Habitat preferences of *Comarum palustre* L. in the peatlands of eastern Poland

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SUMMARY

The aim of this study was to analyse groundwater quality and botanical species composition in natural stands of purple marshlocks (*Comarum palustre* L., synonym *Potentilla palustris* L.), which is common in Central and Western Europe but little known in that region as a medicinal herb. The prospective role of *C. palustre* (owing to efforts to disseminate knowledge of its importance) and the possible need for organic cultivation of this species (due to degradation of its natural habitats) necessitate analysis of its habitat preferences. The methods used in the study included chemical analysis of shallow groundwater and botanical inventory, as well as comprehensive statistical analyses. We concluded that lower values of NH_4 = 0.1–0.21, Ca = 6.8–25.3, Mg = 0.77–2.79, DOC = 25.9–52.9 concentrations (mg dm^{-3}), EC = 72.2–107.6 µS· cm^{-1}, and pH = 5.3–5.7 were conditions promoting the abundance of *C. palustre*. Based on the analysis of physicochemical habitat factors and botanical characteristics, as well as the statistical analyses, the species was found to possess a broad ecological amplitude with respect to most of the factors investigated, but within a range typical for its habitats. The species compositions of the phytocenoses with *C. palustre* were typical for nutrient-poor peatland habitats and also associated with fertile habitats which are considered optimal for the occurrence of *C. palustre*.

KEY WORDS: ecological amplitude, medicinal plant, physicochemical factors, populations, *Potentilla palustris* L., purple marshlocks

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INTRODUCTION

The balance of matter in surface waters and groundwater is determined by physical, geographical and climatic factors, as well as by numerous anthropogenic conditions (Grzywna 2014, Kowalik *et al.* 2014). Among anthropogenic factors, industry, municipal management, transportation, tourism and recreation make the greatest contribution to water pollution (Geertsema *et al*. 2002, Soomers *et al*. 2013). In the case of mires and peatlands, nearby agricultural areas are the primary source of biogenic substances and post-production pollutants, causing a change in the fertility of this type of environment towards eutrophy (Misztal *et al.* 1992).

An increase in the trophic status and productivity of habitats caused by changes in the chemical characteristics of groundwater leads to a qualitative transformation and simplification of the species composition of biocenoses (Serafin *et al*. 2018) and the loss of biological diversity (Wassen *et al*. 2005, Banaszuk & Kamocki 2016). A further consequence of these habitat transformations is the loss from local sites of plant species that fail to withstand revolutionary changes in the environment (Grandcolas *et al*. 2014).

Purple marshlocks (*Comarum palustre* L.), a perennial plant of the Rosaceae family, is one of the lesser known herbal (medicinal) plants in Central and Western Europe but extremely popular in Eastern Europe (Sozinov & Grumno 2016, Kashchenko *et al*. 2017). In Poland, this species is found at many sites and is obtained from natural habitats. However, in recent decades, a decrease in the number of sites and a marked decrease in the number of individuals in these sites have been observed (Zarzycki *et al*. 2002), mostly due to degradation of its natural stands associated with lowered water levels, land drainage, eutrophication, peat extraction, and changes in land use (Herbich 2001, Zarzycki *et al*. 2002, Michalska-Hejduk & Kopeć 2010).

Comarum palustre has a sprawling and erect stem, lignified at the base; odd-pinnate, nearly palmate, serrate, dark green leaves (5–7); and purple 5–6-petal flowers borne in corymbs. It produces red aggregate nut fruits, sometimes called aggregate achenes

(Podhajská & Rivola 1992). In a temperate humid climate, *C. palustre* blooms from May to July (Lambinon *et al.* 2004, Kurtto *et al*. 2018). It is found in North America, Central and Northern Europe, Siberia, Greenland and Iceland, and is associated with shallow marshes, peatlands and acidic wetlands (Olesen & Warncke 1992, Podhajská & Rivola 1992, Somme *et al*. 2012). The parts of *C. palustre* used for pharmaceutical purposes are the rhizome and aerial parts, which are collected from spring to autumn. They contain gallic, ellagic, quinic, catechinic, isobutyric and isovaleric acids, tannins, phlobaphenes, phenolic and triterpene phytoncides, phenolic glycosides, essential oil and quercetin (Ovodova *et al.* 2005, Popov *et al.* 2005a, 2005b; Tomczyk & Latté 2009, Kashchenko *et al.* 2017). Herbal medicines made from purple marshlocks are claimed to exhibit significant astringent, anti-hemorrhagic, antiseptic, anti-inflammatory, antioxidant and anti-exudative activity. They are purported to protect and detoxify the liver and kidneys, inhibit sweat and sebum secretion, block allergic reactions, accelerate wound healing and prevent fermentation in the intestines; and additionally to exert a regenerative effect on the intestinal mucosa and to support the condition and elasticity of the osteoarticular system. The phenolic acids contained in *C. palustre* also have a protective effect on epithelial tissue and hepatocytes (Kiosev 2001, Popov *et al*. 2005a, 2005b; Buzuk *et al*. 2008, Yerschik *et al*. 2009). In terms of commercial use, owing to its high content of tannins, *C. palustre* was formerly used for tanning hides and dyeing wool red (Podhajská & Rivola 1992).

Purple marshlocks is a perennial chamaephyte that prefers habitats on organic soils, mostly in mesotrophic mires, but also in poor and rich fens offering cool and wet conditions with moderate light, as typical of the Holarctic and the circumboreal region (Zarzycki *et al.* 2002, Bacler-Żbikowska 2012).

Natural habitats of herbal plants that are not subject to significant human disturbance provide the optimum content and proportions of biologically active substances (Lyutikova & Turov 2011). This results in herbal materials of high quality and enables the production of natural plant medicines in accordance with the Good Manufacturing Practice (GMP) principles recommended by the WHO (Borkowski 1994, Drozd 2012). This is particularly important at a time when people are seeking mechanisms and products with a limited negative impact on the environment and with health-promoting properties.

Many plants with high healing potential are adapted to life in peatlands. Due to intensifying reclamation, which destroys the natural habitats of these plants, ecological farming is becoming an important alternative. Cultivation also helps to secure the supply and standardise the content of pharmacologically active components, thus making the medical effects predictable, which is very important in the phytopharmaceutical industry (Franke 1999).

The peatlands of Polesie Podlaskie (central Eastern Poland), with minimal human disturbance, are optimal habitats for the natural growth and development of *C. palustre* populations, and hence for the collection of herbal material with natural properties. On the other hand, these habitats are subject to regional hydrotechnical transformations associated with drainage and coal mining.

There is potential for the use of *C. palustre* in phytotherapy across Europe, though it is currently under-appreciated. At the same time, there may be a need for effective organic cultivation of the species, due to the risk of loss of its natural habitats. The aim of this work was to analyse the habitat conditions of *C. palustre* growing in peatlands located in the central part of Eastern Poland (Polesie Podlasie) by investigating selected physical and chemical properties of shallow groundwater and through botanical surveys at sites with *C. palustre*.

METHODS

Study area

The study was carried out on peatlands in the central part of Eastern Poland, within the Łęczna-Włodawa Lakeland in Polesie Podlaskie, which is the western part of Polesie - a physiographic unit extending to the Pripyat River in Ukraine. As a criterion for selection, the sites chosen were located in areas with different levels of nature conservation status, and were not subject to significant human disturbance which enables the occurrence of many environmentally valuable and economically useful plant species, including herbs.

Site selection was based on preliminary studies supported by a topographic analysis and observations of plant communities. Six representative sites were selected, where the presence of *C. palustre* populations was recorded. Four of the sites were lake-bog complexes, namely: Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D) and Lake Moszne (M). These four sites and the mid-forest bog Blizionki (BZ) were all located within the Polesie National Park or its vicinity. The sixth site was the Dekowina (DK) wetland sanctuary in Sobibór Landscape Park (Figure 1).

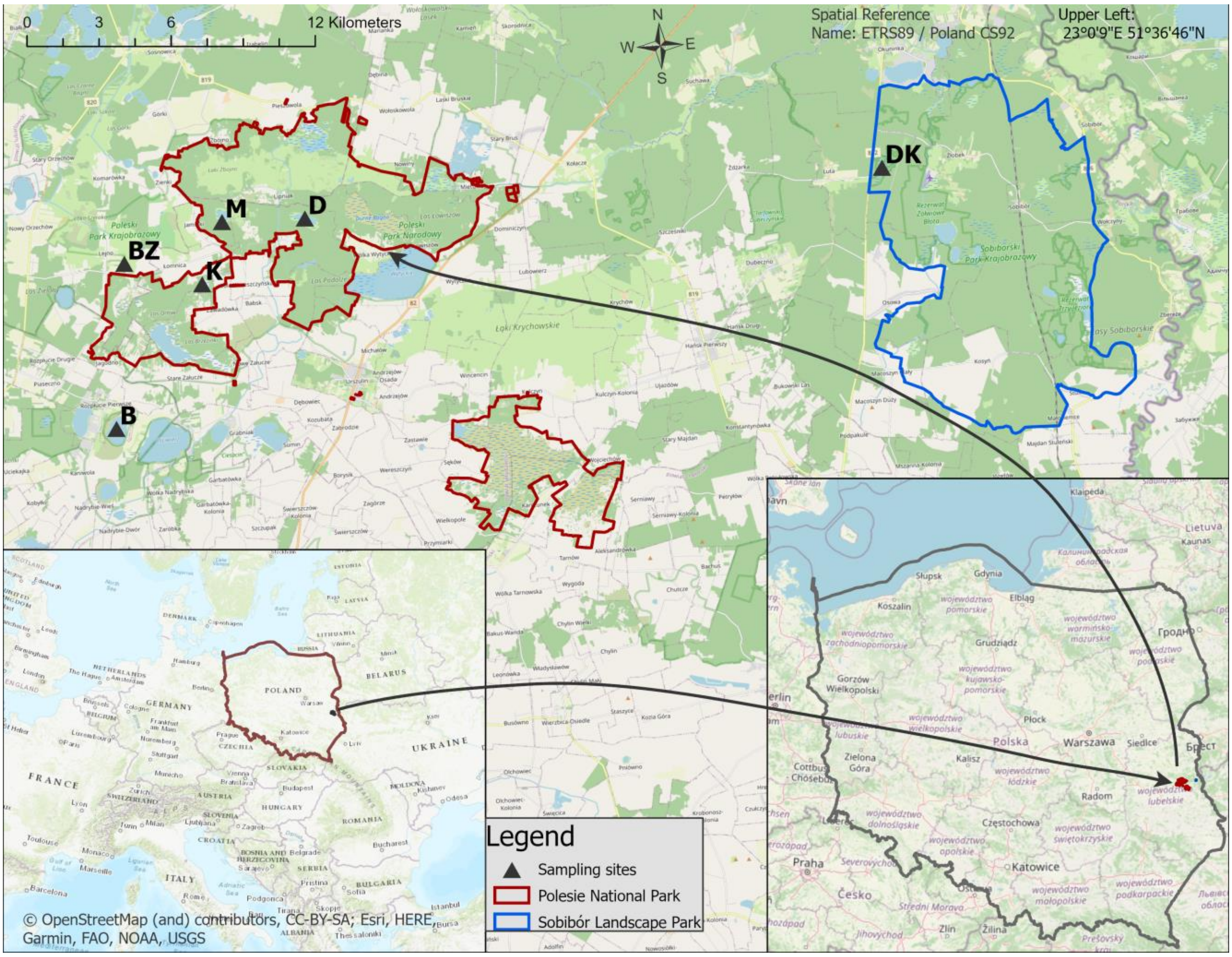

Figure 1. Location of the study and sampling sites in the Polesie Podlaskie region: Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D), Lake Moszne (M), the mid-forest bog Blizionki (BZ) and the Dekowina (DK) wetland sanctuary.

Sampling and water analysis

A soil piezometer (a 1 m length of 10 cm diameter perforated PVC pipe) was installed in the central part of each study site, meaning there were six piezometers in total. Over the period 2011–2013, laboratory chemical analyses of shallow groundwater samples were carried out seven times each year, between April and September. To reflect the range of natural conditions, the samples were collected in various weather conditions. At each site, all available water was taken from the piezometer using a weight-loaded container, the piezometer was allowed to re-fill, then another 1.5 dm^3 of water was immediately collected. The timings of sampling and delivery to the laboratory were in accordance with applicable standards procedures. As the quality of shallow groundwater is important for the functioning of peatland phytocenoses, 14 physicochemical factors were determined, using standard methods, at the Central Agroecological Laboratory (CLA) of the University of Life Sciences in Lublin. These were pH - potentiometric method (PN-EN ISO 10523); electrolytic conductivity (EC) - conductometric method (PN-EN ISO 27888); amount of dissolved organic carbon (DOC) - spectrometric method (PN-EN 1484); content of nitrogen fractions, i.e. total nitrogen (TN) -flow analysis method (ISO 29441), ammonium nitrogen (NH_4) - flow analysis method (PN-EN ISO 11732), nitrate (NO_3) and nitrite (NO_2) - flow analysis method (PN-EN ISO 13395); content of phosphorus fractions, i.e. total phosphorus (TP) and phosphate (PO_4) - spectrophotometric method (PN-EN ISO 6878); concentration of sulfate (SO_4) - ion chromatography method (PN-EN ISO 10304-1); and (analysed less frequently) basic cations by atomic absorption spectrometry, i.e. potassium (K) and sodium (Na) - PN-EN ISO 9964-2, calcium (Ca) and magnesium (Mg) - PN-EN ISO 7980.

Botanical survey

At each site, three representative research plots with an area of 100 m^2 were established. The plots had varying abundance of *C. palustre*, measured as the percentage share of its individuals in the flora. Botanical studies were conducted 3–4 times during the growing season of each year of the study (2011–2013), and the results were aggregated and averaged for each site prior to further analysis.

Detailed plant species abundance and the percentage share (percentage cover) of the species in the phytocenosis on the representative research plots were determined. Due to their significant indicator value, only vascular plants were included in the botanical analyses. The Braun-Blanquet method of observations of flora was used, and phytosociological relevés were made in each research plot. The species were identified according to Rutkowski (2006), and the species nomenclature followed Mirek *et al.* (2002).

Data analysis

The distribution of values of the physicochemical variables for the sites was analysed statistically. The values were not normally distributed and showed heterogeneity of variance, so the nonparametric Mann-Whitney-Wilcoxon test was used for the distribution analysis. Basic order statistics, including the p-value obtained using the Mann-Whitney-Wilcoxon test, were displayed as box plots.

To evaluate relationships between the selected environmental variables, the occurrence of plant species, and individual study sites, a redundancy analysis (RDA) was conducted. Due to the readability of the charts, we decided to exclude those plant species that appeared with a low degree of coverage (maximum 1 %) on only one study site. Therefore, RDA statistical analyses were performed for 34 plant species. When analysing the data for all 43 of the plant species, the percentage of explained variances on the RDA axes was significantly reduced.

The direct ordination method (RDA) was chosen on the basis of the result of detrended correspondence analysis (DCA, SD < 1.6). The analysis included the sites and the plant species (single individuals were excluded from the species lists). Due to the requirements of this method (Blanchet *et al*. 2008, Borcard *et al*. 2011), the set of environmental variables was also limited. The factors for which the Mann-Whitney-Wilcoxon test showed significant difference at a significance level of 0.05 were used in the analysis. The selected environmental variables (NH_4, Ca, DOC and EC) were standardised, and in the case of NH_4 the transformation $\sqrt{y}$ was first applied (Legendre & Gallagher 2001).

All statistical analyses were performed in the R computational environment (R Core Team 2019) using the following libraries: vegan (ver. 2.5-3; Oksanen *et al*. 2018), nortest (ver. 1.0-4), MASS (ver. 7.3-48), and ggdendro (ver. 0.1-2.0).

In a case of the botanical survey, the Jaccard's similarity coefficients (Piernik 2008) were calculated for phytocenoses on all representative plots.

A hierarchical classification analysis was performed both for the sites and for species composition. In both cases, Jaccard's similarity coefficient and the agglomerative minimum variance algorithm (Ward clustering) were used as a measure of similarity.

The results of the laboratory tests were statistically analysed. In the first stage, the sites with *C. palustre* were divided into two groups according to the percentage share (percentage cover) of this species in individual phytocenoses. Study sites B, D and M were included in the first group (with a high percentage cover of *C. palustre*, 6–10 % participation) and the other sites, i.e. K, BZ and DK, were included in the other group (with a low percentage cover of *C. palustre*, ≤ 1% participation).

RESULTS

Water analysis

The water table is an important factor in shaping the habitat properties of peatlands. Fluctuations of peat water levels were not, however, a limiting factor for local populations of *C. palustre*, at least during the period of research. At most sites the water level in the peat remained at a level similar to the peat layer height (visual observations only), showing seasonal fluctuations only (peat water level higher after spring thaws and slightly lower in summer due to evapotranspiration). Similar fluctuations were noted for the sites Dekowina (D) and Blizionki (BZ), which are not directly associated with a lake system, where the peatland water level was slightly lower.

A visualisation of the probable habitat ranges of *C. palustre* for all sites is provided by the analysis of physicochemical properties of the water samples (Figure 2). Significant deviations between individual results were recorded (e.g. for Na, Ca, DOC, EC, TN, TP and SO_4).

Numerous populations of *C. palustre* were found in the Bikcze, Moszne and Długie lake-bog complexes (B, M and D - group I), where the maximum percentage in the phytocenoses reached 10 % (B). Despite the common occurrence of *C. palustre* in the remaining sites K, BZ and DK (group II), the population numbers were relatively low and did not exceed 1 %.

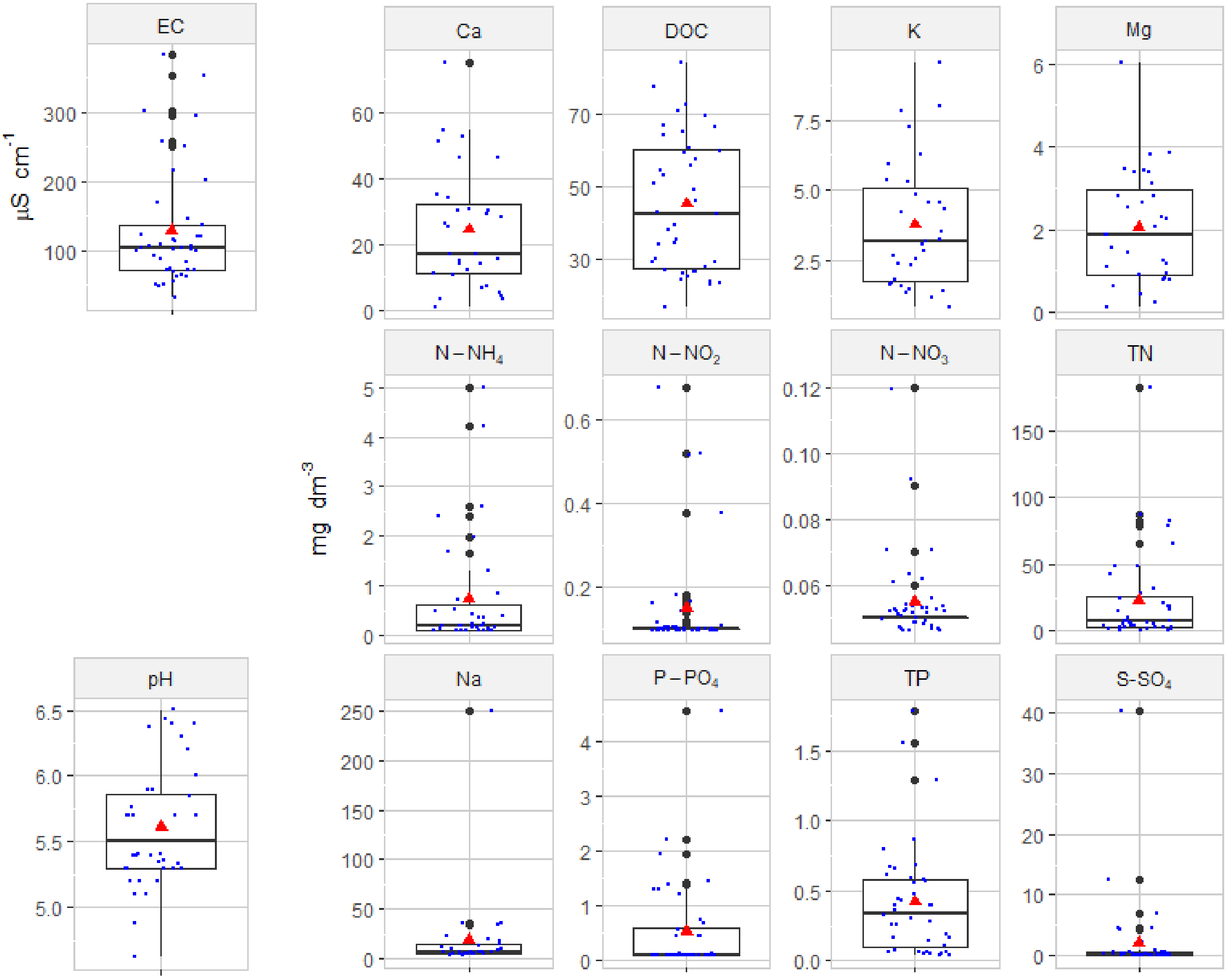

Figure 2. Distributions of values of the investigated physicochemical properties of piezometric groundwater at the sampling sites in 2011–2013. The small blue dots represent values observed on individual sampling dates at Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D), Lake Moszne (M), the mid-forest bog Blizionki (BZ) and the Dekowina (DK) wetland sanctuary. In each boxplot, the box represents the first and third quartiles and the horizontal line dividing the box represents the median. The mean is indicated by the filled red triangle. The whiskers extend to the most extreme observations that are located no more than 1.5 times the interquartile range away from the box. Any observation not included between the whiskers is considered an outlier and is represented by a black dot along the whisker-axis. When there are no outliers, the whiskers terminate at the minimum and maximum values.

The value ranges of some of the physicochemical factors (NH_4, DOC, EC, Ca, Mg, and to a lesser extent pH) varied between the two groups (I, II) of sites distinguished on the basis of difference in population abundance of *C. palustre*, indicating that this species has broad ecological tolerance limits (Figures 3 and 4). At the sites where *C. palustre* was more abundant in the phytocenoses (the lake-peatland sites B, M and D), lower values were found for NH_4, Ca, DOC, EC, Mg and pH compared to the peatland group of sites (K, BZ, DK), which had a smaller proportion of the species but higher values for TN, NO_2, NO_3 and PO_4 (Figures 3 and 4). The values of the other factors (TP, Na, K and SO_4) showed similar value ranges for both groups of sites (Figure 4). The boxplots in Figures 3 and 4 show the Mann-Whitney-Wilcoxon test results for the two site groups and the individual physicochemical properties. Significant differences ($p < 0.05$) in the distributions were found for NH_4 ($p = 0.002$), Ca ($p = 0.0057$), DOC ($p = 0.0273$) and EC ($p = 0.0325$). In the case of Mg and pH, significant differences were observed at $p < 0.1$. Lower values in both of these groups of factors are particularly important for the size of *C. palustre* populations and, therefore, their distribution statistics (upper and lower quartiles) may

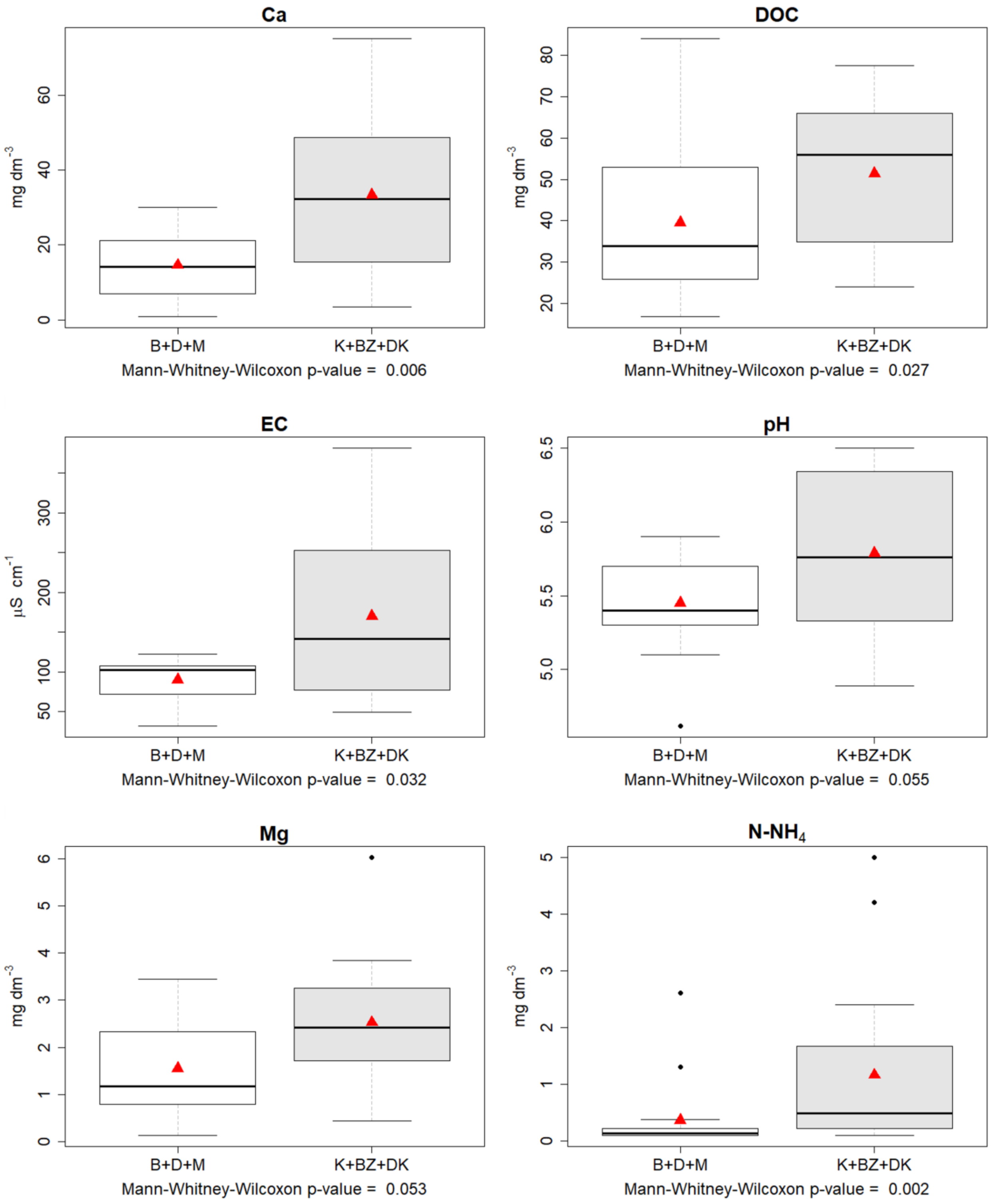

Figure 3. Distribution of values of the investigated physicochemical properties of piezometric groundwater which may have a limiting effect on *Comarum palustre* in 2011–2013, at the study sites Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D), Lake Moszne (M), the mid-forest bog Blizionki (BZ) and the Dekowina (DK) wetland sanctuary. Mann-Whitney-Wilcoxon p-values indicate significance level of differences between site groups. In each boxplot, the box represents the first and third quartiles and the horizontal line dividing the box represents the median. The mean is indicated by the filled red triangle. The whiskers extend to the most extreme observations that are located no more than 1.5 times the interquartile range away from the box. Any observation not included between the whiskers is considered an outlier and is represented by a black dot along the whisker-axis. When there are no outliers, the whiskers terminate at the minimum and maximum values.

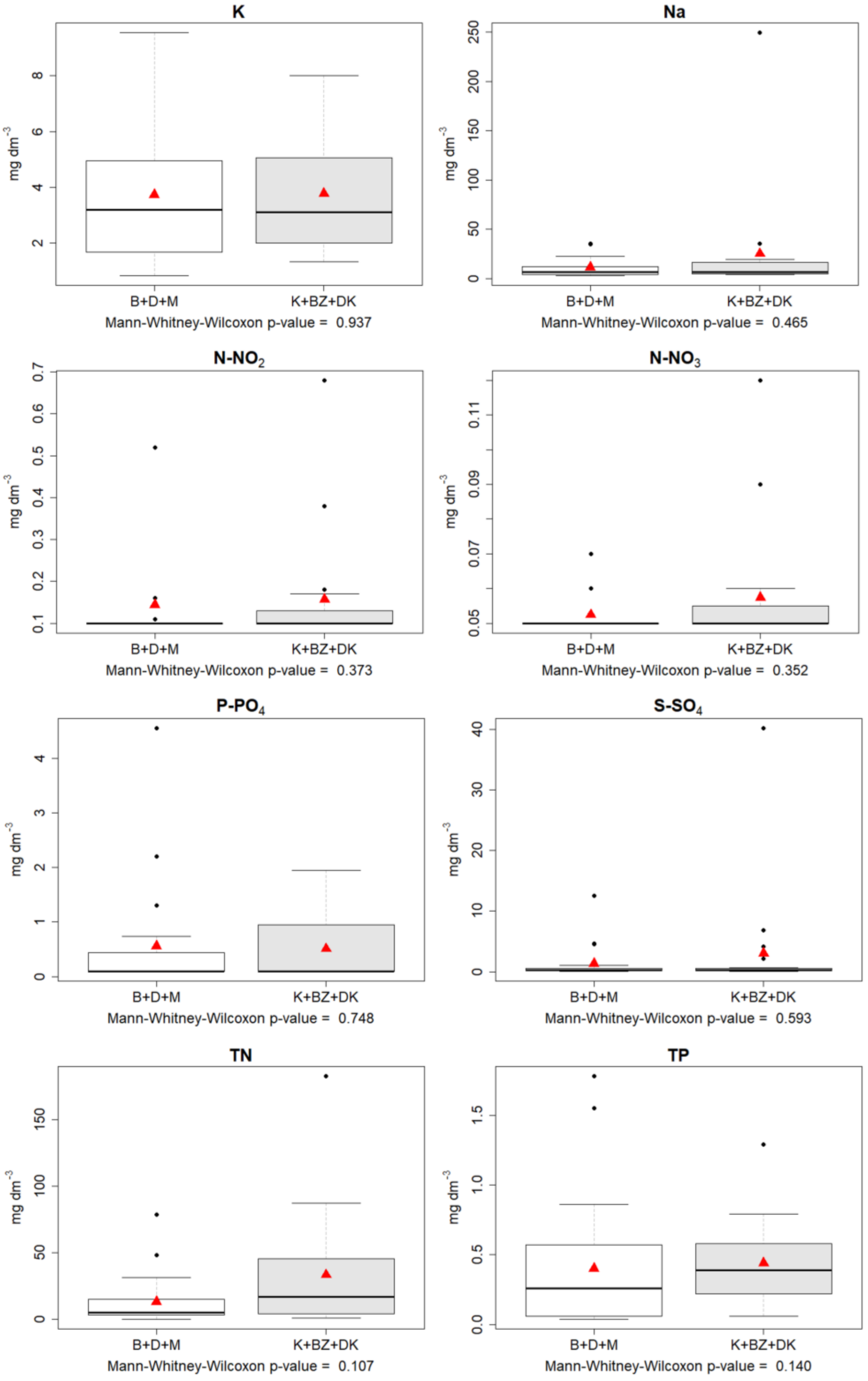

Figure 4. Distribution of values of the other investigated physicochemical properties of piezometric groundwater at the *Comarum palustre* study sites in 2011–2013, at the study sites Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D), Lake Moszne (M), the mid-forest bog Blizionki (BZ) and the Dekowina (DK) wetland sanctuary. Mann-Whitney-Wilcoxon p-values indicate significance level of differences between site groups. In each boxplot, the box represents the first and third quartiles and the horizontal line dividing the box represents the median. The mean is indicated by the filled red triangle. The whiskers extend to the most extreme observations that are located no more than 1.5 times the interquartile range away from the box. Any observation not included between the whiskers is considered an outlier and is represented by a black dot along the whisker-axis. When there are no outliers, the whiskers terminate at the minimum and maximum values.

indicate the ranges for functioning of its populations in the wild: NH_4 = 0.1–0.21; Ca = 6.8–25.3; Mg = 0.77–2.79; DOC = 25.9–52.9 (all $mg \cdot dm^{-3}$); EC = 72.2–107.6 $\mu S \cdot cm^{-1}$; and pH = 5.3–5.7 (Figure 3). For the other factors, i.e. TN, TP, NO_2, NO_3, SO_4, PO_4, Na and K, no significant differences were found in the distribution with respect to the two groups of sites (Figure 3 and 4).

The relationships between environmental variables, plants species composition and individual study sites were evaluated using redundancy analysis (RDA). All environmental variables together explained 78.16 % of the total variation. The first two RDA axes cover 68.28 % of the total explained variance (RDA1: 44.38 %; RDA2: 23.91 %). The RDA results are visualised in triplot ordination diagrams (Figures 5a, 5b) using two scaling methods (scaling=1 and scaling=2) (Borcard *et al*. 2011). An outcome for scaling=1 is a distance triplot. The distances between sites approximate to the Euclidean distances. Sites B, K and BZ and, also, Sites D and M can be expected to have similar values for the environmental variables and similar species composition (Figure 5a). For scaling=2, we obtained a correlation triplot where angles between all environmental vectors reflect linear correlation. It should be emphasised that distances between sites are not approximate Euclidean distances. The environmental variables are represented by vectors whose direction shows the direction of the largest variation. The positive part of the first axis (RDA1) is well correlated with the highest NH_4 values, while the negative part is strongly correlated with EC and Ca. The positive part of RDA2 is well correlated with the highest levels of DOC (Figure 5).

As seen in Figure 5a, sites M and D are both scattered along RDA2, with the lowest levels of all environmental variables. Sites B, K and BZ have the highest EC and Ca values. Site DK is not similar to any of the other sites and has high levels of NH_4 and DOC. In addition, the triplot (Figure 5b) indicates a strong positive correlation between EC and Ca as well as a slightly weaker correlation between NH_4 and DOC. We also observed that *C. palustre* is negatively correlated with all of the environmental variables considered, most strongly with DOC.

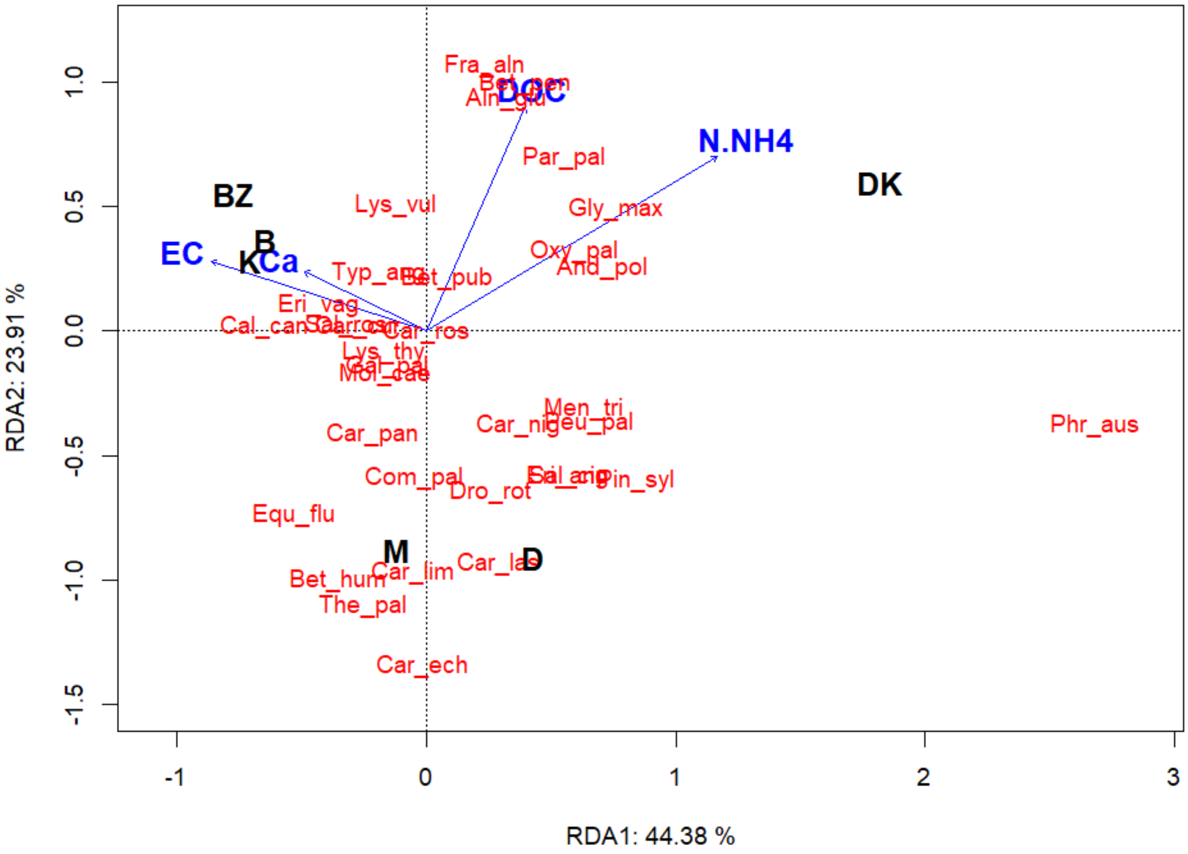

Figure 5. RDA triplot with fitted site scores (black), 43 species (red), and environmental variables (blue) as arrows. Two methods of scaling were used: (a) scaling = 1 and (b) scaling = 2. Key to abbreviations for site names: Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D), Lake Moszne (M), the mid-forest bog Blizionki (BZ) and the Dekowina (DK) wetland sanctuary. Species names according to the caption for Figure 6.

Botanical survey

Botanical analyses were performed to determine the influence of anthropogenic pressure on the biocenotic structure of the ecosystem. In total, 43 vascular plant species were identified in all studied phytocenoses (description in Figure 6); they belonged to 19 botanical families and were differently configured phytocoenotically. Jaccard's similarity coefficient for the phytocenoses exceeded 0.5 occasionally and to a small extent, which may indicate greater variation in flora between the site groups (I and II) and within the groups.

The hierarchical classification analysis for the sites and plant species composition provides more detailed information. The dendrograms are shown in a joint plot (Figure 6) supplemented with a heatmap, which allowed us to perform a simultaneous classification analysis for the sites and species

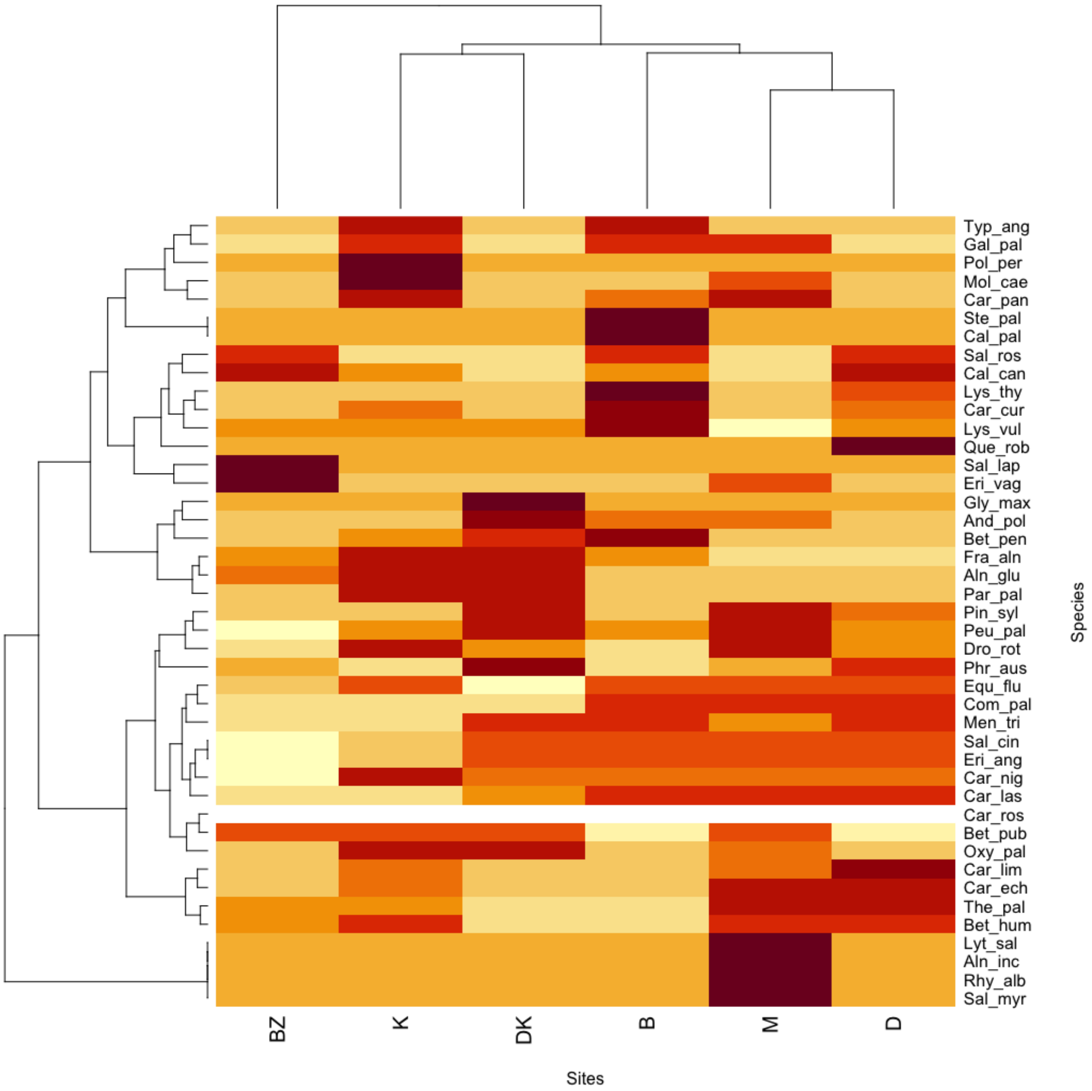

Figure 6. Dendrogram and heatmap of hierarchical agglomerative cluster analysis, based on Jaccard's coefficient and the Ward method, of 43 species and the study sites Lake Bikcze (B), Lake Karaśne (K), Lake Długie (D), Lake Moszne (M), the mid-forest bog Blizionki (BZ) and the Dekowina (DK) wetland sanctuary. Species composition of phytocoenoses: *Alnus glutinosa*, *A. incana*, *Andromeda polifolia*, *Betula humilis*, *B. pendula*, *B. pubescens*, *Calamagrostis canescens*, *Calla palustris*, *Carex curta*, *C. echinata*, *C. lasiocarpa*, *C. limosa*, *C. nigra*, *C. panicea*, *C. rostrata*, *Comarum palustre*, *Drosera rotundifolia*, *Equisetum fluviatile*, *Eriophorum angustifolium*, *E. vaginatum*, *Frangula alnus*, *Galium palustre*, *Glyceria maxima*, *Lysimachia thyrsiflora*, *L. vulgaris*, *Lythrum salicaria*, *Menyanthes trifoliata*, *Molinia caerulea*, *Oxycoccus palustris*, *Quercus robur*, *Parnassia palustris*, *Peucedanum palustre*, *Phragmites australis*, *Pinus sylvestris*, *Polygonum persicaria*, *Rhynchospora alba*, *Salix cinerea*, *S. lapponum*, *S. myrtilloides*, *S. rosmarinifolia*, *Stellaria palustris*, *Thelypteris palustris*, *Typha angustifolia*.

composition. The colour saturation in the heatmaps is analysed directly for each species and in relation to a specific study site. The rule is that the decrease in colour intensity from dark through shades of bronze to bright colour illustrates the increase in the degree of surface coverage for each species. This gives better information about the phytocoenotic relationships at the research sites.

Despite the typological similarity of the study sites, a division into three groups is clearly seen in the hierarchical classification. Group “a” comprises sites B, D and M, located in similar lake-bog complexes with highly similar plant species composition and the highest percentage of *C. palustre* in the phytocenosis. Group “b” comprises sites DK and K, both with a small percentage of *C. palustre*, which exhibit greater similarity of species composition to one another than to group “a”. These two sites differ in origin, Dekowina (DK) being a bog complex that is not directly associated with a water body, whereas K is a bog complex associated with the disappearing Lake Karaśne. Site BZ (Blizionki), with the lowest similarity in species composition to the other two groups, is the sole member of group “c”. It is the remnant of a shallow water body, and although its typology suits the habitat preferences of *C. palustre*, the phytocenosis has a low percentage of the species (Figure 6).

Analysis of the heatmap reveals several correlations, based on the principle that the more intensive the colour, the greater the likelihood of a higher percentage contribution and joint occurrence. For example, see the grouping *Lythrum salicaria*, *Alnus incana*, *Rhynchospora alba* and *Salix myrtylloides* at site Moszne (M) (Figure 6).

The affinity dendrogram of plant species illustrates their tendencies to co-occur. Although the peatlands at the research sites were of the same type, corresponding to the preferences of *C. palustre*, high differentiation was observed both within groupings and between individual sites. This indicated greater differences in species composition and percentage contribution of individual species in both groups of study sites. At each site analysed separately, there are plants with stronger and weaker preferences for joint occurrence. At the sites with abundant purple marshlocks (B, M and D), this species is grouped with *Equisetum fluviatile*, *Menyanthes trifoliata*, *Salix cinerea*, *Eriophorum angustifolium*, *Carex nigra* and *C. lasiocarpa*, whose percentages vary slightly depending on the site. For example, these species can be seen grouped together at the Długie (D) site (Figure 6).

In syntaxonomic terms, the plant species found in the phytocenoses are characteristic for various associations of the classes *Scheuchzerio-Caricetea* and *Alnetea glutinosae*. Thus, they contribute to the appropriate composition for phytocenoses directly associated with the occurrence of *C. palustre*, which most frequently accompanies communities of the order *Molinetalia*, the class *Scheuchzerio-Caricetea fuscae*, less frequently *Alnetea glutinosa*, and in great numbers communities of the class *Oxycocco-Sphagnetea*.

At the sites with lower percentage contributions of *C. palustre* (DK, BZ and K), relative to the sites with higher percentages (B, D and M), differences were noted in the co-occurrence of the above-mentioned group of plant species (*Menyanthes trifoliata*, *Salix cinerea*, *Eriophorum angustifolium*, *Carex nigra* and *Carex lasiocarpa*); particularly in relation to K and BZ, where differences in the percentage share of these species in the phytocenoses were also observed. In the case of DK, the differences were minor (Figure 6).

DISCUSSION

Water analysis

The application of mineral and organic fertilisers increased the amount of nitrogen and phosphorus in agricultural soils and in adjacent ecosystems, including peatland ecosystems; while eutrophic groundwater, increased vehicle traffic, and deposition from the atmosphere were additional sources of nutrients (Koerselman *et al*. 1990). Nutrients may therefore be the most important factors for the habitat quality of shallow groundwater in peatlands (Foy & Withers 2002).

Groundwater quality is also closely linked to point pollution sources in rural settlements, which are responsible for the highest concentrations of organic pollutants, total phosphorus (TP), phosphates (PO_4), ammonium nitrogen (NH_4) and chlorides (Cl). Moreover, villages have been recognised as having the greatest impact on the quality of surface waters, probably due to the frequent discharge of untreated municipal sewage directly into the ground and watercourses (Orzepowski *et al*. 2014).

Mobilisation of plant nutrients within the peatland system itself is an additional source of eutrophication of the environment (Smolders *et al.* 2006).

The potential for secondary eutrophication of peatland ecosystems can also be determined by their degree of acidification (pH) and content of sulfur compounds in the soil solution (Sapek 2014, Serafin *et al.* 2020), as well as the concentrations of sodium, potassium, calcium, and magnesium - the main basic elements determining the reaction of soil solutions (Ligęza & Smal 2004, Serafin *et al.* 2020). Their amount is regulated by sorption or release from the sorption complexes of peatlands (Stolarczyk *et al.* 2017, Serafin *et al.* 2020). On the other hand, electrolytic conductivity (EC) values are an indirect measure of the mineralisation and contamination of groundwater. Another indicator of groundwater eutrophication associated with humic compounds in peatlands is the quantity and quality of dissolved organic matter (DOC), which determines the availability of easily digestible forms of nitrogen and phosphorus associated with humic substances. Its amount depends on drainage of the peat deposit (Górniak 1996), which is determined by changes in hydrographic conditions associated with hydrotechnical modifications in wetland ecosystems.

Drainage measures have been causing major transformations in the balance of biogenic substances in the entire Łęczna-Włodawa Lakeland since the mid-20th century (Wilgat 1991). The construction (1954–1961) and drainage of the Wieprz-Krzna Canal alone caused an increase in the rate of water outflow and a decrease in the groundwater level by an average of 50–80 cm. Moreover, the Wieprz-Krzna Canal carries water with chemical characteristics alien to the region, contributing to changes in the habitat conditions of local peatlands. Another anthropogenic element causing water levels to fall throughout the region is the operation of the Lublin Coal Mine (Janiec 1984, Serafin *et al*. 2018).

A decrease in the groundwater level results in the dehydration of peat layers, which in turn leads to further nutrient pollution in surface formations. Previously deposited organic matter gradually disappears, releasing considerable amounts of biogenic substances (primarily nitrogen and sulfur). This causes acidification of hydrogenic soils through oxygenation of compounds of these elements, as well as the loss of base cations due to retention by flora and leaching from the soil profile (Maciak 1995, Sapek 2014). A decrease in pH can further lead to the gradual release of phosphorus due to the increased solubility of its compounds, e.g. apatite, strengite, or variscite, which in the latter case causes mobilisation of aluminum, also toxic for plants (Stumm & Morgan 1981).

An increase in the abundance and mobility of phosphorus in hydrogenic soils poses a serious threat due to its dispersion to water resources, resulting in changes in chemical properties and affecting the trophic status of many ecosystems (Foy & Withers 2002).

Botanical survey

Changes in groundwater chemical characteristics (e.g. through over-fertilisation) lead to changes in habitat conditions for many plant species, which can be a limiting factor for their occurrence and physiological condition (Falińska 2004). They directly or indirectly determine the rate of metabolic transformations and affect biomass quantity and the content of active substances in plant organs (Kazimierczak *et al*. 2010, Sozinov & Grummo 2016). This is particularly important in the case of plants used for therapeutic purposes, i.e. for herbs. *Comarum palustre* is a native bog species that is undervalued in Central and Western Europe as a plant with medicinal properties. It is known in Western Europe mainly by the synonym *Potentilla palustre* L., yet a compendium devoted to the healing properties of the genus *Potentilla* contained little information about it (see Tomczyk & Latté 2009). It occurs quite commonly and consistently within the Łęczna-Włodawa Lakeland, as confirmed both by the preliminary botanical research carried out in 2011–2013 and by literature data (e.g. Fijałkowski 1991, Pogorzelec & Serafin 2010, Serafin & Pogorzelec 2011). However, the population size of this species in the phytocenoses occurring in the peatlands of the central part of Eastern Poland is relatively low, reaching only 10 % (Serafin *et al.* 2017, 2018). This confirms the decreasing population size of *C. palustre* noted in Poland (according to Zarzycki *et al.* 2002), which is probably due to direct and indirect (climate change) human pressure. This has implications for the future form of legal protection for this species and/or for areas where it occurs. In these circumstances, the interests of phyto-pharmaceutical companies may necessitate organic cultivation of *C. palustre*. In this eventuality, a knowledge of the habitat characteristics favouring its natural occurrence will be valuable and important because this information can support the creation of a medicinal drug with a natural composition of biologically active ingredients in accordance with WHO recommendations (Drozd 2012, Serafin *et al.* 2017, 2018).

In the years 2011–2013, the physical and chemical properties of the groundwater at the study sites did not reflect the intensification of processes associated with drainage of the peatlands in Eastern Poland. Indeed, they confirmed the natural character of the habitats and the stable hydrographic conditions of the research sites. The average values of most of the abiotic factors investigated did not exceed the typical values preferred by *C. palustre* (according to Zarzycki *et al.* 2002), confirming that *C. palustre* possesses a wide ecological amplitude.

Compared to other properties of the study sites, lower values of pH and EC, along with lower concentrations of NH_4, DOC, Ca and Mg, are particularly important for the size of *C. palustre* populations and may, therefore, indicate the ranges for the functioning of these populations in the peatlands of Eastern Poland.

The variation in the condition of populations of *C. palustre* - as measured by its percentage contribution to the phytocenoses - indicates that, among the habitats representative of the region, the chemical composition of the surface water at sites Bikcze (B), Długie (D) and Moszne (M) was most beneficial for abundant *C. palustre*. The range of values for the physical and chemical factors tested at these locations should, therefore, be regarded as preferable for the growth and development of populations of the species.

Investigation of the influence of biotic and abiotic features on the growth of *C. palustre* individuals in various habitats showed that the physiognomy of the surrounding vegetation best explains its growth, although abiotic factors have an indirect influence on this process (Macek & Lepš 2008). Despite the fact that species composition was found to be less important than the physiognomy of the vegetation in that study, it is worthy of comment in relation to the population size of *C. palustre*. The species composition of communities and, thus, the presence of companion plants is of major importance for the occurrence of medicinal plants in naturally preserved habitats (Drobnik *et al*. 2004). The plant species co-occurring in phytocenoses with *C. palustre* are predominantly characteristic of various associations of the classes *Scheuchzerio-Caricetea*, *Oxycocco-Sphagnetea* and *Alnetea glutinosae*, which generally prefer habitats in bogs and transitional peatlands (Sozinov & Grummo 2016). Therefore, these phytocenoses are typical for the study sites and representative of the region and the peatlands of the central part of Eastern Poland (Matuszkiewicz 2001, Zarzycki *et al.* 2002).

The habitat transformations in the peatlands of the Łęczna-Włodawa Lakeland cause considerable disturbances in the water chemistry and plant species composition of habitats. This affects the presence and condition of the populations of many bog plants (Chmielewski *et al*. 1996) and hence probably the natural occurrence of herbs and their content of bioactive substances. It may also reduce the number of sites where *C. palustre* occurs as well as the number of local individuals and populations of the species.

In the years 2011–2013, the botanical variation and differences in water chemistry observed between the study sites are most likely linked to the internal metabolism of the peatlands. Therefore, the ranges of values for the physical and chemical factors of the shallow groundwater and the nature of the vegetation accompanying *C. palustre* should be considered natural. The study sites (particularly those with greater representation of the species, i.e. Bikcze, Moszne and Długie) are suitable for growing and harvesting valuable herbal material. It is worth adding that in Poland the perspective of ecological cultivation of plants on peatlands owned by private persons is not subject to legal regulations, as long as they are not protected areas.

Unfortunately, the difficult terrain conditions in peatlands, which often limit the accessibility of natural resources, are an obstacle to obtaining herbal materials. In protected areas, legislation regarding the protection of biocenotic resources is a further impediment. Therefore, the possibility of organic farming of selected herb species under conditions that are as close to natural as possible (e.g. semi-natural wet meadows) provides a necessary alternative in the long term. This study of habitats at sites with low anthropogenic pressure provides valuable information about habitat factors which can be used to support the effectiveness of organic farming of this type of crop. We should welcome the interest of phytopharmaceutical companies in using the research results to undertake semi-natural cultivation of *C. palustre* as a species with high therapeutic potential.

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AUTHOR CONTRIBUTIONS

Conceptualisation: AS; data curation: AS; formal analysis: AS, MP; investigation: AS, MP, KS; methodology: AS, MP; resources: AS, MP; software: UB-M; supervision: AS; validation: AS, MP, UB-M; visualisation: UB-M; writing (original draft): AS; writing (review and editing): MP.

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