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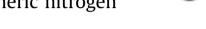
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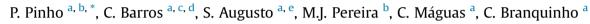
Invited paper

## Using nitrogen concentration and isotopic composition in lichens to spatially assess the relative contribution of atmospheric nitrogen sources in complex landscapes \*



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### ABSTRACT

Reactive nitrogen (Nr) is an important driver of global change, causing alterations in ecosystem biodiversity and functionality. Environmental assessments require monitoring the emission and deposition of both the amount and types of Nr. This is especially important in heterogeneous landscapes, as different land-cover types emit particular forms of Nr to the atmosphere, which can impact ecosystems distinctively. Such assessments require high spatial resolution maps that also integrate temporal variations, and can only be feasibly achieved by using ecological indicators. Our aim was to rank land-cover types according to the amount and form of emitted atmospheric Nr in a complex landscape with multiple sources of N. To do so, we measured and mapped nitrogen concentration and isotopic composition in lichen thalli, which we then related to land-cover data. Results suggested that, at the landscape scale, intensive agriculture and urban areas were the most important sources of Nr to the atmosphere. Additionally, the ocean greatly influences Nr in land, by providing air with low Nr concentration and a unique isotopic composition. These results have important consequences for managing air pollution at the regional level, as they provide critical information for modeling Nr emission and deposition across regional as well as continental scales.

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#### 1. Introduction

Reactive nitrogen (Nr) includes all nitrogen forms capable of readily reacting and causing a number of cascading effects in the environment: reduced nitrogen as ammonia and ammonium (NH<sub>3</sub> and NH<sub>4</sub>), oxidized nitrogen oxides (NO<sub>x</sub>), nitrous oxide (N<sub>2</sub>O), nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>). Reactive nitrogen is released from human activities and its levels already exceed those of naturally fixed N forms. This excess has negative impacts on ecosystem structure and functionality (Erisman et al., 2007; van den Berg et al.,

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2016). Knowing where excessive Nr is being deposited is important in order to take appropriate management actions. However, mapping Nr deposition is not trivial (Hertel et al., 2012). On the one hand, Nr has multiple sources that can be diffuse (such as agriculture or urban areas) or point sources (such as barns or chimneys). These different Nr sources co-occur with other areas that emit little or no Nr (such as forests) – Nr sinks. On the other hand, some Nr forms such as atmospheric ammonia (NH<sub>3</sub>) have a short dispersion range (albeit large impacts on biological systems; Sutton et al., 1998; Pinho et al., 2011), generating high spatial heterogeneity in Nr deposition (Pinho et al., 2014a). Using monitoring stations to map Nr deposition is very costly due to the large number of stations required to capture the spatial heterogeneity of Nr sources. Conversely, using passive samplers is not practicable, as they need to be frequently replaced to allow for temporal integration. To overcome these issues, modeling approaches have provided



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spatially explicit estimations of Nr deposition. Yet, so far these models have been bounded to mapping very small areas with one or few sources of Nr at high resolutions or to mapping large regions at very coarse spatial resolutions (EEA, 2007). Moreover, modeling Nr deposition requires knowing all sources, which is not feasible in large regions where multiple sources and sinks coexist, and have different and unknown relative strengths. This is the case in European Mediterranean landscapes, where different land-cover types co-occur at relatively small spatial scales and act as distinct Nr sources or sinks.

Lichens can be used as ecological indicators of Nr deposition, providing an excellent tool to overcome the issues of spatial resolution (Ribeiro et al., 2014) and temporal integration stated above. Lichens are the result of a symbiosis between algae and fungi and both lichen diversity (Giordani, 2007; Pinho et al., 2004, 2008a, 2008b) and lichen accumulation of pollutants (Augusto et al., 2010; Branquinho et al., 2008) are indicators of atmospheric pollution. In particular, lichens can be used as indicators of Nr concentration (Olsen et al., 2010), as Nr concentration in lichens reflects the amount of Nr deposition from the atmosphere (Liu et al., 2008; Pinho et al., 2014b; Zechmeister et al., 2008). In fact, repeated sampling of a species in a given area with a single Nr source has shown that lichen Nr concentration (N%) reflects Nr deposition in that area (Branquinho et al., 2010; Gaio-Oliveira et al., 2001, 2005).

Yet, multiple sources of Nr are likely to co-occur at larger spatial scales, and emit distinct forms and amounts of Nr. such as reduced (NH<sub>v</sub>) and oxidized (NO<sub>x</sub>) nitrogen (Boltersdorf and Werner, 2013). Identifying the possible sources of pollutants, i.e. sourceapportionment, is thus essential for mapping Nr deposition at large spatial scales without losing resolution. This can be achieved by analyzing nitrogen isotopic composition ( $\delta^{15}N$ ) of the emitted pollutant, as different Nr forms carry a different isotopic signal, i.e. more or less depleted in the heavier isotope (<sup>15</sup>N; Felix and Elliott, 2014; Felix et al., 2014). Source apportionment using stable isotopes is usually done with mixing models (Phillips and Gregg, 2003). However, this is a complex approach and becomes impractical when many different sources are present. This is impractical because to run such models requires knowing the isotopic composition of all sources of Nr in the area. However not all sources are known a priori, and in large areas this can never be achieved. To overcome this problem, Nr deposition in ecosystems can be used as a nitrogen deposition mapping tool. Plants have been shown to respond to different Nr sources by displaying different isotopic composition (Hellmann et al., 2016); yet, they exhibit a limited range of isotopic composition values (although larger variations exist when nitrogen uptake is mainly foliar; see Fogel et al., 2008). Unlike vascular plants, lichens and bryophytes exibit a large variation in nitrogen isotopic composition, and for a given area  $\delta^{15}N$ values are more negative than those for vascular plants (Fogel et al., 2008). Importantly, N isotopic composition in lichens and bryophites has been shown to be related to surrounding Nr sources. For instance,  $\delta^{15}N$  values of lichens and bryophytes collected in agricultural areas tend to be more negative, while those located in areas without important Nr sources tend to be more positive (Boltersdorf and Werner, 2013; Varela et al., 2013). Moreover, lichens should reflect closely the <sup>15</sup>N/<sup>14</sup>N ratio of the atmosphere, even under rather depleted atmospheric values (~-19%; Fogel et al., 2008). However, the characteristic N% and isotopic composition ( $\delta^{15}$ N) of each land-cover type remain poorly characterized in lichens, especially in areas with multiple sources. Thus, we confidently assume that the (relative)  $\delta^{15}$ N measured in lichens along an environmental gradient of Nr sources reflects the relative composition of the main Nr sources in that area.

Here we aimed to support the use of Nr emission/deposition

models, by using lichens as ecological indicators to rank land-cover types according to the amount and form of emitted atmospheric Nr. To demonstrate the potential of this approach we chose a complex study area with multiple Nr sources, including industries and agriculture, for each of which we identified the typical isotopic signature. This information can be used in Nr emission/deposition models to account for the form and amount of nitrogen emitted by each land-cover type, therefore having important implications for air pollution management at the regional level.

#### 2. Methods

#### 2.1. Study area and sampling design

This study covers a coastal area in southwest Europe of c. 380 km<sup>2</sup> (Portugal, Alentejo Litoral region). Climate in this region is typically Mediterranean, with average annual temperatures ranging from 15.7 °C to 17.1 °C and a total annual precipitation of c. 600 mm (1950-2000 average) (Hijmans et al., 2005). The area hosts a population of c. 34 565 inhabitants (INE, 2011) and important industrial facilities (in Sines) that include petrochemical industries, an oil refinery, an industrial harbor, a coal-fueled power plant and an industrial water treatment plant. Apart from the industrial facilities, there are also three cities and many smaller settlements in a matrix of forested areas (including cork oak woodlands and pine and eucalyptus plantations) and shrubland. Agriculture can be found in the remaining areas, including orchards and small-scale animal farms, but these areas are mainly constituted by non-irrigated extensive agriculture for grain cultivation and irrigated high-intensity agriculture for vegetables and rice, most of which heavily fertilized (Fig. 1).

Sampling was distributed evenly across the study area in 43 sampling points (average distance between points 2300 m, max = 4900 m, min = 650 m), where in situ thali of Parmotrema hypoleucinum (Steiner) Hale were collected for analysis. By collecting in-situ lichens we ensured that the samples had been exposed to Nr deposition for a long period, thus representing a temporal integration of Nr deposition. We selected P. hypoleucinum because it can be found abundantly across this region due to its mild tolerance to a range of disturbances. It is a green algae species only, thus not know to be able to fix Nr from atmospheric N<sub>2</sub>. Lichen samples were collected in a single day on February 7th, 2011. To ensure that the conditions were adequate for Nr volatilization from agriculture, we verified weather data of the period prior to the sampling date (from November 1st, 2010 to February 7th, 2011) from the nearest official climate monitoring station (IPMA). During this 99-day period there were 56 days without precipitation, which ensured both that the soil was wet enough for Nr volatilization, but also that there were no barriers for Nr dispersion. Also during this period, there were a total of 65 days with a temperature above 15 °C, which also ensures Nr volatilization from agriculture fields. One 10 g composite sample was collected from 1 to 5 trees (using the available tree species - mostly pines and cork oaks). Lichens were collected from at least 1 m from the ground (to ensure a prevalent effect of atmospheric conditions, rather than an influence of the soil) and only from living trees.

Lichen samples were cleaned from debris, dried at room temperature and powdered in a Reitsch MM2000 ball-mill. Stable isotope ratio analyses were performed at the Stable Isotopes and Instrumental Analysis Facility (SIIAF), at the Faculdade de Ciências, Universidade de Lisboa - Portugal. Sample isotopic composition,  $\delta^{15}$ N, was determined by continuous flow isotope mass spectrometry (CF-IRMS) (Preston and Owens, 1983), on a Sercon Hydra 20–22 (Sercon, UK) stable isotope ratio mass spectrometer, coupled to a EuroEA (EuroVector, Italy) elemental analyser for Download English Version:

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