Nitrogen Deposition in Different Mediterranean Forest Types along the Eastern Adriatic Coast

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ABSTRACT
Mediterranean forests along the eastern Adriatic coast have an important ecological role. However, few studies have been conducted on nitrogen deposition so far. To improve this knowledge, the main aims of our study were: (i) to estimate nitrogen inputs and determine differences among the four Mediterranean forests, (ii) to determine the seasonal behaviour of N deposition compounds, and (iii) to discuss the results in relation to forest type and precipitation. Measurements were carried out over a two-year period on four plots in two regions: holm oak and pubescent oak in Istria, Aleppo pine and black pine in Dalmatia. Bulk open field and throughfall deposition were sampled with continuously exposed collectors. Measurements, analyses and data validation of precipitation and N compounds were carried out. The results showed that the highest average monthly precipitation was recorded in the black pine plot and the lowest in the Aleppo pine plot. Nitrate and ammonia in conifer plots in throughfall samples were lower than in bulk open field samples, indicating possible retention by the tree canopy. The results revealed a higher amount of N deposition collected in broadleaved forests than in conifer forests indicating the washing out of N compounds previously deposited and accumulated in forest canopy. The chemistry of N deposition was strongly influenced by local and anthropogenic sources as well as neighbouring countries. Our results may fill the knowledge gap in understanding the influence of precipitation and seasonality of N compounds in different Mediterranean forest types along the eastern Adriatic coast.

Keywords: precipitation; nitrogen deposition; nitrate; ammonium; Mediterranean forests; pubescent oak; holm oak; black pine; Aleppo pine

INTRODUCTION
The emission of air pollution in Europe has significantly increased in the course of the twentieth century. Large amounts of nitrogen oxides (NOx) are released by human activities, mainly fossil fuel combustion in industry, power plants, heating systems and transport, while ammonia (NH3) from agriculture and farming. This increase has affected N cycling in ecosystems worldwide (Erisman et al. 2013) and is considered one of the threats to Mediterranean sustainability, along with climate change and ozone (De Marco et al. 2019, Jakovljević et al. 2021).

Nitrogen, as well as other air pollutants, is carried by air masses and rain from regional and long-range transport to the rural areas (Perez et al. 2008, Pey et al. 2009, Aguilarme et al. 2017). Atmospheric deposition and its transformation in contact with vegetation are of great importance in understanding its effects on forests. It has an impact on forest ecosystems through eutrophication by nitrogen and soil acidification, thus altering soil properties and processes (Clark et al. 2007). Changes in the soil chemistry may lead to imbalances in nutrient supply and subsequently to unbalanced nutrition of the trees. Nutrient imbalance will affect canopy photosynthesis and in turn decrease forest vitality (Lu et al. 2008, de Vries et al. 2014). For example, critical loads for nutrient nitrogen and their exceedances were both important for defoliation (De Marco et al. 2014).

It is known that cycling and deposition of N in Mediterranean-type ecosystems are highly seasonal processes conditioned by the Mediterranean climate...
For airborne total nitrate (NO$_3^-$) and ammonium (NH$_4^+$), the spatial coverage is slightly better in the western Mediterranean than in eastern regions (Ochoa-Hueso et al. 2011). However, the potential ecological effects of N deposition in Mediterranean-type climates have been less investigated (Balestrini et al. 2007, Bobbink et al. 2010, De Marco et al. 2014, Ferretti et al. 2014, Aguillaume 2015, García-Gómez et al. 2018, Jakovljević et al. 2019) even though they are usually recognized as hotspots of biodiversity (Myers et al. 2000).

Mediterranean forest ecosystems along the eastern Adriatic coast are of very high significance because of ecological and social value and they are invaluable to the human and ecological functions they provide (Matić et al. 2011, Topić and Butorac 2011, HAZU 2013, Topić et al. 2020). These ecosystems are very sensitive and degraded due to numerous wildfires causing the reduction of the vegetative cover and enlarged flow of tourists during the summer season and the constant increase in human activity (Topić and Butorac 2011, Topić et al. 2020).

Nitrogen compounds in Mediterranean forests are transferred from other rural parts of Croatia or from neighbouring countries (Skevin-Sovic et al. 2012, HAOP 2015). The highest exceedances of NH$_3$ ceilings (25%) in Mediterranean part of Croatia was found in 2017 (EEA 2019) while the exceedance of NO$_x$ was not recorded in 2017 and 2018 (AZO 2018, AZO 2019).

Considering the described peculiarity and fragility of the Mediterranean environment, there is an increasing need to improve the knowledge of Mediterranean forest ecosystems (Jakovljević et al. 2019).

The aims of our research were (i) to estimate nitrogen inputs and identified differences between the forests, (ii) to determine the seasonal behaviour of N deposition compounds, and (iii) to discuss the results in relation to forest type and precipitation.

![Figure 1. Location of the study area: pubescent oak (PO) and holm oak (HO) plots in Istria and Aleppo pine (AP) and black pine (BP) plots in Dalmatia.](image-url)
MATERIALS AND METHODS

Study Area

The measurements were performed in different Mediterranean forest ecosystems along the eastern Adriatic coast (Figure 1) on the most dominant deciduous broadleaves species: pubescent oak (Quercus pubescens Wild.) and evergreen holm oak (Quercus ilex L.), and conifer species: black pine (Pinus nigra J.F. Arnold.) and Aleppo pine (Pinus halepensis Mill.).

According to the Köppen climate classification, the plots are distributed in the hot-summer Mediterranean climate subtype (Csa) (Köpen et al. 2011). Average annual precipitation ranges from 879 mm (AP-Vrana plot) to 1277 mm (BP-Split plot), while the mean air temperature varying from 12.4°C (BP-Split plot) to 13.4°C (PO-Poreč plot). Description of the sampling plots are shown in Table 1.

Sampling and Analysis

Sampling, measurements and analyses on the plots were all carried out according to the ICP Forests (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests) manuals (Clarke et al. 2016, König et al. 2016). The study period for N deposition was from January 2017 to December 2018. Deposition was sampled using continuously exposed, randomly placed collectors, comprising a 1-L graduated polyethylene bottle with a funnel with a 14 cm diameter. Nine collectors were placed beneath the forest canopy to collect throughfall deposition (THR) samples (Figure 2a, b). Bulk open field deposition (BOF) was sampled using three collectors, continuously exposed (Figure 2c, d). Samples were collected biweekly for the whole period from January 2017 to December 2018. The analyses of ion concentrations were performed on filtered samples (0.45 µm). Samples of atmospheric deposition were collected biweekly (Clarke et al. 2016, König et al. 2016). Ion chromatography was used to determine concentrations of two N compounds (NO$_3^-$, NH$_4^+$) (ISO 10304, ISO 14911).

After samples were collected and analysed, some samples were excluded due to contamination or precipitation especially related to precipitation problems included theft, vandalism and instrument failure due to extreme storms.

Statistical analyses were performed using programs Microsoft Office Excel 2010 and Statsoft Statistica 13. Data in the tables are expressed as the mean ± standard deviation (±SD). The annual mean concentrations of the measured N compounds were calculated as volume-weighted means. The monthly deposition fluxes were obtained following the same procedure. The Wilcoxon signed test was used to test whether BOF and THR depositions of N ions were significantly different. A significant difference was considered at the level of p<0.05. Data normality was tested using the Kolmogorov–Smirnov test, where the data was considered normally distributed if the D value was insignificant at p<0.05.

RESULTS AND DISCUSSION

The measurements were conducted on four different Mediterranean forest ecosystems to identify the amount and differences in seasonal precipitation and N deposition.

Total precipitation in the studied period at all plots was generally lower in THR, ranging between 1047 mm in Aleppo pine (AP) to 2193 mm in black pine (BP) (Table 2). The amount of rain collected in BOF for all four plots ranged between 1272 mm in AP to 2578 mm in BP. Average monthly precipitation differed markedly between the studied sites. The highest average monthly precipitation was determined at BP plot (123 mm) and the lowest at AP plot (85 mm) (Table 2).

However, exceptionally in summer on AP plot in July 2018, the value of THR was higher than BOF, 135 and 95 mm, respectively (Figure 3e, f). Several factors can influence the difference in rain volume between BOF and THR, such as maximum precipitation rate, vapour pressure deficit or wind speed (Muzylko et al. 2012, Aguillaume et al. 2017). In our case, the smaller amount of precipitation in BOF could be due to water evaporation from the collector due to high temperatures and wind drying during the summer months.

Furthermore, difference between precipitation in the BOF and THR samples indicated that the water quantity

Table 1. Description of sampling plots in two regions along the Adriatic coast.

| Code | PO | HO | AP | BP |
|------|----|----|----|----|
| Region | Forest type | Pubescent oak | Holm oak | Aleppo pine | Black pine |
| Latitude, N | Istria | Istria | Dalmatia | Dalmatia |
| Longitude, E | 45°14'59'' | 44°51'41'' | 43°53'23'' | 43°41'59'' |
| Elevation (m) | 264 | 3 | 20 | 550 |
| Distance to the sea (km) | 9.78 | 0.50 | 2.90 | 17.19 |
| Nearest town | Poreč | Pula | Zadar/Šibenik | Split |
| Distance to the nearest town (km) | 19 | 10 | 38/36 | 24 |
was intercepted by the canopies (Aguillaume et al. 2017, Avila et al. 2017). The interception of rainwater by the canopy depends on the dominant forest type of the plot. In pubescent oak (PO) plot interception was 8%, in holm oak (HO) plot it was 12% and in pine plots interception was 15% in BP and 18% in AP plot, respectively (Table 2). Similar interception values were found on deciduous plots in Italy where values ranged from 10% to 15%, and for coniferous species ranged from 23% to 24% (Balestrini et al. 2007). On the other hand, in Spanish holm oak forests higher interception values up to 34% were found (Aguillaume 2015).

Average monthly N deposition differed between broadleaved and conifer plots (Table 2). In broadleaves plots, the N deposition of both compounds (N-NH$_4^+$ and N-NO$_3^-$) was higher in THR than in BOF.

Ammonium and N-NO$_3^-$ depositions were higher in HO than in PO plot, 8.22 meq m$^{-2}$ and 6.27 meq m$^{-2}$, and 3.81 meq m$^{-2}$ and 3.21 meq m$^{-2}$ respectively. The same was found for N-NH$_4^+$ for BOF samples (5.29 meq m$^{-2}$ in HO and 3.28 meq m$^{-2}$ in PO). Regarding N-NO$_3^-$, values were slightly lower in HO plot (2.04 meq m$^{-2}$) than in PO plot (2.41 meq m$^{-2}$) (Table 2). On the contrary, on plots with conifer species lower N-NH$_4^+$ and N-NO$_3^-$ were found in THR than in BOF (Table 2). Deposition of N-NH$_4^+$ in THR and BOF were higher in BP plot than in AP plot (3.51 meq m$^{2}$ and 1.43 meq m$^{2}$, 3.99 meq m$^{2}$ and 2.81 meq m$^{2}$, respectively).

The same pattern for deposition of N-NO$_3^-$ in THR and BOF samples between BP and AP plot was found. In THR samples, N-NO$_3^-$ deposition on BP plot was 2.43 meq m$^{-2}$ and on AP plot 1.65 meq m$^{-2}$, and in BOF samples on AP and BP plot 1.96 and 3.05 meq m$^{-2}$, respectively (Table 2). Considering N-NO$_3^-$ deposition in AP plot, it originates from wet air masses coming from the Adriatic Sea, and the fact that most of this land area is suitable for agricultural production also contributes to its concentration. Regarding N-NH$_4^+$, highest N-NH$_4^+$ concentrations were measured on the HO and PO plots in THR. These plots are also influenced by the intense agricultural activities in its surroundings, and this is highlighted by the high amount of ammonium (Jakovljević et al. 2019).

Considering throughfall N deposition, it reflects both wash-off of the dry deposited particles on tree canopies and the exchange with the leaf surfaces (absorption and leaching) (Balestrini et al. 2007, Jakovljević et al. 2013, Ferretti et al. 2014, Avila et al. 2017). Usually, it is expected to be higher as it was evident in the oak plots. However, N-NO$_3^-$ and N-NH$_4^+$ in THR samples were lower than in BOF samples on conifer plots (Table 2), indicating possible retention from the canopy (Jakovljević et al. 2019). Forest canopies can intercept the deposited N before it reaches the forest floor. In general, the effectiveness of particle uptake by trees is increased if their leaf and bark surfaces are rough or sticky (Becket et al. 1998). Using the canopy N budget method, Gaige et al. (2007) found that the canopy of the mature conifer forest retained more than 70% of N deposition. Uptake efficiencies by the canopy in the conifer forest was very high, around 90% for N-NH$_4^+$ and 70–80% for...
N-NO$_3^-$, resulting in smaller THR deposition compared to the wet deposition (Sievering et al. 2007).

The Wilcoxon signed test showed that there was a significant level of differences between the average monthly precipitation in BOF and THR samples on the HO and AP plots (Table 3). There are number of reasons affecting the difference between precipitation, such as extreme weather (e.g., rainstorms, hailstorms, etc.) and human activities (e.g., forest management and forest planting) (Cisneros et al. 2018). Processes of rainfall interception of forest canopy depend on the various properties of the rainfall characteristics; especially the rainfall amount and intensity and drop properties such as the number of drops, their velocity, diameter, and median volume diameter. The vegetation periods and leaf area index values can also influence the spatial variability of THR (Zabret et al. 2018, Zabret and Šraj 2018).

In PO and BP plots there were no significant differences between average monthly precipitation of BOF and THR (Table 3).

Although it was expected that a vegetation period would significantly affect the precipitation amount between BOF and THR in the deciduous forest, this was not the case on our PO plot. Our results in PO plot showed slight differences in rainfall amount between leafed and leafless periods (Figure 3). Similar observations were found by Mužylko et al. (2012).

Furthermore, significant differences between BOF and THR deposition of N compounds can be observed at oak plots. In AP plot significant difference for N compounds was not found, while in the BP plot statistical analyses revealed a significant difference in deposition between BOF and THR for N-NO$_3^-$ (Table 3).

The precipitation volume and monthly deposition of N compounds in BOF and THR in 2017 and 2018 on four selected plots are presented in Figure 3.

In all studied plots precipitation mainly occurred in autumn and winter. The trend could be seen on all plots except on AP plots (Figure 3e, f). This plot also had the smallest amount of precipitation.

It was expected that in the summer months the precipitation would be the lowest, or there would be no precipitation at all that could be observed on PO, AP and BP plots in 2017 (Figure 3a, b, e, f, g, h). These results are not uncommon for the Mediterranean climate that is characterised by seasonal incidence of precipitation (FAO and Plan Bleu 2018). A similar trend was observed in western Mediterranean (Izquieta-Rojano et al. 2016). Moreover, the precipitation amount was identified as an important meteorological factor affecting the amount and annual distribution of N deposition. Changes in the N behaviour of ecosystems are driven by fluctuations of physical drivers (i.e., weather conditions) and biological factors (Shibata et al. 2015).

The results obtained indicated a higher amount of N deposition collected in broadleaved forests than in conifer forests (Figure 3). This fact suggested that the N compounds previously deposited and accumulated in the forest canopy

### Table 2. Total amount of precipitation (V), average monthly precipitation (AV), interception between BOF and THR, average monthly deposition of N compounds (N-NO$_3^-$, N-NH$_4^+$) in bulk open field (BOF) and throughfall (THR) at the experimental plots during the study period (2017 and 2018).

| Plot (Sample type) | Study period | V (mm) | AV (mm) | Interception (%) | N-NH$_4^+$ (meq m$^{-2}$) | N-NO$_3^-$ (meq m$^{-2}$) |
|--------------------|--------------|--------|---------|------------------|----------------------------|----------------------------|
| Pubescent oak (PO) | BOF 2017-2018 | 2013   | 88      | 8                | 3.28±3.95                  | 2.41±2.14                  |
|                    | THR 2017-2018 | 1863   | 81      |                  | 6.27±5.83                  | 3.21±2.24                  |
| Holm oak (HO)      | BOF 2017-2018 | 1969   | 94      | 12               | 5.29±11.6                  | 2.04±1.98                  |
|                    | THR 2017-2018 | 1742   | 83      |                  | 8.22±6.01                  | 3.81±3.31                  |
| Aleppo pine (AP)   | BOF 2017-2018 | 1272   | 85      | 18               | 2.81±2.40                  | 1.96±1.83                  |
|                    | THR 2017-2018 | 1047   | 70      |                  | 1.43±1.07                  | 1.65±1.30                  |
| Black pine (BP)    | BOF 2017-2018 | 2578   | 123     | 15               | 3.99±8.36                  | 3.05±3.19                  |
|                    | THR 2017-2018 | 2193   | 104     |                  | 3.51±6.84                  | 2.43±2.73                  |

### Table 3. Significant level of the differences between average monthly precipitation bulk and throughfall fluxes according to the Wilcoxon signed test (marked as bold as significant for p<0.05).

| Plot (BOF-THR) | V   | N-NH$_4^+$ | N-NO$_3^-$ |
|----------------|-----|------------|------------|
| Pubescent oak (PO) | 0.4654 | 0.0043 | 0.0152 |
| Holm oak (HO) | 0.0021 | 0.0019 | 0.0004 |
| Aleppo pine (AP) | 0.0277 | 0.0546 | 0.3967 |
| Black pine (BP) | 0.0766 | 0.6051 | 0.0276 |
were washed out in subsequent rain events. Although rainfall amount may affect these peaks, it is likely that other factors were involved, since variations in the size of the peaks are not proportional to the precipitation amount.

In the Mediterranean area, N deposition accumulates on plant surfaces during dry periods, becoming available as high N concentration in pulses with rainfall events (Meixner and Fenn 2004, Ochoa-Hueso et al. 2011, Aguillaume et al. 2016). These pulses could be observed in THR on all plots. They could be explained by a major contribution from dry-deposited particles, washed out after the driest period of the year. It was more evident on PO and HO plots where the collected deposition during August 2017 was washed out in September 2017 (Figure 3a-d).

The highest monthly deposition of N compounds can be observed in THR and BOF throughout the year for PO and HO plots located in Istria (Figure 3a-d) unlike BP and AP plots in Dalmatia (Figure 3e-g). Istria is a region located on the border with Slovenia and Italy. It was expected that the plots on the Istrian peninsula (PO and HO) would have higher N depositions influenced also by urbanistically and industrially developed neighbouring countries.

Figure 3. Monthly bulk (BOF) and throughfall (THR) deposition of nitrogenous compounds (kg N ha⁻¹ mn⁻¹) and precipitation volume (mm) at the four monitoring plots: PO–pubescent oak (a, b), HO–holm oak (c, d), AP–Aleppo pine (e, f) and BP–black pine (g, h).
CONCLUSION

The atmospheric inputs of nitrogen compounds in different Mediterranean forest types along the eastern Adriatic coast were estimated. The results showed that throughfall N deposition was higher in oak forests rather than pine forests, indicating possible wash-off from the oak canopies and possible retention from pine canopies. High N concentration amounts in THR samples were present during the summer period as a result of major contribution from dry-deposited particles washed out after the driest period of the year. Our results revealed in pubescent oak forest that slight differences in rainfall amount between leafed and leafless periods were present. Depending on the study site, different anthropogenic activities and possible influence of neighbouring countries were identified as potential sources of N deposition. Further research is desirable to monitor the potential effects of N deposition on the forests of eastern Adriatic coast.

Author Contributions

TJ and LB conceived and designed the research, LB and IL carried out the field measurements, LL and IL processed the data and performed statistical analysis; project administration TJ and LL, TJ funding acquisition, TJ and LL performed laboratory measurements, TJ secured the research funding, TJ, LB supervised the research, LL and IL wrote the manuscript, TJ and LB helped to draft the manuscript.

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Conflicts of Interest:

The authors declare no conflict of interest.

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