduction, Blachnik and Saller [67] showed that the competition from graminoid species such as *Holcus mollis* limited the development and establishment of seedlings and young plants. Conversely, the *F. ovina* and *A. capillaris* grasses recorded in the studied plots (Fig. 2) are typical species accompanying arnica in the nutrient-poor, seminatural grasslands of the *Violion caninae*, alliance typical for Central and Western Europe [17,68]. Moreover, *A. capillaris*, a very frequent floristic component of arnica forest habitats, is also a natural component of AM-comprising plant communities in the Eastern Carpathians [63]. The two grass species mentioned above occupy the space in a different way than *H. mollis*. They do not form a compact turf, but leave many gaps; therefore, they are not serious competitors for AM. Moreover, *C. arundinacea* is a natural component of the pine forest of NE Poland and southern Lithuania [6–8] and grassland communities in the Eastern Carpathians [21,22,63].

Calamagrostis arundinacea can compete with arnica in view of its form of growth; however, unlike *H. mollis*, it requires full sun exposure and prefers much more fertile soils and a substrate with neutral pH. Therefore, it is not surprising that it rarely occurs together with arnica in the phytocoenosis of the Knyszyn and Augustów forests [50,51]. The frequency and cover of these graminoids were low (a few percent), which do not compete with arnica in the forest habitats studied, but rather play an accompanying role. However, attention should be paid to the relationship between *A. montana* and *V. myrtillus*. This dwarf shrub, besides *V. vitis-idaea*, is also a natural component of pine forests [66]. Nevertheless, as demonstrated in the present study, the low frequency and cover of *V. myrtillus* (Fig. 5) suggest that arnica prefers sites without or with very low cover of this dwarf shrub. An increase in *V. myrtillus* cover can threaten AM. The observations presented in this paper are consistent with recent reports. For example, in pine forests, arnica individuals are shadowed by *V. myrtillus* dwarf shrubs [7]; however, excessive grazing in the mountain grasslands has facilitated the invasion of arnica sites by *V. myrtillus* [63]. Therefore, the relationship between arnica and blueberry should be considered in terms of active protection. Under the conditions of the study area, *V. myrtillus* was a very effective competitor of AM. Competitive relationships between species have been discussed in detail in the text. The development of the dense cover of dwarf shrubs, mainly *V. myrtillus*, is one of the aspects of regenerative changes in pine forests, observed in recent decades. Indisputably, such natural processes are a threat to the AM population.

The increased frequency of grass species among the total number of vascular plants in *Violion caninae* arnica sites is problematic in Western Europe. During the last 60 years of the twentieth century, the frequency of monocots increased from over 20% to approximately 45% in Germany and from 28% to 40% in the Netherlands, This was due to the enrichment of atmospheric nitrogen in grassland ecosystems accompanied the by loss of species richness, especially dicots [17]. Our results confirm these relationships. Fennema [10] found that the number of dicots was around twofold higher, the number of herb species was approximately 30%, and the total number of species per stand was approximately 30% higher in the present arnica stands than in the former. However, in that study, there were no differences in the percentage cover of herbs and bryophytes, whereas we observed significantly lower cover of the herb layer in the sites with AM.

Environmental properties, such as soil pH, are related to the distribution and diversity of plant species within terrestrial ecosystems [69]. Under the conditions of heathlands in Western Europe, arnica grows on weak-to-moderately acidic soils (pH $H_2O > 4.4$) with a relatively high base cation concentration and relatively low Al:Ca ratios [10,69,70]. The findings of the present study are consistent with these data. Here, the positive correlation between pH and population characteristics confirmed the significance of this factor for arnica persistence. Small populations (11–88 rosettes) were characterized by a pH of 4.32–4.55, whereas large populations (>1,200 rosettes) were characterized by a pH of 4.72–4.87. At first glance, these differences appear negligible; however, an increase in pH by 0.2 and 0.3 leads to an increase of 50% and almost 100% in the H^+ concentration, respectively. Therefore, the soils of the small populations are characterized by a twofold higher H^+ concentration than the soils of the large populations. The visible differences between the pH values of 1 M KCl (potential reaction) and pH in water (active reaction) indicate a significant share of exchangeable aluminum; in turn, hydrolysis contributes to acidification of the soil environment. The properties of the studied soils are characteristic of dystrophic environments [71].

In the present results, the positive correlation between the population characteristics and Ca and K indicated an important role for these macroelements in flower head production (Tab. 6). Arnica is a valuable medical plant, which contains the following compounds: flavonoids, sesquiterpene lactones, polysaccharides, carotenoids, tannins, phenolic acids, and essential oils [25–33]. Therefore, these macroelements can influence not only the flower head production, but also the accumulation and quality of bioactive constituents in the plant.

Arnica is a typical calcifuge species. Plants in this group are less sensitive to Al than calciphilous species because the low Ca-demand in plants appears to be a precondition for their successful growth on acidic, low-Ca soils [72]. Nevertheless, in extremely poor habitats (i.e., very low in calcium), such as the studied pine forest sites, Ca can play a crucial role in the persistence of arnica individuals. A very low Ca concentration, (i.e., lower than the plant demand), can limit growth and flowering. The present findings are largely consistent with previous studies conducted in Western Europe [10]. For example, almost 30 years ago, soils where *A. montana* still occurred on the heathlands were found to be richer in Mg, Ca, K, and Na, and exhibited a higher soil pH value, higher cation/N ratios, and a lower Al/Ca ratio than soils where *A. montana* had disappeared. In the present study, no correlations were observed between the population characteristics and Al^{3+} and the Al/Ca ratio.

The results indicated a positive correlation of the population characteristics (NR, NFR, and PSFR) with the silt fraction and a negative correlation with the sand fraction. The soil texture in arnica habitats has not been formerly studied, despite this typical mountain species inhabiting different soil types: loamy leptosols and loamy cambisols in mountain conditions [67], cambisols and podzols in lowland areas [8,73], and podzols in pine forest habitats [5,6]. Fennema [10] indicated that TEB is a crucial factor for the existence of AM. The mineralogical composition of the clay fraction and pedogenic processes influence the sorption capacity and the share of basic cations in the sorption complex [74]. In the present study, there was no clay fraction in most of the studied soil samples; therefore, TEB is probably associated with organic matter [75], which was confirmed in the presented study (Tab. S1).

Previous studies have highlighted the sensitivity of arnica to air pollution and, consequently, eutrophication and acidification [10–12,76] as the main cause of arnica

decline in nonforested habitats in Western Europe. As noted by Fabiszewski and Wojtuń [16], the main cause of the decreasing populations in the Karkonosze Mountains in the south of Poland (Bohemian Massif) is the change in the soil environment associated with anthropogenic nitrogen fertilization. In the present study, the effect of natural soil processes was emphasized, as the studied arnica habitats are located in NE Poland with a climate characterized by a predominance of precipitation over evaporation, resulting in the transfer of basic cations, mainly Ca^{2+} and Mg^{2+}, and a decrease in the absorption of most nutrients [75,77]. In addition, there are secondary effects of soil acidification, which include reduced durability of mineral bundles, breakdown of the structure of secondary clay minerals, reduction of sorption capacity, and the emergence of large amounts of aluminum and manganese, which are toxic to plants [74,77]. The studied area is situated in the northeastern region of Poland (Podlasie region), which is termed the "green lungs of Poland" because of very low level of urbanization and industrialization. Therefore, the content of heavy metals (Tab. 3) in the analyzed soils is evidently low and does not exceed natural or even boundary values established for mineral soils [74]. Moreover, in the studied region, the weighted average pH of precipitation samples was close to the natural value for all directions of air mass inflow, while they are usually below 5.6 in other regions of Poland [78]. The concentration of NO_x was half, and the concentrations of Cd and Pb were several times lower in atmospheric precipitation than in other regions of the country. Air monitoring data from the analyzed region show that the concentration of dust in the air is several times lower, and the concentration of heavy metals is the lowest of all other regions in Poland [79,80]. Therefore, the low content of heavy metals [74] probably indicates that air pollution does not exert a significant effect on the arnica sites. Although the content of nitrogen ion forms was not tested in the present study, the content of heavy metals, which are a frequent element of air pollution, is very low. It is probable that the acidity of the studied soils is created by processes related to climatic conditions where rainfall exceeds evapotranspiration [81].

Since the Neolithic and early Middle Ages, Scots pine forests in NE Poland have been shaped by various types of human activity. Forest burning (conducted by beekeepers) and litter raking were performed for centuries, and resulted in the permanent removal of nutrients from the upper parts of the soil, as well as highly competitive species of herbaceous plants and shrubs, and led to the promotion of habitat conditions suitable for AM populations. This type of traditional forest use ended in the first decades of the twentieth century. In this period, regeneration processes of mixed forest were initiated, resulting in a growing share of deciduous trees and shrubs as well as the eutrophication of habitats. Regeneration of the lower forest layers resulted in biomass deposition and accumulation of humus and nutrients in the upper levels of the soil profile. Despite this, the negative effect of nitrogen compound deposition from agricultural areas on AM habitats and populations should not be neglected.

Acidification is one of the main effects of nitrogen deposition on terrestrial biodiversity in Europe [82]. The soil reaction determines the availability of nutrients for the plants. For example, soil pH affects the availability of P, K, and other macronutrients [69]. In the present study, the positive correlation between pH and population characteristics indicates that this factor plays a key role in the persistence of arnica in pine forest habitats. However, this is an indirect determinant of the content of potentially bioavailable and exchangeable forms of K, K^+, Ca^{2+}, and other characteristics, such as TEB and BS. This dependence was confirmed by the strong positive correlation between pH and these soil properties (Tab. S1). In the present results, the concentration of potassium (K, K^+) in soils is positively correlated with the population characteristics (TNR, NFR, and PSFR) (Tab. 6). Simultaneously, these forms of potassium are strongly positively correlated with C, TEB, and silt, and highly negatively correlated with sand. In very poor soils, such as those analyzed in the present study, in the absence of a clay fraction, the silt fraction can play an important source of macroelements during weathering of the parent rock. A higher TOC content was observed in soils with a high silt content (Tab. S1). The sum of base cations depends on the organic carbon content and particle-size distribution related to the parent rock type [74]. It is possible that in the absence of the clay fraction, which guarantees the presence of mineral colloids, organic (humus) colloids lead to higher values for TEB, Ca^{2+}, and BS, which are positively correlated with population characteristics (Tab. 6). Moreover, organic matter plays a crucial role

in the absorption of heavy metals such as Cr and Cu [74]. This was confirmed by the positive correlation of TOC with Cr and Cu (Tab. S1). We found that TNR and PSFR in the studied population were not directly affected by the percent share of silt (positive correlation) and sand (negative correlation), but were indirectly affected by the K, Ca^{2+}, and TEB of the studied soils, which were correlated with the silt and TOC contents (Tab. S1).

Conclusions

Acidity, K, Ca, TEB, and BS play crucial roles in the persistence of arnica populations in pine forests. Under the conditions of pine forests in NE Poland, a $pH(H_2O) > 4.5$ and related values of other factors dependent on the soil reaction appear to be crucial for the persistence of arnica populations. Therefore, active protection should be preceded by the accurate assessment of soil conditions, especially those mentioned above. In extremely poor (oligotrophic) habitats, as in the studied pine forest sites, calcium, which is the main component of TEB, can play an important role in the persistence of arnica individuals. The positive correlation between population characteristics, such as the share of flowering ramets, and Ca, may indicate that the concentration of this macroelement affects flowering, resulting in making generative propagation of this plant species more likely. The level of acidity and subsequent consequences in the studied forest arnica habitats are related to the effects of soil-forming processes (leaching of alkaline cations, displacement of mineral particles into the soil) determined by the characteristic vegetation (pine needle domination in acidic litter) and the climatic conditions (rainfall exceeding evapotranspiration) rather than air pollution.

Active protection of *A. montana* in NE Poland is necessary. Assessment of soil conditions and species relationships is an important step towards greater knowledge of the ecology of the species and the first step towards the protection of this endangered and highly valued medicinal plant species. When planning active protection scenarios, selection of sites for arnica introduction and reintroduction, and selection of the population for reinforcement, particular attention should be paid to the frequency and cover of *V. myrtillus*, which in forest habitats can act as a competitor. In the near future, we are planning to take a second step towards the active protection of arnica by performing genetic analyses of the investigated populations, which is necessary for the implementation of a conservation strategy.

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Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3592/0:

Tab. S1 Correlation between the studied soil properties.

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