

## Changes in the epiphytic lichen biota in Scots pine (*Pinus sylvestris*) stands affected by a colony of grey heron (*Ardea cinerea*): a case study from northern Poland

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**Abstract:** Bird colonies affect all elements of inhabited ecosystems, such as the soil, floristic composition and phytocoenosis structure, including the lichen biota. To date, the few papers focusing on changes in the composition of lichen vegetation caused by bird colonies are concerned with saxicolous ornithocrophilous communities. The aim of this study was to define the impact of the grey heron in two breeding colonies on epiphytic lichens on Scots pines presently inhabited by birds, as well as those recently abandoned. Analysis of the lichen biota showed that the species composition and number of lichens were significantly modified in the functioning colony and the post-colony areas when compared with the control plots never inhabited by grey heron. Within the functioning and post-colony areas, mainly species with a wide ecological amplitude and those characteristic of fertile habitats dominated, while acidophilous and ubiquitous taxa occurred in the control plots. Multivariate analyses (for species abundance and ecological characteristics) showed that lichens growing within the functioning colony and post-colony areas differed significantly from those in the control area in their habitat requirements, as they demanded nutrient-rich, low or moderately moist and deacidified bark. Within the control plots, lichens preferring a relatively acidic and slightly nitrified substratum occurred. The direct impact of bird excrement and the fertilized bark could cause significant modifications in qualitative and quantitative species composition compared to the epiphytic lichen biota usually occurring on Scots pines.

**Key words:** corticolous lichens, eutrophication, heronry, Wirth's indicators

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### Introduction

The phenomenon of intense nutrient enrichment of habitat caused by bird colonies is well known and documented (Sobey & Kenworthy 1979; Ishida 1996; Ligeza & Smal 2003; Ellis 2005; Laiviņš & Čekstere 2008). It has been thoroughly studied and described in the case of bird colonies affecting soil and flora of treeless oceanic and sea islands and seashores in various regions of the world (Breslina & Karpovich 1969; Sobey & Kenworthy 1979; Hogg & Morton 1983; Hogg *et al.* 1988; Paradis & Larenzoni 1996; Norton *et al.* 1997; Vidal *et al.* 1998; Abbot *et al.* 2000; Ellis 2005). However, despite the

commonness of bird colonies in trees, only very few papers discuss the impact of nesting on forest ecosystems (Olech 1990; Maesako 1991; Ishida 1996; Mun 1997; Laiviņš & Čekstere 2008; Żółkoś & Meissner 2008, 2010; Adamonytė *et al.* 2013) and no study has been undertaken on the influence of such colonies on epiphytic lichen biota.

Lichens are very sensitive to environmental changes, caused by natural factors as well as human impact, especially the increase or decrease in air pollution (van Dobben 1996; Rusu *et al.* 2006; Wolseley *et al.* 2006, 2010; Motiejūnaitė 2007; Sparrius 2007; Otnyukova & Sekretenko 2008; van Herk 2009; Olsen *et al.* 2010). However, little has been published on the influence of bird colonies on the occurrence and species composition of lichen biota, and what has been published focused mainly on the detection of changes

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in thallial growth caused by the deposition of bird droppings or the development of ornithocoprophilous lichen communities on rocks (e.g. Grønlie 1948; Barkman 1958; Armstrong 1984, 1994, 2000; Seppelt *et al.* 1988; Olech 1990; Valladares & Sancho 1993; Nash 2008). The impact of bird colonies on the epiphytic lichen biota does not appear to have been studied previously, although some research has focused on myxomycetes in the great cormorant colony in Lithuania (Adamonytė *et al.* 2013).

When studying the distribution of the grey heron (*Ardea cinerea*) in Poland and its influence on the plant vegetation within its colonies (Żółkoś & Markowski 2006; Żółkoś & Meissner 2008), considerable changes in the lichen composition of epiphytic communities in most of the investigated stands were observed. In two cases it was clear that the lichen biota changed not only on the trees inhabited by birds, but also on those which had been abandoned a few years before. The bird colonies studied at both localities were established in the same type of pine forest and thus a more detailed investigation on the impact of nutrient enrichment derived from bird excrement on epiphytic biota was undertaken.

In this paper, the substantial modifications in the species composition and abundance of lichens on Scots pine caused by the deposit of bird droppings, as well as the changes after the cessation of that process, are described.

## Material and Methods

### Study area

In northern Poland, 33 of 76 breeding colonies of grey heron were settled within the Scots pine tree stands (Żółkoś *et al.* 2010). All of them were visited, but only two colonies could be clearly divided into two parts: 1) an area with a currently functioning colony and 2) a fragment abandoned by birds 2–3 years earlier. Therefore, only those two colonies of grey heron near Przesieki (53°00'59"N, 15°58'59"E) and Skrzyszewo (54°24'39"N, 17°49'29"E) villages (both in the Pomerania region) were taken into consideration. Both colonies are located at the periphery of the pine tree stands planted in post-agricultural grounds, on slightly inclined slopes with southern (Skrzyszewo) and south-western (Przesieki) exposure.

In Skrzyszewo, the major part of the tree stand consisted of 40-year-old pines, but a group of *c.* 90-year-old pines mixed with oaks was also present at the margin of the existing colony. The trees studied were separated from the adjacent meadow by an undergrowth of oaks and rowans. Herons firstly inhabited only the group of older pines, but when the younger ones reached a height similar to existing pines, birds also used them for nesting. In 2008 the colony comprised of 24 nests.

In Przesieki, the whole tree stand was *c.* 40 years old. Herons initially inhabited trees growing on the upper part of the slope, and then subsequently inhabited other trees further down. There were 57 nests observed in 2007.

### Methods

The research was carried out in the late summers of 2007 and 2008. The age of the Scots pines in both colonies was established by counting annual growth rings of wood collected with the aid of a Pressler drill.

In both forest complexes, three plots were marked out: 1) a control area, 2) a colony (with currently functioning nests) and 3) a post-colony. In each of these, 10 pines were randomly chosen. In Przesieki, one of the trees investigated in the colony plot was excluded from analyses as it did not support any epiphytic lichens; in Skrzyszewo, within the post-colony area, only 8 trees had been inhabited by grey heron in the past. All lichen taxa on the selected trees were listed, from the trunk base to a height of 2 m, and the cover of each species was estimated according to the following 5-point scale: 1 = <1%, 2 = 1–10%, 3 = 11–30%, 4 = 31–50% and 5 = >50%.

No lichen species found on the trees studied reached the fifth cover degree, and on one tree in the colonized area in Przesieki no lichen was observed at all. Most of the lichen species were noted directly in the field, but for some taxa (e.g., *Fuscidea pusilla*, *Lepraria* spp.) it was necessary to collect samples for determination by thin-layer chromatography (TLC) according to Orange *et al.* (2001). *Micarea prasina* was regarded in a wide sense.

Habitat conditions were characterized using the indicator values estimated for lichen species by Wirth (2010), and according to the method of ecological indicator values developed for vascular plants by Ellenberg (1992). The indicator values are a suitable tool for quantitative estimation of environmental changes, as shown primarily for vascular plants (e.g., Schaffers & Sýkora 2000; Żółkoś & Meissner 2008), but also for lichens (Berthelsen *et al.* 2008).

Multivariate analysis was performed to explore relationships among species, samples representing three distinguished investigated areas and the environmental variables. Firstly, Detrended Correspondence Analysis (DCA) was used to identify the length of the ordination axis. The gradient length of the first axis was 3.86 SD and this justified the use of Canonical Correspondence Analysis (CCA) (Jongman *et al.* 1995) to characterize

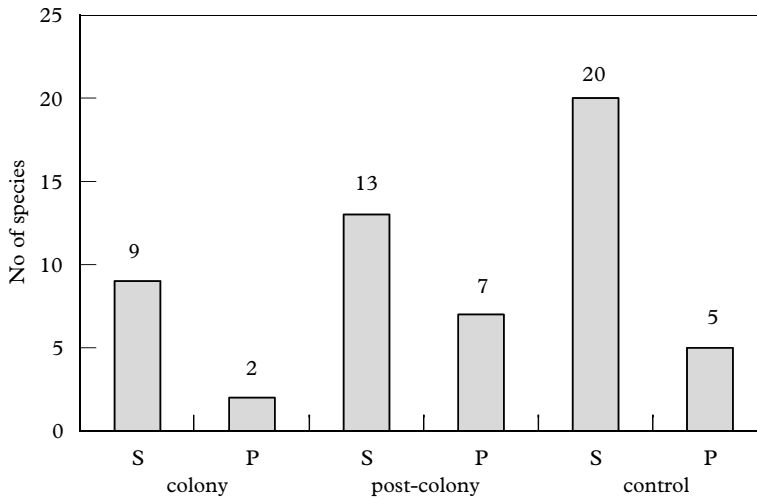


FIG. 1. Number of species within particular plots in Skrzyszewo (S) and Przesieki (P).

variation among the 26 lichen species in 57 samples constrained by 4 traits. Values of the 5-point cover scale were treated as the species data. For each sample, the weighted average values of Wirth's (2010) indicators for moisture (F), light (L), eutrophication (N) and reaction (acidity) (R) were calculated. In the CCA, apart from Wirth's indicator values used as environmental variables, information about three investigated areas (colony, post-colony and control) was also included as supplementary variables. The significance of the environmental variables for explaining variation in species distribution was tested using a Monte-Carlo permutation test with 499 unrestricted permutations. Multivariate analysis was conducted to identify traits used in CCA, of which absolute  $t$ -values  $> 2.1$  were used to indicate important canonical coefficients (ter Braak & Šmilauer 2002). Significant differences among samples within the three areas were detected by a one-way ANOVA and the Tukey post-hoc test, with the use of sample scores for the first canonical axis. Data were square-root transformed to normalize variables and a constant value of 3 was added to bring minimum values after transformation to above zero. Thus, the transformation followed the equation:  $x' = \sqrt{(x+3)}$ , where  $x$  is the original value and  $x'$  the value after transformation. All tests for significance were set at  $P < 0.05$ .

In order to demonstrate the diversity of lichen species composition in the defined areas, the DCA analysis, with a 5-degree scale of species abundance used as species data and the three investigated areas as supplementary variables, was performed.

The analyses were carried out using CANOCO ver. 4.5 (ter Braak & Šmilauer 2002), while other calculations were performed in Statistica software ver. 10.

## Results

The epiphytic lichen biota of the localities studied was diverse. In total, 26 taxa were noted. The number of taxa found on each plot are presented in Figure 1.

Ordination of samples in the CCA diagram (Fig. 2) shows the differences in the lichen biota inhabiting particular trees in the three areas studied. The first four ordination axes accounted for 90% of the variation in the species versus environment relationship. The overall inertia was 2.811. Eigenvalues of the axes show that the first and second axes had the greatest input in the variation (66%) of the data set analyzed (eigenvalues of the first and second axes are 0.55 and 0.22, respectively).

The first ordination axis shows a strong positive correlation with the nutrient-enrichment and a negative correlation with the moisture indicator. The second axis has the strongest positive correlation with the moisture indicator and negative correlation with light (Table 1). A Monte-Carlo permutation test confirmed that the first canonical axis ( $F = 0.55$ ,  $P = 0.002$ ) and the overall model ( $F = 5.93$ ,  $P = 0.002$ ) were statistically significant. Moreover, the averages of sample scores

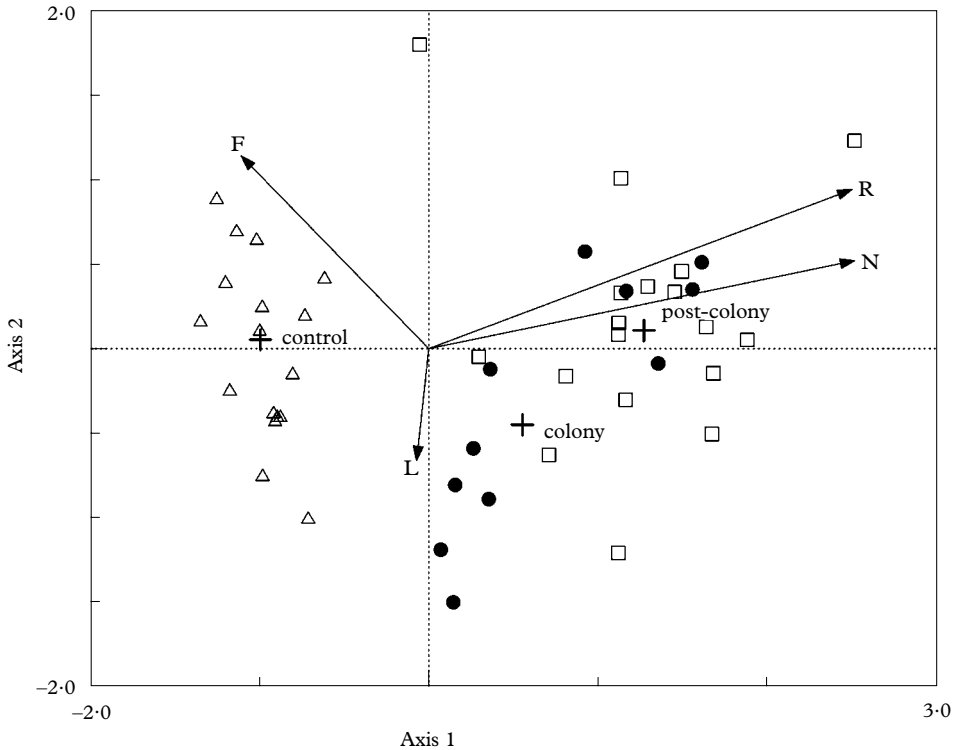


FIG. 2. Samples and environmental variables on the biplot of canonical correspondence analysis (CCA) of the first and second axes. The symbols represent plots: ●, colony; □, post-colony; △, control. Arrows represent supplementary variables (Wirth's indicators): F = moisture, L = light, N = eutrophication (nitrogen), R = reaction (acidity).

in the defined groups (control, colony and post-colony) differed significantly (ANOVA;  $F_{2,54} = 86,98$ ;  $P < 0.0001$ ), and these differences were statistically significant among all groups (Tukey post-hoc test;  $P < 0.04$  in all cases).

Samples from the colony and post-colony areas are grouped to the right of the CCA diagram, in the range of highest values of reaction and fertility, while trees from both control areas are recorded on the left-hand side of the diagram.

Ordination of samples in the DCA diagram (Fig. 3) points at changes in the lichen biota inhabiting particular trees in the three plots. Four ordination axes explain 28% of the total variation in the data set. Eigenvalues of the axes show that the first and second axes had the greatest input in the explanation of variation in the data set analyzed (eigenvalue of the first axis is 0.62 and of the second

is 0.20). The first one, placed on the left-hand side of the diagram, contains taxa connected with the post-colony and colony plots. These are nitrophytes, such as *Physcia tenella* and *Xanthoria* spp. (van Herk 1999), as well as species typical for deciduous trees, such as *Parmelia sulcata* and *Amandinea punctata* (however, excluding *Coenogonium pineti*, which can occur on pines but grows in strongly shaded places) (Fig. 3, Appendix 1). The opposite group consists of species associated with pines. These are acidophytes such as *Lepraria jackii*, *L. elobata*, *Parmeliopsis ambigua* and *Pseudevernia furfuracea*. Between those two groups there are taxa found within all three areas studied (control, post-colony and colony), although with varied abundance. Among them, the most frequent are *Hypogymnia physodes* and *Hypocenomyce scalaris*. The species most commonly occurring in the whole study area is *Lepraria incana* (Appendix 1).

TABLE 1. Statistics for Wirth's values used in the detrended correspondence analysis (DCA) of the first and second axes. Bold values indicate variables with significant correlation and canonical coefficients ( $t$ -value  $> 2 \cdot 1$ ).

Variable	Inter-set correlation		Canonical coefficient		$t$ -value	
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
F (moisture)	-0.3830	-0.3893	<b>-0.1425</b>	<b>0.1516</b>	<b>-2.7859</b>	<b>2.3801</b>
L (light)	-0.0248	-0.2255	0.0618	<b>-0.2031</b>	1.1980	<b>-3.1602</b>
N (nitrogen)	0.8693	0.1780	<b>0.4418</b>	<b>-0.4221</b>	<b>3.0780</b>	<b>-2.3602</b>
R (reaction)	0.8641	0.3217	0.0182	<b>1.0931</b>	0.1162	<b>5.6005</b>

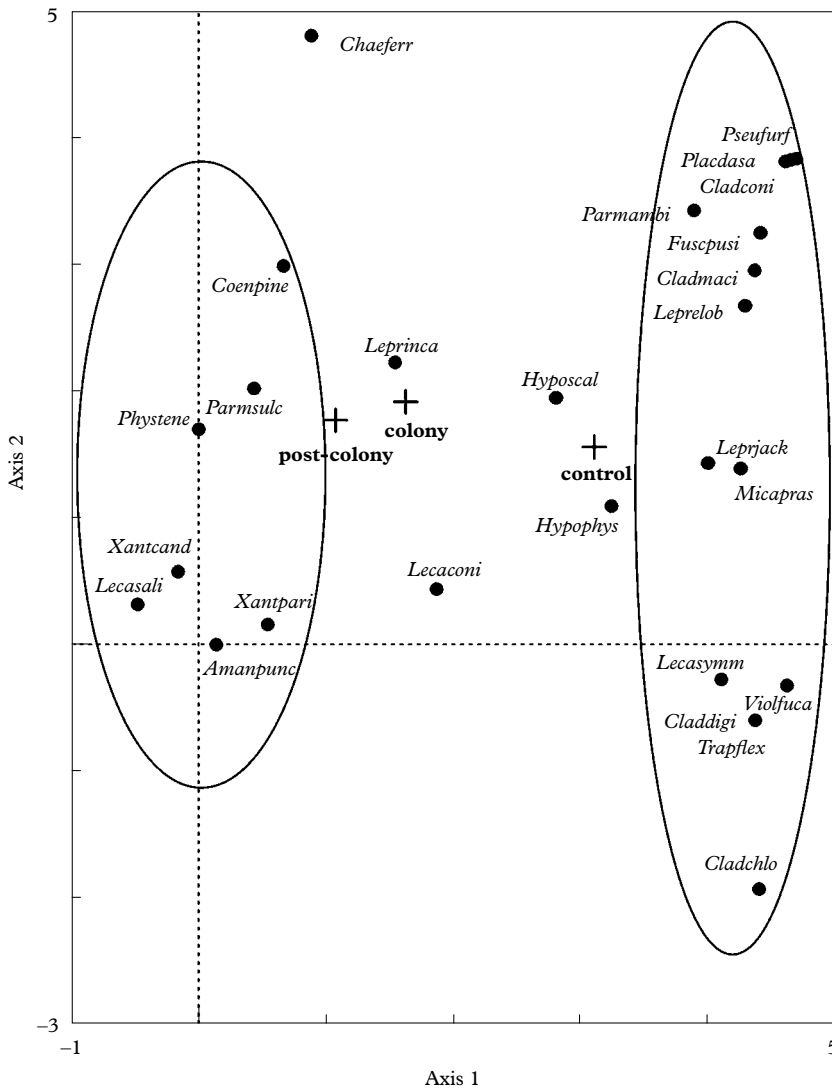


FIG. 3. Lichen species on the diagram of detrended correspondence analysis (DCA) of the first and second axes. The circle on the left-hand side encloses nitrophytes and that on the right-hand side encloses acidophytes. Abbreviations of species names are given in Appendix 1.

### Discussion

The results of multivariate analyses confirm that changes in the lichen biota inhabiting pine trunks observed in the functioning colony and post-colony plots, compared to the control plots, were connected with the direct and indirect influences of the colonies. Heron droppings falling from the tree crowns and flowing down the trunks cause changes in the acidity and fertility of the tree bark (Adamonytė *et al.* 2013), resulting in the appearance of nitrophyte species and lichen epiphytes which occur typically on deciduous trees.

The biodiversity of lichens within the functioning colony area was always the lowest and their cover was minimal, compared to the other areas. This was connected with the change in habitat conditions, and the elimination of lichen thalli could be the direct effect of the toxic influence of bird excrement. The main factor was most probably the urea, which is a highly toxic nitrogen product that generally inactivates proteins by affecting hydrogen bonds, but also the ammonia level and bark pH influence the lichen biota (Nash 2008 and literature cited therein). Only some lichens, such as *Xanthoria parietina* (Gaio-Oliveira *et al.* 2004), are able to survive in such highly modified habitats due to their high urease activities (Rai 1988) and their tolerance of high concentrations of nitrogen substances; this explains the occurrence of such lichens in the post-colony plots.

Differences in the lichen species composition between functioning colonies might be explained by the fact that the rate and quality of changes to the ecosystem caused by the colony are connected not only with the species of nesting bird, but also with the density of nests and the age of the colony (Sobey & Kenworthy 1979; Maesako 1991; Ishida 1996; Mun 1997; García *et al.* 2002; Żółkoś & Meissner 2008, 2010). This probably also applies to the lichen biota studied. The lower number of taxa growing in Przesieki than in Skrzyszewo could be connected with higher nest density and, in consequence, a stronger influence of the colony.

In the post-colony plots of both localities, the most frequent species were from nutrient-rich habitats (nitrophytes), which appear as the result of still high nutrient enrichment and less acidic bark. On the other hand, the occurrence of species typically occurring on pines (acidophytes) in both post-colony plots might be connected with the termination of the bird droppings on trunks, the dilution and flush of accumulated substances by the superficial flow of rain, as well as by the disappearance of the modified fragments of bark, which have peeled from the trees. In such places, conditions on the trunks were similar to those on trees never inhabited by herons, and probably for that reason lichens typical for pine appeared.

The DCA clustered some species in between the groups of acidophytes (the right-hand side of the diagram) and nitrophytes (the left-hand side of the diagram). All those taxa are considered as acidophytes (van Herk 1999), and in the localities studied they occur in two or three plot types (i.e. colony, post-colony and control), although usually with a different frequency. The only species common to all the plots studied at both localities is *Lepraria incana*, which has a relatively wide habitat amplitude and often grows on pines (Kukwa 2006). In the control area it is a typical taxon, while its occurrence in the colony and post-colony areas is probably not a result of its resistance to excrement deposition. *Lepraria incana*, as many other representatives of that genus, occurs in habitats not directly exposed to rain or water-flow down the trunk (e.g. Laundon 1992). This lichen species grows in bark fissures, and thus its occurrence in the colony and post-colony areas is probably the result of its survival in places unchanged by the heron droppings. *Hypogymnia physodes*, *Hypoconomyce scalaris* and *Lecanora conizaeoides* were also commonly recorded, but the last was absent in the colony plots. *Chaenotheca ferruginea* was found in all three plot types, but in each case only on single trees. Their occurrence in the control plot is not surprising, and the presence in other plots can be explained only by reasons stated in the previous paragraph.

There are many examples in the literature describing the bark or soil enrichment in phytocoenoses influenced by bird colonies, as well as showing qualitative and quantitative changes in the flora of vascular plants; that is, significantly higher numbers of nitrophilous species, nitrophytes and species of wide ecological amplitude (Sobey & Kenworthy 1979; Bukaciński *et al.* 1994; Ishida 1996; Mun 1997; Laiviņš & Čekstere 2008; Żółkoś & Meissner 2008). Bird colonies also affect community structure, shown by the occurrence of a highly dense shrub layer (Żółkoś & Meissner 2008; Żółkoś *et al.* 2013). This indirect impact of strong shading of pine trunks explains the common appearance of the hygrophilous and shade-preferring *Coenogonium pineti* (see Wirth 2010) in the post-colony areas.

To conclude, grey heron colonies have a significant impact on the epiphytic lichen biota associated with Scots pine. Firstly, the number of taxa and their coverage decreases, as species typical of Scots pine disappear, and those with wide ecological amplitude remain; next, the nitrophilous species untypical for pines appear. After a bird colony is abandoned, taxa indicating strong local deacidification and nutrient enrichment of the bark start to occur. The colonization of trunks by species typical for pines in the post-colony plots is also observed, which is probably connected with the disappearance of the modified bark. Furthermore, the indirect influence of the colonies caused by changes in the forest structure due to the appearance of a shrub layer, favours the appearance of shade-preferring species.

Changes in lichen biota of the colony and post-colony areas in Skrzyszewo and Przesieki were very similar to each other. One may assume that such a model of the influence of a grey heron colony on epiphytic lichen biota of Scots pines is a recurrent phenomenon elsewhere.

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**Appendix 1. List of epiphytic lichen species and their abundance in three plot types (colony, post-colony and control area) in the bark of trees in studied areas in Poland. Abbreviations are those used in the detrended correspondence analyses (DCA).**

Species	Abbreviations	control <i>n</i> = 20	colony <i>n</i> = 19	post-colony <i>n</i> = 18
<i>Amandinea punctata</i> (Hoffm.) Coppins & Scheid.	<i>Amanpunc</i>	0	4	9
<i>Chaenotheca ferruginea</i> (Turner ex Sm.) Migula	<i>Chaeferr</i>	1	1	1
<i>Cladonia chlorophaea</i> (Flörke ex Sommerf.) Spreng.	<i>Cladchlo</i>	1	0	0
<i>C. coniocraea</i> (Flörke) Spreng.	<i>Cladconi</i>	2	0	0
<i>C. digitata</i> (L.) Hoffm.	<i>Claddigi</i>	1	0	0
<i>C. macilenta</i> Hoffm.	<i>Cladmaci</i>	6	0	0
<i>Coenogonium pineti</i> (Ach.) Lücking & Lumbsch	<i>Coenpine</i>	1	1	15
<i>Fuscidea pusilla</i> Tønsberg	<i>Fuscpusi</i>	5	0	0
<i>Hypocenomyce scalaris</i> (Ach.) M.Choisy	<i>Hyposcal</i>	18	4	5
<i>Hypogymnia physodes</i> (L.) Nyl.	<i>Hypophys</i>	20	4	7
<i>Lecanora conizaeoides</i> Nyl. ex Cromb.	<i>Lecaconi</i>	15	0	5
<i>L. saligna</i> (Schrad.) Zahlbr.	<i>Lecasali</i>	0	0	2
<i>L. symmicta</i> (Ach.) Ach.	<i>Lecasymm</i>	1	0	0
<i>Lepraria elobata</i> Tønsberg	<i>Leprelob</i>	10	0	0
<i>L. incana</i> (L.) Ach.	<i>Leprinca</i>	19	17	14
<i>L. jackii</i> Tønsberg	<i>Leprjack</i>	9	1	1
<i>Micarea prasina</i> Fr.	<i>Micapras</i>	10	0	1
<i>Parmelia sulcata</i> Taylor	<i>Parmsulc</i>	0	0	5
<i>Parmeliopsis ambigua</i> (Wulfen) Nyl.	<i>Parmambi</i>	2	0	0
<i>Physcia tenella</i> (Scop.) DC.	<i>Phystene</i>	0	6	12
<i>Placynthiella dasaea</i> (Stirt.) Tønsberg	<i>Placdas</i>	1	0	0
<i>Pseudevernia furfuracea</i> (L.) Zopf	<i>Pseufurf</i>	1	0	0
<i>Trapeliopsis flexuosa</i> (Fr.) Coppins & P.James	<i>Trapflex</i>	4	0	0
<i>Violella fucata</i> (Stirt.) T.Sprib.	<i>Violfuca</i>	1	0	0
<i>Xanthoria candelaria</i> (L.) Th.Fr.	<i>Xantcand</i>	0	2	4
<i>X. parietina</i> (L.) Th.Fr.	<i>Xantpari</i>	0	0	3