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# Ozone foliar damage and defoliation monitoring of *P.cembra* between 2000 and 2016 in the southeast of France☆

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## 1. Introduction

Climate change is now internationally recognized at a global level, and its long-term impacts are widely studied. Some of these impacts, such as glacier melting, are very well documented and clearly visible. Others are less known by the general public even though they are equally significant. The concentration of ozone gas is directly related to global warming and pollutant emissions in the atmosphere.

Tropospheric ozone is the most common air pollutant (UNECE, 2016) and the third most powerful greenhouse gas if considering radiative forcing (GIEC, 2007; WHO, 2008; Stevenson et al., 2013; Kulkarni et al., 2015). It contributes to climate change (Screpanti and De Marco, 2009). It is a secondary pollutant created by a chemical reaction between nitrogen oxides ($NO_X$) and volatile organic compounds (VOC) enhanced by shortwave radiation and a reversible reaction. Both components are mostly produced by car traffic and industrial and domestic activities. Peak concentrations of ground-level ozone are reached during periods of higher temperatures and solar radiation by photochemical transformation of ozone precursors (The Royal Society, 2008). Global warming will probably increase ozone production in the future (Meleux et al., 2007). By 2050, the regions that are most impacted by this gas, usually dense urban zones, will suffer very high concentration levels during summer periods (AQEG, 2007; Jacob and Winner, 2009). The Mediterranean region, which is a very sunny area, is one of the most affected areas by climate change in Europe, especially during summer (IPCC, 2014). In the south of France, the important road traffic of Marseille, Toulon and Nice conurbations is responsible for a high level of ozone precursors. By 2080, the average temperature in the Provence-Alpes-Côte d'Azur region could increase by 5,2 °C (GIEC, 2013). The mountainous rural areas close to the industrial and tourist cities of this region still have the highest levels of ozone concentration (Sicard and Dalstein-Richer, 2015; Dalstein-Richier et al., 2005). In addition, during summer, this problem is often combined with droughts, causing an important water stress for plants (Alonso et al., 2014). This phenomenon is well-known among various European countries; the highest levels of ozone concentration are always observed during extreme hot weather periods (IPCC, 2014), which are expected to be more frequent in the future (GIEC, 2013).

Ozone has a visible impact on vegetation (Schaub et al., 2005; Paoletti et al., 2007; Fares et al., 2013; Feng et al., 2014) and is especially harmful to forest health (Dalstein et al., 2004; Dalstein and Vas, 2005). Subepidermal plant cells are directly damaged by oxidizing reactions as the gas penetrates the foliar tissue through stomatal openings (Tiwariet et al., 2016). The symptoms are materialized at the foliar level as these injuries provoke leaf necrosis, which is clearly visible to the naked eye; premature falling of the leaves; a modification of the stomatal aperture; and a reduction of photosynthetic activity (Dalstein et al., 2002; Ulrich et al., 2006; Vollenweider et Günthardt-Goerg, 2006). Consequently, tree species affected by ozone are generally weakened and more sensitive to parasitic pathologies and climatic variations. At a global scale, including Europe, Asia and America, foliar damage caused by ozone have been observed on tree species, shrubs and herbaceous plants, in crops and in the natural environment (ICP-Vegetation Annual Report 2015/2016). Ozone injuries have been especially studied, both on broadleaved trees (Schaub and Calatayud, 2013; Feng et al., 2014) and conifers (Dalstein et al., 2001; Dalstein and Vas, 2005; Sicard and Dalstein-Richier, 2015). Among the latter, pine trees are always the most sensitive to ozone, especially *Pinus cembra.* It's an alpine pine, considered a remnant of the Siberian pine glacial area, growing between 1100 and 2500 m above sea level where it is often associated with spruce (*Picea abies*), pine with hooks (*Pinus mugo*) and European larch (*Larix decidua*). This species has been able to cope with difficult climatic and edaphic conditions specific to

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subalpine montane stages: significant temperature differences (−40 °C) (G. Caudullo and D. de Rigo, 2016), acidic soils and harsh ecological conditions from this environment. This species owes it to a particularly well-adapted morphology and physiology. Conical in shape and similar to a candelabra, it can reach 25 m in height as an adult and live up to four or five centuries. It adapts to difficult soil conditions, such as scree, which is why it has a central pivot point and prominent tracing roots. South of the Alps, it evolves in a limited altitudinal area and extends to the Carpathian, Tetra and Transylvanian Alps massifs. Its 4–8 cm triangular section needles are grouped by five. Its mode of reproduction is peculiar, depending essentially on a bird, the jay of the mountains, which feeds on its seeds, very rich in lipid. This bird buries these seeds in the ground, in caches, in order to have reserves to use during the winter. Thus, when the bird dies or when it forgets its caches, *P.cembra* often develops in bouquets of small trees.

*P.cembra* reacts almost as a bio-indicator to ozone (Sicard and Dalstein-Richier, 2015; Dalstein et al., 2008). Visible symptoms observed on plants have been clearly related to ozone concentrations in various European countries (Gottardini et al., 2016). The fact that these visible symptoms are present reflects that there is a significant concentration of ozone in the atmosphere (EPA, 2007).

Defoliation on trees has also been monitored and gives an indication of the trees' strength. The more transparent the crown of a tree appears, the poorer its health status; ozone contributes to this situation in addition to the natural detrimental effects of water stress and mineral deficiency (Ferretti and Fischer, 2013; Gottardini et al., 2016). Studies have clearly shown that ozone contamination plays a part in foliar defoliation of tree species, such as beeches Ferretti et al., 2007). However, it is important to note that leaf deficiency of trees is caused by various factors, and direct relation to ozone contamination is not always established. Droughts, and more particularly, hydric stress, are known to increase foliar defoliation (Paoletti, 2006). Among the Mediterranean region, and precisely in the south of France, drought periods have been more frequent in the past years. Summers are hotter and dryer (Sicard and Dalstein-Richier, 2015). It is through the stomata that ozone enters foliar tissues, and the conditions of external temperature, soil temperature, and the age of the needles more or less facilitate penetration (Day et al., 1991).

Ozone concentrations should thus increase in the future. Considering this situation, this study will present the forest damages caused by ozone and their trends from 2000 to 2016 (i). It will then specify ozone concentration levels during this period and the AOT 40 (Accumulated Ozone exposure over a Threshold of 40 Parts Per Billion) levels that are derived from such ozone levels. These data were monitored using seven physicochemical analysers located on the French Riviera, close to Nice, Monaco, and Italy (ii). Other measurements of ozone concentrations were recorded by eight passive sensors placed in the Mercantour mountain massif (iii), approximately 70 km north from the littoral (iv). During the same period, defoliation, discolouration and other specific ozone damages of *P.cembra* were monitored in 13 forest plots located in the Mercantour mountain massif. During this study, correlations between average ozone concentrations recorded at the shore and close mountain areas and meteorological parameters (temperature, rainfall, relative humidity and shortwave radiation) were evaluated. Correlations with foliar symptoms specific to *P.cembra*, average defoliation and foliage discolouration, were also assessed.

## 2. Equipment and methodology

### 2.1. Study area

The study area is located in the Alpes-Maritimes county in the southeast of France, near the Italian border. The 13 forest plots of *P.cembra* are located in the Mercantour National Park (Fig. 1), at an altitude between 1700 and 2200 m. *P.cembra* is a pioneer species, which has a strong presence in alpine highlands and in the Mercantour mountain massif. It is known to be particularly sensitive to ozone contamination (Sicard and Dalstein-Richier, 2015).

### 2.2. Ozone concentration

#### 2.2.1. Ozone passive sensors

In the Mercantour National Park, eight ozone passive sensors located close to the 13 forest plots recorded average monthly ozone concentration from May to September between 2000 and 2016 (Fig. 1, Table 1). During April months, the amount of snow was still very important in this mountain area and did not permit the collection of data before May.

These kinds of sensors allow the monitoring of ozone concentration in remote areas, far away from any power line (Krupa and Legge, 2000; Dalstein et al., 2004), and provide spatial and temporal information (Gottardini et al., 2016). They are built in Sweden by IVL (Svenska Miljöinstitutet AB), a recognized environmental monitoring institute in Europe. The sensors were positioned at 1,8 m high above the ground, close to the studied plots but out of the forest cover. They were protected from weather conditions by a metal plate and contained a filter impregnated with a solution that absorbs ozone. The gas entered by an open tube and passed through the filter by molecular diffusion. The exposure time was four weeks long (twenty-eight days). The date and hour of the day were recorded each time the sensors were changed, and the used filters were sent monthly to IVL. The ozone concentration was finally determined by ionic chromatography.

#### 2.2.2. Physicochemical analysers

In addition to remote passive sensors, physicochemical analysers were installed to monitor hourly ozone concentrations using the UV absorption method ("OZONE 41M") close to the French Riviera coastline and in the countryside near the city of Nice (Fig. 1). These data were provided by the Surveillance Network for Air Quality (AASQA for its French acronym) which is administered by the French Agency for Energy Management (ADEME). All in all, five analysers were installed close to the shore at an altitude between 20 and 360 m, and two in the villages of Cians and Adréchas, at elevations of 1450 and 1500 m, respectively, close to the forest plots in the Mercantour (Table 1). These analysers provided hourly ozone concentrations, which allowed for the determination of the AOT40 indicator. This latter parameter was defined at the European Union level for vegetation protection (Directive 2002/3/CE). AOT40 indicates the sum of differences between hourly ozone concentrations superior to 40 ppb and the threshold of 40 ppb, taken between 8 h and 20 h during the growth period of plants, defined as from the 1st of April to the 30$^{th}$ of September for trees. For AOT40 measures, the ratio of hourly data must be equal or over 75% for validation. From these data, the annual ozone concentration was calculated considering all of the studied stations between 2000 and 2016.

### 2.3. Assessment of visible ozone symptoms on leaves

In the 13 forest plots of *P.cembra* situated in the Mercantour National Park (Fig. 1, Table 1), ozone leaf damage was assessed according to the European protocol established in the ICP-Forest manual (Programme International Coopératif d'évaluation et de surveillance de la pollution atmosphérique des forêts; Schaub et al., 2010; Michel et al., 2014). In each plot, 5 trees of adult size that were well exposed to sunlight were selected for ozone foliar

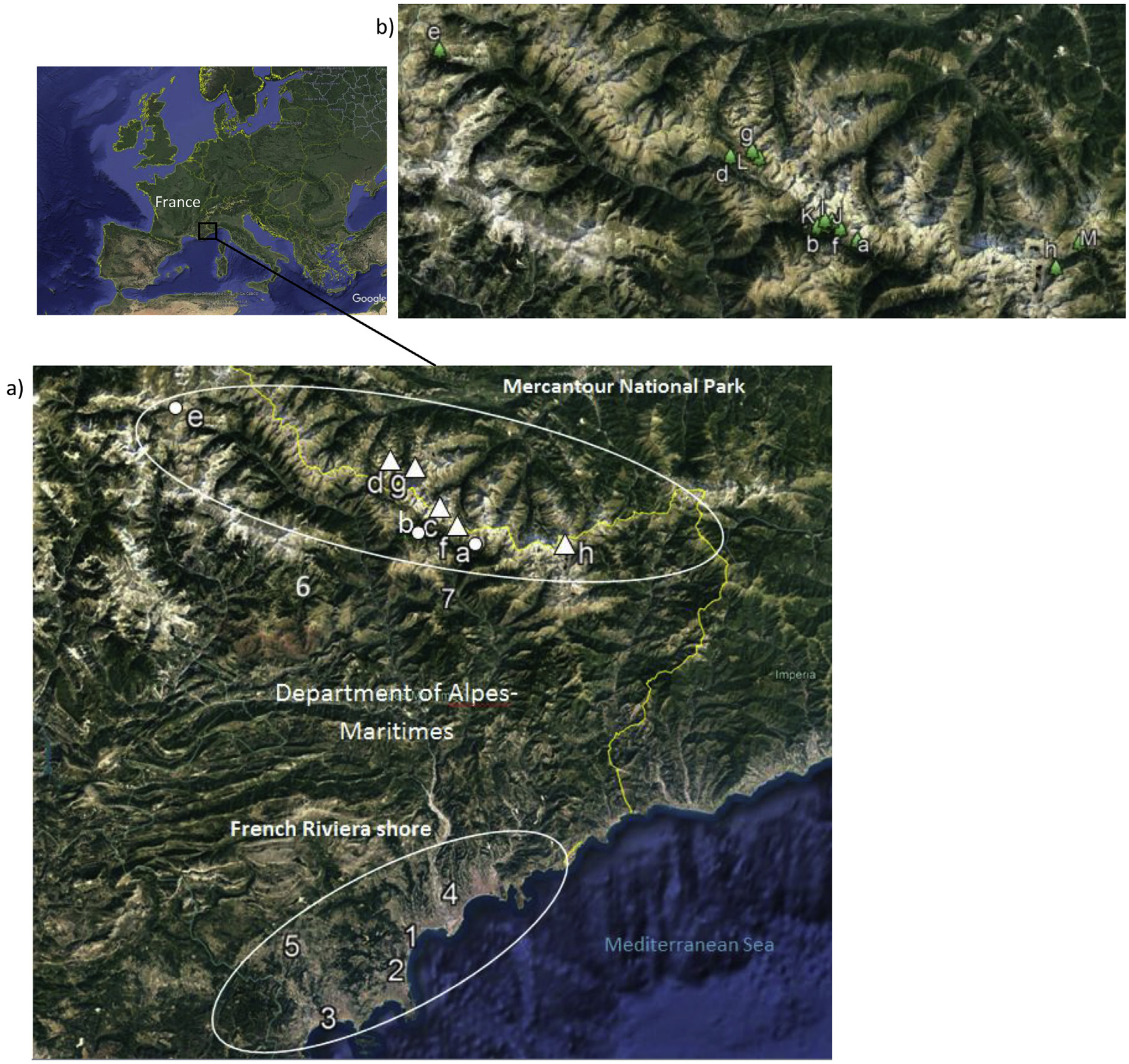


**Fig. 1.** a) Localisation and code of physicochemical analysers and ozone passive sensors situated on mountain ridges and in the bottom of valleys; b) localisation of the 13 forest plots in the Mercantour National Park.

damage monitoring between 2000 and 2016. Thick leaves of adult trees (e.g., *P.cembra*) intercept more light at low angles of incidence than at high angles of incidence (Jordan and Smith, 1993). This has an impact on the appearance of ozone symptoms.

From each tree, five branches were collected on the third upper part of the crown. The percentage of ozone damages were assessed on 25 needles per branch for both 1 year old (C+1) and 2 year old leaves (C+2). The oldest leaves generally presented more symptoms. All in all, the percentage of foliar damages was determined on 65 trees over 16 years. For pine trees, the specific symptoms due to ozone are small pale yellow mottles, called chlorotic spots or ‘mottling’. These symptoms were recorded from mid-July to mid-August when ozone concentrations are the highest of the year and before the leaf senescence period (Tagliaferro et al., 2005; Schaub et al., 2010). The observations were conducted by two experts from the GIEFS (Groupe International d’Études des Forêts Sud-Européennes). The National Forestry Service (Office National des Forêts - ONF) appointed the GIEFS as a national expertise organization to assess visible ozone foliar symptoms. It participates in intercalibration sessions organized by the expert group of the ICP-Forest network, which works on air quality.

### 2.4. Defoliation and discolouration

Defoliation and discolouration were recorded by the GIEFS experts every year from 2000 to 2016 on the 20 trees located in each plot. The general colour of each tree was determined by the importance of the discolouration. According to the European protocol defined in the ICP-Forest network (ICP Forest's protocol), each tree was graded on a scale from 0 (completely green) to 4 (completely yellow). On the other hand, the defoliation indicator reflects the transparency of the crown of a tree and is used to assess its health status (Rossini et al., 2006; Fischer and Lorenz, 2011; De Marco et al., 2014). The ‘transparency’ of the crown of a tree was evaluated by comparison to a standard one, called the “normal tree,” whose foliage was not altered (Fig. 2). The healthy tree must have the same site conditions as the studied ones (Rossini et al., 2006). The leaf loss was determined by classes of 5% of the whole

**Table 1**
GPS coordinates altitude and code of ozone passive sensors and physicochemical analysers located in the department of Alpes-Maritimes. Rural environment is defined as a place without road or habitation.

| Physicochemical analysers | | | | | |
|---|---|---|---|---|---|
| Location | Environment | Code | GPS coordinates | | Altitude (m) |
| | | | Latitude | Longitude | |
| Cagnes | Urban | 1 | 7,1578 | 43,6583 | 20 |
| Antibes | Urban | 2 | 7,0947 | 43,5997 | 80 |
| Cannes | Urban | 3 | 7,0072 | 43,5625 | 80 |
| Nice | Urban | 4 | 7,2119 | 43,6861 | 185 |
| Grasse | Urban | 5 | 6,9194 | 43,6569 | 360 |
| Cians | Rural | 6 | 6,9892 | 44,0883 | 1450 |
| Adréchas | Rural | 7 | 7,2280 | 44,1056 | 1500 |
| Passive sensors | | | | | |
| Entrée PNM | Rural | a | 7°15′14.18″ | 44° 7′42.35″ | 1650 |
| Vacherie du Collet | Rural | b | 7°12′46.22″ | 44° 8′27.73″ | 1880 |
| Pont d'Ingolf | Rural | c | 7°13'46" | 44°8'36" | 1980 |
| Lausetta | Rural | d | 7° 7′31.00″ | 44°11′55.00″ | 1820 |
| Sestrières | Rural | e | 6°49′28.00″ | 44°17′34.00″ | 1980 |
| Col de Salèse | Rural | f | 7°14′11.00″ | 44° 8′18.00″ | 2050 |
| Col de la Lombarde | Rural | g | 7° 8′55.84″ | 44°12′4.34″ | 2350 |
| Cabanes de Julie | Rural | h | 7°27′34.55″ | 44° 5′54.99″ | 2100 |

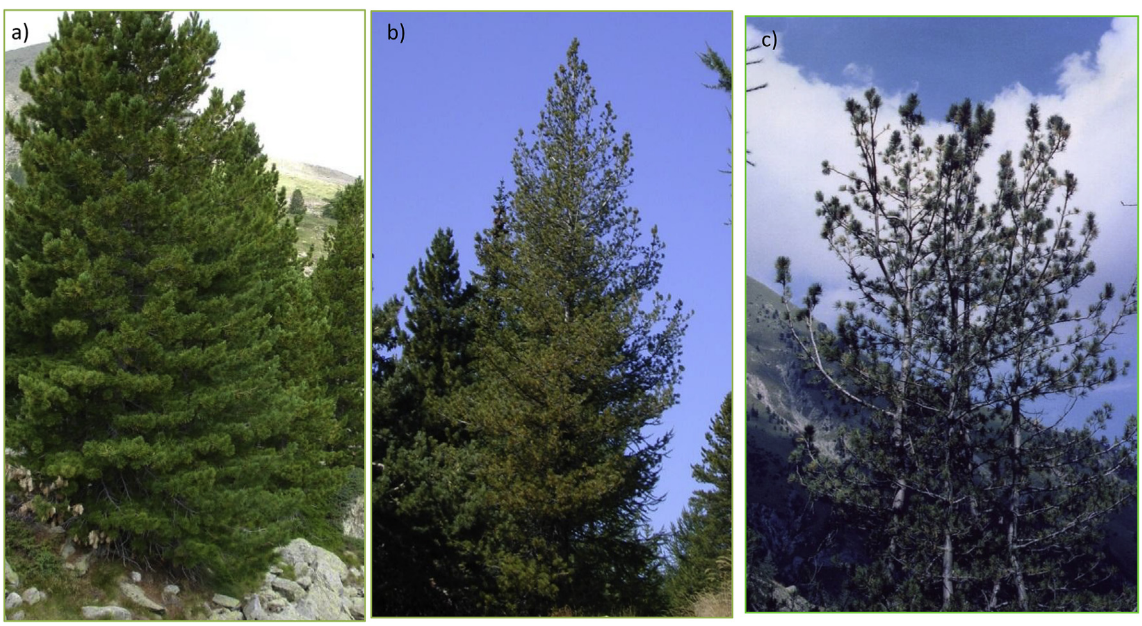


**Fig. 2.** Crown defoliation of *Pinus cembra* in the Mercantour National Park: a) Healthy tree; b) Tree severely injured with 30% of defoliation; c) Tree close to mortality with 60% of defoliation.

crown. An alert phase was reported if the percentage of affected foliage was between 10 and 25%. When it was over 25%, the tree was considered severely injured (Fischer and Lorenz, 2011; Michel et al., 2014) (Fig. 2). Damages due to biotic causes, such as pests or insect plagues, were recorded as well on every studied tree. Indeed, defoliation can also be enhanced by these factors and other abiotic ones, such as climatic or site conditions. To a lesser extent, visible foliar injuries specific to atmospheric ozone are added up to defoliation (Günthardt-Goerg and Vollenweider, 2007).

### 2.5. Meteorological parameters

Modelled hourly temperature meteorological data, relative humidity, solar radiation, and precipitation accumulation at Isola 2000 for the period from 2000 to 2016 were downloaded from the Meteoblue website (www.meteoblue.com). These data permitted the calculation of monthly averages from May to September in order to match the measurements of ozone concentration, which were mentioned previously, in the Mercantour National Park. Data from Isola 2000 had been selected because of their closeness to the meteorological conditions of the forest plots. These data were used in order to establish a possible link between meteorological parameters, atmospheric ozone concentration, and the average percentages of leaf symptoms, defoliation and discolouration that were assessed on each plot every year between 2000 and 2016.

### 2.6. Statistical data

The Mann Kendall test was applied in order to detect significant trends for the thirteen forest plots using average annual data for leaf symptoms, defoliation, discolouration and ozone concentrations recorded from 2000 to 2016. This test was also applied to annual emissions of ozone precursors (NOx, COVNM, CO) using data recorded from 1990 to 2015 on the entire French territory. For the period from 2000 to 2015, the CITEPA (Interprofessional Technical Centre for Studies on Air Pollution) provided these annual data. This test was also applied to AOT40 data and annual ozone concentrations that were recorded by the physicochemical analysers located in the coastline area. The Mann Kendall test is a statistical nonparametric test used to detect an upward or downward trend of a variable of interest over time. It has four different levels of significance (0.1, 0.05, 0.01 and 0.001). The Mann Kendall test is associated with Sen's slope calculation and can be applied to every non-static variable with at least 10 samples. A statistically significant trend is observed when the p-value of the test is less than 5%.

The Spearman test was also used in order to determine if ozone concentration and meteorological parameters (temperature, shortwave radiation, relative humidity and precipitation) affected the defoliation, discolouration and the visible leaf symptoms observed. The Spearman correlation is a nonparametric measure of statistical dependence between the rankings of two variables. It is used when two statistical variables seem to be correlated while the relationship between them is not linear and tries to find a rank correlation coefficient. It evaluates how well the relationship between two variables can be described using a monotonic function. If there are only two variables, a perfect Spearman correlation (+1 or −1) is obtained when one is a monotonic function of the other. Nonparametric statistical tests, unlike parametric ones, can be applied when there are missing data, non-normal distributions, or extreme values that are frequent in environmental time series. In addition, they can also be applied to data with small sample sizes. The Spearman correlation was applied to the average data of defoliation, discolouration and the visible leaf symptoms for each year of the studied period. In addition, it considered the average of ozone concentration data collected each year from 2000 to 2016 in every forest plot, and the modelled meteorological information recorded at Isola 2000.

## 3. Results

### 3.1. Ozone concentrations, ozone precursors and AOT40 calculation

A significant decrease ($p$-value< 0.001) of the quantity of the three ozone precursors (NOx, COVNM and CO) has been observed in the entire national territory over the past 25 years (1990–2015) or the past 15 years (2000–2015) (Table 2). NOx decreased 54 kt year$^{-1}$, COVNM 71 kt year$^{-1}$ and CO 245 kt year$^{-1}$ from 2000 to 2015.

Among eight forest plots, only two sites showed an important decrease in recorded ozone concentrations (Le Collet, p-value< 0.001; Lausetta, p-value< 0.01) (Table 3). Considering all of the plots, the average ozone concentration decreased significantly during the past 16 years. The data recorded in the forest plots located in the Mercantour did not always show significant downward trends contrary to those observed at the national scale for ozone precursors.

**Table 2**
National emissions and annual trends (kilotonne (kt) year$^{-1}$) obtained by the Mann Kendall test from CITEPA data over the 1990–2015 and 2000–2015 periods (p-value = 0.001***).

| | 1990–2015 | | 2000–2015 | |
|---|---|---|---|---|
| | Average | Trend (kt year$^{-1}$) | Average | Trend (kt year$^{-1}$) |
| NOx | 1468,13 | −46,534*** | 1244,23 | −54,005*** |
| VOCMN | 1435,58 | −81,257*** | 1027,18 | −70,911*** |
| CO | 6303,21 | −320,343*** | 4621,72 | −245,444*** |

From 2000 to 2008, ozone concentration stayed approximately 90–95 μg m$^3$, with a maximum recorded in 2003 (101 μg m$^3$) because of a heat wave during that summer. Since 2008, the ozone concentration has been closer to 82–84 μg m$^3$, except in 2012, when it increased up to 88 μg m$^3$. The baseline level of average concentrations is still mostly high.

Ozone concentration significantly increases ($R^2 = 0.72$) with altitude (Fig. 3). The highest ozone concentration was recorded at the Col de la Lombarde site (Table 3) every year. However, the mountain passes of the Mercantour National Park do not suffer any local contamination. Thus, it is clear that the high Col de la Lombarde ozone concentration is the result of a remote pollution. The average concentration recorded at the “Lausetta” plot (1820 m) located in a mountain ridge is far higher than the concentrations observed at sites such as “Vacherie du Collet” (72 μg m$^3$) or the entrance of the National Park (63 μg m$^3$), both situated at the bottom of the valley at a similar altitude (Table 3).

The physicochemical analysers located at the coastline area did not record a significant decrease or increase of ozone concentration over the period from 2000 to 2016 (Table 3). Only the Grasse station, which is located slightly more distant from the shore and slightly higher than the coastline sites, showed a substantial increase of ozone concentration. The AOT40 recorded at the Nice stations significantly decreased (p-value< 0.01), such as in Cians (p-value< 0.05) and Cagnes (p-value< 0.05). However, the physicochemical analyser of Adréchas located at 1500 m height recorded the highest ozone concentration (94.1 μg m$^3$), which can be explained because of its higher altitude (Fig. 4).

These results generally confirm the previous results; altitude plays a major role in explaining these data at sites close to urban areas.

### 3.2. Defoliation and discolouration

In the high mountain area, a significant increase of defoliation of *P.cembra* was observed in five of the thirteen plots between 2000 and 2016 (Table 4). The highest augmentations of leaf loss were recorded at the Valmasque (+0.7% year$^{-1}$), Lausetta (+1.6% year$^{-1}$) and Col de Salese (+0.2% year$^{-1}$) sites.

In all thirteen plots, *P.cembra* suffered the most severe defoliation at the Lausetta station (38.42%). Considering discolouration, only Cabanes de Julie site, located at a high altitude, presented a strong yellowing (0.03% year$^{-1}$). The global average discolouration score reach the 2nd level (Table 4).

### 3.3. Visible ozone leaf symptoms

During the past sixteen years, a significant decrease of foliar injuries directly related to ozone contamination has been observed in 8 plots, and particularly in the one year old needles (C+1). This decrease was also observed for two year old needles (C+2) in 9 sites (Table 4). The downward trend of foliar damage in *P.cembra* is thus confirmed and is directly related to the small decrease of ozone concentrations. The most important decrease was recorded at the Col de la Lombarde site located at an altitude of 2350 m (−1.6% year$^{-1}$ for C+2 and −1.3% year$^{-1}$ for C+1) and at Route de la Lombarde situated at 2250 m height close to the first one (−1.22% year$^{-1}$ for C+2) (Table 4). These forest plots are still the most sensitive to ozone contamination because of their altitude (Fig. 5).

### 3.4. Correlation between visible foliar ozone injuries, meteorological parameters and ozone concentrations

Foliar ozone specific symptoms are significantly correlated to

**Table 3**
Ozone concentration, AOT40 for forest protection (AOT40 values at sites equipped with physicochemical analysers) and annual trends obtained by the Mann Kendall test from over the 2000–2016 period (p-value = 0.001***, 0.01**, 0.05*, 0.1+, >0.1). AOT40 values at sites equipped with physicochemical analysers.

| Plots | Ozone concentration (passive sensors) | | | |
|---|---|---|---|---|
| | Average (μg·m$^3$) | Trend (μg·m$^3$ year$^{-1}$) | No AOT40 values | |
| Parking PNM | 62,97 ± 6,63 | −0,389 | – | – |
| Collet | 72,01 ± 7,45 | −1306*** | – | – |
| Lausetta | 91,59 ± 6,57 | −0,968** | – | – |
| Sestriere | 80,93 ± 9,06 | −0814+ | – | – |
| Pont Ingolf | 84,33 ± 5,96 | −0,744 | – | – |
| Col de Salese | 95,17 ± 7,82 | −0,578 | – | – |
| Valmasque | 98,48 ± 6,90 | −0,74 | – | – |
| Col Lombarde | 105,33 ± 7,12 | −0,568 | – | – |
| All plots | 87,20 ± 5,93 | −0,911** | – | – |
| Ozone concentration (physio-chemical analysers) | | | | |
| Plots | Average (μg·m$^3$) | Trend (μg·m$^3$year-1) | Average AOT40 (ppb) | Trends of AOT40 (ppb.h year-1) |
| Adréchas | 94,1 ± 5,9 | 0,088 | 27829 | −0,378 |
| Cians | 82,8 ± 5,6 | 0,107 | 23038 | −0,429* |
| Grasse | 67,2 ± 10,7 | 0,76* | 16878 | −0,055 |
| Antibes | 48,6 ± 5,2 | 0,225 | 21491 | −0,111 |
| Cannes | 57,2 ± 5,1 | −0,62 | 17725 | −0500+ |
| Nice ouest | 70,0 ± 4,6 | −0,025 | 22748 | −0,560** |
| Cagnes | 53,4 ± 3,0 | −0,089 | 20161 | −0,644* |

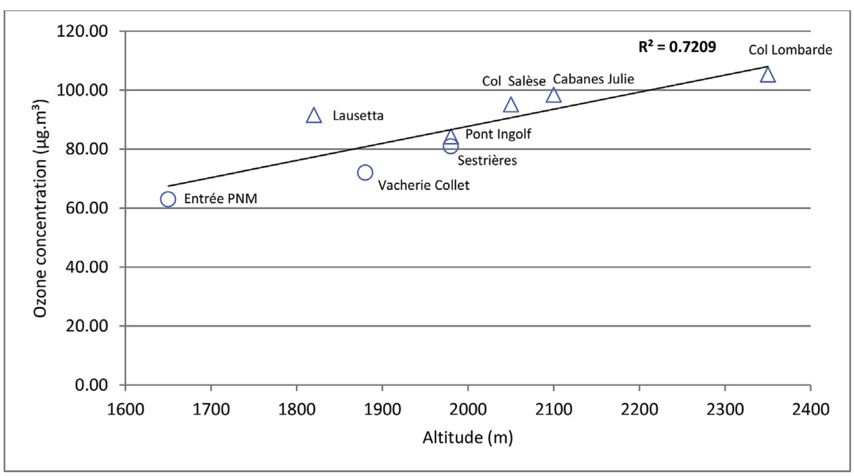


**Fig. 3.** Average ozone concentration (μg.m$^3$) recorded by passive sensors from 2000 to 2016 at each site in function of the altitude of sites (site located at ridge area Δ or at bottom of the valley ○).

ozone concentration for one and two year old needles (p-value< 0.001 and p-value< 0.01, respectively). To a lesser degree, they are correlated to shortwave radiation (p-value< 0.05 for C+1 and C+2) (Table 5). It is logical that ozone concentration is the triggering factor of specific injuries, even more as shortwave radiation, which enhances the photochemical production of ozone from its precursors, is important.

Considering defoliation or yellowing, there is no relationship that could be established with meteorological parameters. Only leaf loss was negatively correlated to ozone concentration (p-value< 0.05). Other factors, such as low light levels and deficiencies, may intervene as well.

## 4. Discussion

During the past 25 years, the decrease of ozone precursors over the entire national territory is the result of policies for the reduction of atmospheric contamination. This strategy was put in place by the European Union since the beginning of the 1990s. Currently, it is possible to see its consequences. The research of Sicard et al. (2011) confirmed this downward trend for ozone precursors during the period from 1990 to 2008. In France, curiously, even though NOx and COVNM decreased by 45% and 61%, respectively, between 2000 and 2014 (CITEPA, 2016), the average levels of ozone concentrations increased by 8% during the period 2000–2011. At a global scale, the same trend was observed with the augmentation of baseline levels in Europe, the United States and Japan (UNECE, 2010).

In the Mercantour National Park, the decrease of average ozone concentrations in 8 sites is only explained because two of them recorded significant decreases of this pollutant. Most of the studied stations do not explain this downward trend. As is the case at the national scale, the baseline level of ozone concentration among all of the plots remains high.

During this research, the physicochemical analysers located close to the French Riviera coastline recorded ozone concentrations much lower than rural stations situated at altitude in the Mercantour. Ozone production not only depends on the quantity of its precursors but also on its formation or destruction as air is moving, and it is also subject to the importance of solar radiation. In urban areas, road traffic, which produces high levels of NO, causes the

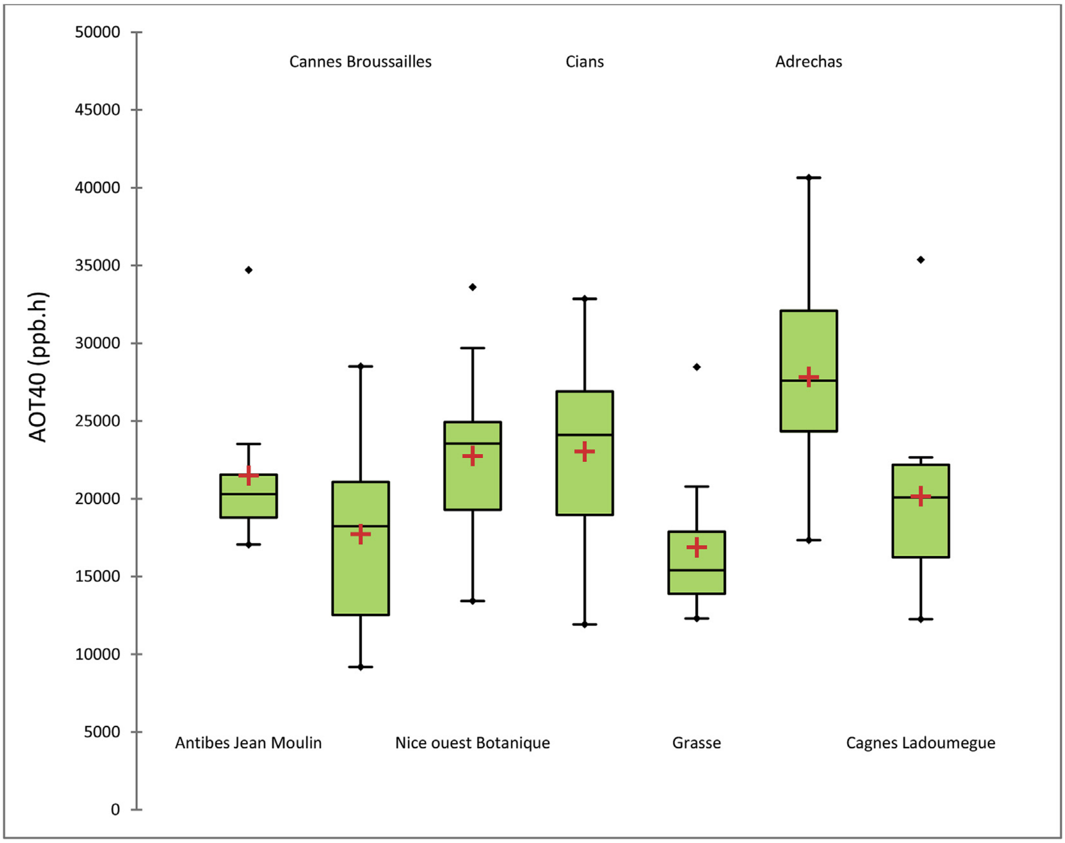


**Fig. 4.** Tukey boxes for AOT40 (ppb.h) recorded by physicochemical analysers at coastline and close countryside sites.

**Table 4**
Crown defoliation, discolouration score averages (±standard deviation), foliar surface percentage averages (±standard deviation) of foliar surface affected by chlorotic mottles and annual trends (% year$^{-1}$) obtained by Mann-Kendall test from forest plots in the Mercantour National Park, over the 2000–2016 period (p-value = 0.001*** 0.01**, 0.05*, 0.1+, >0.1).

| Plot | Altitude (m) | Crown defoliation | | Discolouration | | Average percentage of foliar surface affected by specific ozone chlorotic mottles | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Average (%) | Trend (% year-1) | Average (%) | Trend (unit year-1) | One-year-old needle (C+1) | | Two-year-old needle (C+2) | |
| | | | | | | Average (%) | Trend (%.year$^{-1}$) | Average (%) | Trend (%.year$^{-1}$) |
| Entrée PNM | 1650 | 26,45 ± 5,59 | 0661+ | 1,03 ± 0,58 | 0,012 | 10,88 | −0,4*** | 21,14 | −0,900** |
| Lausetta | 1820 | 38,42 ± 13,12 | 1633*** | 1,45 ± 0,38 | 0,025 | 12,45 | −0,5*** | 23,67 | −1044*** |
| Valmasque | 1820 | 27,97 ± 7,71 | 0,709*** | 1,47 ± 0,41 | 0,025 | 13,86 | 0,08 | 25,50 | −0,300 |
| Collet | 1880 | 29,29 ± 7,37 | 0,981* | 1,06 ± 0,62 | 0,045 | 10,69 | −0,323 | 20,55 | −0,350 |
| Germas | 1950 | 30,01 ± 5,32 | 0,398 | 1,23 ± 0,49 | 0,05625+ | 11,41 | −0,2 | 22,70 | −0,667** |
| Sestrière | 1980 | 22,93 ± 3,22 | −0,025 | 0,88 ± 0,32 | 0,015 | 11,60 | −0,2 | 21,61 | −0,727* |
| Pont Ingolf 1 | 1980 | 31,33 ± 2,80 | 0,228 | 1,58 ± 0,32 | 0,016 | 14,13 | −0,629** | 25,64 | −0,700 |
| Pont Ingolf 2 | 1990 | 23,73 ± 6,20 | 0,477 | 1,17 ± 0,59 | 0,064 | 15,58 | −0453+ | 28,23 | −0,613* |
| Pont Ingolf 3 | 2020 | 26,12 ± 4,84 | 0,696* | 1,30 ± 0,46 | 0,033 | 13,38 | −0,65* | 24,17 | −0,952* |
| Col Salèse | 2050 | 29,36 ± 2,94 | 0,226** | 1,43 ± 0,63 | 0076+ | 18,66 | −0,855* | 32,31 | −0,300 |
| Cabanes de Julie | 2100 | 34,85 ± 4,88 | 0,201 | 2,00 ± 0,33 | 0,034* | 20,51 | −0,707* | 34,37 | −0,840* |
| Col de la Lombarde | 2350 | 27,09 ± 4,37 | 0,152 | 1,68 ± 0,33 | 0,032 | 19,80 | −1308*** | 32,78 | −1600*** |
| Route de la Lombarde | 2250 | 32,66 ± 3,31 | −0,167 | 1,69 ± 0,43 | 0,021 | 20,19 | −0,9** | 34,51 | −1220*** |
| All stations | – | 30,24 ± 3,53 | 0504+ | 1,41 ± 0,38 | 0,043* | 14,89 | −0,483*** | 26,82 | −0,652*** |

destruction of ozone. In that way, in this study, urbanized coastal areas recorded lower ozone concentration levels. The difference between rural and urban zones is thus important. Within the entire national territory, this gap was approximately 10 to μg·m$^3$ between 2000 and 2012 (DGEC, 2013).

Ozone concentration is not completely and linearly correlated to altitude. The topography plays an important role in the records. Indeed, ozone concentrations are higher in mountain ridge areas than at the bottom of the valley. This clarifies why the average concentration in the "Lausetta" plot (1820 m) was higher than those recorded at "Vacherie du Collet" (1880 m) and at the entrance of the park ("Entrée PNM") (1790 m). This phenomenon is explained by the fact that marine thermal breezes and winds in valleys carry the pollution from urbanized coastal areas to the summits during the day. As the air masses are transported, the primary pollutants, such as NO and COVNM, are subject to photochemical reactions that produce ozone. This is finally "stored" at altitude in atmospheric reservoirs. To resume, ozone is produced

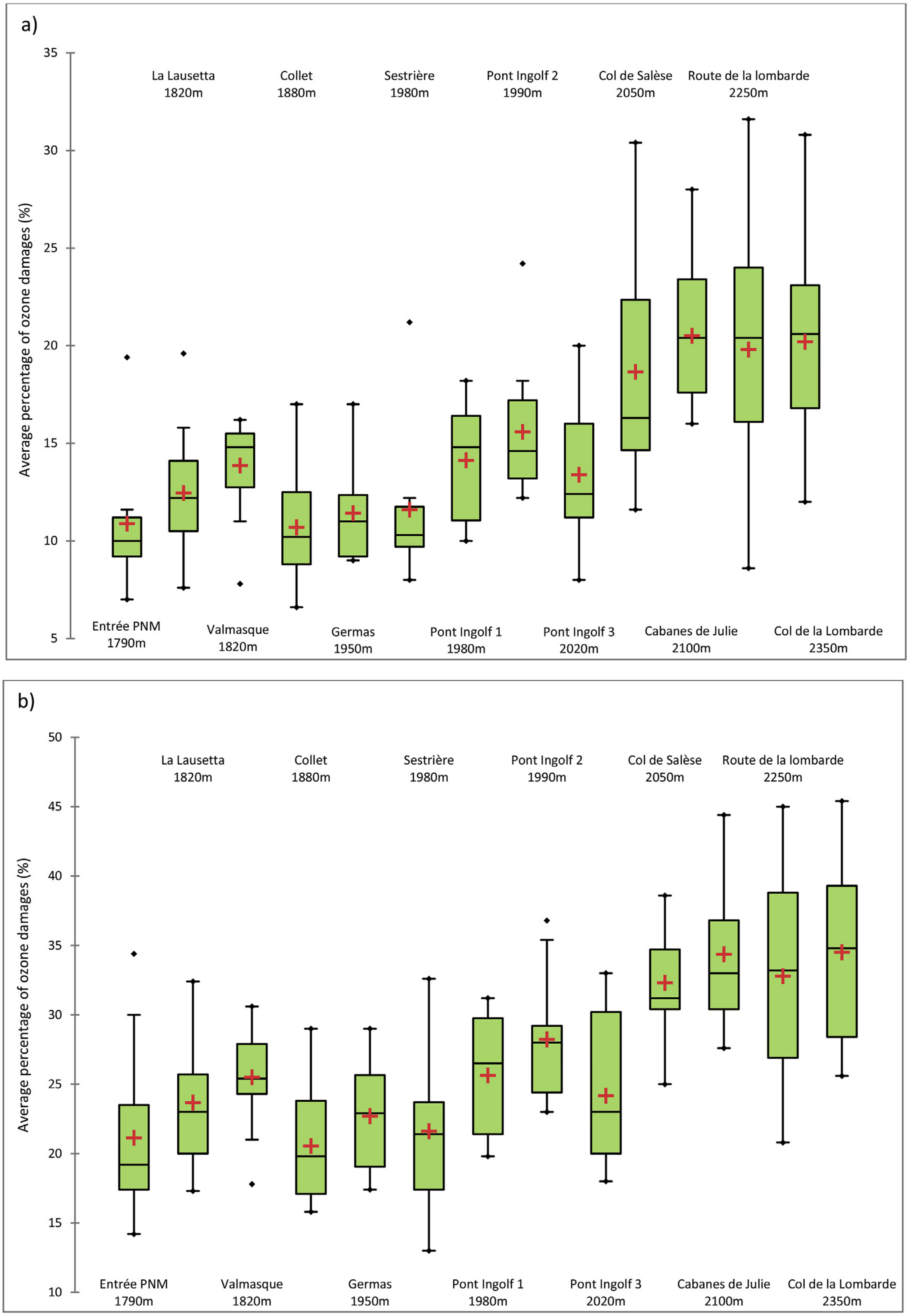


**Fig. 5.** Average percentage of ozone damages observed (a) upon one year old needles (C+1), and (b) upon two years old needles (C+2) in the 13 studied forest plots of the Mercantour.

**Table 5**
Spearman correlation and p-value (p-value = 0.001**, 0.01*,0.05*) between average ozone visible injuries, discolouration, defoliation recorded in all plots, meteorological parameters (temperature, relative humidity, shortwave radiation, precipitation) and average ozone concentration from May to September in all plots (over the period 2000–2016).

| | Temperature (°c year$^{-1}$) | Relative humidity (% year$^{-1}$) | Shortwave radiation (w.m$^2$ year$^{-1}$) | Total precipitation (mm year-$^1$) | Ozone (µg·m$^3$ year$^{-1}$) |
|---|---|---|---|---|---|
| O3 visible injury C+2 | 0,136 | - 0,632* | 0,618* | −0,221 | 0761** |
| O3 visible injury C+1 | 0,054 | - 0,496 | 0632* | −0,293 | 0829*** |
| Discolouration | 0,236 | - 0,039 | 0079 | 0,311 | - 0,439 |
| Defoliation | 0,189 | 0,05 | - 0,036 | 0221 | - 0,539* |

from precursors emitted by coastal conurbations, and then it is transported long distances to mountain summits, increasing its concentration.

The Mediterranean region is densely populated along the shore and receives strong sunlight, so ozone photochemical formation is very important during summer. This explains why only a few sites recorded a significant decrease in ozone concentration and why the baseline level remains high. Furthermore, it would be interesting to monitor the pollutant emissions in order to know if, as it occurs at a national scale, there is an upward trend of ozone concentration despite the decrease of its precursors. Tropospheric ozone does not only come from photochemical transformation of anthropic pollution, 10–20% is also produced by local terpene emissions from vegetation (Fehsenfeld et al., 1992; Fuentes et al., 2000; Calfapietra et al., 2009). Thus, the dense, large conifer forests of the Mercantour National Park contribute to local ozone formation in addition to the gases coming from the shore.

The maximum ozone concentration that was observed by this study during the past sixteen years was in 2003, when very high temperatures had been recorded throughout Europe (Matyssek et al., 2006). This particular year was characterized by a strong ozone pollution in the southeast of France (Prev'air, INERIS), confirming the tangible impact of a combination of chemical precursors produced in the littoral area, with shortwave radiation and high temperatures. In addition, the increase in temperature and water stress caused an increase in terpenic emissions, allowing the plants to cope with stress conditions (Sharkey and Loreto, 1993).

Eleven of the 13 studied forest plots showed a significant decrease of ozone concentrations during the past 16 years. Foliar symptoms are related to the amount of ozone that penetrates into the plant through the stomata. When the weather is hot and dry, which occurs frequently in the Mediterranean region, the stomata stay closed to prevent the plant from dehydration (Paoletti, 2006). Thus, a water deficit in the soil is known to lessen ozone penetration into the vegetation (Alonso et al., 2008), protecting it from damages induced by the gas (Bohler et al., 2013). Experimentation in controlled conditions demonstrated a decrease of visible injuries on poplars with water stress (Feng et al., 2014). During past years, especially in the Mediterranean region, higher temperatures during summer as well as drought conditions have been observed (GIEC, 2013). It is thus probable that stomata closure duration would have increased, reducing the ozone captured. This statement, in conjunction with decreasing levels of ozone, can explain the significant decrease observed in the percentages of foliar injuries. The presence of visible symptoms on leaves not only depends on the quantity of ozone but also on dry soil conditions and stomatal openings. These complementary factors are also subject to exposure and topographic conditions. Thus, tree species located on the south side of the mountains receive higher solar radiations, but they can present a less severe reaction to ozone than the ones situated on the northern slope where soil humidity is more important. In the latter case, stomatal opening can be greater and, even though ozone concentration is less, it will be more harmful (Day et al., 1991).

This research showed that defoliation in *P.cembra* located in the Mercantour National Park has generally increased during the past years, confirming the trend observed since the 1990s (Fischer and Lorenz, 2011; Sicard and Dalstein-Richier, 2015). The Spearman test could not establish a correlation between defoliation and meteorological parameters. This test used data from average ozone concentrations and the average defoliation recorded among all sites from May to September every year from 2000 to 2016. The Isola 2000 weather station was used to collect meteorological modelled data from May to September every year. The lack of real meteorological information close to the sites could explain the fact that no correlation was found between defoliation and the meteorological parameters. As the studied forest plots were located in a mountainous area at different altitudes, the weather conditions can be very distinct from one another. In a future study, it would be interesting to establish meteorological stations next to each forest plot in order to have a better understanding of the influence of these parameters over defoliation. Lots of research has already highlighted the impact of water stress on defoliation (Sicard and Dalstein-Richier, 2015).

Furthermore, climate change has a stronger impact in mountainous zones at higher altitudes. There are thus ideas for new studies. Installing meteorological stations in these places would permit better monitoring and previsions of ozone effects on plants at altitude.

The observed foliar symptoms were very significantly correlated to ozone concentration on both one year-old ($p$-value$< 0.001$) and two year-old needles ($p$-value$< 0.01$), which confirms that this gas is responsible for the mottling of *P.cembra*. Ozone is formed by photochemistry of its precursors, which explains the strong positive correlation ($p$-value$< 0.05$) between visible foliar symptoms and shortwave radiation.

As it penetrates through the plant tissue, ozone can induce an alteration of grana structures and thylakoids (Sutinen et al., 1990), an increase of oxidizing stress inside the chloroplasts (Pell et al., 1997), and a decrease of photosynthesis (Pell et al., 1997; Wittig et al., 2009). Biomass and efficiency losses between 3 and 23% had been observed in crops in various countries (Annual Report ICP-vegetation 2015/2016) in addition to a decrease in radial growth in beeches (19,5%) and spruces (6,6%) in Switzerland during 10 years of study (Braun et al., 2014). It would be interesting to determine dendrometric measurements along with photosynthesis activity observations in order to detect if ozone concentration has an impact on *P.cembra* growth in the Mercantour. It would also be interesting to consider the ozone flux approach to establish a link between the actual ambient ozone concentration, weather conditions, soil conditions and observed damage.

In addition, it might be interesting to use a multiple regression approach, where defoliation, leaf colour and chlorotic mottles are the response variables. Site could be used as an indicator variable and ozone concentration as a regressor variable.

## 5. Conclusion

*P.cembra* of the Mercantour massif in the southeast of France has suffered lower ozone visible injuries during the past sixteen years. Even though the gas concentration has been decreasing, the reduction of visible foliar symptoms was not very important. Summer droughts, which have been more pronounced lately, are the reason that the conditions for ozone formation are optimum. Furthermore, water stress known to increase stomatal closure contributes to the diminution of ozone penetrating into pine needles, reducing the percentage of damaged foliage. The visible foliar injuries that were observed during this study are truly correlated to ozone concentrations, which are much higher in the Mercantour than in the urbanized coastline. The altitudinal factor is also essential to take into account when monitoring forest evolution considering meteorological parameters and newly derived photochemical emissions. These forests at higher altitude are natural laboratories of climate change in the sense that they are more sensitive to the consequences of the human way of life. It would be interesting in the future to focus on these extreme sites, which indicate better than other places the impending global changes.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.10.081.

## References

Alonso, R., Elvira, S., Sanz, M.J., Gerosa, G., Emberson, L.D., Bermejo, B., Gimeno, B.S., 2008. Sensitivity analysis of a parameterization of the stomatal component ofthe DO3SE model for Quercus ilex to estimate ozone fluxes. Environ. Pollut. 155, 473–480.
Alonso, R., Elvira, S., González-Fernández, I., Calvete, H., García-Gómez, H., Bermejo, V., 2014. Drought stress does not protect Quercus ilex L. from ozone effects: results from a comparative study of two subspecies differing in ozone sensitivity. Plant Biol. 16, 375–384.
AQEG, 2007. Air Quality and Climate Change: a UK Perspective. Report Prepared by the Air Quality Expert Group for the Department for Environment, Food and Rural Affairs; Scottish Executive. Welsh Assembly Government; and Department of the Environment in Northern Ireland. PB12489 ISBN 0-85521-172-5.
Bohler, S., Sergeant, K., Jolivet, Y., Hoffmann, L., Hausman, J.F., Dizengremel, P., Renaut, J., 2013. A physiological and proteomic study of poplar leaves during ozone exposure combined with mild drought. Proteomics 13, 1737–1754.
Braun, S., Schindler, C., Rihm, B., 2014. Growth losses in Swiss forests caused by ozone: epidemiological data analysis of stem increment of Fagus sylvatica L. and Picea abies Karst. Environ. Pollut. 192, 129–138.
Calfapietra, C., Fares, S., Loreto, F., 2009. Volatile organic compounds from Italian vegetation and their interaction with ozone. Environ. Pollut. 157, 1478–1486.
Caudullo, G., de Rigo, D., 2016. *Pinus cembra* in Europe: distribution, habitat, usage and threats. European Atlas of Forest Tree Species 120–121.
CITEPA, 2016. Inventaire des émissions de polluants et de gaz à effet de serre au format SECTEN.
Dalstein, L., Vas, N., 2005. Ozone concentrations and ozone-induced symptoms on coastal and alpine Mediterranean pines in southern France. Water, Air, Soil Pollut. 160, 181–195.
Dalstein, L., Teton, S., Cottereau, C., Vas, N., 2001. Niveaux d'ozone et endommagement foliaire sur quelques essences méditerranéennes. Pollut. Atmosphérique 170, 263–277.
Dalstein, L., Vollenweider, P., Vas, N., Günthardt-Goerg, M.S., 2002. L'ozone et les conifères du sud-est de la France. Forêt Méditerranéenne XXIII, 105–116.
Dalstein, L., Vas, N., Ulrich, E., Cecchini, S., 2004. Première approche de la dégradation de la forêt française en relation avec l'ozone. Environnement Tech. 236, 42–44.
Dalstein, L., Vas, N., Ulrich, E., Cecchini, S., 2008. Effets de l'ozone sur quelques peuplements forestiers du réseau RENECOFOR (Office national des forêts). Forêt Méditerranéenne XXIX, 329–336.
Dalstein-Richier, L., Mangin, A., Carréga, P., Gueguen, C., Vas, N., Sanchez, O., Théodore, B., Bérolo, W., 2005. État des forêts d'altitude en relation avec la pollution de l'air par l'ozone dans la région niçoise. Pollut. Atmosphérique 188, 503–519.
Day, T.A., Heckathorn, S.A., DeLucia, E.H., 1991. Limitations of photosynthesis in Pinus taeda L.(Loblolly pine) at low soil temperatures. Plant Physiol. 96, 1246–1254.
De Marco, A., Proietti, C., Cionni, I., Fischer, R., Screpanti, A., Vitale, M., 2014. Future impacts of nitrogen deposition and climate change scenarios on forest crown defoliation. Environ. Pollut. 194, 171–180.
DGEC, 2013. Bilan de la qualité de l'air en 2012.
EPA, 2007. Review of the National Ambient Air Quality Standards for Ozone: Policy Assessment of Scientific and Technical Information. Staff Paper. Office of Air Quality Planning and Standards. Report No. EPA-452/R-07-007a.
Fares, S., Vargas, R., Detto, M., Goldstein, A.H., Karlik, J., Paoletti, E., Vitale, M., 2013. Tropospheric ozone reduces carbon assimilation in trees: estimates from analysis of continuous flux measurements. Global Change Biol. 19, 2427–2443.
Fehsenfeld, F., Calvert, J., Fall, R., Goldan, P., Guenther, A.B., Hewitt, N.C., Lamb, B., Liu, S., Trainer, M., Westberg, H., Zimmerman, P., 1992. Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. Global Biogeochem. Cycles 6, 390–430.
Feng, Z., Sun, J., Wan, W., Hu, E., Calatayud, V., 2014. Evidence of widespread ozone-induced visible injury on plants in Beijing, China. Environ. Pollut. 193, 296–301.
Ferretti, M., Fischer, R., 2013. Forest Monitoring: Methods for Terrestrial Investigations in Europe with an Overview of North America and Asia. Developments in Environmental Science, vol. 12. Elsevier, Oxford.
Ferretti, M., Calderisi, M., Bussotti, F., 2007. Ozone exposure, defoliation of beech (Fagus sylvatica L.) and visible foliar symptoms on native plants in selected plots of South-Western Europe. Environ. Pollut. 145, 644–651.
Fischer, R., Lorenz, M., 2011. Forest Condition in Europe, 2011. Technical Report of ICP Forests and Fut Mon.Work Report of the Institute for World Forestry 2011/1. ICP Forests, Hamburg, p. 212p.
Fuentes, J.D., Lerdau, M., Atkinson, R., Baldocchi, D., Bottenheim, J.W., Ciccioli, P., Lamb, B., Geron, C., Gu, L., Guenther, A., Sharkey, T.D., Stockwell, W., 2000. Biogenic hydrocarbons in the atmospheric boundary layer: a review. Bull. Am. Meteorol. Soc. 81, 1537–1575.
GIEC, 2007. Fourth report, Climate Change 2007: Impacts, Adaptation and Vulnerability.
GIEC, 2013. Fift report, Climate Change 2013: the Physical Science Basis.
Gottardini, E., Calatayud, V., Ferretti, M., Haeni, M., Schaub, M., 2016. Spatial and temporal distribution of ozone symptoms across Europe from 2002 to 2014. In: Michel, A., Seidling, W. (Eds.), Forest Condition in Europe: 2016 Technical Report of ICP Forests. ICP Forests, Eberswalde, pp. 73–82.
Günthardt-Goerg, M.S., Vollenweider, P., 2007. Linking stress with macroscopic and microscopic leaf response in trees: new diagnostic perspectives. Environ. Pollut. 147, 467–488.
IPCC, 2014. In: Barros, V.R., Field, C.B., Dokken, D.J., Mastrandrea, M.D., Mach, K.J., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, p. 688.
Jacob, D.J., Winner, D.A., 2009. Effect of climate change on air quality. Atmos. Environ. 43, 51–63.
Jordan, D.N., Smith, W.K., 1993. Simulated influence of leaf geometry on sunlight interception and photosynthesis in conifer needles. Tree Physiol. 13, 29–39.
Krupa, S.V., Legge, A.H., 2000. Passive sampling of ambient, gaseous air pollutants: an assessment from an ecological perspective. Environ. Pollut. 107, 31–45.
Kulkarni, P.S., Bortoli, D., Domingues, A., Silva, A.M., 2015. Surface ozone variability and trend over urban and suburban sites in Portugal. Aerosol Air Qual. Res. 1–15.
Matyssek, R., Le Thiec, D., Low, M., Dizengremel, P., Nunn, A.J., Haberle, K.H., 2006. Interactions between drought and O3 stress in forest trees. Plant Biol. 8, 11–17.
Meleux, F., Solmon, F., Giorgi, F., 2007. Increase in European summer ozone amounts due to climate change. Atmos. Environ. 41, 7577–7587.
Michel, A., Seidling, W., Lorenz, M., Becher, G., 2014. Forest condition in Europe: 2013 technical report of ICP forests. In: Report under the UNECE Convention on Long-range Transboundary Air Pollution. Thünen Working Paper 19.
Paoletti, E., 2006. Impact of ozone on Mediterranean forest: a review. Environ. Pollut. 144, 463–474.
Paoletti, E., Contran, N., Manning, W.J., Tagliaferro, F., 2007. Ethylenediurea (EDU) affects the growth of ozone-sensitive and tolerant Ash (Fraxinus excelsior) trees under ambient O3 conditions. Sci. World J. 7, 128–133.
Pell, E.J., Schlagnhaufer, C.D., Arteca, R.N., 1997. Ozone-induced oxidative stress: mechanisms of action and reaction. Physiol. Plantarum 100, 264–273.
Prev'air, INERIS. 30th August, 2018. http://dev-prevair.ineris.fr/fr/episode-o3.php#I.
Rossini, M., Panigada, C., Meroni, M., Colombo, R., 2006. Assessment of oak forest condition based on leaf biochemical variables and chlorophyll fluorescence. Tree Physiol. 26, 1487–1496.
Schaub, M., Skelly, J.M., Zhangc, J.W., Ferdinand, J.A., Savage, J.E., Stevenson, R.E., Davis, D.D., Steiner, K.C., 2005. Physiological and foliar symptom response in the crowns of *Prunus serotina, Fraxinus americana* and *Acer rubrum* canopy trees to ambient ozone under forest conditions. Environ. Pollut. 133, 553–567.
Schaub, M., Calatayud, V., Ferretti, M., Brunialti, G., Lövblad, G., Krause, G., Sanz, M.J., 2010. Monitoring of ozone injury, manual part X. In: Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the

Effects of Air Pollution on Forests. UNECE ICP Forests Programme, Hamburg.
Schaub, M., Calatayud, V., 2013. In: Ferretti, M., Fischer, R. (Eds.), Assessment of Visible Foliar Injury Induced by Ozone. Forest Monitoring, vol. 1. Elsevier, UK. Chapter: 11, Publisher.
Screpanti, A., De Marco, A., 2009. Corrosion on Cultural Heritage Buildings in Italy: a role for ozone? Environ. Pollut. 157, 1513–1520.
Sharkey, T.D., Loreto, F., 1993. Water-stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia 95, 328–333.
Sicard, P., Dalstein-Richier, L., 2015. Health and vitality assessment of two common pine species in the context of climate change in southern Europe. Environ. Res. 137, 235–245.
Sicard, P., Dalstein-Richier, L., Vas, N., 2011. Annual and seasonal trends of ambient ozone concentration and its impacts on forest vegetation in Mercantour National Park (South-eastern France) over the 2000-2008 period. Environ. Pollut. 159, 351–362.
Stevenson, D.S., Young, P.J., Naik, V., Lamarque, J.F., Shindell, D.T., Voulgaraki, A., Skeie, R.B., Dalsoren, S.B., 2013. Tropospheric ozone changes, radiative forcing and attribution to emissions in the atmospheric chemistry and climate model inter-comparison project(ACCMIP). Atmos. Chem. Phys. 13, 3063–3085.
Sutinen, S., Skärby, L., Wallin, G., Selldén, G., 1990. Long-term exposure of Norway spruce, *Picea abies* (L.) Karst. to ozone in open-top chambers. II. Effects on the ultrastructure of needles. New Phytol. 115, 345–355.
Tagliaferro, F., Ferrara, A.M., Spaziani, F., Viotto, E., 2005. Sintomi fogliari di tipo ozone like su vegetazione spontaneae ornamentale in Piemonte. Linea Ecologica XXVII, 47–53.
The Royal Society report, 2008. Ground-Level Ozone in the 21st Century: Future Trends, Impacts and Policy Implications. Science Policy Report 15/08, p. 133.
Tiwariet, S., Grote, R., Churkina, G., Butler, T., 2016. Ozone damage, detoxificationand the role of isoprenoids new impetus for integrated models. Funct. Plant Biol. 43, 324–326.
Ulrich, E., Dalstein, L., Günthardt-Goerg, M.S., Vollenweider, P., Cecchini, S., Vas, N., Sjöberg, K., Skarman, T., Karlsson, G.P., 2006. Effets de l'ozone sur la végétation, concentrations d'ozone (2000-2002) et symptômes d'ozone sur la végétation forestière (2001-2003). Office National des Forêts, Direction Technique. Département Recherche, p. 126. ISBN 2-84207-300-2.
UNECE, 2010. Mapping Critical Levels for Vegetation. International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops, Bangor, UK.
UNECE ICP Forest Programme Co-ordingating Centre, 2016. Manual on Methods and Criteria for Harmonized Sampling Assessment Monitoring and Analysis of the Effects of Air Pollution on Forests. Thünen Institute of Forest Ecosystems, Eberswalde.
Vollenweider, P., Günthardt-Goerg, M.S., 2006. Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. Environ. Pollut. 137, 455–465.
WHO, 2008. The World Health Report 2008 - Primary Health Care (Now More than Ever).
Wittig, V.E., Ainsworth, E.A., Naidu, S.L., Karnosky, D.F., Long, S.P., 2009. Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: a quantitative meta-analysis. Global Change Biol. 15, 396–424.