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Performance evaluation and applicability of *Lichens GO*, a citizen science-based protocol for urban air quality monitoring

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ABSTRACT

The *Lichens GO* program is a French citizen science initiative based on the European guidelines that aims to evaluate the lichen diversity for urban air quality monitoring. In this study, we assessed the performance and applicability of the current *Lichens GO* protocol to then propose adaptations to make it more reliable and feasible for citizen science. To achieve this goal, we considered four aspects of the citizen science program: potential protocol simplifications, sampling site availability, observer bias, and volunteer feedbacks. Simulated simplification scenarios from a reference data set highlighted the large influence of reducing the number of sampled trees on taxonomic and functional structure metrics compared to reducing the number of sampled tree exposure sides and considered lichen species list. When considering the *Lichens GO* protocol (i.e., three trees, four exposure sides, *Lichens GO* species list) compared to the reference data set (i.e., five trees, four exposure sides, exhaustive species list), we evaluated an underestimation of lichen species richness (−25%), acidophilous species proportion (−94%), and functional diversity (−21%). In parallel, the maximum distance between sampled trees did not influence the taxonomic and functional structure metrics when considering a homogeneous sampling area (i.e., similar light or shade conditions). Finally, we compared *Lichens GO* relevés from 25 volunteers in the same site to highlight the major identification difficulties that could compromise the ecological interpretation. To improve the quality of data collected by citizens without increasing the sampling effort, we suggest to: (1) increase the maximum distance between trees from 10 to 50 m to extend the sampling site availability; (2) adapt the *Lichens GO* identification key to limit species confusion; and (3) assign an ecological trait to some lichen species groupings to improve the ecological interpretation. The proposed adaptations were tested and showed an improvement in the acidophilous species proportion (from −94 to −13%) and functional diversity (from −21 to −4%).

1. Introduction

Air pollution is a major environmental concern because it may cause up to seven million deaths worldwide every year (WHO, 2021) and has a long-term impact on biodiversity and ecosystem functioning (IPBES, 2019). Assessing air quality is required for identifying and reducing the sources of pollutant emissions and, therefore, limiting the effects on human and ecosystem health. Monitoring networks using sensors have been established for long-term measurements of chemical and physical parameters and for publishing maps of the average air quality state (EEA, 2020). Such robust measurements, however, suffer from a significant cost, require the prior installation of sensors, and do not evaluate the direct impact on human and ecosystem health, which in turn results in a low spatial cover that is unsuitable for local sources detection (Seed et al., 2013). Biomonitoring, which involves sensitive organisms to assess the environmental quality, appears as a complementary approach to collect more data by directly studying biodiversity health in response to various environmental disturbances (Abas, 2021; AL-Alam

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et al., 2019; Giordano et al., 2021; Markert et al., 2003).

In the 19th century, Nylander (1866) observed a relationship between lichen diversity and air pollution in Paris. Indeed, because of their biological features, lichens are sensitive to atmospheric deposition, which promotes their use as environmental biomonitors (Conti and Cecchetti, 2001; Nimis and Purvis, 2002). Moreover, each lichen species is specifically resistant/sensitive to various air pollutants, making it possible to establish bioindication scales (i.e., based on the presence of sensitive species) for each pollutant, such as sulfur dioxide (Hawksworth and Rose, 1970), nitrogen dioxide (Davies et al., 2007), ammonia (Wolseley et al., 2009), ozone (Gombert, 1999), and trace metals (Agnan et al., 2017). Therefore, identifying the abundance of both resistant and sensitive species is a key step in bioindication studies (Llop et al., 2012). In Europe, the standardized guidelines EN 16413 attempt to assess the epiphytic lichen diversity and abundance using a grid method in the four cardinal directions (N, E, S, W) to quantify the frequency of each lichen species (CSN, 2014). This method allows calculating the lichen diversity value (LDV) as an overall index for air quality assessment. Simplified protocols have been proposed to increase data acquisition by non-expert observers in view of a better spatial resolution (Giordani et al., 2009).

Several studies have shown the interest of citizen science to improve both spatial and temporal resolutions of ecological data (Dickinson et al., 2012, 2010; Kullenberg and Kasperowski, 2016). The use of non-expert collected data, however, requires an adapted data processing to reduce the noisy signal (Isaac et al., 2014; Will-Wolf et al., 2002). Some citizen science programs have already focused on lichens to evaluate both lichen diversity and environmental quality (Casanovas et al., 2014; Gilbert, 1974; Maréchal et al., 2019). For instance, the OPAL Air Survey is a citizen science protocol to assess nitrogen pollution in the United Kingdom based on nine macrolichen species (Tregidgo et al., 2013). It has been carried out between 2008 and 2015 and results in a relationship between lichen distribution and nitrogen deposition (Seed et al., 2013; Welden et al., 2018). These promising findings confirm the interest of developing citizen science programs using lichens for air quality assessment in urban areas. In addition, citizens show a growing interest in these organisms (Munzi and Giovanetti, 2021) despite some potential identification difficulties (McMullin and Allen, 2022).

The *Lichens GO* program was developed in 2017 by Sorbonne University and the Muséum national d'Histoire naturelle (Paris, France) to evaluate the overall air quality in urban environments (Abensour et al., 2020). *Lichens GO* is a simplified version of the European protocol, including only 41 common lichen morphospecies sampled on three trees in urban areas. However, to use this citizen-based protocol for environmental purposes, the data must first undergo a relevance evaluation. In this context, our main objective was to assess the performance and applicability of the current *Lichens GO* protocol to then propose adaptations to make the protocol more reliable and feasible in a citizen science context. We thus specifically considered: (1) simplifications of the protocol (i.e., reduction of the sampling effort) to facilitate its use by simulating scenarios from a reference data set collected following the European guidelines; (2) improvements in the number of potential urban sampling sites by increasing the maximum distance between trees; (3) the observation bias to adapt the lichen identification key by comparing identification performance from participants with different taxonomic skills; and (4) the applicability of the current protocol and the perception of any possible modifications by performing a citizen feedback.

2. Materials and methods

2.1. Lichens GO protocol

Lichens GO (www.lichensgo.eu) is a citizen-science initiative for biodiversity and air quality assessment in urban areas based on the European guidelines EN 16413 (CSN, 2014). The simplified protocol specifies sampling three trees (of the same species as far as possible) selected by the volunteer. The three trees should: (1) not be coniferous or peeling bark species (e.g., birch or plane tree); (2) be spaced no >10 m apart; (3) have a single straight trunk with a minimum circumference of 50 cm; and (4) have limited presence of other epiphytes (e.g. moss or ivy). Station information (spatial coordinates and weather conditions), tree species (to consider any potential influence of the bark), and lichens species observed in the station are reported by the participant on the *Lichens GO* field sheet. The volunteers place a grid composed of five vertical quadrats of 10 cm × 10 cm on each tree trunk at 1 m above ground and in the four cardinal directions (N, E, S, W). The frequency of each lichen species (between 0 and 1) is calculated by counting the number of quadrats where the species is present divided by the total number of sampled quadrats. For species identification, the participants use a dedicated and simple identification key that requires only macroscopic field observation (using a ×10 magnifying glass). This *Lichens GO* identification key only considers 41 lichen morphospecies frequently encountered in urban areas, including 31 species or groups of species (e.g., *Amandinea punctata/Lecidella elaeochroma*), three genera, and seven broader categories (such as "other crustose lichen", allowing each encountered species to be considered).

2.2. Protocol simplifications

2.2.1. Sampling sites and sampling protocol

To assess the performance of the *Lichens GO* protocol and potential simplifications to reduce the sampling effort, we simulated different scenarios by drawing observations from a reference data set collected following the European guidelines (EN 16413; CSN, 2014). Each draw involved decreasing the number of sampled trees and/or the number of sampled tree exposure sides and/or the size of the sampled species list (considering only lichen morphospecies of the *Lichens GO* identification key). This reference data set was sampled between April 2009 and April 2012 by a lichenologist in 114 urban and *peri*-urban sampling sites (i.e., without forested areas within 50 m) along the Rhône valley (south of Lyon, France). Within each site, five trees were sampled using the grid method (Asta et al., 2002). All lichen species occurring inside the grid were identified.

2.2.2. Simulations

The protocol performance was assessed using the 114 sampling sites from the Rhône valley data set. We considered 120 different scenarios (4 × 15 × 2) consisting of all possible combinations of the following factors: number of sampled trees per site (from 4 to 1, including four modalities), number of sampled exposure sides (from 4 to 1, including the fifteen possible combinations), as well as two possible lists of sampled species (exhaustive species list or *Lichens GO* species list). For each scenario, the average values of different ecological metrics (see section 2.6) were computed across all sampling sites (e.g., average lichen species richness per site). For representativeness purposes, we randomly simulated 100 simplified data sets for each scenario and summarized them by computing the median and 95% confidence interval (i.e., the 0.025 and 0.975 quantiles).

2.3. Distance between trees

The effect of distance between trees on the *Lichens GO* relevé was tested in Louvain-la-Neuve, Belgium (50.6659°N; 4.5863°E), along a 12 m wide road running northwest-southeast. The road was bordered by wood on the northern part, while the southern part consisted of open grassland. The *Lichens GO* protocol was applied on 16 trees (*Tilia* sp.) on either side of the road and approximately 15 m from each other, including nine trees on the southwest side and seven trees on the northeast side with similar age and circumferences to limit any additional influence (Lie et al., 2009; Llewellyn et al., 2020; Nascimbene et al., 2009). We generated all 3-tree combinations on each roadside (southwest and northeast sides, respectively) and grouped these

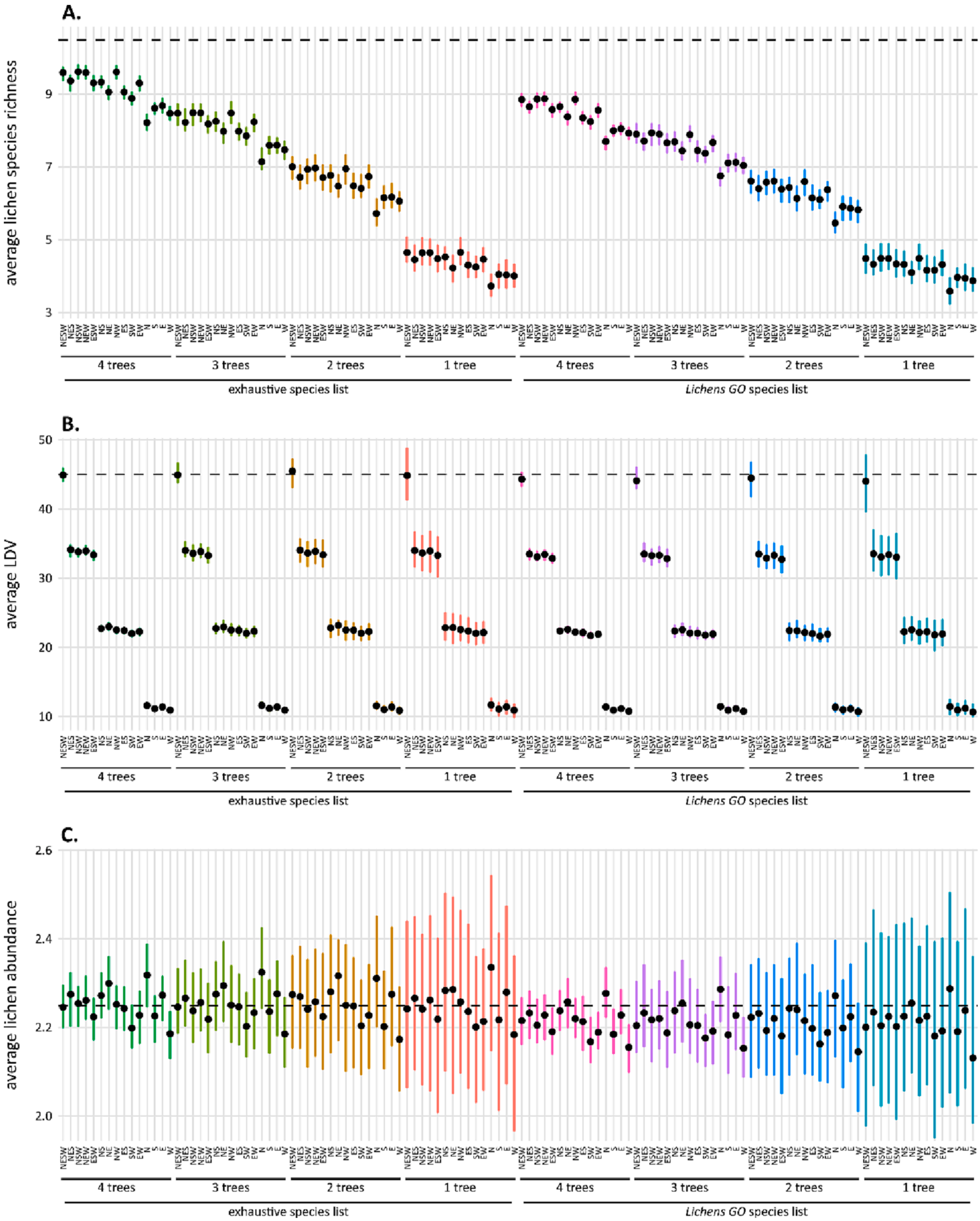

Fig. 1. Influence of scenarios (number of sampled trees, number of sampled tree exposure sides, considered lichen species list) on lichen species richness (A), LDV (B), and lichen abundance (C). Letters indicate the sampled tree exposure sides (N: north; E: east; S: south; W: west). Results indicate median values (black dots) and 95% confidence interval (colored bars) of the averaged metrics after 100 simulations. Average values from the reference data set (i.e., 5 trees, 4 exposure sides, exhaustive species list) are presented by the dashed line.

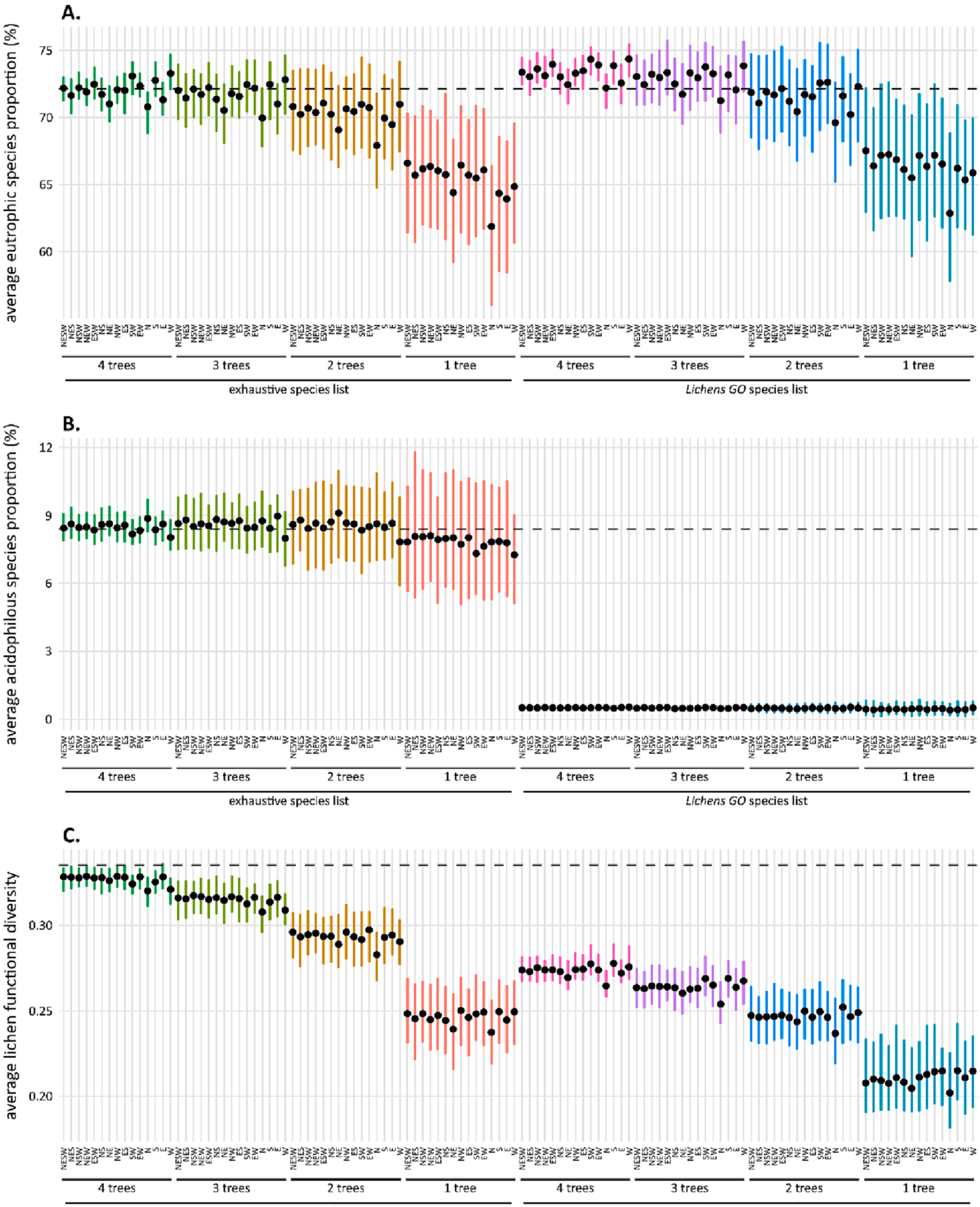

Fig. 2. Influence of scenarios (number of sampled trees, number of sampled tree exposure sides, considered lichen species list) on proportions of eutrophic (A) and acidophilous (B) species and lichen functional diversity (Rao coefficient; C). Letters indicate the sampled tree exposure sides (N: north; E: east; S: south; W: west). Results indicate median values (black dots) and 95% confidence interval (colored bars) of the averaged metrics after 100 simulations. Average values from the reference data set (i.e., 5 trees, 4 exposure sides, exhaustive species list) are presented by the dashed line.

combinations following maximum distance constraints: <20 m (only adjacent trees; n = 7 and 4 combinations, respectively), <35 m (including one tree out of two; n = 24 and 14, respectively), <50 m (including one tree out of three; n = 45 and 24, respectively), and <200 m (all possible combinations; n = 84 and 35, respectively).

2.4. Observation bias

The observation bias was tested in the UCLouvain campus (Louvain-la-Neuve, Belgium; 50.6656°N; 4.6209°E). Twenty-four participants applied the *Lichens GO* protocol on the same three trees (two *Tilia* sp. and one *Sorbus aucuparia*) independently and without cooperation. According to their ecological knowledge, we distinguished 19 adult neophytes (with no *a priori* lichen knowledge) and two experienced observers (with good lichen identification skills), as well as three classes of high school students aged 14 to 18 (with no *a priori* lichen knowledge) who completed one relevé by groups of 12 (i.e., four students by tree). All of these participants constitute the different target audiences of the *Lichens GO* program. Each of them received a 1.5 h-training (training proposed for new participants in the *Lichens GO* program in addition to having access to online documents; www.lichensgo.eu), including general information about lichen biology and ecology, lichen identification, protocol procedure, and an identification exercise of six common species in urban areas (*Punctelia subrudecta*, *Evernia prunastri*, *Ramalina farinacea*, *Physcia tenella*, *Flavoparmelia caperata*, and *Physconia grisea*) to practice the identification key.

2.5. Citizen feedback

Finally, we conducted an online survey among current *Lichens GO* participants to get their feedback on the protocol applicability and potential simplifications. We distributed the questionnaire via the *Lichens GO* mailing-list and the Tela Botanica (French-speaking network of amateur botanists) newsletter. The selected questions concerned the motivation to participate and carry on, as well as feelings and perceptions about the protocol implementation (cf. Supplementary Material SM1). Briefly, specific aspects of the protocol have been addressed, such as the time required to set up the protocol or the use of the identification key and other material items.

2.6. Data processing and statistical analyses

To assess the performance of the *Lichens GO* protocol and potential simplifications, we considered several ecological metrics: (1) taxonomic metrics (lichen species richness, LDV, and lichen abundance); (2) functional structure metrics (eutrophic and acidophilous species proportions); and (3) functional diversity (Rao's quadratic entropy). The LDV was calculated for each tree and averaged to obtain a single LDV by sampling site (Asta et al., 2002). For a given site, the lichen abundance represents the sum of lichen species frequencies. Unlike the LDV, this metric is not affected by the number of quadrats (i.e., normalized frequencies) that allows comparing scenarios with different numbers of tree exposure sides. Ecological trait data were extracted from the ITALIC 7.0 online database (Nimis and Martellos, 2022), including substrate pH, solar irradiation, aridity, eutrophication, poleotolerance (i.e., tolerance to pollution), and altitudinal distribution. Maximum scores of each ecological trait were considered (Llop et al., 2012). For functional structure metrics, we only considered eutrophic (if eutrophication score ≥ 4) and acidophilous (if substrate pH score ≤ 2) species proportion to characterize the potential impact of atmospheric pollution (eutrophying and acidifying pollutants, respectively) by computing the ratio between the abundance of those ecological groups and the total lichen abundance. The Rao's quadratic entropy (Botta-Dukát, 2005; Rao, 1982), which represents the co-occurrence of ecologically contrasted species in a sampling site, was calculated using the frequency of each lichen species and the maximum values of the six ITALIC's ecological traits (standardized between 0 and 1).

To estimate the participant performance, results were compared with those of an expert (considered as the reference), who sampled the same trees, by calculating a similarity index as follows (eq. (1):

$$\text{similarity index} = \frac{\sum_{t=1}^{3}\sum_{e=1}^{4}\sum_{s=1}^{41}\min(P_{tes}, E_{tes})}{\sum_{t=1}^{3}\sum_{e=1}^{4}\sum_{s=1}^{41}\max(P_{tes}, E_{tes})} \quad (1)$$

with: *t*, the tree (from 1 to 3); *e*, the tree exposure side (from 1 to 4); *s*, the species (from 1 to 41, following the 41 *Lichens GO* species); P_{tes}, the frequency of the species *s* on the exposure side *e* of the tree *t* observed by the participant; and E_{tes}, the frequency of the species *s* on the exposure side *e* of the tree *t* observed by the expert. A similarity index of 1 indicates that the relevé is identical to that performed by the expert. Conversely, a value of 0 indicates that the relevé is entirely different from that performed by the expert (i.e., no common species on the same tree exposure side). Note that this similarity index considers the identification correctness, the reported frequency of each species, and the grid position on the tree trunk.

Statistical analyses were performed using the R 4.2.1 software for statistical computing. The Rao coefficient was computed using the SYNCSA package (1.3.4) and statistically significant differences between modalities of the distance between trees were tested using the non-parametric Kruskal-Wallis test and Mann-Whitney *U* test ($\alpha = 0.05$).

3. Results and discussions

3.1. Potential protocol simplifications

The protocol performance was assessed by comparing different scenarios following a decrease in number of sampled trees, sampled tree exposure sides, and considered lichen species. In this section, we evaluate the response of these scenarios on average values of taxonomic metrics (lichen species richness, LDV, and lichen abundance; Fig. 1), functional structure metrics (proportions of eutrophic and acidophilous species; Fig. 2A-B), and functional diversity (Rao coefficient; Fig. 2C).

3.1.1. Number of sampled trees

The first parameter we assessed was the number of sampled trees (from 5 in the reference data set to 1 in the simplest simulated data set). All investigated indicators showed decreasing values along with the decrease in number of sampled trees, except for LDV and lichen abundance (Fig. 1B-C). The average lichen species richness decreased from 10.5 (median value, with 5 trees) to 9.6 with 4 trees (i.e., −9%), 8.5 with 3 trees (−19%), 6.9 with 2 trees (−34%), and 4.6 (−56%) when only one tree was sampled (Fig. 1A). This is related to the representativeness of sampled trees: the higher the number of sampled trees, the higher the number of recorded lichen species (Giordani et al., 2011). The major gap was observed from 2 to 1 tree with a median loss of 2.3 lichen species, i.e., 22% of the total estimated richness with 5 trees. Conversely, average LDV and lichen abundance were not affected by the decrease in number of sampled trees, resulting from their weighting by the sampling effort (trees or quadrats, respectively; Fig. 1B-C). However, the heterogeneity of both indicators largely increased (i.e., the 95% confidence interval was 3.9-times broader for both indicators when decreasing from 4 to 1 tree).

Concerning the functional structure metrics, the decrease in number of sampled trees negatively influenced the average eutrophic species proportion with a maximum of −8% from 5 to 1 tree (Fig. 2A), while no obvious effect was evidenced for the average acidophilous species proportion because of the large confidence interval (Fig. 2B). Despite the larger average proportion of eutrophic species (average value of 72%) compared to the acidophilous species (8%), this indicates that eutrophic lichens can be missed following a decrease in number of sampled trees due to a more heterogeneous distribution among trees in one sampling site, unlike acidophilous species. The average Rao coefficient (Fig. 2C)

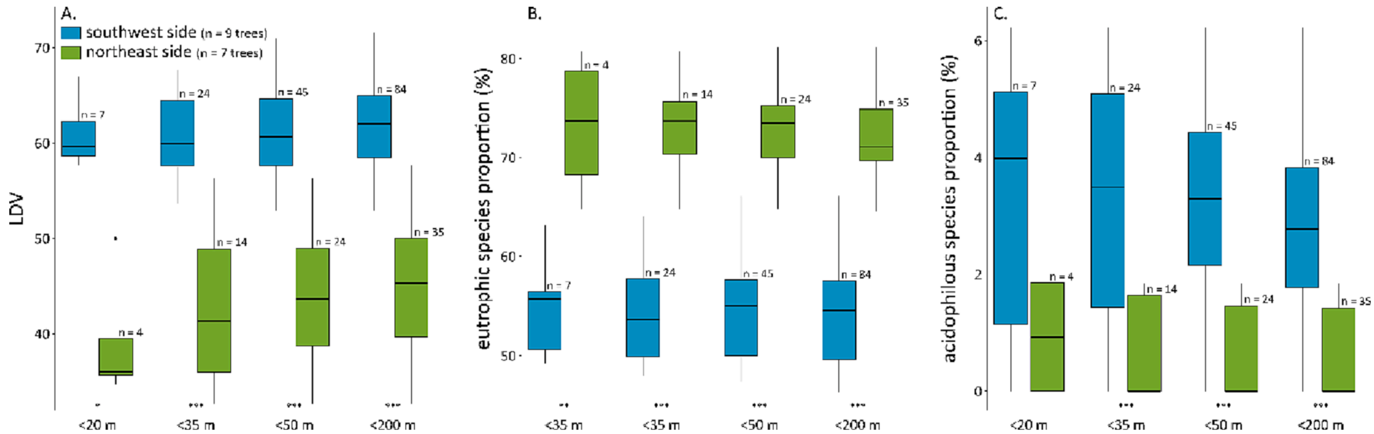

Fig. 3. Influence of roadside and maximum distance between trees (exposed to sunlight in southwest side or shaded in northeast side) on LDV (A) and proportion of eutrophic (B) and acidophilous (C) species. Mann-Whitney *U* test between the two roadsides: *p < 0.05, **p < 0.01, and ***p < 0.001.

was considerably impacted by the number of sampled trees, with a median decrease between 2 (from 5 to 4 trees) and 26% (from 5 to 1 tree). This results from the loss of the lichen species richness reducing the functional diversity in the sampled lichen communities and primarily affecting ecologically contrasted scarce species (Botta-Dukát, 2005).

3.1.2. Number of sampled tree exposure sides

Then, we assessed the influence of a decrease in number of sampled tree exposure sides, from 4 (NESW) to 1 (N, E, S, or W). Results showed a negative effect on the lichen species richness (between −12 and −15% from 4 to 1 exposure side according to the tree number; Fig. 1A), while no influence was evidenced on the lichen abundance (Fig. 1C). As observed for the decrease in number of sampled trees, this results from the representativeness of the sampled area, becoming limited when the number of sampled tree exposure sides is reduced. Both lichen species richness and abundance indicated important heterogeneity in the single-exposure side scenarios, (on average, north exposure sides showed higher abundance and lower diversity than south ones), likely resulting from distinct light exposure or regional specificities (north–south wind direction in the studied region). However, no consistent pattern was observed in the intermediate scenarios (i.e., 2- and 3-exposure sides): for example, no higher abundance and lower diversity in the scenarios including north exposure side (i.e., NE, NW, and NEW). Interestingly, sampling a single exposure side always showed better results than sampling four exposure sides with one less tree. According to the calculation method (Asta et al., 2002), the LDV was mainly impacted by the decrease in number of sampled tree exposure sides: it decreased by the same factor as the number of sampling grids (up to −75% with only one sampled tree exposure side; Fig. 1B).

Results also showed a lower effect of the decrease in number of sampled tree exposure sides on the functional structure metrics and functional diversity (Fig. 2), compared to the decrease in number of sampled trees: within a fixed number of sampled trees, the medians of those metrics varied between +1 and −2% from 4 to 1 tree exposure side, except −4% for the proportion of eutrophic species with only one sampled tree. Also, proportions of eutrophic and acidophilous species may be influenced by the regional conditions (e.g., prevailing wind direction), as observed for lichen species richness and abundance between north and south exposure sides (here, lower eutrophic species proportion in the north exposure side).

3.1.3. List of considered lichen species

Finally, we tested the influence of the lichen species considered in the *Lichens GO* identification key on taxonomic metrics, functional structure metrics, and functional diversity. The simulated scenarios showed a slight decrease of the average lichen species richness (between −3 and −8% in median, depending on the number of sampled trees and sampled tree exposure sides; Fig. 1A), resulting from the species grouping into single morphospecies (e.g., *Amandinea punctata* and *Lecidella elaeochroma*) or broader lichen category (e.g., other crustose lichens, powdery crustose lichens). This influence, however, remains small compared to removing one sampled tree (at least −9%, up to −34%) or limiting to a single sampled tree exposure side (about −12%). Both LDV and lichen abundance were almost not influenced by the species grouping (between −1 and −2% in median, depending on the number of sampled trees and exposure sides; Fig. 1B-C). The data heterogeneity was similar to those observed in the respective scenario using the exhaustive species list.

Although the proportion of eutrophic species was almost not affected by the considered lichen species list (+1 to +2%; Fig. 2A), the proportion of acidophilous species (−94 to −95%, depending on the number of sampled trees and exposure sides; Fig. 2B) and the functional diversity (−14 to −17%; Fig. 2C) were largely underestimated. Indeed, the low number of acidophilous species considered in the *Lichens GO* protocol or the fact that several frequent acidophilous species are included as broader lichen categories without ecological trait score (e.g., *Lepraria incana*, *Phlyctis argena*, *Lecanora expallens*, etc.) constrains the ecological characterization. This reduction in the ecological diversity of the described lichen communities thus affects the functional diversity (Fig. 2C).

3.2. Distance between trees

To assess the selection bias, we considered three metrics calculated for each distance constraint in the southwest and northeast sides (Fig. 3): LDV and proportion of eutrophic and acidophilous species. Results showed a statistically significant difference (Mann-Whitney *U* test: $p < 0.05$, except for acidophilous species proportion at <20 m) between the two sides for the three considered indicators, with higher LDV and acidophilous and lower eutrophic species proportions for the southwest side. This results from distinct surrounding contexts: the lighter condition in the southwest part supports a higher lichen diversity characterized by acidophilous species unlike the shaded condition (López et al., 2016). Also, since several crustose lichens (e.g., *Lepraria incana*) do not have an ecological trait score in the *Lichens GO* species list (i.e., included in a broader lichen category), the acidophilous species proportion may be underestimated.

Within the same roadside, the values of the three indicators did not change with the increase in maximum distance limits (Kruskal-Wallis test: $p > 0.05$). This indicates that whatever the maximum distance criteria, the ecological response remains the same if we consider a

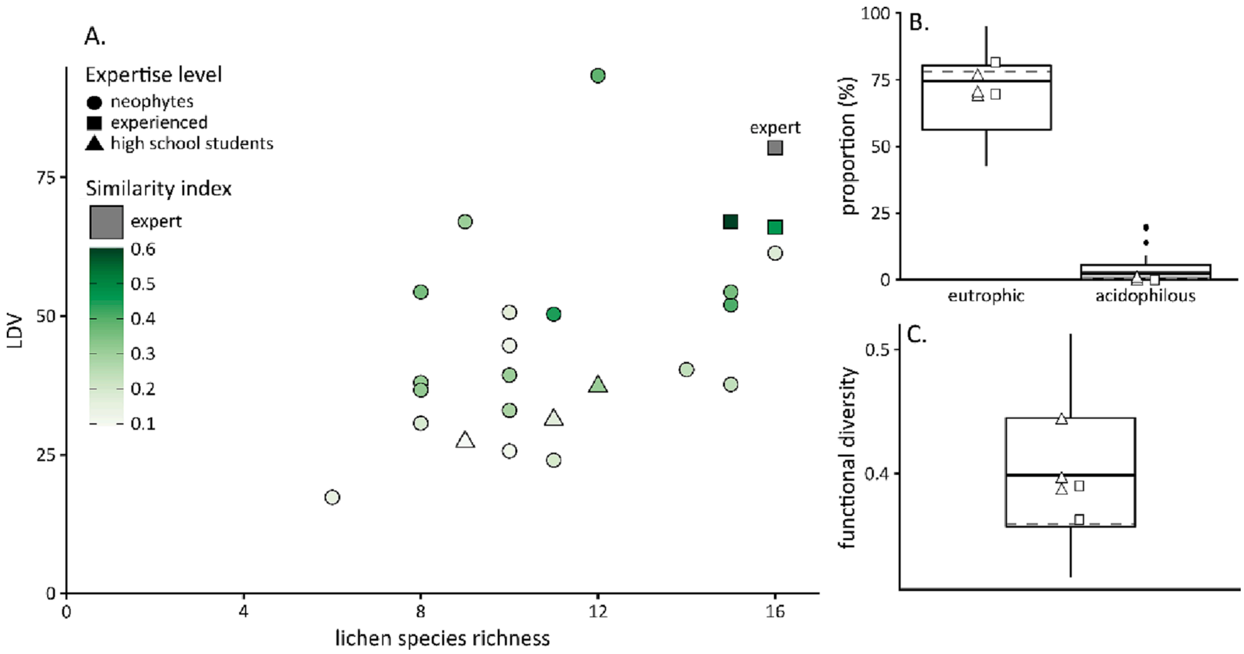

Fig. 4. Observation bias on lichen species richness and LDV according to the participant expertise level (neophytes, experienced, and high school students) compared to the expert (A). Color scale indicates the similarity index, i.e., the proximity of each participant to the expert (from 0 to 1). Ecological influences of this observation bias are considered through the proportions of eutrophic and acidophilous species (B) and functional diversity (Rao coefficient; C).

homogeneous sampling environment (represented by each roadside). However, the maximum distance alone is insufficient to constrain the results: indeed, the width of the road (i.e., 12 m) would allow sample trees on both roadsides. Thus, the distance between two trees could be increased if the sampling site remains as homogeneous as possible to reduce this bias.

3.3. Observation bias

To evaluate the observation bias, we compared three metrics considering the expertise level of the participants (neophytes, experienced, and high school students; Fig. 4A): lichen species richness, LDV, and proximity to the expert (similarity index). A substantial heterogeneity was evidenced in the three metrics calculated, with relative standard deviations of 26.2 and 38.8% for lichen species richness and LDV,

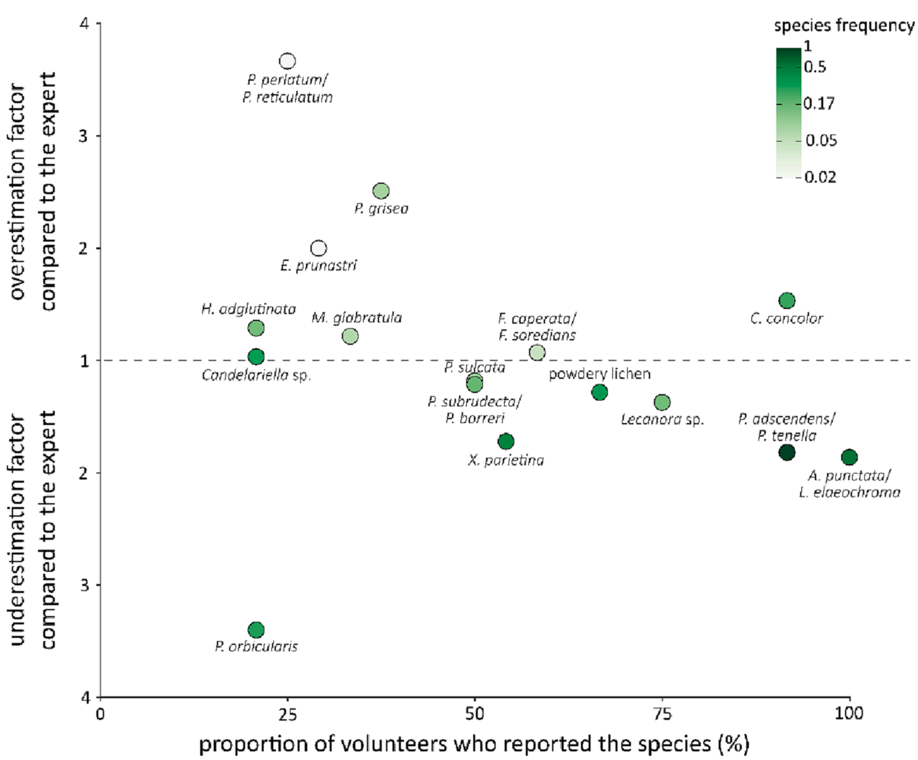

Fig. 5. Under- or overestimation of each species observed by the expert as a function of the proportion of volunteers (n = 24) who have reported it. The underestimation factor corresponds to the ratio of the expert frequency to the average frequency of the volunteers who observed the species. The overestimation factor corresponds to the inverse ratio. The dashed line indicates no under- or overestimation. Color scale indicates the lichen frequency according to the expert (logarithmic scale).

respectively. On average, the neophytes (n = 19) obtained a LDV of 44.8 (from 17.3 to 93.3) with 10.8 species (from 6 to 16), which were underestimated compared to the expert (LDV of 80.3 with 16 species), i.e., almost half of the LDV with 5 missing species. Since the trees were pre-selected, this may result from identification difficulties (Brunialti et al., 2012): for example, most participants missed the juvenile individuals *Physcia adscendens/P. tenella* while present almost everywhere in the sampling site (i.e., frequency of 0.98 according to the expert). In addition, some species are difficult to observe for non-experienced participants because of their small size (e.g., *Hyperphyscia adglutinata*) or their bark-like color (e.g., *Melanelixia* sp.), as already observed by the OPAL observatory (Seed et al., 2013). Only one participant overestimated the LDV because of the consideration of green algae as crustose lichens. Despite little data (n = 2), the experienced participants were closer to the expert for both lichen species richness (average of 15.5 species) and LDV (average of 66.5). Interestingly, the high school students (n = 3) reported the same lichen species richness as the neophytes (average of 10.7 species), but they underestimated the LDV (average of 32). Hence, if the high school students only participate in *Lichens GO* through an academic program, they will not gain in their lichen experience resulting from the high student turnover rate, unlike neophyte volunteers.

However, lichen species richness and LDV that are frequently used in the literature (Brunialti et al., 2012, 2002; Cristofolini et al., 2014; Giordani et al., 2009) do not best reflect the relevé quality. Indeed, these indicators do not consider the correct identification. We thus used the similarity index (eq. (1) that considers potential confusions in species identification on each tree exposure side. The average similarity index obtained by the neophytes reached 0.27 (i.e., 27% of lichen frequency correctly identified by the participant compared to the expert, from 0.12 to 0.45). Note, however, that this index is strict (i.e., drastic decrease in value with a small difference between the participant and the expert) and also integrates the grid position, which explains the relatively low values even for experienced volunteers (average of 0.54, up to 0.62). Indeed, since the participants placed the sampling grid independently, they may include or exclude the species located at the edges according to the grid position (Brunialti et al., 2012). High school students showed similar values as other neophytes, indicating the usability of data provided by school participation.

The bias resulting from both identification and frequency estimation errors was investigated through the proportions of eutrophic and acidophilous species (Fig. 4B) and functional diversity (Fig. 4C). Despite a high heterogeneity among the neophytes, the medians of the three metrics (74.5%, 2.5%, and 0.39, respectively) were close in absolute value to the results obtained by the expert (78.0%, 0.4%, and 0.36, respectively). We attribute the heterogeneity to identification errors of frequent species, such as *Hypogymnia physodes/H. tubulosa* instead of *Physcia adscendens/P. tenella*. Finally, the high school students were generally in the range of neophytes, and the experienced participants remained close to the expert.

To improve the data quality, it is required to identify the major difficulties encountered by the volunteers. Indeed, some species were reported by at least 90% of the volunteers (*Amandinea punctata/Lecidella elaeochroma*, *Physcia adscendens/P. tenella*, and *Candelaria concolor*), while others were almost unnoticed (*Hyperphyscia adglutinata*, *Candelariella* sp., *Phaeophyscia orbicularis*) with <25% of the participants (Fig. 5). Abundant lichen species (frequency > 0.5) were more easily reported by the participants, with the exception of *Xanthoria parietina* (reported by 54% of participants) because of its juvenile specimens (i.e., without apothecia) and therefore confused with *Candelaria concolor*. Note that the incorrect identification of these abundant species is particularly problematic for the ecological interpretation and drastically reduces the similarity index (e.g., *Physcia adscendens/P. tenella*, eutrophic species, identified as *Hypgymnia physodes/H. tubulosa*, acidophilous species that was not present on the site). Also, species that were represented by a single specimen (frequency = 0.02), such as *Parmotrema perlatum* and *Evernia prunastri*, may have been omitted because of a grid position bias (Brunialti et al., 2012) or confusion with other species. Some small but relatively abundant species (0.15 ≤ frequency ≤ 0.30), however, seemed particularly difficult to observe (*Hyperphyscia adglutinata*, *Phaeophyscia orbicularis*, *Candelariella* sp., observed by <25% of the volunteers), while large foliose lichens (*Flavoparmelia caperata* and *Parmelia sulcata*) were identified by more than half of the volunteers despite their low frequency (0.05 ≤ frequency ≤ 0.17).

Beyond the lichen species identification bias, the frequency estimation is another source of potential errors. When observed by the participants, the most abundant species (e.g., *Amandinea punctata/Lecidella elaeochroma* and *Physcia adscendens/P. tenella*) were underestimated compared to the expert (up to 2-times lower; Fig. 5). This may be related to the presence of juvenile specimens or the lack of neophyte experience. *Candelaria concolor* was the only abundant species whose frequency is, on average, overestimated, probably resulting from the confusion with juvenile specimens of *Xanthoria parietina*. In addition to being almost unnoticed, *Phaeophyscia orbicularis* was strongly underestimated (3.4 times), mainly resulting from its small size. Conversely, *Parmotrema perlatum* was overestimated (confusion with *Punctelia* sp.). Finally, the participants reported large foliose lichens (*Flavoparmelia caperata* and *Parmelia sulcata*) with a frequency close to the expert one.

3.4. *Lichens GO* protocol feasibility

The *Lichens GO* protocol feasibility was assessed using an online survey (cf. Supplementary Material SM1). We obtained 41 responses, 14 of which concerned participants who had carried out at least one *Lichens GO* relevé (on average, 45 years old, mainly women and retired people). For 11 of the 14 respondents (71%), the protocol takes no longer than 1.5 h, while for 2 of them (15%), it takes >2 h. These durations were in the same range as observed for similar simplified protocols tested by volunteers (i.e., four trees, between 22 and 47 morphospecies; Giordani et al., 2009). Despite the complexity of the protocol, 11 of the 14 respondents do not wish to reduce the number of sampled trees, and 12 of the 14 do not wish to reduce the number of sampled tree exposure sides. However, 4 of the 14 respondents reported the too high number of species considered in the *Lichens GO* protocol, which also may constitute a hindrance for people who have never performed a lichen relevé.

3.5. Overall performance and potential adaptations for the *Lichens GO* protocol

Following the different evaluations performed in this study, we now assess the overall performance of the *Lichens GO* protocol and propose adaptations to make it more reliable and feasible for citizens. By simulating scenarios from the reference data set (i.e., five trees, four exposure sides, exhaustive species list) to the *Lichens GO* protocol (i.e., three trees, four exposure sides, *Lichens GO* species list), we estimated variable influences on taxonomic metrics: decrease in lichen species richness (−25%), but little effect on LDV (−2%) and lichen abundance (−2%). This results from the species grouping into single morphospecies or broader lichen category. However, functional structure metrics and functional diversity were impacted by the protocol, related to the lichen species considered in the *Lichens GO* identification key: −94% for the acidophilous species proportion and −21% for the Rao coefficient. In agreement with the citizen feedback, we do not want to modify the number of sampling trees or tree exposure sides. To improve the data quality without increasing the sampling effort, we suggest to: (1) increase the maximum distance between trees (from 10 to 50 m) to extend the sampling site availability and data points as long as the homogeneity of the area is guaranteed; (2) adapt the *Lichens GO* identification key to avoid species confusion; and (3) assign an ecological trait to certain broader lichen categories according to the most frequent species present in these categories to limit the loss of ecological interpretation.

From this perspective, the new identification key (cf. Supplementary

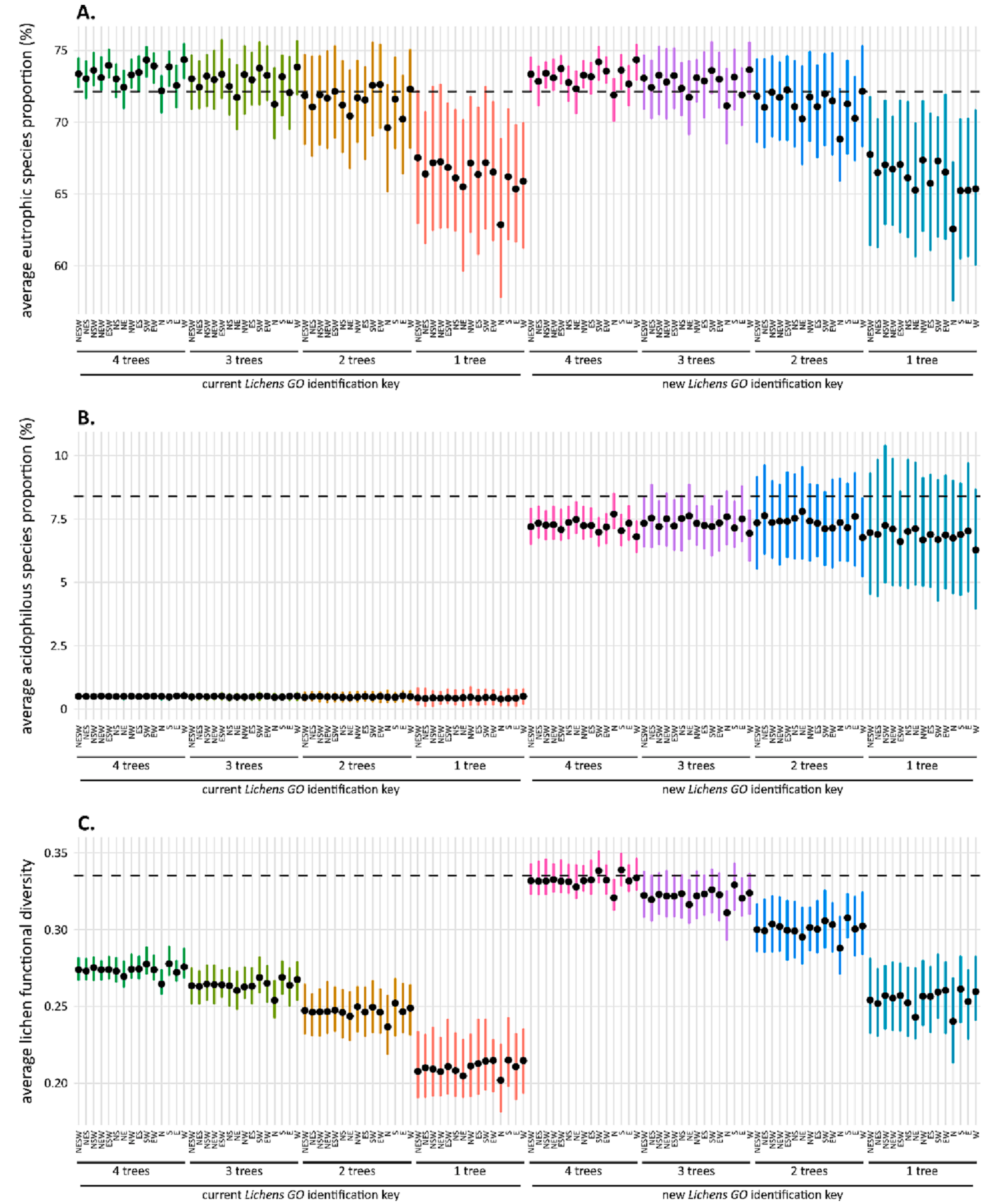

Fig. 6. Influence of the proposed adaptations for each scenario (number of sampled trees, number of sampled tree exposure sides, considered lichen species list) on proportions of eutrophic (A) and acidophilous (B) species and lichen functional diversity (Rao coefficient; C). Letters indicate the sampled tree exposure sides (N: north; E: east; S: south; W: west). Results indicate median values (black dots) and 95% confidence interval (colored bars) of the averaged metrics after 100 simulations. Average values from the reference data set (i.e., 5 trees, 4 exposure sides, exhaustive species list) are presented by the dashed line.

Material SM2) was proposed by: (1) excluding some rare species (such as "other fruticose lichen with cylindric branches"); (2) grouping together morphologically similar species with the same ecological features (e.g., *Punctelia subrudecta/P. borreri* and *Punctelia jeckeri* as *Punctelia* sp. and *Melanelixia glabratula* and "other *Melanohalea*" than *Melanohalea exasperata* as "isidiate brown lichen"); (3) adding other forms of species previously present in the key (such as shaded and juvenile *Xanthoria parietina* and non-sorediate *Parmelia sulcata*); and (4) duplicating certain lichen species (such as *Physcia adscendens* in fruticose lichens and *Hyperphyscia adglutinata* in crustose lichens). Some of these changes also address the citizen criticism regarding the large number of species considered in *Lichens GO*. Moreover, we suggest to classify the "powdery lichens" as acidophilous species since most powdery species are *Lepraria incana* and *Lecanora expallens* in urban area (Davies et al., 2002; Larsen et al., 2007; Poličnik et al., 2008; Zahradnikova, 2010). Note that these changes were made in consultation with lichenologists and volunteers who are involved in *Lichens GO* and aware of the current identification key weaknesses. Considering all the modifications previously proposed, we obtained new simulation results (Fig. 6): both acidophilous species proportion and Rao coefficient were improved compared to the current *Lichens GO* protocol (from −94 to −13% and from −21 to −4%, respectively). Only the eutrophic species proportion did not show modification. This mainly results from the ecological consideration of "powdery lichens".

Furthermore, we suggest to add a second protocol including fewer species (as done for OPAL) or with lower sampling effort that may facilitate new volunteers' involvement. Note that reducing the number of sampled tree exposure sides showed always better results than reducing the number of sampled trees. In case of <4-exposure sides sampling, however, this could introduce a bias in the data set collected in the absence of recommendations on tree exposure side selection, as well as difficulties in interpreting data collected under various climatic conditions (OPAL, 2013; VDI, 1995). A data set collected from the new protocol would be compared to data from the full *Lichens GO* protocol for calibration in some geographical areas to limit the reduction of data quality with only a simpler but less efficient protocol (e.g., lack of precision in low pollution gradients; Tregidgo et al., 2013).

4. Conclusions

Lichens GO is a citizen science-based program that aims to evaluate both lichen diversity and air quality in urban areas. The present study assessed the performance and applicability of the current *Lichens GO* protocol to then propose adaptations to make the protocol more reliable and feasible for citizen science. Using simulated scenarios on a field campaign data set, we estimated a decrease of 25% in lichen species richness and significant biases on functional structure metrics (underestimation of acidophilous species proportion) and functional diversity (Rao coefficient). To improve the quality of collected data without increasing the sampling effort, we suggest to: (1) raise the maximum distance between trees to 50 m; (2) modify the *Lichens GO* identification key to avoid common identification errors; and (3) attribute an ecological trait to some broader lichen categories. These adaptations improved the ecological interpretation, particularly for the acidophilous species proportion and functional diversity. We also propose to develop a second protocol corresponding to a simplified version of *Lichens GO* to encourage volunteers to participate to this citizen science program without starting with a too complex protocol. *Lichens GO* will allow identifying urban areas with low biodiversity due to poor air quality in order to alert public authorities to improve environmental quality.

CRediT authorship contribution statement

Hugo Counoy: Conceptualization, Formal analysis, Software, Visualization, Writing – original draft. **Laure Turcati:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition. **Romain Lorrillière:** Methodology, Formal analysis, Writing – review & editing. **Simon Bénateau:** Methodology, Formal analysis, Writing – review & editing. **Jean-Paul Maalouf:** Software, Formal analysis, Visualization, Writing – review & editing. **Grégory Agnello:** Investigation, Writing – review & editing. **Sébastien Turpin:** Methodology, Writing – review & editing. **Yannick Agnan:** Conceptualization, Methodology, Visualization, Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2023.110269.

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