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Epiphytic Cryptogams as Bioindicators of Air Quality in a Tropical Andean City

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Abstract: Air pollution is one of the main environmental problems in developed and developing countries. Epiphytic cryptogams (bryophytes and lichens) are proposed as a reliable indicator to detect environmental changes, given to their sensitivity to pollutants. In this study we evaluated air quality in the city of Ambato using bryophytes and epiphytic lichens on three land uses (urban, peri-urban and control). In each zone we selected ten trees (a total of 90 trees) for each station (a total of nine stations), where we recorded the frequency and cover of epiphytic cryptogams in a quadrat of 10 × 50 cm that was divided into 5 × 5 cm squares. Differences in richness, index of atmospheric purity (IAP) and diversity were analyzed using a generalized linear model (GLM) and changes in species composition using multivariate analysis. We recorded 39 species of cryptogams (25 lichens and 14 bryophytes). Richness, diversity and index of atmospheric purity were higher in the control zone compared to the urbanized zones. Community composition changed between the different zones, with increasing differences between the control and urban zones. The urban areas of the city of Ambato were identified with high levels of air pollution due to their lower diversity related to higher vehicular traffic and industrial activities (e.g., footwear and textile factories, tanneries). Thus, epiphytic cryptogams are a fast and low-cost method for air quality assessment in tropical areas.

Keywords: alpha diversity; beta diversity; bryophytes; Ecuador; lichens

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1. Introduction

Air pollution is currently one of the main environmental problems in urban and rural areas due to rapidly developing industrialization and urbanization worldwide as well as in Latin American cities [1–4]. Vehicular traffic and industrial activities in emerging economies deteriorate the air quality in large areas where there is no air pollution monitoring network [5], and thus chronic and acute exposures of inhaled pollutants can produce toxic effects in biological systems [6].

Ambato is a city located in the central Andean valley of Ecuador and one the most important cities in terms of productivity (e.g., industrial, commercial and manufacturing engine) and economy at the regional level [7]. Thus, industrial activities (e.g., footwear and textile factories, tanneries) lead to a constant growth in the flow of different types of motorized transport that generate high levels of air pollution [8]. The expansion of the urban areas towards rural areas implies the elimination of green areas, generating environmental impacts in the city, and therefore air pollution becomes one of the main environmental problems [8]. Thus, in the city has carried out evaluations of certain pollutants as sulfur dioxide (SO_2), nitrogen dioxide (NO_2) ozone (O_2), benzene (C_6H_6) [8], carbon dioxide (CO_2) and sulfur oxides (SO_x) related with air pollution. However, there is only one air pollution monitoring station and few studies in this city, which has not made possible the accurate determination of the current state of the air quality in the different zones of city of Ambato [9].

Epiphytic cryptogams, due to their anatomical, morphological, and physiological characteristics (e.g., they obtain nutrients directly from the atmosphere) [10], are very effective as bioindicators of air pollution in many cities around the world [11–20], and also for programs and protocols of air pollution in several countries. These organisms allow early detection of signs of environmental change given that they are fully exposed to airborne contaminants [11,16,21]. Thus, the application of epiphytic cryptogams are a fast and low-cost method for this purpose.

The Index of Atmospheric Purity (IAP) has been a commonly used tool to determine air quality using lichens and bryophytes [16]; however, more studies have been in temperate zones [22–25] than tropical ones [16,26,27]. Previous studies found that higher IAP values had shown better air quality [28–32]. Conversely, low IAP values are related with reduction of the most sensitive species, indicating higher degrees of air pollution [14,16,32]. Thus, studies in tropical areas have documented that urban areas have high levels of air pollutant related with vehicular traffic and industrial activities than areas far from the city [11,14,16,30,33–37].

Studies in Ecuador using bioindicators to detect air pollution are scarce [3,32,37]. Thus, for the first time, epiphytic cryptogams (lichens and bryophytes) were used as biomonitors to assess the air pollution in the city of Ambato. Specifically, we addressed the following questions: (1) Are the richness, IAP and composition of cryptogams (lichens and bryophytes) influenced by land use changes? and (2) Can cryptogams (lichens and bryophytes) be used as indicators of air pollution related to vehicular traffic?

2. Materials and Methods

2.1. Study Area

The present study was carried out at nine stations of the city of Ambato (Figure 1), in the western mountain cordillera of Ecuador, at 2678 m a.s.l. with an approximate area of 1016.454 km^2 and temperature that fluctuates between 12 and 27 °C. Nine sampling stations located in three land uses (three urban stations, three peri-urban stations and three control stations) were selected.

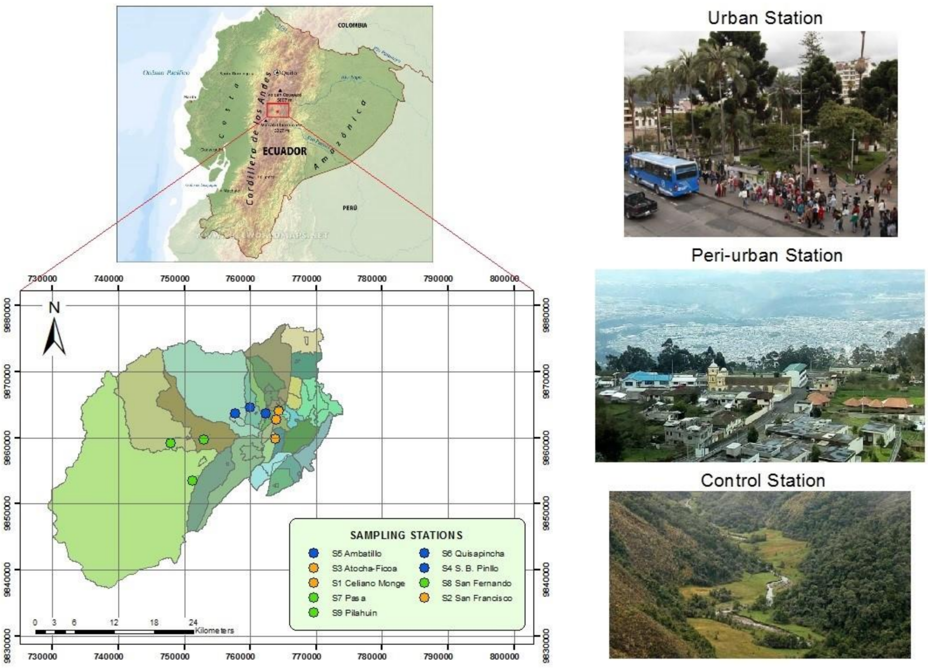

Figure 1. Sampling stations in the city of Ambato. S1 = Celiano Monge, S2 = San Francisco, S3 = AT = Atocha-Ficoa, S4 = SB Pinllo, S5 = Ambatillo, S6 = Quisapincha, S7 = Pasa, S8 = San Fernando and S9 = Pilahuin.

The control zone (Figure 1) was located in the high areas of the rustic parishes of Ambato, represented by a dense canopy layer of evergreen montane tropical vegetation (e.g., *Alnus acuminata*) and very little rural traffic (3 vehicles/h). The peri-urban zone (Figure 1) was located on the periphery of the city with an accelerated advancement of the agricultural frontier, where the disturbed forests are mixed with pastures dominated by planted trees of *Eucalyptus globulus*; and is subject to relative high levels of traffic (1000 vehicles/h) due to the transit between this area and the city. The urban zone (Figure 1) was located within the central area. This part of the city has a very uniform structure and is dominated by a section of grassland with planted trees of introduced species and palms. This area has higher levels of vehicular traffic (2080 vehicles/h) and industrial and commercial activities than the others. The research was conducted during the months of March to September 2020.

2.2. Design and Data Collection

Three stations were selected for each land use (a total of nine stations), 10 trees with similar bark structure and diameter at breast height (DBH) > 10 cm were chosen for each station (90 trees). In each tree, the frequency and cover of bryophytes and epiphytic lichens were recorded with a 10 × 50 cm quadrat that was divided into 20 squares of 5 × 5 cm [30,32], which was placed vertically at 1.20 m.

We obtained vehicular traffic with punctual one-day sampling data related with the number of vehicles for each zone. We considered three different categories: (LV) = light-vehicles: cars and small vans; (HV) = heavy-vehicles: trucks and buses; and (MT) = motorbikes [38].

The specimens were identified using general and specific taxonomic keys for bryophytes [39–41] and lichens [42–45]. Specimens were deposited in the herbarium of the Universidad Técnica Particular de Loja (HUTPL) and were collected with authorization for scientific research No. 02-2020-IC-FLO-DPAT-VS and moved under the wildlife specimens mobilization act No. 17-2020-DPAT-V.S.

2.3. Data Analysis

In order to determine the sampling effort in the three zones, a species accumulation curve based on samples and the Chao 2 nonparametric richness estimator was used. Species richness and diversity (Shannon-Weaver and Simpson indices) were determined. The Index of Atmospheric Purity (IAP) was used to determine air quality in the city of Ambato. The IAP was calculated based on the summary of the frequencies of all the species present in each sampled tree [30].

IAP tree = Summary of the frequency of each species. Values from 0 to 20.

IAP area = Average IAP values of trees for each area.

Box plots were used to visualize changes in richness, diversity, and index of atmospheric purity in the different zones. Changes in richness, diversity, and the index of atmospheric purity of epiphytic cryptogams related with land use and station were analyzed with generalized linear models (GLM), using a Poisson error distribution and a logarithmic link function [46]. We applied non-parametric correlations (Spearman, $p < 0.05$) between richness, IAP, diversity and vehicular traffic.

Non-metric multidimensional scaling (NMDS) was performed to detect patterns of species composition in relation to land uses. The NMDS was run using the Bray-Curtis distance and 999 Monte Carlo permutations. To test whether the three land uses had significantly different compositions of epiphytic cryptogams and to detect the effects of station and vehicular traffic, we performed a permutational multivariate analysis of variance (PERMANOVA). All analyses were performed with R statistical software version 3.6.3 [47] and the statistical package “vegan” [48].

3. Results

3.1. Alfa Diversity

A total of 39 epiphytic cryptogams (25 lichens and 14 bryophytes) were identified in the 90 sampled trees, distributed in 22 families and 29 genera (Appendix A). The accumulation curves and Chao 2 richness estimator showed high values of estimated species for the control area with 39 ± 6.17, follow peri-urban and urban zones with 16 ± 0.48 and 8 ± 0.96, respectively (Figure 2).

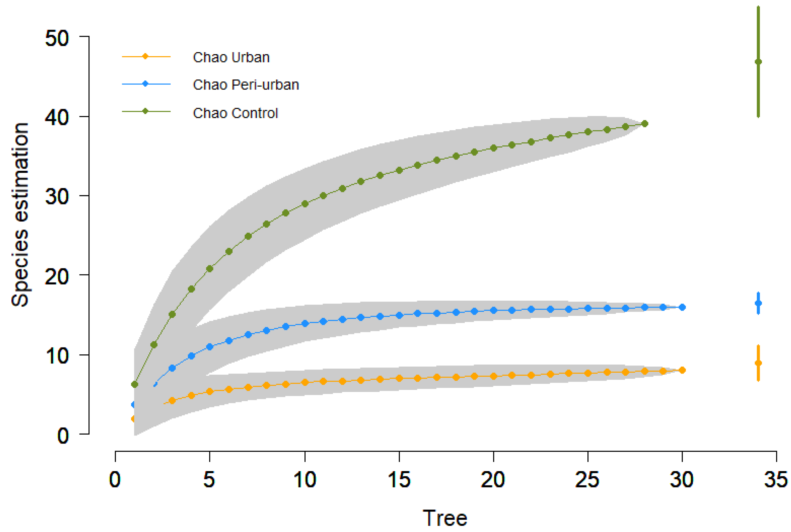

Figure 2. Species rarefaction curve with 95% confidence interval and Chao 2 estimator for three land uses (Control, Peri-urban and Urban).

Species richness at tree level showed a high value for the control zone (14) compared with the peri-urban (9) and urban (5) zones (Figure 3). The mean value of the Shannon-Weaver index was 1.50 for the control zone, 1.10 and 0.70 for the peri-urban and urban zones, respectively (Figure 3). A similar pattern was observed for Simpson's index, with high values for the control zone (0.70 control zone, 0.62 peri-urban zone and 0.45 urban zone). The IAP showed high values for control and peri-urban zones (Figure 3). On the other hand, IAP by zone showed high values for the control zone with 75.7, followed by the peri-urban and urban zones with 67.76 and 44.66, respectively.

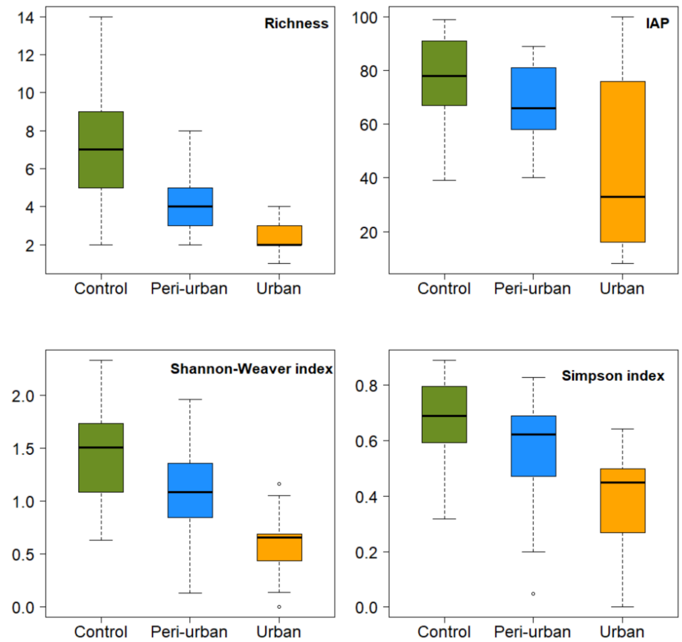

Figure 3. Box and whisker plots of species richness, IAP (Index of Atmospheric Purity) Shannon-Weaver and Simpson index between urban, peri-urban and control areas.

Richness, species diversity and index of atmospheric purity were influenced by zone, where the control zone has a positive effect and the peri-urban and urban zones have a negative effect (Table 1). A similar pattern was observed at the station level, with a positive effect at the stations in the control zone (Table 1).

Table 1. Results of the Generalized Linear Model between richness, index of atmospheric purity and diversity of epiphytic cryptogams as a function of the different land uses and station. S1 = Celiano Monge, S2 = San Francisco, S3 = Atocha-Ficoa, S4 = SB Pinllo, S5 = Ambatillo, S6 = Quisapincha, S7 = Pasa, S8 = San Fernando and S9 = Pilahuin.

	Richness		Index of Atmospheric Purity		Shannon-Weaver Index		Simpson Index	
	Estimate	***p* Value**	**Estimate**	***p* Value**	**Estimate**	***p* Value**	**Estimate**	***p* Value**
Control	1.8245	<0.0001	4.18358	<0.0001	1.3274	<0.0001	0.6523	<0.0001
Peri-urban	−0.7691	<0.0001	−0.22552	<0.0001	−0.3486	0.0212	−0.0190	<0.0001
Urban	−0.7598	0.0007	0.25908	<0.0001	−0.8322	<0.0001	−0.3651	<0.0001
S5	0.1973	0.4939	0.27895	0.0002	−0.3047	0.1501	−0.2654	0.0067
S1	−0.2662	0.3597	−1.5647	<0.0001	0.1188	0.4378	0.1159	0.0980
S7	0.2796	0.0967	0.24962	<0.0001	0.1109	0.4565	−0.0138	0.8384
S9	0.1633	0.3443	0.19344	0.0002	0.2053	0.17	0.0586	0.3870
S6	0.8009	0.0025	0.26586	0.0004	0.5401	0.0118	0.0904	0.3458
S2	−0.1304	0.6226	−1.02642	<0.0001	0.1948	0.1826	0.1428	0.03301

Following this pattern, the results of Spearman correlations showed a strong negative correlation between richness (r = −0.71, *p*-value < 0.0001), IAP (r = −0.51, *p*-value < 0.0001), the Shannon-Weaver index (r = −0.64, *p*-value < 0.0001), r = −0.55, *p*-value < 0.0001) and vehicular traffic.

3.2. Beta Diversity

The NMDS ordination showed that the community composition of epiphytic cryptogams (lichen and bryophyte) is different in the in urban areas compared to the control areas (Figure 4).

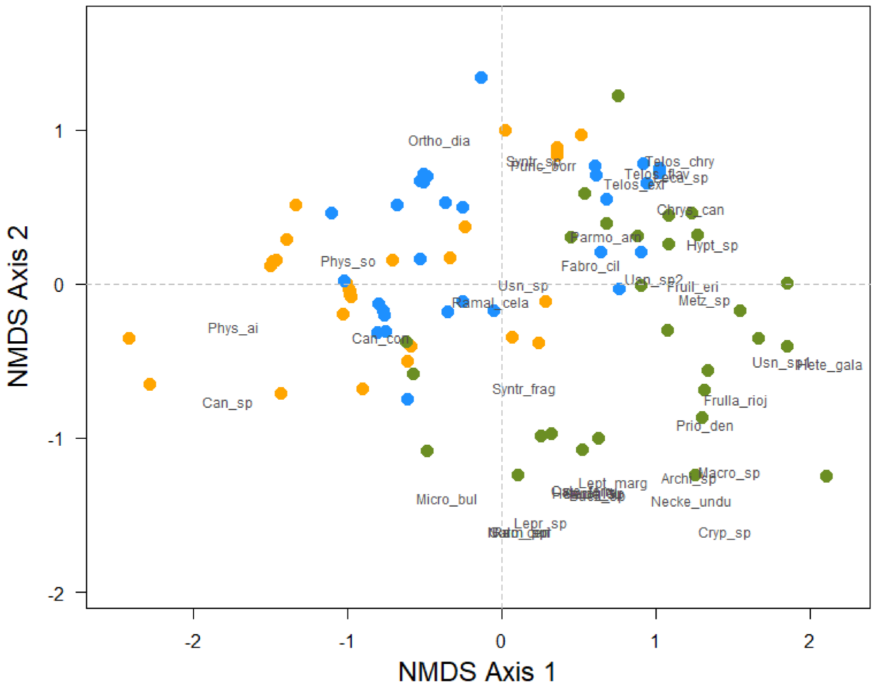

Figure 4. Non-metric multidimensional scaling analysis (NMDS) of the species composition in the three land uses. Urban zone = orange dots, peri-urban zone = blue dots and control zone = green dots.

The multivariate statistical analyses showed that epiphytic composition of cryptogams was structured according to land use changes, with a large component of variation (i.e., 30%) associated with station, followed by zone and vehicular traffic, with 15% and 12 %, respectively (Table 2).

Table 2. PERMANOVA results of species composition at the zone and station level. Df = degrees of freedom; SS = sum of squares; MS = median squares, F = F-statistics; R^2 = coefficient of variation.

Factor	Df	SS	MS	F	R^2	*p* Value
Station	5	10.053	2.0106	12.01	0.3087	0.001
Vehicular traffic	1	3.911	3.9111	23.36	0.1201	0.001
Zone	2	5.038	2.5188	15.04	0.1547	0.001
Residuals	81	13.563	0.1674		0.4165	
Total	89	32.564			1	

4. Discussion

The results indicated changes in the diversity (alpha and beta) and the index of atmospheric purity of epiphytic cryptogam communities as moving from rural (control) to urban areas. Similarly, previous studies found that diversity and IAP decreases in urban areas as a result of land use, industrial activity and increase in the flow of different types of motorized transport that generate a greater amount of polluting emissions [15,26,32,49].

In this context, the index of atmospheric purity, richness, and diversity (Shannon-Weaver and Simpson diversity indices) showed a significant increase in the control zone, indicating better air quality, as reported in previous studies [14,26,28,50,51]. In addition, in the control zone there was a higher richness of species sensitive to contamination (e.g., *Neckeropsis undulata*) and lichen species (e.g., *Leptogium marginellum*). An opposite pattern was observed in the urban area, with low diversity and IAP values related to higher pollution, mainly due to the vehicle fleet and industrial activities in urban zones [26,27,29,30] and the dominance of species resistant to atmospheric pollution, such as *Candelaria concolor*, *Physcia sorediosa* and *Physcia aipolia*.

Following the same pattern, the composition of the cryptogam communities revealed significant changes between control and urban zones. Control zones were dominated by bryophyte species (e.g., *Neckeropsis undulata* and *Syntrichia fragilis*) and some sensitive lichen species (e.g., *Teloschistes chrysophthalmus*) that were classified as sensitive to contamination in previous studies [16,26]. Conversely, in urban areas there is a greater coverage of tolerant species, such as *Candelaria concolor* and *Physcia aipolia* that are closely related to increased vehicular traffic and industrial activities. Supporting this idea, several studies have documented that these species are characteristic of areas with a high degree of pollution [16,26,32].

However, the Atocha–Ficoa urban station showed IAP values and community composition very similar to the control station, which may be related to vegetation cover (nine hectares) with a high diversity of tree species (*Cedrela odorata* L. and *Jubaea chilensis* Baill). Similarly to our results, previous studies have shown that urban areas with large vegetation coverage and native wooded species imply more habitat available for the establishment of epiphytic cryptogams [32]. Thus, it is important to consider that changes in diversity, IAP, and community composition in the epiphytic cryptogam communities in the city of Ambato also occurred at the station level.

Although our study demonstrates the efficacy of cryptogam diversity in biomonitoring of land use changes and air quality in the city of Ambato, we suggest that future work should evaluate the effects of microclimatic changes and host tree traits in epiphytic cryptogams as part of biomonitoring, given that previous studies have shown that urbanization related with microclimatic changes (e.g., canopy cover), host tree traits including bark texture, pH, and tree diameter show significative effects on cryptogam diversity in tropical urban environments [52–54].

5. Conclusions

In conclusion, the index of atmospheric purity, alpha and beta diversity of epiphytic cryptogams were drastically affected by changes in land use (e.g., urbanization), related to increased vehicular traffic and industrial activities (e.g., footwear, tanneries, textile). The urban area of the city of Ambato was identified as having high levels of air pollution due to its high vehicular traffic, low diversity and IAP values. This work showed that epiphytic cryptogams are suitable for monitoring air pollution in Ecuador, and that the combined use of alpha, beta diversity and index of atmospheric purity with epiphytic cryptogams proved to be a fast and low-cost effective method for air quality assessment in tropical areas.

Author Contributions: Conceptualization, Á.B. and J.D.; methodology, Á.B. and J.D.; formal analysis, Á.B. and J.D.; investigation, Á.B. and J.D.; writing—original draft preparation, Á.B., J.D., P.S. and L.M.; writing—review and editing, Á.B., J.D. and L.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Stations on which each species appears. S1–3 = Urban station; S4–6 = Peri-urban station and S7–9 = Control station. S1 = Celiano Monge, S2 = San Francisco, S3 = AT = Atocha-Ficoa, S4 = SB Pinllo, S5 = Ambatillo, S6 = Quisapincha, S7 = Pasa, S8 = San Fernando and S9 = Pilahuin.

Taxa	Code	S1	S2	S3	S4	S5	S6	S7	S8	S9
Bryophytes						Coverage %				
Archilejeunea sp.	Arch_sp	-	-	-	-	-	-	-	0.80	0.90
Cryphaea sp.	Cryp_sp	-	-	-	-	-	-	-	0.40	-
Fabronia ciliaris (Brid.) Brid.	Fabr_cil	-	-	-	0.10	-	1.40	-	-	0.70
Frullania ericoides (Nees) Mont.	Frulla_eri	-	-	-	-	-	1.90	0.90	2.80	-
Frullania riojaneirensis (Raddi) Spruce	Frulla_rio	-	-	-	-	-	-	-	2.70	-
Macromitrium sp.	Macro_sp	-	-	-	-	-	-	-	5.00	6.20
Metzgeria sp.	Metz_sp	-	-	-	-	-	-	0.30	0.50	0.10
Microlejeunea bullata (Taylor) Steph.	Micro_bu	-	-	-	-	-	-	-	-	8.80
Neckeropsis undulata (Hedw.) Reichardt	Necke_undu	-	-	-	-	-	-	-	2.40	5.30
Orthotrichum diaphanum Schrad. ex Brid.	Ortho_dia	-	-	-	18.90	-	1.00	0.80	-	1.50
Prionodon densus (Sw. ex Hedw.) Müll. Hal.	Prio_den	-	-	-	-	-	-	0.10	0.30	-
Sematophyllum sp.	Sema_sp	-	-	-	-	-	-	-	-	2.20
Syntrichia fragilis (Taylor) Ochyra	Syntr_fra	-	2.80	2.40	-	0.10	2.60	2.20	5.80	15.90
Syntrichia sp.	Syntr_sp	-	-	8.30	5.40	-	3.50	2.40	0.10	-

Table A1. *Cont.*

Taxa	Code	S1	S2	S3	S4	S5	S6	S7	S8	S9
Lichens										
Buellia sp.	Buell_sp	-	-	-	-	-	-	-	0.10	1.40
Candelaria concolor (Dicks.) Arnold	Can_col	5.40	8.90	0.20	1.50	47.10	-	-	-	18.90
Candelaria sp.	Can_sp	-	-	2.60	-	-	-	-	-	1.50
Caloplaca aff. *ferruginea* (Huds.) Th.Fr.	Calo_ferr	-	-	-	-	-	-	-	-	2.90
Caloplaca sp.	Calo_sp	-	-	-	-	-	-	-	-	2.10
Chrysothrix candelaris (L.) J. R. Laundon	Chry_can	-	-	-	-	-	-	2.50	-	-
Heterodermia galactophylla (Tuck.) W.L.Culb.	Het_gala	-	-	-	-	-	-	-	0.10	-
Hypotrachyna sp.	Hyp_sp	-	-	-	-	-	-	3.50	1.70	-
Lecanora sp.	Leca_sp	-	-	-	-	-	2.80	2.70	0.10	-
Lepraria sp.	Lepr_sp	-	-	-	-	-	-	-	-	0.90
Leptogium marginellum (Sw.) Gray	Lept_mar	-	-	-	-	-	-	-	-	0.10
Leucodermia leucomelos (L.) Kalb	Leu_leuco	-	-	-	-	-	-	-	-	0.40
Normandina pulchella (Borrer) Nyl.	Norm_pul	-	-	-	-	-	-	-	-	0.10
Parmotrema arnoldii (Du Rietz) Hale	Parm_arn	0.20	-	39.50	-	0.80	12.30	16.30	9.50	5.40
Physcia aipolia (Ehrh. ex Humb.) Fürnr.	Phys_aip	0.10	6.20	12.70	1.60	-	-	-	-	0.80
Physcia sorediosa (Vain.) Lynge	Phys_sor	13.10	12.30	0.60	21.40	17.10	-	-	-	2.60
Punctelia borreri (Sm.) Krog	Punc_borr	0.50	-	18.20	16.90	1.00	24.30	39.00	1.60	-
Ramalina celastri (Spreng.) Krog & Swinscow	Ramal_cel	-	-	-	-	2.10	-	1.80	1.50	-
Ramalina sp.	Ramal_sp	-	-	-	-	-	-	-	-	0.10
Teloschistes chrysophthalmus (L.) Beltr.	Telos_chry	-	-	-	-	-	-	2.20	-	-
Teloschistes exilis (Michx.) Vain.	Telos_exi	-	-	-	-	-	3.80	2.70	-	-
Teloschistes flavicans (Sw.) Norman	Telos_flav	-	-	-	-	-	12.10	3.80	0.10	-
Usnea sp.	Usn_sp	-	-	-	-	1.00	1.50	1.50		0.20
Usnea sp. 1	Usn_sp1	-	-	-	-	-	1.10	0.80	30.00	-
Usnea sp. 2	Usn_sp2	-	-	-	-	-	-	0.60	0.20	-
Species richness		5	4	8	7	7	12	18	20	23

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