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Assessing photosynthetic biomarkers in lichen transplants exposed under different light regimes

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ABSTRACT

The present work is a methodological study testing alternative exposure conditions of lichen transplants for biomonitoring studies. We evaluated the effects of different light regime on the vitality of lichen by analysing the photosynthetic efficiency of transplanted thalli of the fruticose species *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea*. In particular, we tested the hypothesis that shading lichen transplants is necessary to ensure sufficient lichen vitality during the exposure time. Thalli of *P. furfuracea* were exposed in a polluted area within the harbour of Genoa (N-Italy) for 4 weeks under two experimental conditions: 5 thalli were covered with a shade cloth, whereas 5 thalli were kept in the light. We showed that, under the same environmental conditions (i.e., climate and pollution regime), shading the thalli minimised the photoinhibition and prolonged the vitality of the lichen. Thus, we recommend shading lichen transplants during biomonitoring surveys to better detect the effects of pollution on the lichens.

1. Introduction

Lichens have been widely used to assess the effects of atmospheric pollution, owing to their sensitive to anthropogenic disturbances (Nimis et al., 2002). Atmospheric pollutants may affect lichens at different levels of biological organisation, e.g., determining alteration of community diversity and composition (Giordani, 2007), accumulation of trace elements within the lichen thalli (Bargagli and Mikhailova, 2002) or causing eco-physiological effects, such as changes on their metabolism (Jensen and Kricke, 2002; Mikhailova, 2002). These latter physiological responses have been used as biomarkers of stress in biomonitoring studies of atmospheric pollution (Manrique et al., 1993; Cuny et al., 2002). From an applicative perspective, in many urban areas where in situ lichens do not occur, biomarkers are typically evaluated on transplanted lichen material collected from pristine sites and transferred to polluted target areas. Among different biomarkers, changes in lichen photosynthetic process, measured as chlorophyll fluorescence by the ratio F_V/F_M, may be considered as sensitive indicators of stress and gives important information on their vitality (Niewiadomska et al., 1998). It has been widely demonstrated that the primary photochemical efficiency changes in response to air pollution and climatic factors (Calatayud et al., 1996; Maxwell and Johnson, 2000; Piccotto et al., 2011).

Among climatic variables, light plays a key role in photosynthesis process. However, high solar radiation may cause damages to photosystem II (PSII) in lichens. The damaging of physiological process induced by light may affect the vitality of lichens to similar extent than gaseous pollutants. An excess of light flux compared to the photon requirement of photosynthesis can cause photoinhibition, which consists of a reduction of the optimal photon yield and the capacity of photosynthetic CO_2 fixation (Krause, 1988). Photoinhibited samples display an alteration in the fluorescence emission that reflects the inactivation of the electron transport system in the thylakoids through damage to the reaction centres (Krause, 1988; Manrique et al., 1993; Gauslaa and Solhaug, 2000). As a result of photoinhibition, the primary photochemical efficiency is significantly reduced. Hence, both photoinhibition and air pollution have comparable effects on the vitality of lichens and can hamper the interpretation of photosynthetic parameters as biomarkers of atmospheric pollution.

The present work is a methodological study testing alternative light exposure conditions of lichen transplants for biomonitoring studies. We carried out a 1-month experiment in a polluted area (Genoa industrial harbour), which can be considered as a typical

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context for application of biomonitoring using lichen transplants. Particularly, we studied the effects of different solar intensity on the vitality of lichen by analysing the photosynthetic efficiency of transplanted thalli of the fruticose lichen *Pseudevernia furfuracea* (L.) Zopf. var. *furfuracea*. We tested whether, under the same environmental conditions (i.e., climate and pollution regime), the vitality of transplanted lichen thalli varies, depending on the light conditions to which they are subjected. In particular, we hypothesise that shading lichen transplants is necessary to ensure sufficient lichen vitality during the exposure time.

2. Materials and methods

2.1. Lichen species

Thalli of *P. furfuracea* (L.) Zopf var. *furfuracea* were collected from a humid sub-Mediterranean area of the Ligurian Apennine (NW-Italy), far from any local source of air pollution. They were sampled from northerly exposed barks of *Abies alba* Mill. *P. furfuracea* is a fruticose lichen with *Trebouxia* algae as a photobiont widely used in biomonitoring studies with transplants (Sorbo et al., 2008; Tretiach et al., 2007, 2011). The species is reported to be a meso-xerophytic lichen, growing on barks and rocks at sites with diffuse light, and is scarce at sites with very high direct solar irradiation (Nimis and Martellos, 2008).

2.2. The study area

Thalli of *P. furfuracea* were exposed in the harbour area of Genoa. Data on the main climatic variables were continuously recorded over the entire exposure period by a weather station located close to the transplanted thalli. The exposure period was characterised by low rainfall limited to the first week (12.8 mm), temperatures ranging from 15.3 and 19.5 °C and solar radiation between 209.8 and 287 W/m^2. Given the proximity of the study area to the sea, the relative humidity was high (>70%), except in the second week during which it was 54.5%.

Atmospheric concentrations of the main gaseous pollutants were obtained from an automatic gauge of the Liguria regional monitoring network, located close to the exposure site. Hourly average SO_2 concentration during the entire period was 7.4 $\mu g/m^3$, ranging from 0.6 $\mu g/m^3$ and 59.9 $\mu g/m^3$. Hourly average NO_2 concentrations was 67.6 $\mu g/m^3$ ranging between 18.3 $\mu g/m^3$ and 148.3 $\mu g/m^3$.

2.3. Experimental condition

Prior to exposure, samples were cleaned from any traces of debris. Before being transplanted, all samples were subjected to a pre-conditioning period of two days in wet chambers with low light (5 $\mu mol\, m^{-2}\, s^{-1}$), to minimise a rise in the F_V/F_M caused by recovery from natural photoinhibition (Gauslaa and Solhaug, 2004). Afterwards, the thalli were exposed for one month at the exposure facilities of ISMAR-CNR experimental marine station (EMS) in the port area of Genoa close to the sea and near shipyards. To test the effect of shading on the photosynthetic efficiency of *P. furfuracea*, two different experimental conditions were assessed: 5 thalli (afterwards reported as 'Shady') were shaded with a shade cloth (UV stabilised HDPE agro shade net, shade factors 40%), and 5 thalli (Light) were kept in the light. Samples for each experimental condition were chosen randomly. Samples of the two experimental conditions were fixed on two networks and attached nearby to the railing of the climatic station. The shade cloth upon the Shady thalli was fixed to the four corners of the network to ensure a constant airflow and the maintenance of comparable microclimatic conditions with respect to Light thalli, except for solar radiation. A detailed scheme of the sample exposition is reported in Fig. 1.

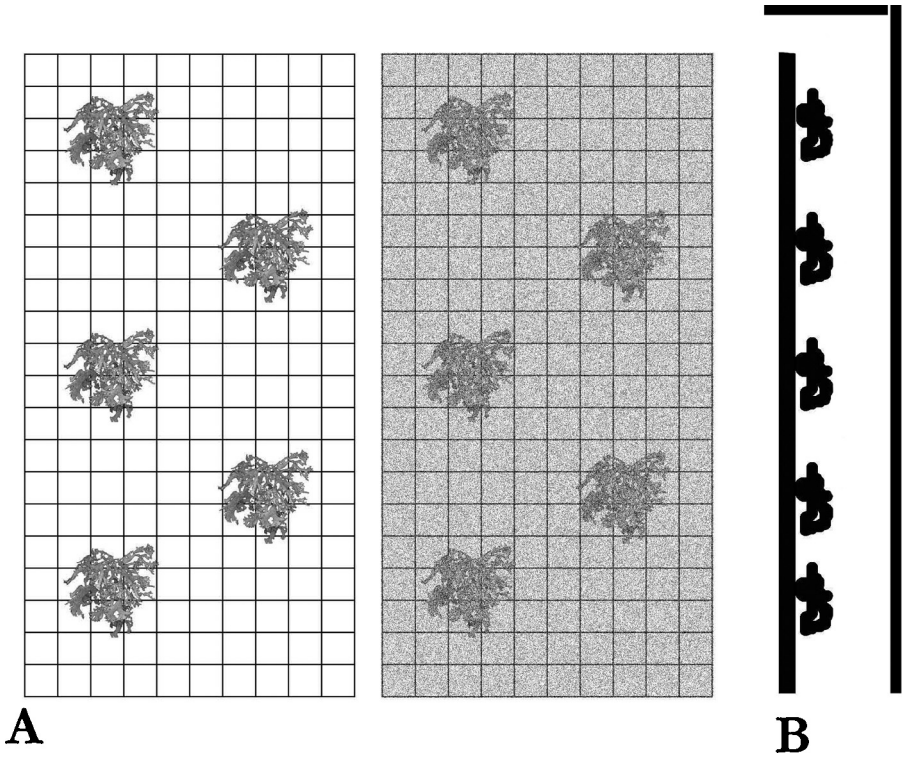

Fig. 1. Schematic arrangement of experimental conditions. (A) Front view: Shady (grey) and Light (white) transplanted lichens. Light and Shade samples were fixed on the two different nets, exposed at 20 cm from each other. On each net, thalli were 5 cm distant from each other. (B) Transversal vision: the cloth was placed at 40 cm from the lichen and fixed at the four corners of the network.