LTER montado
Monitoring Protocols

epiphytic lichens

PARAMETER **Biotic - Fungi - Lichens**

AIM To monitor changes in epiphytic lichens diversity and abundance

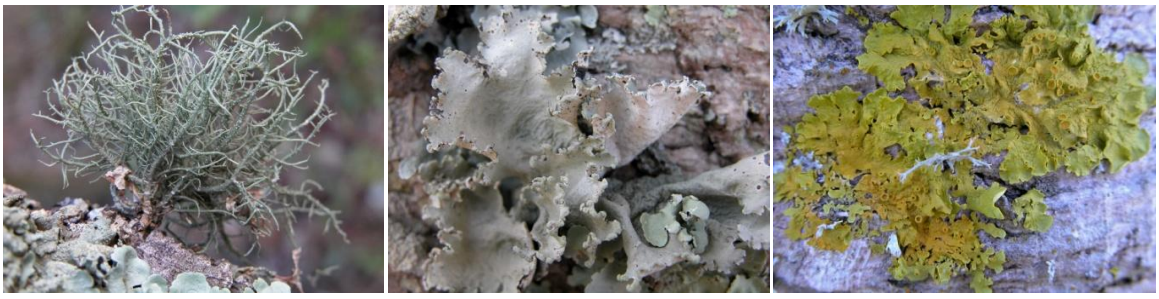
RATIONALE Lichens are organisms that result of a symbiosis between a photosynthetic partner (green algae and/or cyanobacteria) and a fungus. Lichen physiological features (absence of cuticle and root), and other characteristics (stable physiognomy, widespread occurrence) make them excellent ecological indicators and sensitive to small changes on the atmospheric environment. Under a given disturbance, the most sensitive species disappear, while the most tolerant remain. For that reason, lichen diversity is frequently used as an ecological indicator of the effects of natural and human-driven environmental changes: atmospheric pollution, climate, land-use intensity, and forest fragmentation (Matos et al., 2015; Munzi et al., 2014; Pinho et al., 2008; Ribeiro et al., 2013). This protocol aims at monitoring epiphytic lichen diversity within LTsER-montado sampling sites. It is based on the European Protocol to map lichen diversity as an indicator of environmental quality (Asta et al., 2002). Adaptations regarding tree selection were done to include *Quercus suber* specificities (its bark is harvested every 9 years, thus sampling can only be done in the unharvested area of the trunk (Pinho et al., 2012).

ECOSYSTEM SERVICES Air quality.

VARIABLE Frequency.

KEYWORDS biodiversity; epiphytic lichen; richness; frequency; air quality; climate; atmosphere;

AUTHOR(S) Pedro Pinho, Paula Matos, João Pedro Santos, Joana Vieira, Cristina Máguas, Silvana Munzi, Sofia Augusto, Laura Concostrina, Cristina Branquinho. Contact: ppinho@fc.ul.pt



Examples of epiphytic lichen species common on montado (*Usnea rubicunda*, *Parmotrema hypoleucinum*, *Xanthoria parietina*).

epiphytic lichens

METHOD Epiphytic lichens (lichens growing on tree bark) diversity, in a sampling grid.

Material

Field: sampling grid, magnifying lens, knife, chemical reagents for spot test, envelops, GPS, measuring tape, survey sheets. The sampling grid is composed of five 10*10 cm squares vertically disposed on a rectangular grid. It can be built with two rigid 50 cm long segments, united by 6 lose 10 cm perpendicular segments.

Laboratory: chemical reagents (iodine (I), calcium hypochlorite (C), potassium hydroxide (K), para-phenylendiamine (P)); stereoscope (minimum range × 10 to × 60) and optical microscope (× 400 up to × 1 000) with an eyepiece micrometer.

Location

Tree selection within the sampling site: all trees must be selected within 50 m from the centroid, ensuring homogenous conditions (e.g. the same land-use intensity). Six trees must be sampled per site and all trees must:

- belong to the same species (either *Quercus suber* or *Quercus ilex*, one species per sampling site);
- be healthy with no visible signs of disease (e.g. defoliation);
- have a tree trunk not obstructed by other plants (e.g. shrubs growing on the tree base that might obstruct light reaching the trunk);
- be sampled either on the main tree-trunk (preferably) or a main branch. In either case, the portion of the tree to be monitored must:
  - have a circumference between 50 and 250 cm;
  - be at more than 100 cm from the ground;
  - have an inclination <20°;
  - have no damaged areas (decortication, branches, knots);
  - have less than 20% of the portion of the tree to be monitored covered by other epiphyte or climbing plants (sum of the 4 aspects);

(note that, accordingly to these rules, for *Q. suber*, the portion of the tree to be monitored cannot have been decorticated; if that is the case, the portion of the tree to be monitored must then be raised either in the main trunk (preferably) or a main branch must be selected).

Sampling

The grid is placed according to the rules stated above, vertically on the trunk and on the four main aspects (north, east, south and west). For each aspect, all species found within the grid are identified and the number of squares in which a species occurs is noted as its frequency. A single species frequency on each tree can thus vary between 0 and 20. For example if *Evernia prunastri* is found on 12 out of the 20 possible squares, it will have a frequency of 12 for that tree.

All unidentified species must be collected for identification in laboratory, but for these species a frequency must be noted (e.g. as unknown Usnea1, frequency=8). Additionally, a typical example of each identified species should be collected outside the sampling area and deposited in a herbarium (e.g. LISU). A collection envelope should be used in both cases.

Frequency and period

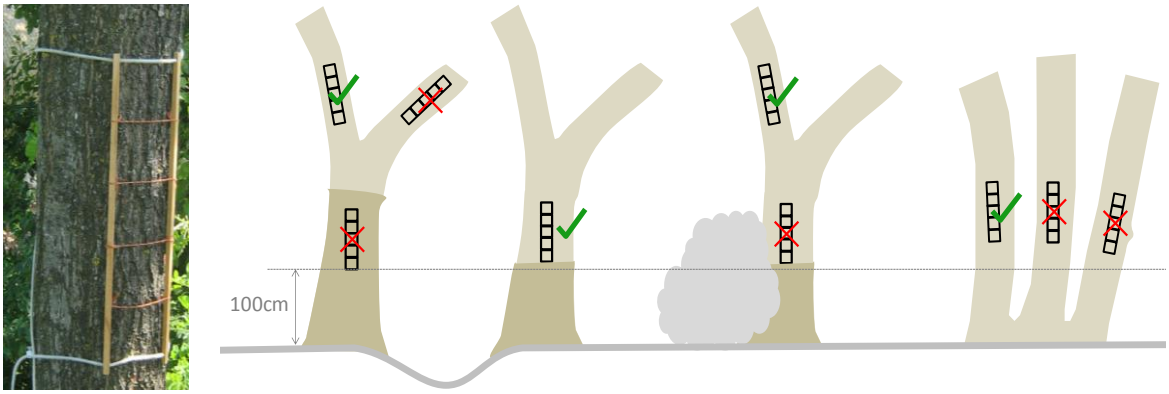
Every 5 years, on the same plots but not necessarily on the same trees; adapt

the sampling period to the most favorable weather for field work.

Estimators

Species richness (number of species), LDV-Lichen Diversity Value. The values are calculated summing all species frequency (e.g. LDVtotal) and dividing species according to morphological and response functional groups (e.g. LDVfruticose).

Species richness corresponds to the total number of species found within the grid for all the sampled trees. LDV is the sum of the frequency of all species found within the grid divided by the number of sampled trees. Species should be further divided into morphological and response functional groups (e.g. calculating the richness of fruticose species or the LDV of species with an eutrophication tolerance of 1). Response functional groups are based on the classification in ITALIC (http://dbiodbs.univ.trieste.it, Nimis and Martellos, 2008).



Left: placement of the sampling grid on the trunk; this placement is then repeated on the other 3 aspects. Right: possible areas to place the grid (at at least 100 cm from the ground) in a tree excluding: areas where the bark was harvested (dark brown) or damaged, or areas with more than 20% inclination; areas where other vegetation shadows the sampling area, like shrubs or other trunks; and avoid areas with bumps or injuries in some of the aspects.

REFERENCES

Asta, J., Erhardt, W., Ferretti, M., Fornasier, F., Kirschbaum, U., Nimis, P.L., Purvis, O.W., Pirintsos, S., Scheidegger, C., van Haluwyn, C., Wirth, V., 2002. Mapping lichen diversity as an indicator of environmental quality, in: Nimis, P., Scheidegger, C., Wolseley, P. (Eds.), Monitoring with Lichens- Monitoring Lichens. Kluwer Academic Publisher, The Netherlands, pp. 273-279.

Matos, P., Pedro, P., Gregorio, A., Isabel, M., Alice, N., Amilcar, S., Cristina, B., (2015). Lichen traits responding to aridity. Journal of Ecology 103:451-458.

Munzi, S., Correia, O., Silva, P., Lopes, N., Freitas, C., Branquinho, C., Pinho, P., 2014. Lichens as ecological indicators in urban areas: beyond the effects of pollutants. Journal of Applied Ecology 51, 1750-1757.

Nimis, P., Martellos, S., 2008. ITALIC - The Information System on Italian Lichens v. 4.0. University of Trieste, Dept. of Biology, IN4.0/1.

Pinho, P., Augusto, S., Martins-Loucao, M.A., Pereira, M.J., Soares, A., Maguas, C., Branquinho, C., 2008. Causes of change in nitrophytic and oligotrophic lichen species in a Mediterranean climate: Impact of land cover and atmospheric pollutants. Environmental Pollution 154, 380-389.

Pinho, P., Bergamini, A., Carvalho, P., Branquinho, C., Stofer, S., Scheidegger, C., Maguas, C., 2012. Lichen functional groups as ecological indicators of the effects of land-use in Mediterranean ecosystems. Ecological Indicators 15, 36-42.

Pinho, P., Llop, E., Ribeiro, M.C., Cruz, C., Soares, A., Pereira, M.J., Branquinho, C., 2014. Tools for determining critical levels of atmospheric ammonia under the influence of multiple disturbances. Environmental Pollution 188, 88-93.

Ribeiro, M.C., Pinho, P., Llop, E., Branquinho, C., Sousa, A.J., Pereira, M.J., 2013. Multivariate geostatistical methods for analysis of relationships between ecological indicators and environmental factors at multiple spatial scales. Ecological Indicators 29, 339-347