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Mapping pollution in a megalopolis: the case for atmospheric biomonitors of nitrogen deposition

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Abstract

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. However, deployment of automated networks can be excessively costly for most cities so the utilization of widely distributed biological species suitable for biomonitoring may be a good alternative. The aim of this work was thus to assess the suitability of different atmospheric organisms as biomonitors of nitrogen deposition, by means of an extensive sampling of a lichen, two mosses, and one bromeliad throughout the Valley of Mexico, the basin where the megalopolis of Mexico City (population 20 million) is located, and subsequent measurements of nitrogen metabolism parameters. In all cases significant responses of nitrogen content, C:N ratio and $\delta^{15}N$ were found for the lichen *Anaptychia* sp. the mosses *Grimmia* sp. and *Fabronia* sp., and the bromeliad *Tillandsia recurvata* in response to season and collected site. In turn, $\delta^{15}N$ for the mosses responded linearly to the wet deposition (R^2= 0.7 for *Grimmia* sp. and R^2=0.2 for *Fabronia* sp.). 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, latter species was not found in sites with NOx concentrations above 212 ppm. These biomonitors can be utilized in tandem to determine the status of nitrogenous pollution in regions without monitoring networks.

Capsule:

An ensamble of atmospheric biomonitors of nitrogen deposition can be usefull for determining pollution in urban areas. In particular, tissue nitrogen content, C:N ratio, and $\delta^{15}N$ of mosses responded to wet deposition, while those of *Tillandsia recurvata* were able to track NOx.

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Introduction

Nitrogen deposition, which has increased by more than 50% since the second half of the 20th century, is one the most predominant pollutants in the atmosphere (Vitousek et al., 1997; Galloway et al., 2004; Steffen et al., 2015). This phenomenon results from the release of nitrogenous compounds to the atmosphere, both in cities by industrial and automobile activities and the countryside by agriculture and livestock. These activities cause soil and water acidification and eutrophization, changes in ecosystem structure, i.e., causes the decrease of the species richness and proliferation of some invasive species, representing a severe threat for global biodiversity (Sala et al., 2000; Galloway et al., 2004; Phoenix et al., 2006; Duprè et al., 2010; Scherer-Lorenzen et al., 2007; Silva et al., 2015). Nitrogenous pollution not only represents a risk for global biodiversity, but it also is an issue for human health worldwide. In highly populated areas jammed-traffic motorways produce high accumulations of NOx at ground level that can be haemful by direct inhalation. Additionally, NOx is a precursor of ozone, another dangerous compound. For example, in Mexico City, a megapolis with more than 20 million habitants, at least 9600 deaths are attributable to atmospheric pollution annually, resulting from, respiratory and heart diseases, and pollutant build up in the nervous system and the brain (Samet and Utell, 1990; Stevens et al., 2008; Instituto Nacional de Estadística y Geografía 2011; Lim et al., 2012; Maher et al., 2016). Harmful effects on human health are also observe, in the countryside, where ammonia emissions quickly react with other atmospheric pollutants to form airbone particulate matter that can go into the respiratory system. However, despite of the known effects caused by the particulate matter, the precise effects of ammonia are uncertain (Harrison and Yin, 2000; Schwartz et al., 2002; Samet and Bell, 2004; Reay, 2015). Yet nitrogen is often neglected in public policies and the prevalent discourse of global environmental change.

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Monitoring nitrogenous pollution is an important issue. However, the deployment of air quality monitoring networks is cost prohibitive in many regions of the planet, including countries such as Mexico with developing economies. In this respect, according to Mexican law, cities with populations above 500,000 must have air quality monitoring networks, but most of them lack of such systems (SEMARNAT 2012). To fill this gap, a cost effective alternative is the use of biomonitors. For example, throughfall nitrogen deposition can be determined by means of the thallus nitrogen content of lichens (Root et al., 2013). Also, mosses are widely used and reliable biomonitors that have been utilized over various years, because they allow covering vast areas of territory at low cost and can reflect the rates of deposition by means of their nitrogen content, as well as the possible sources of pollution by their isotopic composition (Pitcairn et al., 2001; Harmens et al., 2011). This results from their lack of cuticle, which allows the uptake of nutrients directly from the atmosphere. In addition, their lack of an active mechanism to mobilize chemicals results in a low isotopic fractionation (Peñuelas and Filella, 2001; Bragazza et al., 2005; Liu et al., 2012a). Another potential group of biomonitors for nitrogen pollution are atmospheric bromeliads, whose CAM photosynthesis enables them to be physiologically active round year and uptake pollution regardless of environmental conditions. In particular, the bromeliads of the genus *Tillandsia* that are amply distributed in the Americas in a wide variety of ecosystems, has been utilized for monitoring NOx pollution (Figuereido et al., 2004; Reyes-Garcia and Griffiths, 2009; Zambrano et al., 2009; Felix et al., 2016).

The combined use of these groups of biomonitors can provide reliable information and an approximate picture of atmospheric pollution in regions without air quality monitoring networks. For this reason the aims of this study were to: 1) determine the spatial distribution of nitrogen content and the isotopic composition of the lichen *Anaptychia* sp., the mosses *Grimmia* sp., and

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Fabronia sp., and the atmospheric bromeliad *Tillandsia recurvata* throughout the Valley of Mexico; 2) evaluate the suitability of these organisms for monitoring the response to the various chemical species comprising nitrogen deposition and appraise possible use in vast areas of the territory where no monitoring networks are available.

Materials and methods

Study area

The Valley of Mexico is located in central Mexico where it covers an area of 7500 km^2 and is surrounded by the Neo-Volcanic Axis Mountains that reach elevations above 3500 m, while the mean elevation of the Valley averages 2240 m (Fig. 1; Calderón and Rzedowski, 2001). Annual precipitation, which occurs seasonally from May to October, ranges from 600 mm in the lower part of the Valley to 1300 mm up in the mountains. The mean annual temperature ranges from 15 to 18 ^{o}C thorough the interior of the valley, and is lower than 10 ^{o}C in the mountains. Predominant winds blow from the northeast and northwest (Servicio meteorologico nacional 2016).

In the southern portion of the Mexico Valley sits Mexico City, one of the largest cities in the world, whose population exceeds 20 million. At the north of the Valley sits Pachuca the capital of the state of Hidalgo, whose population reaches 3 million. Also, within the Valley occur small towns dedicated to industry and fields dedicated to agriculture, adding to a total population of the Valley of Mexico that reaches over 30 million (Instituto Nacional de Estadística y Geografía 2011).

Nitrogen deposition data

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The Mexico City environmental authority has deployed an air quality network of 16 monitoring stations for wet deposition (Fig. 1). This network collects data from May to November, during the rainy season. 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 is the so called dissolved inorganic nitrogen (DIN), which is biologically available (Liu et al., 2012a). For this study, the rates of wet deposition measured by this monitoring network during 2014 were utilized for the analyses described below. The Mexico City air quality network also has 27 monitoring stations that measure the atmospheric NOx concentration year round, during 24 hours at day. We utilized the sum of the atmospheric NOx concentration during the 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 (Sistema de Monitoreo Atmosférico, Ciudad de México. 2015).

Plant material and sampling

Four species of three types of atmospheric organisms were selected for this study: the lichen *Anaptychia* sp., the mosses *Grimmia* sp. and *Fabronia* sp., and the epiphytic bromeliad *Tillandsia recurvata* (L). These types of organisms have been utilized separately in various studies as indicators of nitrogen deposition (Zambrano et al., 2009; Harmens et al., 2011; Jovan et al., 2012). These species are widely distributed in the tropical and subtropical Americas, and are particularly abundant in the Mexico Valley, growing in numerous substrates such as, tress of the genus *Acacia, Casuarina, Fouquieria, Pinus, Quercus,* and *Schinus*. Additionally, they can be found growing on plants of the genus *Opuntia* and other cacti. The bromeliad is also commonly found attached to electricity cables of many cities and roads, meanwhile, the moss

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and the lichen are also commonly found on concrete constructions and bare rocks (Root et al., 2013; Cardenas and Delgadillo 2009; Zambrano et al., 2009).

Tissue samples of the above mentioned species were collected from 36 sites throughout the Valley of Mexico in May and November 2014. For each site and season samples were collected from 5 individuals of each species. The bromeliad was only found in 24 sites of the Valley. Samples were collected from different phorophytes such as *Casuarina*, *Pinus*, *Schinus*, *Opuntia* and some cacti, in urban parks, agricultural sites and protecting natural areas throughout the Valley. 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 made it difficult to collect samples on a regular grid (Wang and Pataki, 2010).

Isotopic analysis

The tissues of the different biomonitors from the Valley of Mexico 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, the carbon and nitrogen content, as well as their isotopic proportions, 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 the atmospheric air standards.

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

$$\delta^{15}N_{(‰\ versus\ air)} = (R_{sample}/R_{standard} - 1) \times 1000$$

where, R is the ratio of $^{15}N/^{14}N$ for nitrogen isotope abundance for a given sample (Ehleringer and Osmond, 1989; Evans et al., 1996).

Statistical analysis

Multiple regression models were utilized 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 evaluated. The same analysis was conducted for the concentration of the atmospheric NOx. Data passed a normality test in both cases (Skudnik et al., 2015). To determine differences among the nitrogen content (% dry weight), the C:N ratio as well as the $\delta^{15}N$ values of the species studied from different sites and season throughout the Valley two way ANOVAs was performed followed by a post hoc Holm–Sidak analysis tests ($p \leq 0.05$) for multiple comparisons to identify significant pairwise differences. Statistical analyses were conducted with Sigmaplot 12 (Systat Software Inc. USA).

Geostatistical analysis

The geographical distribution of the nitrogen content, C:N ratio and the $\delta^{15}N$ values in the lichen, the mosses and the bromeliad, as well as the data obtained from the monitoring network were mapped in ArcMap 10 (part of ArcGIS® Esri, Redlands, USA). The ordinary Kriging method (a geostatistical gridding tool for irregularly spaced data; Cressie, 1988) was utilized to determine the rates of wet deposition, and the concentration of NOx in those samples sites where

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a monitoring station network was not available inside of the area covered by the monitoring network.

Results

Anaptychia sp.

Nitrogen content of *Anaptychia sp.* ranged between 1.3 and 5.0% (dry weight), significant differences were found among sites. In particular, the differences were clearer when comparing sites located in the city and the countryside. For example, lichens growing in rural areas such as PNC had the nitrogen content in the lower part of the range lichens growing in Mexico City where the nitrogen content reached the high value of 5.0% (Table 1; Fig. 3A).

The nitrogen content was also affected by the season (Table 1). However, in the most polluted areas of Mexico City the tissue nitrogen content was not as high as it was in sites with intermediate nitrogen pollution, e.g. PEX with 31.5 Kg N ha^{-1} $year^{-1}$ led to a nitrogen content of 5.0% (Fig. 3 A). The interaction between site and season was significant for all parameters measured (Table 1).

The C:N ratio ranged from 7.5 and 39.1. It was affected by the season, and the site (Table 1). Similarly, the isotopic composition was affected by the season, and the site with $\delta^{15}N$ ranging between –9.4 and 5.2‰, they tended to be more positive in northeast and downtown Mexico City and in southeast Pachuca, which contrast with values from rural areas like PNC, where they were very negative (Fig. 3 B; Table 1). Multiple regression showed no direct relationship between the wet deposition and the nitrogen content nor the $\delta^{15}N$ values. Also, multiple regression showed that the atmospheric concentration of NOx had no effect on the nitrogen content, the C:N ratio and the isotopic composition (see appendix).

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Grimmia sp.

The lowest nitrogen content of 1.3 ± 0.1% was found for mosses growing in PNC during both seasons (Fig. 3 C). At this site, the rates of wet deposition were below to 5 Kg N ha^{-1} $year^{-1}$. The nitrogen content was statistically different for mosses growing in VCM where the rates of wet deposition reached 39.9 Kg N ha^{-1} $year^{-1}$ and it reached 3.8 ± 0.1% (Fig. 2 A, Fig. 3 C; Table 1). The nitrogen content throughout the Valley averaged 2.8 ± 0.1% (dry weight). Despite the high rates of wet deposition recorded in Mexico City, mosses were not affected by the rates of wet deposition that ranged from 27.14 to 48.26 Kg N ha^{-1} $year^{-1}$ of total nitrogen (NH_4^+ + NO_3^-) during 2014. There were no significant differences between the nitrogen content of mosses collected at the end of the dry season and mosses growing at the end of the rainy season (Table 1). Also, the atmospheric concentration of NOx had no effect on the nitrogen content of this moss (see appendix).

The C:N ratio of *Grimmia* sp. was directly affected by the site where it grew (Table 1). In particular, it was higher in rural areas and decreased significantly in the urban portion of the Valley, ranging between 9.5 and 33.6, with an average of 14.6 ± 0.4. The rates of wet deposition had no effect on the C:N ratio, while the relationship between C:N ratio and the NOx concentration inside the monitoring network was very low ($R^2 < 0.01$; see appendix).

The $\delta^{15}N$ of *Grimmia* sp. was affected by the site, in particular they were negative in rural areas and were positive in urban areas where the wet deposition were lower than 30 Kg N ha^{-1} $year^{-1}$. However, when the wet deposition exceeded this threshold, the $\delta^{15}N$ became negative (Table 1; Fig. 3 D). In turn, the season showed no effect on the $\delta^{15}N$ of this moss. The $\delta^{15}N$ of *Grimmia sp.* was strongly affected by the wet deposition. In particular, the natural abundance of ^{15}N of the moss decreased with increasing rates of NO_3^+ during both seasons (Fig 4). The most

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positive $\delta^{15}N$ value of 6.6‰ was found on FZM where the total wet nitrogen deposition reached 27.14 Kg N ha^{-1} $year^{-1}$ during 2014 (Fig. 2 A, Fig. 3 D). The lowest $\delta^{15}N$ value recorded for *Grimmia sp.* in Mexico City was –5.3‰ in DDL, where the total wet deposition reached 40.1 Kg N ha^{-1} $year^{-1}$ during 2013. However, the most negative value recorded for this moss across the Valley was –7.0‰ at LZM, at this semi-rural site there is no monitoring station. Similar to the nitrogen content and the C:N ratio, the concentration of NOx showed no effect on the isotopic composition of this moss (See appendix).

Fabronia sp.

The nitrogen content of *Fabronia sp*. ranged between 1.7 and 3.4% (dry weight) across the Valley, on average it reached 2.6 ± 0.1% (dry weight), no statistical differences were found between seasons, but significant differences were found between the countryside and some regions inside the urban area (Table 1). Multiple regression showed that wet deposition had no effect on the nitrogen content of this moss. For example, the lower deposition of 24.13 Kg N ha^{-1} $year^{-1}$ recorded at BTM had a corresponding nitrogen content of 3.1%, while a higher deposition of 48.26 Kg N ha^{-1} $year^{-1}$ in TSC had a corresponding nitrogen content of 3.4% (Fig. 2 A, Fig. 3 E). This lack of response was also observed between the concentration of atmospheric NOx and the nitrogen content of this moss. The C:N ratio that ranged between 9.0 and 29.5 was not affected by the wet deposition nor by the atmospheric concentration of NOx. The site and the season were statistical significant (Table 1).

The $\delta^{15}N$ across the Valley ranged from –6.6 to 7.8‰, the most negative value was found in ZUT, in this site there is not a monitoring network. In turn, a $\delta^{15}N$ of –6.0 ± 0.2‰ was found in DDL where nitrogen deposition reached 37.65 ± 0.01 Kg N ha^{-1} $year^{-1}$ (Fig. 2 A; Fig 3 F), the

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most positive value was found in FZM, where the nitrogen deposition was 33.69 and 27.14 Kg N ha^{-1} $year^{-1}$ during 2013 and 2014 respectively. Significant differences were found between rural areas and the city, on the contrary season showed no effect on the isotopic composition of this moss (Table 1).

Tillandsia recurvata

The nitrogen content of *Tillandsia recurvata* ranged between 0.8% and 3.6% (dry weight) with an average of 1.8 ± 0.2% during the dry season throughout the Valley. While during the wet season it ranged between 1.0 and 2.2% (dry weight) averaging 1.4 ± 0.1%, significant differences were found between plants of the countryside and the city (Table 1; Fig. 3 G). No relationship was found between nitrogen content and the rates of wet deposition inside the atmospheric monitoring network of Mexico City. In contrast, a positive relationship between the nitrogen content of *T. recurvata* and the atmospheric concentration of the NOx was found between the plants collected during the dry and the rainy season (Fig. 6A). The highest nitrogen content of 3.6% was found in PNT where the atmospheric concentration of NOx reached 212.7 ppm (Fig. 2B). The lowest nitrogen content of 0.8% was found in ZUT where there is no monitoring station, but a nitrogen content of 1.2% was found in TZY where the concentration of NOx reached 153.6 ppm during the dry season. This pattern was also observed during the rainy season when the nitrogen content of 1.1% was found at RPM where the NOx reached 119.3 ppm. Meanwhile, a nitrogen content of 1.9% was found in plants collected at PNT where the concentration of NOx reached 134.5 ppm.

The C:N ratio of *Tillandsia recurvata* was not affected by wet deposition, but it was strongly influenced by the concentration of NOx during both seasons (Fig. 6B). The site had a

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strong influence in the C:N ratio being higher at rural areas and lower in the Mexico City area (Table 1). The lowest C:N ratio was 15.9 at CLM where the NOx was 185.7 ppm during dry season. During the rainy season the lowest C:N ratio of 21.5 was measured for plants from BJA, where the NOx concentration reached 131.7 ppm. The highest C:N ratio of 40 and 37.6 was found at TZY where the concentration of NOx reached 153.6 ppm and 109.5 ppm during the dry and rainy season respectively. (Figure 6 B).

The $\delta^{15}N$ of the *Tillandsia recurvata* growing in rural areas were negative, contrasting with the positive values found in the city, also significant differences were found between seasons (Table 1; Fig. 3 H). No relationship between the $\delta^{15}N$ and the wet deposition during both seasons was observed. The relationship between atmospheric NOx concentration and the $\delta^{15}N$ of the plants collected in both seasons was weak. However, when data were analyzed excluding RPM and PEV, the relationship becomes stronger for data of plants at both seasons (Fig. 6 C). Outside of the area where the atmospheric monitoring network is deployed there was a clear relationship between the $\delta^{15}N$ of the plants and the human activity associated with each site. For instance, in the city of Pachuca at the northern portion of the Valley, the $\delta^{15}N$ are positive, like in Mexico City, while in the rural areas between these cities the $\delta^{15}N$ were negative.

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Discussion

Anaptychia sp.

The nitrogen content of lichens has been effectively utilized as indicator of nitrogen deposition. For example, three species of lichens in the United States can detect deposition inputs less than 10 Kg N ha^{-1} $year^{-1}$ (McMurray et al., 2013; Root et al., 2013). Here, the nitrogen content of the lichen´s thallus responded to the nitrogen deposition in the Valley of Mexico. In particular, it was lower at rural areas and was higher at intermediate nitrogen deposition sites, but it decreased again in sites where nitrogen deposition reached 49 Kg N ha^{-1} $year^{-1}$ of wet deposition. Lichens that were exposed to different nitrogenous compounds from dry and wet deposition reached a threshold beyond which they cannot take up more nitrogen, this explains why no relationship was found between nitrogen content and wet deposition, nor for the NOx concentration. The same was observed for the $\delta^{15}N$ values, which were negative in rural areas, positive in the city, but become negative under the highest rates of deposition (Jovan et al., 2012). Owing to the lack of a direct relationship between the nitrogen content and the isotopic composition of the lichen with the dry or the wet deposition in the Mexico City area, it was difficult determine which type of deposition caused the major effect on this organism.

Grimmia sp. and *Fabronia sp.*

The nitrogen content of mosses is directly affected by the increase of nitrogen deposition rates and, as a result, it is a function of the distance to urban centers (Solga et al. 2005; Liu et al. 2008a; Liu et al. 2008b Zechmeister et al. 2008; Harmens et al 2011). In particular, it has been observed for 6 species of mosses that their nitrogen content increases by 0.01–0.06% (dry weight) per each 1 Kg N ha^{-1} $year^{-1}$ of wet deposition (Solga et al. 2005; Pitcairn et al. 2006).

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Here, the effect of different rates of wet deposition on the nitrogen content of mosses was most evident when comparing mosses growing in remote natural protected areas of the Valley with mosses growing in different areas of Mexico City. However, this response was not observed for the mosses growing within the area where the monitoring network is deployed, where the high rates of wet deposition ranged between 24.13 and 49.91 Kg N ha^{-1} $year^{-1}$ during 2014. This lack of response suggests that the mosses reached a threshold of saturation of 24 Kg N ha^{-1} $year^{-1}$, similar to what occurs with mosses collected across Europe whose point of saturation is 20 Kg N ha^{-1} $year^{-1}$, but contrasts with mosses from the Arctic where the saturation is reached at 10 Kg N ha^{-1} $year^{-1}$ (Gordon et al. 2001; Harmens et al. 2014).

Plants growing on sites with low rates of deposition typically have negative $\delta^{15}N$ values, while for plants growing on sites with higher rates of deposition their $\delta^{15}N$ values can be either positive or negative depending of the prevalent nitrogenous pollutant (wet NH_4^+ or NO_3^-; dry NHx or NOx,) and the amount of deposition (Stewart et al., 2002; Xiao et al. 2010; Liu et al. 2008a, 2012a; Schröder et al. 2014; Díaz-Álvarez et al., 2016). Such a variation was also observed here. In particular, the negative $\delta^{15}N$ found for mosses from rural areas of the Valley of Mexico can be attributed to the uptake of NH_4^+ derived from NH_x emissions of fertilizers and livestock waste, whose $\delta^{15}N$ values typically are negative (Xiao et al. 2010, Liu et al. 2012a; Delgado et al., 2013; Skudnik et al., 2015). Mosses from some areas of the Valley such as the city of Pachuca, as well as some areas of Mexico City with rates of wet deposition below 35 Kg N ha^{-1} $year^{-1}$ presented positive $\delta^{15}N$, suggesting that they likely uptake NO_3^- derived from NO_x of fossil fuel burning and industrial activities which $\delta^{15}N$ typically are positive (Felix et al., 2012, 2014ab).

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Mosses take up NH_4^+ preferentially over NO_3^- because less energy is needed in its assimilation (Tcherkez and Farquhar, 2006; Liu et al 2012a, 2013a). Also, high rates of nitrogen deposition can cause the inhibition of the nitrate reductase (Liu et al. 2012a; Liu et al 2013a). Indeed, when nitrogen deposition rates reach 10 Kg N ha^{-1} $year^{-1}$ the NO_3^- assimilation is reduced significantly and when it exceeds 30 Kg N ha^{-1} $year^{-1}$ the nitrate reductase is completely inhibited (Gordon et al. 2002; Forsum et al. 2006; Liu et al. 2012a,b). This causes that the NO_3^- from deposition cannot be uptaken and possibly lost by leaching from the tissues of the mosses, as a result they cannot increase their nitrogen content (Pitcairn et al. 2006). The inhibition of the nitrate reductase by increasing rates of deposition also has an important effect on the isotopic composition of mosses. In particular, in the Valley of Mexico, their $\delta^{15}N$ became negative when the rates of wet deposition exceeded 35 Kg N ha^{-1} $year^{-1}$, which is a strong indicative that nitrate reductase had been inhibited and that the main compound uptake was NH_4^+, a pollutant that in 2014 represented 35% of the total wet deposition in Mexico City (Pitcairn et al. 2006; Wiedermnn et al 2009).

Positive $\delta^{15}N$ values found for mosses growing in areas with rates of deposition below 35 Kg N ha^{-1} $year^{-1}$ suggest that nitrate reductase was not inhibited or not completely inhibited by the prevailing deposition. This also occurs with the moss *Hypnum plumaeforme* subjected to watering with NO_3^-, whose positive $\delta^{15}N$ values show no inhibition of the nitrate reductase (Liu et al 2012c). The differential uptake of NH_4^+ has shown to be harmful to mosses, this can lead to loss of the less resilient species in areas with high rates of deposition (Paulissen et al 2004).

In the Mexico City region, the main nitrogenous pollutant gas was NOx (NO + NO_2). This gaseous component of pollution in different concentrations has no effect on the nitrogen content of 8 species of mosses growing in England (Pearson et al. 2000). Here, differences

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between nitrogen content from mosses growing in rural areas and mosses from highly polluted areas were evident, suggesting the inhibition of the nitrate reductase by high concentrations of NOx, as occurs for 4 species of mosses (Morgan et al. 1992). However, no relationship was found here between the NOx concentrations and the nitrogen content of the mosses, contrary to what was found in mosses from UK whose nitrogen content responds differently to dry and wet deposition, being higher in sites dominated by dry deposition (Pitcairn et al. 2006). Also, the isotopic composition was not affected by the concentration of NOx. These results from both species suggest that wet deposition is the major factor that influences the nitrogen content and the $\delta^{15}N$ of the mosses considered here for the Valley of Mexico. The gaseous NOx is easily dispersed by wind into the atmosphere, it has low deposition rates and a relatively short atmospheric lifetime. Additionally, much of the NOx can be uptaken by the tress, which may preclude the NOx from reaching mosses at such rates that could enable biomonitoring (Padgett et al., 2009; Hertel et al., 2011; Redling et al., 2013). Instead, the wet deposition is a constant source of nitrogen that reaches mosses during at least 6 months at year in the Valley of Mexico.

Tillandsia recurvata

Despite the high rates of wet nitrogen deposition recorded in Mexico City that can reach 50 Kg N ha^{-1} $year^{-1}$, neither the nitrogen content nor the C:N ratio of *Tillandsia recurvata* were directly affected by this type of deposition. This could be due to the fact that raindrops cannot be absorbed by the non-absorptive roots of these plants, whose main function is to attach the plant to the phorophyte, contrary to what occurs with other epiphytic plants such as orchids (Benzing, 1976; Schmitt et al., 1989; Diaz-Alvarez et. al., 2015). Also, raindrops cannot be directly absorbed by the stomata of most plants because the size of the drops and their high surface

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tension (Trejo-Tellez & Rodriguez-Mendoza, 2007). *Tillandsia recurvata* absorbs water by means of foliar trichomes. However, the nitrogen content of this bromeliad did not increase with the increasing rates of wet deposition in Mexico City, so it is possible that the absorption of rain water is lower than the absorption of the water vapor from the air when it condenses on the leaf surface. *Tillandsia recurvata* showed a strong reliance on the gaseous nitrogen in form of NOx present in the atmosphere of the Valley, suggested by the direct relationship found here between nitrogen content and NOx concentration (Schmitt et al. 1989). The NOx emitted in the Valley was absorbed by the leaves of *T. recurvata*. This is possible because *T. recurvata* can absorb particles and humidity of the air by means of the trichomes present in the leaves surface (Schmitt et al., 1989). However, despite the direct relationship found here, it was difficult to determine the portion of pollution present in particles and humidity that was absorbed by this bromeliad.

The close relationship between the concentration of NOx and the nitrogen content of *Tillandsia recurvata* found here was similar to what occurs for *Tillandsia capillaris*, another atmospheric bromeliad, whose nitrogen content is higher for plants growing on power lines close to a highway, where the NOx concentration is high, than for plants attached to trees away from the highway with lower NOx concentrations (Abril and Bucher 2008). The atmospheric NOx concentration is so important for the nutrition of *T. recurvata* that there is a higher abundance on the power lines from regions with higher NOx concentrations (Santos et al., 2014).

The differences found between the nitrogen content of the bromeliads collected in either season are the result of two main factors. First, the growth of *T. recurvata* occurs mainly after the rainy season, the tissues formed during this time of the year already have inherently higher nitrogen content than older tissues. The second reason is that precipitation drags the NOx from the atmosphere to the ground surface reducing the NOx available for these plants during the

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rainy season (Taiz and Zeiger 2010; Secretaría del Medio Ambiente del Gobierno del Distrito Federal 2015).

Because the wet deposition had no effect on nitrogen content for *Tillandsia recurvata* it is likely that nitrate reductase was not inhibited by the rates of wet deposition, as has been observed for other plants such as mosses, as discussed above. Instead, the positive $\delta^{15}N$ found here for *T. recurvata* that was directly related with high concentrations of atmospheric NOx, suggests that the gaseous nitrogen did not inhibit nitrate reductase. This tolerance of nitrate reductase has also been observed in plants of *Lycopersicon esculentum* and *Nicotiana tabacum* (Vallano and Sparks 2007). It is likely that NOx concentrations higher than 221 ppm measured during the dry season as was observed in Mexico City may produce inhibition in this enzyme. This could be the reason why *T. recurvata* was not found in sites with concentrations of NOx higher to 221.2 ppm. However, more studies must be conducted in order to determine the concentrations that may cause inhibition of nitrate reductase on *T. recurvata* because of its high capacity as biomonitor of NOx concentrations.

The direct relationship found here between $\delta^{15}N$ and the atmospheric concentration of NOx in Mexico City has also been observed for *Tillandsia recurvata* in the Mezquital Valley, state of Mexico, where its $\delta^{15}N$ are positive near industrial areas and is negative in rural areas (Zambrano et al., 2009). In this respect the air of industrialized and densely populated areas typically has NOx with positive $\delta^{15}N$ owing to its fossil fuel origin. Also, vehicle exhaust does not represent a relevant source of NH_3 emissions, even close to high-traffic roads that are the main source of pollution in the countryside (Frati et al., 2006). In turn, the $\delta^{15}N$ of NOx of biogenic emissions from the soil and emissions from livestock waste tend to be negative (Redling et al. 2013; Felix and Elliott 2013, 2014). Here, this was evidenced by the $\delta^{15}N$ of *T.*

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recurvata growing outside of the area with the monitoring network, where the land use is a combination of rural, forest and small towns.

The exclusion of two sampling points (RPM and PEV) from the analysis produced a better relationship between NOx concentration and the $\delta^{15}N$ found on *Tillandsia recurvata*. This could be the effect of the topographic conditions at each site. For example, in RPM *T. recurvata* was surrounded by a dense layer of vegetation that did not allow the NOx to diffuse through it and reach plants collected there, this effect of the distance to the source of pollution has been observed in forest in Norway (Ammann et al., 1999). Meanwhile, *T. recurvata* collected in PEV was located inside a micro-valley surrounded by hills, thus a natural barrier the flow of NOx from outside into it and reach the plants. Instead, the layer of NOx remained over this micro-valley. In both cases the negative $\delta^{15}N$ values found in *T. recurvata* were affected by the nitrogenous emissions from the soil, whose $\delta^{15}N$ values can be as negative as –30‰ (García-Méndez et al. 1991; Felix and Elliott 2013, 2014). Another factor that affected the $\delta^{15}N$ of these plants was their location inside the micro-valley no to far from a river contaminated with human wasted, and close to animal farms whose $\delta^{15}N$ are mainly negative (Felix and Elliott 2014).

The nitrogen content and the isotopic composition of the atmospheric bromeliad *Tillandsia recurvata* is determined by the predominant anthropogenic activity where this bromeliad establishes. In fact, due to the close relationship between *Tillandsia recurvata* and the concentration of NOx, increasing concentrations of NOx in sites forests and other natural protected areas can be an issue for such ecosystems where this bromeliad growths. Because, the increase of the population of *T. recurvata* (as an invasive plant) can cause the reduction of the reproductive success and germination of some species of trees, this has already been observed in some ecosystems in Mexico (Castellanos-Vargas et al., 2009; Flores-Palacios 2014, 2015).

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The CAM bromeliad *Tillandsia recurvata* remains physiologically active year round recording the atmospheric pollution by gaseous nitrogen during the entire year. But, because *T. recurvata* does not record effectively the wet deposition predicting possible rates of deposition with only this bromeliad is difficult. The combined use of *T. recurvata* with another biomonitor like the mosses that record the wet deposition, is a useful tool for determining the status of nitrogenous pollution in regions where air quality monitoring networks are not available, especially during for early stages of deposition when these organisms better respond. This methodology can work on mid-sized cities like Pachuca where these organisms did not reach the threshold point of response to atmospheric nitrogen pollution. Finally, the utilization of these organisms can be an early alert for avoiding health problems for ecosystems and humans, and bring this approach to the policy maker for reducing emissions.

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

			%N (dry weight)		C:N ratio		$\delta^{15}N$ (‰)	
		d.f.	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

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	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

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Figure legends

Figure 1. Localization of the Valley of Mexico. Red and yellow dots represent the spatial distribution of the air quality network stations of the wet deposition and the automatic monitoring network for NOx, respectively. This network is located mainly in the Mexico City region, at the south of the Valley (http://www.aire.cdmx.gob.mx/). Green triangles represent the sites of collection for the different organisms throughout the Valley. The red line delimits the basin, the white line is the state division, and the blue line shows Mexico City.

Figure 2. Spatial distribution of the total wet deposition Kg N ha^{-1} $year^{-1}$, mainly in the Mexico City area at the south portion of the valley, during 2014 (A) and atmospheric concentration of NOx ppm in the middle and norther portion of Mexico City during the dry season (B). Data are from the Mexico City government air monitoring network (Fig. 1).

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).

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

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

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.*

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Figure 1.

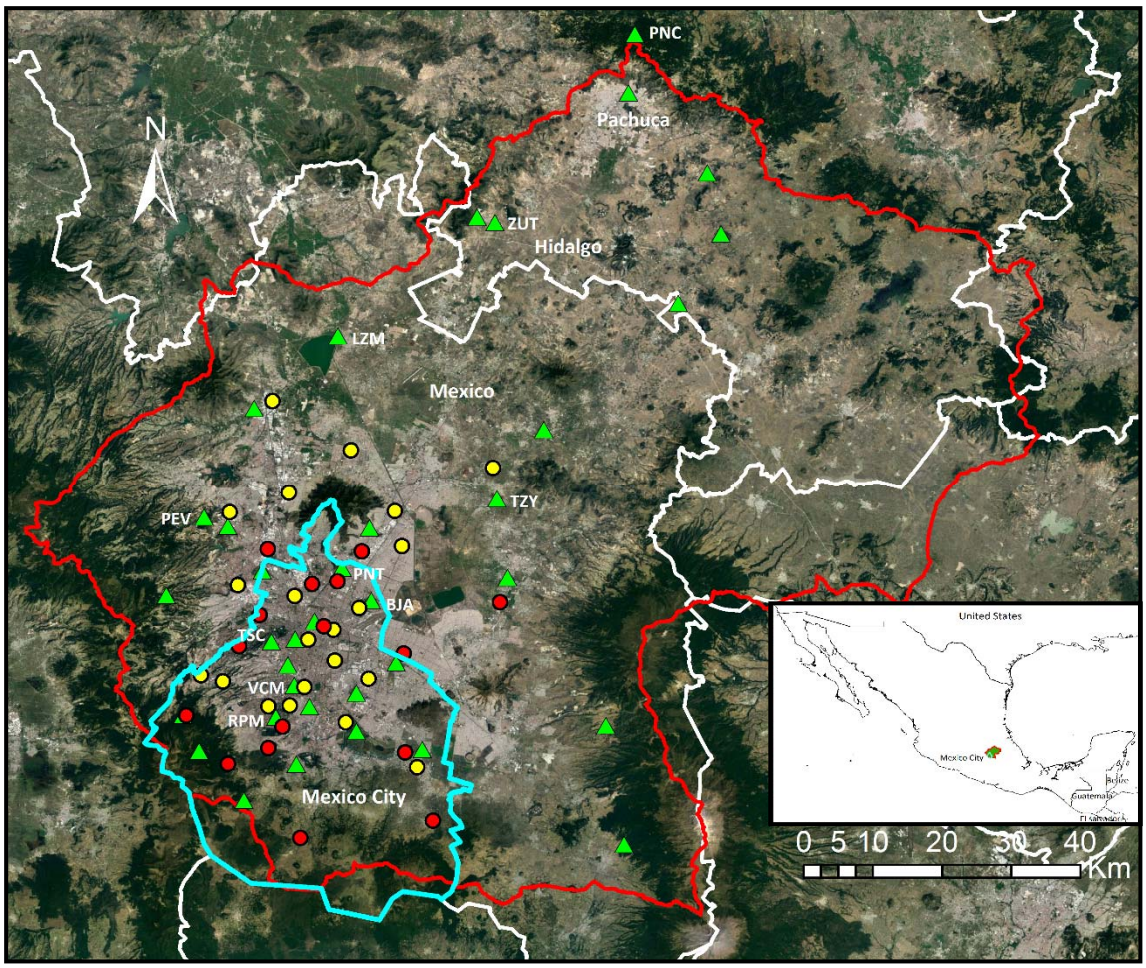

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Figure 2.

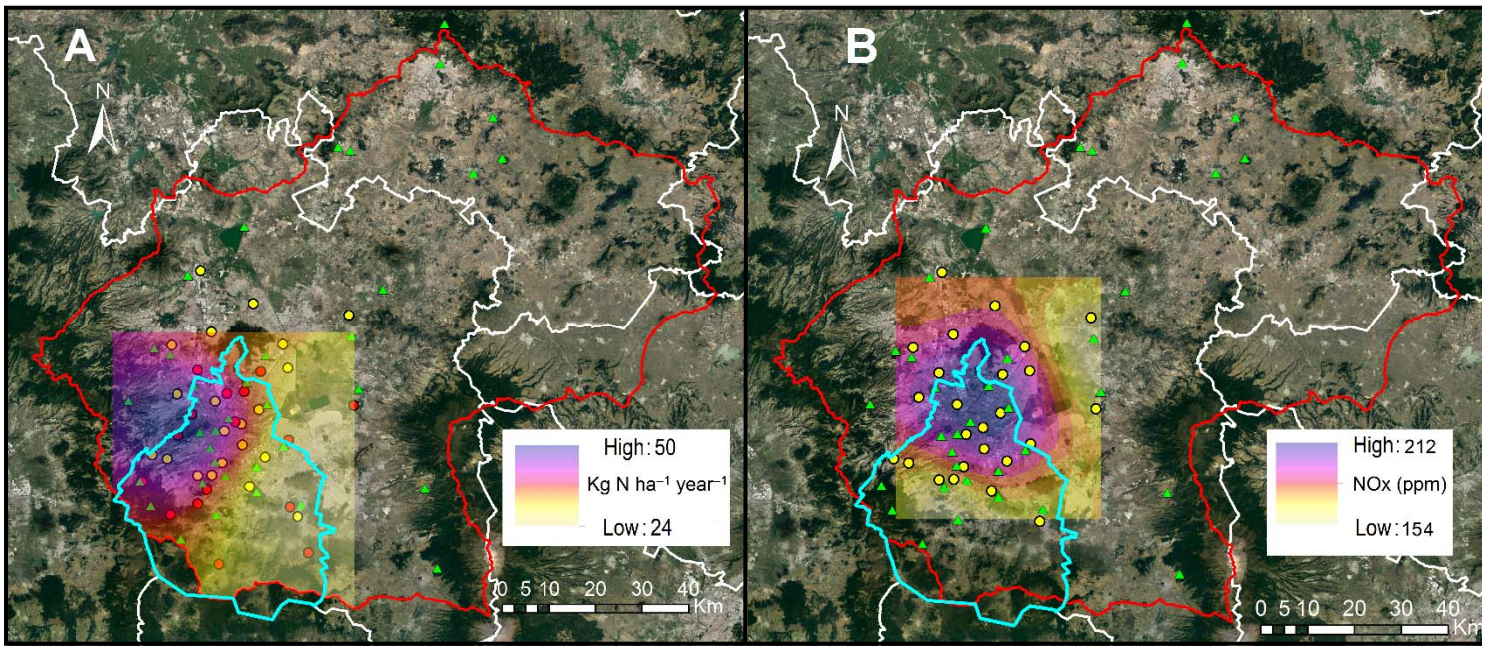

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Figure 3

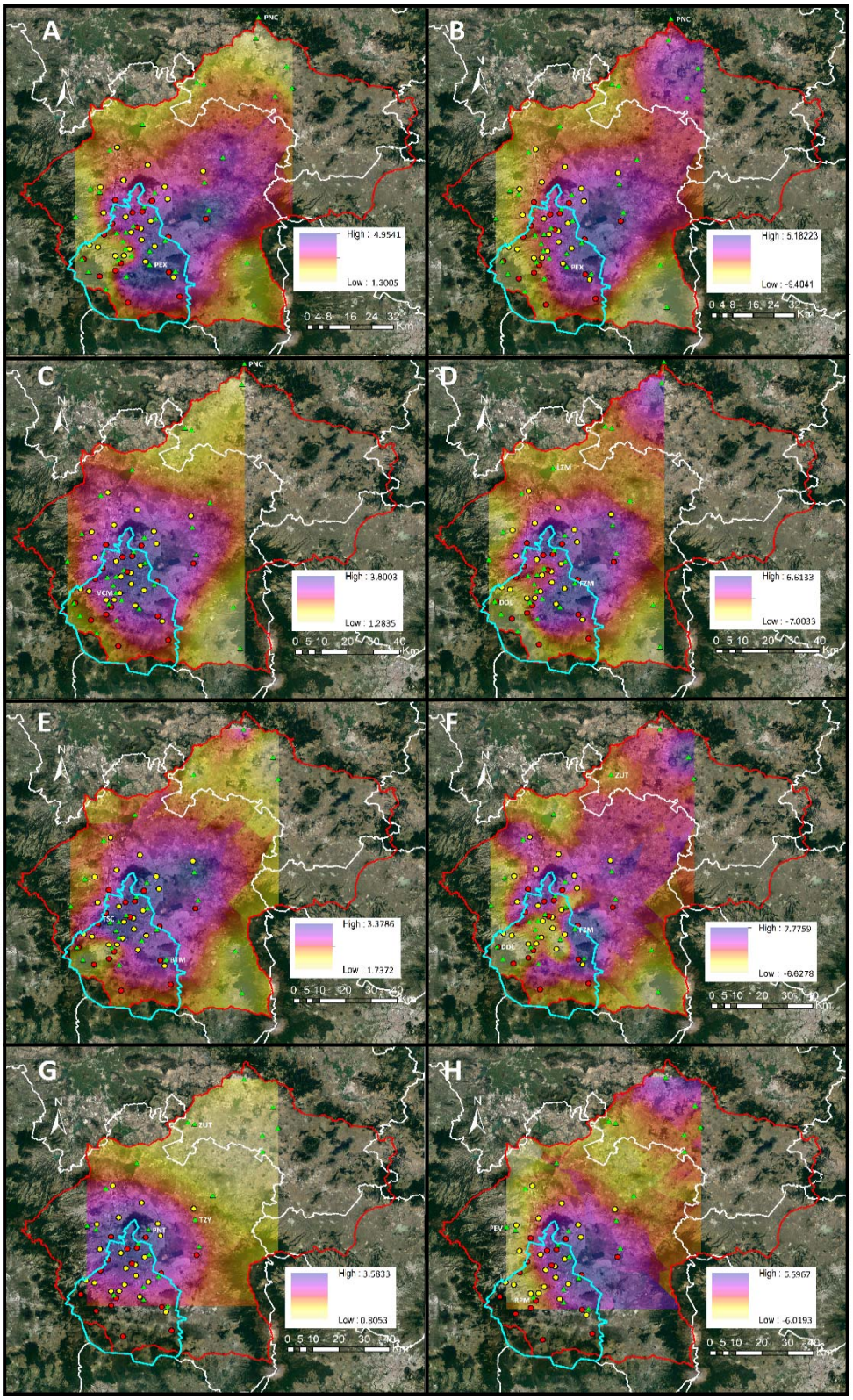

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Figure 4.

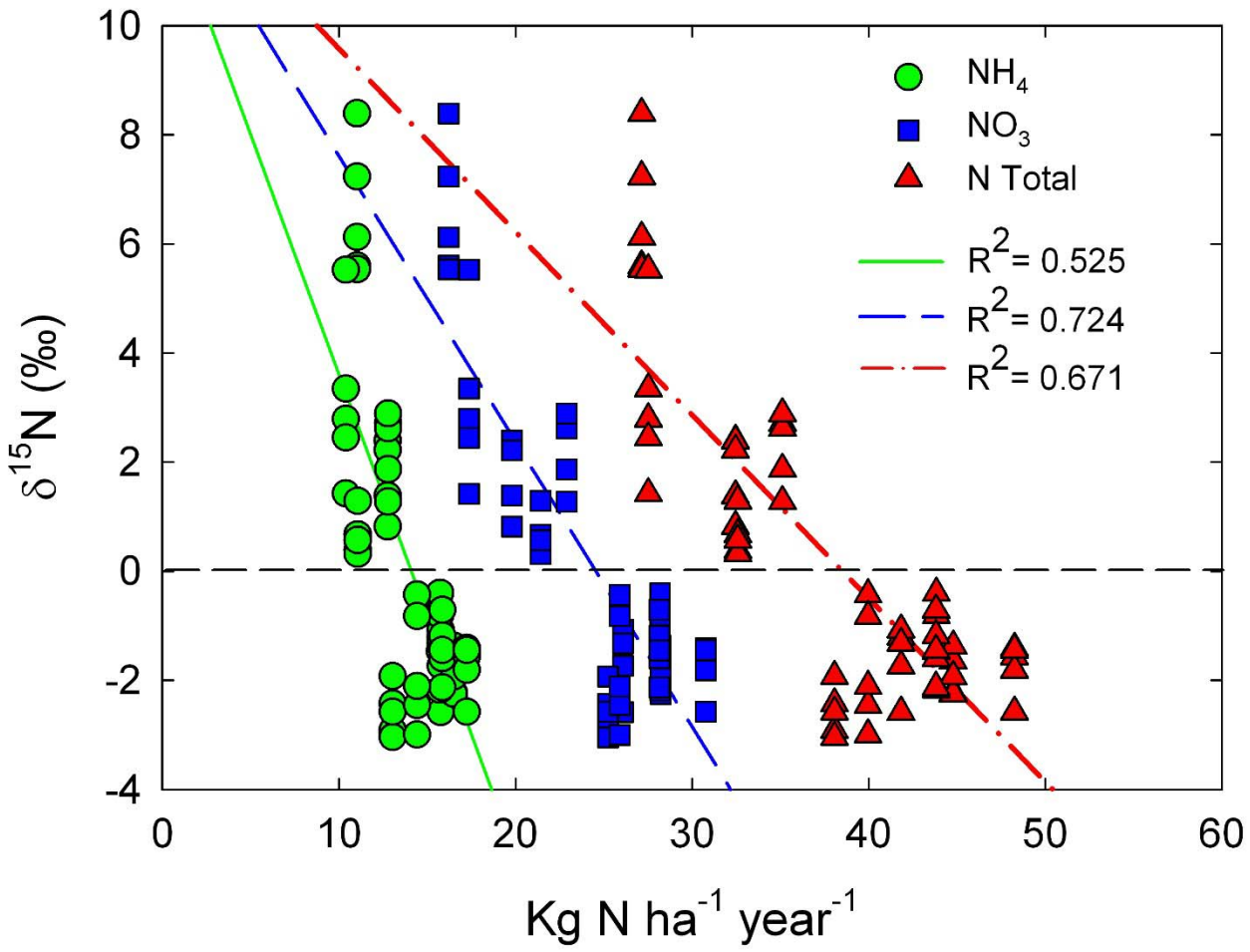

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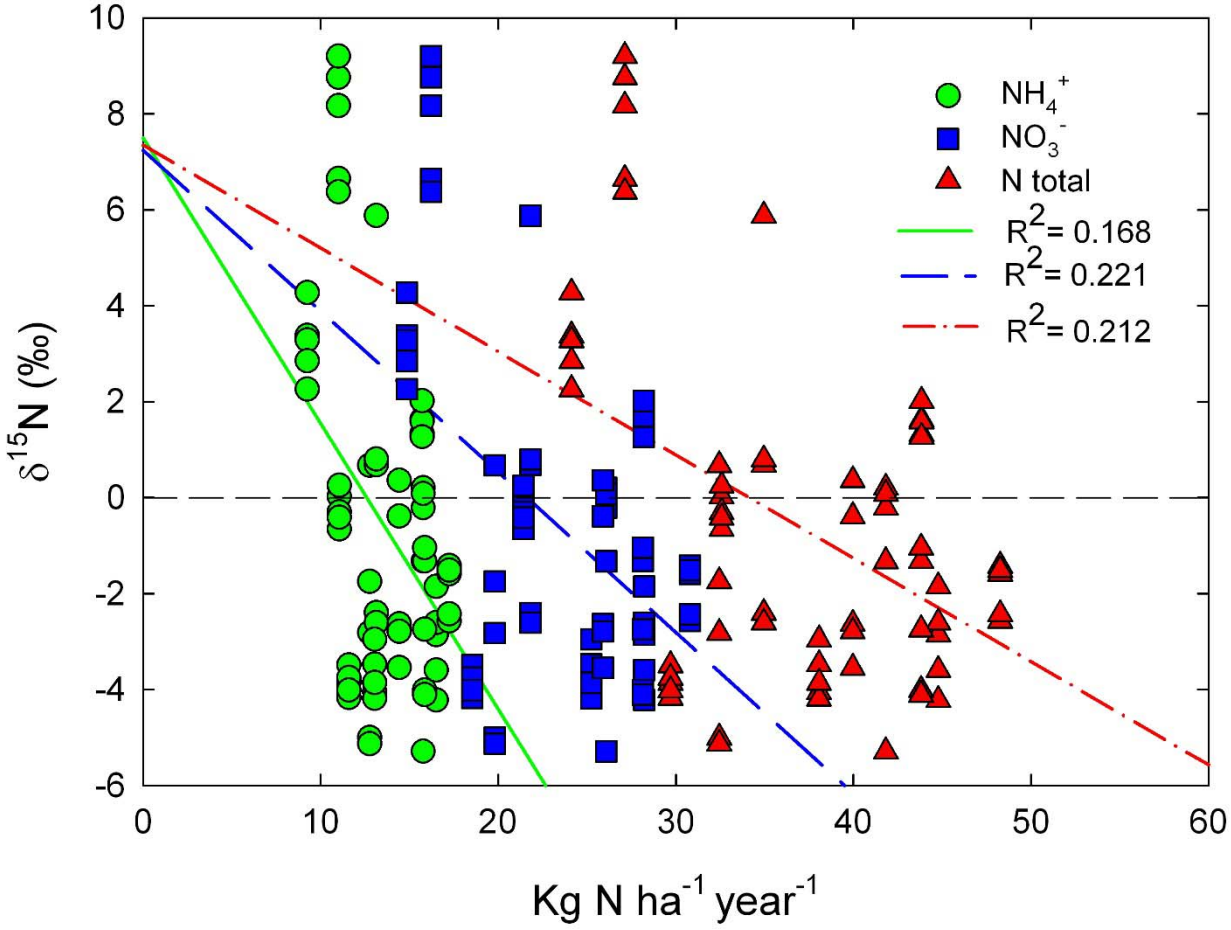
Figure 5.



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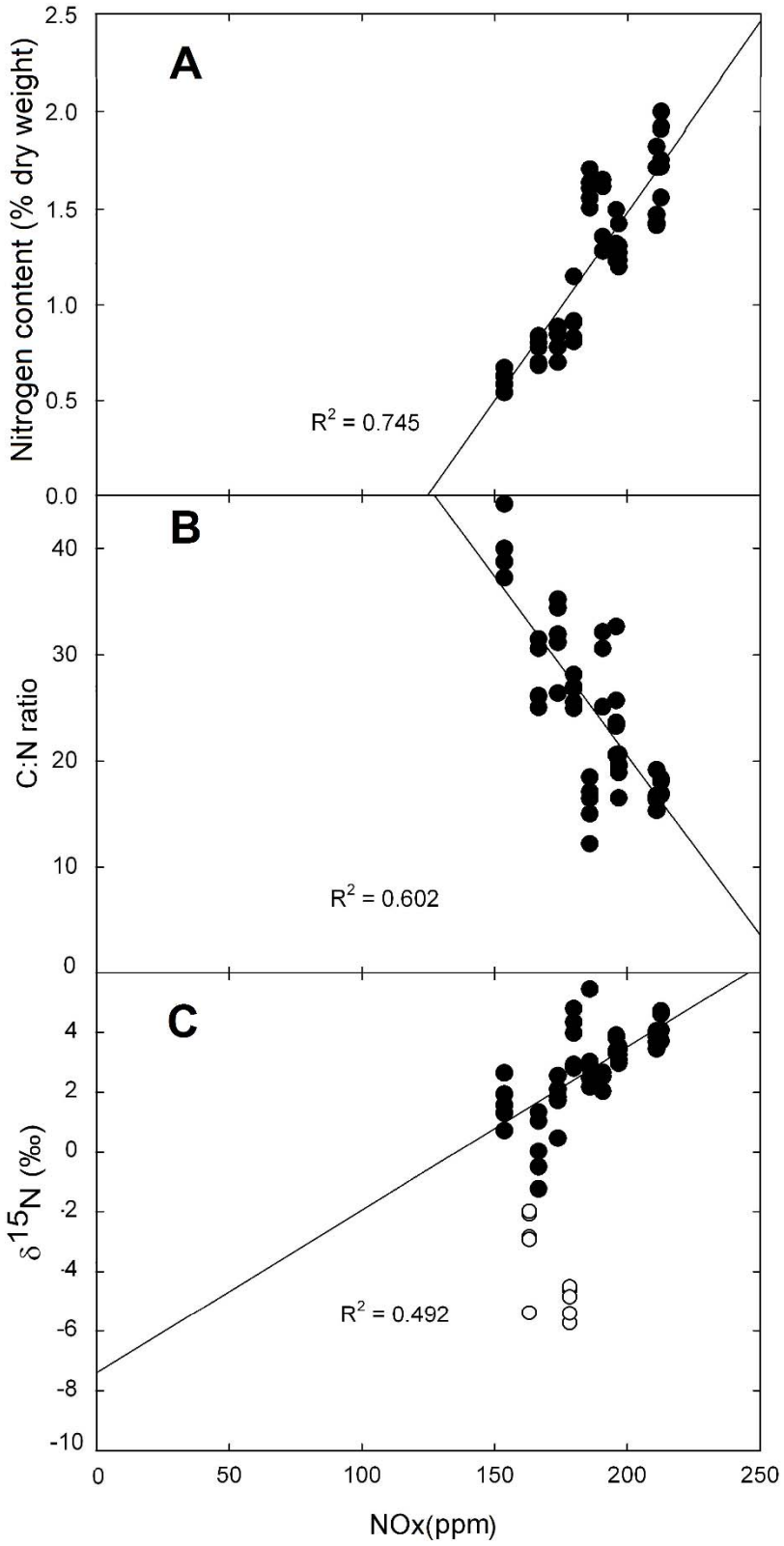
Figure 6.