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Effects of ammonia from livestock farming on lichen photosynthesis

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ABSTRACT

This study investigated if atmospheric ammonia (NH_3) pollution around a sheep farm influences the photosynthetic performance of the lichens *Evernia prunastri* and *Pseudevernia furfuracea*. Thalli of both species were transplanted for up to 30 days in a semi-arid region (Crete, Greece), at sites with concentrations of atmospheric ammonia of ca. 60 μg/m^3 (at a sheep farm), ca. 15 μg/m^3 (60 m from the sheep farm) and ca. 2 μg/m^3 (a remote area 5 km away). Lichen photosynthesis was analysed by the chlorophyll *a* fluorescence emission to identify targets of ammonia pollution. The results indicated that the photosystem II of the two lichens exposed to NH_3 is susceptible to this pollutant in the gas-phase. The parameter PI_{ABS}, a global index of photosynthetic performance that combines in a single expression the three functional steps of the photosynthetic activity (light absorption, excitation energy trapping, and conversion of excitation energy to electron transport) was much more sensitive to NH_3 than the F_V/F_M ratio, one of the most commonly used stress indicators.

1. Introduction

The atmospheric concentration of ammonia (NH_3) is often high in countries with intensive agriculture and animal husbandry (Graedel et al., 1995). The major sources of atmospheric NH_3 in Europe are livestock farming, fertilizers and some industrial activities (Asman, 1992). Emissions from livestock farming contain a cocktail of compounds: NH_3, CO_2, gaseous amines and dust, including nutrient-containing particles, such as N, P and K, which may modify plant communities (Cape et al., 2009). In the atmosphere, ca 30% of NH_3 is converted to ammonium (NH_4^+), which may further react, be dispersed with aerosols or be removed from the atmosphere mainly by wet deposition (Asman and Janssen, 1987).

NH_3 is the main source of dry deposition of atmospheric N around livestock husbandries, and as a gas it may influence lichen communities composition (Frati et al., 2008). Owing to the fact that atmospheric NH_3 has been widely measured in areas of northern Europe with intensive agriculture and livestock farming (e.g. van Herk, 1999; Sutton et al., 2003, 2009), it has been possible to recognize that nitrophilous epiphytes are positively correlated with NH_3 (Sparrius, 2007) and that NH_3 enhances nitrophilous- and decreases acidophilous epiphytes mainly indirectly by rising bark pH (van Herk, 2001). In particular, prolonged exposures (years) to atmospheric NH_3 concentrations >3 μg/m^3 promotes nitrophilous lichens (van Herk et al., 2003) and rise bark pH of local trees within 2–3 km from a point source (van Herk, 2001).

In the Mediterranean area, also dust and dry conditions lead to an increase in bark pH enhancing nitrophilous lichens, most of which are also species of xeric environments (Loppi and De Dominicis, 1996; Loppi et al., 1997), complicating the detection of the effects of N compounds (Frati et al., 2008).

Concern for the increasing deposition of NH_3 is mainly focused on biological effects at community level, i.e. when changes or damages to the environment have already occurred. On the other hand, monitoring changes at physiological level may help to detect early stress symptoms. Lichen transplants proved to offer rapid information on occurring stress by the analysis of selected physiological parameters (Paoli and Loppi, 2008).

The aim of the present study was to test the hypothesis that NH_3 pollution in the Mediterranean area directly influences the photosynthetic performance of lichens.

2. Materials and methods

2.1. Study area

A point source of NH_3 (35°18′47″N, 25°01′40″E), represented by a sheep farm, was selected in the lowlands of Northern Crete (Greece), where climate is xerothermic and more than 125 dry days occur per year. During the experiment (see

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below) there was no rainfall, the weather was dry and warm, with temperatures up to 38 °C. Dueing to these conditions, lichens could only be partially hydrated by dew in the night or humidity from the sea.

Farming is a traditional and important sector of Crete's agricultural production and the island as a whole hosts more than one million sheep and goats that are mostly farmed by small landholders (Volanis et al., 2007).

The selected farm has around 150 heads, sheep were kept outdoors for most of the year and feeding was based chiefly on the available spontaneous vegetation. Flocks were free to graze and regularly gathered in traditional small stocks, e.g. for milking.

2.2. Lichen material and experimental design

Twigs (ca. 30 cm in length) carrying respectively a minimum of 5 thalli of the lichens *Evernia prunastri* and *Pseudevernia furfuracea* were harvested in May 2005 from a rural area in Mt. Olympos (Northern Greece) far from any local pollution source and let to acclimate 3 weeks at ambient light and temperature in Northern Crete (Greece). The species were selected being extensively used in biomonitoring surveys. Both have *Trebouxia* algae as a photobiont and a fruticose growth form, but have a slightly different ecology: *P. furfuracea* is considered a xero-mesophytic acidophilous species and *E. prunastri* more hygro-mesophytic and less acidophilous. On the 15th of June 2005 lichen samples were exposed in the neighbourhood of the sheep farm and retrieved respectively after 15 and 30 days of exposure.

Lichens were exposed at the farm, i.e. 0–5 m from the boundary of the stocking area and at a radial distance of 60–80 m. Another batch of samples was exposed in a remote area 5 km from the farm and was used as a control. Lichens were transplanted together with their carrying substrate using a fishing-net (mesh size 2.5 cm) containing twigs of both species, at 2 m above ground on the north side of local trees, tied directly to the brunches or protected in plastic cages to prevent possible damage by grazing. Ten lichen-nets were exposed at each site as replicates. Experimental conditions were therefore similar at all sites, except for distance from the farm.

2.3. Ammonia monitoring

Atmospheric NH_3 was measured by passive samplers using diffusion tubes (Radiello®, Aquaria). At each site, 5 samplers were placed together with lichen thalli at a height of around 2 m above ground for one 7-day period in the middle of the experiment (between June, the 30th and July, the 7th). Samplers contained a filter impregnated with phosphoric acid which adsorbs gas-phase NH_3 as NH_4^+, that can be easily measured spectrophotometrically by the indophenol blue method (Allen, 1989). The detection limit was 0.6 μg/m^3; uncertainty was 6.5%.

2.4. Chlorophyll fluorescence analysis

After each retrieval, lichen samples were air dried and stored at −20 °C. A physiological recovery of the lichens was carried out prior to measurements. To avoid any osmotic stress by air humidity after the freezing, samples were left 15 min in dry ambient conditions. They were subsequently sprayed with water until wet and the excess water was removed by hand-shaking. Samples were then stored at 4 °C in the dark for 24 h. The outermost 2 cm of the thalli were then randomly selected for measurements, but avoiding areas with excess of vegetative structures (isidia and soredia). Measurements were carried out with a Plant Efficiency Analyser (PEA, Hansatech Ltd, UK). After dark-adaptation for 10 min, samples were lightened for 1 s with a saturating excitation pulse (1800 μmol s^{-1} m^{-2}) of red light (650 nm) from a LED into the fluorometer sensor and fluorescence emission recorded. All the fluorescence transients were recorded with a time span from 10 μs to 1 s. Data were acquired with a time resolution of 10 μs for the first 2 ms and later on the instrument automatically turned to slower registration rate. Ten fluorescence emission curves were recorded for each retrieval in each site for both species.

Chlorophyll *a* fluorescence emission was analysed as follows:

I) to assess if NH_3 emitted from a sheep farm affects lichen photosynthesis, we first used the classical physiological indicator of photosynthetic efficiency F_V/F_M, representing the potential quantum yield of primary photochemistry (Maxwell and Johnson, 2000).

II) Secondarily, we described the effects of NH_3 on fluorescence kinetics. The fast fluorescence kinetic typically outlines a transient curve: when the curve is plotted on a log-time axis a sequence of steps called O–J–I–P, each corresponding to its changing inclination, is apparent (Strasser et al., 2000).
The minimal fluorescence F_0 is measured at 50 μs and corresponds to O, the J-step is recorded at 2 ms, the I-step at 30 ms and P at about 300 ms, generally in correspondence of maximal fluorescence F_M. The value of fluorescence emission recorded at these points is used to calculate a series of parameters, which serve to translate original data to biophysical parameters that quantify energy fluxes and their ratios, physiological states, conformation and overall performance of the samples (Strasser et al., 2000).

III) On a third step we therefore evaluated the effects of NH_3 on the functioning of PSII through the JIP-test. Information concerning the theory and derivation of the formulae is described e.g. in Strasser et al. (2000, 2004).

The energy cascade from light absorption by PSII to electron transport involves the absorption of photon flux (ABS) by antenna pigments, creating excited chlorophyll. The excitation energy is partly dissipated (DI_0) as heating and fluorescence emission, and in part is profitably addressed to the reaction centre (RC) as trapping flux (TR_0); in the RC the excitation is converted into redox energy by reducing the electron acceptor Q_A to Q_{A-} which is then reoxidised to Q_A leading to the electron transport (ET_0) and later to CO_2 fixation (Strasser et al., 2004).
To quantify energy fluxes we used the following parameters that refer to time zero, when all reaction centres of PSII are open:

- $ABS/RC = (M_0/V_J)/[1 - (F_0/F_M)]$ is the equation representing the absorbed energy per RC of PSII
- $TR_0/RC = M_0/V_J$ is the specific trapping flux at time zero per RC
- $DI_0/RC = ABS/RC - TR_0/RC$ is the energy flux which is dissipated chiefly as heat
- $ET_0/RC = M_0/VJ\cdot(1 - VJ)ET_0/RC = M_0/V_J\,(1 - V_J)$ is the energy flux corresponding to the effective electron transport per RC
where $V_J = (F_{2\ ms} - F_0)/(F_M - F_0)$ and $M_0 = 4\cdot(F_{300\mu s} - F_0)/(F_M - F_0)$ is the initial slope of the curve, representing a measure of Q_A reduction in the first 250 μs, multiplied by 4 to give the value at 1 ms.

To quantify phenomenological energy fluxes per excited cross-section (CS):
- ABS/CS for light absorption
- TR_0/CS for excitation energy trapping
- DI_0/CS for heat dissipation
- ET_0/CS for electron transport.

The fraction of active RCs per excited cross-section (RC/CS) and the total number of active RCs per absorption (RC/ABS) were also considered.
To quantify flux ratios:
- $\Psi_0 = ET_0/TR_0$ expresses the probability that a trapped exciton, a quantum of electronic excitation, enters the transport chain and moves an electron further than Q_A;
- $\varphi P_0 = TR_0/ABS$ expresses the probability that an absorbed photon will be trapped by the reaction centre of PSII, it represents the maximum quantum yield of primary photochemistry and roughly corresponds to F_V/F_M;
- $\varphi D_0 = DI_0/ABS$, expresses the probability that excitation energy will be dissipated in the antenna chlorophyll, being $\varphi P_0 + \varphi D_0 = 1$;
- $\varphi E_0 = ET_0/ABS$ is the maximum yield of electron transport ($=\psi_0\times\varphi P_0$).

Some technical parameters are referred to the area above the transient curve:
- $Sm = Area/(F_M - F_0)$ is a measure of the energy needed to close all the RCs, accounting the multiple turnover in the closure of the RCs, where Area is the area growth between the fluorescence curve and the maximal fluorescence signal;
- the expression $N = S_m\cdot M_0/V_J$ indicates how many times Q_A has been reduced to Q_A in the time span from t_0 to t_{Fmax};
- the ratio S_m/t_{Fmax} expresses the average redox state of Q_A^-/Q_A, that means the average fraction of open RCs during the time needed to complete the closure of all the RCs.

IV) The performance index PI_{ABS}, a global indicator that resumes the contribution of all parameters, was used to express the overall vitality of the samples:

$$PI_{ABS} = RC/ABS\cdot\varphi P_0/(1 - \varphi P_0)\cdot\psi_0/(1 - \psi_0)$$

2.5. Statistical analysis

Significance of differences ($P < 0.05$) among various treatments and controls was checked by one-way analysis of variance (ANOVA), using the Bonferroni test for post-hoc comparisons. Prior to analysis data not matching a normal distribution (Kolmogorov–Smirnov test at the 95% confidence interval) were treated with Box-Cox transformation.

3. Results

3.1. Concentrations of NH_3 around the sheep farm

Atmospheric concentration of NH_3 recorded by passive samplers for 7 days in the middle of the experiment was 62.4 ± 4.3 μg/m^3 at the sheep farm, 15.0 ± 1.5 μg/m^3 at a radial distance of 60 m and 1.3 ± 0.9 μg/m^3 at a neighbouring remote control area.

3.2. Effects of NH_3 on the potential quantum yield of PSII

Lichens transplanted in close proximity of a point source of NH_3 showed a marked decrease in the potential quantum yield of PSII already after 15 days, as indicated by the low values of the