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Characterization of nitrogen deposition in a megalopolis by means of atmospheric biomonitors

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An increase of nitrogen deposition resulting from human activities is not only a major threat for global biodiversity, but also for human health, especially in highly populated regions. It is thus important and in some instances legally mandated to monitor reactive nitrogen species in the atmosphere. The utilization of widely distributed biological species suitable for biomonitoring may be a good alternative. We assessed the suitability of an ensemble of atmospheric biomonitors of nitrogen deposition by means of an extensive sampling of a lichen, two mosses, and a bromeliad throughout the Valley of Mexico, whose population reaches 30 million, and subsequent measurements of nitrogen metabolism parameters. In all cases we found significant responses of nitrogen content, C:N ratio and the $\delta^{15}N$ to season and site. In turn, the $\delta^{15}N$ for the mosses responded linearly to the wet deposition. Also, the nitrogen content ($R^2 = 0.7$), the C:N ratio ($R^2 = 0.6$), and $\delta^{15}N$ ($R^2 = 0.5$) for the bromeliad had a linear response to NOx. However, the bromeliad was not found in sites with NOx concentrations exceeding 80 ppb, apparently of as a consequence of excess nitrogen. These biomonitors can be utilized in tandem to determine the status of atmospheric nitrogenous pollution in regions without monitoring networks for avoiding health problems for ecosystems and humans.

Nitrogen deposition is one the most predominant forms of atmospheric pollution[1]. This phenomenon results from the release of nitrogenous compounds to the atmosphere, both in cities, and in the country, including oxidized (NOx) and reduced (NHx) species, which are highly reactive. Nitrogen deposition causes acidification and eutrophication of aquatic and terrestrial ecosystems, contributes to the proliferation of invasive species leading to changes in ecosystem structure; it is thus an important factor for climate change[1–6]. Nitrogenous pollution is also an issue for human health worldwide. Indeed, in traffic-jammed motorways, high accumulations of NOx at ground level are harmful by direct inhalation[7]. NOx emissions also contribute to the formation of secondary compounds including tropospheric ozone and particulate matter which are responsible in part for 9 million annual deaths worldwide[8]. For the case of Mexico City (Fig. 1), with more than 20 million habitants, at least 9600 deaths can be attributed annually to atmospheric pollution, resulting from respiratory and heart diseases, as well as pollutant build up in the nervous system and the brain[9,10]. In turn, ammonia emissions from agricultural sources quickly react with other atmospheric pollutants to form airbone particulate matter that can be readily inhaled[7]. Yet nitrogen is mostly neglected in public policy despite its importance in the prevalent discourse of global environmental change.

While monitoring nitrogenous pollution is an important issue, the deployment of air quality monitoring networks can be cost prohibitive in regions with developing economies. To fill this gap, an affordable alternative is the use of biomonitors[11]. For instance, throughfall deposition has been determined by the nitrogen content of lichen thalli[12]. Mosses are also widely used as biomonitors that allow covering vast areas, given that their nitrogen content reflects the rates of deposition and their isotopic composition can reflect the possible sources of pollution[13,14]. A group of special promise for biomonitoring in the neo-tropical region are atmospheric bromeliads, whose succulent tissues allow for yearlong physiologically activity, taking up pollution regardless of the seasonal environmental conditions. This is the case for the genus *Tillandsia*, amply distributed in the Americas, which has been utilized for monitoring NOx pollution given that its nutrition depends exclusively from atmospheric sources[15,16]. However, physiological limitations of these atmospheric organisms can confuse the observed

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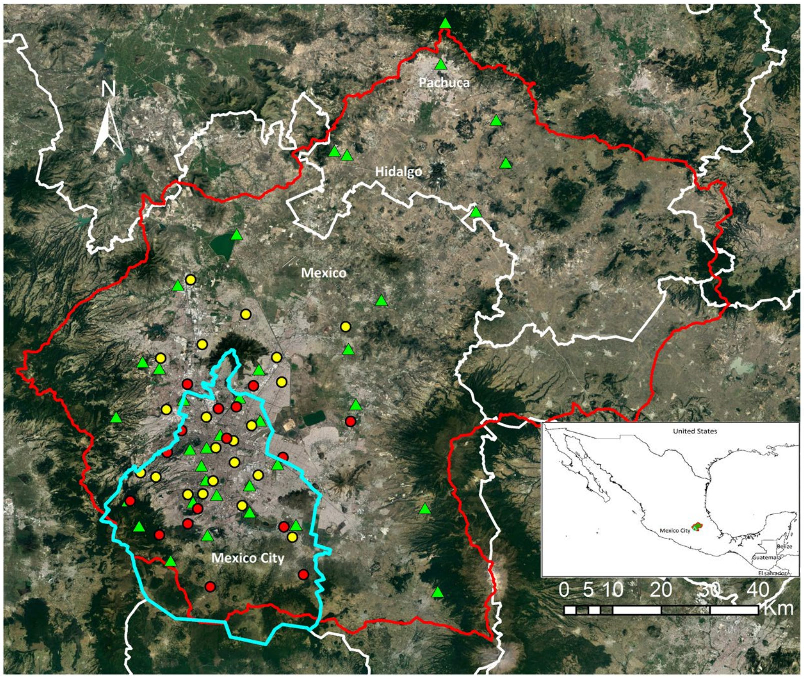

Figure 1. Localization of the Valley of Mexico. Red and yellow dots represent the spatial distribution of the air quality network stations for wet deposition and the automatic monitoring network for NOx, respectively. This network is located mainly in Mexico City and its metropolitan area. Green triangles represent the sites where biomonitors were collected throughout the Valley. The red line delimits the basin, the white line indicates state division, and the blue line shows Mexico City limits. The map was created with ArcGIS 10 (Esri, Redlands, California, USA). Image data: Google Earth; image date: 5 September 2016.

deposition, therefore it has been proposed that an ensemble of various biomonitors for tracking this type of atmospheric pollution improves biomonitoring[11].

To assess whether the combined use of various biomonitors can provide reliable information on nitrogen deposition we: (1) determined the spatial distribution of nitrogen content and the isotopic composition for the lichen *Anaptychia* sp., the mosses *Grimmia* sp., and *Fabronia* sp., and the bromeliad *Tillandsia recurvata* throughout the Valley of Mexico; and (2) evaluated the suitability as biomonitors of these organisms by comparing their nitrogen with an existing automated atmospheric monitoring network.

Results

Spatial distribution of nitrogen deposition. The total wet deposition in Mexico City increased from east to west, it ranged from 23 to 45 kg ha^{-1} $year^{-1}$ during 2013, and between 24 and 50 kg ha^{-1} $year^{-1}$ during 2014 (Fig. 2a). We found the highest rates of wet deposition in northwestern Mexico City. The rest of the valley lacks monitoring stations. The only existing record is from a natural protected area at the north of the Valley, whose wet deposition is always below 5 kg ha^{-1} $year^{-1}$ [17]. For NOx concentrations, the northern part of Mexico City is the zone with the highest concentrations reaching 78.8 ppb during the dry season and 40.6 ppb during the rainy season (Fig. 2b). The lowest concentrations of NOx were found in the south and the east part of Mexico City which reached 21.8 ppb and 9.2 ppb during dry and rainy season respectively (Fig. 2b).

Nitrogen relations of biomonitors. *Anaptychia sp.* The nitrogen content of *Anaptychia sp.* ranged from 1.4% (dry mass) in a natural protected area at the north of the Valley to 5.0% in an urban site in Mexico City during the dry season (Fig. 3a). We observed the same pattern during the rainy season, in which the nitrogen content ranged from 1.3 to 4.6%. The interaction between site and season was significant for all parameters measured (Table 1). A notable finding was that the highest nitrogen deposition of 50 kg N ha^{-1} $year^{-1}$ did not produce the highest nitrogen content in this lichen, which was observed in a site whose nitrogen deposition reached 31.5 kg N ha^{-1} $year^{-1}$.

The C:N ratio ranged from 7.5 to 39.1, increasing from the urban to the rural sites during the dry season; it ranged from 9.4 to 34.1 during the rainy season. Similarly, the $\delta^{15}N$ values ranged between −9.4 and 5.2‰, being more positive in northeastern and downtown Mexico City and in southeastern Pachuca, contrasting with negative values from rural areas (Fig. 3b). A linear regression showed no direct relationship between nitrogen deposition and the parameters measured for this lichen ($R^2 = 0.14$ for nitrogen content, $R^2 = 0.07$ for the C:N ratio and $R^2 = 0.16$ for the $\delta^{15}N$).

Grimmia sp. We found significant differences between the nitrogen content of mosses from a natural protected area at the north of the valley that reached 1.3% where the deposition was 5.0 Kg N ha^{-1} $year^{-1}$, with those of a

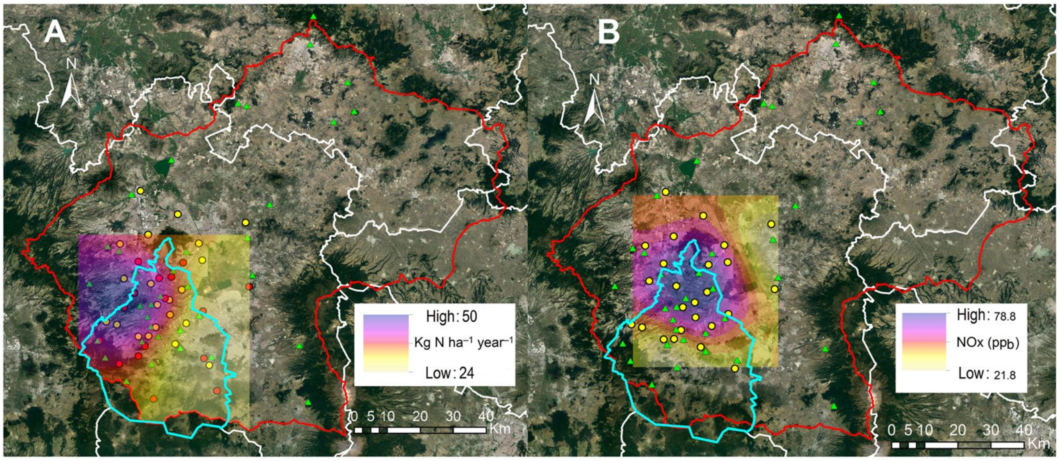

Figure 2. Spatial distribution of the total wet deposition in Kg N ha^{-1} year^{-1} during 2014 (**A**), and atmospheric concentration of NOx in ppb (**B**). Data is available for public access in the website of the Mexico City government (http://www.aire.cdmx.gob.mx/default.php). The map was created with ArcGIS 10 (Esri, Redlands, California, USA). Image data: Google Earth; image date: 5 September 2016.

site at the north of Mexico City of 3.8% where the deposition reached 40 Kg N ha^{-1} year^{-1} (Fig. 3c). We observed no differences in the nitrogen between for the dry and rainy season (Table 1). A linear regression showed that the atmospheric concentration of NOx had no effect on the nitrogen content ($R^2 < 0.01$). The C:N ratio was higher in rural areas and decreased significantly in the urban ones, it ranged between 9.5 and 33.6. On the contrary, NOx concentration had no effect on the C:N ($R^2 < 0.01$).

We found the most negative δ^{15}N value of −7.0‰ in a rural area and the most positive one of 6.6‰ in an urban site where the wet deposition was lower than 35 Kg N ha^{-1} year^{-1}, exceeding this point δ^{15}N became negative reaching −5.3‰, as occurs with mosses growing at 40 Kg N ha^{-1} year^{-1} (Figs. 3d; 4). We also observed positive values in the city of Pachuca at the north of the Valley. Season and NOx concentration showed no effect on the δ^{15}N of this moss (Table 1).

Fabronia sp. The nitrogen content ranged between 1.7 and 3.4% (dry weight) across the Valley. We found no differences between the seasons, but significant differences between the rural areas and some sites in Mexico City (Table 1; Fig. 3e). Wet deposition had no effect on the nitrogen content for the mosses from Mexico City. For example, it only increased by 0.3% when nitrogen deposition when from 24.1 to 48.3 Kg N ha^{-1} year^{-1}. The site and the season were significant (Table 1). The NOx concentration had no effect on the nitrogen content of this moss. In addition, neither the wet deposition, nor the NOx concentration resulted in alterations on C:N ratio, which ranged from 9.0 and 29.5.

The δ^{15}N ranged from −6.6 to 7.8‰. We found the most negative value in a semi-rural site at the north of the Mexico City (Fig. 3f), while a δ^{15}N of -6.0 ± 0.2‰ was observed at the south, where the deposition reached 37.6 Kg N ha^{-1} year^{-1}. The most positive value was found in the eastern border of Mexico City, where the nitrogen deposition was 33.7 and 27.1 Kg N ha^{-1} year^{-1} during 2013 and 2014 respectively. The urban environment in the city of Pachuca also had a considerable effect on the isotopic composition of this moss, as positive values were the rule. Significant differences were found between rural areas and the urban ones. In contrast, the season showed no effect on the isotopic composition of this moss (Table 1). We found a weak relationship between the rate of nitrogen deposition and the δ^{15}N values ($R^2 = 0.2$; Fig. 5).

Tillandsia recurvata. The nitrogen content for *Tillandsia recurvata* ranged from 0.8 to 3.6% during the dry season and from 1.0 to 2.2% (dry weight) during wet season (Fig. 3g). The highest content was observed in the northern part of Mexico City and was significantly different from the lowest value found in a semirural site in the central part of the Valley. At the same time, bromeliads collected in the city of Pachuca had a similar nitrogen content as those from semirural areas of the Valley (Fig. 3g). However, this variation was not due to wet deposition. Instead, it responded positively to the NOx concentration (Fig. 6a). For example, the nitrogen content reached 3.6% in an urban site where the NOx reached 57.4 ppb, contrasting with the lowest nitrogen content that reached 0.8% in a rural site where the NOx concentration presumably was lower than 5 ppb, based on the lowest recorded concentration in Mexico City. Wet deposition had no effect on the C:N ratio of this bromeliad, but it was strongly affected by the concentration of NOx during both seasons (Fig. 6b). For example, we found the lowest C:N ratio of 15.9 where the NOx reached 50.3 ppb, and the highest C:N of 40 where the NOx concentrations were likely lower than 5 ppb. The δ^{15}N values for *T. recurvata* were negative in rural areas and became positive in the cities, varying from −5.0 to 4.4‰ during the dry season and −7.7 to 5.1‰ during the wet season (Fig. 3h). The δ^{15}N values responded positively to the NOx concentration ($R^2 = 0.49$; Fig. 6c), while the wet deposition had no effect on δ^{15}N values ($R^2 = 0.01$). The data from two sites (Fig. 6c open circles) were excluded from the analysis because topographic and exposure to pollutants were non-representative, clearly skewing the isotopic signature. In particular,

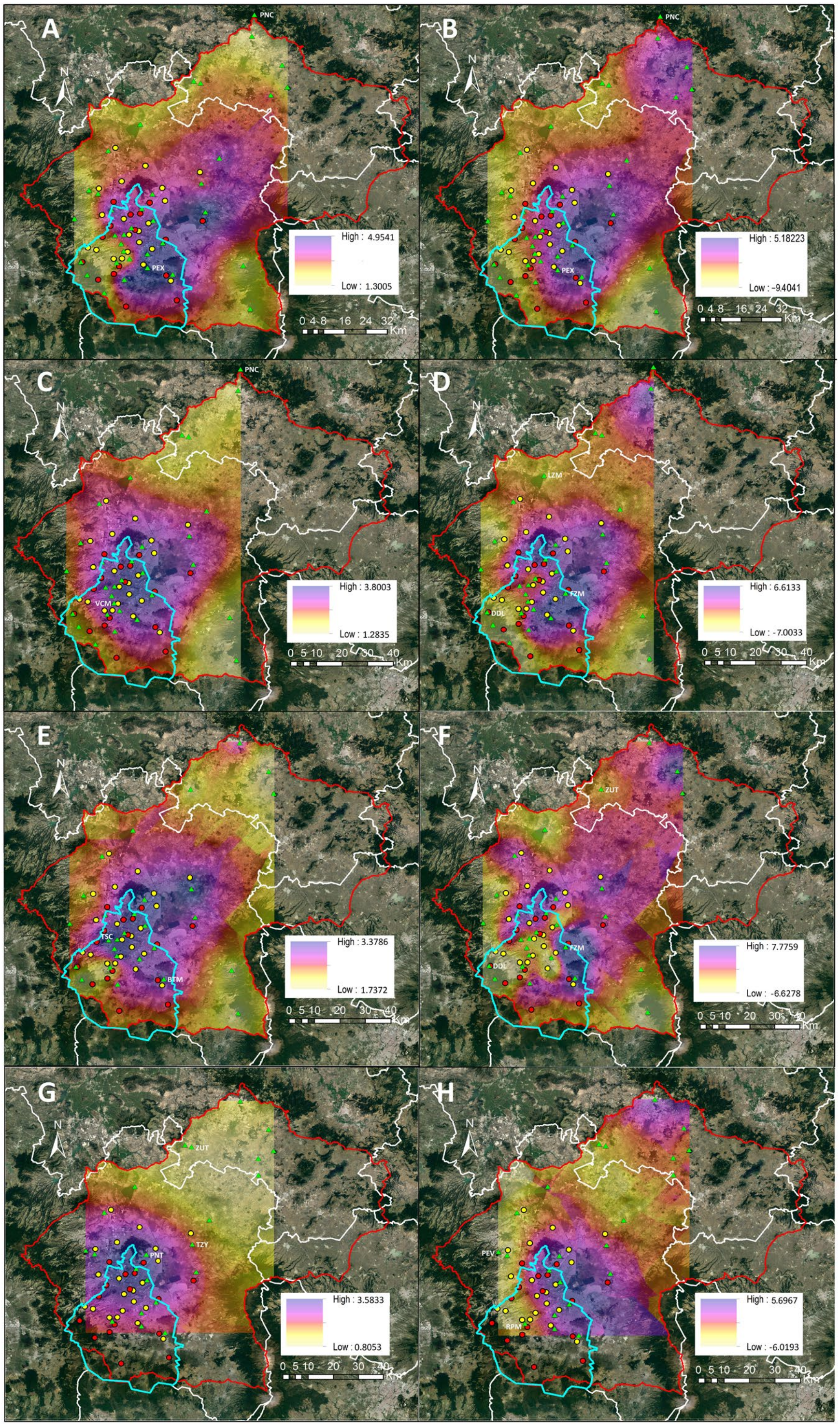

Figure 3. Spatial distribution of the nitrogen content (**A,C,E,G**) and $\delta^{15}N$ values (**B,D,F,H**) for *Anaptychia* sp. (**A,B**), *Grimmia* sp. (**C,D**), *Fabronia* sp. (**E,F**) and *Tillandsia recurvata* (**G,H**). The map was created with ArcGIS 10 (Esri, Redlands, California, USA). Image data: Google Earth; image date: 5 September 2016.

one of the sites is a zoological park with large mammals that is crossed by a stream that was visibly polluted. The second site was a small ravine with dense vegetation, where the diffusion of NOx was probably reduced and where biogenic emissions were negative.

		d.f.	%N (dry weight)		C:N ratio		$\delta^{15}N$ (‰)	
			F	P	F	P	F	P
Anaptychia sp.	Site	33	26.16	<0.001	34.78	<0.001	29.04	<0.001
	Season	1	7.83	0.005	10.14	0.002	0.99	<0.001
	Site × Season	33	3.18	<0.001	3.22	<0.001	2.34	<0.001
Grimmia sp.	Site	31	34.33	<0.001	40.73	<0.001	123.04	<0.001
	Season	1	1.47	0.226	30.02	<0.001	2.95	0.087
	Site × Season	31	5.27	<0.001	3.25	<0.001	4.95	<0.001
Fabronia sp.	Site	29	24.92	<0.001	15.50	<0.001	46.87	<0.001
	Season	1	2.57	0.11	6.14	0.014	1.44	0.232
	Site × Season	29	5.80	<0.001	3.93	<0.001	6.62	<0.001
Tillandsia recurvata	Site	21	34.82	<0.001	33.92	<0.001	57.47	<0.001
	Season	1	96.05	<0.001	6.53	0.011	136.29	<0.001
	Site × Season	21	16.02	<0.001	3.97	<0.001	7.56	<0.001

Table 1. Two-way ANOVA for responses of potential biomonitoring organisms growing in the Valley of Mexico.

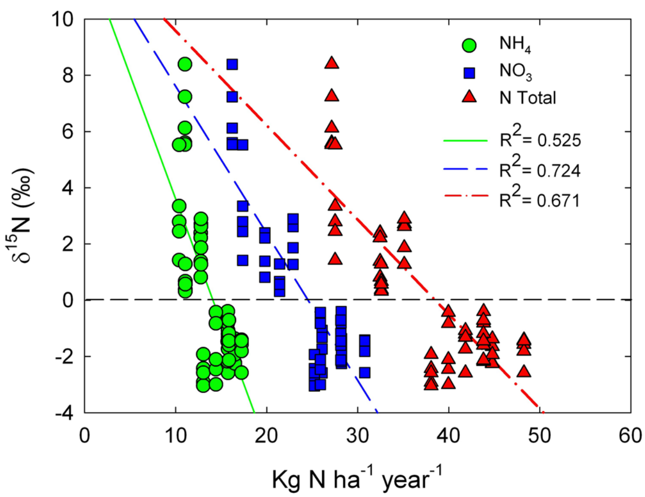

Figure 4. Relationship between wet deposition of ammonium (green circles), nitrate (blue squares), and total deposition, $NH_4^+ + NO_3^-$ (red triangles) during 2014 and the $\delta^{15}N$ values of the moss *Grimmia* sp.

Discussion

The distribution of nitrogenous pollution and deposition in Mexico City generally matched that of the sources of emission. For instance, the highest values were measured in the northern part of the city, where the majority of the manufacturing industries are located along numerous important and busy motorways[18]. Indeed, motor vehicles, followed by industrial emissions, are the most important source of NOx in México City, whose concentration is directly measured by the city government from the air[18,19]. In addition, nitrogenous emissions from the agricultural zone between Mexico City and Pachuca substantially contribute to the observed pattern, given predominant north-to-south winds[18]. In this case, NHx originated from both agricultural and industrial activities are measured from wet deposition.

The nitrogen content of lichens has been utilized as an effective indicator of nitrogen deposition. For example, three species of lichens can record nitrogen inputs of wet deposition below 10 Kg N ha^{-1} $year^{-1}$, point at which they saturate[12,20]. We observed a threshold of nitrogen tolerance for lichens from the Valley, when the deposition reached 31.5 kg N ha^{-1} $year^{-1}$ above which they could not take up additional nitrogen. We observed a similar pattern for the $\delta^{15}N$. While the negative values from rural areas were the result of low rates of deposition, high rates of deposition in urban areas resulted in positive isotopic values. However, above a threshold of saturation the isotopic values turned negative, as has been already observed in other species of lichen[21]. Their isotopic composition responded more to wet than to dry deposition as was observed for mosses. Additionally, different studies show a point of saturation when studying throughfall deposition[20,21].

The nitrogen content of moss tissues is affected by the rate of deposition and responds to the distance to urban centers[14,22–24]. We also observed an important influence of urban centers for the nitrogen content of mosses from the Valley. Their $\delta^{15}N$ values are affected by the rates of deposition and the prevalent pollutant (e.g. wet NH_4^+ or NO_3^-; dry NHx or NOx,), being more commonly negative in mosses from rural areas[25–28]. The observed negative

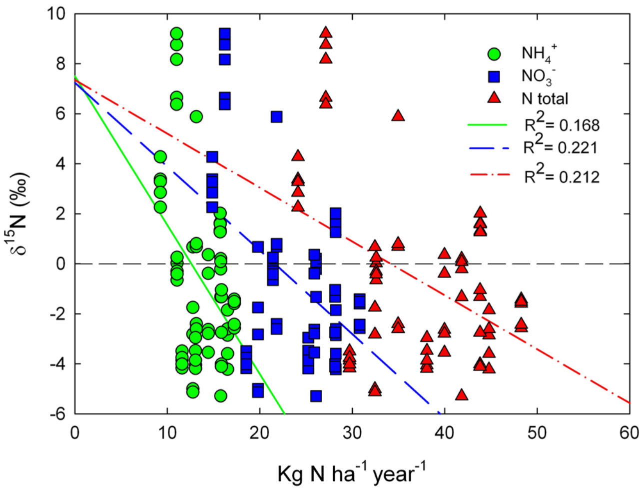

Figure 5. Relationship between wet deposition of ammonium (green circles), nitrate (blue squares) and total deposition, $NH_4^+ + NO_3^-$ (red triangles) during 2014 and the $\delta^{15}N$ values of the moss *Fabronia* sp.

$\delta^{15}N$ for mosses from rural areas resulted from uptake of NH_4^+ derived from fertilizers and livestock emissions which have characteristic negative $\delta^{15}N$ values[27,29,30]. Mosses from urban areas of the Valley such as Pachuca, as well as some areas of Mexico City where the wet deposition was below 35 Kg N ha^{-1} year^{-1} had positive $\delta^{15}N$, suggesting that they most likely take up NO_3^- derived from NO_x of fossil fuel burning and industrial activities which typically display positive $\delta^{15}N$[29]. Another possible mechanism influencing the isotopic composition of these mosses is the gas-particle conversion process, in which isotopic enrichment occurs for the aerosol nitrogen resulted, producing positive $\delta^{15}N$[31].

Mosses take up NH_4^+ preferentially over NO_3^- because less energy is needed in its assimilation[32]. Additionally, high deposition rates can cause the inhibition of nitrate reductase, reducing NO_3^- assimilation[27,33,34]. This occurs to some degree when the deposition reaches 10 Kg N ha^{-1} year^{-1}, but when it exceeds 30 Kg N ha^{-1} year^{-1} the nitrate reductase becomes completely inhibited not only precluding NO_3^- uptake, but even leading to its loss by leaching[27,35,36]. Nitrate reductase inhibition also has an important effect on the isotopic composition of mosses because it favors the uptake of NH_4^+ that typically has negative $\delta^{15}N$ values[30]. In the Valley of Mexico, moss $\delta^{15}N$ became negative when the rates of wet deposition exceeded 35 Kg N ha^{-1} year^{-1}, which suggests that nitrate reductase was indeed inhibited, allowing NH_4^+ uptake, a pollutant that represented 35% of the total wet deposition in Mexico City during 2014[18,36,37]. The atmospheric concentration of NOx had no effect on the nitrogen content nor the isotopic composition of either moss considered here because when NH_3 is the prevalent nitrogenous pollutant in the atmosphere, the nitrogen content of the mosses can increase more than from wet deposition. However, in Mexico City the prevalent nitrogenous gas pollutant is NOx, suggesting that nitrogen content and the $\delta^{15}N$ of the mosses responded more to wet deposition than to gaseous pollutants[27,38].

Despite the high rates of wet deposition recorded in Mexico City which exceeded 50 Kg N ha^{-1} year^{-1} in some areas, neither the nitrogen content nor the C:N ratio of *Tillandsia recurvata* were directly affected. This occurs because raindrops cannot be absorbed by the non-absorptive roots of these plants[39]. Instead, nitrogen was taken up as NOx by the leaves of this bromeliad because it can absorb particles and gasses from the air thanks to stomatal gas exchange and the trichomes present in the surface[39]. This is evident from the close relationship found here between the nitrogen content and the NOx concentration in the Valley. This is similar to what occurs for other atmospheric bromeliads, whose nitrogen content is higher in the vicinity of highways than further away[40–42]. The seasonal differences found for the nitrogen content appeared to respond to the phenology of this bromeliad which grows after the rainy season. Indeed, the NOx were dragged from the atmosphere to the ground surface during rainy season reducing its biological availability[18,43].

Both the nitrogen content and the isotopic composition of *T. recurvata* were determined by the predominant anthropic activity of the sites where the plant was collected. Bromeliads take up NOx with positive $\delta^{15}N$ from industrialized and densely populated areas, contrasting with the negative emissions originated from biogenic sources, such as the soil and livestock waste[44,45]. The positive $\delta^{15}N$ found for *T. recurvata* suggests no nitrate reductase inhibition, but it is likely that NOx concentrations higher than 80 ppb may result in some inhibition of this enzyme. This could be one of the reasons why *T. recurvata* was not found in sites with concentrations of NOx higher than 80 ppb.

The distinct responses observed for each biomonitor evaluated here are a result of the ecophysiological traits of each functional type. Indeed, the lack of a cuticle and a vascular system for mosses, allows a rapid/ready assimilation of incoming pollution, mainly from wet deposition. However, their weak response to NOx levels could be an effect of plant phenology, as the mosses remain dormant during the dry season, so the uptake of the gas is not physiologically possible. For the case of the tillandsia, the fact that it is adapted to a water and nutrient-limiting environment was the cause for its suitability for monitoring NOx, as all its nutrition is atmospheric and their water is intercepted from the atmosphere[46]. In addition, their potential uptake of wet deposition is inherently

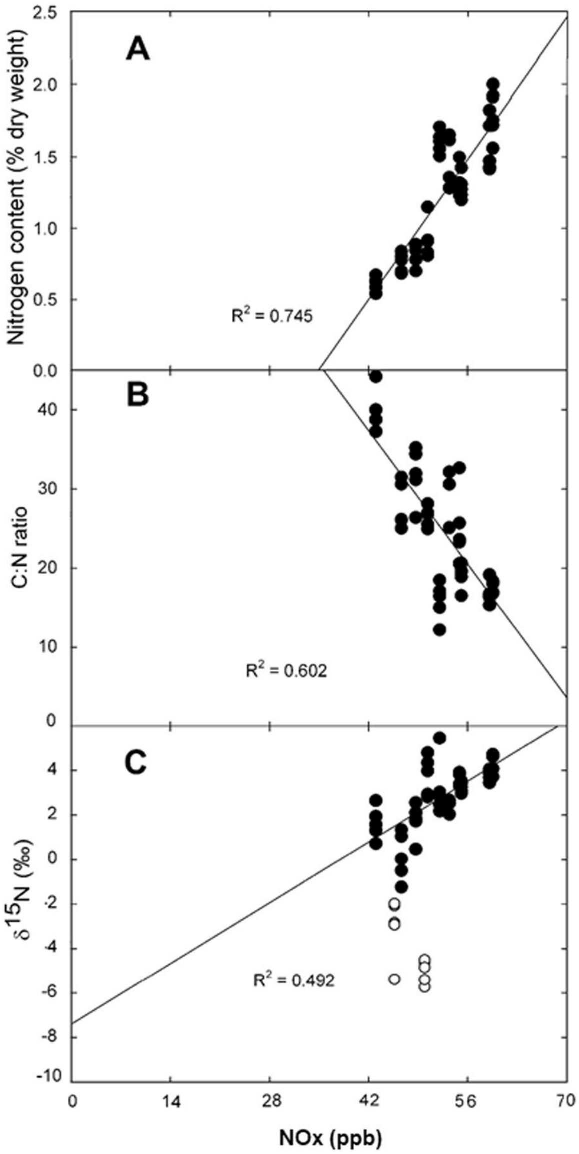

Figure 6. Relationship between NOx concentration during the 2014 dry season and the nitrogen content (**A**), C:N ratio (**B**), and the $\delta^{15}N$ values (**C**) of the bromeliad *Tillandsia recurvata*. Open circles were excluded from the regression analysis because they were collected from non-typical environmental conditions that skewed the isotopic signatures of *T. recurvata* to very negative values.

limited by their small area to volume ratio that in turn reduces water loss[47]. Also, their rates for taking up water are low, so exposure to more benign environments does not lead to increased water uptake as we have found experimentally[48].

Of course, caution must be taken when implementing a biomonitoring method utilizing spontaneously occurring plants, as their nitrogen parameters can also respond to various factors other than nitrogenous pollution. For instance, if dewfall is substantial during the dry season, mosses can remain physiologically active, and thus be able to assimilate NOx. Also, water availability and extreme temperatures can have an effect on the N relations of plants. For instance, high daytime temperatures and a very low relative humidity during the dry season or a very cloudy sky during the rainy season can both lead to stomatal closure during the night for the CAM bromeliads. In either case, uptake of NOx will not occur. Other factors to consider are pathogens and herbivores[49]. For these reasons, specific biomonitors need to be developed for each region of interest, taking in consideration the particular environmental conditions and the ecophysiological traits of potential biomonitors[11].

For the case of the Mexico valley, and by possible extension to the Trans-Mexican Volcanic Belt, *T. recurvata* can be utilized for monitoring NOx along with a moss that records wet deposition. This approach can become a useful tool for determining the status of nitrogenous pollution in regions where air quality monitoring networks are not available. Especially in mid-sized cities and surrounding areas where the saturation thresholds of these organisms has not been reached. Finally, the utilization in tandem of these organisms can inform an early alert for avoiding health problems for both ecosystems and humans.

Methods

Study area. The Valley of Mexico is located in central Mexico spanning 7500 km^2, with an average elevation of 2240 m[50]. Annual precipitation ranges from 600 mm at the center of the valley to 1300 mm in the surrounding mountains. The predominant winds blow from the northeast and northwest[51]. Mexico City sits at the southern edge of the Valley with a population of 20 million. At the northern edge, Pachuca, the capital of the state of Hidalgo, has a population of 3 million. Additional, settlements interspersed in the valley with industrial or agricultural activities comprise the rest of the 30 million inhabitants of this basin[10].

Nitrogen environment. The Mexico City environmental authority has deployed an air quality network of 16 monitoring stations for wet deposition (Fig. 1). This network collects data during the rainy season from May to November. The nitrogen collected consists of dissolved NO_3^- and NH_4^+, so that the total nitrogen is the sum of both forms of deposition ($NO_3^- + NH_4^+$). This dissolved inorganic nitrogen (DIN) is biologically available[27]. For this study, the rates of wet deposition measured by this monitoring network during 2014 were utilized for the analyses described below. This air quality network also has 27 monitoring stations for measuring the atmospheric NOx concentration ($NO + NO_2$) year-round, 24 hours a day. We utilized the mean seasonal concentration in ppb during dry season (November 2013 to April 2014) and during the rainy season (from May 2014 to October 2014). Data were obtained from the website of the environmental authority of the Mexico City (http://www.aire.cdmx.gob.mx/default.php).

Biomonitoring. We selected four species of three types of atmospheric organisms, the lichen *Anaptychia* sp., the mosses *Grimmia* sp. and *Fabronia* sp., and the bromeliad *Tillandsia recurvata* (L). These organisms have been utilized as monitors of nitrogen deposition and are abundant in the Valley of Mexico[14,15,21]. We collected tissue samples from 5 individuals of each species growing on different substrates from 36 sites throughout the valley, including urban parks, agricultural sites, and natural protected areas, for the dry season on (8–20 May) and, the wet season 3–15 November of 2014. The distribution of the sites within the basin was determined by the occurrence of the biomonitoring species and by the complex nature of the landscape, which precluded the collection of samples from a regular grid[52].

The tissue samples were dried at 60 °C in a gravity convection oven until reaching constant weight. The dried tissues were ground to a fine powder in a ball mill (Retsch MM300; Retsch, Vienna, Austria), wrapped into tin capsules (Costech Analytical, Inc. Valencia, California, USA), and weighed with a microbalance (0.01 mg, Sartorius, Göttingen, Germany). For each sample, both the nitrogen content and their isotopic signature were determined at the Stable Isotope Facility, University of Wyoming (Laramie, Wyoming, USA), with a Carlo Erba EA 1110 elemental analyzer (Costech Analytical Inc., Valencia, CA, USA) attached to a continuous flow isotope ratio mass spectrometer (Finnigan Delta Plus XP, Thermo Electron Corp, Waltham, MA). Nitrogen isotope ratios, reported in parts per thousand, were calculated relative to atmospheric air standards.

The analytical precision for the $\delta^{15}N$ was $0.3 \pm 0.07‰$ (SD). The natural abundances of ^{15}N were calculated as:

$$\delta^{15}N(‰_{\text{versus air}}) = \left(R_{\text{sample}}/R_{\text{standard}} - 1\right) \times 1000$$

where, R is the ratio of $^{15}N/^{14}N$ for nitrogen isotope abundance for a given sample[53,54].

Statistical analyses. We utilized linear regressions to determine the relationship between total wet nitrogen deposition and the nitrogen content (% dry weight), the C:N ratio as well as the $\delta^{15}N$ of the organisms considered. The same was done for the concentration of atmospheric NOx. Data accomplished normality in both cases[30]. We performed two way ANOVAs (factors were site and season) followed by a post hoc Holm–Sidak tests ($p \le 0.05$) to determine differences in the nitrogen content (% dry weight), the C:N ratio, as well as the $\delta^{15}N$ values of the tissue samples. All statistical analyses were conducted with Sigmaplot 12 (Systat Software Inc. USA).

Geostatistical analyses. We utilized the ordinary Kriging method (a geostatistical gridding tool for irregularly spaced data[55]) to determine the rates of wet deposition, and the concentration of NOx in the sites where no monitoring station was available for the area covered by the monitoring network. Likewise, we modelled the geographical distribution of the nitrogen content, C:N ratio, and the $\delta^{15}N$ values for the biomonitors, as well as the data from the monitoring network with ArcGIS 10 (Esri, Redlands, California, USA).

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

Both authors planned the study, conducted field work, took part in the preparation of the manuscript, have reviewed the results, and have approved the final version of this manuscript. E.A.D.A. analyzed the data, prepared all of the figures, and wrote the initial version of the manuscript. E.d.l.B. procured funding, revised and approved the manuscript, and drove in the field and planned a gastronomic tour in the Valley of Mexico.

Additional Information

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