Seasonal patterns and related microclimatic drivers of soil inorganic nitrogen in an urban montane cloud forest

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Abstract

Understanding the matter and energy dynamics in environments with strong human influence is essential since it allows us to know relevant ecological drivers in urban green land areas. It has been hypothesized that biogeochemical cycles in urban forests are more open (susceptible to nutrients soil losses) with respect to rural forests near cities. However, it is not clear if this ecosystem function occurs in the same way in systems from different latitudes. Soil nutrient dynamics and microclimatic conditions of an urban and a nearby rural montane cloud forest were registered from January 2016 to July 2017. Our objective was to compare edaphic and micro-climatic factors that drive soil inorganic N dynamics in these forests. Climate was slightly cooler and drier, and soil C, N, P and organic matter were lower in the urban than the rural forest. Seasonal soil inorganic N forms were related to above ground conditions in the urban forest, but to the belowground conditions in the rural forest. Consistently low $\text{NH}_4\text{:NO}_3$ ratio indicated high susceptibility to N soil loss in the urban forest. Our results support the hypothesis that urban cloud forests are functioning as open ecosystems in contrast to the rural forests.

Introduction

Nitrogen (N) often limits primary productivity in natural terrestrial ecosystems, and it is more energetically costly to obtain and use than other nutrients (Vitousek and Howarth 1991; Gutschick 1981). In ecosystems N exists in different forms, including inorganic like ammonium (NH_4) and nitrate (NO_3) and organic like amino acids and nucleic acids. Inorganic N is directly available for plants and microorganism, but organic N must be mineralized. Biological N fixation is the main source of atmospheric income, and NO_3 and gaseous losses are the main outputs for N in terrestrial ecosystems. However, anthropogenic activities present considerable additional sources that modify N inputs and outputs (Chapin et al. 2011). When outputs become too large, the ecosystems start to be “open” (susceptible to nutrients soil losses), and becomes a source of N and other elements (Chapin et al. 2011; Adler and Tanner 2013). An ecosystem is considered to be open when the outputs of nutrients and / or energy released are higher (e.g. nitrate solubilized in water or surface heat flux) than the inputs, and there is no return of the nutrients or energy to the ecosystem (towards vegetation or soil microbial biomass, etc.). On the contrary, an ecosystem is considered to be closed when matter and / or energy is constantly reused or cycled rather than lost (Lavelle and Berhe 2005; Schlesinger and Bernhardt 2013).

Since more than 4 billion inhabitants are currently located within cities around the world (55% of the world's population; World Bank 2020), and this is expected to increase in size, it is important to know the influence of human activity on ecosystem biogeochemistry and nutrient transformation processes (Kaye et al. 2006). Urban expansion could trigger environmental alterations that can affect some biogeochemical processes by incorporation of substances, particles and gases into the soil, mainly from fossil fuels burning and some solid, liquid and gaseous wastes (Grimm et al. 2008; Adler and Tanner 2003). Green areas within cities such as urban forests and parks can be directly affected through the modification of some stocks, flows, and processes of transformation of matter and energy in the soil (Pouyat et al. 2003; Adler and Tanner 2013). Little is known about the difference in the transport and

transformation of matter and energy in soils of urban ecosystems, compared to natural ones and if models developed for natural environments significantly differ in urban environments (Kaye et al. 2006; Pavao-Suckerman 2008; Trammel et al. 2017). However, assumptions can be made based on natural models. Hypothetically, urban forests may have a loss of nutrients and changes in their productivity due to the fact that biogeochemical cycles may be more susceptible to opening-up, compared to natural ecosystems without, or little, human disturbance (Chapin et al. 2011). If the above is true and given the conditions in which urban forest soil develops, it is expected that urban ecosystems will present different strategies for nutrients conservation, compared to natural ecosystems. While research has been conducted on N cycling mechanisms in temperate urban forest soils (Pavao-Suckerman 2005; Kaye et al. 2006; Trammel et al. 2017), there is very little information on the functioning of green areas in other latitudes, and even less in regions of montane cloud forests. It should be noted that cloud forests are among the most biodiverse in the world but suffer a great threat from changes of coverage and land use (Bubb et al. 2004).

Comparing an urban forest to another with little human influence in terms of soil N conservation, would allow us to assess the intensity and importance of human activities on soil inorganic N dynamic. In addition, it would help us to identify the functioning of urban ecosystems compared to natural ecosystems and with this, recognize the potential of urban ecology to propose new theoretical models (Kaye et al. 2006).

The objectives of this work were to know the seasonal patterns of soil inorganic N forms of an urban cloud forest completely surrounded by a city and a nearby native cloud forest, and determine the edaphic and micro-climatic factors that drive soil inorganic N dynamics. The relationship $\text{NH}_4:\text{NO}_3$ will provide information about the nutritional state of the soil, potential energy stored, and movements of N in the ecosystem taking into account the low solubilization and high conserved energy of ammonium (NH_4) as a reduced form of N in contrast with nitrate (NO_3) as an oxidized form of N (Robertson and Groffman 2007; Abbasi et al. 2017; Recous et al. 1990; Hughes et al. 2004; Emmett 2007). Higher nitrate concentrations have been reported for urban environments in contrast to rural areas in other temperate zones, (Goldman et al. 1995; Raciti et al. 2011; Zhu et al. 2006; Enloe et al. 2015; Enloe 2014). If the differences in the potential of energy stored and movements of N between temperate urban and rural temperate forest are similar in warmer ecosystems, we hypothesized that the relation $\text{NH}_4:\text{NO}_3$ will be lower in the urban than in rural cloud forest.

Materials And Methods

Study site

Two sampling sites were established. The predominant vegetation corresponds to elements of the tropical montane cloud forest within the central mountainous area of the Gulf of Mexico, but under conditions of different human influence (Fig. 1). The Cerro de Macuiltépetl Ecological Park (19–32'51.26"N and 96 – 55'12.66"W; 1560 m asl) is a 30 ha fragment of cloud forest under protection for

more than 40 years and completely surrounded by the city of Xalapa, Veracruz, Mexico (Government of the State of Veracruz 2020). The city has about 600 thousand inhabitants and the ecological park vegetation is typical of the cloud forest, with tree species such as *Oreopanax xalapensis*, *Clethra mexicana*, *Quercus xalapensis*, *Turpinia insignis*, *Liquidambar macrophylla*, *Liquidambar styraciflua*, *Citharexylum mocinnii*, *Carpinus Carolinian*, *Eugenia xalapensis*, *Miconia glaberrima*, among others (Castillo-Campos 1991).

The average annual temperature in the city of Xalapa is 18.6 ° C, with an average annual maximum and minimum of 27.4 ° C in May and 10.4 ° C in January, respectively. The average annual rainfall is 1433 mm, with June being the wettest month with 275.4 mm and December the driest with 37.1 mm (SMN 2020). Around the city of Xalapa there are sugarcane crops, coffee trees and secondary forests of conifers and oaks (Allan-Ellis and Martínez-Bello 2010; CONABIO 2020).

The sampled rural forest has very little human activity and is located in the private area called El Riscal (19–28'54.04"N, 96 – 59'44.80"W to 1570 m asl), 10 km in straight line from the urban forest. Given the relative short distance between the sampled sites and the same altitude, both forests are influenced by the same regional climatic frontal systems in winter and tropical systems in summer (Ruiz-Barradas et al. 2010). The rural forest presents typical cloud forest vegetation with a larger forest area compared to the urban forest. Dominant species in both sites are *Liquidambar styraciflua*, *Quercus xalapensis*, *Q. leiophylla*, *Q. germana*, *Clethra mexicana*, *Turpinia insignis*, *Cinnamomum effusum*, *Carpinus caroliniana* and *Oreopanax xalapensis*, and shrub species such as *Ocotea psychotrioides*, *Miconia glaberrima*, *Palicourea padifolia* and *Eugenia xalapensis*.

Soil sampling and analysis

Soil collections were made by composite samples taken by triplicate on a plot of 1 m² at a depth of 15 cm (n = 13 plots with composite samples were obtained, distributed in an area of 2.8 ha) at each study site, urban and rural. Soil collections were obtained seven times during the period between January 2016 and June 2017. The soil was stored in black bags at 4° C for further analysis in the Water and Soil Laboratory of the Institute of Ecology approximately 24 hours after sampling.

Micro-climatic variables

In each plot the surface floor (T0) and 15 cm deep (T15) temperatures were measured, as well as the depth of the litter found above them. At one point located in the center of each study site, an automatic weather station (Davis Instruments, USA) was installed at a height of 5 m above the ground with which temperature (Tair) and relative air humidity (RH) were recorded every 15 minutes during the sampling season (January 2016-June 2017). Solar radiation (Rad), dew point temperature (Td), precipitation (Rain) and potential evapotranspiration (ETo) were also obtained.

We used the meteorological data between 10:00 and 15:00 hours since it was the time during which sample collection and measurements of the edaphic variables were made. The VPD was quantified by the difference between the saturation vapor pressure (*es*) and the current vapor pressure (*e*). The *es* was

calculated using the Equation of Clausius-Clapeyron and e was obtained through the relationship of RH to e_s (Borgnakke and Sonntag 2013).

Edaphic variables

To determine the soil inorganic N concentration, we used the method of Maynard and Kalra (1993), obtaining the ammonium concentration (NH_4) by shaking the sample with KCl 2.0 N. Nitrate (NO_3) was extracted with the same extract as ammonium because it is soluble in water. Concentration of NH_4 was determined by selective ion electrodes, and NO_3 by capillary electrophoresis. Concentration of soil inorganic N (SINC) was determined by the sum of NH_4 and NO_3 . The available phosphorus was determined in a fresh soil solution (P-Bray) by extracting it with a mixed solution of ammonium fluoride (NH_4F 0.03N) and HCl 0.025 M (Bray and Kurtz 1945). In the case of total P, digestion was performed with nitric and perchloric acid, while the quantification was performed with the vanadomolibdate reagent and the determination was performed on a visible light spectrum photometer (Model Spectronic 21.D). Soil pH was measured in a solution 1:5 with water. Soil water content (SWC) was obtained gravimetrically from the relationship between wet soil weight loss and dry weight. Total C and N concentration were determined through complete combustion in a Truspec analyzer (Leco, St. Joseph, MI). Organic C and organic matter were quantified with the Walkley and Black technique (Sollins et al. 1999). Inorganic carbon was calculated as the difference between total soil carbon and total organic carbon. The concentration of total sulfur in soil was determined with the turbidimetric method (Standard Methods For the Examination of Water and Wastewater, 4500-SO42 – SULFATE 2017).

Data analysis

To explore whether the amount of soil inorganic N and environmental microclimatic conditions are modified by the seasonality and urbanization of the cloud forest, we performed an ANOVA model with a split-plot design (Montgomery 2013), forest as a two level factor (urban and rural forest) as the main plots, and seasonality as a seven level factor (months of sampling) as subplots. Multiple regressions were performed to determine the relations of soil N concentration (ammonium and nitrate) with edaphic and microclimatic variables. Variables such as NO_3 , NH_4 and SINC were square root transformed to satisfy normality criteria. Adequate minimum models were reported for the significant variables evaluated (Crawley 2013). All statistical analyses were performed with R version 3.6.1 (R Core Team 2020). The alpha value for all cases was 0.05.

Results

Edaphic and microclimatic variables

Soil of rural cloud forest showed significantly higher total C, N and P concentrations, soil organic matter and organic C, inorganic N and NH_4 and was more acidic than the urban forest (Table 1). On the other hand, soil of the urban forest showed higher concentrations of NO_3 , P-Bray, and S total.

Table 1

Mean (standard deviation) values of micro-climate and soil variables from January 2016 to June 2017.

<i>Soil variable</i>	<i>Urban Forest</i>	<i>Rural Forest</i>	<i>P value</i>
<i>NH₄ (mg/kg)</i>	10.8 (9.4)	38.4 (33.4)	< 0.001
<i>NO₃ (mg/kg)</i>	28.3 (21.2)	20.1 (19.2)	< 0.001
<i>Soil Inorganic N (mg/kg)</i>	39.1 (24.1)	57.3 (34.5)	< 0.001
<i>P-Bray (mg/kg)</i>	2.4 (2.6)	0.6 (0.9)	< 0.001
<i>Total P (mg/kg)</i>	504.9 (159.8)	721.9 (137.5)	0.01
<i>Total C (%)</i>	6.9 (1.2)	16.1 (2.7)	< 0.001
<i>Total N (%)</i>	0.9 (0.1)	1.56 (0.2)	< 0.001
<i>Total S (%)</i>	1.8 (0.3)	1.3 (0.1)	< 0.001
<i>Soil Organic Matter (%)</i>	11.4 (1.8)	23.5 (3.9)	< 0.001
<i>Organic C (%)</i>	6.6 (1.1)	13.63 (2.3)	< 0.001
<i>Micro-climatic variable</i>			
<i>15 cm Soil Temperature (C)</i>	16.7 (2.7)	17.9 (2.7)	< 0.001
<i>Surface Soil Temperature (C)</i>	18 (2.7)	18.5 (2.7)	0.004
<i>Soil Water Content (%)</i>	33 (5.9)	51.4 (5.5)	< 0.001
<i>pH</i>	5.8 (0.6)	4.5 (0.6)	< 0.001
<i>Air Temperature (C)</i>	19.7 (2)	21.4 (2.6)	< 0.001
<i>Dew Point Temperature (C)</i>	13.1 (2)	16.5 (2.3)	< 0.001
<i>Accumulated Precipitation (mm)</i>	1665	1688	-
<i>Accumulated Potential Evapotranspiration (mm)</i>	632	573	-
<i>Solar Radiation (W/m²)</i>	529.9 (103.5)	486.2 (160.7)	0.15
<i>Relative Humidity (%)</i>	57.9 (5.9)	65.7 (5.5)	< 0.001
<i>Water pressure (hPa)</i>	15.2 (1.6)	19 (2.3)	< 0.001
<i>Saturated Water Pressure (hPa)</i>	22.7 (2.8)	25.7 (3.6)	< 0.001
<i>Vapor Pressure Deficit (hPa)</i>	7.4 (1.6)	6.5 (1.7)	< 0.001

Precipitation was not significantly different between forests (Fig. 2). However, urban forest showed greater solar radiation, potential evapotranspiration and pressure vapor deficit but lower air and soil

temperature than rural forest (Table 1). The SWC did not show marked seasonal differences but the urban forest showed the lowest mean SWC compared to the rural forest (Fig. 2). The water output (evapotranspiration) and VPD from the urban forest were greater than in the rural forest, with higher values in the rainy season, in June (Fig. 3). The annual soil temperature at surface and at 15 cm depth showed the highest and lowest values in summer and winter, respectively, with a mean difference of 1 °C at the surface and 0.5° C at 15 cm of depth (Fig. 4).

Soil inorganic N concentration

The urban forest was lower in SINC compared to the rural one (Table 1). The predominant form of N was NH_4 in the rural forest, twice what was found of NO_3 (Table 1). On the other hand, urban forest had higher concentration of NO_3 compared with NH_4 concentration. Throughout the year, in the urban and rural forest the behavior of N available forms follows a pattern with high values in the warm and rainy season (summer) and low values in the dry and cold winter season, (Fig. 5). The maximum and minimum values of NH_4 in the urban forest were 17.12 and 3.9 mg N/kg respectively, while those of NO_3 were 40.49 and 4.9 mg N/kg. In the rural forest the highest NH_4 value was 79.94 mg N/kg, while the minimum was 11.69 mg N/kg. On the other hand, at this same site, maximum and minimum NO_3 values were 29.3 mg N/kg and 7.23 mg N/kg, respectively.

Concentrations of soil NO_3 and NH_4 in the rural forest were significantly related to soil temperature at 15 cm depth (Table 2). The NH_4 and SINC in rural forest showed a significant relationship only with the temperature below soil (Table 2, Fig. 6). In the urban forest the relationships were different to the rural forest since SINC and NO_3 were significantly related to T0 and VPD, while NH_4 was significantly related only to e ($R^2 = 0.27$; Table 2, Fig. 7).

Table 2. Multiple regressions for ratios to soil N concentration and soil and/or micro-climate variables that were significant ($p < 0.05$. SINC = Soil Inorganic N concentration, T15 = Soil temperature at 15cm depth, T0 = Soil surface temperature, VPD = Water vapor deficit, e = Water vapor pressure.

<i>Study site</i>	<i>Regression</i>	<i>R²</i>	<i>P</i>
<i>Rural forest</i>	$NO_3 = -32.2276 + 3.0517 \cdot T15 - 0.2653 \cdot NH_4$	0.19	<0.001
	$\sqrt{NH_4} = -4.9219 + 0.5798 \cdot T15$	0.26	<0.001
	$\sqrt{SINC} = -3.8129 + 0.6145 \cdot T15$	0.45	<0.001
<i>Urban forest</i>	$\sqrt{NO_3} = -2.7742 + 0.2447 \cdot T0 + 0.4371 \cdot VPD$	0.30	<0.001
	$NH_4 = -35.2852 + 3.025 \cdot e$	0.27	<0.001
	$\sqrt{SINC} = -3.0174 + 0.0387 \cdot T0 + 0.4525 \cdot VPD$	0.43	<0.001

The behavior of N concentrations (NO_3 , NH_4 , and SINC) in relation to edaphic and microclimatic variables is shown in Figs. 6 and 7. The increase in SINC was directly related to soil temperature at 15 of depth in the rural forest (Fig. 6); however, soil inorganic N concentrations in the urban forest increased with the increment with surface soil temperature and VPD (Fig. 7).

NH₄:NO₃ ratio

The urban forest showed lower NH_4 : NO_3 ratio than the rural forest and both forests showed significant ($F = 3.078$, $p = 0.007$) seasonal variations during the year (Fig. 8). The rural forest presented minimum and maximum NH_4 : NO_3 ratio values in February and March and in June and August, respectively. The maximum NH_4 : NO_3 ratio values of the rural forest coincided with the rainy and warm season (summer season). As for the urban forest, the seasonal pattern of the NH_4 : NO_3 ratio values was similar to that of the rural forest, but with significant differences between forests ($p < 0.001$), showing minimum values in March and February, and maximum values in June, with a peak in January 2016. The extreme peak of the NH_4 : NO_3 ratio in January coincides with the coldest and driest month of the period evaluated in the urban forest.

Discussion

Edaphic and micro-climatic conditions between forests and soil inorganic N dynamics

Inorganic forms of N in the rural forest were related to below ground conditions whereas above ground conditions seem to drive soil inorganic N dynamics in the urban forest. Although soil ammonium is the

main substrate for nitrate formation by autotrophic or heterotrophic nitrification processes (Robertson and Groffman 2007), very little is known about the microclimatic and edaphic conditions of this process.

Our hypothesis was supported, N inorganic forms differ between forests, the rural forest showed the highest ammonium concentration, while the urban forest showed the highest nitrate concentration. Thus, the urban forest showed a low potential of energy stored and potentially the highest movements of N in the ecosystem, in contrast to the rural forest. Edaphic and micro-climatic conditions associated with the dynamics of inorganic N between forests suggest that heterotrophic nitrifiers predominated in rural forests, while autotrophic nitrifiers predominated in urban forests.

Rural forest

Rural forest conditions were characterized by higher soil C and N concentration and lower soil pH compared to the urban forest. Although the existence of nitrification in acid soils (as was founded in the rural forest) has been recorded, little is known about its physiological bases (Robertson and Groffman 2007). A high potential net C and N mineralization rate (Dominguez-Eusebio et al. under review), and higher soil C in contrast to the urban forest suggests that heterotroph nitrifiers are carrying out the nitrification process in the rural forest using C as energy source, although further research must be done to confirm this hypothesis. Commonly, autotrophic nitrifiers carry out the nitrification process in the soil, but they are relatively poor competitors for ammonium when there is great abundance of heterotrophic microbes in the soil community (Robertson and Groffman 2007).

Nitrate and ammonium association with soil temperature at 15 cm deep in the rural forest can be explained not only because the temperature is an important driver of microbial activity including the nitrificant community (Bardgett 2005; Robertson and Groffman 2007; Smith et al. 2015), but also due to the fact that temperature drives N mineralization and immobilization processes in the soil (Robertson and Groffman 2007). A greater capacity for retention of radiative energy associated with a lower albedo, and lowest N microbial immobilization compared to the urban forest have been reported in a previous study (Dominguez-Eusebio et al. under review). Nutritional needs of the microbial community in the rural forest could be supported by inputs of organic substrates with low C:N ratio, allowing the increase of ammonium and nitrate in the soil solution produced by N mineralization of the organic substrates (Robertson and Groffman 2007). Ammonium may be related to soil N conservation because it has greater energy storage capacity, is less soluble in water, with less potential to be lost by leaching compared with nitrate.

Urban forest

The urban forest showed lower concentrations of C and N in the soil than the rural forest. Also, lower potential net C and N mineralization rate, but greater potential net N microbial immobilization and net nitrification have been recorded in the urban forest in comparison with the rural forest (Dominguez-Eusebio et al. under review). The positive association between nitrate concentration and surface temperature in the soil of the urban forest could indicate that the main nitrifier activity may be taking

place at the soil superficial level. According to our results, increases in soil surface temperature in the urban forest could stimulate nitrate production. It is possible that the nitrification process may be performed as autotrophic nitrification in this forest. Autotrophic nitrifiers are forced aerobes and obtain their C from CO₂ or carbonates, rather than soil organic matter (Robertson and Groffman 2007). A superficial position of the community nitrifiers could give them access to the atmospheric CO₂. An autotrophic nitrification would imply access to ammonium generated by N inputs through an atmospheric deposition processes. Ammonium in soil solution is a ionized form of ammonia (NH₃) and a great source of substrate to autotrophic nitrification (Robertson and Groffman 2007). Given that solar energy is one of the main factors of water evaporation, the movements of soil surface water in the urban forest could play an important role in the conservation or loss of inorganic N at the soil surface. Soil water availability is an important factor in soil nitrification and N mineralization processes (Linn and Doran 1984), specifically the ammonium oxidation linked to water concentration in soil pore spaces (McGill 2007). In addition, there are processes of NH₃ deposition and volatilization into and from the soil solution in response to the difference in vapor pressure between the solution and ambient air (Mosier 2008). It is possible that processes of NH₃ deposition and volatilization at soil superficial level may be linked with an autotrophic nitrifier community in the soil urban forest, but further research is needed.

VPD was also positively correlated with nitrate production in urban forest. This was related to a lower SWC in this forest, as well as greater potential and real evapotranspiration (Dominguez-Eusebio et al. 2019). Smaller SWC values in the urban forest were also related to greater VPD, since it is a force that drives the movement of water in the soil-plant-atmosphere system (Rawson et al. 1977; Yuan et al. 2019). The relationship of NO₃ to atmospheric variables (such as VPD) suggests that the urban forest could be susceptible to the deposition of some components suspended in the air. Especially the atmospheric NH₃, of which much higher concentrations have been reported in cities compared to relatively pristine environment (Mosier 2008). Thus could affect vegetation growth, productivity, tolerance to drought and frost, and response to herbivores (Krupa 2003). It should be noted that the increase in atmospheric NH₃ has been related to anthropic activities so urban forests could be strongly influenced by surrounding human activities (Vitousek et al. 1997). It is known that the biogeochemistry of urban forests is strongly influenced by the urban environment (Michopoulos 2011).

It is not common to observe a relationship between inorganic N and VPD in forest soils, but in agricultural soils or with burning of biomass and fossil fuels. A portion of the N contained in plant biomass or fossil fuels is converted to NH₃ and emitted to the atmosphere (Mosier 2008). Such relationship can also be observed in soils influenced by domestic or wild animal excreta and crops with decomposing organic waste (Mosier 2008). It has been estimated that domestic animal excreta contribute at least with 21.6 Tg of N to global ammonia emissions, synthetic fertilizers with 9 Tg of N, plant biomass burning or plant residues decomposition with 5.9 Tg of N, and burning fossil fuels with at least 0.1 Tg of N (Mosier 2008). Our results suggest that human activities such pet transit, conglomerations of wild animals in the city that spend the night in the forest, in addition to those related to the plant residues and fossil fuels burning, may be influencing the inorganic N dynamic in this urban forest.

NH₄: NO₃ ratio as a trace of soil inorganic N dynamic in urban ecosystems

Similar to three oak forests and one desert in North America, the tropical cloud forest showed higher NO₃ concentrations in the urban forest compared to the rural forest. Both forests presented higher NO₃ concentration than temperate ecosystems (Table 3).

Table 3

Urban and rural forest comparison in soil inorganic N concentration. Data showed are from: *Goldman et al. (1995); ** Raciti et al. (2011); ***Zhu et al. (2006);**** Enloe et al. (2015, 2014).

Latitude	Dominated Vegetation	Site	Status	NO ₃	NH ₄	NH ₄ :NO ₃	pH
40°	Oak	New York, USA*	Urban	1.3 mgkg ⁻¹	11.7 mgkg ⁻¹	9.0	4.6
			Rural	0.6 mgkg ⁻¹	19.4 mgkg ⁻¹	32.3	4.7
39°	Tulip-Oaks	Maryland, USA**	Urban	3.8 g m ⁻²	3.5 g m ⁻²	0.92	-
			Rural	0.7 g m ⁻²	8.1 g m ⁻²	11.6	-
33°	Scrubland	Arizona, USA***	Urban	6.18 mgkg ⁻¹	2.17 mgkg ⁻¹	0.35	-
			Rural	4.1 mgkg ⁻¹	1.89 mgkg ⁻¹	0.46	-
29°	Oak-Pine	Florida, USA****	Urban	113.05 g ha ⁻¹ d ⁻¹	66.3 g ha ⁻¹ d ⁻¹	0.57	3.8
			Rural	16.3g ha ⁻¹ d ⁻¹	67.3 g ha ⁻¹ d ⁻¹	4.12	3.5
19°	Montane cloud forest (This study)	Veracruz, Mexico	Urban	28.3 mg g ⁻¹	10.8 mg g ⁻¹	0.7	5.8
			Rural	20.4 mg g ⁻¹	38.4 mg g ⁻¹	2.9	4.5

Soil nitrate can be a direct source of atmospheric gases and water pollutant (NO_x and N₂O) by denitrification or leaching (Robertson and Groffman 2007). Since nitrate is more mobile and soluble than ammonium, it can be easily transported out of the root zone when precipitation exceeds

evapotranspiration (Robertson and Groffman 2007). On the other hand, the ammonium concentration, being the reduced form of N, gives us valuable information about soil N conservation due to its lower mobility and solubility, and higher energy storage capacity. We showed that the urban forest had lower soil ammonium concentration in contrast to the rural forest. This was similar to the soil N concentration pattern for urban and rural forests in more temperate ecosystems (Table 4). According to the $\text{NH}_4\text{:NO}_3$ ratio, it can be observed that great soil N conservation occurs in temperate soils followed by tropical and subtropical soils, but in deserts less ammonium conservation in rural than in urban soil is observed (Table 3).

Seasonal ammonium and nitrate patterns showed a peak in the nitrate concentration for the urban forest in June and in the ammonium concentration for the rural forest in August (Fig. 5). Both months correspond to high precipitation, temperature and the growing season in the study area. Monitoring stoichiometric patterns of soil $\text{NH}_4\text{:NO}_3$ ratio could help us to generate public urban health policies and could give us clues about the energy stored in these ecosystems.

Soil N losses in urban forests: Challenges for the urban ecology in N cycle studies

According to Kaye et al. (2006) it is important to know urban biogeochemistry, among other things, in order to know whether ecological knowledge developed in natural systems can explain the environment where humans live, and the relationship between urbanization and functioning of ecosystems structure. If urban and non-urban ecosystems are working in a similar way, then urban ecology results in an extension of existing ecological theory. However, if they are fundamentally different, then the urban research has the potential to enrich the field of ecology with new theoretical advances (Kaye et al. 2006). In our case, soil inorganic dynamics in the urban forest suggest a different biogeochemical model from that of the rural forest.

A challenge for tropical urban forests function as the one studied here is to know the mechanisms and microclimatic conditions of the system in terms of N losses by volatilization of NH_3 and denitrification processes. From an ecosystem management perspective, denitrification is advantageous when it is desirable to remove excess NO_3 from soil prior to its movement to the ground and water bodies or surface waters (Robertson and Groffman 2007). However, the denitrification process can lead to soil acidification (Robertson and Groffman 2007) so, depending on soil pH, they could be vulnerable if the soil is already acidic as in the case of tropical and subtropical urban forests (Table 3). In the case of ammonia volatilization, when the soil does not have enough adsorption capacity to retain ammonium, ammonia is not fixed and returned to the atmosphere by volatilization. In contrast, when there is enough fixed ammonium in soil solution, the ammonia is not volatilized (Mosier 2008). Physically attached to soil particles, ammonium that originates from ammonia deposition fixes ammonia and controls ammonia volatilization (Mosier 2008). Soil organic material influences this process by regulating the soil structure and adsorption capacity of soil clays (Mosier 2008).

Our results suggest a strong functional role of the soil-atmosphere system in the biogeochemistry of the urban forest studied. Understanding the soil-plant-atmosphere system in urban environments could help us to understand the dynamics of soil inorganic N and its effect on the microbial and plant community. Specifically, VPD seems to play an important role in this functioning. It has been shown that plant nutrient uptake by some species is affected by the rate of transpiration, which could be limited by the VPD (Lihanvainen et al. 2016; McDonald et al. 2002; Cernusak et al. 2009; Shrestha et al. 2015; Plhák 2011). Increases in the VPD will affect vegetation growth and mortality, and the relationship between vegetation and N losses. The VPD and water movements in the soil-plant-atmosphere system could be another challenge for N cycle studies in urban ecology (Yuan et al. 2019).

Conclusions

Micro-climatic variables such as soil surface temperature conditions, air vapor pressure, and vapor pressure deficit, which are directly related to evapotranspiration, were associated with soil inorganic N concentration in the soil of the urban forest. However, temperature at 15 cm depth was associated with the concentration of soil inorganic N in the rural forest. The plant-soil system seems to have greater relevance in the soil inorganic N dynamic of the rural forest. In contrast, feedback processes aboveground suggest greater relevance of the soil-atmosphere system in soil inorganic N dynamics in the urban forest.

Differences in the ratio of $\text{NH}_4\text{:NO}_3$ between urban and rural forests have several important implications. First, its value may result from processes in the soil that sustains conservation of N (high value of $\text{NH}_4\text{:NO}_3$ ratio) or losses of N (low value of $\text{NH}_4\text{:NO}_3$ ratio) in the system. Second, it can be an indicator of the importance of microbial activity that transforms N in the soil (autotrophic nitrification or heterotrophic nitrification). And third, it can be easily measured and monitored in order to track the changes in the dynamics of inorganic N. The $\text{NH}_4\text{:NO}_3$ ratio of our urban mountain cloud forest suggests high atmospheric inputs of inorganic N via deposition, and also large losses via NO_3 by leaching. On the other hand, for our rural forest this ratio suggests a high potential for soil N conservation in form of NH_4 .

In a world with increasing urbanization, urban biogeochemistry could give us clues to better understand the mechanisms of energy and matter exchange in the conservation of N, and how different it is from the biogeochemistry of undisturbed systems.

Declarations

Not applicable.

Ethics approval, consent to participate and consent for publication

All authors declare our ethical approval and consent to participate in the research and publication of the manuscript.

Availability of data and material

We also declare our consent to the availability of data and materials.

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Conflicts of interest/Competing interests

We have no competing interests.

Authors' contributions

All authors contributed to the conception and design of the study, planned the questions, objectives and analysis of the work. CADE performed the preparation of materials and data collection, carried out the analyses and wrote the first draft of the manuscript. OLB and YP reviewed and provided feedback on the manuscript in its final version. All authors read and approved the final version of the manuscript.

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Figures

Figure 1

Distribution of the cloud forest in Mexico (Left, red dots represent the study sites) and close up of sample sites (right). The urban forest (30 ha) is located in the center of the city of Xalapa in the state of Veracruz in Mexico. Source: CONABIO (2020). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Seasonal behavior of (a) surface and soil temperature at 15 cm depth in an urban and rural cloud forest from January 2016 to June 2017. Mean $\pm$ 1 standard deviation are shown.

Figure 3

Seasonal behavior of precipitation and soil water content (SWC) in an urban and rural cloud forest from January 2016 to June 2017. Dark and clear bars indicate precipitation and light triangle and dark diamonds indicate SWC for the urban and rural forest.

Figure 4

Seasonal potential rate of evapotranspiration (ETo) and vapor pressure deficit (VPD) in an urban and rural cloud forest from January 2016 to June 2017. Bars indicate VPD and lines indicate ETo (triangles represent the urban forest and diamonds the rural forest). Mean $\pm$ 1 standard deviation are showed.

Figure 5

Seasonal behavior of soil inorganic N concentrations in an urban and rural cloud forest from January 2016 to June 2017. Mean $\pm$ 1 standard deviation are shown.

Figure 6

Relationship between soil inorganic N, edaphic and micro-climatic conditions of the rural forest. Red points are the fitted data obtained from models (see, Table 2). NO3 = Nitrate, NH4 = Ammonium, SINC = Soil inorganic N concentration (NH4+NO3), T15 = Soil temperature at 15 cm depth.

Figure 7

Relationships between soil inorganic N concentration, edaphic and micro-climatic conditions of the urban cloud forest. Red points are the fitted data obtained from models (see, Table 2). NO3 = Nitrate, NH4 =

Ammonium, SINC = soil inorganic N concentration ($\text{NH}_4 + \text{NO}_3$), T_0 = Surface soil temperature, e = Water vapor pressure, VPD = Vapor pressure deficit in the air.

Figure 8

Seasonal pattern of the $\text{NH}_4:\text{NO}_3$ ratio in a rural (grey) and urban (white) cloud forest from January 2016 to June 2017. Mean $\pm$ 1 standard deviation are shown.