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The Concentration of Nitrogen in Nitrophilous and Non-Nitrophilous Lichen Species

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Abstract

The purpose of this work was to compare nitrogen concentrations in *Xanthoria parietina*, a nitrophilous lichen species, with non-nitrophilous species (*Ramalina fastigiata, Parmelia caperata* and *Evernia prunastri*). In order to understand the differences between nitrophilous and non-nitrophilous lichen species, total nitrogen concentrations, the organic and inorganic nitrogen fractions, the deposited nitrogen on thallus surface and the cation exchange capacity were determined. The nitrophilous species *X. parietina* presented always higher nitrogen concentrations than the other studied species, when collected at the same sites. The spatial variation in nitrogen concentration in this lichen species reflects the amount of deposited nitrogen, particularly ammonium. No deposited nitrogen was detected on the thallus surface of both *X. parietina* and *R. fastigiata*. The scarce amount of nitrogen on the thallus surface was probably hampered by the level of organic nitrogen from the whole thallus. The proportion of inorganic nitrogen in relation to the total concentration seems to reflect nitrogen sources. The lowest cation exchange capacity

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found for *X. parietina* can be a contribution to the understanding of the tolerance mechanisms of this nitrophilous species. Moreover, *E. prunastri*, an acidophilous lichen species, presented the highest cation exchange capacity of all the studied species.

Keywords: *Xanthoria parietina*, nitrogen deposition, nitrophilous lichens, non-nitrophilous lichens

1. Introduction

The effect of atmospheric pollution on lichens has been reported since the end of the 19th century (Gries, 1996). Among the different types of atmospheric pollution affecting lichens, SO_2 is one of the best studied (e.g. Hawksworth and Rose, 1970). Nevertheless, in recent years, SO_2 emissions have been decreasing in several countries, such as The Netherlands (Van Dobben and De Bakker, 1996), England (Hawksworth and McManus, 1989), France (Letrouit-Galinou et al., 1992) and United States (Shannon, 1999; Lynch et al., 2000). This reduction has been generally followed by increased nitrogen emissions, as observed in The Netherlands (Van Dobben and De Bakker, 1996) and in the United Kingdom (Skinner et al., 1997). Nitrogen pollution increase has been observed in urban areas, in areas with intensive industrial activities (oxidized forms of nitrogen: NO_x and N_2O) and in agricultural areas due to fertilizers and livestock excrements (reduced forms of nitrogen: NH_3 and NH_4^+) (Bytnerowicz and Fenn, 1996). In these regions the levels of atmospheric nitrogen deposition can be one or two orders of magnitude higher than in areas where these activities are minimal (Nash, 1996). The progressive increase in nitric oxide release in the past years is attributable mainly to the increasing number of motor vehicles, but on the other hand does not persist in the atmosphere (Fellenberg, 1999).

The lichen flora from these areas has also been changing, with a general decrease of acidophilous species, such as *Evernia prunastri* and *Hypogymnia physodes* and an increase of nitrophilous species, such as *Physcia adscendens* and *Xanthoria parietina* (De Bakker, 1989; Van Dobben and Ter Braak, 1998; Van Herk, 1999). This, together with the fact that some lichen species have a different capacity for nitrogen uptake in nature (Søchting, 1995) and that nitrogen levels in lichens decrease with increasing distances from the emission source (Søchting, 1995; Ruoss, 1999), has made them potential biomonitors for nitrogen deposition.

Under field conditions, nitrogen is deposited to the lichens as either wet or dry deposition. In wet deposition ions such as ammonium and nitrate are deposited, whereas dry deposition can take place as either ammonia gas or as particles. In these organisms nitrogen occurs either in organic and inorganic

form. Those in an inorganic form might be: i) soluble or particulate located on the surface of the lichen thallus and/or on intercellular spaces; ii) soluble bound to the lichen wall and on the outer surface of the cell membrane through cation exchange processes; iii) soluble located within the cell.

With this study we aimed to compare total nitrogen concentration in a nitrophilous lichen species (*Xanthoria parietina*) with non-nitrophilous species (*Ramalina fastigiata, Parmelia caperata* and *Evernia prunastri*). Additionally, we intend to understand how the total nitrogen concentration varies among sites with different nitrogen emission sources. To fulfil these objectives, the total nitrogen concentration, the organic and inorganic nitrogen fractions, the deposited nitrogen on thallus surface and the cation exchange capacity were determined.

2. Materials and Methods

Sites and pollution sources characterisation

Lichen species were collected at least three days after rainfall events, in different sites with different pollution sources: site A is an intensive industrial and urban area where the main nitrogen sources are the oxidised ones, NO_x and N_2O. Surrounding villages present large exploitation of pigs production, where ammonia emission may be considerable. Site B is within an intensive urban area nearby a main road and located in a large race-course. Besides NO_x and N_2O, a major source is reduced nitrogen (NH_3). Site C is a rural area with only two ha of low abandoned farms surrounded by pine and oak forests. Nitrogen emissions are mainly from natural sources, particularly soil microbiological processes: N_2O and NO_x (Mosier and Kroeze, 1998). Site D is located in the centre of a small village surrounded by an oak forest from a natural park and by an olive grove area. There is no intensive farming and similarly to what has been indicated for the previous site, mainly nitrogen emission sources are related to microbiological soil processes (N_2O and NO_x). Site E is located in a natural park nearby intensive livestock exploitation. Nitrogen emission sources are mainly a reduced nitrogen form (NH_3). Site F is located in an urban site nearby an intensive road. Motor vehicles are the major emission sources of NO_x and N_2O.

Comparison between nitrogen concentration in marginal and central parts of Xanthoria parietina

At least ten thalli of *Xanthoria parietina* (L.) Th. Fr. were collected at each of sites A, B and C in April 1998. In the laboratory, lichen thalli were cleaned

from detritus. Marginal areas, at least with one centimetre, were separated from central ones. The total nitrogen concentration was measured in marginal and central parts after dried for 48 hours at 60°C. The total nitrogen was quantified in a Dumas combustion elemental analyser (EuroVector, Italy).

Total nitrogen concentration in different lichen species

The total nitrogen concentration was compared in four different lichen species: *Xanthoria parietina*, a nitrophilous species and *Evernia prunastri* (L.) Ach., *Ramalina fastigiata* (Pers.) Ach. and *Parmelia caperata* (L.) Ach., non-nitrophilous species. Three thalli of each lichen species were collected at site D on the same day of October 1999. *X. parietina* thalli were collected from roof tiles, *E. prunastri* thalli were collected from tree branches of *Quercus faginea* and *R. fastigiata* and *P. caperata* thalli were collected from tree trunks of Olea europaea. In the laboratory, lichen thalli were cleaned from detritus. The total nitrogen content was measured in thalli after dried for 48 hours at 60°C. The total nitrogen was quantified in a Dumas combustion elemental analyser (EuroVector, Italy).

Total nitrogen concentration in X. parietina collected at different sites

Thalli of *X. parietina* were collected in April 1998 from roof tiles at three different sites: A, B and C. Twenty in the first site and around 140 lichen samples in the two other sites were collected. The procedures described above were applied to the thalli in order to quantify the total nitrogen content.

Comparison of thallus nitrogen fractions between X. parietina and R. fastigiata

The purpose of this experiment was to determine within the cell both the organic and the inorganic fraction using the method of Miller and Brown (1999), adapted from Brown and Wells (1988). *X. parietina* thalli were collected from roof tiles in sites E and F. *R. fastigiata* thalli were collected from tree branches of *Olea europaea* in site D. Thalli from site F were collected one day before than those from sites D and E, in March 2000. One set of eight samples was immediately used for nitrogen quantification, the total nitrogen. Deposited nitrogen fraction was estimated by the difference between total nitrogen and the remained nitrogen obtained after washing the thallus with running distilled and deionised water (DDW) for approximately 3 min. This fraction represented the amount of unbound nitrogen intercepted by the lichen surface. The organic nitrogen fraction was determined measuring the total nitrogen in the thallus after submitting the samples to a sequential elution technique as

described in Miller and Brown (1999). The sequential elution consisted of washing initially the samples in running distilled and deionised water (DDW) for approximately 3 min, followed by shaking these washed thalli for 40 min in 20 mM $NiCl_2$ (10 ml), and then by an overnight drying at 80°C which ruptures the cell membrane and finally by a second washing with fresh 20 mM $NiCl_2$ solution (10 ml) for 30 min. These washed solutions represent: deposited nitrogen fraction, extracellular inorganic-bound nitrogen and intracellular inorganic nitrogen, hereby called inorganic nitrogen fraction. The organic nitrogen fraction contains organic structural nitrogen and probably also some insoluble particles rich in nitrogen that might have become entrapped by the hyphae of the lichen fungus.

Comparison of the cation exchange capacity among lichen species

In order to evaluate the cation exchange capacity of the four lichen species, eight samples of those species were submitted to saturated concentrations of Pb, an element with the greatest affinity for the lichens' wall binding sites (Neiboer and Richardson, 1980). All lichen samples were collected in site D. In the laboratory, lichen thalli were cleaned from detritus and stored in a high relative humidity atmosphere for 48 hours, over water, in a closed box, in order to restore their physiological activity and reduce membrane permeability (Buck and Brown, 1979). Samples of each species (ca. 4 g of lichen dry weight in 1 l) were immersed and agitated separately by air bubbling for 2 h in a solution of 20 mM $PbNO_3$ concentration. Samples were then rinsed with running distilled and deionised water (DDW) (ca. 4 g of lichen dry weight in 2 l) for approximately 3 min, to remove unbound Pb. Extracellular Pb was determined using a sequential elution technique reported by Branquinho and Brown (1994) with Na_2-EDTA at pH 4.5 as displacing agent. Samples of each species (30–80 mg) were shaken for 40 min in 20 mM Na_2-EDTA (10 ml) followed by a second washing with fresh 20 mM Na_2-EDTA solution (10 ml) for 30 min. Lichen metal concentrations were expressed on a total dry weight basis. Each fraction was analysed by atomic absorption spectrophotometry (SpectrAA 50 Varian) using an air/acetylene flame. Standards were prepared with DDW.

Sampling and data analysis

As much as possible similar thallus sizes were sampled for each lichen species for all experiments. After the previous experiment of comparison between nitrogen concentration in marginal and central parts of *X. parietina*, the whole thallus has always been considered in all experiments.

For each experiment a one-way ANOVA was performed followed by a

multiple comparison Tukey test (for $P<0.05$) using STATISTICA package software. All data are present as means ± standard deviations.

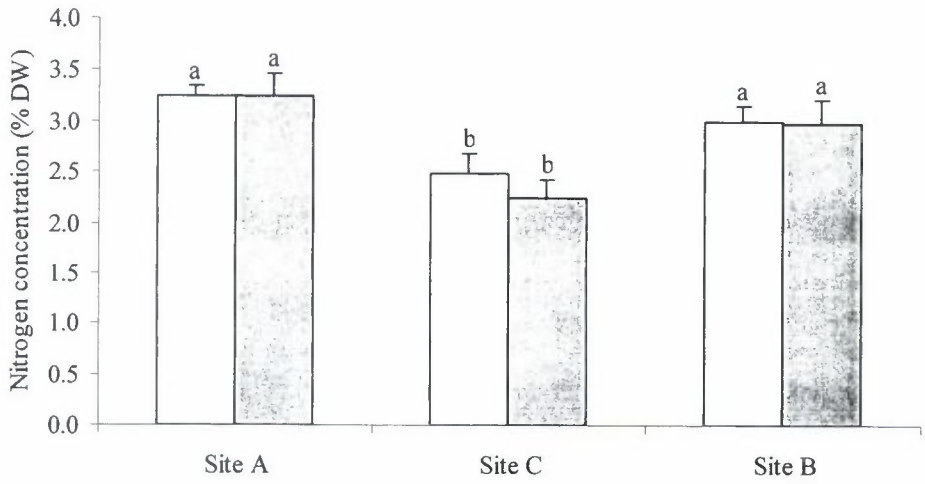

Figure 1. Nitrogen concentration (% DW) in marginal (white bars) and central parts (shaded bars) of *Xanthoria parietina* collected in sites A, B and C at the same time of the year. Columns with the same letters are not significantly different.

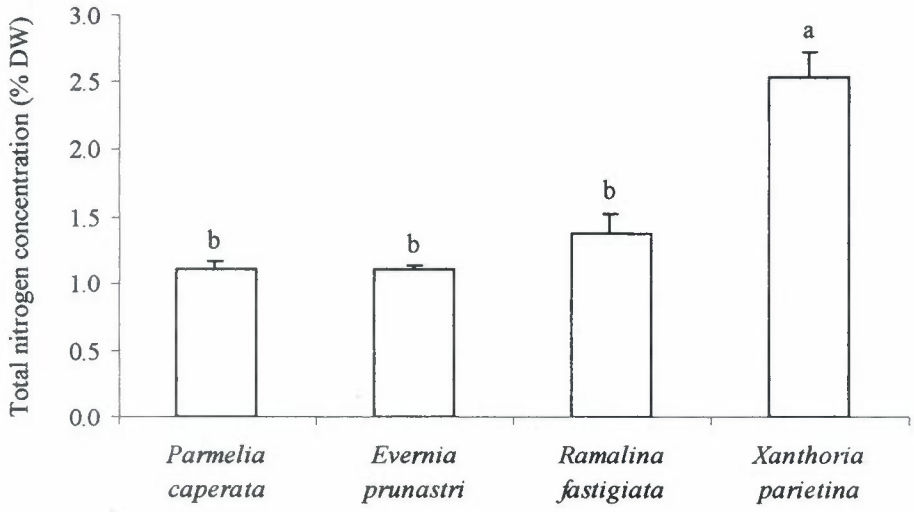

Figure 2. Total nitrogen content (% DW) in four different lichen species collected in site D at the same time of the year. Columns with the same letters are not significantly different.

3. Results

No differences were found in total nitrogen concentration between marginal and central areas of *X. parietina* (Fig. 1). The only significant differences were observed between sites.

When comparing the total nitrogen concentration among different lichen species in site D, *X. parietina* had the highest value (2.53% DW, Fig. 2), significantly different from all the other species. Total nitrogen concentration in the other species ranged from 1.10% DW and 1.37% DW (Fig. 2).

Thalli of *X. parietina* collected in sites A, B and C, showed different values of total nitrogen concentration (Table 1). The lowest value was found in the rural site and the highest in the industrial and urban site (Table 1).

Deposited nitrogen fraction and total nitrogen fraction in samples from sites D, E and F were compared in *X. parietina* and *R. fastigiata* (Table 2). No significant differences were found for both species.

The data in the sequential elution technique (Fig. 3) showed that the organic nitrogen fraction was the major component of the total nitrogen in the lichens. Lichens collected in sites with the same sources of nitrogen pollution (sites D and F) had the same percentage of organic nitrogen fraction (c. 75%), as compared with the lichens collected in site E, influenced by urban pollution (c. 87%) (Table 3).

The lowest cation exchange capacity was shown by *X. parietina* (Fig. 4), significantly different from the other species. On the contrary, *E. prunastri* showed the highest (Fig. 4). The cation exchange capacity of *P. caperata* and *R. fastigiata* were in the middle of the other two species.

4. Discussion

The results showed that *X. parietina*, a nitrophilous species, has always higher nitrogen concentrations than the other studied species, when collected at the same sites (Fig. 2). This high value of nitrogen concentration stays within the range found for lichen species (Crittenden, 1975). Within the group of non-fixing nitrogen lichens, where *X. parietina* is included, the range of nitrogen concentration is much narrow, varying between 0.2 to 1.0% DW (Crittenden et al., 1994). The high nitrogen concentration found in *X. parietina* resembles concentrations found in nitrogen fixing lichens (Crittenden et al., 1994). Thus, *X. parietina* is an outlier lichen and those values may reflect its nitrophily.

The spatial variation in nitrogen concentration in this lichen species (Table 1) reflects the amount of deposited nitrogen. A correlation between nitrogen concentration of *Cladonia portentosa* and nitrogen deposition in the British

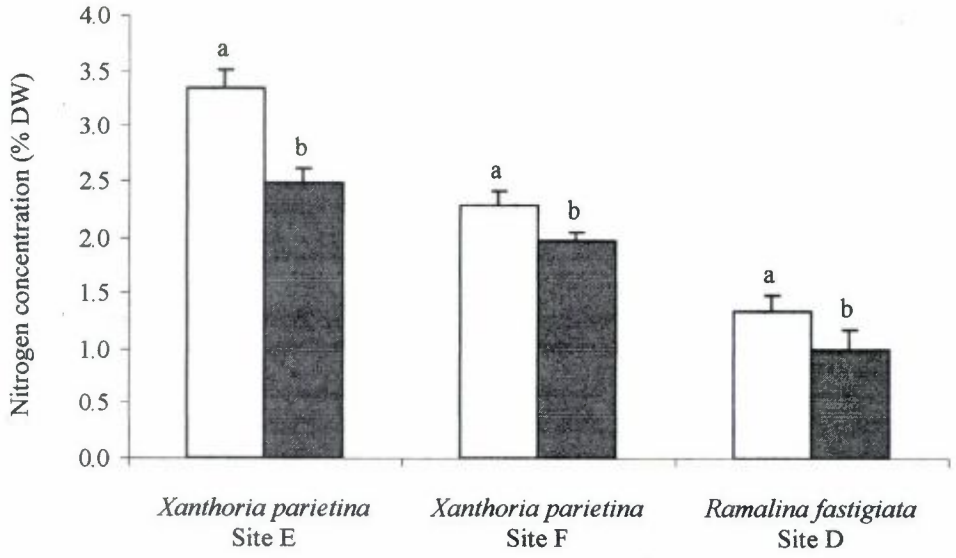

Figure 3. Total nitrogen fraction (white bars) and organic nitrogen fraction (shaded bars) (% DW) in *Xanthoria parietina* collected in sites E and F and *Ramalina fastigiata* collected in site D. The difference between columns of total nitrogen fraction and organic nitrogen fraction corresponds to the inorganic extracellular and intracellular fractions. Columns with the same letters are not significantly different.

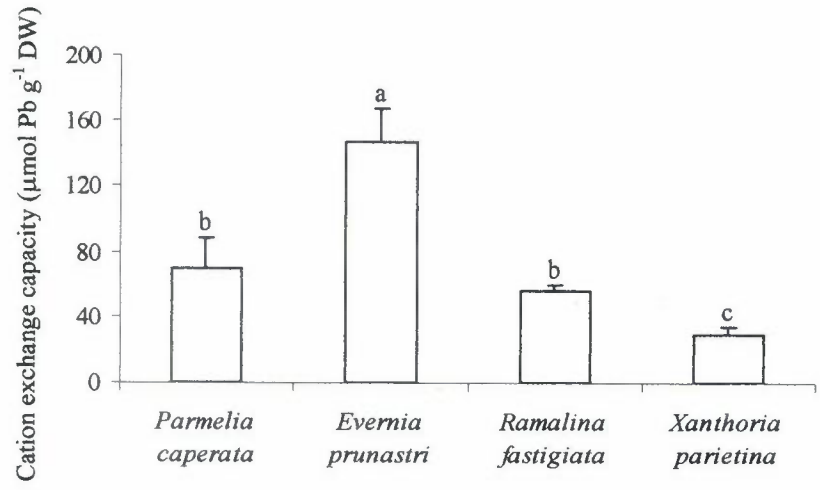

Figure 4. Comparison of the cation exchange capacity (µmol Pb g^{-1} DW) in four different lichen species collected in site D at the same time of the year. Columns with the same letters are not significantly different.

Table 1. Comparison of nitrogen total concentration (% DW) in *Xanthoria parietina* collected from different sites. Values with different letters are significantly different.

Sites	Sources of pollution	Total nitrogen concentration
A	Industrial and urban	3.25 ± 0.22 a
B	Urban and livestock	3.01 ± 0.20 a
C	Rural	2.36 ± 0.23 b

Table 2. Comparison of nitrogen concentration (% DW) between total nitrogen and deposited nitrogen fractions of *Xanthoria parietina* collected in sites E and F and *Ramalina fastigiata* collected in site D. Data with the same letters are not significantly different.

Nitrogen fractions	*Xanthoria parietina* Site E	*Xanthoria parietina* Site F	*Ramalina fastigiata* Site D
Total nitrogen	3.34 ± 0.17 a	2.27 ± 0.13 a	1.34 ± 0.15 a
Deposited nitrogen	3.38 ± 0.12 a	2.30 ± 0.08 a	1.40 ± 0.35 a

Table 3. Comparison of total nitrogen, organic and inorganic nitrogen fraction concentrations (% DW) between *Xanthoria parietina* collected in sites E and F and *Ramalina fastigiata* collected in site D. The percentage of inorganic nitrogen in relation to total nitrogen is also shown.

Species	Site	Total nitrogen	Organic nitrogen	Inorganic nitrogen	Inorganic/total nitrogen
X. parietina	E	3.34 ± 0.17	2.48 ± 0.13	0.86 ± 0.21	25.8
X. parietina	F	2.27 ± 0.13	1.98 ± 0.06	0.30 ± 0.14	13.1
R. fastigiata	D	1.34 ± 0.15	1.00 ± 0.17	0.34 ± 0.23	25.5

Isles has been found (Hyvärinen and Crittenden, 1998) only for NO_x and not for ammonia (NH_3). On the contrary, in this work we found a major relationship between nitrogen concentration in *X. parietina* and ammonia deposition. Nitrogen concentrations in *X. parietina* from sites A, B and E, affected mainly

by ammonia sources, present similar and significantly higher values than those in sites D and F, affected mainly by N_2O and NO_x sources. Similar results have already been reported for this species (Capelão et al., 2000). This result is also in accordance with the fact that N_2O and NO_x are not stable in the atmosphere being very quickly transformed in other gaseous forms (Fellenberg, 1999). This could be an explanation for lichens being more influenced by ammonia sources.

Within the non-nitrophilous species, all the three lichens, *R. fastigiata*, *P. caperata* and *E. prunastri*, had similar lower total nitrogen concentrations than *X. parietina* collected in the same site (Fig. 2). These low values, within the range of non-fixing lichens described by Crittenden et al. (1994), reflect the non-nitrophilous characteristics of these species. It would be useful to correlate different nitrogen sources with nitrogen concentration values in all the studied species. However, the number of automatic stations for nitrogen deposition is clearly infrequent, causing serious difficulties in obtaining official nitrogen deposition values for the studied areas.

From all the studied species *X. parietina* can be the best candidate for a good biomonitor of nitrogen deposition, particularly in the case of ammonia sources: (i) it presents a large distribution, being present in urban, rural and natural preserved sites (e.g. Table 1); (ii) it presents uniform nitrogen concentrations independently of the analysed thallus, since no variation between central and marginal parts were observed (Fig. 1); (iii) it reflects the variation of nitrogen pollution sources, justifying the variation of nitrogen concentration between sites (Fig. 3 and Table 1).

In a previous study performed in Portugal and using *R. fastigiata*, it was possible to conclude that ammonium concentration in lichens was highly correlated to the dry atmospheric deposition (Capelão et al., 2000). This result, together with the one in this study, show that nitrogen concentration in these lichen species is better correlated with dry deposition than with wet deposition and that NH_3 has a stronger influence on nitrogen concentration in the lichens than NO_x. In other studies performed in the northern Europe, nitrogen concentration in lichens is better correlated with wet deposition and nitrate influenced markedly stronger nitrogen concentration in lichens than ammonium (Bruteig, 1993; Hyvärinen and Crittenden, 1998).

The highest levels of total nitrogen concentration in *X. parietina* raised the question of where was the nitrogen located. Understanding the cellular location of nitrogen in nitrophilous and non-nitrophilous lichens is an important step for understanding the apparent tolerance of nitrophilous species to high levels of nitrogen both under laboratory and natural conditions (Miller and Brown, 1999). If the dry deposition could contribute significantly to the total nitrogen in lichens, different levels of inorganic nitrogen should be expected before and after washing the lichen thallus, which was not the case (Table 2). Moreover, nitrogen in lichens are mainly present in the organic form in a order of 20–30 mg

per g dry weight (Fig. 3), whereas the amount of ammonium or nitrate on thallus surface is in the range of µg (Capelão et al., 2000). Thus, large differences in ammonium concentration are hampered by the level of organic nitrogen present in lichens. Further studies on dry deposition, particularly under different conditions will be crucial to clarify possible surface nitrogen dry deposition.

Inorganic nitrogen fractions are most probably derived from environmental sources. The proportion of inorganic nitrogen in relation to the total concentration in different lichens species at different sites was similar, though the absolute amounts differ markedly (Table 3). Thus, the same proportion may reflect the same nitrogen sources (Table 3).

Lichens can scavenge nitrate and ammonium simultaneously (Crittenden, 1996) but they absorb preferentially ammonium when both nitrogen sources are present (Greenfield, 1992). This can explain the high nitrogen concentrations in sites A, B and E (Table 1 and Fig. 3) and, additionally, may also explain the observed higher amount of the inorganic nitrogen fraction by an oversaturation of the organic nitrogen fraction (Fig. 3). At the urban site the mixture of other pollutants together with prevalent nitrogen oxidised forms might also increase the competition for the lichen wall binding sites reducing the proportion of the inorganic nitrogen, as compared with rural one (Table 3).

Even though we cannot explain the nitrogen uptake mechanisms just in terms of cation exchange capacity, this parameter may contribute to the understanding of the tolerance mechanisms of a nitrophilous species like *X. parietina*. Although *X. parietina* showed the highest levels of total and organic nitrogen it showed the lowest cation exchange capacity (Figs. 2–4). This fact may be one of the reasons why this species is present in areas where the levels of nitrogen are high. This would mean that the ability of nitrogen to bind with the lichen cell walls is limited, and, as a consequence, *X. parietina* may be protected against the possible toxic effects of nitrogen in environments where nitrogen is too high. However, the results concerning cation exchange capacity and nitrogen concentration may be seen as contradictory (Figs. 2 and 4). One possible explanation for this apparent contradiction is that *X. parietina* was always collected from roof tiles, a more exposed location than the tree trunks where the other species were collected. Because roofs are strongly exposed to winds, *X. parietina* may have received larger doses of nitrogen than the other species that were sheltered by the trees canopy. This could explain the fact that *X. parietina* had higher levels of total nitrogen concentration despite its cation exchange capacity was the lowest. Thus, we need to compare the total nitrogen concentration of *X. parietina* with the other lichen species, all collected from the same habitat, for example, all from tree trunks, in order to better understand how cation exchange capacity could partially explain *X. parietina* tolerance to nitrogen. It is interesting to see that *E. prunastri*, one of

the lichen species that disappears first when nitrogen levels increase (De Bakker, 1989; Van Dobben and Ter Braak, 1998; Van Herk, 1999) has the highest cation exchange capacity. We also need to know more about the relationship between intracellular uptake and metabolism in order to understand the high tolerance of *X. parietina* to nitrogen and the differences between nitrophilous and non-nitrophilous species.

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