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Lichen biomonitoring of ammonia emission and nitrogen deposition around a pig stockfarm

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The diversity of strictly nitrophytic lichen species can be used to monitor and map NH_3 pollution in the Mediterranean area.

Abstract

Effects of high ammonia emissions and nitrogen deposition were investigated on lichens around a pig stockfarm (ca. 7,000 animals) in central Italy. Four sites were selected along a transect at 200, 400, 1000 and 2500 m from the stockfarm, the diversity of epiphytic lichens was measured and transplanted thalli of *Xanthoria parietina* and *Flavoparmelia caperata* exposed, together with passive NH_3 (diffusion tubes) samplers. Ammonia dramatically decreased from the centre of the stockfarm to the sampled sites, where it was correlated with bark pH. Total lichen diversity was not associated with either NH_3 concentrations or bark pH, but the diversity of strictly nitrophytic species was highly correlated with both parameters. *Physconia grisea* was the best indicator species for NH_3 pollution. Total N accumulated in *X. parietina* and *F. caperata* was correlated with NH_3 concentrations.

Keywords: Air pollution; Ammonium; Bark pH; Bioaccumulation; Biodiversity; Eutrophication

1. Introduction

During the last decades, the supply of reactive nitrogen to global terrestrial ecosystems has doubled as a consequence of human activity (Galloway, 1998). Release to land results in eutrophication of both fresh and coastal waters, while emission to the atmosphere results in regional eutrophication and acidification, crop damage and impacts on human health, (Erisman et al., 2003). The major sources of deposited atmospheric N are ammonia (NH_3) and nitrogen oxides (NO and NO_2). On a global scale, and especially in Europe, emissions of reduced N, mainly resulting from livestock management and fertilizer application, exceed those of oxidised N (Pitcairn et al., 2003).

Several studies carried out in central and northern Europe recognized NH_3 air pollution as an important factor affecting epiphytic lichen vegetation (Søchting, 1995; Van Herk, 1999; Ruoss, 1999). In The Netherlands, during the last 10 years, an increase in nitrophytic lichen species, paralleled by a decrease in acidophytic ones, has occurred in areas with high cattle density. This phenomenon was especially apparent on acid-barked trees, on which nitrophytes were previously scarce or absent (Van Dobben and Ter Braak, 1998). A similar shift in species composition was also observed in the UK (Wolseley and James, 2002) and Switzerland (Ruoss, 1999). All these authors attributed changes to the rise in atmospheric NH_3 concentration.

In Italy, NH_3 emission reaches a significant amount, of about 448,000 t/y, 70% of which is from livestock and 18% from the use of N-based fertilizers. However, in this country,

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to the best of our knowledge, no investigation has yet been attempted to assess effects of this pollutant on vegetation. This could be partly because in areas with a Mediterranean climate, like Italy, dust can mask the effects of NH_3 (Loppi and De Dominicis, 1996; Loppi et al., 1997).

The aim of the present study was to investigate the effects of high ammonia emissions and nitrogen deposition on lichens, along a transect from a pig stockfarm in Italy. At each site, the epiphytic lichen diversity was measured and lichen-bags of *Xanthoria parietina* and *Flavoparmelia caperata* were exposed, together with passive NH_3 samplers.

2. Materials and methods

2.1. Site description

The farm, situated at an altitude of ~50 m, is located in a rural area, 40 km SE from Grosseto (Tuscany, central Italy). It has ca. 7400 pigs, which, according to the emission factor of Doorn et al. (2002), are estimated to emit about 52 t/y of NH_3. Climate is sub-Mediterranean, with a mean annual temperature of 15.2 °C and a mean annual rainfall of 854 mm, which is concentrated in autumn and winter; summer is fairly dry.

Four sites were selected along a transect (200, 400, 1000, 2500 m) downwind of the stockfarm in the dominant wind direction. Transect length and sampling sites were selected according to diffusion models. In each site, 5 *Quercus pubescens* trees were selected for biomonitoring.

2.2. Ammonia monitoring

Monitoring was carried out using passive diffusion tube samplers (Radiello®, Aquaria). Samplers were placed twice for a period of two weeks (13–27 August 2003 and 31 October–12 November 2003) on *Quercus pubescens* trees at a height of 3 m from ground (to reduce vandalism). For each tree, 4 passive samplers were placed each time, 2 on the side facing the stockfarm (SE side) and 2 on the opposite side (NW side). On each occasion, 2 passive samplers were placed in the centre of the stockfarm to assess source emissions.

Samplers contain 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). Detection limit was 0.4 μg/m^3 and uncertainty 6.5%.

2.3. Diversity of epiphytic lichens

On both sampled sides of each tree selected for ammonia monitoring, the epiphytic lichen diversity was measured using a 30 × 50 cm sampling grid divided into 10 rectangles of 10 × 15 cm, placed on tree trunks at 1 m above ground. The sum of lichen frequencies within the sampling grid was measured as an index of lichen diversity (ILD). The following derivative indices were also calculated (Loppi, 2004):

ILDn = sum of frequencies of nitrophytic species;
ILDsn = sum of frequencies of strictly nitrophytic species;
ILDnn = sum of frequencies of non-nitrophytic species.

Selection of nitrophytic species was accomplished by using the "floristic query interface" of the on-line checklist of Italian lichens (Nimis, 2003). Strictly nitrophytic species were selected by asking for species having an indicator value for eutrophication from 4 (rather high eutrophication) to 5 (very high eutrophication).

2.4. Bark analysis

Bark samples were collected beneath the sampling grid used for biodiversity counts for pH and NH_4^+ measurements.

To measure bark pH, 0.5 g of the surface 2 mm were ground and soaked in vials with 10 ml deionized water and vigorously shaken by hand. Samples were left for 30 min, shaken again and centrifuged for 5 min at 4,000 rpm; the clear fluid fraction was used for analyses. Bark pH was measured with a pH-meter (Crison Basic 20).

To measure ammonium concentrations, 0.2 g of the surface 2 mm were ground, soaked in vials with 10 ml deionized water and shaken for 1 h. Samples were then centrifuged for 10 min at 4,000 rpm and the clear fluid fraction was filtered with sterile filters (porosity = 0.45 μm) and used for analyses. Ammonium concentrations, expressed on a dry weight basis, were measured spectrophotometrically by the indophenol blue method (Allen, 1989). Detection limit was 2.5 μg/g. Internal standards added during the extraction indicated recoveries >90%.

2.5. Bioaccumulation

Samples of the lichens *Xanthoria parietina* (nitrophytic species) and *Flavoparmelia caperata* (acidophytic species) were collected from oak trees in a remote area of Tuscany far from the pig stockfarm and any other local pollution sources. Two lichen-bags, each containing 5–6 thalli of *X. parietina* or *F. caperata*, were exposed for two months (13 August–12 October 2003) together with passive NH_3 samplers at the centre of the stockfarm and at the 4 sampling sites. Five thalli were immediately analyzed for their N content, to provide a reference level at the beginning of the experiment, while five additional thalli were resuspended in the original site to evaluate any possible effect of transplanting on nitrogen accumulation.

In the laboratory, lichen samples were air-dried to constant weight and carefully cleaned with nylon tweezers under a binocular microscope to remove as much extraneous material as possible. Samples were not washed since washing may remove nitrogen deposited on the lichen surface (Gombert et al., 2003). Unwashed samples were ground and the nitrogen content of 3 sub-samples, expressed on a dry weight basis, was determined by a nitrogen-carbon-sulphur analyser (CHNS-O Perkin Elmer 2400). Analytical quality was checked by analyzing Acetanilide (C_8H_9NO) with a certified N content of 10.36%. Precision of analysis was estimated by the coefficient of variation of 3 replicates and was found to be within 5%.

2.6. Statistics

The significance of differences in lichen and chemico-physical parameters of the two sides of the sampled trees was tested by the Kolmogorov–Smirnov two-sample test. Correlation analysis was carried out by the Spearman rank coefficient.

3. Results and discussion

All parameters investigated within this study were measured on both tree sides facing and opposite the livestock (SE and NW respectively). This choice was justified by the fact that Wolseley and James (2002) reported a marked variation in bark pH around the trunk of *Quercus* trees near an intensive poultry unit in Britain, where a pH of 6.2 on the N side contrasted with a pH of 3.9 on the S side. However, in our case no investigated parameters showed statistically significant ($P > 0.05$) differences between the two sides, and results were pooled for subsequent statistical analysis.

3.1. Ammonia monitoring

Atmospheric ammonia concentrations measured by passive samplers are shown in Fig. 1. Since NH_3 is either readily converted to NH_4^+ or subjected to fast dispersal and dilution, high concentrations are only found close to emission sources

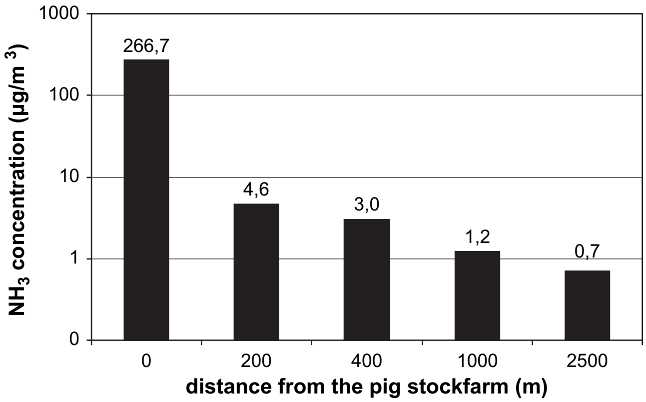

Fig. 1. Atmospheric ammonia concentrations measured by passive samplers at the centre and at increasing distances from a pig stockfarm. Note logarithmic scale on the y-axis and artificial regular spacing on the x-axis.

(Ferm, 1998). In the present study, the NH_3 concentration was negatively correlated with distance from the pig stockfarm ($R^2 = 0.95$, $P < 0.001$) according to a logarithmic function. In the centre of the farm, concentrations peaked 267 µg/m^3 but declined rapidly with distance, reaching a regional background of 0.7 µg/m^3 at 2,500 m. A 98% reduction was already achieved in the first 200 m from source (4.6 µg/m^3). A similar decline in NH_3 concentrations around a poultry building, with background values at 270 m from the source, was reported by Fowler et al. (1998). Pitcairn et al. (2002) also reported a 95% reduction in NH_3 concentrations at 650 m from a poultry farm in the UK, with a sharp decrease within the first 200 m.

3.2. Bark properties and diversity of epiphytic lichens

A total of 27 epiphytic lichen species was found; 16 species were nitrophytic and 3 strictly nitrophytic (Table 1). The most common species (percentage occurrence >75%) were *Physcia adscendens*, *Hyperphyscia adglutinata* and *Phaeophyscia hirsuta* (nitrophytic species) and *Phaeophyscia orbicularis* and *Physconia grisea* (strictly nitrophytic species). All these lichens are widespread in Italy and are typical components of *Xanthorion* vegetation, which includes nitrophytic, photophilous and rather xerophytic communities (Nimis, 2003). This vegetation reaches its optimum on trees with a bark pH in the range 5–7 and normally does not colonize the acid bark of *Quercus* trees, with a typical pH range of 3.7–5 (Barkman, 1958).

Van Herk (2001) reported bark pH values for *Quercus robur* in the range 3.7–4.4 in forest environments and in the range 3.8–5.0 in urban areas, but also noted higher values (5.4–6.4) in areas characterized by intense agricultural activities and high NH_3 immission. On the contrary, the bark NH_4^+ content in these latter sites was not markedly high (about 1.13 mg/g). As these are agricultural areas dominated by nitrophytic lichen communities, it was concluded that the effect of airborne NH_3 on nitrophytic lichens is probably determined by a rise in bark pH rather than by increased bark NH_4^+ availability.

Table 1
Mean frequency of all lichen species found in each sampling site

	S1	S2	S3	S4
*Amandinea punctata**	0	5	0	4
*Caloplaca cerinella**	5	1	0	0
*Candelaria concolor**	0	8	10	0
*Candelariella reflexa***	2	9	0	4
*Diploicia canescens**	0	1	0	0
*Hyperphyscia adglutinata**	8	9	10	5
*Lecanora chlarotera**	0	0	1	5
*L. hagenii**	5	1	0	0
*Lecidella elaeochroma**	0	0	0	6
Lepraria sp.	0	0	0	6
Flavoparmelia caperata	0	1	1	0
F. soredians	0	1	0	7
Punctelia subrudecta	1	3	1	1
Parmelia sulcata	0	0	0	1
Parmelina tiliacea	1	3	2	1
Parmotrema chinense	0	0	0	2
*Phaeophyscia hirsuta**	10	6	10	0
*P. nigricans**	1	1	0	0
*P. orbicularis***	7	3	6	0
*Physcia adscendens**	4	1	7	5
*P. aipolia**	0	0	4	0
*P. biziana**	5	1	1	1
*P. biziana var. leptophylla**	0	0	2	0
*Physconia distorta**	0	0	1	1
*P. grisea***	10	9	5	0
P. perisidiosa	0	0	1	0
*Xanthoria parietina**	8	4	10	0

* = nitrophytic species; ** = strictly nitrophytic species.

In the Mediterranean area, bark pH values of *Quercus* trees are often higher than those measured in central and northern Europe. For the Mediterranean basin, high oak bark pH values have been reported for *Q. robur* by Ilijanovic et al. (1989) in Croatia (range 4.0–6.3), for *Q. pubescens* by Loppi and De Dominicis (1996) and Loppi and Putortì (1995) and for *Q. ilex* by Loppi and Frati (2004) in central Italy (ranges 5.6–6.8, 5.6–6.2, 5.9–6.7 respectively). A possible explanation is that in the Mediterranean area, characterized by a warm and dry climate, dust is one of the main causes for the rise in bark pH of *Quercus* trees (Loppi and De Dominicis, 1996). This hypothesis was formerly postulated by Barkman (1958) to explain the presence of *Xanthoria* and *Physcia* spp. vegetation on *Q. ilex*, a Mediterranean oak that normally has an acid bark (4.9–5.7). According to this author, strong impregnation with calcareous dust makes *Q. ilex* bark neutral or basic. Gilbert (1976) similarly found that alkaline dust contamination raises bark pH and causes bark hypertrophication, thus allowing *Xanthorion* species to develop on normally acid-barked trees. This phenomenon seems to be fairly common in the Mediterranean area (Loppi and De Dominicis, 1996; Loppi et al., 1997). Moreover, dust impregnation, independently of dust chemistry, also makes the bark drier (Loppi and Pirintsos, 2000), and since nitrophytic species are also xerophytic, such interactions should be taken into account. Furthermore, in the Mediterranean area, synergistic effects of light must also be considered since most nitrophytic species are also photophilous and a higher light influx leads to drier conditions.

Bark pH and bark NH_4^+ values, as well as ILD values measured during the present survey are shown in Table 2. Bark pH ranged between 4.9–6.7 and correlated positively with NH_3 concentrations ($R^2 = 0.62$, $P < 0.05$) and negatively with distance from the pig stockfarm ($R^2 = 0.86$, $P < 0.001$; Tab. 3). Mean bark NH_4^+ was 28.1 ± 14.4 μg/g, with a range of 7.6–56.5 μg/g. These values are much lower than those reported by Van Herk (2001) for the Netherlands, but are well within the range of NH_4^+ concentrations of bark samples measured in several European countries (Schulz et al., 1999).

The diversity of nitrophytic lichens (ILDn), in the same way as the diversity of non-nitrophytic lichens (ILDnn) and the total lichen diversity (ILD), did not show any association with NH_3 concentrations. On the contrary, the diversity of strictly nitrophytic species (ILDsn) was positively correlated with both NH_3 ($R^2 = 0.74$, $P < 0.01$) and bark pH ($R = 0.62$, $P < 0.05$). These results are in line with a study on the effects of air pollution, nitrogen deposition, agriculture and dust by the diversity of epiphytic lichens in central Italy (Loppi, 2004). By calculating ILD values only with strictly nitrophytic species, a map was produced which highlighted two peaks in the vicinity of two big sheep and pig stockfarms, and the latter was so big that it was coupled with a NH_4 production plant. This map was supposed to reflect NH_3 emissions in the study area.

Among strictly nitrophytic lichens found, *Physconia grisea* was the best indicator species for NH_3 pollution. In fact, the frequency of this species was positively correlated with both NH_3 concentrations ($R^2 = 0.69$, $P < 0.01$) and bark pH ($R^2 = 0.88$, $P < 0.001$), and negatively correlated with distance from the pig stockfarm ($R = 0.92$, $P < 0.001$). Bark NH_4^+ was not correlated with any investigated parameter, especially atmospheric NH_3 and ILDsn. This result agrees perfectly with data reported by Van Herk (2001), suggesting that the effect of ammonia on lichens is not due to its toxicity or increased availability of ammonium bark ion concentrations, but rather through its effect on bark pH. However, our studies differ somewhat from those in central and northern Europe. In our case, NH_3 pollution does not cause a shift in species composition of lichens growing on oak from acidophytic to nitrophytic species, but from neutrophytic-nitrophytic to strictly nitrophytic species.

3.3. Bioaccumulation of nitrogen

Results of analytical determinations are reported in Table 3. Since the mean N concentrations in control lichens analyzed at the beginning and the end of the experiment (resuspended samples) did not differ significantly ($P < 0.05$), the results were pooled and used as a reference level. This result indicates that the influence, if any, of the transplanting process was negligible and that N concentrations are not affected by the transplanting procedure.

The mean total nitrogen contents of *X. parietina* and *F. caperata* thalli collected in the control site, expressed as percent of dry weight, were respectively 1.67% and 1.91%. Gaio-Oliveira et al. (2004) measured N contents in *X. parietina* in the range 1.34–3.34% and Rai (1988) reported a value of 1.41%. The range of concentrations of nitrogen in *Parmelia* sp. is 0.5–2.1 (Palmqvist et al., 2002; Rai, 1988).

Both *X. parietina* and *F. caperata* exposed along the transect accumulated nitrogen in their thalli. However, samples of *F. caperata* exposed in the centre of the pig stockfarm showed visible signs of injury, confirming that this is a sensitive species to NH_3 pollution. Similar observations have also been reported by Søchting (1995) for thalli of *H. physodes* transplanted close to a pig farm in southern Jutland, and by Kauppi (1976) for the same lichen species transplanted near a fertilizer factory. On the contrary, stress symptoms were not evident in *X. parietina* thalli, which maintained a healthy appearance even at high NH_3 concentrations. This is in agreement with the results of Gaio-Oliveira et al. (2001) which found that the nitrophilous species *X. parietina* has a much lower cation exchange capacity for nitrogen compared to the non-nitrophilous species *F. caperata*. A low cation exchange capacity implies a limited possibility of nitrogen to bind with the cell walls of *X. parietina*, protecting this species against the possible toxic effects of nitrogen in environments where this element reaches high concentrations. Furthermore, an experimental study on the effects of intensive fertilization on *X. parietina* showed damage only to thalli treated with a very high concentration of NH_4Cl (0.69 M), confirming that this species has a very high tolerance to N pollution (Gaio-Oliveira et al., 2004). The same study also showed that the accumulation of nitrogen by *X. parietina* is subjected to saturation, probably by the saturation of the cation exchange capacity of the thallus. This latter result is in line with the N concentrations measured in the present study in the centre of the pig stockfarm, which, despite being higher compared with those measured at the other sites, are not directly related with the very high NH_3 concentrations. Treating the data at the

Table 2
Mean (±standard deviation) bark pH values and bark NH_4^+ concentrations, and ILD, ILDn, ILDsn, ILDnn (see text for explanations) measured in each sampling site

Sampling site	Bark pH	bark NH_4^+ (μg/g dw)	ILD	ILDn	ILDsn	ILDnn
S1	6.6 ± 0.1	18.9 ± 10.8	63 ± 28	62 ± 30	19 ± 8	1 ± 1
S2	6.2 ± 0.1	37 ± 9.7	63 ± 13	55 ± 9	21 ± 2	8 ± 5
S3	6.2 ± 0.1	24.4 ± 12.3	68 ± 14	64 ± 9	10 ± 0	4 ± 6
S4	5.2 ± 0.3	32.1 ± 19.1	46 ± 16	30 ± 5	4 ± 6	17 ± 11

Table 3
Mean (±standard deviation) concentrations of total nitrogen (% dry weight) measured in thalli of the lichens *Xanthoria parietina* and *Flavoparmelia caperata*, in control samples and in samples transplanted for two months at the centre and at increasing distances (sites S1–S4) from a pig stockfarm

Sampling site	*Xanthoria parietina*	*Flavoparmelia caperata*
Control	1.67 ± 0.10	1.91 ± 0.16
Stockfarm	2.35 ± 0.04	2.56 ± 0.02
S1	1.95 ± 0.18	2.06 ± 0.19
S2	2.08 ± 0.41	1.87 ± 0.20
S3	1.77 ± 0.13	1.78 ± 0.21
S4	1.66 ± 0.23	1.55 ± 0.10

centre of the stockfarm as outliers, nitrogen concentrations were negatively correlated with distance from the stockfarm ($R^2 = 0.90$; $P < 0.05$ for *F. caperata*, and $R = 0.79$; $P < 0.05$ for *X. parietina*) and positively correlated with atmospheric NH_3 concentrations ($R = 0.94$; $P < 0.05$ for *F. caperata*, and $R = 0.72$; $P < 0.05$ for *X. parietina*). Similarly, Søchting (1995) reported increased levels of nitrogen with decreasing distance from a pig farm in transplanted thalli of *H. physodes*, with values that ranged from 1.4 in the control site to 2.9 close to the farm. Likewise, Kauppi (1980) collected samples of *H. physodes* at different distances from a fertilizer factory, and found that the nitrogen content in the samples closest to the factory was 4–5 times higher compared to the control value (3.2% vs. 0.8%), and declined sharply with increasing distance from the nitrogen source. Nitrogen accumulation was reported also by Ruoss (1999) in *Physcia caesia*, a widespread and N-tolerant species in central Switzerland, with values in the range 1.7–1.8% in control sites and 4.2–4.4% near intensive NH_3 emission. Compared to control samples, an increase in N content of *Pseudevernia furfuracea* thalli exposed in bags was also observed by Vingiani et al. (2004) in the urban area of Naples, suffering from NO_X pollution. Interestingly, samples exposed in an agricultural area NW of Naples had a N content higher than that of samples exposed in urban parks or along busy roads. A study in Grenoble (SE France) to monitor NO_X pollution, indicated that traffic load and proximity to roads influenced the nitrogen content of the nitrophytic species *Physcia adscendens*, but not that of the acidophytic *H. physodes* (Gombert et al., 2003).

4. Conclusions

Ammonia air pollution is an important factor affecting epiphytic lichen vegetation (Van Herk, 1999; Van Dobben and Ter Braak, 1998). However, the influence of NH_3 has been largely investigated in central and northern Europe (Van Herk, 1999; Søchting, 1995; Ruoss, 1999). Studies in the Mediterranean area are very scanty since dust is a complicating factor (Loppi and De Dominicis, 1996; Loppi et al., 1997).

Results of the present survey confirmed that NH_3 does not directly influence the lichen vegetation through an increased availability of bark NH_4^+, but rather by increased bark pH. However, in the Mediterranean area, dust impregnation, especially during sunny, and dry conditions also raises bark pH causing bark hypertrophication, allowing nitrophytic species to develop on normally acid-barked trees. This fact implies that, in contrast to N Europe, ammonia pollution does not cause a shift in species composition from acidophytic to nitrophytic species, but from neutro-nitrophytic to strictly nitrophytic species. As a consequence, the diversity of strictly nitrophytic species (ILDsn) can profitably be used to monitor and map NH_3 pollution. Among nitrophytic lichens surveyed in this study, *Physconia grisea* was the best indicator species for NH_3 pollution, being positively correlated with airborne NH_3 and bark pH, and negatively correlated with distance from the pig stockfarm.

Exposed thalli of both *X. parietina* and *F. caperata* accumulated nitrogen, with concentrations negatively correlated with distance from the stockfarm and positively correlated with atmospheric NH_3 concentrations. Furthermore, samples of *F. caperata* exposed in the centre of the pig stockfarm showed visible signs of injury, while these symptoms were not evident in *X. parietina* thalli, confirming that the former is a sensitive species and the latter a resistant species to NH_3 pollution.

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