# 2 Sampling and Interpreting Lichen Diversity Data for Biomonitoring Purposes

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## Contents




**Abstract**

Although lichen diversity values are broadly used as bioindicators, mainly for air pollution, lichen communities can be substantially influenced by other ecological factors, such as tree species and forest structure, and microclimatic conditions. In particular, species composition may be a suitable indicator for climate and land-use effects as well. For effective utilization of lichen diversity data in biomonitoring studies including air pollution, ecosystem functioning, and forestry studies, standardized sampling procedure and avoiding sampling and non-sampling errors are the important aspects to be considered. Further interpretation of lichen diversity data requires careful data analysis for providing affirmative results related to ambient air quality. In any lichen biomonitoring program, expected deliverables are based on a hypothesis, which may be achieved by standardization of the sampling procedures based on the functional requirement of the dependent environmental variables. The chapter discusses the procedures and methodology for sampling and interpreting lichen diversity data for biomonitoring purposes.



**Keywords**

Air pollution · Ecosystem functioning · Quality assurance · LDV



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## 2.1 Introduction

Due to their physiology, lichens are sensitive to a series of environmental parameters (namely light, air humidity, UV-B radiation, temperature, and airborne chemicals such as $SO_2$ and $NO_x$ making them useful indicators for air pollution and climate change (Poikolainen et al. 1998; Tarhanen et al. 2000; Cornelissen et al. 2001; Kricke and Loppi 2002; Castello and Skert 2005). Biomonitoring methods based on the diversity of epiphytic lichens are among the most used worldwide. Apart from assessing the effects of gaseous pollutants, these approaches were recently extended to a suite of other anthropogenic disturbances (Nimis et al. 2002). Several aspects of lichen diversity (e.g., species richness and abundance, species composition, indicator species, functional traits and groups) are usually considered, each of them for a particular reason (Nimis et al. 2002). In simplified terms, it is possible to identify three main purposes to perform lichen monitoring:

1. Air quality monitoring. The sensitivity of lichens to phytotoxic gases (mainly $SO_2$ and $NO_x$) underlies their use to assess and monitor the effects of atmospheric pollution (Hawksworth and Rose 1970; Richardson 1993). They have been used as long-term biomonitors of air pollution both for small-scale (Nimis et al. 1990; Loppi et al. 2004; Giordani 2007) and large-scale surveys (Nimis et al. 1991; Van Dobben and DeBakker 1996; Bennett and Wetmore 1999; Frati and Brunialti 2006; Giordani 2006). Lichen biomonitoring surveys are often used to integrate instrumental data of atmospheric pollution (Nimis et al. 1990; Giordani et al. 2002; Giordani 2007; Pinho et al. 2004) and for developing forecasts in connection with human health (Cislaghi and Nimis 1997).
2. Sustainable forestry. Epiphyte diversity is related to forest structure and dynamics. Forest management, directly or indirectly, affects several environmental factors relevant to dispersal, establishment, and maintenance of lichen species. Studies on lichen diversity clearly demonstrate dramatic losses of species caused by forest management in European temperate and boreal forests (Hauck et al. 2013). In general, higher lichen diversity is related to lower management intensity, even though in some cases non-intensively managed forests may provide better conditions for epiphytic lichens than recently abandoned forests. For instance, several studies suggest that selective cutting is less detrimental to forest lichens than the shelterwood system or clear cutting (Nascimbene et al. 2013b).
3. Ecosystem functioning. Epiphytic lichens play an important role in both the forest water cycle, increasing canopy interception of precipitations and forest–nutrient cycling, above all for N-fixing ‘cyanolichens’ (lichens with a cyanobacterial symbiont; for a review see Ellis 2012). In addition to nutrient cycling, lichens are a focal point in forest food webs. They influence the ecological success of forest-dwelling animals in a wide variety of ways, e.g., for macrofauna in nest building, and as forage (Ellis 2012). In some cases, indicator species have been proposed as proxies for lichen species richness more generally. As an example, forest stands with the flagship species *Lobaria pulmonaria* tended to have more red-listed species and more continuity indicator species than stands without this lichen (Nilsson et al. 1995), tentatively pointing to covariation among continuity indicators. Similarly, comparing stands with different abundance and fertility attributes for *L. pulmonaria*, these attributes were associated with higher tree and stand-level macrolichen species richness and with significant differences in species composition among *L. pulmonaria* site-types, i.e., cyanolichens and rare species skewed to the ‘*L. pulmonaria* stands’ (Nascimbene et al. 2010).

In this chapter, we intend to explore a series of key issues on the topic in order to provide a framework of the subject with an overview of recently published researches.

In this regard, Fig. 2.1 provides a step-by-step flow diagram of operations required to address a lichen monitoring program. As a rule, these are

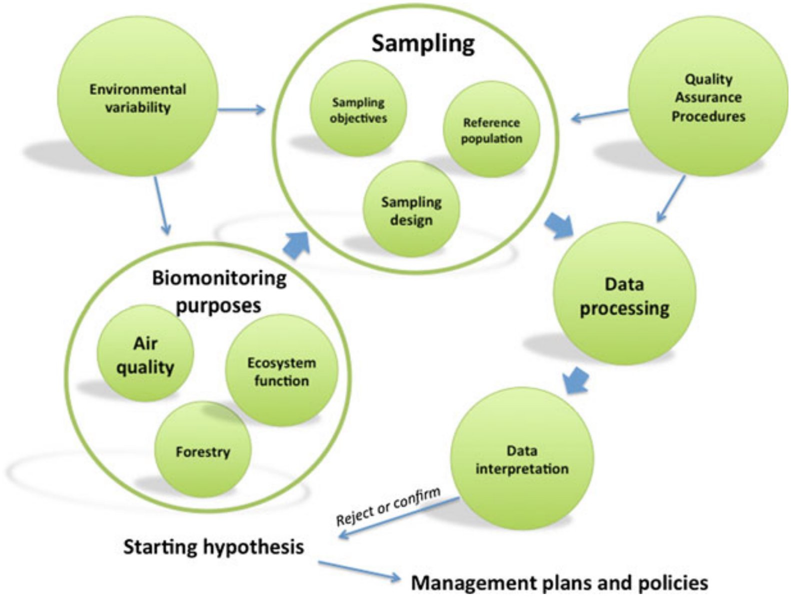



**Fig. 2.1** Schematic diagram of the operations required to address a lichen monitoring program

the typical questions to be answered: does air quality affect lichen diversity in our study area? Or else, does forest management (or fragmentation) influence lichen communities in our investigated forests? Or even, do lichen diversity play a role in ecosystem function of our natural park?

The first step in the organization of a lichen monitoring program is to establish the initial null versus alternative hypotheses. For example, we may state the basic hypothesis that the expected lichen diversity is equal for impact and control sites (with respect to air pollution or to forest management). However, this is where environmental variability comes into play. Thus, the second step is to qualify, quantify, and control the main ecological factors that can affect our response variable, both including those of our interest and also the ones which may represent a source of noise (Brunialti et al. 2010a). Indeed, the effect of large-scale (such as climate) or local scale (as tree substrate) natural factors may represent relevant sources of variability and noise in relation to the phenomenon we are studying (Giordani et al. 2013; Ellis 2012). Also, some of these effects may be cumulative (Jovan and McCune 2004; Pinho et al. 2004; Giordani 2006; Caruso and Thor 2007). For instance, we should study air pollution effect on lichen diversity in forest sites with different forest management: We will have to set the study in order to disentangle the effect of individual factors and determine which of the two acts as main driver affecting lichen diversity or the presence/abundance of indicator species (Giordani 2007; Giordani et al. 2014a).

Our opinion is that this issue may have a central role in biomonitoring studies. For this reason, an entire Sect. 2.2.1 of the chapter focuses on these aspects, where we explore the main sources of variability affecting lichen diversity at different spatial scales. This variability being considered to adequately respond to our initial question, and the third step of our scheme concerns the adoption of an appropriate sampling design, from the selection of objectives and target population to the development of an adequate sampling strategy. We address this topic in Sect. 2.2.2, where we explore the main standardized methods for lichen diversity assessment, and analyze their suitability for various purposes.

Section 2.2.3 focuses on quality assurance procedures that are closely linked to sampling design and should be taken into account to obtain reproducible, reliable, and defensible data (Ferretti 2011). In this respect, we must remember that there is a strong link between data quality, information quality, and decision-making processes (Crumbling 2002).

The fourth step is then the phase of data processing. A broad range of statistical tools may be adopted to test the initial hypothesis. A subsequent interpretation of the results is essential to verify that the statistical significance of the test has a meaning even from an ecological point of view (Sect. 2.3). Starting from raw data, it is possible to adopt a series of interpretative devices that allow analyzing various aspects of the phenomenon under investigation. Only in this way, we may obtain useful information for management and decision making.

In this regard, the last step of our process involves the rejection or acceptance of the starting null hypothesis. On the basis of this result, we get essential information to proceed with the process of planning and management (policies to reduce air pollution, forest and landscape management, etc.). Indeed, without reliable information on changes to ecological systems and on the causes of those changes, decision making cannot deal effectively with these issues (Ferretti 2011).

The main objectives reported above are addressed in most cases with management and decision-making intent. In this perspective, if biomonitoring should be taken as a serious basis for decision making, it needs to produce robust, defensible data and documented quality (Brunialti et al. 2004). With this in mind, it is essential to approach the issue by means of a suitable sampling design and also appropriate statistical and interpretative tools that should be carefully selected to address properly each of these purposes (Ferretti and Erhardt 2002).

## 2.2 Sampling Lichen Diversity

There is no doubt that ecological monitoring has a fundamental role in providing baseline data on the status and trend of environmental resources. However, as underlined by Legg and Nagy (2006), in several cases, ecological conservation programs suffer from the lack of details of goal and hypothesis formulation, survey design, data quality and statistical power at the start. As a result, sometimes, they are likely to fail to reach the necessary standard of being capable of rejecting a false null hypothesis with reasonable power. This is mainly related to the adoption of an unsuitable sampling design, and this is basically the reason why sampling has frequently been considered as the weakest point of monitoring programs. This is unfortunate because failure to provide convincing evidence of the overall quality of environmental information can have serious consequences in environmental decision making. Indeed, we must consider that the defensibility of the data supporting the decision is fundamental (Ferretti 2011). To avoid this and to promote a correct process, the selection of a suitable sampling design represents the first step to reduce data variability due to sampling error. When selecting the proper design, the objectives (biomonitoring purposes, Fig. 2.1) of the survey and environmental variability should be taken into account carefully (Brunialti et al. 2004).

With respect to lichen biomonitoring, environmental variability acting at different spatial scales has been taken into account during the standardization process of the sampling protocols (Giordani et al. 2001; Brunialti and Giordani 2003; Nimis et al. 2002), thereby providing useful information for the selection of suitable sampling designs.

This section provides useful insights on several issues relevant to environmental variability, sampling design, and quality assurance procedures, which are the basis of a proper environmental monitoring program.

### 2.2.1 Variability of Lichen Diversity Across Scales

Variability is an inherent property of ecological systems and every attempt to measure and interpret the environment should consider it (Brunialti et al. 2004). Thus, in the assessment of environmental quality by means of biomonitors, it is important to understand the environmental processes driving the variability of biological data, since this may affect the forecasting precision of these techniques (Laskowky and Kutz 1998). Environmental factors such as geomorphology, climatic variables, and substrate could have a great impact on the ecosystem property being studied in order to assess environmental quality, such as the rate of indicator species, the biodiversity of a community, or the presence of injuries on organisms. In simple terms, environmental heterogeneity may control the variability of lichen diversity and the composition of lichen communities at least at three spatial scales: (1) tree level; (2) plot level, and (3) landscape level. In this section, we highlight the possible effect of these ecological factors on lichen diversity by referring to the main studies conducted so far.

#### 2.2.1.1 Spatial Scale of Variation in Lichen Monitoring Data

Quantifying spatial and temporal variation is a key element for understanding mechanisms and processes that structure the species assemblages for assessing the environmental impact and for identifying an appropriate scale of sampling (Levin 1992; Underwood et al. 2000). Nonetheless, biodiversity assessments are strongly dependent on observation scales (Gaston 2000; Purvis and Hector 2000), and natural spatial variation or the spacing of sampling units may decisively affect results (Dungan et al. 2002).

Although several studies have investigated spatial variation in plants (see, e.g., Palmer 1990; Gunnar and Moen 1998), lichens are still poorly investigated from this point of view (Ferretti et al. 2004), even though descriptors of lichen diversity are widely used in applicative studies for assessing alterations of the environment (Nimis et al. 2002), covering areas ranging from some dozen to some thousands square kilometers (e.g., Giordani et al. 2002). In particular, bioindication techniques usually focus on between-site variability in epiphytic lichen diversity using a high sampling density and do not sufficiently consider possible within-site variability, probably because this would imply sampling more trees, thus increasing the cost of the survey (Ferretti et al. 2004; Brunialti et al. 2010a). Controlling the spatial variation at macroscales is necessary but not sufficient, if the variation at more detailed levels (e.g., considering areas <1 km$^2$) remains unknown.

A recent study explored the variability of lichen composition throughout the spatial levels using sound sampling protocols, along biogeographic, climatic, and pollution gradients (Giordani et al. 2013). The authors showed that the distribution of variation in lichen diversity was impressively similar across the spatial scales (Giordani et al. 2013). In particular, they found that it was fairly constant despite climatic variation, landscape complexity, air pollution, and general anthropogenic pressure. Moreover, the predictability of lichen bioindication methods, as estimated by the between-plot variability/total variability ratio, was limited to less than 40 %, thus calling for some refinements in sampling and interpretation phases (Fig. 2.2). This finding suggests the adoption of specific experimental designs, such as a stratified random sampling or a tree-based sampling to improve the detection of the sources of variation.

#### 2.2.1.2 From Tree-level to Landscape-level Variability

Epiphytic lichens distribution depends on a complex set of environmental and substrate-related explanatory variables, acting from the tree to the landscape level (Nimis et al. 2002). Several tree-level environmental factors are important for the growth of epiphytic lichens and drive the diversity and composition of their communities (Ellis 2012). Figure 2.3 summarizes the main ecological factors acting at this level on a hypothetical tree with a different canopy in winter and summer periods or a deciduous and an evergreen tree, respectively.

We can distinguish at least two main categories of ecological factors that have been explored by many studies (see Ellis 2012): microclimatic variables (light availability, temperature, and relative humidity) and bark properties (pH, texture, exfoliation, water-holding capacity). In this respect, both substrate eutrophication, due to the grazing of cattle and wild animals and also to soil re-suspension (Loppi and De Dominicis 1996; Frati et al. 2007) and circumference, and

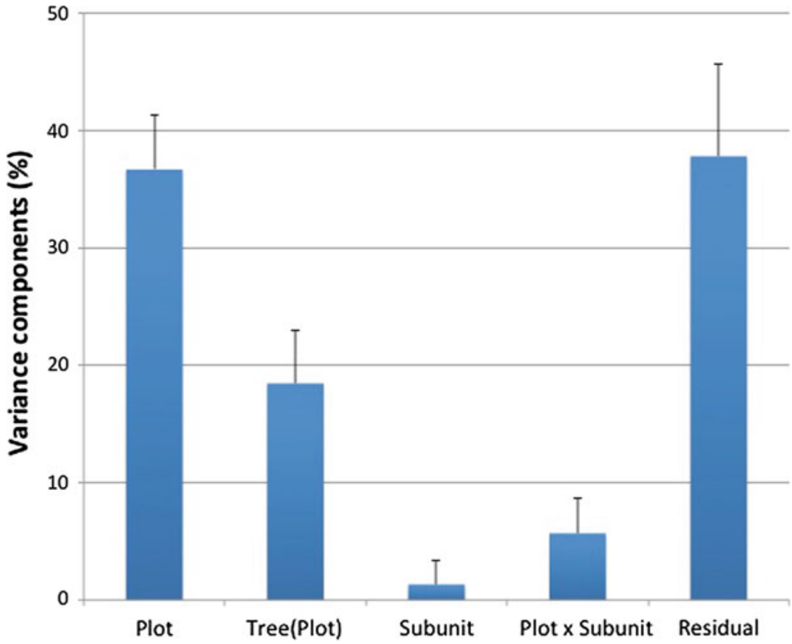



**Fig. 2.2** Estimated components of variance (%) in lichen diversity values, calculated for each spatial scale (modified from Giordani et al. 2013)

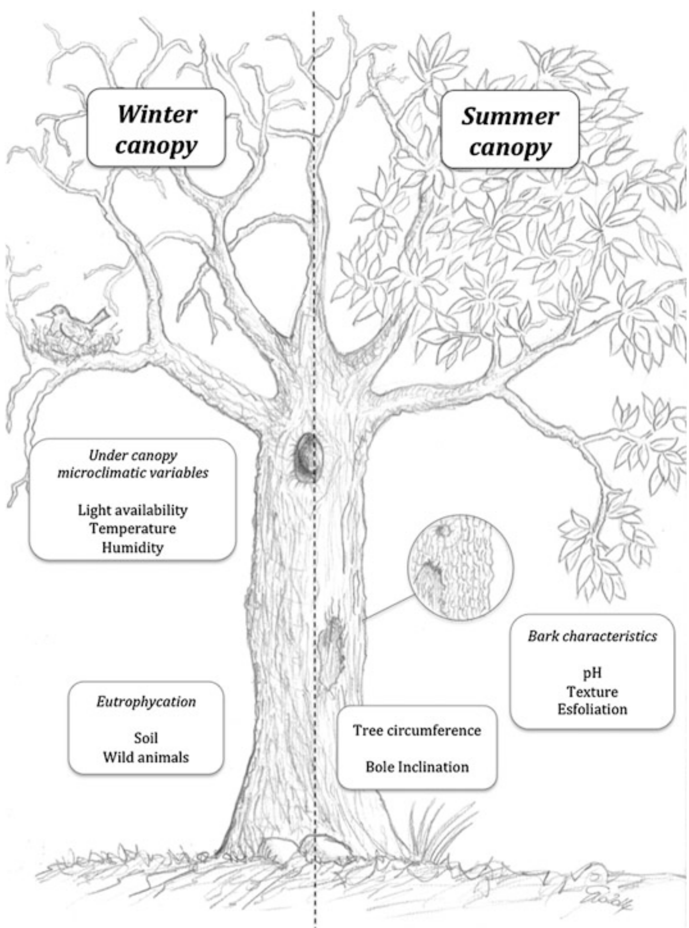



**Fig. 2.3** Conceptual model illustrating the main ecological factors potentially affecting lichen communities under the canopy of a broad-leaved tree in winter and summer periods (see explanation in the text)

inclination of the bole may contribute indirectly to modify these ecological factors (see, e.g., Giordani et al. 2001).

Figure 2.4 summarizes the main ecological factors affecting lichen communities at plot and landscape levels. They are mainly related to the distribution and density of trees (e.g., the gradient from isolated trees to near-natural forest stands) and to the land-use and geomorphological variability (e.g., altitudinal range, agricultural, or forested landscapes). Light and humidity availability generally play the role of direct factors in this context.

Basically, all these aspects are very interconnected and show that the effects largely covariate. Canopy structure decisively controls light conditions at the lower bole (Moe and Botnen 1997; Fritz et al. 2009), and this may result as a negative effect of canopy shading on species richness, both at tree level (Loppi and Frati 2004) and at plot level (Brunialti et al. 2010b). While focusing on edge effects in fragmented boreal and Mediterranean forests, several authors pinpointed light as a main driving factor for lichen colonization, affecting lichen abundance, diversity, and composition at tree level (Esseen and Renhorn 1998; Belinchón et al. 2007; Boudreault et al. 2008; Brunialti et al. 2012b). In particular, higher lichen species richness was found at the side of the trunk facing the edge (Brunialti et al. 2012b), or in correspondence with the first light peak (Belinchón et al. 2007). Loppi and Frati (2004) found higher lichen diversity values on *Tilia platyphyllos* compared to *Quercus ilex.* These findings were explained by significantly higher winter light conditions and water-holding capacity of the bark for *Tilia* trees. Bark-pH was not found as discriminant parameter among the two tree species. On the contrary, many studies have demonstrated a strong epiphyte response to bark-pH, explaining epiphyte community variation compared among tree species in both boreal and temperate systems (Hyvärinen et al. 1992; Gauslaa 1995; Kuusinen 1996; Jüriado et al. 2009; Lewis and Ellis 2010; Leppik et al. 2011). Moreover, intraspecific studies, among boles of the same tree species, have indicated that bark-pH/nutrient status may be modified by an interaction with the soil environment (Gauslaa 1995; Gustafsson and Eriksson 1995; Kermit and

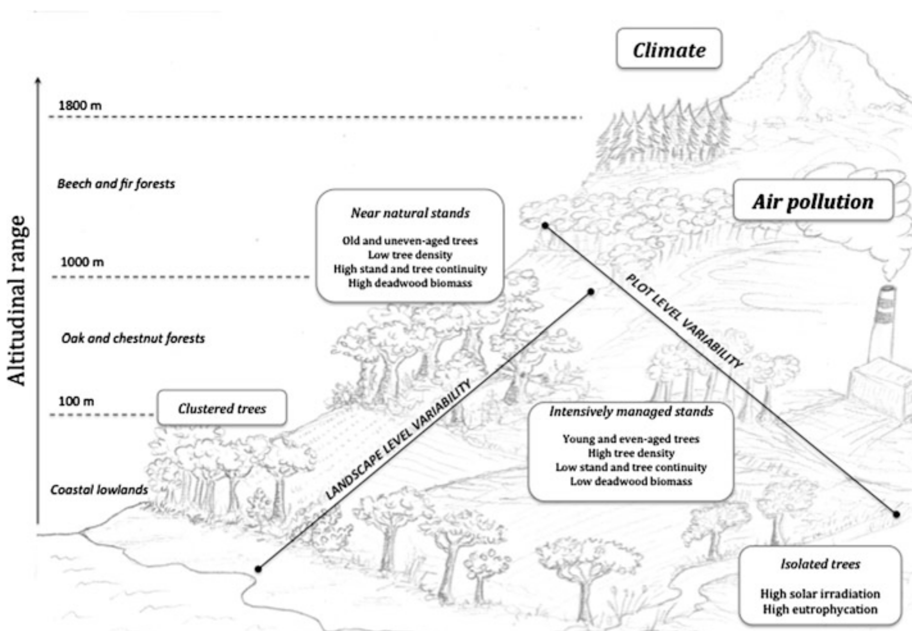



**Fig. 2.4** Conceptual model illustrating the main ecological factors potentially affecting lichen communities at plot and landscape levels (see explanation in the text)

Gauslaa 2001) and with a subsequent effect on epiphyte community composition. Interestingly, differences in tree age/size (dbh) correlate with bark-pH among individual boles of the same species, though the direction of this relationship cannot be generalized. Bark-pH may decrease with increasing tree circumference (Bates 1992; Kuusinen 1994) and/or age (Ellis and Coppins 2007), or pH may increase with circumference (Jüriado et al. 2009) and/or tree age (Fritz et al. 2009). Also, bark texture was found to be an important tree species factor influencing lichen diversity (Bates 1992; Cácares et al. 2007; Ranius et al. 2008; Fritz et al. 2009). This is subject to an effect of tree age/size (dbh) and an interaction with vertical height on the trunk (Johansson et al. 2007; Ranius et al. 2008; Fritz 2009), and it is related with a species-specific relationship between bark roughness and tree age/size (Uliczka and Angelstam 1999).

The standardization process of air quality biomonitoring method took into account all these sources of variability acting on lichen diversity at tree level, making it possible to isolate environmental gradients in a simplified system (Asta et al. 2002; EN 16413 2014; see paragraph interpreting lichen diversity). For this reason, the standard features of the sampling trees (slope, pH of the bark, etc.) have been selected carefully (Table 2.1).

With respect to plot-level variability, Giordani (2006) carried out a hierarchical evaluation of the effects of substrate- and environmental-related variables on lichen diversity. Elevation, mean annual temperature, and latitude were the main factors influencing epiphytic lichen distribution, together with disturbances (such as atmospheric pollution, forest fires and agricultural practices) and habitat heterogeneity that covary along the strongest gradient of lichen community composition. Lichen species richness was positively associated with mean annual rainfall and longitude, negatively associated with harvesting, and positively associated, though weakly, with the occurrence of past forest fires (these burnt sites were characterized by recolonization processes).

In a study of epiphytic lichens of forest ecosystems of Tuscany, Loppi et al. (1999) concluded that habitat characteristics are more important than phorophyte properties and there is evidence that the epiphytic lichen vegetation of deciduous *Quercus* trees follows a distribution which is related to elevation and climate, with great differences in community structure along the altitudinal gradient (Loppi et al. 1997).

As for the aspects affecting lichen distribution at landscape level, many authors pointed out the role of climatic factors (McCune et al. 1997; Goward and Spribille 2005; Hauck and Spribille 2005; Giordani 2006, 2007). In this respect, Giordani and Incerti (2008) found that the distribution of more than 30 % of epiphytic species was associated to macroclimatic variables. A significant subset of epiphytic lichens in the study area has been proved to be efficient bioclimatic indicator for montane, humid sub-Mediterranean, and Mediterranean units.

Further, also land-use intensity drives the local variation of lichen diversity, both in Mediterranean (Giordani et al. 2010) and in boreal ecosystems (Holt et al. 2008). Land-use intensity

**Table 2.1** List of features to of a standard tree (see EN 16413 2014; Asta et al. 2002)

| Feature | Description |
|---|---|
| Suitable tree species | The sampling tree belongs to one of the groups with similar bark physicochemical properties (EN 16413 2014; Asta et al. 2002). Indicatively, species belonging to the same group can be used interchangeably |
| Trunk circumference | The sampling tree has a trunk circumference (at 130 cm from the ground level) between 50 and 250 cm |
| Trunk inclination | Each exposition (N, E, S, W) has an inclination (at the center of each grid) <20° |
| Bark damage | The area of the trunk that is unsuitable for recording (damage, decortication, branching, knots and/or other epiphytes or climbing plants such as ivy, preventing growth of lichens) within each of the 4 grids when summed <20 % |

was detected as a good proxy for describing the lichen distribution and abundance under anthropogenic pressures. In particular, land-use categories, mainly based on vegetational features, differed in epiphytic and epilithic lichen communities, and the strongest differences were observed among the forested sites versus managed agroforestry lands. These latter were mainly characterized by common lichen vegetation (mainly xerophytic–nitrophytic species).

Similarly, in Scotland, lichen communities differed between natural ancient pine forests and those of trees within managed sites both in forest and agricultural areas (Wolseley et al. 2006). The alteration was mainly due to increasing nitrophytes, most evident for epiphytes, but also significant for saxicolous communities. According to Bergamini et al. (2005), the trend of modification of lichen communities under changing land use is nearly constant along large latitudinal gradient, ranging from northern Europe to the Mediterranean. Also, at this large scale, strong differences were detected between the forested and the more open land-use types, especially for epiphytic crustose lichens.

### 2.2.2 Standardizing Lichen Diversity Sampling

Differences between methods, difference in the application of the same method, measurement error, sampling and non-sampling error, and errors related to model applications are all terms of the whole error budget that inevitably affects environmental surveys (Gertner et al. 2002). In this perspective, the extent to which the objective of the survey is matched depends very much on the ability to manage the various sources of variability (Khol et al. 2000; Wagner 1995) by adopting suitable standard operating procedures (Brunialti et al. 2004, 2012b).

A recent example of standardization process relates to the sampling protocol to assess epyphitic lichen diversity for air quality. In fact, the European Committee for Standardization (CEN, Comité Européen de Normalization) has recently published an European standard, reporting the sampling protocol for lichen diversity assessment (EN 16413 2014). The process of standardization started in 2007 and took into account the previous European and national guidelines (Asta et al. 2002; VDI 2005; AFNOR 2008). In the meantime, some field tests have been performed to obtain information on the type and size of errors and the uncertainty of the methodologies under standardization (Brunialti et al. 2012a, Cristofolini et al. 2014). To fill this gap, the tests dealt with the entire process from survey design to field measurements. In particular, the comparative tests, consisting in multiple exercises, were run with the aim of comparing the results obtained by different, well-experienced operators faced with the same problem, at the same time, under the same field conditions and following the same standard operating procedures (SOPs).

A similar standardization sampling procedure has been adopted in forest monitoring. An example is represented by the sampling protocol of the EU project ‘Forest BIOdiversity Test phase Assessments’ (ForestBIOTA), carried out by 12 European countries in the framework of the ICP Forests Expert Panel on Biodiversity and Ground Vegetation Assessments (Fisher et al. 2009). In this context, Stofer et al. (2003, 2012) prepared a standardized sampling protocol for lichen diversity assessment that takes into account all the main steps of the sampling from the tree- to the stand level.

#### 2.2.2.1 Sampling Objectives

A clear definition of objective is important; otherwise, any statement about data quality remains elusive (Ferretti 2011). As a consequence, although it is not a typical source of error per se, ambiguous monitoring objectives can be great promoters of errors: On the one hand, the monitoring design cannot be properly addressed if the objective is not clear, and incorrect design may jeopardize the whole monitoring. For this reason, objective must be explicit with respect to target population and its attributes, spatial and temporal domains, desired precision level and minimum detectable change, and type I and II acceptable error rates.

With respect to the CEN standard for biomonitoring ambient air, the sampling objective is to obtain an estimate of the parameter of the response variable (e.g., mean species richness or mean lichen diversity value, LDV) over the study domain with a given precision (EN 16413 2014). The precision level should be expressed in terms of confidence intervals for a defined probability level. It is required that the sampling objective is defined for each study. For example, we may want to obtain an estimate of the mean LDV for the study domain with a confidence interval ±10 % of the mean value at a probability (*P*) level of 95 %.

Since the computation of estimates and confidence intervals depends on the sampling design adopted, each study shall define precision and probability levels, taking into account the requirements of the study framework and considering the available resources (EN 16413 2014).

#### 2.2.2.2 Target Population

In statistical terminology, the target population refers to a different concept than that associated with the community, population, individual, and genetic concept of biological systems. The target population is the collection of elements about which information is wanted (Cochran 1977). Both the 'target population' for which information is wanted and the 'elements' that make up the target population must be rigorously defined. As clearly stated, target population should be carefully selected before starting each monitoring program. It must be representative of the whole population in order to extend the results obtained by the selection of a sample of it to the entire population. In fact, we must consider that data obtained outside a formal sampling design cannot be considered representative for the entire population, and therefore, conclusions cannot be extended without making assumptions about it (Ferretti 2011). Similarly, models built upon non-representative data can be seriously biased. A classical example is the biodiversity data collected by taxonomists who are inclined to concentrate their efforts in the localities that guarantee success in the collection of as many species as possible, thus resulting in species-richness bias (Sastre and Lobo 2009).

For instance, a recent comparative test to identify critical issues in lichen biomonitoring demonstrated that different teams may select different target populations when planning the work (Brunialti et al. 2012a). Although all of them may be formally correct, these differences are a source of inconsistency in the results and could potentially lead to differing conclusions. It is therefore extremely important that every step of the design process is properly documented and reported in survey documents so that future repetitions may be made.

#### 2.2.2.3 Sampling Design and Sampling Schemes

For broader applications (e.g., at the European level), a standard protocol should be flexible enough to preserve the representativeness of the data under different ecological conditions. In general, it is difficult, if not impossible, to find a single sampling design that is suitable for each situation. Different options of sampling schemes may be adopted, mainly in relation to the complexity of the survey area and with the distribution of the trees to be sampled (Elzinga et al. 2001; EN 16413 2014; Ferretti and Erhardt 2002). With respect to air monitoring pollution, the recent CEN standard proposes the adoption of different schemes in relation to ecologically homogeneous and heterogeneous areas. In particular, in the first case, we may have tree options:

1. When standard trees are abundantly and homogeneously distributed over the study domain, a simple random or systematic design is recommended. Plot sampling is recommended, with sample plots allocated according to a regular grid, with the starting point of the grid chosen at random (e.g., Giordani et al. 2002).
2. When standard trees are abundantly scattered in clusters over the study domain, tree-based cluster sampling or two stage sampling is recommended. A criterion to identify clusters should be initially set, then identify, and list

all the clusters and obtain a random sample of them. It should be noted that a two-stage sampling requests a subsampling of the sampling unit, and it introduces a further source of variability which may affect the quality of the data. It is important to take this into account when performing statistical analysis of the data (EN 16413 2014).

3. When standard trees are infrequently scattered over the study domain, a simple tree-based random sampling is recommended. It is possible to obtain a list of the individual trees on the basis of the aerial photo and to select randomly the sample trees.

Similar options may be selected also in ecologically heterogeneous areas. In this case, however, a stratified random sampling design is recommended both with standard trees homogeneously distributed and scattered in clusters over the study domain. A tree-based stratified random design is suitable when we have standard trees infrequently scattered over the study domain.

Notwithstanding this great variability of solutions, it is not so obvious that each of these is properly taken into account when a monitoring program is designed, since the experts in the field may be more influenced by examples from the past (seen as best practices) that by newly suggested options. The results of a comparative test carried out of operators on the same area and with the same standard procedures by five independent groups partly confirm this statement. In this study, in fact, most teams adopted a stratified random sampling (Brunialti et al. 2012a). Although they largely agreed on selection of the sampling scheme, considerable differences occurred in subsequent steps of the sampling design: For example, the number of selected land cover categories ranged from 2 to 8 and the sampling density 23–43. The authors observed that training courses on sampling design and basic statistics may be successful in reducing this source of variability in routine field studies.

#### 2.2.2.4 Sampling Units

As reported above, both plot-based and tree-based sampling schemes can be adopted. According to the latter option, the sampling units are represented by the standard trees available in our survey area. Instead, when considering the plot-based sampling, the selection of the optimal shape and size of the plot remains an open question. In this case, the goal is to find an acceptable trade-off between a good representativeness of the study area and a cost-effective sampling effort.

Concerning the shape of sampling units, circular or square plots are used in most cases. In general, the former are suggested in air pollution monitoring programs (Giordani et al. 2002), while the latter are mainly adopted in forest surveys (Giordani 2006). The basis for a proper selection of the shape of sampling units relies both on probabilistic issues and on practical questions (Elzinga et al. 2001). For rectangular sampling units, the associated edge error is larger than for circular plots. Moreover, a circular plot requires only one measurement (radius) to be installed. In special cases, such as for the assessment of the effect of forest fragmentation, rectangular transects were taken into account, as they could fit better with the natural shape of forest fragments, which were further divided into subplots (Brunialti et al. 2012b).

As for the plot dimensions, McCune and Lesica (1992) found trade-offs between species capture and accuracy of cover estimates for three different within-site sample designs for inventory of macrolichen communities in forest plots. On average, whole-plot surveys captured a higher proportion of species than did multiple microplots, while giving less accurate cover estimates for species. The reverse was true for microplots, with lower species captures and much better cover estimates for common species. Belt transects fell between the over two sampling designs.

Ravera and Brunialti (2013) showed that a probabilistic sampling based on the selection of only three trees within small circular plots (14 m diameter) can be effective for assessing the occurrence of species of conservation concern in old growth forests in an Italian National Park. They found that most of the species were present in a few sampling sites and only a small group of species were common to more than 50 % of the plots. This may suggest that the adopted sampling design allowed not only to determine the most local common species but also to detect the presence of sporadic species (Ravera and Brunialti 2013). To confirm this, their lichen list reported several new and interesting species and represented 30 % of the indicators of forest continuity known from the region. This is a considerable result if we consider the nature of the adopted sampling and also the fact that regional floras mainly report records from preferential surveys. We must keep in mind, however, that floristic surveys, mainly carried out by means of a preferential sampling, are biased by the fact that they are primarily carried out to discover rare species in specific habitats or ecological niches. Humphrey et al. (2002) obtained similar results in a study carried out to compare lichen and bryophyte communities between planted and seminatural stands. A high percentage of species was recorded only once, and very few species were common to more than half the plots. This 'local rarity' phenomenon has been noted in other studies (Vitt et al. 1995; Collins and Glenn 1997; Qian et al. 1999; Humphrey et al. 2000) and is partly related to sampling area. The authors of that study observed that it is possible that a 1-ha sampling plot used is too small to capture a representative sample of lower plant diversity in forest stands. For example, Rose (1993) recommends a minimum sampling area of 1 $km^2$, but this depends on the objective of the survey. Hence, these findings suggest to adopt one of the following approaches for future investigations: (1) extend the surveys to plot with a broad area and, at the same time, detect a higher number of trees and/or substrates (rocks, soil, etc.). Although this approach could be very time consuming, it should ensure the finding of a large number of species; (2) improve the number of small plots in the study area. In this way, with a lower sampling effort at the individual plot, it is possible to obtain information on a wider territory. Moreover, this second approach should be mostly useful for preliminary lichen surveys in poorly studied wild areas (Ravera and Brunialti 2013).

#### 2.2.2.5 Sampling Density

Optimal sampling density is an essential requirement of lichen biomonitoring surveys for obtaining precise and unbiased estimates of population parameters and maps of known reliability (Ferretti et al. 2004). This aspect is often a sore point of biomonitoring projects and should be carefully addressed to respond correctly to the sampling objective and to select a sample with a proper sample size to avoid jeopardizing the effectiveness of the investigation. Apart from catching a sufficient amount of variability, a decision on a proper sample size forcely considers the sampling effort and its costs and it often happens to reduce the number of sampling sites in order to limit the costs.

Both the ecological complexity of a given area and the desired level of precision (in terms of confidence interval) drive the sampling design. In heterogeneous areas, a greater number of sampling units and more detailed land cover stratification are needed to obtain accurate estimates. For broader application (e.g., at the European level), a standard protocol should be flexible enough to preserve the representativeness of the data under different ecological conditions (Brunialti et al. 2012a). In this respect, Ferretti et al. (2004), starting from the results of two large-scale surveys undertaken in Italy, carried out a study on the effects of different sampling densities (the number of sampling units in the study area) and on the reliability (in terms of confidence intervals and relative error in the mean values) of the estimates of the lichen diversity values of a given area and of lichen diversity maps. An iterative approach was taken into account to generate subsets with lower sampling density with respect to the original sampling units (ordinary kriging interpolations). Obviously, a higher sampling density may lead to a low error rate but may be financially unsustainable. A very low sampling density, on the other hand, may provide uncertain data as to be of no real use (Khöl et al. 1994; Ferretti and Erhardt 2002). Their findings suggested that a large-scale lichen diversity spatial pattern can be detected with a much more relaxed grid density than those originally applied (Ferretti et al. 2004). This is extremely important, since reducing the sampling effort can result in considerable savings in resources that can be diverted to additional, more detailed investigation with denser sampling in those areas identified as problematic by the study based on the low sampling density. These results were also confirmed by a similar study (Frati and Brunialti 2006), within a long-term monitoring program, that showed the possibility to reduce the sampling effort in future monitoring surveys, resulting in a considerable reduction of the sampling effort maintaining a good data quality.

#### 2.2.2.6 Lichen Survey

As discussed in the previous sections, a series of micro- and macroenvironmental variables can affect the composition and diversity of epiphytic lichen communities. For this reason, a standardized sampling strategy to count lichen diversity is extremely important to ensure comparable and accurate results. The recently developed European standards for lichen biomonitoring (Stofer et al. 2012; EN 16413 2014) have been developed as the result of a standardization process carried out in the last few years starting from the Index of Atmospheric Purity (IAP) approach (Hawksworth and Rose 1970; Nimis et al. 1990, 1991) and upgrading it with previous guidelines (Asta et al. 2002; VDI 3957 2005). Furthermore, also field experience of several European researchers and the results of recent comparative tests (Brunialti et al. 2012a; Cristofolini et al. 2014) were useful to obtain the current sampling strategy for lichen survey. At the end of this process, to reduce the effect of several possible sources of error (e.g., different size of explored area on the trunk, subjectivity in the positioning of the sampling grid, etc.), several parameters have been standardized. Hence, the abundance of each lichen species is currently sampled by means of a sampling grid consisting of a 10 × 50 cm ladder divided into 5 10 × 10 cm quadrants. This ladder grid is placed systematically on the N, E, S, and W side of the bole of each tree (4 per tree), with the top edge 1.5 m above ground, following the standards suggested by Asta et al. (2002). Summary measurements of species richness and abundance are usually calculated for each plot: mean number of species per tree; total number of species within the plot; and mean lichen diversity value (LDV—Asta et al. 2002) calculated as the sum of the abundance of each species within the sampling grids on a tree, averaged for all trees within a plot.

### 2.2.3 Quality Assurance

Biomonitoring investigations are subjected to a variety of error sources that need to be acknowledged and documented in order to be managed properly (Brunialti et al. 2004). The quality of the data originating from biological measurements depends heavily on at least three factors (see, e.g., Kovacs 1992; Brunialti et al. 2004): (1) variability of the biomonitoring organisms (interactions between the organisms and environmental factors); (2) type of sampling (sampling design, density of sampling points); and (3) operators involved in assessing lichen diversity, which is a method requiring a relatively high taxonomic knowledge.

The first issue has already been discussed above (Sect. 2.2.1), and we have seen that this source of variability can be controlled with a good knowledge of the ecological characteristics of biological indicators considered and adopting proper sampling designs. Regarding the type of sampling, the sampling errors are closely associated with the sampling design adopted and its quality. As for the third factor, the effect of operators' subjectivity and expertise has been widely addressed within lichen biomonitoring so as to obtain useful information to further improve standardized protocols.

To respond to these issues and to take all the steps of the monitoring survey into account, the adoption of quality assurance procedures is strongly recommended. QA is an organized group of activities defining the way in which tasks are to be performed to ensure an expressed level of quality. The main benefit of a QA plan is the improved consistency, reliability, and cost-effectiveness of a program through time (Ferretti 1998). A QA plan is essential since it forces program managers to identify and evaluate most of the factors involved in the program. In addition, the assessment of data quality enables mathematical management of uncertainty due to the method used (Ferretti 1998; Cline and Burkman 1989).

It is therefore important that environmental biologists and field ecologists consider QA as a key attribute of their work in order to provide robust and defensible data to decision makers (Brunialti et al. 2004).

#### 2.2.3.1 Sampling Errors

Sampling errors are generated by the nature of the sampling itself and by the degree of variability in the target population. As reported above, such kinds of errors always occur but can be controlled by appropriate sampling design (Cochran 1977; Köhl et al. 2000; Ferretti and Erhardt 2002).

A good sampling design is essential either to collect data amenable to statistical analyses and to control errors in relation to the costs (Brunialti et al. 2004). An important issue, in this respect, is selectivity, which seems particularly important in ecological measurement. A protocol is selective if the response provided as a measurement depends only on the intended ecosystem property (Olsen et al. 1999). Regarding this aspect, Yoccoz et al. (2001) suggest that quantitative state variables characterizing the system well should be privileged. For example, when defining management objectives in terms of changes of densities of indicator species, the program should incorporate tests to ensure that selected species are indeed indicators of the process and variables of interest (Yoccoz et al. 2001). For this reason, it is important to establish a priori the variables of interest in a sampling protocol. The criteria for this selection should be based on data quality, applicability, data collection, repeatability, data analysis and interpretation, and cost-effectiveness. In particular, the use of quantitative state variables is recommended in order to reduce the error in data collecting due to the subjectivity of the operators.

#### 2.2.3.2 Non-sampling Errors

Non-sampling errors include measurement, classification, and observer errors, which are rooted in how the standard operating procedures (SOPs) are prepared and applied and how well-trained and skilled the field crews are (Ferretti 2009). In general, non-sampling errors can occur when the methodology is poorly standardized, when teams have insufficient skills or insufficient care is taken in applying the method, or when there are problems with instrument calibration (Ferretti and Erhardt 2002). Many papers have focused on these topics and have shown that non-sampling errors can be a significant source of variability in monitoring studies (Gertner and Köhl 1992; McCune et al. 1997; Giordani et al. 2009; Francini et al. 2009; Gottardini et al. 2009; Marchetto et al. 2009; Sastre and Lobo 2009).

In the specific case of epiphytic lichen assessment, non-sampling error may basically occur at two stages (Brunialti et al. 2012a): the identification of standard trees and the counting of lichen species. As for the former case, an imprecise definition of suitable trees in the SOPs might be one of the main reasons for differences in the number of suitable trees found by the different teams. As for the counting of lichen species, floristic knowledge is a crucial issue that needs to be addressed in lichen biomonitoring where the protocol is based on assessment of all lichen species, including groups of lichens which are hard to identify in the field, such as crustose lichens (Asta et al. 2002). Differences in the floristic skills of the teams can cause serious errors (Brunialti et al. 2002; Giordani et al. 2009). Brunialti et al. (2012a) confirmed that considerable underestimation of species richness may occur even when sampling within an a priori positioned grid. Floristic skill is even more important when assessing crustose-dominated communities, where poorly developed thalli often occur (Giordani et al. 2009). Undoubtedly, variability among crews could be reduced with intercalibration courses and harmonization procedures (McCune et al. 1997; Brunialti et al. 2002, 2004). In fact, there is evidence that operators often improve in accuracy during the same test and that their accuracy improves with taxonomic training and, above all, continuous fieldwork (Brunialti et al. 2002).

As far as precision is concerned, very high levels are usually registered among operators, suggesting a high reproducibility of the lichen diversity counts (Brunialti et al. 2002). This is extremely important for correct evaluation of time series in biomonitoring studies. However, it should be borne in mind that changes in operators in long-term monitoring of permanent plots can give misleading results (McCune et al. 1997) and should be carefully addressed.

## 2.3 Interpreting Lichen Diversity

As described in the previous paragraphs, a considerable effort has been made in the recent years for standardizing the sampling design and strategies of lichen biomonitoring. In the following paragraphs, we will describe some of the most used approaches for interpreting lichen diversity data in terms of effects of various anthropogenic disturbances. In general, the interpretation of geographic patterns and temporal trends of lichen diversity may be assisted by using ecological indicator values (Hawksworth and Rose 1970; Wirth 2010; Nimis and Martellos 2001, 2002), multivariate statistics, such as numerical analysis of matrices of species (Giordani et al. 2002; Giordani 2006), nonparametric models (McCune and Mefford 2004; Giordani 2007), or other statistical tools.

### 2.3.1 The Concepts of Biomonitoring

We can define monitoring as the process of gathering information about some system–state variables at different points in time for the purpose of assessing the state of the system and making inferences about changes in state over time (Yoccoz et al. 2001). If our focus is on the monitoring of biological diversity, the systems of interest to us are typically ecosystems or components of such systems (communities and populations), and the variables of interest include quantities such as species richness, species diversity, biomass, and population size.

#### 2.3.1.1 Lichen Diversity Value, α-Diversity

According to the recently developed standards for lichen biomonitoring (Stofer et al. 2012; EN 16413 2014), the basic results of lichen diversity sampling are aggregated matrices of the species frequencies at nested spatial levels of sampling, i.e., a matrix of species at aspects of each tree; at trees of each sampling units; and, finally, at sampling unit level. Several diversity indices are calculated a posteriori basing on these basic matrices. This recommendation comes to the fact that several interpretative tools may be applied to basic data, which are used in a various manner in different countries.

As a simple but effective approach, α-diversity (number of species at plot level) is a robust parameter for interpreting patterns of epiphytic lichen communities along gradient of anthropogenic effects, such as pollution or forest management.

Among the possible descriptors obtained from the basic species × sampling unit matrix, the lichen diversity value (LDV) by Asta et al. (2002) is by far one of the most used in applicative biomonitoring surveys. The value for a given sampling unit is calculated as the sum of the frequencies of all lichen species found on each tree within the unit and averaged by the number of sampled trees. Relevant differences in lichen growth may be expected on different cardinal aspects of the trunks; therefore, it is suggested that frequencies are also summed separately for each aspect, and possibly, additional analysis might be carried out in this sense. LDV in its basic definition has been extensively used in applicative studies all over Europe (Paoli et al. 2006; Svoboda et al. 2010; Giordani et al. 2014a), and its relationships with pollution and other environmental factors had been analyzed in details. In the Mediterranean, together with pollution, the mean annual rainfall of the survey area seems to be the best predictor for LDV variability (Giordani 2006). However, the decisive variables affecting the lichen diversity are apparently different in urban versus forested areas (Giordani 2007). In these latter, harvesting and forest fires showed a predominant effect. Contrarily, in urban areas, air pollutants, mainly $SO_2$, are the main limiting factors, even if this relationship is lowering under ameliorating conditions of atmospheric pollution. Similarly, Svoboda et al. (2010) found that lichen diversity in Central Europe responded differently to environmental predictors depending on different human impact. These authors observed that in industrial regions, air pollution was the strongest factor affecting lichen diversity, whereas in agricultural to highly forested regions, LDV was mainly influenced by forest age and forest fragmentation.

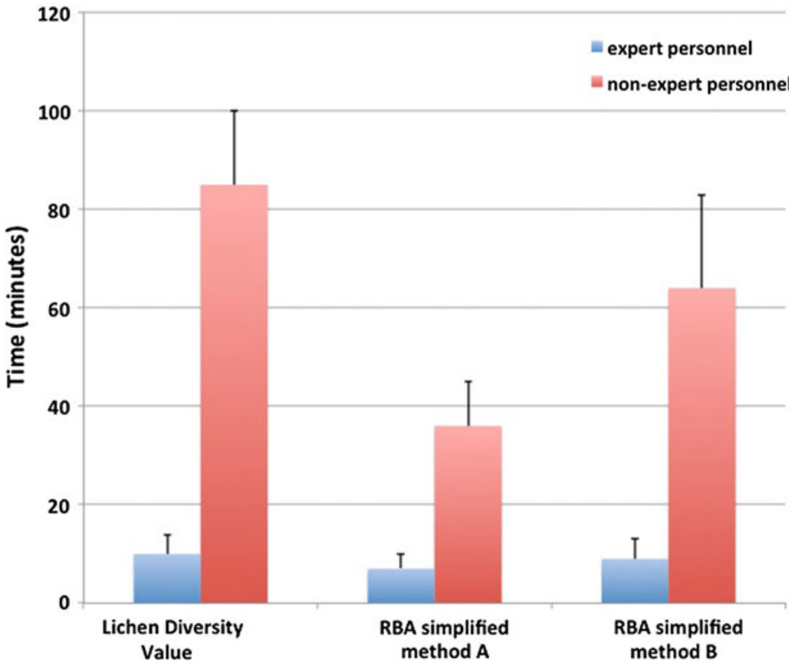
Starting from the basic species × sampling unit matrix, further parameters can be derived from the data set of the species frequencies, including the relative LDVs of morphofunctional groups of lichens associated to particular sources of atmospheric pollution (e.g., nitrophilus versus acidophilus species—see Sect. 3.2.3).

#### 2.3.1.2 Rapid Biodiversity Assessment (RBA) Based on Morphospecies

The application of biomonitoring methods based on high levels of taxonomic knowledge, such as the recently standardized lichen biomonitoring method (EN 16413 2014), requires an adequate number of specialists that are not always available, especially in large-scale biodiversity assessments (Wilkie et al. 2003). The use of guilds or morphological groups as indicators of changes in ecosystem function has been considered by several authors as a good compromise between the need for specialized knowledge and rapid field procedures employing non-specialist technicians, thus providing a possible shortcut in assessing total species richness (see, e.g., Pharo et al. 2000; Giordani et al. 2009). This issue has been considered in several ecological monitoring fields to explore the possibility of using surrogate species for estimate total biological diversity. As an example, several studies have been performed to assess the congruence among vascular plants, vertebrate, invertebrate, bryophytes, and lichens within large-scale biomonitoring surveys using simplified assessment methods (see Oliver et al. 1998; Pharo et al. 2000; Wilkie et al. 2003; Santi et al. 2010). However, some conflicting results obtained from the works listed above suggest that this is not always the ideal solution and several critical issues emerge: Among the others, there are sources of variation coming from the fact that the communities do not always behave in a linear and unambiguous manner. Also, the data quality in surveys involving non-specialist crews may vary at such a level which could drastically compromise the reliability of the results. In this respect, Giordani et al. (2009) carried out a study to compare data obtained by non-specialists through simplified methods based on morphospecies (RBA), with those collected by specialists using the lichen diversity value (LDV) method. They found that lichen diversity estimated by means of Rapid Biodiversity Assessments (i.e., based on morphospecies) showed good correlations with the results of a classical, systematic identification of species only when performed by operators with high taxonomic knowledge. Furthermore, the use of sampling lists based on highly simplified morphospecies did not lead to significant advantages in terms of time needed for fieldwork (Fig. 2.5).

In some other cases, Rapid Biodiversity Assessments of lichen biodiversity led to interesting results even at large scale. Recently, citizen science approaches have been applied to lichen biomonitoring of the effects of atmospheric pollution. These are voluntary schemes engaging members of the public in the collection of scientific information. The OPAL Air Survey in the UK (Davies et al. 2011) used presence and abundance data for 9 selected lichens, collected by more than 4,000 volunteers in a public survey of lichens on trees, to examine the response of individual species and groups of indicator lichens to air pollution and climate drivers on a national scale. The use of these macrolichen indicators has shown to have robust relationships with

**Fig. 2.5** Time (minutes) required for each lichen diversity assessment (tree level), using different sampling lists. Lichen diversity value (sampling of lichens at species level) was compared with two different simplification methods of rapid biodiversity assessment (*RBA*, simplified methods A and B). Data from Giordani et al. (2009)




modeled nitrogenous pollutants at the national scale (Seed et al. 2013). In the USA, Casanovas et al. (2014) proposed a citizen scientist-based survey methodology for macrolichen diversity in which parataxonomic units (PUs), as identified in lichen photographs, served as species surrogates to estimate lichen diversity. Although in most cases the authors showed that the observed and estimated cumulative richnesses from both techniques were not statistically significantly different from each other, the extensive use of these approaches in biomonitoring surveys should be carefully evaluated, as misidentifications of morphologically similar species could led to wrong interpretation of data, e.g., in terms of relative abundance of functional groups. At this regards, it has been shown that the use of electronic devices and identification tools may help to increase the quality of RBA. The project Dryades developed interactive identification keys in the form of applications for mobile devices. Keys were generated from databases of morphoanatomical characters. The applications were tested Europe wide during the project KeyToNature and have proved to be useful in education and in projects of citizen science (Nimis et al. 2012).

#### 2.3.1.3 β-Diversity

Most biomonitoring applicative surveys and researches using epiphytic lichens have focused on analyzing plot-level species richness (e.g., alfa-diversity), abundance, and/or composition patterns along ecological gradients. Recently, it has been suggested that also analyses of β-diversity may provide insights into mechanisms and drivers influencing lichen communities, thus contributing to a better interpretation of the results. Beta-diversity has been interpreted mostly as the extent of change in community composition (Whittaker 1960). Basically, beta-diversity patterns are originated from two distinct processes, the replacement and the loss of species (Carvalho et al. 2012). With the aim of differentiating the relative influences of these components on beta-diversity, various measures have been proposed, which relied on an additive rather than multiplicative approach (Baselga 2010). As a first application to lichen communities, Nascimbene et al. (2013a) used the conceptual scheme by Podani and Schmera (2011) to evaluate the relative importance of β-diversity, nestedness, and agreement in species richness in presence–absence data matrices via partitioning pairwise gamma diversity into additive components. Podani and Schmera (2011) considered three complementary indices that measure similarity (S), relative species replacement (R), and relative richness difference (D) for given pairs of observations. In particular, according to these authors, β-diversity is defined as the additive result of R and D, whereas other descriptors, namely nestedness and richness agreement, result from the additive effects of similarity with the other two complementary components (S + D and S + R). By analyzing the diversity in *L. pulmonaria* communities in Italian forests, Nascimbene et al. (2013a) showed that both species replacement and similarity were generally associated with forest structure predictors, such as the number of trees in the plot and the distance between trees, while richness difference was mainly associated with geographic predictors, with special reference to longitude and altitude. Giordani et al. (2014b) coupled the analysis of β-diversity with the approach based on the relative abundance of functional groups. These authors explored the shift in functional groups for nitrogen tolerance along a gradient of increasing cattle load in epilithic lichen communities of alpine pasturelands. An increasing cattle load caused a decreasing replacement of oligotrophic species and consequently a decrease in β-diversity. Conversely, when considering a data set with only N-tolerant species, there was very high pairwise similarity among sampling plots, irrespectively by the cattle load gradient to which they were exposed.

#### 2.3.1.4 Indicator Species

Detecting species that best characterize some set of sites is an important step in evaluating classifications in community ecology. With reference to lichen biomonitoring, this approach has been mostly applied for detecting the effects of forest management or to assess the ecological continuity of ancient woodlands. In some cases, lists of indicators species were compiled on the basis of expert assessments (e.g., Rose 1976). As for other biologically based approaches, also for biomonitoring, the use of proper methods for measuring the explanatory power of species is essential, whenever priority must be given to species that best reflect environmental quality (Dufrêne and Legendre 1997; Podani and Csányi 2010).

Whittet and Ellis (2013) tested 29 indicator species of forest continuity, as proposed by the current suite of British lichen indicators (Coppins and Coppins 2002) for different biogeographic regions in the UK. In accordance with previous studies (e.g., Sætersdal et al. 2005; Giordani and Incerti 2008), these authors confirmed that indicator species are likely to have a restricted geographic scope. Moreover, they suggested that only some of the studied taxa could be actually accurate indicators of ecological continuity through a dependency on the long-term persistence, whereas in other cases, several species may possibly be associated with specialist microhabitats under a sub-optimal climate, whereas they would not be significantly associated with ancient woodlands. Giordani (2012) compared the performance of four potential indicators for monitoring the effects of forest management on epiphytic lichens in broad-leaved Mediterranean forests. Indicators included total lichen diversity (LDV) and the abundances of species associated with intensive management, species associated with aged coppiced woodlands and indicator species ratio (ISR). ISR was defined as the ratio between the difference between the species associated to aged coppice forests and those associated to intensively managed forests and the total abundance. At each of 50 sampling sites, the four indicators were calculated using indicator value analysis (Dufrêne and Legendre 1997) and compared through correspondence analysis. By balancing the partial information provided by both sensitive and resistant species, ISR was shown to be a more effective indicator, being independent of floristic composition and the occurrence of rare species. The main drawback of the indicator species approach is that evaluation of the effects of a given stress (e.g., forest management) is possibly biased due to the fact that lichen species are strongly threatened by several anthropogenic disturbances occurring at the same time (e.g., high levels of air pollutants or forest fires).

### 2.3.2 Interpretative Tools

#### 2.3.2.1 Interpretative Scales of Alteration

Following the definition of Nimis (1999), biomonitoring techniques estimate the degree of alteration from normal conditions resulting from the effects of pollution on the reactive components (e.g., lichens) of the ecosystems. However, defining 'normal conditions' in ecology is extremely critical and can only rely on an operational basis. At this regard, some authors proposed an interpretation of lichen diversity data in terms of percentile deviations from an observed maximum diversity. Loppi et al. (2002a, b) sampled lichen diversity on about 3,000 trees in northern Tyrrhenian, Italy, and considered as 'natural' those values ≥ the 98° percentile of their frequency distribution. The average of these values was taken as an operational definition of 'naturality,' which represents a 'maximum potential lichen diversity' in a given area. These authors considered that a 25 % deviation from 'normal conditions' could still be regarded as 'natural,' owing to natural fluctuations of lichen diversity. Starting from this point, an interpretative scale of naturality/alteration was build basing the different degrees of naturality/alteration on progressive 25 % deviations from 'normal conditions.'

As lichen distribution strongly depends on macro- and mesoclimatic factors (see Ellis 2012), such interpretative scales of lichen diversity in terms of deviation from maximum potential conditions information should be referred to a regional level. Thus, direct comparisons between biomonitoring surveys carried out in different bioclimatic regions are often poorly informative and can also led to misleading interpretations. A similar approach of regionalization has been followed and debated in the case of other biomonitoring techniques (e.g., Moog et al. 2004). For example, the EU Water Framework Directive suggested qualitative reference for evaluating the ecological status of water bodies, assessing the highest potential quality, based on the composition of aquatic communities. It was possible to predict how the response of diatom communities to anthropogenic pressure in each French hydroecoregion was predicted (Tison et al. 2007), in relation to the topology of running waters and validated with benthic macroinvertebrates from reference sites (Wasson et al. 2002).

The delimitation of eco-regions or bioclimatic regions bases upon differences in the composition of their lichen flora. At this regards, Giordani and Incerti (2008) applied a nonparametric multiplicative regression model to the lichen flora of a climatically heterogenous area and detected 59 species which were significantly associated to macroclimatic variables (i.e., annual rainfall and temperature). A cluster analysis grouped the taxa into four subsets that were related to different climatic niches (warm–humid, cold–humid, mesothermic–humid, warm–dry) corresponding to distinct bioclimatic regions.

As an example of regionalization of interpretative scales, Castello and Skert (2005) provided evaluation scales of environmental alteration based on lichen diversity in the North Adriatic sub-Mediterranean bioclimatic region. These authors sampled deciduous oaks in 11 reference sites in open stands within natural woods or near very small isolated villages in rural or natural areas, far from large urban areas, industrial zones, and long-distance transport of air pollutants. As a result of relatively dry condition occurring in North Adriatic, the LDV threshold for naturality class was slightly lower than the one calculated for the more humid Thyrrenian Italy (Giordani 2004). Regionalized interpretations are also strongly recommended by the German guidelines (VDI 3957 2005), which states that a comparison of surveys of different regions is only possible if the surveyed areas have a similar climate and, therefore, a comparable lichen flora, as is the case with Central Europe (excepting the Alps) for which the evaluation scale was calibrated.

In some cases, ad hoc interpretative scales have been calculated for local situations. Due to the lack of an interpretative scale for semiarid Mediterranean bioclimatic region, Paoli et al. (2006) developed a calibrated scale for a small area (36 $km^2$) according to the protocol suggested by Loppi et al. (2002a, b) for the assessment of environmental deviation from natural conditions. Macroregional- and local-based interpretations of lichen diversity are not mutually exclusive and can provide integrate information on the actual environmental conditions.

Thought that nitrogen is becoming one of the most relevant limiting factors for lichen communities, especially in Central Europe, the German standard for lichen biomonitoring (VDI 3957 2005) proposed an interesting integrate approach for interpretation, which is basically based on the relative diversity of functional groups for nitrogen requirements. According to the German guidelines, nitrophytic species which respond positively and oligotrophic species which respond negatively to eutrophication (referred to as ‘reference species’) are calculated separately. The two partial LDVs of a sampling unit are combined to form the ‘air quality index’. The LDV of the reference species is entered along the ordinate axis, whereas the LDV of the indicators of eutrophication is entered along the abscissa. The quality class assigned to the field in which the crossing point of the lichen diversity values comes to lie gives the evaluation of the air quality of the sampling unit. According to the German approach, the thresholds of the air quality classes in the matrix are chosen so that the class width is approximately equal to three times of standard deviation of the LDV in the study area. In this way, the lichen cover of sampling units belonging to different air quality classes is significantly different if the respective classes are separated by at least one other class.

The use of interpretative scales of lichen diversity based on deviation from maximum potential diversity also presents some weak points. From a theoretical point of view, it is well known that natural situations are not necessarily associated with maximum values of biodiversity. According to influential ecological theories, such as the intermediate disturbance hypothesis (Connell 1978), intermediate levels of disturbance, in terms of frequency and/or intensity of the phenomena, will maximize species diversity. The question has been shown to be more complex and less generalizable than this, with recent studies demonstrating that diversity could show monotonic, unimodal, or even flat response to disturbance, depending on the studied organisms and on the disturbance aspects considered (Hall et al. 2012). In an applicative perspective, the lacking of undisturbed reference situations in some eco- or bioclimatic regions (e.g., the Po Plain in Italy or many anthropized areas of Central Europe and North America) makes difficult to define properly the values associated to the highest classes of the scale, thus affecting the entire interpretation process.

#### 2.3.2.2 Mapping Lichen Diversity

Mapping lichen diversity is an attractive approach, which allows an immediate representation of the results. Showing spatial distribution patterns of the studied descriptors, maps of lichen biodiversity, or abundance enable a quick and clear identification of areas with different levels of disturbance (Pinho et al. 2004; Asta et al. 2002). Spatial mapping of lichen diversity or associated measurements had been extensively used both in research and applicative lichen biomonitoring works (Giordani et al. 2002; Pinho et al. 2004, 2008a; Geiser et al. 2010). A large suite of GIS softwares provide tools for interpolating (i.e., estimating) the values of the response variable (e.g., lichen diversity) in non-measured parts of the survey area, basing on the data collected at sampling sites. In this chapter, we do not review the technical aspects of this approach. Basically, it follows the principle of geostatistical modeling theory originally developed for applied geology and more recently applied to ecology (see, e.g., Perry et al. 2002 for a detailed description). However, when applying these techniques, lichenologists should be aware that incorrect settings of the geostatistical model could lead to misleading results and interpretation and that the error associated to a poor spatial model might be also larger than those imputable to other sources of errors, such as sampling design or taxonomic misidentification.

Estimated values in lichen biodiversity maps are related to sampling densities (the number of sampling units in the study area), which in turn affect the reliability of the collected data in terms of confidence intervals and relative error in the mean values (Geiser and Neitlich 2007). By analyzing two large surveys of lichen mapping carried out in Italy (Nimis et al. 1991; Giordani et al. 2002), Ferretti et al. (2004) showed that even a considerable reduction (up to 50 %) of the original sampling effort led to a much smaller increase in mapping errors (<18 %). These data suggested that reducing the sampling effort ensures a considerable saving in resources that can be diverted to additional and/or more detailed investigation with denser sampling in those areas identified as problematic by the study based on the low sampling density.

Despite the fact that the interactions of pollution with other confounding factors in determining the distribution of lichens has been largely ascertained (Nimis et al. 2002), surprisingly, this source of variation was not always explicitly considered when building a spatial model for lichen biomonitoring data. A good example of integrate interpretation of lichen data, accounting the variability of both pollution and climate, was provided by Geiser and Neitlich (2007). Analyzing the effects of air quality and climate on epiphytic lichens in the US Pacific Northwest, they produced a kriged map of air scores, which were derived by the coordinates on the main axis of an NMS ordination of sampling plots located along gradients of pollution and climate. The estimated values of the air scores in non-sampled cells of the study area were calculated using the Gaussian semi-variogram model and a variable search radius including 20 sampled points. The spatial scale of analysis of a geostatistical model is often determined a priori. Pinho et al. (2008a) warned that, using this approach, some relationships between indicator and environment may be overlooked. Ribeiro et al. (2013) faced this problem investigating the relationships between ecological indicators and underlying environmental factors in Portugal. They used a multivariate geostatistical method, a linear model of coregionalization, to analyze the joint distribution of biodiversity variables in their study area. They were able to assess the strength of the relationship between each environmental factor and ecological indicator at several spatial scales. Basically, they related information on land cover and climatic variables with the abundance of fruticose lichen species, which were expected to be very sensitive to multiple environmental drivers. Their analysis implied the calculation of a nested variogram function to quantify the intensity and direction of correlations between the abundance of fruticose lichens and environmental factors at relevant spatial scales. These authors found that at medium scales (c. 15 km), open-space areas (considered as a proxy variable for particle emissions) were more important for shaping the abundance of this lichen group, whereas at larger scales (c. 45 km), open artificial areas (as a proxy for gaseous pollutants) and climate were preponderant.

#### 2.3.2.3 The Use of Lichen Functional Groups to Detect Critical Loads and Critical Levels of Pollutants

'Functional groups' is the term used to describe sets of species exerting a comparable effect upon a particular process or responding in a similar manner to changes in their external constraints (Lévêque and Mounolou 2003). In particular, species' functional groups have been proved to be a valuable tool for comparing lichen diversity across diverse regions where high levels of floristic variation may occur (Giordani et al. 2012). Interestingly, recent revisions of the critical loads (CLOs) and critical levels (CLEs) for a large number of ecosystems are based on the response of lichens to main pollutants (e.g., Cape et al. 2009; Fenn et al. 2008). CLOs and CLEs have been defined to set sustainable thresholds for the protection of ecosystems from the effects of pollutants. The CLO is defined as "a quantitative estimate of deposition of one or more pollutants below which significant harmful effects on specified elements of the environment do not occur according to present knowledge" (Posthumus 1988), whereas the CLEs refer to the concentration of pollutants in the atmosphere above which adverse effects occur (Cape et al. 2009). Pinho et al. (2014) recently proposed a new tool to calculate CLEs by stratifying ammonia concentrations into classes and focusing on the highest diversity values. Based on the significant correlations between ammonia and biodiversity, the CLE of ammonia for Mediterranean evergreen woodlands was found to be 0.69 μg $m^{-3}$, below the currently accepted pan-European CLE of 1.0 μg $m^{-3}$.

Concerning the detection of CLOs, the occurrence of oligotrophic lichen species provided information on the actual impact of reduced nitrogen compounds (mainly ammonia) in the forest plots of the European network ICP Forests (Giordani et al. 2014a). The critical load causing a significant change of the expected composition of epiphytic lichen vegetation occurred at nitrogen deposition = 2.4 kg N $ha^{-1}$ $year^{-1}$. Lichen functional groups for eutrophication and/or, more specifically, nitrogen tolerance have been extensively used to assess the critical level and critical load of nitrogen compounds in several forest ecosystems all over the world (Fenn et al. 2008; Geiser et al. 2010; Pinho et al. 2008b, 2011). Interestingly, the results for European plots were in accordance with those of other areas of the world. For example, in conifer forests of the Pacific North West of USA, Geiser et al. (2010) found, for wet deposition, a critical load ranging from 0.7 to 4.4 kg $ha^{-1}$ $year^{-1}$, depending on the amount of precipitation. The concept of critical load for lichen communities has been also applied to other sources of disturbance. Giordani et al. (2014b) established the cattle critical load in alpine pasturelands, in terms of Adult Cattle Units (ACU) per hectare per year. These authors showed that the relative frequency of oligotrophic epilithic lichen species significantly decreased as ACU increased. The cattle critical load was set for ACU = 0.12 ACU $ha^{-1}$ $year^{-1}$.

## 2.4 Open Questions

Despite the relevant number of researches in the field of lichen biomonitoring carried out in the last 25 years, there are still open points which have not yet been sufficiently addressed. In some cases, these questions are far from being minor and responses are urged in order to make the interpretation of lichen biomonitoring more robust. Among the others, the insufficient knowledge on the timescale of the response of epiphytic lichen communities to disturbances and the lacking of an adequate integration about lichen biomonitoring and other standard biomonitoring techniques particularly call for more attention by researchers and stakeholders. According to its technical definition, monitoring is the collection and analysis of repeated observations or measurements to evaluate changes in condition and progress toward a management objective (Elzinga et al. 2001). Thus, also for lichen biomonitoring studies, an accurate knowledge on the temporal variation of the observed phenomenon is crucial for getting reliable results. However, despite the huge literature on physiological effects of disturbances on lichen thalli, the time span between the disturbance (e.g., pollution) and the alteration on epiphytic lichen communities in terms of species loss, recover, or changes in species composition has not been fully explored. Such effects result from complex interactions between temporal trends of limiting factors (e.g., phytotoxic gases) and natural dynamics of the communities, which include re-colonization processes driven by dispersal, substrate availability, establishment of propagules, and intra- and interspecific competition (Werth et al. 2006).

Data from long observation periods showed clear trends in lichen diversity (Lisowska 2011). The diversity of epiphytic species in London has continued to increase from the 1970s to 2004 as a response to decreasing $NO_x$ atmospheric concentrations (Hawksworth and Rose 1970; Davies 2007). In the urban area of Turin (N-Italy), contrasting trends of the numbers of both lichen species presence and abundance were observed over a period of 200 years as a result of changing pollution scenarios (Isocrono et al. 2007). A dramatic species loss was detected in seminatural broadleaved forests in northwestern Germany from the nineteenth century to date (Hauck et al. 2013). Up to 70 % of the species became rarer during the 100- to 150-year long observation period, and an extinction rate of 28 % was estimated.

Against these robust evidences of long-term variations, information on short-term trends of lichen diversity and composition are surprisingly scarce and urge more detailed researches. Loppi et al. (2004) carried out 5 repeated surveys of lichen from 1993 to 2000 with time spans ranging from 1 to 3 years. They showed that despite their slow growth rate, lichens respond rapidly to decreasing concentrations of air pollutants, allowing annual changes to be detected. Total species richness increased from 1993 to 1999 and then decreased again in 2000, while the mean number of species per station increased from 1993 to 1999 and remained constant in 2000. The β-diversity decreased linearly from 1993 to 1999, indicating that sampling stations became floristically more similar in time.

From an applicative perspective, when the aim is the evaluation of change between subsequent measurements, there are several implications related to the statistical analysis for detecting changes, which should carefully considered (EN 16413 2014). Among them, one should decide whether to make the sampling units temporary or permanent. When sampling units are temporary, the random sampling procedure is carried out independently at each sampling period (Elzinga et al. 2001). The principal advantage of using permanent instead of temporary sampling units is that the statistical tests for detecting change from one time period to the next in permanent sampling units are much more powerful than the tests used on temporary sampling units.

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