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Review

Recent Trends and Future Challenges for Lichen Biomonitoring in Forests

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Abstract: Currently, forest ecosystems are often located in remote areas, far from direct sources of air pollution. Nonetheless, they may be affected by different types of atmospheric deposition, which can compromise their health and inner balance. Epiphytic lichens respond to air pollution and climate change, and they have been widely adopted as ecological indicators, mainly in urban and industrial areas, while forest ecosystems are still underrepresented. However, in recent years, their use has become increasingly widespread, especially in the context of long-term monitoring programs for air pollution in forests. In this review, we provide a critical analysis of the topic from the point of view of the different methodological approaches based on lichen responses adopted in forest ecosystems. Further, we discuss the main challenges posed by the current global change scenario.

Keywords: air pollution; climate change; lichen diversity; functional traits; indicator species; bioaccumulation

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1. Introduction

Despite the great progress that has been made in achieving better air quality standards over recent decades, reducing global air pollution still represents one of the main challenges in our society, and air pollution continues to harm people's health and the environment [1].

Currently, forest ecosystems are often located in remote areas, far from direct sources of air pollution. Nonetheless, many natural and anthropogenic substances are transported over long distances from their emission sources, and they mainly spread through wet and dry deposition. Indeed, forests may be affected by different types of atmospheric deposition, which can compromise their health and inner balance. The deposition of sulfur and nitrogen compounds is a major driver of various changes in forest ecosystems and can be monitored through long-term programs [2]. This underlines the need to identify a series of sensitive and reactive forest components able to provide sharp indications of the interactions between air pollution, deposition, climate change, and extreme events.

In this regard, lichens are usually quite sensitive to environmental changes and can play a role as early-warning indicators of impacts better than other forest components [3]. These organisms are formed through a symbiotic relationship between a fungus (mycobiont) and one or more photosynthetic partners—algae or cyanobacteria (photobiont). Unlike most vascular plants, which obtain nutrients mainly from the soil through their roots, lichen metabolism strictly depends on interactions with the atmosphere. The absence of a protective cuticle makes them poikilohydric organisms, the water status of which varies passively with the surrounding environmental conditions [4]. In addition, several lichen species can accumulate trace elements far beyond their physiological requirements [5]. For these reasons, they respond directly to air pollution and climate change, and they have been widely adopted as biomonitors, mostly in urban and industrial areas [4,6–8] rather than in forest ecosystems [9].

In forested areas, which usually experience low direct impacts from airborne pollutants, lichens are more often studied for the monitoring of sustainable forestry (e.g., [10–13]) or ecosystem functioning (e.g., [8,14–16]) rather than air pollution. However, in recent years, their use has become increasingly widespread, especially in the context of long-term monitoring programs for air pollution in forests (ICP Forests [17]; ICP Integrated Monitoring [18]; United States Forest Service—Forest Inventory and Analysis [19]; Finnish air quality epiphytic macrolichen survey method [20]).

In this literature review, we provide a critical analysis of the topic from the point of view of the main methods adopted in various contexts. We performed a screening of current and emerging research by using an expert assessment approach, selecting the most relevant literature from the main scientific databases (e.g., Scopus and WOS; keywords: lichen, monitoring, forest). We considered the more recent works (from approximately the last 25 years) above all and focused on those studies, which allowed us to outline a logical framework on which to base our discussion.

We mainly focused on lower-trunk epiphytic lichens, as they are the most studied in forest ecosystems. The review followed the rationale of the conceptual diagram describing the relations between the main pollution drivers and the different methodological approaches based on lichen responses adopted in forest ecosystems (Figure 1).

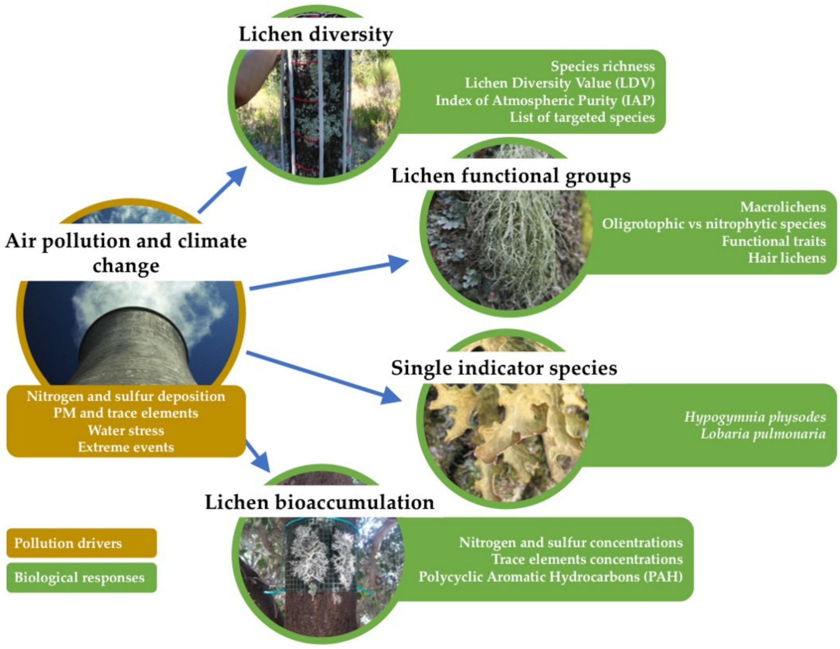

Figure 1. Conceptual diagram describing the relations between the main pollution drivers and the methodological approaches based on lichen responses adopted in forest ecosystems. Photos: air pollution and climate change (cooling tower of a geothermal power plant); lichen diversity (sampling grid for lichen diversity value monitoring [17]); lichen functional groups (the fruticose lichen *Ramalina farinacea*); single indicator species (the air pollution-sensitive foliose species *Lobaria pulmonaria*); bioaccumulation (samples of the foliose lichen *Pseudevernia furfuracea* exposed on a tree during a bioaccumulation survey). Photos by Giorgio Brunialti.

Starting from the key drivers of air pollution and climate change, we classified lichen biomonitoring studies in terms of their approaches in four main categories based on:

1. Several aspects of lichen diversity: the assessment of (i) lichen diversity indices based on lichen presence and abundances, such as the lichen diversity index (LDV) and the index of atmospheric purity (IAP); and (ii) lichen diversity with other methods;
2. The responses of lichen functional groups of target species: macrolichens, oligotrophic vs. nitrophytic species, functional traits, hair lichens;
3. The viability of single indicator species, such as *Hypogymnia physodes* (L.) Nyl. and *Lobaria pulmonaria* (L.) Hoffm.;

4. The bioaccumulation of several airborne pollutants, such as nitrogen and sulfur concentrations and other potentially toxic elements (including trace elements and PAHs).

These issues are discussed in detail in the following sections. It was not uncommon for the research and applied studies found in the literature to cover more than one of the aspects listed above.

2. Lichen Communities—Lichen Diversity Indices

Biomonitoring methods based on the study of epiphytic lichen communities represent excellent tools for monitoring the effects of air pollutants over time, especially sulfur and nitrogen compounds and atmospheric particulate matter (for a review, see, for example, [6,8]). Most of them detect lichen diversity within a sampling grid placed on tree trunks and consider richness and abundance indices, such as the index of atmospheric purity (IAP) [21–24] and the lichen diversity value (LDV) [17,25,26]. The IAP combines the number of species at the site with their sensitivity towards environmental stressors, primarily air pollution. The LDV is the most recent methodology, and it is strongly standardized to allow easier comparisons throughout Europe; it is not related to any specific pollutant but can be considered an indicator of general environmental quality. Although most of the research has been conducted in urban and industrial areas (for a review, see [9]) where air pollution represents one of the biggest threats to human health, there are also numerous examples of scientific studies and monitoring programs that adopt the LDV or IAP in forest ecosystems. Most of them concern sites in Europe [7,20,27–35], North America [36,37], and South America [38].

Here, we consider for discussion only some of the relevant papers on the topic ranging from 2005 to 2021 (Table 1).

Table 1. Lichen communities and lichen diversity indices: list of selected papers.

	Index of Atmospheric Purity (IAP)	Lichen Diversity Value (LDV)	Other Lichen Diversity Methods
Description	The IAP detects lichen diversity within a sampling grid placed on tree trunks. It combines the number of species at the site with their sensitivity towards environmental stressors, primarily air pollution	The LDV is the most recent methodology, and it is strongly standardized to allow easier comparisons throughout Europe; it is not related to any specific pollutant but can be considered an indicator of general environmental quality	In these papers, lichen communities were studied at the tree or plot level without calculating the IAP or LDV
Standard methods	LeBlanc and De Sloover [21], Ammann et al. [22], VDI Richtlinie 3799 [24]	Stofer et al. [17], Asta et al. [25], EN 16413 [26]	IM Programme Centre [18], USFS [19]
Review papers	Nimis et al. [6], Abas [9], Kricke and Loppi [23], Conti and Cecchetti [39]	Nimis et al. [6], Giordani and Brunialti [8], Kricke and Loppi [23]	Nimis et al. [6], Ellis [14]
Year			
2005			Jovan and McCune [40]
2006		Giordani [27]	
2007		Giordani [28], Svoboda [29]	Geiser and Neitlich [41]
2008	Poličnik et al. [30], Blasco et al. [42]	Cristofolini et al. [31]	
2009	Mayer et al. [20]	Brunialti et al. [43]	
2010		Svoboda [32]	Marmor et al. [44], Gadsdon et al. [45]
2012		Pinho et al. [33], Giordani et al. [34]	
2013	Gibson et al. [36]		Pinho et al. [46], Mayer et al. [47]

Table 1. *Cont.*

	Index of Atmospheric Purity (IAP)	Lichen Diversity Value (LDV)	Other Lichen Diversity Methods
2014		Giordani et al. [7]	Geiser et al. [48]
2015			McDomough et al. [49]
2017	Agnan et al. [35], McMullin et al. [37]	Agnan et al. [35]	
2018			Degtjarenko et al. [50]
2019		Papitto et al. [51]	Geiser et al. [52]
2020	Correa-Ochoa et al. [38], Tanona and Czarnota [53]		Geiser et al. [54]
2021			Morillas et al. [55]

Forests are usually far from direct emissions sources and, therefore, they are subject to lower pollution conditions than urban and industrial areas. Thus, while in the latter, atmospheric pollution is the main limiting factor for lichen communities (see, for example, [27,28,32], in forest environments, habitat-related variables may play a major role in lichen diversity, leading to the possibility of incorrect interpretation of diversity indices [28,30,32]. Some authors agree that the IAP may be a suitable air pollution indicator even in forests and with low pollution levels. A study conducted in Finnish and Russian spruce- and birch-dominated forests in Finland and Russia [20] showed a clear correlation between modeled air pollution and IAP values based on epiphytic communities. The authors demonstrated that the IAP also does not seem to be influenced by forest structure characteristics or forest type in areas with low air pollution and can detect fine gradients in sulfur and nitrogen air pollutants. Similarly, Gibson et al. [36] showed that the IAP can reveal sites where ecological degradation occurs, which may be associated with air pollution, even at low levels. However, many authors suggest carefully interpreting lichen diversity data in terms of the direct effects of pollution in forest areas. For example, in the Western Carpathians forests, IAP values were negligibly related to air pollution depositions and better reflected forest ecological conditions (Tanona and Czarnota [53]). In a study carried out in the province of Genova (Italy), Giordani [28] showed that, in forested areas, harvesting and forest fires have the predominant effects on lichen diversity, suggesting the need to develop a more defined sampling protocol to estimate atmospheric pollution in such environments. In forested areas lacking air pollution, Brunialti et al. [43] found that lichen diversity strictly depends on the structural characteristics of the managed forests, mainly tree substrates, forest types, and forest management. These results confirm the strong influences of land use and forest management on LDVs, which may represent confounding effects for data interpretation in the context of atmospheric pollution assessment. For this reason, the authors suggested adopting interpretative scales for LDV scores in different environmental conditions (forested and non-forested areas). Similarly, Svoboda et al. [32] found that forest age and fragmentation strongly influence lichen diversity, and, in some cases, several natural factors can obscure the effect of human influence.

Many studies have provided interesting biomonitoring results regarding the relationships between lichen communities' compositions and modeled or measured values of nitrogen and sulfur deposition within forest national monitoring networks in several different European countries (Estonia [44,50], the UK [45], Portugal [46,55], Finland [47], Italy [51], and the USA [40,41,48,49,52,54]). Forests are remote ecosystems of less interest from a risk assessment point of view because of their low population density. However, studying the effects of atmospheric pollution in forest environments may be a good approach for obtaining a reference for the background values in remote areas. Moreover, these studies can provide information on the long-range dispersion of pollutants. For this purpose, the Convention on Long-range Transboundary Air Pollution (the Air Convention, formerly CLRTAP) of the United Nations Economic Commission for Europe (UNECE) launched the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) in 1985. This network monitors forest conditions at two monitoring levels to gain insight into the spatial and temporal variations in forest conditions (level I) and to clarify cause–effect relationships (level II). In this context, the study of lichen diversity was also introduced with the adoption of a specific survey manual [17]. The protocol refers to the European standard [25,26], but the sampling plan (selection of trees within the plot) is adapted to the ICP Forests context. The method was applied in the Forest Biota project [56] with the objectives of (i) monitoring the richness and frequencies of lichen species in EU/ICP Forests level II plots; (ii) evaluating the relationship between lichen diversity and influencing factors (e.g., stand structure and composition, deposition); (iii) testing a methodology for biodiversity assessment specifically for EU/ICP Forests level II plots; and (iv) setting a baseline for monitoring future changes at the plot level. In total, 83 plots (1155 trees) across ten European countries were considered. The results showed that species' richness and evenness were not significantly dependent on sulfur and nitrogen depositions but highly correlated with stand structure, geographical location, and altitude. Functional traits, such as growth form, are more effective in describing pollutant depositions; specifically, nitrogen input [7]. In addition, the data collected in the Italian plots confirmed that lichen species' richness is influenced by, among other factors, longitude and latitude. In contrast, functional traits (growth forms, types of photobiont, types of reproductive strategy) are more related to factors that may be independent of geographic position [34].

Recently, to respond to EU Directive 2016/2284 (NEC), the Italian NEC network was set up, and it includes several sites from the ICP Forests network. This directive commits the Member States to: (i) reducing anthropogenic emissions of sulfur, nitrogen, non-methane volatile organic compounds, ammonia, and particulate matter (PM) through the elaboration and implementation of national air pollution control programs; and (ii) monitoring the effects of air pollutants on ecosystems. The network currently comprises six sites. The first two surveys (2019 and 2020) found the lowest LDVs for the sites in the Po and the pre-Alpine belt, which have historically been more subject to high levels of air pollutants. In contrast, the Apennine plots from central and southern Italy showed the highest values [51,57]. The LIFE MODERnNEC Project (project website: https://lifemodernec.eu/) was recently funded to improve this monitoring system, both by introducing new sites belonging to different bioclimatic regions, as required by the NEC guidelines [58], and by adopting new methods based on functional traits and the presence of indicator species.

We can thus state that lichen diversity indices are also very useful for monitoring forest areas. However, a future challenge is to improve the interpretation of the results based on natural alteration scales developed ad hoc for environments, such as those for the urban and industrial contexts. Furthermore, the development of rapid biodiversity assessment (RBA) methods could facilitate the monitoring of large areas with the involvement of non-expert personnel, which would be useful in the context of national forest monitoring networks. In this regard, image analysis and artificial intelligence could be promising tools. One of the future directions for the Italian monitoring network is to use artificial intelligence algorithms to recognize key groups or single lichen species starting from photographic images of lichens on tree trunks. The subsequent statistical processing of the collected data will make it possible to obtain information on lichen communities and, consequently, on air quality trends in Italian forests [59].

3. Lichen Functional Groups

Functional diversity is a component of biodiversity that generally concerns the range of functions organisms have in communities and ecosystems [60]. It can provide a mechanistic link between changes in ecosystems and the functional roles of specific groups of organisms (functional groups) distinguished according to their functional traits. Monitoring of the functional traits of animals, plants, and fungi has increased considerably in recent decades [60]. Concerning epiphytic lichens, functional traits such as growth forms, photosynthetic partners, reproductive strategies, and sensitivity to pollutants (e.g., oligotrophic and nitrophytic species) have been extensively studied in the context of forest monitoring (e.g., [16,44,45,48,50,55]). Here, we consider for discussion only some of the relevant papers on the topic ranging from 1999 to 2021 (Table 2).

Table 2. Lichen functional groups: list of selected papers.

Functional Groups	Oligotrophic vs. Nitrophytic Species	Macrolichens	Other Functional Traits
Description	The proportions of these two functional groups in the lower trunks of forest trees have been confirmed as a suitable indicator of the impact of oxidized and reduced nitrogen compounds. These studies have been used to establish the nitrogen critical loads for epiphytic lichen communities in North America and Europe	Several monitoring protocols consider the responses of macrolichens (foliose and fruticose species) as a suitable tool for assessing the effects of nitrogen and sulfur depositions. Most of these papers also focus on nitrogen-tolerant and -sensitive species	These papers deal with air pollution and climate change and take into account additional functional traits, such as photobiont type, structures for sexual (ascomata type and pigmentation) and asexual reproduction (vegetative propagules, such as pycnidia, sporodochia, isidia, or soredia), and secondary metabolites
Review papers	Ochoa-Hueso [3], Giordani and Brunialti [8], Ellis [14], Nascimbene et al. [15], Ellis et al. [16]		
Year			
1999	Wolseley et al. [61]	Wolseley et al. [61]	
2003		Kinnunen et al. [62]	
2004	Pitcairn et al. [63]	Pitcairn et al. [63]	
2005	Jovan and McCune [40]	Jovan and McCune [40]	
2006	Wolseley et al. [64]	Will-Wolf et al. [65]	
2007	Geiser and Neitlich [41]	Geiser and Neitlich [41]	
2009	Wolseley et al. [66]	Wolseley et al. [66]	
2010	Marmor et al. [44], Gadsdon et al. [45]	Marmor et al. [44]	
2011			Marini et al. [67]
2012	Pinho et al. [33,46], Giordani et al. [34], Jovan et al. [68]		
2014	Giordani et al. [7], Geiser et al. [48]	Geiser et al. [48]	
2015	McDonough et al. [49]	McDonough et al. [49], Root et al. [69]	
2018	Degtjarenko et al. [50]		
2019	Geiser et al. [52]	Nascimbene et al. [15], Geiser et al. [52]	
2020			Hurtado et al. [70,71]
2021	Morillas et al. [55]	Geiser et al. [54]	Łubek et al. [72]

In general, most studies suggest that functional diversity can provide additional information in the study of lichen diversity, allowing early-warning responses to forest environmental changes; above all, in response to nitrogen compounds, which are now the predominant air pollutants [7]. One example is the composition and ratio of oligotrophic and nitrophytic species in the lower trunks of forest trees. Several studies suggest that the proportions of the two functional groups may be a suitable indicator of the impact of oxidized and reduced nitrogen compounds [31,33,45,46,68].

This line of research includes the study by Gadsdon et al. [45], which supports the use of lichen communities growing on the twigs of acid-barked tree species as sensitive indicators of NH_3 and NO_2 air pollution. This approach is based on the fact that the bark pH of the twigs is naturally higher than that of the trunk, and lichens on twigs are more sensitive to these compounds than those living on the tree trunk [61,63,64,66]. These results are further supported by Marmor et al. [44], who studied the relationship between the vertical distribution of macrolichens on trunks and alkaline dust pollution. Considering a subset of ten lichen species, they showed that their composition in the upper canopy was a more informative indicator of air pollution in forests compared to the lichen composition in the lower part of the trunk on trees (first 2 m). However, the authors pointed out that the sampling of canopy lichens is complicated due to methodological difficulties. For this reason, most studies have focused on lichens growing on the lower trunk, which are easier to detect and can give equally promising results. A long-term study (1993–2010) carried out at a remote site of the European Alps (Austria) showed a significant decrease in epiphytic lichen diversity in the presence of high levels of S and N transported by fog and rain due to the decrease in oligotrophic species rather than the increase in nitrophytic ones [47]. The authors explained these results in terms of the differential time lag in the responses: nitrogen-sensitive species could have already vanished, while nitrophytic ones had not yet started to colonize the monitoring plot. In addition, in the USDA Forest Service Air Program, Root et al. [69] confirmed that, when nitrogen deposition increases, nitrogen-loving eutrophic lichens become dominant over the oligotrophic species that thrive in nutrient-poor habitats.

Among the functional groups, macrolichens have been widely considered in both European [20,62,73] and United States [19,40,41,48,49,54] forest-monitoring programs. The objectives of these studies were to determine the presence and abundance (from rare to abundant, four-class ordinal scale) of epiphytic macrolichen species (i.e., foliose and fruticose) on living and dead trees (including recently fallen branches and logs) in each plot. Compared to the assessment of the total lichen diversity (including crustose species), the main advantage of this method was that the crew members were required to collect lichen samples and send them to be identified by lichen specialists. Since expert lichenologists were not directly involved in the field, this made it possible to survey many plots (e.g., the USDA Forest Service surveyed approximately 1500 sampling sites). Furthermore, this simplified method is reliable and suitable for explaining air pollution trends, as the results of several field surveys carried out in recent decades highly correlate with N and S depositions [48,52,54]. In particular, the abundance of nitrophilous lichens correlates with the deposition of nitrogen compounds in both reduced and oxidized forms [40,68]. In this context, functional groups have been widely used to establish the nitrogen and sulfur critical loads for epiphytic lichen communities in North America and Europe [7,33,68]. Such lichen-based critical loads, tested by means of multiple linear regression models, are important tools that can help managers, regulators, and policymakers meet the goals of protecting biodiversity and sustaining the health and productivity of forests [48,52,54]. Similar results were obtained in Europe [7,33], confirming the relations between macrolichens and the main nitrogen compounds. In the context of the ICP Forests monitoring program (Forest Biota project), Giordani et al. [7] showed that the percentage of macrolichens is the most effective indicator of nitrogen compounds, with 57% of the variation explained by nitrogen depositions.

The assessment of lichen functional groups may also represent an interesting tool in the field of climate change monitoring. Indeed, several studies have underlined the influence of climate change on lichen communities and the potential application of bioclimatic models to lichen species as indicators of climate change risk [74]. Geiser and Neitlich [41] modeled epiphytic macrolichen community responses to air quality and climate gradients at 1416 forested plots in western Oregon and Washington. They showed that the gradients were responsive to regionally increasing nitrogen availability and to temperature changes predicted by climate models. A study carried out in the interior forested mountain ecosystems of the Pacific Northwest (USA) offered a different picture, highlighting that climatic moisture deficit and continentality, independently of air quality impacts, are the main drivers for macrolichens [69].

Among macrolichens, hair lichens represent a promising functional group for the monitoring of climate change and air pollution depositions, particularly in the context of high-elevation mountain areas, as reported in a recent literature review by Nascimbene et al. [15]. This morpho-functional group includes pendulous epiphytic lichens with fruticose-filamentose thalli mainly belonging to the genera *Alectoria*, *Bryoria*, *Evernia*, *Ramalina*, and *Usnea*. The authors provide a starting point for the development of predictive tools to study the effects of global change in the Alps.

Other functional traits can be considered suitable indicators for monitoring forest pollution and climate change (e.g., [67,70,71]). In a study carried out in Italy, Marini et al. [68] suggested that the impacts of global change on lichens are likely influenced by the photobiont type (i.e., the synthetic partner of the lichen symbiosis), on which the differential responses in lichen species' richness to various environmental drivers depend. In Polish forests, Łubek et al. [72] considered a broader set of functional traits, including growth form, photobiont type, structures for sexual (ascomata type and pigmentation) and asexual reproduction (vegetative propagules, such as pycnidia, sporodochia, isidia, and soredia), and secondary metabolites. Furthermore, these researchers also examined the ecological requirements for temperature, moisture, light, continentalism, and eutrophication among lichen species. They suggested that the concurrent study of the functional diversity and the ecological optima of species, rather than considering shifts in species composition alone, can help in better monitoring directions in lichen biota changes over time.

We can, therefore, conclude that the study of the functional diversity of lichens represents a promising research field for the development of interpretative approaches to the trends in lichen community responses to current and future air pollution and climate changes in forest ecosystems.

4. The Focus on Single Indicator Species

The study of single indicator species, such as air pollution-sensitive or more tolerant lichens, can provide useful information on air pollution and climate change impacts on forest sites. This approach builds on the assumption that air pollutants can cause physical and physiological alterations to lichens, resulting in visible injuries on their thalli (e.g., damage or discoloration, reduction in the production of apothecia and soredia; for a review, see [4]). Here, we consider for discussion only some of the relevant papers on the topic ranging from 1995 to 2022 (Table 3).

Table 3. Indicator species: list of selected papers.

Indicator Species	*Hypogymnia physodes*	*Lobaria pulmonaria*
Description	This species is considered an indicator species that is rather tolerant to air pollution. Its abundance is determined by counting the number of dots in a sampling grid placed on tree bark. A five-class scale of damage is used to assess the most damaged individuals observed at heights of between 50 and 200 cm above ground	This large foliose species is very sensitive to air pollution and declining heavily throughout Europe. Several studies have demonstrated its suitability both as a flagship and as an umbrella species for nature conservation, and it is associated with many other rare and endangered forest-dwelling organisms
Year		
1995		Nilsson et al. [75]
2003	Kinnunen et al. [62]	
2004		Campbell and Fredeen [76]

Table 3. *Cont.*

Indicator Species	*Hypogymnia physodes*	*Lobaria pulmonaria*
2006	Will-Wolf et al. [65]	
2007	Jeran et al. [77]	
2009	Mayer et al. [20]	
2010		Nascimbene et al. [78]
2013	Mayer et al. [47]	Nascimbene et al. [79]
2015		Brunialti et al. [80,81]
2020		Paoli et al. [82]
2022		Di Nuzzo et al. [73]

In this context, the Finnish epiphytic lichen method aims to monitor air quality by focusing on the presence of a set of macro lichen species and the abundance of and degree of damage suffered by two species with known pollution sensitivity: the highly sensitive *Bryoria* spp. and the more tolerant *Hypogymina physodes* [73]. The abundances of the two species are obtained by counting the numbers of dots in a sampling grid placed on tree bark. A five-class scale of damage is used to assess to the most damaged individuals observed between heights of 50 and 200 cm above ground. However, when adopting this method in southern Finland and northwestern Russia, Mayer et al. [20] showed that the responses of single species may not be reliable bioindicators at low air pollution concentrations. Indeed, the sensitive lichen *Bryoria* spp. were rare in their forest sites, thus not providing information, while the abundance of and damage suffered by *H. physodes* were correlated with forest structure but not with air pollution, as already suggested in a previous study by Will-Wolf et al. [65].

Another lichen widely used in forest monitoring is *Lobaria pulmonaria*. This large foliose species is very sensitive to air pollution and declining heavily throughout Europe. Several studies have demonstrated its suitability both as a flagship and as an umbrella species for nature conservation, and it is associated with many other rare and endangered forest-dwelling organisms (e.g., [75,76,78,79,83]). Further, being easy to identify in the field, it can be successfully sampled by non-expert lichenologists, such as personnel trained in appropriate training courses. These features make *L. pulmonaria* an excellent indicator of air quality and forest continuity, especially when setting up rapid biodiversity assessments (RBAs) in monitoring programs carried out over large forest areas. In particular, studying its abundance and the viability of its populations in forest plots may represent a suitable method for obtaining early-warning responses to environmental changes in the medium-to long-term periods. In this respect, many authors not only consider the occurrence and abundance of *L. pulmonaria* but also assess its conservation status and health in terms of active growth (presence of meristematic lobes) and dispersion capacity (presence of juvenile thalli, vegetative propagules, and fruiting bodies) (e.g., [80,81,83]). However, most of these studies investigate the effects of forest management and old-growth structural attributes. Although promising, the use of this lichen as an air pollution bioindicator is still under-explored. In a recent study by Paoli et al. [82], *L. pulmonaria* was adopted as a model to test if the translocation of this sensitive species is only effective under low-pollution conditions. They transplanted fragments and whole thalli in beech and oak forests in Central and Southern Europe (Slovakia and Italy). The transplantation was successful in remote areas with low or negligible metal contamination. On the other hand, the translocation of the thalli in the most polluted sites did not ensure their survival. These results show that air quality still limits the recolonization of *L. pulmonaria* in the sites where it disappeared in the past.

Therefore, using single species in forest monitoring can provide additional information on the overall framework of air pollution in these ecosystems.

Furthermore, these species are usually easy to identify in the field, even by non-expert personnel, so they can be adopted in large-scale monitoring networks using simplified detection methods. However, studies have shown that focusing on a single species can also have some drawbacks. Indeed, the distribution of single forest lichen species, as well as being affected by air pollution, may also be affected by microclimatic and/or biogeographic variables, thus reducing their effectiveness as bioindicators (e.g., [80,83]).

The adoption of this approach together with the methods for lichen biodiversity assessment (both species richness and functional diversity) discussed in the previous sections is therefore suggested. Future challenges in this research field include obtaining further insights into ecophysiological aspects to distinguish the effects of air pollution from other environmental factors.

5. Bioaccumulation Approach

Lichens are among the most widely used organisms for the biomonitoring of airborne pollutants (including N and S, trace elements, and polycyclic aromatic hydrocarbons). This is due to their metabolism, which is strictly dependent on atmospheric exchanges, and their resistance to these pollutants, which they can accumulate at high levels, far above lichens' physiological requirements [5,84–87].

Lichen bioaccumulation approaches are usually adopted for monitoring urban and industrial areas (for a review, see [9]) and, despite their potential, these methods are still not widespread in forest ecosystems. However, such studies could be very useful to investigate how long-range transport can promote global dispersion and deposition of air pollutants in remote ecosystems. Here, we consider for discussion only some of the relevant papers on the topic ranging from 1998 to 2022 (Table 4).

Table 4. Bioaccumulation approach: list of selected papers.

	Nitrogen and/or Sulfur Content	Trace Elements	PAHs
Description	These papers consider nitrogen and sulfur content in lichen thalli	These papers deal with the bioaccumulation of trace elements, including potentially toxic elements	Epiphytic lichens are also useful biomonitors for persistent organic air pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs). These papers represent a selection of these kinds of studies
Review papers	Wolterbeek [84], Augusto et al. [85], Van der Wat and Forbes [86], Bargagli [87]		
Year			
1998	Loppi et al. [88]	Loppi et al. [88]	
2003		Loppi and Pirintsos [89]	
2006			Blasco et al. [90]
2007	Otnyukova [91]	Jeran et al. [77], Otnyukova [91]	
2008			Blasco et al. [42]
2009	Conti et al. [92]	Conti et al. [92]	
2011			Blasco et al. [93]
2013	Root et al. [94]		
2014			Nascimbene et al. [95]
2015	Root et al. [96]	Agnan et al. [97]	
2017		Agnan et al. [35]	
2018	Manninen [98]	Kłos et al. [99], Cecconi et al. [100]	

Table 4. *Cont.*

	Nitrogen and/or Sulfur Content	Trace Elements	PAHs
2019		Benítez et al. [101]	Shukla et al. [102]
2020		Paoli et al. [82]	
2021	Root et al. [96]	Ancora et al. [103]	
2022		Carrillo et al. [104]	

With regard to biomonitor species, epiphytic foliose and/or fruticose lichens are usually sampled. Among the foliose species, the most widely adopted in forest monitoring belong to the family Parmeliaceae, such as *Flavoparmelia caperata*, *Parmotrema arnoldii*, and *Hypogymnia physodes* (e.g., [77,88,89,98,99,101,103,104]), while *Letharia vulpina*, *Pseudevernia furfuracea*, and *Usnea* are the most widely used fruticose species (e.g., [69,91,92,94,96,100]).

From the point of view of the pollutants, some studies have compared nitrogen and sulfur concentrations in lichen thalli with the deposition of these elements in various forest sites belonging to the USA forests monitoring program [69,94,96]. At 84 forest sites in Western North America, Root et al. [94] estimated the throughfall inorganic N deposition from N concentrations in the lichens *Platismatia glauca*, *Letharia vulpine*, and *Evernia mesomorpha*. They concluded that lichen N concentration can predict throughfall N deposition, and it is especially useful for the identification of areas that exceed critical loads for sensitive ecosystem components. Similar results were obtained when considering N concentrations in *L. vulpina* and ambient-air nitrate concentrations in fine particulate matter ($PM_{2.5}$) at more than 500 sampling sites in the Pacific Northwest in the USA [69]. For S lichen content, the high within-plot variability, the complexity of lichen nutrient absorption, and a short gradient in S deposition are the main factors limiting the possibility of calibrating lichen S concentrations with S throughfall deposition [96].

Most of the lichen bioaccumulation studies carried out worldwide in forests focus on trace elements/metals (e.g., [9,39,87]. In general, relatively low concentrations of these pollutants were monitored in these studies, thus making the results useful for detecting background levels [35,89,92,100]. For example, Cecconi et al. [100], by collecting samples of *Pseudevernia furfuracea* at remote mountain sites, reported specific background element content (43 elements) for use in reference datasets for biomonitoring applications in the context of three macro-regions of Italy (the western Alps, the eastern Alps plus northern Apennines, and the central and southern Apennines). Similarly, Conti et al. [92] evaluated the bioaccumulation of 26 elements in the fruticose lichen *Usnea barbata* to define their background levels in the province of Tierra del Fuego (Southern Patagonia, Argentina). It should be considered that knowledge about background values is essential for correctly assessing pollution levels in terms of deviations from unaltered (natural) references [105,106].

Another key issue in bioaccumulation surveys is the capacity to sharply detect local peak concentrations of trace elements associated with specific natural or artificial air pollution from local sources [97,99,103]. Working at a certain altitudinal range in a volcanic area of Central Italy (Mt. Amiata; between 120 and 1730 m a.s.l.), Ancora et al. [103] showed enhanced accumulation of Pb, Cd, Hg, and Zn in samples of epiphytic lichens (*Parmelia* species) and epigeic mosses (*Hypnum cupressiforme*) collected at altitudes above 1300 m, where beech forests are frequently shrouded in clouds and fog and receive more snow and rain than the lower chestnut and oak woods.

Using the epiphytic lichen *F. caperata* as a biomonitor in six oak and beech forests in Central Italy, Loppi and Pirintsos [89] detected overall low levels of trace elements, except for at two sites showing local accumulations of some heavy metals. High levels of Pb measured in a beech forest with high tourist influence were related to vehicular traffic, whereas an oak forest site showed a local peak for pollutants originating from a steel mill (Mn, Cr, and Ni).

In a study carried out in the mountain forests of Poland, Kłos et al. [99] analyzed the concentrations of several trace elements (Mn, Ni, Cu, Zn, Cd, Hg, and Pb) in samples of the epiphytic lichen *Hypogymnia physodes* collected in spring, summer, and autumn. Both regional and seasonal differences in element concentrations, deriving from nearby territorial emissions and unidentified local emission sources, were identified. Similarly, Agnan et al. [97], in a bioaccumulation study at 21 French forest sites, observed local contributions from anthropogenic activities and local lithology. Their results showed improved accumulation of lithogenic elements deriving from natural dusts (e.g., Al, Fe, and Ti) where the density of the forest canopy was lower. On the other hand, a denser canopy allowed for greater bioaccumulation of elements deriving from foliar leaching, such as Mn and Zn, and more mobile elements, such as Cd.

Epiphytic lichens are also useful biomonitors for persistent organic air pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs) and polychlorodibenzeno-dioxins and -furans (PCDD/Fs). In this context, Augusto et al. [85] and Van der Wat and Forbes [86] have published two interesting reviews on the factors influencing lichen accumulation, biomonitoring guidelines, and the levels of PAHs and PCDD/Fs found in lichens. However, most studies have been carried out in urban and industrial areas, whereas forested areas are widely underrepresented. For example, in mountain areas of the Pyrenees, Blasco and his coworkers [42,90,93] found that vehicular traffic was the main source of PAHs affecting the concentrations of these compounds in foliose (*Parmelia sulcata* and *Lobaria pulmonaria*) and fruticose lichens (*Evernia prunastri*, *Ramalina farinacea*, *Pseudevernia furfuracea*, and *Usnea* sp.). The authors showed differing accumulation behavior among the lichen species. Further, all species showed high levels of 3-ring PAHs and low levels of 6-ring PAHs. Nascimbene et al. [95] also confirmed that traffic due to touristic pressure was the main source of PAHs along the roads leading to seven passes in the Dolomites (southeastern Alps). Upon considering the concentrations of PAHs in samples of the epiphytic lichen *Pseudevernia furfuracea*, they concluded that traffic PAH pollution may impact natural ecosystems and lichen diversity at relatively long distances away from the emission source. Finally, a study conducted in the Garhwal Himalayan forests using the lichen *Heterodermia diademata* indicated that the influences of long-range transport of polychlorinated biphenyl (PCB) congeners, polycyclic aromatic hydrocarbons (PAHs), and nitro-PAHs (N-PAHs) as well as local combustion practices, were the main factors for air pollution in the region [102].

Although lichens are useful biomonitors for PCDD/Fs in urban and industrial areas [85,86], to the best of our knowledge, no monitoring studies of these pollutants in forest environments have been conducted to date.

Finally, lichen bioaccumulation methods are widely used for monitoring point sources of air pollution, especially in urban and industrial areas. In contrast, as far as we know from this analysis, their use in forest ecosystems is still not widespread. However, it is evident that, when these methods have been applied in such environments, they have proved effective in identifying both background levels of pollutants in remote areas and local deposition of pollutants in relation to their long-distance transport. Therefore, the study of epiphytic lichens as early-warning systems to detect signs of a changing environment in forest ecosystems can be confirmed as effective.

6. Conclusions and Perspectives

The close interconnection between the aspects explored in this review provides a complex and multifaceted picture of the topic. Further research is, therefore, needed to follow the relationship between the trends for air pollutants and their long-range transport and, at the same time, distinguish them from other sources of variability. Our findings show how the key to success for the extensive use of lichens as forest indicators relates to the following main features: (i) they are versatile in their responses, both in terms of biodiversity and bioaccumulation; (ii) they are relatively easy to detect, leading to the possibility of adopting simplified procedures; (iii) they respond to different environmental drivers, thus offering the possibility of adopting numerous approaches depending on the issue being explored.

This framework offers some interesting food for thought regarding setting up future research and operational studies to effectively address the future challenges posed by the current global change scenario.

1. With regard to biodiversity methods, well-suited interpretative tools that can better describe air quality and climate changes induced by human activities in forests are still lacking. They could be elaborated based on the previous experience already acquired in the context of studies carried out in urban and industrial areas;
2. With regard to functional diversity and traits, research should be encouraged and strengthened, especially delving into the ecophysiological responses of individual lichens or groups of species;
3. Using single indicator species could be insufficient as a tool if not supported by biodiversity studies. However, simplified methods based on single forest species, sensitive or resistant to pollution, can be helpful in forest-monitoring programs conducted over large territories and involving many non-expert personnel for the identification of whole lichen communities. Furthermore, since these forest-dwelling species respond not only to air pollution but also to other variables, such as the structure of the forest, it is necessary to improve the interpretation of the results to distinguish the effects of the various environmental drivers;
4. Regarding lichen bioaccumulation in forest ecosystems, further efforts to improve the detection of transboundary air pollutants and to promote the adoption of standard methods are needed. Moreover, epiphytic lichens could be effective indicators supporting the use of epigeic mosses, which have been already adopted by the European ICP Forests program to assess heavy metals, nitrogen, microplastics, and persistent organic pollutants (ICP Vegetation Manual [107]). These latter pollutants (POPs) are currently neglected in bioaccumulation studies with lichens in the forest environment. However, this kind of investigation could provide interesting information on the deposition of these pollutants in remote areas.

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