BIOLOGICAL EFFECTS OF AMMONIA RELEASED FROM A COMPOSTING PLANT ASSESSED WITH LICHENS

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Abstract

In this study we investigated whether ammonia emissions from industrial composting of organic waste may influence the surrounding environment, using lichens as bioindicators. To this purpose, samples of N-tolerant and N-sensitive lichens, namely *Xanthoria parietina* and *Evernia prunastri*, were transplanted for 1–3 months along transects at increasing distance (0–400 m) from a composting facility in Tuscany, Italy. Atmospheric concentrations of ammonia were measured using passive samplers. The physiological response of lichen transplants was investigated by means of the photosynthetic efficiency (measured as chlorophyll *a* fluorescence emission), the integrity of cell membranes (measured as electrolyte leakage), and sample viability (measured as enzymatic activity of dehydrogenase). Epiphytic lichen communities were investigated using biodiversity indices. The results showed decreasing concentrations of ammonia, from 48.7 μg/m^3 at the composting facility, to 2.7 μg/m^3 at 400 m. The N-tolerant *X. parietina* was not affected and some physiological parameters even showed a higher performance, while the N-sensitive *E. prunastri* showed a reduced performance with increasing atmospheric concentrations approaching the source. A shift from lichen communities composed by meso-acidophilous species (actual condition) to more nitrophilous communities in the near future, approaching the composting facility is suggested. It is concluded that lichens can provide useful data for decision-makers to establish correct science-based environmentally sustainable waste management policies.

Keywords: Ammonia, Bioindicators, Industrial composting, Lichens, Photosynthetic performance, Transplants

1. Introduction

The application of "friendly" environmental technologies to reduce landfilling of organic wastes, improving recycling of organic matter and nutrients, is a key point within the Integrated Pollution Prevention and Control plan foreseen by the EU legislation. As a matter of fact, the European Commission (1999) targeted a decrease in the amount of organic waste being landfilled by 20 % in 2010 and by 50 % in 2050.

The industrial aerobic treatment of organic wastes (composting) utilizes a naturally-occurring biochemical process of controlled aerobic decomposition of organic material and transforms crop residues, organic municipal wastes, industrial organic wastes and animal faeces into compost. To obtain high-quality compost, the piles of organic material are watered and turned over a period of weeks or months and by controlling the levels of oxygen and balancing the C:N ratio through proper mixing of green and brown materials. The end-product of composting is a moist soil-like product, with earthy odour (Kumar et al., 2011).

Composting is regarded as an environmental friendly process, but it is by no means free from causing environmental concern. A composting facility may involve several pollution and odours sources e.g. reception of the material and handling, forced aeration, stock piling (Cadena et al., 2009) and the process of waste decomposition releases odours, airborne particles and bioaerosols (Park et al., 2011). Compost emissions may include volatile organic compounds (VOCs), sulphur- (S) and nitrogen- (N) based compounds, as well as greenhouse gases such as nitrous oxide (N_2O) and methane (CH_4) (Hellebrand, 1998; Kumar et al., 2011). In addition, a certain amount of N can be locally released to the environment as ammonium/ammonia (NH_4^+/NH_3) (Kumar et al., 2011; Zeng et al., 2012). In fact, NH_3 is considered the main N gas emitted during the composting process (Clemens and Cuhls, 2003; Ko et al., 2008; Cadena et al., 2009). Therefore, different techniques, based on oxidation, absorption, adsorption and biofiltration are used to yield an effective NH_3 removal and reduce the N loss during composting of different organic wastes (Busca and Pistarino, 2003; Pagans et al., 2006; Wu et al., 2011).

At the ecosystem level, lichens are among the most sensitive organisms to NH_3 pollution (Cape et al., 2009), having a differential response depending on their functional response group (Pinho et al., 2008; 2009). Oligotrophic species are extremely sensitive to NH_3 pollution and react by retreat, while nitrophilous species are tolerant and may spread abundantly in N-rich environments (Pinho et al., 2008).

After a prolonged exposure to NH_3, the epiphytic lichen vegetation is influenced both by the toxic effect of NH_3 pollution on acidophilous species, and by the rising of bark pH determined by N depositions, which indirectly enhances nitrophilous lichens (Van Herk, 2001). Furthermore, nitrophilous species respond to multiple N deposition products (Jovan et al., 2012), that affect lichens especially when internalized by the thallus (e.g. under humid conditions). It has been shown that the photosynthetic apparatus of lichens exposed to ecologically relevant NH_4^+/NH_3 concentrations is susceptible to these pollutants (Paoli et al., 2010; Munzi et al., 2012b).

In the present study we investigated whether NH_3 emissions from industrial composting influence the lichens in the surrounding environment. Our working hypothesis is that, in N-rich environments (e.g. around point sources of NH_3), nitrophilous species would be rapidly enhanced and correspondingly, non nitrophilous ones would be negatively affected, allowing early signs of stress to be rapidly detected at physiological level. To the purpose, we transplanted a N-sensitive species (*Evernia prunastri*) and a N-tolerant species (*Xanthoria parietina*) around a point source of NH_3 represented by a composting facility in Tuscany (central Italy). The aims of the study were to detect early signs of environmental changes around point sources of NH_3 by means of lichens and to foresee future variation in lichen communities caused by NH_3.

2. Materials and Methods

2.1 Study area

The study area is located along a narrow valley in proximity of the Apennines in Tuscany, Italy (44°02'33" N, 10°42'20" E, 350 m asl), where a composting facility is operating since 2010. The composting facility treats 31,000 tons/y of organic waste originating from forest management, cutting and pruning from private and public gardens and parks (4,500 tons/y); sewage sludge (9,500 tons/y); organic matter form municipal waste (16,000 tons/y). The production of compost starts by mechanical mixing of the selected shredded material. The following step may last up to 27 days and consists in oxidation and sanitation under controlled conditions (temperature of the material ca. 67 °C, optimal moisture 60–65 %). After an intermediate mechanical screening (mesh 70 mm), other 44 days may be necessary for maturation. Finally, refining and

screening (mesh 14 mm) complete the production. The composting plant is the sole main source of NH_3 pollution in the area. The vegetation of the area is characterized by forests of *Castanea sativa* with scattered *Quercus cerris*. Prevailing winds flow along the narrow valley, chiefly towards NW. The hottest month is July (average T = 20.6 °C) and the coldest is January (average T = 3.9 °C). Average annual rainfall is 1,460 mm, with the main raining events occurring during October – December and March – April.

2.2. Experimental design

The study was based on four transects in the directions NE, SE, SW, NW centred on the composting facility. For each transect, a first level of sampling sites was set up at a distance of 0–50 m from the source (along the border of the facility), a second level at ca. 200 m, and a control site at a distance of ca. 400 m. These distances were selected according to several studies in the literature reporting a sharp decrease (up to 98%) of atmospheric NH_3 within the first 200 m from the source (Frati et al., 2007; Fowler et al., 1998; Pitcairn et al., 2002).

2.3 Atmospheric ammonia

Atmospheric NH_3 was measured by diffusion tubes passive samplers (Radiello®, Aquaria). At each site, two samplers were placed at a height of ca. 2 m above the ground, during two working weeks of the composting plant (i.e. 18–25 March 2011 and 18–25 May 2011). Samplers contained a filter impregnated with phosphoric acid which adsorbs gas-phase NH_3 as NH_4^+, which can be measured spectrophotometrically by the indophenol blue method (Allen, 1989). Detection limit was 0.7 $\mu g/m^3$, uncertainty was 6.5%.

2.4 Lichen transplants

To investigate the biological effects of NH_3 emissions from industrial composting we collected samples of the lichens *Xanthoria parietina* (N-tolerant) and *Evernia prunastri* (N-sensitive) from a background area (Murlo – 43°11'06" N, 11°21'40" E 310 m asl). Samples were kept 4 days in a climatic-chamber, ensuring standardised pre-conditioning at 16±1 °C, RH 55+5 %, photoperiod of 12 hours at 40 $\mu mol\ m^{-2}\ s^{-1}$ photons PAR and then exposed in the study area.

Xanthoria parietina (L.) Th.Fr. is a foliose lichen with wide ecological amplitude, forming yellow-orange rosettes, with lobes broadening towards the apices. For the transplant experiment we selected *Quercus* branches, each carrying at least 10 thalli. *Evernia prunastri* (L.) Ach. is a fruticose (shrub-like) lichen with lobes up to 5 mm wide, palmately branched, green-grey upper surface and white lower surface. Due to the high surface-volume ratio and the easiness of preparation for analyses, the species is often used in monitoring studies (Paoli et al., 2011). For the transplant experiment we selected thalli of 5–7 cm. Both species have a green-algal photobiont (*Trebouxia*). They are characterized by a different sensitivity to N: *E. prunastri* grows in sites with no or weak eutrophication and is sensitive to excess N in the environment, whereas *X. parietina* may grow in sites with high levels of eutrophication (Nimis and Martellos, 2008).

Lichen samples were transplanted at a height of ca. 2 m above the ground together with their carrying substrate, i.e. simulating their natural conditions, on the 18th of February 2011; one set of samples remained exposed for 1 month and another for 3 months.

Microclimatic data were recorded at each site (Tab. 1) to ensure that lichen transplants have been exposed under similar environmental conditions, except for the distance from the source.

After each retrieval, lichen samples were air dried and stored at -18 °C for later physiological measurements, since freezing of air dried samples for a few weeks does not alter the status of the thalli (Paoli et al., 2013a).

2.5 Cell membrane damage

A simple test to check the integrity of the plasma membrane enclosing lichen cells is to place a piece of lichen thallus in deionized water and measure the variation in electrical conductivity (Marques et al., 2005). In damaged cell membranes, permeability is altered and electrolyte leakage occurs, mainly K^+ ions, which are the most abundant (McKersie et al., 1982).

Subsamples of ca. 100 mg were taken from each sample and soaked shaking for 1 h in 50 mL of deionized H_2O. The electrical conductivity of the water (expressed in μS cm^{-1} at a normalized temperature of 25 °C) was measured before and after lichen immersion using a conductivity-meter (Basic 30, Crison Instruments). Thalli were then boiled for 10 min to cause total rupture of cell membranes, and conductivity measured

again. Relative conductivity (EC%) was expressed as % between the ratio of conductivity after 1 h soaking to that after boiling, after accounting for the initial conductivity of deionized water.

2.6 Viability

Reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) is directly linked to the activity of the mitochondrial respiratory chain (Ruf and Brunner, 2003). In particular, this conversion is driven by dehydrogenase systems and is a good indicator of dehydrogenase activity (Bačkor and Fahselt, 2005), and has being used for a long time to indicate normal functioning of plant seeds and other plant tissues. The lichen material (ca. 15 mg) was incubated in the dark for 20 h in 2 mL of 0.6% TTC and 0.005 % Triton X 100 solution in 50 mM phosphate buffer (pH 6.8). Solutions were then removed and samples rinsed in distilled water until bubbles of Triton X were produced. Water-insoluble formazan was extracted with 6 mL of ethanol at 65 °C for 1 hour. Tubes were then centrifuged at 4000 g for 10 min and absorbance red at 492 nm. Results were expressed as absorbance units/g (dw).

2.7 Photosynthetic efficiency

The classical indicator of chlorophyll fluorescence, F_V/F_M, representing the potential quantum yield of primary photochemistry, was measured. In addition, the performance index (PI_{ABS}), a global indicator of the photosynthetic performance (Strasser et al., 2000), was calculated. In order to analyse each sample, the selected lichen material was hydrated and then dark-adapted with a clip for 10 min to allow full dark adaptation of the photosynthetic pigments. Lichens rested on a foam pad whilst in the clip to minimize damage to the thalli. Samples were then lightened for 1 sec with a saturating 3,000 μmol m^{-2} s^{-1} light pulse. Fluorescence emission was recorded for 1 sec with a Plant Efficiency Analyzer (Handy PEA, Hansatech).

2.8 Lichen diversity

The lichen diversity was measured on 12 trees of *Castanea sativa* and *Quercus cerris*. Trees were deemed suitable if well lit, with girth >60 cm, trunk almost straight (deviation from the vertical <20 °), not damaged and without parts with >25 % bryophyte cover. Lichens were sampled with a grid consisting of four 50×10

cm^2 ladders, each divided into five 10×10 cm^2 squares. For each relevé (corresponding to a cardinal exposure) the grid was positioned systematically on the N, E, S and W aspects of the trunk of each tree, between 1 and 2 m from the ground (Asta et al., 2002).

All lichen species growing within the grid were identified and the number of squares in which each species was found was recorded. An Index of Lichen Diversity (ILD) was calculated as the sum of frequencies of epiphytic lichens on each sampled tree (Asta et al., 2002). Measurements were made in autumn 2010, i.e. at the beginning of the operations of the composting facility, and repeated in spring 2013, exactly on the same surfaces. Only stations at 200 and 400 m from the source were sampled, since at the composting facility suitable trees were lacking. The ILD values were interpreted in terms of effects of air pollution according to the following scale (Paoli et al., 2012): 0 = very high (lichen desert), 1–40 = high, 41–80 = moderate, 81–120 = low, >120 = negligible. Lichen frequencies were also evaluated by grouping the species according to the functional traits of biodiversity in response to eutrophication, in particular grouping nitrophilous species according to the classification of the lichens of Italy by Nimis and Martellos (2008). Species with maximum score 4 or 5 were considered as nitrophilous and used to calculate the ILD_{nitro} (Loppi, 2004). The ILD_{nitro} values were interpreted in terms of effects of N air pollution according to the following scale (Ruisi et al., 2005): 0 = negligible, 1–20 = low, 21–40 = moderate, 41–60 = high, >60 = very high.

2.8 Statistical analysis

After checking the normality of data distribution (Shapiro-Wilk, 95% confidence interval) and in case log-correct for skewed distributions, a two-way analysis of variance (ANOVA) was run separately for *E. prunastri* and *X. parietina*, as well as for ILD values, to investigate the effects of distance from the facility and duration of the exposure (2010 vs. 2013 for ILD data) on the investigated parameters, using the Tukey test ($P<0.05$) for post hoc comparisons.

3. Results

3.1 Atmospheric ammonia

Atmospheric concentrations of NH_3 measured with passive samplers amounted to 48.7±18.9 μg/m^3 at the composting facility and sharply decreased to 8.1±0.9 μg/m^3 at 200 m, and went further down to 2.7±0.6 μg/m^3 at 400 m.

3.2 Physiological response of the transplants

Prior to the transplant experiment, all investigated physiological parameters were measured in control samples (Tab. 2) and corresponded to values of healthy lichens (Munzi et al., 2010; Paoli et al., 2011; Pisani et al., 2011).

Table 3 summarizes the results of ANOVA. In *E. prunastri* the investigated parameters significantly changed as a function of the distance from the composting plant, while cell membrane integrity and viability were also influenced by the time of exposure; no time effect was found for photosynthetic efficiency parameters. On the contrary, in *X. parietina* photosynthetic efficiency was the only parameter that changed and only according to the exposure time.

In detail, after the transplant, the N-sensitive *E. prunastri* (Fig. 1) exposed for three months showed a decrease of the overall thallus viability (expressed as dehydrogenase activity), a decrease of cell membrane integrity (expressed as EC%) reflecting an alteration of membrane permeability and a decrease of the photosynthetic performance of the photobiont (expressed as F_V/F_M and PI_{ABS}) comparing thalli at the composting facility and samples exposed at 200 m and at 400 m. EC% at the composting facility was significantly higher than at the other sites already after the first month of exposure and in general it increased in time in the whole area, i.e. comparing thalli retrieved after 1 and 3 months of exposure. Similarly, viability of samples decreased at the facility comparing thalli collected after 1 and 3 months (P<0.05).

As far as the N-tolerant *X. parietina* is concerned (Fig. 2), no significant difference among sites was found comparing EC%, viability and photosynthetic performance after both 1 and 3 months of exposure. However, a temporal increase of the photosynthetic performance (in particular PI_{ABS}) at the composting facility and at 200 m comparing thalli collected after 1 and 3 months (P<0.05) was observed.

3.3 Lichen diversity

Table 4 summarizes the results of lichen diversity. The average ILD_{tot} of the ~~whole~~ study area was 73±14 in 2010 and 75±12 in 2013, indicating that accounting the area as a whole, the overall condition ~~of the study area~~ did not change over the 3 years of study. The results of the statistical analysis indicated a significant difference in ILD_{tot} at 200 and 400 m from the composting facility, both in 2010 and 2013. Values of ILD_{tot} indicated a condition of moderate air pollution at 200 m and low air pollution at 400 m (Paoli et al., 2012). The distance effect could result from a pre-existing pollution gradient, since the facility is close to the main road of the area. Despite the overall ILD_{nitro} for the whole study area did not change in time, a significant (P<0.05) temporal difference emerged for sites at 200 m, with higher values measured in 2013 and a spatial difference in 2013, with higher values at 200 m. ILD_{nitro} values indicated a situation of moderate eutrophication at 400 m (both in 2010 and 2013) and at 200 m in 2010, rising to high eutrophication in 2013 (Ruisi et al., 2005).

4. Discussion

4.1 Atmospheric ammonia

During compost production, NH_3 is released from both the aerobic and anaerobic decomposition of proteins and amino acids (Haug, 1993). NH_3 and other odour emissions are chiefly released in the first weeks of material handling (Schlegelmilch et al. 2005), in particular when thermophilic temperatures (> 45 °C) are associated to a high (>9) compost pH (Beck-Friis et al., 2001). Cadena et al. (2009) estimated that each Mg of the organic fraction of municipal solid waste treated in a composting plant may release up to 3.9 kg of atmospheric NH_3. During the aerobic composting of biowaste, concentrations of NH_3 in the waste gas up to 227 mg/m^3 were reported (Smet et al., 1999), while NH_3 concentrations up to 700 mg/m^3 were found in exhaust gases from sludge composting (Haug, 1993). Despite these huge concentrations, studies on the dispersion of bioaerosols and associated odour emissions from composting plants showed that background concentrations are generally achieved within 200–300 m from the source (Herr et al., 2004; Prasad et al., 2004). Our results are perfectly in line with these findings and suggest that in our study concentrations of NH_3 are highly fluctuating, with peaks during outdoor handling and maturation of the compost. In fact, the

residence time of NH_3 in the atmosphere may vary from few hours to 4 days, depending on the season, source and site characteristics (Krupa, 2003). As a matter of fact, our mean atmospheric NH_3 concentration of 48.7 $\mu g/m^3$ at the composting facility is perfectly in line with the 45.4 $\mu g/m^3$ measured at a cow farm in Italy (Frati et al., 2008) and the 62.4±4.3 $\mu g/m^3$ recorded at a sheep farm in Greece (Paoli et al., 2010). Also the background NH_3 concentrations of 2.1 and 1.3 $\mu g/m^3$ respectively reported in these latter studies are consistent with the 2.7 $\mu g/m^3$ found in the present study at 400 m.

4.2 Ammonia and lichen physiology

Transplanting lichens with different sensitivity to NH_3 allowed detection of early physiological indications of potential biological changes before consequences are apparent at the community level. The N-tolerant species *X. parietina* was not affected by the proximity to the facility and some parameters even suggested a better performance, while on the opposite, the N-sensitive species *E. prunastri* showed reduced performances approaching the NH_3 source.

Evernia prunastri is known for being a N-sensitive lichen, decreasing or even disappearing in presence of excess N in the environment (Van Herk, 1999; 2001) and reappearing in sites experiencing decreasing N pollution (Lackovičová et al., 2013).

The physiological bases for the higher sensitivity of *E. prunastri* to excess N compared with *X. parietina* depend, at least in part, on its higher (ca. 5-fold) cation exchange capacity (Gaio-Oliveira et al., 2001), the higher surface-volume ratio (being a fruticose lichen) and the higher uptake capacity of N compounds as an adaptation to deal with low N concentrations in the environment (Gaio-Oliveira et al., 2005a). Moreover, in *E. prunastri* physiological parameters have been shown to respond gradually to increasing N concentrations, with more marked effects either increasing the N concentration or the duration of the exposure (Munzi et al., 2012).

When released into the environment, NH_3 is readily converted to NH_4^+ or subject to dry deposition (Fangmeier et al., 1994). Under dry conditions, lichen metabolism is kept at minimal level and NH_3 would dry deposit onto the lichen surface. Then, that deposited NH_3 may have an effect when internalized by the lichen, the next time sufficient moisture (humidity or precipitation) is available. Hence, NH_3 should correlate

well with lichen responses especially where it dominates N emissions and depositions. Humidity facilitate lichen exposure to air pollutants and during the experimental period in the study area, lichens encountered chiefly humid conditions. Toxicity by NH_4^+/NH_3 has been extensively studied in higher plants (Fangmeier et al., 1994; Britto and Kronzucker, 2002) and detrimental effects can be early detected at physiological level, involving alteration of secondary metabolism and changes due to increased N uptake and assimilation (Fangmeier et al., 1994). Since NH_4^+ is cytotoxic and cannot be stored without damage, it should be converted into aminoacids (Neuhäuser et al., 2007). However, NH_4^+ assimilation requires C skeletons and the lichen *E. prunastri* cannot compensate for elevated NH_4^+ levels with higher C assimilation (Munzi et al., 2013). The lower cation exchange capacity of *X. parietina*, which allows avoiding excess N uptake, and the ability to provide C skeletons for N assimilation, might explain the higher N-tolerance of this species (Gaio-Oliveira et al., 2005a).

Although the N critical load in forest lichens of Europe has been set to 2.4 kg ha^{-1} yr^{-1} (Giordani et al., 2013), it was estimated that *X. parietina* can tolerate a N deposition load of 1,000 kg ha^{-1} yr^{-1} (Gaio-Oliveira et al., 2004). In fact, only weekly treatments over a 10 months period with extreme N concentrations (0.34 M NH_4Cl and above), lead to damages to both the photobiont and the mycobiont, as evidenced by reduced chlorophyll *a* and ergosterol concentrations in the thalli (Gaio-Oliveira et al., 2004).

In case of higher N availability in the environment, the photosynthetic capacity should be increased to the same extent as the energy demand following an enhanced N assimilation (Turpin, 1991). Nitrophilous species such as *X. parietina* are able to increase their photosynthetic capacity at NH_4^+ levels detrimental to acidophilous species (Gaio-Oliveira et al., 2005b). This finding is coherent with our observation that samples of *X. parietina* exposed at the composting facility and at 200 m increased their photosynthetic performance (in particular PI_{ABS}) when compared with samples exposed at 400 m, already after one month of exposure.

Although it may be argued that is the different growth form that may influence the differential response to NH_3, with *X. parietina* being a foliose lichen and *E. prunastri* a fruticose one, there is evidence that it is not (only) a matter of growth form. Munzi et al. (2012a) collected samples of *X. parietina* from both a N-rich and a N-poor site and in this latter site also the N-sensitive *E. prunastri* and *Usnea sp*. were collected. All samples were treated with excess N. The results showed that *X. parietina* from the N-poor site behaved more

similarly to *E. prunastri* and *Usnea* sp. than to *X. parietina* from the N-rich site (Munzi et al., 2012b). The Authors elegantly showed that the response of *X. parietina* to excess N was primarily determined by its native N environment.

The tolerance of lichens to reactive N may also be related with their specific metabolic characteristics. Pirintsos et al. (2009) suggested that polyamines may play an important role in modulating lichen tolerance to N stress and found that external supply of polyamines increased the resistance of *E. prunastri*, while inhibitors of polyamine biosynthesis reduced the tolerance of *X. parietina*.

Relevant NH_4^+/NH_3 levels may result toxic to the photosynthetic apparatus as they function as electron acceptors, uncoupling electron transport (Losada and Arnon, 1963). Therefore, also the photosynthetic performance of the lichen photobiont can be a suitable indicator of the biological effects of NH_4^+/NH_3 in the environment (Munzi et al., 2010; Paoli et al., 2010). Our results indicated that photosynthetic performance, integrity of cell membranes and dark respiration (as indicated by dehydrogenase activity) were altered in *E. prunastri* exposed at the composting facility, despite the measured values did not really reflect a dramatic change. In fact, our samples still did not show visible symptoms of damage induced by the release of NH_3. Frati et al. (2007), assessing the biological effects of NH_3 released from a pig farm in a Mediterranean environment of central Italy, found visible signs of injury in samples of *Flavoparmelia caperata* exposed in the centre of the pig farm (where a peak of 267 $\mu g/m^3$ NH_3 was measured), while these symptoms were not visible in *X. parietina* thalli. This indicates that the former is a more sensitive species and confirms that the latter is in general a resistant species to NH_3 pollution. Our results, concerning the viability of the samples assessed by TTC reduction, suggested that NH_3 may impair the dark respiration of the lichen *E. prunastri*. Decrease of respiratory dehydrogenase activity has been similarly reported, e.g. in the aposymbiotically grown mycobiont of the lichen *Cladonia cristatella* exposed to copper excess (Bačkor et al., 2006) and in the lichen *X. parietina* treated with boron (Pisani et al., 2009) arsenic (Pisani et al., 2011) and antimony (Paoli et al., 2013b).

High levels of NH_4^+/NH_3 may damage membrane lipids of plant cells (Fangmeier et al., 1994), and in lichens are known to cause the alteration of membrane permeability (Munzi et al., 2009). The damage endured by cell membranes of *E. prunastri* can be used as a suitable parameter for monitoring the effects of acute N

pollution (Munzi et al., 2009). However, in *E. prunastri*, long-lasting treatment with ecologically relevant NH_4^+ concentrations (50–500 μM) did not cause a significant alteration of membrane permeability, but decreased the photosynthetic performance of the photobiont (Munzi et al., 2012b).

The above evidences of a decreased photosynthetic performance, increased cell membrane damage and decreased viability allow to hypothesize that our samples were likely exposed to episodes of acute NH_3 release from the composting plant, which affected both symbiotic lichen partners in the sensitive *E. prunastri* but were ineffective in the resistant *X. parietina*.

4.3 Ammonia and lichen diversity

Atmospheric NH_3 is an important driver of the epiphytic lichen vegetation (Van Dobben and Ter Braak, 1998; Hauck, 2010). Comparing our biodiversity data measured in 2010 and 2013, we observed biological indications of an increasing eutrophication in the surroundings of the composting plant, as suggested by the diffusion of nitrophilous lichens in sites concerned by NH_3 deposition. It is known that in areas with high atmospheric NH_3 the absorption of NH_3 rises bark pH and consequently enhances nitrophilous lichens and disadvantages acidophilous ones (Van Herk, 1999). This phenomenon is particularly evident on acid substrates, where nitrophilous species are naturally scarce (Van Dobben and Ter Braak, 1998; Frati et al., 2008). Correspondingly, it was also shown a decrease of nitrophilous lichens in areas experiencing falling NH_3 levels (Sparrius, 2007). However, it should be pointed out that besides bark pH, other bark chemical and physical properties related to tree species and age are important in determining modifications of lichen communities (Spier et al., 2010).

The long-term (several years) critical level for atmospheric NH_3 in Europe, which was 8 $\mu g/m^3$ as annual average concentration, has been actually revised for lichens and bryophytes down to 1 $\mu g/m^3$ (Cape et al. 2009). This critical level indicates that the lichen communities around our composting facility are likely to be changed in the future if atmospheric level of NH_3 will remain constant. Moreover, it suggests that also the lichen communities at 400 m from the composting facility, which are exposed to average NH_3 concentrations ca. 2–3 times higher of this new critical level, may be concerned.

The effects of atmospheric depositions of NH_3 on vegetation have been reviewed by Fangmeier et al. (1994). Generally, in agricultural areas, NH_3 levels sharply decrease with distance from high-intensity point-sources, with a parallel shift in lichen communities from nitrophilous to oligotrophic ones. Pinho et al. (2012) investigated the biological effects of NH_3 around a cow-barn in Portugal and found an exponential decrease of both NH_3 concentrations and N deposition already within a distance of 130 m from source, in the direction of prevailing winds. Moving toward the cow-barn, the average NH_3 increased from 1.5 $\mu g/m^3$ (background) to 34 $\mu g/m^3$ (with a peak of 78 $\mu g/m^3$). Concerning lichen diversity, increasing NH_3 led to a complete replacement of oligotrophic species by nitrophilous ones within 65 m from the NH_3 source (Pinho et al., 2011). Frati et al. (2007) found out that although the total diversity of epiphytic lichens on *Q. pubescens* barks around a pig farm was not associated with atmospheric NH_3, the frequency of strictly nitrophilous lichens clearly increased with increasing level of NH_3 in the atmosphere and the rising of bark pH, and in parallel, the frequency of non-nitrophilous species decreased. Moving away from the pig farm, where a peak of 267 $\mu g/m^3$ NH_3 was measured, a 98% reduction of NH_3 was achieved already in the first 200 m from the source (4.6 $\mu g/m^3$). Similarly, Fowler et al. (1998) measured NH_3 around a poultry farm in the UK and found concentrations up to 63 $\mu g/m^3$ within 15 m from the source, declining to local background levels of 1–2 $\mu g/m^3$ within a distance of 270 m. More recently, Jones et al. (2013) measured NH_3 around a poultry farm in the UK, to investigate its potential impact on an adjacent sand dune Natura 2000 reserve, and found that mean NH_3 concentrations declined rapidly from 60.1 $\mu g/m^3$ at the poultry unit, down to 6.3 $\mu g/m^3$ at 300 m, dropping to 1.2 $\mu g/m^3$ at 800 m. In a study carried out around cow farms in central Italy, Frati et al. (2008) measured NH_3 concentrations in the air up to 45 $\mu g/m^3$ close to the stocking areas and observed that the distribution of nitrophilous species such as *H. adglutinata* and *X. parietina* clearly increased on local pine trees with proximity to the stock farm and with the rise of bark pH caused by both NH_3 and dust pollution. Similarly, in our study, after 3 years of operations of the composting facility, nitrophilous lichens increased on tree barks within 200 m from the source.

Several epiphytic lichens are known to be indicator species for elevated environmental N levels (e.g., *Amandinea punctata*, *Hyperphyscia adglutinata, Phaeophyscia orbicularis*, *Physcia adscendens*, *P. tenella*, *X. parietina*), which are often replacing acidophilous or subneutrophilous species in eutrophicated and light-

flooded habitats (Hauck, 2010). In habitats where trees with a smooth bark (e.g., *Castanea*) are colonized by acidophilous or subneutrophilous species such as *Graphis scripta*, *Opegrapha atra*, *O. rufescens,* such as in our study area, eutrophication may lead to the replacement of such species by nitrophilous ones in light-flooded conditions, or, in very shaded conditions, to their replacement by free-living algae (Hauck and Wirth, 2010). Interestingly, similar results were observed by Nascimbene and Marini (2010) and Nascimbene et al. (2012), which investigated the responses of lichen communities to habitat changes caused by the invasion of *Robinia pseudoacacia* (black-locust), an alien tree with N-fixing activity, which showed that black-locust invasion led to a shift in lichen communities from assemblages composed by acidophilous, shade tolerant and N-sensitive species to lichen communities mainly formed by N-tolerant species. The reasons can be the excessive thinning and consequent canopy openness and its bark features, such as the high buffering and water retention capacity and the nutrient content, likely enhanced by an interaction with the soil, whose chemistry is influenced by the nitrogen fixation activity of the bacteria associated with the roots (Nascimbene et al., 2012).

The data collected at our composting plant suggest that a likely shift from oligotrophic to nitrophilous lichens will occur in the near future approaching the source. The above evidences indicate that when investigating the effects of NH_3 pollution around a point source, the functional traits of epiphytic lichen diversity are more helpful than the total diversity. Therefore, concerning species diversity, the main signal to be searched both in space (approaching the source) and time (after a prolonged exposure) is the increase of the frequency of nitrophilous lichen species.

5. Conclusions

This study clearly showed that N-tolerant and N-sensitive lichens react differently when exposed for 1–3 months to atmospheric NH_3. The N-tolerant *X. parietina* was not affected and some physiological parameters even showed a higher performance, while the N-sensitive *E. prunastri* showed a reduced performance with increasing atmospheric concentrations approaching the source. These evidences suggest a likely shift from lichen communities composed by meso-acidophilous species (actual condition) to more nitrophilous communities in the near future, approaching the composting facility. The use of physiological responses of

lichen transplants provides a qualitative tool for the assessment of environmental conditions around similar point sources. Reflecting the effects of air pollution on the ecosystems, these bioindicators can provide useful data for decision-makers to establish correct science-based environmentally sustainable waste management policies, also in consideration of the possible health effects related with waste management (Porta et al., 2009).

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Table 1. Microclimatic parameters at sampling sites measured during the transplant experiment, between 10:30 and 11:30 a.m. and 12:00 and 1:00 p.m. (March 2011, 18th, 22th, 25th, 29th); solar radiation (SR, µmol s^{-1} m^{-2}), temperature (T, °C), relative humidity (RH %). Cumulative rainfall during the experiment (18 February 2011 – 18 May 2011) was 350 mm.

Microclimatic parameters	at the composting facility	200 m from the composing facility	400 m from the composing facility
SR (10:30 - 11:30 am)	1,020 – 1,370	860 – 1,270	850 – 1,180
SR (12:00 - 1:00 pm)	1,350 – 1,540	1,145 – 1,490	1,080 – 1,415
T (10:30 - 11:30 am)	24.0 – 24.6	23.5 – 26.7	23.2 – 25.0
T (12:00 - 1:00 pm)	24.9 – 26.5	24.0 – 25.6	24.4 – 25.5
RH % (10:30 - 11:30 am)	15.8 – 20.5	13.6 – 24.5	15.0 – 26.0
RH % (12:00 - 1:00 pm)	13.2 – 18.3	13.1 – 22.4	13.5 – 24.5

Table 2. Physiological parameters measured in samples of the lichens *Evernia prunastri* and *Xanthoria parietina* prior to exposure (n = 10). Cell membrane damages (EC %), sample viability (A_{492}, absorbance units at 492 nm / g dw), photosynthetic efficiency (F_V/F_M), performance index (PI_{ABS}).

	Evernia prunastri	*Xanthoria parietina*
EC %	3.0 ± 0.8	3.0 ± 1.0
A_{492}	3.6 ± 0.1	15.4 ± 1.9
F_V/F_M	0.64 ± 0.08	0.71 ± 0.06
PI_{ABS}	0.26 ± 0.11	0.34 ± 0.10

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Table 3. Results of ANOVA. *F* and *P* values in bold are significant (95 % confidence interval). Cell membrane damages (EC %), sample viability (A_{492}, absorbance units at 492 nm/g dw) photosynthetic efficiency (F_V/F_M), performance index (PI_{ABS}).

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ANOVA effects	Distance	Time	Interaction
Evernia prunastri			
EC %	***F* = 22.92 *P* = 0.000**	***F* = 34.22 *P* = 0.000**	***F* = 4.991 *P* = 0.011**
A_{492}	***F* = 4.944 *P* = 0.015**	***F* = 8.052 *P* = 0.009**	*F* = 2.621 *P* = 0.092
F_V/F_M	***F* = 3.691 *P* = 0.028**	*F* = 0.035 *P* = 0.852	*F* = 2.461 *P* = 0.091
PI_{ABS}	***F* = 5.714 *P* = 0.004**	*F* = 0.825 *P* = 0.366	***F* = 6.533 *P* = 0.002**
Xanthoria parietina			
EC %	*F* = 3.309 *P* = 0.060	*F* = 0.674 *P* = 0.422	*F* = 1.124 *P* = 0.347
A_{492}	*F* = 1.542 *P* = 0.234	*F* = 2.024 *P* = 0.168	*F* = 0.172 *P* = 0.843
F_V/F_M	*F* = 2.321 *P* = 0.103	***F* = 17.87 *P* = 0.000**	*F* = 1.092 *P* = 0.340
PI_{ABS}	*F* = 2.312 *P* = 0.104	***F* = 25.18 *P* = 0.000**	*F* = 0.552 *P* = 0.576

Table 4. Lichen species recorded during the study and Index of Lichen Diversity (ILD) measured in 2010 and 2013. P % = percentage occurrence, F % = frequency; nitro = nitrophilous species (Nimis and Martellos 2008). ILD_{tot} = total ILD, ILD_{nitro} = ILD by nitrophilous species. Values in bold are significantly different according to the year ($P<0.05$); values followed by different letters indicate a significant variation according to the site (capital letters in 2010, small letters in 2013, $P<0.05$). Results of ANOVA. *F* and *P* values in bold are significant (95 % confidence interval).

	P %		F %	
Lichen taxa	**2010**	**2013**	**2010**	**2013**
Lecidella elaeochroma (Ach.) M.Choisy [nitro]	100	100	92	91
Physcia adscendens (Fr.) H.Olivier [nitro]	92	92	48	53
Flavoparmelia caperata (L.) Hale	75	83	36	35
Normandina pulchella (Borrer) Nyl	67	58	40	34
Parmotrema perlatum (Huds.) M.Choisy	58	50	19	19
Parmelia sulcata Taylor	50	50	36	26
Candelariella reflexa (Nyl.) Lettau [nitro]	42	67	54	47
Graphis scripta (L.) Ach.	42	42	63	64
Candelariella xanthostigma (Ach.) Lettau	42	33	21	52
Pertusaria leioplaca DC.	33	42	19	13
Porina aenea (Wallr.) Zahlbr.	33	33	30	32
Melanelixia fuliginosa (Duby) O. Blanco *et al.*	33	25	25	15
Lecanora carpinea (L.) Vain.	25	50	10	13
Candelaria concolor (Dicks.) Stein [nitro]	25	25	17	24
Opegrapha atra Pers.	25	25	92	82
Evernia prunastri (L.) Ach.	25	17	12	13

Xanthoria parietina (L.) Th.Fr. [nitro]	25	17	22	5
Caloplaca ferruginea (Huds.) Th.Fr.	17	17	8	5
Opegrapha rufescens Pers.	17	17	38	40
Pertusaria albescens (Huds.) M.Choisy & Werner	17		8	
Hyperphyscia adglutinata (Flörke) Mayrhofer & Poelt [nitro]	8	25	10	19
Lecanora expallens Ach.	8	25	35	24
Phaeophyscia chloantha (Ach.) Moberg [nitro]	8	25	15	10
Arthonia radiata (Pers.) Ach.	8	17	5	5
Lecanora argentata (Ach.) Malme	8	8	25	25
Lecanora symmicta (Ach.) Ach.	8	8	5	5
Punctelia borreri (Sm.) Krog	8	8	5	5
Ramalina farinacea (L.) Ach.	8		15	
Lepraria sp.	8		10	
Usnea sp.	8	8	5	5
Physcia biziana (A.Massal.) Zahlbr. v. *biziana* [nitro]		8		15
Phlyctis argena (Spreng.) Flot		8		5
Physconia servitii (Nádv.) Poelt		8		5

	distance 200 m		distance 400 m	
Index of Lichen Diversity	2010	2013	2010	2013
ILD_{tot}	67 ± 8 B	72 ± 12 b	91 ± 6 A	86 ± 7 a
ILD_{nitro}	**36 ± 7**	**42 ± 6** a	32 ± 15	27 ± 8 b

ANOVA effects	Distance	Year	Interaction
ILD_{tot}	***F* = 14.77 *P* = 0.001**	*F* = 0.07 *P* = 0.799	*F* = 2.06 *P* = 0.166
ILD_{nitro}	***F* = 6.936 *P* = 0.016**	*F* = 0.25 *P* = 0.876	*F* = 1.869 *P* = 0.187

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Figure 1. Physiological parameters in transplanted *Evernia prunastri* (N-sensitive) as a function of distance from the NH_3 source (0, 200, 400 m) and time of exposure (white blocks = 1 month, black blocks = 3 months). **A**) Cell membrane damages (EC %), **B**) sample viability (A_{492}, absorbance units at 492 nm/g dw), **C**) photosynthetic efficiency (F_V/F_M), **D**) performance index (PI_{ABS}). * = significant difference according to
630 the time of exposure (Tukey test, $P<0.05$); different letters indicate a significant variation according to the distance from the composting plant (Tukey test, $P<0.05$).

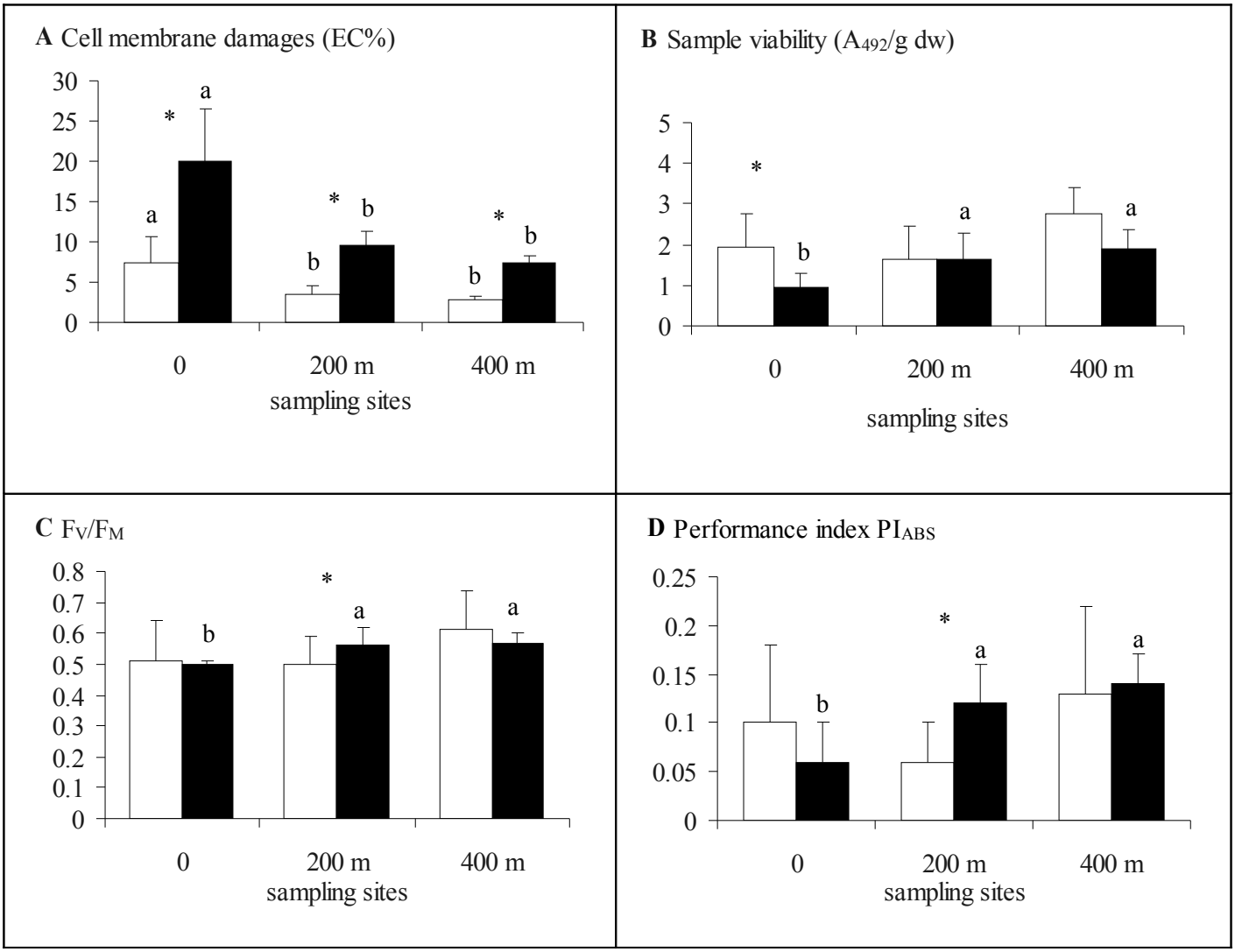

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Figure 2. Physiological parameters in transplanted *Xanthoria parietina* (N-tolerant) as a function of distance from the NH_3 source (0, 200, 400 m) and time of exposure (white blocks = 1 month, black blocks = 3 months). **A**) Cell membrane damages (EC %), **B**) sample viability (A_{492}, absorbance units at 492 nm/g dw), **C**) photosynthetic efficiency (F_V/F_M), **D**) performance index (PI_{ABS}). * = significant difference according to the time of exposure (Tukey test, $P<0.05$); different letters indicate a significant variation according to the distance from the composting plant (Tukey test, $P<0.05$).