

# LIFE MODERN NEC

Air quality, the response of ecosystems

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## **LIFE MODERn (NEC)**

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## **DELIVERABLE**

### **ACTION A.1**

#### **Analysis and evaluation of existing data**

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## Contents

1. Introduction	4
2. Screening the timely and emerging research on the topic	4
<b>2.1 Forest ecosystems</b>	5
2.1.2 Foliar analysis	11
2.1.3 Soil solution	12
2.1.4 Ground vegetation	16
2.1.5 Epiphytic lichens	19
2.1.6 Ozone injury	27
2.1.7 Crown condition	30
2.1.8 Forest growth	41
2.1.9 Plant phenology	45
<b>2.2 Freshwater ecosystems</b>	48
<b>2.3 Impacts of air pollution and climate change on forests</b>	55
2.3.1 The NEC Directive	56
2.3.1 Meteorological data	59
2.3.2 Visibility	62
3. Collection of time series of data	65
4. Data processing and synthesis of the state of the art	66
<b>4.1 Data characterization</b>	66
<b>4.2 Conceptual scheme</b>	70
<b>4.3 Selection of the most suitable data processing procedure</b>	71
<b>4.4 Results of the univariate and multivariate analysis for each indicator</b>	71
<b>4.5 Results of the multiple linear regression models (MLRM)</b>	97
5. Conclusion	106

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### Summary

As a preparatory action of the LIFE MODERn (NEC) project, Action A.1 is dedicated to the collection and evaluation of existing data, to obtain a clear baseline for the project activities, thus providing starting information for Actions B.1 and B.2. Further, the output of this Action will be discussed in the context of the Working Panel (Action B.5) and will be useful for implementing the activities of Action B.3.

For this purpose, i) the timely and emerging research on the topic was collected and critically revised, obtaining almost 600 papers (forests: 535 papers; freshwaters: 44 papers); ii) times series of data coming from the main Italian networks on air pollutants and their effect of the last 20 years (period 2000-2020) were acquired; iii) a data processing, with a multivariate approach was put in place.

The results can be summarised as follows:

- Modelled concentrations and depositions: in these last 20 years a general decreasing trend is evident for sulphur and nitrogen oxides, both in our forest and freshwater sites, while for reduced nitrogen a decreasing trend is less evident. Both concentrations and depositions show a clear correlation with altitude, with the sites at higher altitudes showing the lower air pollution levels.
- By analysing the single indicators, both for forests and for freshwaters, a clear trend in ecosystem responses is not evident, which seem to be more influenced by site-specific features, such as altitude or the proximity to atmospheric emissions.

The predictive models provide an overview that can be summarised as follows:

- Forest ecosystems. Only some response variables belonging to three indicators are influenced by the considered drivers (crown condition, soil solution, and ground vegetation). Damage to branches and  $\text{NO}_3$  concentrations in the soil solution are negatively affected by relative humidity. The herbaceous layer cover is negatively affected by nitrogen depositions and positively influenced by the depositions of  $\text{SO}_4$ , K and alkalinity.
- Freshwater ecosystems. Most of the water chemistry variables are positively influenced by depositions, both nitrogen and sulphur compounds, and by precipitation.

The results of this Action will be extremely important to obtain a baseline for Actions B.1 (selection of the new monitoring sites), B.2 (definition of a new set of indicators) and B.3 (implementation and testing; point 3).

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### 1. Introduction

In the first phase of the project, the collection and evaluation of existing data are of key importance to finetune a suitable overview of the topic. Based on the screening of data series of the last decades in urban, peri-urban and remote areas, the main objective of this action is to identify the trends of atmospheric pollutant emissions and of reactive components (indicators) of forest and freshwater ecosystems.

Specific activities will be devoted to:

1. **Screening the timely and emerging research on the topic.** To explore recent studies documenting the current trends and changes in air pollution and their potential effects on forest and freshwater indicators.
2. **Collection of time-series of existing data** coming from the main Italian networks on air pollution and its effects.
3. **Data processing and synthesis of the state of the art.** A comprehensive data screening will be carried out to find the main relationships among air pollution drivers and ecosystems' responses.

The activities of Action A.1 are essential to obtain an overall framework on the relationships between emissions and their impacts on ecosystems. Currently, a solid network of monitoring sites in urban and peri-urban areas is well structured, providing a clear picture of air pollutants emissions. However, the collection of data from remote-rural areas and the study of the impacts of air pollution on ecosystems is still missing and lacking integration of results. Action A.1 will also document the shift of the CLRTAP ICP programs from the topic of acidification to the major current concern about eutrophication and ozone impacts on ecosystems, thus supporting Action A.2 in the revision of protocols. Action A.1 will also study how the LIFE MODERn (NEC) will contribute to the enrichment and evolution of the NEC template for data reporting.

### 2. Screening the timely and emerging research on the topic

The aim of this activity is to collect and critically assess the scientific papers and technical publications about air pollution and its impacts on forest and freshwater ecosystems. Moreover, recent reports by ISPRA, EEA and EC concerning emissions and air quality in Europe will be taken into account. It is important to underline that most of the project partnership is participating in international peer-review publications regarding the mentioned research topic.

A total of 579 papers has been collected and examined, focusing on the main aspects of forest (535 papers) and freshwater (44 papers) monitoring (Figure 2.1).

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1

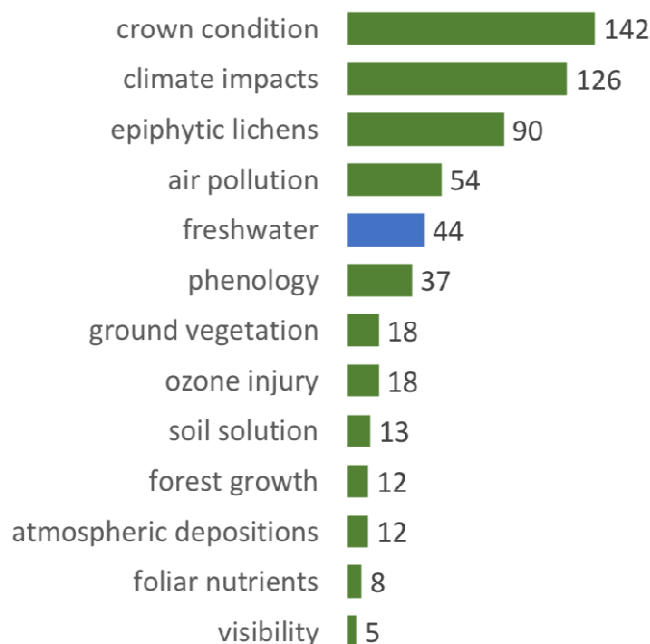


Figure 2.1 – Number of papers obtained by the screening for each aspect of forest (green bars) and freshwater (blue bar) ecosystems.

## 2.1 Forest ecosystems

### 2.1.1 Atmospheric depositions

#### Introduction

The atmospheric deposition of pollutants and their derivatives is a major concern for freshwater and forest ecosystems. In fact, human activity altered the nitrogen (N) cycle and presently dominates the creation of reactive N in Europe, America and Asia (Canfield et al., 2010). Total atmospheric emissions of reactive N in the forms of  $\text{NO}_x$  and  $\text{NH}_3$  increased from 23  $\text{Tg yr}^{-1}$  in 1860–1893 to 93 in the early 1990s and is expected to reach 189  $\text{Tg yr}^{-1}$  in 2050 (Galloway et al., 2004), with a consequent increase of N concentration in atmospheric precipitation (Brimblecombe & Stedmann, 1982), and a resulting increase of deposition of reactive N (Denman et al., 2007).

High N deposition on forests may affect tree growth and health, vegetation, soil, biota, soil, soil water and run-off (McNulty et al., 2005; Lu et al., 2009; Janssens et al., 2010; Bleeker et al., 2011). Furthermore, N availability can stimulate growth and enhance carbon uptake (e.g., Mac Donald et al., 2011, Erisman et al., 2011), by accelerated photosynthesis (e.g., Fleischer et al., 2013), the decreased C allocation to roots and increased wood formation (“allocation shift”), and decreased decomposition, leading to accumulation of surface litter and soil organic matter (Janssens & Luysaert, 2009; Janssens et al., 2010).

Many temperate forests are N-limited (Oren et al., 2001), and any input of additional N causes enhanced tree growth. This growth stimulation, however, may be not supported by other nutrients (e.g., Emmett, 1999) and/or can be counteracted by possible detrimental effects on tree health due to increased

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



sensitivity to pest/pathogens (e.g., Roelofs et al., 1985; Braun et al., 1999) and to extreme weather conditions, which may in turn have negative effects on growth (e.g., Dobbertin, 2005).

In Italy, N depositions range from 7 to 24 kg N ha<sup>-1</sup> yr<sup>-1</sup>; Marchetto et al. (2008) and Ferretti et al. (2014) found that N deposition affected both soil and tree nutrition: topsoil exchangeable base cations (BCE) and pH decreased with increasing N deposition, and foliar nutrient N ratios (especially N : P and N : K) increased. Comparison between deposition in the open field and below tree canopy (throughfall) suggested possible canopy uptake of N, levelling out for bulk deposition >4–6 kg ha<sup>-1</sup> yr<sup>-1</sup>. They also found that N depositions stimulate a positive growth response in all sites, in spite of signals of possible recent N saturation. This may suggest a time lag for detrimental N effects, but also that, under continuous high N input, the reported positive growth response may be not sustainable in the long-term.

In Italy, atmospheric deposition has been sampled since 1997 in 6 to 22 forest areas, depending on the available budget, following the ICP Forests protocol and data quality control procedures. The highest deposition loads were recorded in the Po Plain, where most of Italian industry and agriculture is concentrated, and in the surrounding foothills and mountains, such as the pre-alpine area in North-Western Italy (Rogora et al. 2006; Cecchini et al., 2021). Trend analysis shows a widespread decrease in the acidity of precipitation in the last 1990s and 2000s, as a consequence of the reduced emission of S compounds (Rogora et al. 2006). On the other hand, nitrate and ammonium depositions did not decrease until recently (Rogora et al. 2016).

Even if deposition sampling and analyses seems relatively simple, when applied in forest sites a number of problems arise, which still require the improvement of the protocol used. The first problem is to assure reliable sampling, considering that concentration values in rain, and particularly in snow, are very low, and N compounds, such as nitrate and ammonium, can be affected by microbial metabolism and evaporation. The low concentration also requires special attention in the laboratory and strict procedure for quality assurance and control.

In forest sites, atmospheric deposition is measured both in the open field and in the plot, below the tree canopy. The former measurement is used to estimate wet deposition, i.e., ionic content of rain and snow. The latter is intended to also include dry deposition collected on tree leaves and washed out by rain and snow. Comparing modelled and measured sulphur deposition (e.g., Marchetto et al., 2021) shows that this protocol works well for sulphur deposition. However, nitrogen deposition strongly interacts with tree canopy, so that for low N deposition levels throughfall (under canopy) deposition is frequently lower than open field deposition, because of foliar uptake. Attempts to evaluate canopy uptake were discussed by several authors (e.g., Draaijers & Erisman, 1995; Staelens et al., 2008; Talkner et al., 2010), but this topic is still in development and most of the studies deals with forests of the temperate regions, while Mediterranean species are relatively neglected.

### Recent literature review

In this chapter, we discuss recent development in deposition monitoring focusing on recent papers concerning deposition monitoring in forest sites published in the last 5 years. Literature review of the effects of nitrogen deposition on forests will be included in a further chapter.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### I. Measurement and trend analyses

Lovreskov (2021) reports deposition measurements along the eastern Adriatic coast, an area relatively close to Italy; they found that the amount of nitrogen deposition was strongly influenced by local anthropogenic sources as well as transport from neighbouring countries.

Vuorenmaa et al. (2018) estimated deposition trends in the International Long-Term Ecological Research Network (ILTER), which includes the Italian forest and freshwater sites among hundreds of other long-term research/monitoring sites. In most sites, they found a significant decrease in sulphate, nitrate and ammonium deposition.

Chung-Te Chang (2022) found similar results in Europe and North America, based on the EMEP and NADP networks, respectively. However, they could not find significant decreases in ion deposition in South Eastern Asia.

Karlsson et al. (2021) also found decreasing N deposition in Sweden, together with a strong deposition gradient from the southern sites, closer to N emission sources, to the remote northern and eastern sites. They developed a novel sampling method for the indirect measurement of total N deposition in forest sites.

Marchetto et al. (2021) found a good agreement (bias <25%) between modelled and measured deposition in Europe, (based on the EMEP/MSC-W model and on both ICP-Forests and EMEP deposition networks) for sulphate and nitrate open field deposition, while larger differences are more evident for ammonium deposition, likely due to the greater influence of local ammonia sources. Modelled sulphur total deposition compares well with throughfall deposition measured in forest plots, while the estimate of nitrogen deposition is strongly affected by the tree canopy. However, marked differences were found in some sites, underlying the importance of site-specific measurement.

Similar results were also found by Schwede et al. (2018) who used a chemical transport model to provide estimates of atmospheric N inputs to forest ecosystems around the globe. Highest total N deposition occurs in eastern and southern China, Japan, Eastern U.S. and Europe while the highest dry deposition occurs in tropical forests. They found that differences between the grid-average and forest specific could be as much as a factor of two and up to more than a factor of five in extreme cases. This suggests that consideration should be given to using forest-specific deposition for input to ecosystem assessments such as critical load determinations.

Forsius et al. (2021) compared measured deposition with the critical loads, i.e., deposition thresholds used to describe the sensitivity of ecosystems to atmospheric deposition. They computed the critical loads for eutrophication and acidification for 17 forested ecosystems in northern and central Europe using long-term measurements (1990–2017) for open field deposition, throughfall and runoff water chemistry. They found stronger reductions of S deposition as compared to N and a relation between calculated exceedance of the critical load and measured runoff water concentrations and fluxes.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### II. Canopy effect on throughfall deposition

Several attempts were made to model and estimate the “canopy effect”.

Ahrend et al. (2020) compare different modelling approaches developed to estimate total N deposition to forests. Three common methods are the (i) “canopy budget model,” (ii) “inferential method,” and (iii) “emission-based estimates” using a chemical transport model, which are reported to show considerable and site-specific differences. In this paper they used data from more than 100 German intensive forest monitoring sites over a period of 16 years for a cross-comparison of these approaches. Even if wet deposition estimates of the emission-based approach were in good agreement with wet-only corrected bulk open field deposition measurements used by the other two approaches. larger discrepancies were observed when dry deposition estimates are compared between the emissions-based approach and the other two approaches, which appear to be related to a combination of meteorological conditions and tree species effects.

On the contrary, Thimonier et al. (2019) found good agreement among three different approaches for determining the wet and dry deposition of nitrogen at 17 forest sites in Switzerland, namely (1) measurements of bulk deposition and throughfall, (2) measurements of bulk deposition and measurements of air concentrations of ammonia (NH<sub>3</sub>) and nitrogen dioxide (NO<sub>2</sub>), to which deposition velocities were applied, and (3) an emission-based model with high spatial resolution. The three approaches generally yielded comparable estimates of total deposition, with some notable differences at some sites. The minimum N deposition estimated from throughfall exceeds the lower limit of critical load at all sites except those in the Central Alps, while deposition estimated with the methods (2) and (3) exceeds the lower limit of critical load at all sites and even exceeds the upper limit at several locations.

Avila et al. (2021) tried to assess canopy effects on some Mediterranean tree species, measured N deposition and gas N concentrations at four Spanish holm oak (*Quercus ilex*) forests. They found that tree canopies retained 20-60% of N compounds, with larger retention in remote rural sites than in sites close to a large urban area.

To improve the evaluation of the canopy effect, Garcia-Gomez et al. (2018) developed an innovative approach, combining the empirical inferential method or surface N deposition with stomatal uptake of N gas from the DO3SE (Deposition of Ozone and Stomatal Exchange) model. The estimated total deposition varied among the sites and matched the geographical patterns. On average, dry N deposition represented 77% of total N deposition of N, including dry deposition of gaseous and particulate atmospheric N (58%), stomatal deposition (19%).

Finally, Guerrieri et al. (2019) evaluated the importance of microbial activity nitrogen cycling in forests, and in particular in Mediterranean holm oak forests, using microbial community analysis, functional gene amplification and stable isotope analysis. They found that during the transition from hot dry summer to cool wet winter throughfall nitrate deposition was higher than in the open field, deriving from both dry depositions, but nitrification by epiphytic leaf microbes was an important contributor (20%) during dry summer. They also put in evidence different microbial communities between tree canopies and throughfall samples on one hand and open field samples on the other hand.

# LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



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LIFE20 GIE/IT/000091

Delivarable, action A1



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LIFE20 GIE/IT/000091

Delivarable, action A1



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### 2.1.2 Foliar analysis

#### Introduction

The aims of forest condition assessments are the monitoring of the health state of forests, and the detection of time trends and spatial patterns. Insight in the causes of changes can only be achieved if additional parameters from other ecosystem compartments are available. The nutritional state of trees is often indicative of processes at the ecosystem level. Inadequate nutrient supply may be a direct cause of low tree vitality or a factor which increases adverse air pollution effects. High concentrations of certain elements in the leaf or needle tissues may be the effect of intoxication or of high air-pollution levels. Unfavourable chemical conditions in the rooting zone of the soil may also lead to imbalances in the nutrient supply and subsequently to unbalanced nutrition of the trees. Thus, sampling and analysis of needles and leaves are essential. The analyses have to be performed at regular time intervals in order to establish potential relationships between changes in the stand condition and changes of the nutritional status. Sampling must be frequent enough to detect trends in the mineral nutrition of trees, not biased by interannual fluctuations in element concentrations.

For the sampling and analysis of leaves and needles is important to perform harmonized and standard procedures, harmonisation is necessary to permit trans-national studies on the spatiotemporal trends of nutritional status and the impact of air-pollutants of forest trees.

Parameters measured within the foliage survey in LIFE MODERn (NEC) are N, S, P, Ca, Mg, K and C. Investigating these elements and the ratio of some of them enables to estimate the nutritional status of trees and the impact of air pollutants at the monitoring sites, the detection of time trends and spatial patterns and to contribute to the understanding and quantification of forest condition.

The main objectives of foliar analysis in LIFE MODERn (NEC) are i) quantification of the mean element concentrations (N, S, P, Ca, Mg, K and C) and ii) detection of temporal trends of mean element concentrations of nutrients with a documented statistical precision.

#### Methodological approach

Sample trees are randomly selected in the plots to represent the population of trees and the same sample trees should be sampled over the years; the trees in the plots are all numbered. Five trees are sampled from the main species within a stand. The samples are individually preserved in bags for analysis. Sampling is performed biannually, the sampling are performed in uneven years (2021, 2023, 2025 etc.). For deciduous species (including larch), sampling is performed during the second half of the growing season and before the very beginning of the autumnal yellowing and senescence. For evergreen species, sampling must be performed during the dormancy period. As trees must not be felled, the trees will be sampled by a tree climber (no crampons).

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



The national laboratories have to participate annually in the needle/leaf inter-laboratory test organized by Forest Foliar Coordinating Centre of ICP Forests. The laboratory results are considered of sufficient quality when the laboratory received a qualification for the concerning parameter(s) after participation in the Interlaboratory Comparisons

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### 2.1.3 Soil solution

#### Introduction

High nitrate ( $NO_3^-$ ) and sulphate ( $SO_4^{2-}$ ) concentrations in atmospheric deposition can be related to water and soil acidification (Ronse et al., 1988; Norton et al., 2004; De Schrijver et al., 2006; Oulehle et al., 2010). Long-term excesses of N and S compound deposition cause unfavourable conditions in forest soils (Verstraeten et al., 2012). Despite the positive effect of N deposition, as a nutrient, on forest growth rates, a potential negative effect at sites with high N deposition has been recognized at continental scale in Europe (Etzold et al., 2020). Further, high nitrate concentrations in surface and ground waters can lead to eutrophication (Aber et al., 1998; Fenn et al., 1998; Rabalais, 2002) and potential health problems (Briand et al., 2017). Critical limits for N concentrations in soil solution were frequently exceeded between the early 1990s and 2006 in Europe (Iost et al., 2012).

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Delivarable, action A1



### Evidences in Europe and in Italy

European scale studies on time trends of soil solution DOC (Camino-Serrano et al., 2016) and pH (Johnson et al., 2018) outlined several investigation limits. The high complexity of the factors involved in determining DOC concentration, most of which depend on internal dynamics of the forest-soil system, prevented any extrapolation of the pollution impact. The size, complexity and encompassed variability of the network prevented the extraction of common signals of soil solution eutrophication and acidification, suggesting that no such thing as a common signal exists across so large a variability. These results clearly suggest the utility of similar investigations across narrower fields. Schmitz et al. (2019), reviewing studies on the responses to N deposition loads, observed varied results at national and regional scale. For example, in the Netherlands and Flanders soil solution  $NO_3^-$  declined in response to decreasing N deposition (Boxman et al., 2008; Verstraeten et al., 2012). In contrast, an intensive study in Germany found  $NO_3^-$  leaching constant from a spruce (*Picea abies*) stand and increasing at a beech (*Fagus sylvatica*) stand, despite decreasing N deposition between 1973 and 2013, indicating a reduction of the N retention capacity of the soil over time (Meesenburg et al., 2016). Other studies found no trends in  $NO_3^-$  soil solution concentrations during periods of stable N deposition (e.g. Alewell et al., 2000 - Germany; Johnson et al., 2013 - Ireland; Pannatier et al., 2010 - Switzerland; Vanguelova et al., 2010 - UK). At a heavily acidified forest in the Czech Republic,  $NO_3^-$  concentrations in soil solution declined despite no decrease in N deposition (Oulehle et al., 2011).

In Italy, inorganic N in soil solutions showed no significant trends in response to decreasing nitrogen deposition in most of the sites. The decreasing trends of  $NO_3^-$  deposition seem too slight to act on mineral N in soil solution. The existence of actual nitrate leaching out of the soil were established for several sites of the CONECOFOR network, pointing out the fundamental, and hitherto underestimated, weight of nitrogen deposition, especially ammonium deposition, on soil pH changes in time (Cecchini et al., 2019, 2021). Although complete retention of the ammonium form was observed,  $NH_4^+$  further contributes to acidification through nitrification, a central soil N process that also regulates hydrologic N losses (Zhang et al., 2016). Soil solution results for Italian forests demonstrate high relevance for the aims of the NEC network and clearly suggest the opportunity of studying actual water fluxes through the soil, in order to quantify the actual N export from forest soils.

Although recovery from acidification, under decreasing acidifying deposition, is not uniform across European countries, soil acidification has been widely found to be an ongoing process (Petrash et al., 2019; Achilles et al., 2021), while simultaneous decreases in BCE deposition appear to inhibit soil recovery (Likens et al., 1996; Alewell et al., 2000; Folster et al., 2003; Vanguelova et al., 2010; Meeseburg et al., 2016); this phenomenon is being thoroughly assessed by an European-wide study (Johnson et al., 2018). BCE deposition can also be due to industrial activities, such as mining (Watmough et al., 2014; Davidson et al., 2020) or quarrying (Oulehle et al., 2006) and thus the general decrease of “heavy” industrial activities in the developed world is a likely cause of BCE deposition decrease.

Italy, due to its geographical position, receives large amounts of BCE deposition from marine aerosol and Saharan dust (Lequy et al., 2012; Costantini et al., 2018; Cecchini et al., 2019) and is subjected to a component of atmospheric deposition which is lesser or absent in northern European countries. The most represented chemical species in this Mediterranean pattern of deposition are base cations and chloride, for which a significant gradient of decrease from south to north is observed (Ehrmann et al., 2017; Cecchini et

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LIFE20 GIE/IT/000091

Delivarable, action A1



al, 2019). The acidification of forest soils appears to be prevented in south-central Italy and markedly mitigated in northern Italy by the present regime of BCE atmospheric deposition, in synergy, in many areas of Italy, with evapotranspiration in excess over precipitation, a condition that reduces water exceedance and flow throughout the soil.

A most evident and generalised trend, in Italy, was the concomitant decrease of  $SO_4^{2-}$  from atmospheric deposition and in soil solution, confirming the effectiveness of the emission abatement policies concerning S compounds. This result agrees with observations at the European scale (Johnson et al., 2018) and from individual forest plots (i.e. Karlsson et al., 2011; Vanguelova et al., 2010; Verstraeten et al., 2012; Waldner et al., 2014).

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# LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Zhang, J., P. Tian, J. Tang, L. Yuan, Y. Ke, Z. Cai, B. Zhu, and C. Müller (2016), The characteristics of soil N transformations regulate the composition of hydrologic N export from terrestrial ecosystem, *J. Geophys. Res. Biogeosci.*, 121, 1409–1419, doi:10.1002/2016JG003398.

## 2.1.4 Ground vegetation

### Introduction

European forests cover more than 40% of the continental land surface (European Environment Agency, 2016) and provide essential ecosystem services, such as carbon sequestration, recreation, biodiversity conservation, timber production. For millennia humans have exploited European forests to satisfy the increasing demands of a more complex society (Warde, 2006). Still, the rising pressure on ecosystems due to global changes (i.e., climate change, land use change, pollution; Ammer et al., 2018) has alarmed scientists and policymakers, leading to the elaboration of several national and international policies and regulations (e.g., NEC Directive; Sustainable forest management principles (SFM); Strategia Forestale Nazionale, Decree 23/12/2021). Despite the principle of diversity maintenance is often cited in these policies (e.g., see the criterion n. 4 of the SFM), many diversity components are still not adequately considered (e.g., vascular plants of the understorey, bryophytes, lichens, fungi, etc.; see Burrascano et al., 2021). Here we focus on the understorey vegetation.

One of the most important components of forest biodiversity is represented by the understorey plants. Despite representing less than 1% of forest biomass, the understorey accounts for more than 80% of plant diversity in temperate forests and significantly contributes to ecosystem functions (Blondeel et al. 2021), including the capacity to influence tree regeneration and affect litter quality (Landuyt et al. 2019).

Therefore, understanding the effect of environmental factors on the vascular plants of the understorey is fundamental (Naqinezhad et al. 2022).

Forest understoreies are claimed to be particularly sensitive to changes in macro- and microenvironmental changes generated by climatic, soil, forest structure and management drivers. These drivers can act directly (i.e., through forestry roads, harvesting machinery or charcoal kiln platforms; Cervellini et al., 2017) or indirectly, by modifying environmental conditions of the ground layer (e.g., light, temperature, soil moisture and nutrient status; Decocq et al., 2004; Cervellini et al., 2017; Douda et al., 2017). Despite this, the understorey layer has been rarely considered in monitoring activities and management decisions across Europe and is often neglected in regional and local planning (Blondeel et al., 2021; Cutini et al., 2021; Chelli et al., 2021).

Here, we report the results of a screening on the timely and emerging research on the topic to explore recent studies documenting the current trends and changes in air pollution and their potential effects on the understorey vegetation. We have mainly addressed reviews and articles dealing with the effects of atmospheric pollution and climate change on plant community composition and functioning (i.e., by using plant functional traits or functional groups) in forest ecosystems.

We mainly focused on recent (2014-2022) papers and review articles in order to provide an updated picture of the existing scientific knowledge, and then selected the most informative ones for a deeper evaluation potentially relevant to the NEC Directive and to the LIFE MODERn(NEC) project.

## LIFE MODERN (NEC)

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### Results and discussion

We selected a total of 18 timely and emerging research papers on the topic, ranging from 2014 to 2022 (see Table in Annex). They deal with several aspects of plant understorey monitoring, including experimental (e.g., N addition) and observational approaches, as well as different spatio-temporal scales. Additionally, several interactive factors were considered (e.g., climate variability, forest management), especially in long-term observational studies where the decoupling of the different understorey drivers is not an easy task. At broad scales, climate variables were found to be the main drivers of understorey (e.g., Temperature-related factors; Chelli et al., 2019a,b). However, several studies highlighted the need to integrate at least soil properties as potentially important drivers of trait variation in broad scale studies of forest systems (Ewald & Ziche, 2017). Indeed, climate-soil interactions showed a certain importance in modulating understorey functioning, including traits and functions related to both the above- and belowground plant compartments (Chelli et al., 2019a,b). In detail, soil N and P availability were the edaphic explanatory variables most correlated to trait variation at community level.

Observational approaches at broad scale (North-South gradients in Europe) based on monitoring sites found a general pattern of gradual replacement of oligotrophic species by eutrophic species as a response to N deposition (Dirnbock et al., 2014). However, despite the future projections of N deposition decrease, the oligotrophic species are expected to further decrease according to models (Dirnbock et al., 2018). This is probably due to interactions with climate change.

When focusing on studies at stand level including local factors the results are more controversial. On the one hand, Bernhardt-Romermann et al. (2015) found a considerable among-site variation partly explained by temporal changes in light availability and density of herbivores, while baseline levels of N deposition (and not annual variations) determined subsequent diversity changes. This finding was confirmed by long-term local experiments which manipulated temperature, light and N input in temperate broadleaved forest understorey. In fact, these studies demonstrated that warming and increased light availability were the main drivers of plant community changes both in terms of species composition and traits (Govaert et al. 2021,a,b). In particular, generalists species, such as *Rubus fruticosus*, benefitted from the warming and light treatments and outcompeted forest specialists. This might ultimately led to biotic homogenization (Govaert et al., 2021b). On the contrary, N input was less important (Govaert et al., 2021b; Roth et al. 2021a).

On the other hand, other studies found that altered nutrient supply (due to atmospheric N deposition), together with changes in light regimes and management-related disturbance, were the main drivers of vegetation changes (Forster et al., 2017). Regarding the direction of changes, Simkin et al. (2016) found that negative relationships between N deposition and species richness are common, albeit not universal, and that local processes can moderate vegetation responses to N deposition

The different responses above described suggest a context-dependent sensitivity of understorey vegetation to atmospheric deposition. The high number of factors affecting the vegetation response to deposition (e.g., forest type, baseline soil conditions, landscape conditions, historical and current management, etc.) and their potentially complex interactions complicate our efforts to make predictions (Simkin et al., 2016; Perring et al., 2018a,b; Roth et al. 2021a,b; Kirby et al., 2022). In addition, other two important aspects must be considered; first, the potentially different response provided by different indicators (e.g., species composition and abundance, species richness, plant functional traits, productivity; Kirby et al., 2022);

# LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



second, the combined effect of different types of deposition (e.g., nitrogen and phosphorus; Hedwall et al., 2017).

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### 2.1.5 Epiphytic lichens

#### Introduction

Due to their physiology, lichens are sensitive to a series of environmental parameters (namely light, air humidity, UV-B radiation, temperature, and airborne chemicals such as SO<sub>2</sub> and NO<sub>x</sub>) making them useful indicators for air pollution and climate change (see for a review Giordani and Brunialti 2015; Abas 2021). Biomonitoring methods based on the diversity and on the accumulation capacity of epiphytic lichens are among the most used worldwide (Garty 2001; Nimis et al. 2002). Several aspects of lichen diversity (e.g., species richness and abundance, species composition, indicator species, functional traits and groups) are usually considered, each of them for a particular reason (Nimis et al. 2002).

At the same time, lichens are among the most widely used organisms for the biomonitoring of airborne trace elements, due to their resistance to heavy metals and to their metabolism strictly dependent on atmospheric exchanges. They receive nutrients directly from the atmosphere and lack roots, waxy cuticle and stomata and they are able to accumulate trace elements to very high levels, far above their physiological requirements (for reviews see Richardson 1992, Bargagli 1998, Garty 2001, Wolterbeek 2002).

Although most of the studies addressing atmospheric pollution have been carried out within urban and industrial areas (see Conti and Cecchetti 2001; Abas 2021), there are also numerous examples of scientific

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



studies and monitoring programs that use lichens as indicators of air pollution in the context of forest ecosystems (Ellis 2012). In simplified terms, it is possible to identify three main purposes to perform lichen monitoring (see Giordani and Brunialti for a detailed description): i) air quality monitoring (both lichen diversity and bioaccumulation methods; monitoring the effects of sulphur and nitrogen oxides, and the accumulation of PAH, and major and trace elements); ii) sustainable forestry (lichen diversity and indicator species; effects of forest structure and dynamics, forest continuity, etc.) and iii) ecosystem functioning (lichen diversity and biomass, indicator species; forest-nutrient cycling and food webs, forest water balance, etc.).

In this document, we report the results of a screening on the timely and emerging research on the topic to explore recent studies documenting the current trends and changes in air pollution and their potential effects on lichens as indicators. We have mainly addressed reviews and articles dealing with the effects of atmospheric pollution and climate change on lichen communities and on mapping trace elements in forest ecosystems. In addition, to obtain a wide picture of forest variables and drivers affecting lichens (e.g., linked directly or indirectly to forest structure and continuity), we also took into account papers dealing with these aspects and not directly addressing atmospheric pollutants deposition. In fact, the research articles focusing on lichen diversity and forest management are very important to identify any disturbing factor with respect to biological responses due to the effect of pollution, and must be carefully considered in the interpretative phase of monitoring programmes.

Finally, we try to make a critical analysis of this topic in order to understand the state of research and applications in this field. The aim is to take stock of the situation at present and to give a boost to the scientific community to improve the method at national and international level.

### Methodological approach

In this work, we focused on the articles dealing with lichen monitoring in forest ecosystems, mainly addressing the following aspects:

- the effects of air pollution and climate change on species assemblages and sensitive species;
- the bioaccumulation and mapping of trace elements;
- the environmental variables (both structural and climatic) affecting lichen diversity (both species richness and abundance and functional diversity) in relation to different forest management intensities.

A database of the main works carried out in the last 30 years (from 1991 to 2021) was prepared, based on a bibliographic research on the major search engines in the field of scientific publications: [www.sciencedirect.com](http://www.sciencedirect.com); [www.tandfonline.com](http://www.tandfonline.com); [www.mdpi.com](http://www.mdpi.com) (query: lichen AND monitoring AND forest AND pollution). This first 'rough' and 'uncorrected' screening was used as a starting point to have a wide idea of the amount of literature on the subject (more than 2,000 results). The resulting list was then drastically cleaned up, by excluding local, not-pertinent, not-original papers and including also those major review and research articles that can be considered relevant to the NEC Directive and to the LIFE Modern(NEC) project.

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

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### Results and discussion

We selected a total of 90 timely and emerging research papers on the topic, ranging from 1992 to 2021 (see Table in Annex). They deal with several aspects of lichen monitoring in forest ecosystems, from bioaccumulation of several air pollutants (trace elements and heavy metals, PAH, nitrogen), to the assessment of lichen diversity and/or functional diversity in relation to both air pollution (mainly nitrogen and sulphur oxides) and forest management.

The list includes fifteen review articles that can represent a useful baseline to explore lichen biomonitoring studies, including: i) the bioaccumulation of trace elements (see e.g., Conti and Cecchetti 2001; Bargagli 2016; Abas 2021) and persistent organic pollutants (see e.g., Augusto et al. 2013; Van der Wat and Forbes 2015), ii) the effects of atmospheric pollution and climate change on lichen diversity and indicator species (Carter et al. 2017; Ochoa-Huseo et al. 2017; Ellis 2019; Nascimbene 2019); iii) the main drivers of forest management affecting lichen communities and their functional diversity (Gao et al. 2015; Łubek et al. 2021; Oettel and Lapin 2021; Ellis et al. 2021); iv) the procedures and methodology for sampling and interpreting lichen diversity data for biomonitoring purposes (Giordani and Brunialti 2015).

By analysing the remaining 75 research articles, 29% (17 papers) deal with **bioaccumulation of trace elements or PAH** by means of epiphytic foliose and/or fruticose lichens in forest ecosystems. Among the foliose species, the most represented biomonitors adopted in these studies belong to the family Parmeliaceae, such as *Flavoparmelia caperata*, *Parmotrema arnoldii* and *Hypogymnia physodes* (see e.g., Loppi et al. 1998; Loppi and Pirintsos 2003; Jeran et al. 2007, Manninen 2018; Kłos et al. 2018; Benitez et al. 2019; Ancora et al. 2021), while *Pseudevernia furfuracea* and *Usnea* are the mostly used fruticose ones (see e.g., Otnyukova T. 2007; Conti et al. 2009; Cecconi et al. 2018). These studies considered from 4 to 26 elements, with Cd, Cu, Hg, Mn, Ni, Pb and Zn being the most commonly analysed.

Twenty-seven articles out of 75 (36%) do not address directly air pollution, but they explore several aspects of **forest management affecting biodiversity patterns** in lichen communities, group of species (e.g., functional traits) or single sensitive species (e.g., *Lobaria pulmonaria*). In particular, several studies focus on the effect of sustainable forest management (see e.g., Brunialti et al. 2020; Cutini et al. 2021; Frati et al. 2021) and forest structure and variability (see e.g., Giordani et al. 2001; Giordani 2006; Moning et al. 2009; Brunialti et al. 2009, 2010, 2012, 2013, 2015a; Svoboda et al. 2010; Ellis and Coppins 2010; Li et al. 2013) on lichen diversity. Other articles deal with the presence, abundance or vitality of indicator species, such as the foliose lichen *Lobaria pulmonaria*, in relation to forest continuity (Nascimbene et al. 2010, 2013; Brunialti et al. 2015b; Bianchi et al. 2020). The remaining studies represent the timely and emerging research on the role of lichen functional diversity within forest ecosystems: functional traits and groups (growth forms, reproductive strategies, photobiont) are adopted as tools to study the patterns of environmental drivers and climate change (see e.g., Marini et al. 2011; Pinho et al. 2012a; Benítez et al. 2018; Aragón et al. 2019; Hurtado et al. 2020a, 2020b; Łubek et al. 2021; Brunialti et al. 2021; Boch et al. 2021).

Although these papers could seem not directly linked with the topic of LIFE Modern(NEC), their results can be extremely useful to discern the effects on lichen diversity due to air pollution from those due to forest structure and continuity, because these studies focus on direct and indirect effects of the main forest variables linked to forest management intensity. Above all, the approach based on functional diversity of epiphytic lichen communities may result useful in the development of new indicators in the context of

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Action B2. At the same time, several aspects on variables affecting lichen functional diversity and indicator species can be useful for data processing and for the development of interpretative tools in Action B4.

The remaining thirty-one papers out of 75 (41%) focus directly on **atmospheric pollution** by exploring several aspects of lichen diversity: lichen species richness, the assessment of lichen diversity indexes based on lichen presence and abundance, such as the Lichen Diversity Index (LDV) and the Index of Atmospheric Purity (IAP).

Most of the papers (19) adopt LDV or IAP to monitor the effects of nitrogen and sulphur oxides in forest sites in Europe (Svoboda 2007; Giordani 2007; Poličnik et al. 2008; Cristofolini et al. 2008; Mayer et al. 2009; Giordani et al. 2012, 2014; Agnan et al. 2017; Brunialti et al. 2020), Northern America (Geiser et al. 2010; Jovan et al. 2012; Gibson et al. 2013; Mc Mullin 2017; Geiser et al. 2019) and Southern America (Correa-Ochoa et al. 2020). The list includes also four **field manuals**, namely the European guidelines for mapping lichen diversity by means of the Lichen Diversity Value (LDV; Asta et al. 2001), the Finnish lichen diversity method (Kinnunen et al. 2003), the European standard (EN 16413 2014) and the ICP Forests field manual for the assessment of epiphytic lichen diversity (Stofer et al. 2016). This latter method is currently used within the Italian NEC Network (Papitto et al. 2019; Brunialti et al. 2020) and in the context of Action B4 of LIFE Modern(NEC).

The remaining 12 research articles provide interesting biomonitoring results on the relationships of lichen communities composition and modelled or measured values of nitrogen and sulphur deposition, in the context of national monitoring networks carried out respectively in several European countries (Estonia: Marmor et al. 2010, Degtjarenko et al. 2018; UK: Gadsdon et al. 2010; Portugal: Pinho et al. 2012b; Morillas et al. 2021; Finland: Mayer et al. 2013; Italy: Papitto et al. 2019) and in the USA (Jovan and McCune 2005; Geiser and Neitlich 2007; Geiser et al. 2014, 2021; McDonough et al. 2015).

Some of these latter articles approach the monitoring of functional traits of epiphytic lichens, such as the abundance and/or frequency of functional diversity (growth forms, photosynthetic partner, reproductive strategies, nitrophytes and nitrophobes species; see e.g., Gadsdon et al. 2010; Marmor et al. 2010, Degtjarenko et al. 2018 Geiser et al. 2014; Morillas et al. 2021). This is a promising field of research that can be effectively applied to forest monitoring programmes as the NEC Directive Network. For this reason, in the context of the LIFE Modern(NEC) project, they can be useful for Actions A2, B2 and B4.

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## 2.1.6 Ozone injury

### Research Background

Tropospheric ozone (O<sub>3</sub>) is recognized as a widespread phytotoxic air pollutant and the third most important greenhouse gas after CO<sub>2</sub> and CH<sub>4</sub> (Mills et al., 2018). In particular, the Mediterranean basin is an area where high O<sub>3</sub> episodes are often observed due to high solar radiation and temperature, low precipitation, and recirculation of the polluted air mass during summer seasons (Ochoa-Hueso et al., 2017).

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



When forest plants are exposed to O<sub>3</sub>, O<sub>3</sub> damage becomes visible on the leaves (O<sub>3</sub> visible foliar injury) as necrotic stippling, yellowing and bronze red dots in interveinal areas on upper leaf surfaces. It has been known that O<sub>3</sub> visible foliar injury is an only easily detectable biomarker to assess potential phytotoxicity of O<sub>3</sub> especially in a field condition.

In Europe, during the 1980s, with growing concern about the problems of air pollution, the Convention on Long-range Transboundary Air Pollution (CLRTAP) within the framework of United Nations Economic Commission for Europe (UNECE) has been initiated to target the reduction of key harmful air pollutants. During the 1990s, CLRTAP has focused on a reduction of harmful effects of O<sub>3</sub> on vegetation through targeting an establishment of legislative standards. To achieve this aim, a pan-European scale protocol has been launched to monitor O<sub>3</sub> visible foliar injury for the ICP-Forests intensive forest monitoring (Level II permanent observation plots) across Europe in order to contribute to a better understanding of the effects of air pollution and climate change factors on forest ecosystems. The assessment of O<sub>3</sub> visible foliar injury considers both the dominant tree species of each plot and the natural vegetation in light exposed sampling sites (LESS) at the forest edge. Specific information for the O<sub>3</sub> injury assessment is available in the ICP-Forest website (<http://icp-forests.net/page/icp-forests-manual>) (Schaub et al., 2020).

### Exposure-based and Flux-based O<sub>3</sub> metrics

The critical point when examining O<sub>3</sub> impacts on vegetation is how to calculate the O<sub>3</sub> phytotoxicity metrics, which are the versatile tools serving as a predictor of plant response to O<sub>3</sub> for communicating with policy and decision makers to set environmental standards (Paoletti and Manning, 2007). In fact, in order to assess the potential O<sub>3</sub> risks to vegetation, several metrics have been suggested (Paoletti and Manning, 2007, Lefohn et al., 2018). In the 1980s, scientific studies demonstrated O<sub>3</sub> exposure indices calculated from data sets that used a mean value of O<sub>3</sub> concentrations (M24: daily mean 24-h ozone concentration; M7: daily mean 7-h ozone concentration [9:00 to 16:00 h], Musselman et al., 1994). Obviously, these indices of mean O<sub>3</sub> concentrations provide equal weight to all concentrations and ignore the length of exposure. However, according to a series of manipulative experiments, they also emphasized that higher O<sub>3</sub> concentrations should be weighted and concentrations should be accumulated over time. For this reason, in the 1990s, in Europe AOT40 (accumulated exposure over a threshold of 40 ppb) as a cumulative exposure index was suggested and defined in the third workshop at Bern, Switzerland for setting a critical level for ozone (Fuhrer et al., 1997). Probably, it is suitable for the “Level I approach” to protect the most sensitive known receptor under the most sensitive environmental conditions. The AOT40 is thus currently employed as the European standard for the protection of vegetation (EU Directive 2008/50/EC). Although AOT40 has an advantage of simplicity, thanks to recent scientific studies, it was found that exceedances of the AOT40 critical levels may not match with the O<sub>3</sub> effects recorded as forest-health indicators (Sicard et al., 2016; Feng et al., 2019). Therefore, there is an urgent need to develop a biologically significant metric for the quantitative O<sub>3</sub> risk assessment (Level II approach) considering vegetation type, soil conditions, climate and other factors. In fact, since O<sub>3</sub> is taken up through stomata into leaves and thus causing a damage to plant physiology and biochemistry, scientific evidence suggests that O<sub>3</sub> damage to plants is more strongly related to the stomatal O<sub>3</sub> uptake than to the concentration in the atmosphere around the plants (Hoshika et al., 2018). For example, in Mediterranean Europe with hot and dry climate during summer, drought-induced stomatal closure limits O<sub>3</sub> uptake, thereby expecting a protection of leaf tissues from the oxidative stress caused by O<sub>3</sub>. Therefore, recently, a new “flux-based” metric has been proposed, so-called the phytotoxic ozone dose (PODy), which have incorporated both the importance of accumulated O<sub>3</sub> uptake via stomata and O<sub>3</sub> detoxification using Y as a surrogate for an ozone detoxification threshold (CLRTAP, 2017). This new flux-based metric is considered a promising index for the protection of forests

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



against O<sub>3</sub> following the revised EU National Emission Ceilings Directive (2016/2284/EU, hereafter NEC Directive) where it is stated that “Member States shall ensure the monitoring of negative impacts of air pollution upon ecosystems through a cost-effective and risk-based approach, based on a network of monitoring sites” (Art. 9, NEC Directive) (De Marco et al., 2019). The approach using PODy has been advanced to the point where it employs the species-specific parameters to estimate O<sub>3</sub> impacts because the O<sub>3</sub> sensitivity varies with species (CLRTAP, 2017). A continuous development of species-specific O<sub>3</sub> uptake parameters is currently on-going research to ensure better understanding of biologically meaningful O<sub>3</sub> metrics (Paoletti et al., 2019). In addition, to realize a proper assessment of PODy, O<sub>3</sub> data with high temporal resolution (e.g. one-hour intervals) should be needed and therefore a renewed interest in monitoring systems at forest sites has arisen.

### Ozone injury monitoring sites in Italy

Since 2000, the ICP-Forests has started annual intercalibration courses for the assessment of O<sub>3</sub> visible foliar injury for European forests. After this event, in Italy, O<sub>3</sub> visible foliar injury has been intensively monitored in the intensive monitoring sites such as CONECOFOR (La Rete Nazionale per il CONTROLLO degli ECOSistemi FORestali) (Bussotti et al., 2003; Gottardini et al., 2016) and the recently developed MOTTLES (MONitoring ozone injury for seTTing new critical LEvelS) network (Paoletti et al., 2019). The revised EU National Emission Ceilings Directive (2016/2284/EU, hereafter NEC Directive) has currently set objectives for emission reduction of air pollutants for each Member State as percentages of reduction to be reached by 2030 compared to 2005 levels. For this purpose, the concept of “monitoring and quantifying air pollution impacts” on ecosystems was included into the NEC Directive as an annex. Previously, passive samplers were often utilized for the O<sub>3</sub> monitoring in forests because they have an advantage in remote areas where electrical power is not available (Gerosa et al., 2007; Calatayud et al., 2016). However, the O<sub>3</sub> monitoring now requires dealing with high-temporal resolution data for the proper estimation of stomatal O<sub>3</sub> flux because the risk assessment of O<sub>3</sub> is moving towards a stomatal flux basis for the quantitative O<sub>3</sub> risk assessment (Level II approach). The NEC Italia monitoring network, coordinated by CUFA (Comando unità forestali, ambientali e agroalimentari), has been therefore developed considering the MOTTLES monitoring concept. In fact, a previous LIFE project MOTTLES (LIFE15 ENV/IT/000183) developed an integrated monitoring concept for the continuous O<sub>3</sub> measurement by using active O<sub>3</sub> monitors for up-to-date Italian forest monitoring (Paoletti et al., 2019). To assess O<sub>3</sub> injury to forest trees, MOTTLES network includes 6 Italian forest sites with a continuous monitoring of stomatal O<sub>3</sub> flux and principal indicators of O<sub>3</sub> injury such as O<sub>3</sub> visible foliar injury since 2017.

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### 2.1.7 Crown condition

#### Introduction

Defoliation is the most relevant parameter for assessing the health of trees in large-scale terrestrial surveys, which have been carried out in European forests since 1980ies. Defoliation is defined as leaf loss

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



(fallen and undeveloped leaves, loss of foliar surface following biotic or abiotic events) on the assessable crown when compared to a reference fully foliated tree. The assessable crown includes the presence of recent died branches. The ecological and physiological significance of tree crown defoliation has been widely debated. Defoliation is an unspecific parameter, integrating the intrinsic genetic variability of trees, site effects (soil fertility, climatic features, structure, and composition of the forest stand), and external factors such as abiotic and biotic stresses. Defoliation is not necessarily equivalent to damage and can be considered indicative of the plastic equilibrium of a tree in its own environment.

The assessment of defoliation and general crown conditions (described by an array of parameters, that includes foliar damage and discoloration, branch dieback and trunk injuries) was introduced to monitor the impact of atmospheric depositions (“acid rains”) and air pollution on forest ecosystems, but nowadays it is considered a reliable response parameter in relation to many environmental stress and climate change.

In this document, we report the results of a screening on the historical development and timely and emerging research on the topic to define its significance in relation to the purpose of the protection of forest ecosystems subjected to environmental pollution and climate change.

### Methodological approach

In this work, we focused on the articles dealing with the issue connected to the crown condition assessment, its evolution over time, and its ecological and physiological significance.

A database of the main works carried out in the last 30 years (from 1991 to 2021) was prepared, based on bibliographic research on the major search engines in the field of scientific publications, including major review and research articles, as well international documents and reports that can be considered relevant to the NEC Directive and to the LIFE MODERn(NEC) project.

### Results and discussion

#### *Quality Assurance*

In a first period, a large body of literature was devoted to the problems related to the quality of data and reliability of the assessment process. Some papers addressed the general questions of the European program (Innes et al., 1988a, b, 1993; Ferretti et al., 1997, 2010; Johnson and Jacob, 2010); specific issues were:

- The comparability and harmonization of field crews (Innes et al., 1993; Ghosh et al., 1995; Gertner and Köhl, 1995; Redfern and Boswell, 2004; Ferretti et al., 1999; Bussotti et al., 2009, Solberg and Strand, 1999; Eickenscheidt and Wellbrock., 2012);
- Methods to make the assessment objective as possible (Pouttu and Dobbertin, 2000; Mizoue, 2002; Durrant and Boswell, 2002; Dobbertin et al., 2004, 2005; Nakajima et al., 2011
- The significance of the symptoms assessed (Ferretti et al., 1998; Innes, 1998; Zarnoch et al., 2004)
- The sampling strategy and integration of the ICP Forests network with the National Forest Inventories (Dobbertin et al., 2001; Wulf et al., 2012; Travaglini et al., 2012; Gasparini et al., 2013; Kovac̣et al., 2014)

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### *Levels and trends*

Levels and trends of defoliation are reported on a yearly basis for the whole European program (<http://icp-forests.net/page/icp-forests-technical-report>), and recently reviewed by Potočić et al. (2021). At European level, the proportion of fully foliated trees has declined over the past 30 years, while mean defoliation has increased, particularly since 2010. Temporal trends for specific countries were analyzed by Solberg (1999b); Baeda et al. (2004), Aamlid et al., (2000), Bussotti et al. (1995, 2002, 2003, 2005), Seidling (2004, 2005), Seiling and Mues, 2005; Toigo et al. (2020); Ugarkovic et al. (2021). It is generally recognized that Insects (among biotic factors) and drought (among abiotic factors) are among the most frequently reported causes of tree damage, whereas air pollution may exacerbate the negative effects of such factors. The role of biotic damage was emphasized by Nevalainen et al. (2010).

### *Atmospheric deposition*

Acidic depositions, and soil acidification by sulphur and nitrogen pollutants, were relevant ecological factors in Central Europe at least until the first years of XXI century. Correlative studies between atmospheric depositions and crown conditions at European level were carried out by de Vries et al. (2000a, b), van Leeuwen (2000) and Klap et al. (2000), and updated by de Vries et al. (2014). Among regional studies, the most important were carried out in Lithuania (Augustatis et al., 2007) and Solberg and Tørseth (1997) in Norway. Studies in extra European countries are also available (Wang et al., 2007; Duarte et al., 2013).

### *Ozone and nitrogen*

Atmospheric ozone levels (and stomatal fluxes) and nitrogen deposition are nowadays the most important pollutants potentially impacting forests, therefore many studies explored the possible effects on defoliation on statistical bases, both at continental and local level. The results are often contradictories.

Effects of ozone on defoliation were found by Augustatis et al. (2008), Araminiene et al. (2019), Díaz-de-Quijano et al. (2009), Sicard and Dalstein-Richier (2015), De Marco et al. (2017). No effects, on the other hand, were found by Bussotti et al. (2009); Ferretti et al. (2003; 2007; 2018); Gottardini et al. (2018), Paoletti et al. (2019). Contrasting results can be explained with different tree species sensitivity, climatic features, metrics to estimate ozone levels or fluxes and nitrogen deposition, statistical strategies (Ewald, 2005).

Effects of nitrogen deposition on defoliation were reviewed by Schmitz et al. (2019). Ferretti et al. (2015) found that N-related variables improved defoliation models based on data from 71 plots across Europe. Higher N deposition led to higher percentage of defoliated trees for beech and spruce, while the effect was opposite for pine. Vitale et al. (2014) and De Marco et al. (2014) found aspects of N deposition to be relevant determinants of crown condition for several species across Europe, with varying direction of the effect. Other studies found weak or no relation between defoliation and N deposition (Klap et al., 2000; Solberg and Tørseth, 1997; Staszewski et al., 2012; Jakovljevic et al., 2019).

### *Effects of defoliation on growth*

Recent studies explored the consequences of crown defoliation on the growth. An analysis carried out on *Picea abies* (L.) Karst. in Norway (Solberg, 1999a, Solberg and Tveite 2000) revealed a significant, although

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



weak, relationship between crown density and growth. Seidling et al. (2012) and Tallieu et al. (2020), analysing the annual growth in forest trees, observed that the yearly fluctuations of defoliation and growth are not synchronic, since the sprouting of new leaves and the length of branchlets depend on the climate conditions of the previous year (Ferretti et al. 2014), whereas the radial tree growth depends on the water availability in the soil in the current year (Navarro et al. 2020). Different increment patterns were observed on *Quercus robur* with different defoliation levels (Dobrishev et al., 2006). Tallieu et al. (2020) also observed significant effects on the growth of *Fagus sylvatica* L. when the difference of defoliation was more than 20% with respect to the previous year. Severe crown defoliation and foliar symptoms (leaf browning) were associated with basal area increment (BAI) reduction in *F. sylvatica* trees in the 2018 drought event in Switzerland (Rohner et al. 2020). The relationships between crown conditions and growth, however, seems to be species-specific being conifers more sensitive than broadleaved trees (Ferretti et al., 2021a, b).

### *Integrated evaluation of defoliation*

The relationships between defoliation and physiological responses were recently explored in some papers. It is assumed that the reduction of the photosynthetic surface reduces the overall net photosynthesis at the crown level, but low crown density may also favour the penetration of light into the canopy and enable better exploitation of solar radiation from the residual leaves or trigger compensatory photosynthesis. If more solar radiation reaches the inner parts of a defoliated crown, this may induce a transitory photoinhibition of the photosystem II (PSII) with the reduction of the maximum quantum yield of primary photochemistry (Fv/Fm) (Gottardini et al. 2016, 2020), compensated by the enhancement of the electron transport rate beyond the photosystem I (PSI), with species-specific effects (Pollastrini et al. 2016). The accumulation of photoprotective compounds was detected on defoliated *Quercus ilex* trees (Encina-Valero et al., 2021). Concerning the relationships with water conditions, defoliation may improve the water state of a tree by reducing transpiration (Balducci et al. 2020). Another issue was the response of trees in pure and mixed stands (tree diversity effect). The papers produced by Eichhorn et al. (2005), Sousa-Silva et al. (2018), Bussotti et al. (2018), Iacopetti et al. (2019) showed contrasting trends (species-specific and site-specific), but in general trees growing in mixed stands were less susceptible to defoliation in relation to extreme events.

### *Climatic factors and extreme events*

Climatic factors, with special reference to drought, were the most important predictors for levels and changes of defoliation. Relevant researches were carried out by Solber et al. (2004); Zierl (2004); Seidlin et al. (2007); Carnicer et al. (2011); de la Cruz et al. (2014); Ferretti et al. (2014); Camarero et al. (2015); Popa et al. (2017).

Extreme drought and heat waves are recurrent in Europe from the begin of the XXI century, inducing high levels of defoliation and tree mortality (Breda et al. 2006; Rebetez et al., 2006; Neumann et al., 2017; Pollastrini et al. 2019; Puletti et al. 2019; Schuldt et al. 2020; Brun et al., 2020; Bussotti et al., 2021). High defoliation may compromise the reserves of non-structural carbohydrates (NSC) (Galiano et al. 2012; Wiley et al., 2013; Deslauriers et al. 2015; Puri et al., 2015; Guada et al., 2016; Wang et al. 2020; Barker Plotkin et al., 2021; Senf et al., 2018, 2021), which play a fundamental role in coping with environmental stress (including air pollution) and pest attacks (Hartmann and Trumbore 2016). The expected increase of drought and heat waves in the next decades (Spinoni et al., 2017, 2018; Magno et al., 2018; Moravec et al., 2021) may increase the risks for forests.

# LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



## Conclusions

The assessment of crown conditions and defoliation was launched at European level in the '80 of the XX century to monitor the impacts of air pollutants and acidic depositions on forests. Despite the alleged subjectivity of the scoring system and the methodological problems connected, the method has established itself as reliable and now the ICP Forests Level I network represents an indispensable multipurpose infrastructure (see, for ex., Nussbaumer et al., 2018), whose importance is increasing in condition of climate change and recurrent drought and heat waves that may exacerbate the action of pollutants. In this perspective there is the exigency to improve the informative potential of the survey with the introduction of specific physiological indicators (Bussotti and Pollastrini, 2017a, b; Finch et al., 2021) of impact and responses at tree and plot level (Saura-Mas et al., 2015), according to the concept of fitness-resilience (Bussotti and Pollastrini, 2021). In this perspective the metabolism of carbon (storage and growth) in defoliated trees (as consequence of extreme events) is of particular importance for the prevision of risks connected to the future of forests. A further advancement of the crown condition monitoring will be the combination of the terrestrial survey with aerial images obtained with the most advanced technological advancements (Sentinel, UAV etc).

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## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### 2.1.8 Forest growth

#### Introduction

Tree growth (increment) is a fundamental quantitative indicator of tree vitality and ability to buffer environmental constraints; it is able to provide a sensitive response to impacts across space and time.

In recent years, the growth of forests under changing environmental conditions has been a major concern across the globe. Forest growth is among the parameters measured within the intensive (Level II) forest monitoring of UN/ECE ICP-Forests (International Co-operative Programme on Assessment and Monitoring of Air pollution effects on Forests), aimed at analyzing the effects of air pollution and other stress factors on the conditions of forests in Europe (Lorenz et al. 2004).

The condition of Europe's forests has been heavily studied and discussed during the last three decades. In the beginning of the 1980s a concern for a widespread forest decline in central Europe caused by air pollution was growing. Increasing emissions of sulphur dioxide and oxidized nitrogen were likely to produce soil acidification; catastrophic forest damage was seen in heavily polluted areas while crown thinning and discolouration was also widely seen in less polluted areas.

Trees react to environmental changes within their auto-ecological tolerance and search for a new equilibrium. Climate change acts as a driving force on ecosystem processes with direct and indirect feedback. Further concurrent or counteracting factors, i.e., CO<sub>2</sub> enrichment, ozone level, nitrogen fertilization, sulphates deposition, drive the soil-tree-atmosphere relationships and the specific tree growth within each geographical area.

As a feedback, changes in patterns of tree growth can have a huge impact on atmospheric and biogeochemical cycles, climate change, and biodiversity.

The environmental changes also affect forests indirectly via the occurrence of diseases, pathogens and insect outbreaks.

#### Methodological approach

In this work, we focused on the articles dealing with the issue connected to the tree growth assessment, its evolution over time, and its ecological and physiological significance.

A database of the main works carried out in the last 30 years (from 1996 to 2021) was prepared, based on bibliographic research on the major search engines in the field of scientific publications, including major review and research articles, as well international documents and reports that can be considered relevant to the NEC Directive and to the LIFE MODERn(NEC) project.

#### Results and discussion

Tree growth processes can be ranked by order of importance in foliage growth, root growth, bud growth, storage tissue growth, stem growth and growth of defense compounds, and reproductive growth. Under stress the photosynthesis and the subsequent carbon allocation of a tree may be altered in a way that the less important processes including stem growth are reduced first. Stress factors may either affect the

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



photosynthetic processes directly (for example defoliation by insects or damage of foliage due to frost or air pollution) or indirectly via reduced resources (for example reduced water supply during drought, reduced nutrient uptake due to root damage or nutritional imbalance due to high acidic or N deposition).

Important for any potential vitality indicator is the comparison with a suitable reference. Depending on the aim of the study the references used can be the growth of trees without a presumed stress, growth of presumed healthy trees, growth in a presumed stress-free period or expected growth derived from models. The general disadvantage is that no absolute growth reference is available.

The examples of various reactions of tree growth to environmental stress illustrate that tree growth can serve as a vitality indicator if reference growth or growth trends are available.

Tree vitality is one of the most important indicators of forest condition. Tree vitality, or tree condition, describes the general outer appearance of an individual tree. In addition, tree health reflects the pathological state of a tree. As vitality cannot be measured directly, various indicators can be used to describe it. If tree vitality is used as an indicator of forest condition during forest surveys, it is clear that field-practical, low-cost methods are needed. Although vitality is a theoretical concept and defined differently in a range of studies, it always includes the power to live, grow, and develop. Therefore, indicators of tree life, growth, and development have to be used.

Recent findings are concerning the use of tree-ring stable isotopes not only to reconstruct the impact of past climatic events, such as drought, but also to study forest decline induced by air pollution episodes, and other natural disturbances and environmental stress, such as pest outbreaks and wildfires. They have proven to be useful tools for understanding physiological processes and tree response to such stress factors.

Tree productivity is often considered to be an expression of tree vitality. However, its use is questionable because the most productive trees are not always in good health. For example, tree growth may increase as a reaction to fungal attacks or injury. Trees may also grow very slowly in the shade of other trees for hundreds of years, only becoming truly productive once they are exposed to more light. To assess tree productivity, the carbohydrates being produced, i.e., the carbon fixed through photosynthesis, should be measured. As it is difficult to measure carbohydrate production in the field, tree biomass, as derived from tree volume, is used. Thus, tree volume and biomass are certainly indicators of tree growth, but they are time consuming to estimate, and strongly affected by errors and approximations. Basal area increment may be used as a proxy for volume. The advantage of using basal area is that no data standardization is needed to remove the geometrical age trend, i.e., the decreasing trend in ring width due to the increasing stem circumference with tree age, preserving all other long-term trends.

Variations in atmospheric gas concentrations, such as the increases in CO<sub>2</sub> concentrations, air pollution, and acidic precipitation due to fossil fuel combustion, affect forest ecosystems at individual tree and canopy levels. For example, in several forests, elevated CO<sub>2</sub> concentrations, temperature, and drought increase seem to promote an increase in plant water use efficiency with an enhancement of photosynthesis and a generally modest reduction in stomatal conductance. The influence of each factor is still under debate and difficult to disentangle. Tree-ring  $\delta^{13}\text{C}$  samples from around the globe show an increase in iWUE since pre-industrial times. Exposure to SO<sub>2</sub> and O<sub>3</sub> has been reported to inhibit photosynthesis by inducing stomatal closure. Meanwhile, elevated NO<sub>x</sub> concentrations may increase photosynthetic rates by

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



activating carboxylation enzymes due to the “N fertilization effect” during short-term exposure or in the long-term trend.

Some other analysis and results suggest a different picture, where traditional ecological factors like biotic and abiotic damage to trees, tree nutrition (N ratio to K and Mg), tree size and age, and site factors are, by far, the most important and the only statistically significant drivers of forest health and productivity. (the negative effects of ozone on forest health and growth were probably overestimated in Europe, and perhaps elsewhere, over the past decade).

In order to clarify the causes of the increased forest growth the EU-project RECOGNITION was established, and recently concluded that increased availability of nitrogen is the main driver for increased growth, although they could not definitely determine whether this was due to increased nitrogen deposition or increased availability in the soil.

At Italian level (IT), forest monitoring data collected at permanent plots over the period 2000-2009 were studied to identify the possible impact of nitrogen (N) deposition on soil chemistry, tree nutrition and growth. Growth at IT sites was explained for the most part by factors related to site, management and meteorology. The possible role of N-related variables was evaluated starting from this basis. Measured N deposition was found to exceed CLs at several of our monitoring sites, to affect soil nutrients (reduction of BCE and pH), to augment foliar N-ratios (particularly N : P and N : K) and to promote growth and C sequestration. Coupled with findings from other studies documenting instances of N saturation at some of our sites (e.g., PIE1), our results provide clear evidence of an impact of N deposition on temperate forests in southern Europe. Implications may concern ecosystem chemistry (depletion of nutrients, nitrification of soil water, run-off and stream water), diversity (shift in species, changes in species coverage), health (augmented susceptibility to pests and pathogens) and productivity (positive effect at present, but quite uncertain in the long-term). In this perspective, predictions about the future ability of forest to sequester C and thus mitigate climate change are only possible with great caution.

Mediterranean forests are particularly sensitive to global change. Future scenarios predict an increase in drought stress (with increased temperature and decreased precipitation) throughout Europe (e.g., the 2003 heat wave) with the Mediterranean region being particularly affected. The climatic trends in the area during the last 50 years have been characterized by a rise in mean temperature (2-4° C) and an increase in both frequency and intensity of severe droughts, one of the most important factors triggering both temporary declines and mortality in temperate forests.

The presence of a forest decline caused by air pollution was, however, severely questioned. On the other hand, later studies have also substantiated the increasing forest growth in comparison to the previous 40 years. A literature review on growth rates in natural forests found an increase in forest productivity in three out of four studies, and a decline in productivity in only 10% of the studies since the middle of the last century.

Spiecker et al. (1996) provided four plausible explanations for an increased forest growth: (1) increased availability of nitrogen, (2) increased atmospheric CO<sub>2</sub> concentration, (3) climate change such as increased

# LIFE MODERn (NEC)

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temperature and extended growing seasons, and (4) altered forest management practices such as thinning type and genetic selection.

The major finding of several studies was a positive relationship between higher than normal volume increment on one hand and nitrogen deposition on the other hand.

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## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

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### 2.1.9 Plant phenology

#### Introduction

Phenology is the study of plant and animal seasonal life cycles. Plant phenology is the study of plant cycle phases (e.g., leaf unfolding, flowering, blooming, leaf fall) and their development over time in relation to abiotic and biotic factors (Lieth, 1974). Among these factors, the more influential ones are meteorological drivers such as air temperature, rainfall, humidity, radiation, both quality and amount per day (Baddour and Kontongomde, 2009; Schwartz, 2013). Indeed, observed changes in vegetation phenology (i.e., shift of key phenological stages) has been widely recognized as an indicator of environmental stress factors, such as anthropogenic climate change, since timing of vegetation key phenological stages is driven, above all, by air temperature. As stated by the Intergovernmental Panel on Climate Change (IPCC, 2018), advancement in spring phenology has been observed in plants and animals in recent decades in most Northern Hemisphere ecosystems ( $2.8 \pm 0.35$  days per decade), and these shifts have been attributed to changes in climate (Settele et al., 2014).

Vegetation phenology can be observed on the ground and large datasets are nowadays available as collected by worldwide networks. However, field observations (i.e. visual observations) can cover limited time and spatial scales and, when addressing geo-processes related to climate change, large-area studies are necessary.

Field assessment of plant phenology is carried out by repeated visits in the field throughout the seasons, to detect the various phenological phases of plants by visual observation. This kind of survey (traditional method) needs expert personnel, and it is time-consuming, especially during the key weeks of the growing season (i.e., start of spring and start of autumn, respectively the begin and the end of the growing season in temperate and boreal forests), when more frequent field visits are required to detect the transition from one phenological phase to the next. To partially resolve this time-consuming work and to collect a large volume of good quality data, Red-Green-Blue (RGB) digital cameras sensing visible-light wavelengths, called phenocams (Keenan et al. 2014, Tang et al. 2016), has been used; these cameras allow us to collect and store photos of the vegetation over time. The assessment of plant phenology by these imaging sensors is based on the monitoring of changes of leaf/crown color that is associated with different plant development stages. Several colour indices can be adopted, including the commonly used greenness index. Optical properties of crowns can be analysed also by spectral radiometers, detecting reflected radiance of leaf surface.

More recently, plant phenology has been assessed also by Remote Sensing (RS) techniques that use Earth Observation (EO) data to monitor land surface phenology (LSP) over large areas and over long time periods (de Beurs and Henebry, 2004). Indeed, satellite RS provide regular and frequent observations of the Earth surface with a synoptic view supporting broad scale studies of ecosystems status and changes rather than analysis of individual species. However, field measurements and observations will always be necessary for calibration and validation activities (Beaubien and Hall-Beyer 2003). Frequent observations of forest surface

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LIFE20 GIE/IT/000091

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by RS techniques deliver time series datasets that can be processed with standardized approaches to detect trends of the status of the vegetation and anomalies in expected trends during the season, due to climate factors and/or other abiotic and biotic disturbing agents occurring in forests, like pest attacks, fires.

Time series of image data on land surface, thus forest surface, have been provided in the past years by satellite sensors, such as the Advanced Very High Resolution Radiometer (AVHRR) (e.g. Duchemin et al., 1999) and the Moderate Resolution Imaging Spectrometer (MODIS) (e.g. Testa et al., 2018) with coarse spatial resolution ranging in 250 m to 1 km and very high temporal frequency of acquisition. Medium spatial resolution satellite data suitable for filling the gap between coarser data and field data/observations, are collected by the Landsat mission (with the longest historical archive) and Copernicus Sentinel (more recent, with spatial resolution of 10-20 m and temporal of 5 days). Vegetation phenological metrics are estimated by a time series of vegetation indices that, combining the spectral response of the observed surface in the visible to near infrared spectral wavelengths, are indicators of the status of vegetation cover (Filipponi et al. 2022). Several vegetational indices have been exploited to this aim such as the Normalized Difference Vegetation Index (NDVI) and Enhanced Vegetation Index (EVI). The key phenological metrics are generally estimated with algorithms that are based on, for example, thresholding, inflection points in time-series greenness curves, or rates of change in vegetation index values.

In this document, we report the results of a screening on the timely and emerging research on vegetation phenology to explore recent studies documenting the current trends and changes in air pollution and their potential effects on plant phenology as indicator. We have mainly addressed reviews and articles dealing with the effects of climate change on plant phenology key phases (i.e. bud break, leaf development, flowering, seed production, masting, pollen dispersion, fruiting), especially for forest tree species.

In addition, to obtain a wide picture of forest variables and drivers affecting phenological phases in forest tree species, we also took into account papers dealing with these aspects and not directly addressing atmospheric pollutants deposition.

### Methodological approach

A database of the main works carried out in the last 30 years (from 1991 to 2021) was prepared, based on bibliographic research on the major search engines in the field of scientific publications, including major review and research articles, as well international documents and reports that can be considered relevant to the NEC Directive and to the LIFE ModernNEC project.

### Results and discussion

We selected emerging research papers on the topic (see Table in Annex), dealing with several aspects of plant phenology assessment.

Plant phenology monitoring can be performed by various methods and techniques, characterized by different time- and spatial resolutions. Ground, near-surface and remote sensors are nowadays available allowing to collect data on phenological changes in forest ecosystems, with a high time and spatial resolution, limited in traditional field surveys. Near-surface digital cameras and spectral radiometers, that can be mounted on the ground on tripod/towers and/or sensors carried by low altitude platforms such as Unmanned Aerial Vehicles (UAV), provide frequent data with a very high spatial detail to provide species-specific and /or site level measurements. These data can fill the gap between traditional visual observations

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

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and satellite data that allow a synoptic view over large areas. Each (i.e., visual evaluation by expert personnel, collection and analysis of images recorded in near- or remote surface detection) has advantages and disadvantages. A suitable combination and integration among them could solve some of drawbacks.

### Emerging opportunities and future advancements

Vegetation phenology is the study of recurring plant life cycle stages, seasonality which is linked to many ecosystem processes. It is an important proxy of climate and environmental changes and pressures (Berra and Gaulton, 2021), included air pollution, hence plant phenology can be considered relevant to NEC Directive and to the LIFE Modern(NEC) project.

#### *Vegetation phenology from automatic devices*

Digital automatic repeat cameras are nowadays easily available, with low cost, and able to conveniently and continuously record plant phenology. They should be installed in network sites for long-term monitoring of forest trees phenology. These cameras should be complemented by spectroradiometers to monitor leaf- and canopy-scale ecophysiology to capture additional physiological variables other than leaf color information alone. The development of low-cost hyperspectral cameras along with new spectral indices and new devices to capture solar-induced fluorescence to track in situ vegetation activities is desirable.

#### *Vegetation phenology from Remote Sensing*

Remote Sensing has been playing an important and increasing role in forest, and more in general in natural resources, monitoring and assessment. Its application to vegetation phenology assessment has significantly raised in the last years.

In order to implement time series analysis, collected by satellite records, several tools have been developed for automatic processing and extraction of key phenological metrics and are freely available such as TIMESAT (Jönsson and Eklundh,2004). More recently, the *sen2rts* R library has been developed to extract and manage time series from Sentinel-2 archives (Ranghetti et al. 2021); compared to TIMESAT, *sen2rts* R library has been developed for processing S2 time series.

Not to forget that RS techniques also include data acquired by low-altitude platforms (e.g., air-borne, UAV) that, together with unprecedented computing capacity, open new opportunities for monitoring forest phenology (Berra and Gaulton, 2021).

Concluding, to improve forest monitoring surveys in order to detect and understand promptly tree responses to environmental factors, a synergetic use of multiple approaches and techniques to evaluate plant phenology in forest is desirable. A combination and integration among several remote (i.e. orbital) sensors and various metrics to assess key phenological parameters, with data from new sensors at ground, near-surface and airborne level is feasible. Traditional ground-based observations are highly useful for calibration and validation of the proposed methodologies.

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LIFE20 GIE/IT/000091

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## 2.2 Freshwater ecosystems

Here we present a review of the literature on the topic of atmospheric pollution effects on freshwater, with a focus on acidification and nutrient nitrogen and on indicators selected by Art. 9 of the NEC Directive. Both pan-European and local studies are considered, including previous assessment on ICP WATERS sites in Italy. Studies considering confounding factors (e.g., climate, land use change) in the response of freshwater ecosystems to changing emission and deposition are included.

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### Freshwater ecosystems as indicators of atmospheric pollution

Freshwater ecosystems are considered sensitive indicators of the effects of atmosphere pollution. In particular, in remote or pristine areas, where direct pollutant sources are absent, water quality of freshwater ecosystems is strongly dependent on atmospheric inputs (Lepori and Keck 2012). Deposition may be a relevant source of nutrients (P and N compounds) to these ecosystems, but also a vehicle of atmospheric pollutants (acidifying compounds, POPs, heavy metals) transported with the air masses from source regions (EEA, 2014). Nitrogen deposition has received particular attention since nitrogen may be both an acidifying and eutrophying agent for freshwater ecosystems (Shibata et al., 2015; Kaste et al., 2020). Mountain lakes and streams and more generally water bodies in remote areas i.e., far from point source of pollutants, may be affected by air pollutants from emission sources located in the lowlands or in downwind regions (Rogora et al., 2013; Tiberti et al., 2019). These sites have proved to be useful early-warning indicators of changes in climate and in the chemical composition of the atmosphere. Therefore, they became the focus of monitoring networks and research projects on surface water pollution due to atmospheric sources (Evans et al., 2001; Marchetto & Rogora, 2004; Curtis et al. 2005; Skjelkvåle et al. 2005).

The International Cooperative Programme on Assessment and Monitoring Effects of Air Pollution on Rivers and Lakes (ICP Waters) was established in 1985, with the specific objective of assessing the degree and extent of atmospheric pollution effects on surface waters in Europe and North America ([www.icpwaters.no](http://www.icpwaters.no)). ICP Waters monitoring sites have provided evidence of the negative effects of airborne pollutants on freshwaters but also of the positive response of these ecosystems to decreasing emissions (Skjelkvåle et al. 2005; Garmo et al. 2014). Long term data contributed to the evaluation of policy for emission reduction, such as the Gothenburg Protocol (ICP WATERS, 2011).

Recently, the National Emission Ceilings Directive (NECD) (European Parliament and Council, 2016) set 2020 and 2030 emission reduction commitments for the main air pollutants and introduced in Art. 9 the requirement to member states to ensure the monitoring of negative impacts of air pollution upon ecosystems, including freshwaters. As recommended by ICP WATERS, an effective monitoring of the effects of air pollution on aquatic ecosystems should be based on sensitive sites, for which atmospheric pollutants have been identified as the main stressor (ICP WATERS Manual, 2010) i.e., sites that do not have impacts from local pollution sources such as domestic sewage, industrial wastewater, agriculture. Small headwater catchments in pristine regions e.g., in protected areas, with limited anthropogenic disturbance, are suitable sites to monitor the effects on air pollution. Sites should also be as representative as possible of the diversity of a region (chemically, biologically and geographically).

### Long-term response of freshwater to changing atmospheric inputs

The deposition of acidity and acidifying agents such as  $\text{NO}_3$  and  $\text{SO}_4$  has caused widespread acidification of sensitive water bodies in the 1970s and 1980s; then, extensive recovery has occurred, due to the emission reduction of S and N compounds promoted by international protocols (Skjelkvåle et al. 2005). Several networks have been established at national and international level to define inputs, developing critical loads and monitoring ecosystem response to S and N deposition (e.g., Aherne & Shaw, 2010; Curtis et al., 2005; Cathcart et al., 2016; Forsius et al., 2019). EU projects have documented the recovery of freshwater ecosystems from acidification, with a major role played by the decreasing deposition of  $\text{SO}_4$  (Evans et al., 2001; Prechtel et al. 2001). On the other hand, deposition of N compounds, especially of reduced N,

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

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decreased at a lesser extent and remained high in some areas, with impact on vulnerable ecosystems (Wright et al., 2001; Rogora et al., 2012; Kaste et al. 2020). N deposition in excess of ecosystem demand may cause N saturation of soils and consequence leaching of  $\text{NO}_3$  below the rooting zone, with the ultimate effects of increasing nitrogen in water bodies: this may cause acidification, eutrophication, nutrient imbalance and deterioration of the ecosystem health (Wright et al., 2001). The widespread decrease in the deposition and surface water concentrations of  $\text{SO}_4$ , has caused  $\text{NO}_3$  to become the dominant acidifying anion in some sensitive ecosystems such as high mountain lakes (Rogora et al., 2013). More recently, signs of recovery from N saturation and decreasing levels of  $\text{NO}_3$  in surface waters in response to changing deposition were also documented (Rogora et al., 2012; Garmo et al., 2014).

While there was extended evidence for chemical recovery of freshwaters due to decreasing deposition of atmospheric pollutants, evidence for biological recovery was fragmented (Kowalik et al., 2007; Marchetto et al., 2004; Vrba et al., 2016), prompting for a need to identify and monitor affected freshwaters and quantify their recovery towards a target state (Juggings et al., 2016).

Several studies also highlighted how recovery was delayed with respect to decreasing emission and deposition due to several factors including legacy of  $\text{SO}_4$  stored in soils and wetland (Marx et al., 2017), interactions among nutrients and catchment biogeochemistry (Kopacek et al., 2015; Ohulele et al., 2017), land use change (Krecek et al., 2019; ICP WATERS, 2020) and climate (Wright & Jenkins, 2001; Baker et al., 2021). These factors altogether have been defined as 'confounding factors' i.e., environmental factors other than deposition which can affect chemical and biological recovery of freshwaters in response to reduced acid deposition (ICP WATERS, 2020; Wright & Jenkins, 2001).

Beside describing dynamics of S and N compounds, long-term studies also focused on base cations (BC: Ca, Mg, K), heavy metals (mainly Al) and dissolved organic carbon (DOC). BC may be progressively depleted from soil catchments due to acid deposition, causing soil acidification and contemporary increase of BC concentrations in freshwaters; on the opposite, decreasing BC have been observed at several locations during the recovery process (Garmo et al., 2014): a paradoxical negative effect of the successful remediation of acid deposition is a widespread decline of Ca concentration in freshwater towards critically low levels for many aquatic organisms (Weyhenmeyer et al., 2019). A further important effect of acidic deposition is the mobilization of Al and its shift from organic to inorganic forms that are toxic to terrestrial and aquatic biota: atmospheric deposition has proved to be a dominant source for several metals in small headwater catchments (Burton et al., 2013; Tornimbeni et al., 2012). Increasing trend of DOC have been reported in the early 2000s at several sites and related to decreasing levels of acid deposition (Stoddard et al., 2003; Evans and Monteith, 2001): decreases in acidity and ionic strength were both indicated as chemical mechanisms increasing the solubility of soil carbon and causing positive DOC trends (Evans et al., 2012).

Long term data such as those provided by monitoring networks are an invaluable asset to describe and evaluate patterns of acidification/N enrichment and recovery of surface water bodies in relation to atmospheric pollution deposition, as well as the role of confounding factors.

### Chemical and biological Indicators

Since the earliest studies on acidification of surface waters, loss of alkalinity has been identified as a simple, 'early warning' indicator of the first stage of acidification in lakes and rivers. Therefore alkalinity, or alternatively ANC (Acid Neutralizing Capacity), is commonly used as an index of the acidification status, and

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

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of acid sensitivity, of surface waters. ANC is considered as a key indicator in Annex V of the NEC Directive, with pH, dissolved SO<sub>4</sub>, NO<sub>3</sub> and DOC as supporting indicators.

In Europe the ANC value of 20 µeq L<sup>-1</sup> has been identified as the minimum level required for ecosystem protection under UNECE protocols (Rogora et al., 2013). When considering the biota, critical limits should vary depending on the target group of organisms; and even when considering the same target (e.g., macroinvertebrates), critical limits may vary, depending on the typical fauna of sensitive species and their adaptations to native water chemistry. In the high Alps for instance an ANC limit of 30 µeq L<sup>-1</sup> has been suggested (Raddum and Skjelkvåle 2001).

Beside changes in average levels of key variables or indicators, short term changes must be considered to evaluate episodic acidification or short-term pulses of pollutants (Lepori et al. 2003). Acidic episodes, linked to snowmelt, heavy rain or sea-salt deposition may contribute to explain delayed biological recovery (Schneider et al., 2018; Kowalik et al., 2007). For mountain lakes in particular, a critical period is snowmelt, when a drop of pH may occur due to dilution of the alkalinity pool and the release of previously stored acid anions (Rogora et al., 2013).

For this reason, within ICP WATERS it has been recently proposed to focus on the minimum pH, beside average chemical conditions, to assess the severity of acid stress (ICP WATERS, 2020). pH minima may have a fast effect on the biota, causing sensitive species to disappear (Schneider et al., 2018). More generally, a high sampling frequency and the collection of samples in target moments e.g., at snowmelt, after heavy rainfall etc. may help to identify factors hindering biological recovery.

To assess the extent of biological damage, the NEC directive in Annex V suggests the use of sensitive receptors (microphytes, macrophytes and diatoms) and the evaluation of the loss of fish stocks or invertebrates. Fish, diatoms and macroinvertebrates are the biological indicators which have been used within ICP WATERS to assess biological damage and recovery, and specific indicators have been proposed (ICP Waters Manual, 2010). However, site- or region-specific indicators would be advisable for a proper evaluation of biological response, considering the typical fauna and the sensitive species of different regions or the specific target of the monitoring (Juggins et al., 2016; Vrba et al., 2016).

Target limits of specific indicators may be used to define the deposition level and the point in time (target year) when the critical limit is no longer violated ("target load" concept). To assess the timing of recovery, dynamic modeling has been extensively used, with both chemical (e.g., ANC) and biological target (e.g. recovery of fish populations) (Wright et al., 2005; Larssen et al., 2010; Helliwell et al., 2014; Posch et al., 2019).

### Freshwater sites in Italy

Italy has been contributing data to the ICP WATERS Programme since 1995, when the CNR Water Research Institute of Verbania (CNR IRSA) was appointed by the Italian Ministry of the Environment (presently Ministry for Ecological Transition) as National Focal Point for ICP WATERS (Mosello et al., 1999). CNR IRSA regularly collected and sent to the Coordinating Centre (NVA, Norway) chemical data collected at selected sites (alpine and subalpine rivers and lakes). Biological data were collected on a more irregular basis and on a limited number of sites (Marchetto et al., 2004). Rivers and lakes in Italy were selected based on the following criteria: (i) areas affected by high deposition of atmospheric pollutants; (ii) minimal direct anthropogenic disturbance; (iii) availability of information on the main characteristics of the watersheds; (iv) previously collected chemical and biological data, with protocols close to those adopted in the ICP WATERS (Mosello et al., 1999). The selected sites were located in the area of Lake Maggiore watershed, North-West Italy: this area received high deposition of atmospheric pollutants transported from emission

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



sources in the Po Valley, one of the most urbanised and industrialised areas of Europe. A network of atmospheric deposition sampling sites existed in this area, contributing to the evaluation of pollutant inputs to freshwater (Rogora et al., 2016)- Long-term research on both atmospheric deposition and freshwater chemistry have been performed since the beginning in strict cooperation with the Swiss National Focal Point for ICP WATERS, being Swiss sites located in the same study area /Rogora et al., 2013).

Data collected at Italian sites pointed out an advanced stage of N saturation of some subalpine catchments, with consequent NO<sub>3</sub> release to surface water. A reversal of this condition was detected since the mid 2000s, when decreasing trends of NO<sub>3</sub> were observed in several rivers and lakes in response to changing deposition (Rogora et al., 2012). The situation also improved as regards the acidifying effect of NO<sub>3</sub> and SO<sub>4</sub> on sensitive ecosystems such as high-altitude lakes: most of the lakes indeed recovered from acidification, even though the situation may still be critical at some sites at snowmelt (Rogora et al., 2013). Deposition proved to be important in shaping the chemical characteristics of lakes also in other areas of the Alps (Tiberti et al., 2019; Boggero et al., 2018). Studies evaluating biological recovery highlighted some positive effects, such as change in diatom flora and appearance of sensitive species among benthic insects (Marchetto e al., 2004). In an assessment of oligochaete assemblages of Swiss and Italian high-altitude lakes, the acid status of lake waters and their buffer capacity, in turn related to catchment lithology, proved to be a key driver in determining the species distribution among lakes. However, biological data as a whole were irregular in time and limited to a few sites. Further, the focus of biological investigations has been until now mainly on the effects of acidification, while the possible consequences of N enrichment also need to be evaluated.

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### 2.3 Impacts of air pollution and climate change on forests

Forests cover ~30% of the world's land surface, store 45% of terrestrial carbon (Bonan, 2008), and are home to 80% of global terrestrial biodiversity (IUCN, 2021). Sustainable socio-economic development depends on the proper management of natural resources, including forest ecosystems (Badea, 2013). Air pollution and climate change have major impacts on and complex interactions with forest health and productivity (Augustaitis & Bytnerowicz, 2008; Kozlov et al., 2009). For example, tropospheric ozone (O<sub>3</sub>), which is both a phytotoxic gas and radiative forcer (Myhre et al., 2013), and nitrogen deposition (Du & Vries, 2018), which causes forest decline due to acidification (Augustaitis et al., 2010) and changes in the frequency and intensity of climatic extremes (e.g., heat waves, rainfall, windstorms), may impact the structure, composition and functioning of terrestrial ecosystems. These impacts can directly influence carbon cycling and its feedback to the climate system (Paoletti et al., 2007; Serengil et al., 2011; Matyssek et al., 2012; Frank et al., 2015; Sicard et al., 2020).

The future of global forests is a subject of public and political concern due to extensive forest degradation worldwide (Hao et al., 2018; Liu et al., 2018). Recently, environmental pollution was identified as one of the five main drivers of biodiversity loss (EC, 2020). Although environmental pollution is an integral part of global change (Dale et al., 2000), most of the research addressing the biotic effects of climate change do not consider this issue. Further, most studies on both the distribution of pollutants and the biotic effects of pollution have neglected the issue of climate change (Sicard et al., 2016). As a result, studies exploring the combined effects of air pollution and climate change, remain uncommon.

A Web of Science search conducted in June 2021 identified only 74 peer-reviewed articles containing the keywords "climat\*" and "pollut\*" and "tree\*" or "forest\*" in the title, 59 of which were relevant research papers (Tab. 1S): Eleven studies used modeling to explore combined effects of air pollution and climate, 27 studies were based on observations of forest health in either spatial or temporal gradients of air pollution and climate, and only 1 reported the outcomes of a field experiment. The low number of experimental studies with factorial design involving both airborne pollutants and climate is alarming because it hampers our ability to identify cause-and-effect relationships as well as to decipher the mechanisms underlying the combined or interactive effects of pollution and climate on the health of individual trees and forest ecosystems. As a result, the quality of our predictions of the combined effects of climate change and air pollution on future forest health is uncertain.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### 2.3.1 The NEC Directive

#### Introduction

The Long-Range Transboundary Air Pollution Convention (LRTAP), signed in 1979 within the UNECE (United Nations Economic Commission for Europe), was the first international agreement aimed at the reduction of emissions of atmospheric pollutants dangerous for both human health and the ecosystems. The Gothenburg Protocol (1999, with a revision in 2012) is one of the eight protocols pertaining to the LRTAP Convention: it relates to the abatement of acidification and eutrophication processes and ozone at ground level. Directive 2001/81 / EC, the first “emission ceiling” directive was a direct emanation of the Gothenburg Protocol: it introduced emission values maximums (“ceilings”), not to be exceeded by 2010. Following the implementation of emission reduction policies implemented by the European Union, between 1990 and 2010 the emissions of sulfur dioxide decreased by 82%, emissions of nitrogen oxides by 47%, emissions of organic compounds non-methane volatiles by 56% and ammonia emissions by 28% (*Italian Air Pollution Control Programme, 2021*). However, following 2010, the concentrations of atmospheric pollutants have been still quite high and several EU countries still today cannot achieve the required air quality standards established by law.

On 31 December 2016, the EU Directive 2016/2284 for the reduction of national emissions of certain atmospheric pollutants came into force: it is the so-called “NEC Directive” - National Emission Ceilings Directive, which replaced the 2001/81 Directive.

The current NEC Directive, in order to contribute to the general improvement of air quality in the European Union and protect human health and ecosystems, asks for the achievement of national reduction targets emissions of particulate matter, sulfur oxides, nitrogen oxides, non-methane volatile organic compounds and ammonia by 2020 and 2030 and aims at making a significant contribution to achieving the objectives of Directive 2008/50/ EC on ambient air quality.

Particularly, it is expected that the NEC Directive will:

1. reduce annual anthropogenic emissions of sulfur dioxide pollutants, nitrogen oxides, non-methane volatile organic compounds, ammonia and particulate matter PM<sub>2.5</sub> to meet specific reduction targets by 2020 and 2030, ensuring the reaching intermediate levels by 2025;
2. activate monitoring of emissions of selected substances for which reduction obligations are not foreseen;
3. collect, with a monitoring system, data relating to the impacts of pollution atmospheric on ecosystems.

Point three refers to Art.9 of the NEC Directive. Project LIFE MODERn(NEC) is focused on the requirements of this innovative Art.9, being aimed at the evaluation and measurement of indicators for the study of impacts of air pollution on forest and freshwater ecosystems, within an improved Italian NEC Network of monitoring sites.

We report here the results of a screening of relevant literature concerning the implementation of the NEC Directive in Europe and in Italy.

We found a very huge literature regarding air quality, especially related to the requirements of Directive 2001/81 and 2008/50, crossing different research areas and applications, but we finally selected 7 research papers published between 2014 and 2021 (see attached table, in chronological order), to focus on the time frame immediately before and after the publication of the NEC Directive. The overall concept emerging

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



from this literature collection retraces consistently the design of LIFE MODERn(NEC), moving from a general picture of the status and trends of air quality in Europe between 2002 and 2011 to research articles which analyze whether the measured emission reductions are analyzed at ecosystem level. Finally, other papers focus on the Directive itself with a deepening into the Italian situation. Paper n.7 describes two 2030 modeled scenarios. The papers were collected by search on the internet or suggested by experts involved in LIFE MODERn(NEC).

### Results and discussion

Guerreiro et al. (2014) depict the status and trends of atmospheric pollution in Europe between 2002 and 2011. The main pollutants considered are PM, ground level O<sub>3</sub>, NO<sub>2</sub>, NO<sub>x</sub>, BaP, SO<sub>2</sub>, CO, toxic metals and benzene, for each of those the authors explain sources of emission, precursors as well as potential threats especially for human health. We can learn here that emissions of these air pollutants in Europe have decreased in the considered period, thanks to successful legislation. Despite that, the paper highlights how air concentration of pollutants has not always reflected the decline in emissions and European citizens are still breathing air whose quality is not complying with the World Health Organization Standards yet. For example, while SO<sub>2</sub> had a clear and marked decline, N compounds showed a contradictory behavior, with lowering concentrations but NO<sub>x</sub> emissions in 2002 being 5% higher than the ceiling set for the EU as a whole by the 2001/81 Directive and several exceedances of NO<sub>2</sub> annual limit value in 21 EU countries in 2011. Moreover, the article shows that there is a discrepancy between a cut in the emission of O<sub>3</sub> precursor gases and the change in observed average concentration of O<sub>3</sub> in Europe. This paper was published before the adoption of the NEC Directive, so few hints on the effects of air pollutants on ecosystems, namely vegetation, can be found when discussing eutrophication potential of N compounds and O<sub>3</sub> damages on plants (reduction of crop yields and reduction of forest growth).

Article 9 of the NEC Directive and the need for monitoring effects of air pollution on ecosystems on a national network of sites become relevant points inside De Marco et al. (2019, Fares & Paoletti, (2019), Ferretti (2021), published after the adoption of the NEC Directive. Fares & Paoletti (2019) put in evidence the high biodiversity level of Italian ecosystems and especially of Mediterranean environments, which make it difficult the selection of appropriate sites and indicators for the requirements of the NEC Directive. All of the three articles focus attention on the existing ICP Forests infrastructure, suitable sources for monitoring sites and indicators, having several characteristics that make it a very good basis for developing an advanced forest monitoring network responding to the Directive.

In addition, De Marco et al. give an overview of air pollution in Italy between 1999 and 2017: results show that SO<sub>4</sub><sup>2-</sup> deposition strongly decreased at all monitoring sites, while NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> decreased more slightly; moreover, O<sub>3</sub> concentration (and so AOT40, the metric identified to protect forest from ozone pollution) showed a decrease, while PODy (flux-based metric under discussion as new European legislative standard for forest protection); in this case also (as in the case of Guerreiro et al. 2014), N compounds and O<sub>3</sub> showed an ambiguous behavior.

Van Grinsven et al. (2016) and Serrano et al. (2019) are two interesting articles, going into details in assessing how the successful reduction of emissions following the implementation of legislation can be seen at ecosystem level. It has to be stressed that when the authors cite the NEC Directive, they refer to the 2001/81 Directive, before the revision occurred in 2016. However, Van Grinsven et al. consider the Nitrates

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Directive and the Water Framework Directive also, to assess the effectiveness of policy measures on the protection of aquatic ecosystems, especially ground water quality affected by large use of agriculture fertilizers, in the Netherlands between 1990 and 2012. General benefits of legislation measures on aquatic ecosystems were substantial in decreasing N and P surpluses, even if, concerning limits established by the 2001/81 Directive, NH<sub>3</sub> emission had an effective reduction but critical loads of nitrogen continued to exceed at the majority of the study areas.

Serrano et al. (2019) consider a study area composed of a mixed-land-use industrialized Mediterranean agroforestry system in southwest Europe, in 2002 and in 2011, using lichens as biological indicators of the impacts of N and S compounds on ecosystems. Under the considered air quality Directive (2001/81), the authors find that the reduction of S-emission and, consequently, air concentration were reflected by S-deposition decrease in lichens, but this was not the case for N, with a slight N reduction near industrial observation sites and an increase in N-deposition in lichens close to agricultural sites.

Piersanti et al. (2021) show a different approach to the implementation of air quality legislation by considering two models for NEC Directive 2030 targets, one “with measures” and one “with additional measures”, both presenting a significant reduction of the pollutants concentration. The model assessment was conducted with the MINNI system (National Integrated Model to support International Negotiation on the Atmospheric Pollution), an Integrated Assessment Model (IAM) suite developed by ENEA with ARIANET (private company) and IIASA (International Institute for Applied Systems Analysis) on behalf of the Italian

Ministry for Environment.

From the literature revision carried out we can make the following considerations: i) the need of a focus on certain air pollutants (i.e. O<sub>3</sub>, N compounds), characterized by a less clear behavior with respect to other atmospheric pollutants considered by the NEC Directive; ii) the importance of going beyond the evaluation of emissions or concentration to get into the details of biological responses of living organisms to air pollution impacts and foresees; iii) the potential of modeling to design scenarios close to the targets of the Directive.

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# LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



[https://www.mite.gov.it/sites/default/files/archivio/normativa/PNCIA\\_20\\_12\\_21.pdf](https://www.mite.gov.it/sites/default/files/archivio/normativa/PNCIA_20_12_21.pdf). 165 pagg.

## 2.3.1 Meteorological data

### Introduction

The increasing environmental pressures linked to both natural and anthropogenic factors increased the interest in monitoring forest ecosystems. Among them, Climate change gained great importance because it affects forests' health status both directly and indirectly. There are many aspects that complicate the description of the meteo-climatic condition that insist on a given territory. Among them, the duration and extent of the phenomena, the spatial extension, the possible influence on the forest system at various scales (from local to regional), are of particular importance. Consequently, the methodological approaches to analyse the phenomenon, the variables choice and the validation of possible climate indicators are also varied and multiple. Since 1997, the monitoring of weather conditions has been carried out by CREA-FL, that makes use of automatic monitoring stations within the extensive network of Con.Eco.For forest monitoring areas. The continuous implementation of this network allows to provide a significant amount of climatic, agro-climatic and pedo-climatic variables at a punctual level. Great attention is given to variables that are relevant for forest ecosystems (i.e. precipitation and thermometric regime), on which the evidences, at national and international level, of possible climate change processes in progress are clearer. The continuous monitoring of meteorological conditions also allows to highlight the distribution of some extreme events of potential interest for forests. The integration long-term monitoring of meteo-climatic conditions with other lines of research of the CONECOFOR program represent a significant wealth of information, supportive for inter- or co-disciplinary studies on forest ecology and plant physiology and, more generally, contributes to determining, on a detailed scale, the state of health of Italian forests.

### Materials and methods

Climate data acquisition is foreseen in each permanent area monitored, both under canopy cover (location in the plot) and in a large clearing (open field) within a radius of 2 km, in accordance with the provisions of EU Regulations 1091/94 and 690/95.

The monitoring consists in the automatic acquisition of daily and hourly data of the main variables of meteorological interest: air temperature at 2 m, relative humidity at 2 m, solar radiation, wind speed and direction, precipitation. The activity started in 1997 with 8 areas and 13 motoring stations. All the data collected have been validated and processed. The stations are installed and managed both by CREA-FL and by other bodies, while the data analysis is centralized to the CREA-FL biometeorology laboratory.

The meteorological data are sent to an autonomous database in which the descriptive metadata of the area (geographical and physical characteristics), of the control units (acquisition methods, installed instrumentation), of the sensors (technical characteristics), of the measured parameters and of their processing are collected. In the data entry phase, the database management program provides a series of checks and automatic processing.

Further checks on the operation of the stations and therefore on the validity of the data are carried out by processing the error codes reported by the detection system (data logger) in specific output variables. The

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



procedure for reporting values “out of range” (average values of the measured parameters were determined for previous years in the same area) is also active.

The "control" field indicates both which sensor gave unrealistic values and how long the problem lasted. These values are then used to evaluate the completeness and the quality of the data (as a percentage of good operating time).

Since 2016, thanks to the LIFE projects Smart4Action and Mottles, it has been possible to upgrade the hardware of the weather stations of 6 sites. This update allowed to send weather data (both hourly and daily values) to an FTP server and gave real time access to the data of the stations.

## Results

### *Climate analysis*

The climate of a given territory expresses the average state of the lower atmosphere above. Such a state is defined according to the value of a number of physical quantities, of which the most characteristics are air temperature and precipitation. These values can be calculated through statistical processing, using cumulated, averages or frequency indicators. Since interannual variability is significant, it is necessary to take into account as many years of observation as possible. Within the same year, the trends of climatic variables follow varied seasonal rhythms, which are expressed through curves that indicate, month by month, the variations of these quantities.

A detailed overview is provided by a set of descriptive statistics calculated on five fundamental meteorological variables. The elaboration highlights how the installed stations are able to gather, with precision, the multiplicity of climates observable along the Italian peninsula, both in terms of pluviometric and thermometric regimes. Focusing on precipitation we observed a shift from relatively low annual averages (630 mm in Tuscany, 749 mm in Emilia Romagna), which indicate potentially dry local conditions associated to medium hilltop forests, to much higher averages (1819 mm in Veneto, 1814 mm in Calabria), typical of cool and moist mountain forest areas.

The analysis of the thermometric regime highlights results similar to those already observed for pluviometry, emphasizing the representativeness of the monitoring of typical forest environments with respect to the different climatic conditions present in our country. The thermometric regime is strongly influenced by altimetry and this variable is therefore sufficient to explain the changes observed in both the minimum and maximum temperature on an annual basis and for the entire period. The study of the relationships between climate variables is of great interest for the analysis of climate regimes and their possible variations over time. The analysis, developed through suitable statistical procedures of a descriptive and inferential nature, also through parametric and non-parametric correlation analysis, proceeds at an annual or monthly level, on a large number of stations capable of producing a picture as articulated as possible of the meteorological conditions of a region representative from a geographical point of view.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### *Some characteristic weather events*

The continuous monitoring of meteorological variables allowed to identify some significant meteorological events observed in the first decade of the 2000s. The hottest and coldest years and/or seasons were identified and the territories most exposed to such excursions were associated with them. These trends show how, beyond the possible climate changes, the weather conditions on land are still oscillating from one year to another, from season to season, and this appears to be of particular importance for the analysis of the health status of forest ecosystems.

Considering, for example, the summer period, two consecutive summers represented, in the decade, the two climatic extremes. Summer 2002 was, almost everywhere, cold and rainy with temperatures lower than the averages of the period: e.g., -8% in Passo Lavazè (TRE1) and with increasingly abundant rainfall than the average between + 7% in Tarvisio (FRI2) and even + 160% in Cala Violina (TOS2). In Piedmont this phenomenon is particularly evident: 4 episodes were observed with precipitation exceeding 300 mm per day, in contrast to the other years of the decade, even in the year 2000 in which an alluvial episode was observed against the Po river. Summer 2003, on the other hand, was very hot and dry, with temperatures rising sharply. Deviations from the average for the period were between + 7% in Piano Limina (CAL1) and + 24% in La Thuile (VAL1) and Renon (BOL1). Rainfall was heavily scarce. The loss of precipitation was evident everywhere, ranging from - 8% in Passo Lavazè (TRE1) and - 62% in Renon (BOL1).

For the winter season, the winters 2004-2005 and 2006-2007 represented particularly harsh periods: compared to the periodic averages, the winter 2004-2005 recorded temperatures between -2% in Valsessera (PIE1) and even -72% in Brasimone (EMI2). The last months of the 2009 vintage are a prelude to a new cold season particularly rigid for the rapid lowering of the minimum temperatures observed during the winter months, which reached -22 ° C at Devero (PIE3) in December. This testifies that the normal and wide climatic variability expression of complex atmospheric regimes such as the Mediterranean cannot in itself be considered as a sufficient signal of climate change.

### *The wind regime as an example of a climatic anomaly*

A further example of the analysis of climatic variations and anomalous events is produced by the elaboration of the data of wind direction and intensity that have shown over the last decade a spatial configuration and a rather homogeneous seasonal distribution, except in some particularly relevant periods. From specific graphs of the wind roses it can be observed that both variables are distributed in a rather homogeneous way over the years, except for 2003 (which represents an anomaly). In that year, in the FRI-2 station there was a prevailing direction from the south-west, in contrast to the prevailing direction from the east detected in all other years and which coincided with a generalized thermal increase and a decrease in precipitation.

Comparing a normal year (2002) with the anomalous year (2003), the analysis of the monthly data shows that it was the spring-summer period (from May to September) 2003 that represented a strong anomaly, with wind direction prevailing from the southeast unlike the previous year in which in the same months a prevailing direction from the east had been recorded, as usual, with quite representative intensities also from the north-east and south-east.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### *Hydrological balance*

The hydrological balance of a soil sample in a given time interval can be represented by the formula (eqn. 1):

$$R_f = R_i + P_u - ET - S$$

where  $R_i$  and  $R_f$  are the water reserves, respectively at the beginning and at the end of the period,  $P_u$  represents the useful precipitation,  $ET$  is the evapotranspiration and  $S$  is the surplus, that is, water that comes out of the sample for percolation in the deeper layers.

The concept of “useful precipitation” derives from the consideration that water does not penetrate completely into the ground, but that a fraction of it exits the system by surface sliding. This fraction depends on numerous factors including the slope, the permeability of the soil and the intensity of the precipitation itself. For the latter variable, the data collected showed that the percentage distribution of the total rain by classes of hourly intensity, despite the annual variability, has a trend for each site.

Within the FutMon project, occurred during the last two years of observations (2010-2011), the D3 demonstrative action aimed at setting up and validating models for the estimation of the hydrological balance by measuring the water content of the soil with special sensors (TDR), replicated three times at three levels of depth, was included. The Action involved 5 areas of the network (ABR1, EMI1, FRI2, LAZ1 and TRE1) in addition to the already present monitoring scheme without replication (late 2003-early 2004) in the EMI1, LAZ1 and PIE1 areas.

The balance was carried out on the three depth levels (0-20, 20-40 and 40-60 cm), evapotranspiration was estimated with the Thornthwaite formula and was attributed to the three layers proportionally to the root mass present in the relative layer.

By plotting the balance, it is possible to highlight the risk of drought more or less extensive in the periods July-September. The occurrence of water deficit causes a reduction in the transpiratory activity of plants by closing the stomata, resulting in a lower production of organic matter compared to the amount potentially allowed by other climatic parameters.

As an overall information, it is evident that about 43% of the total rain has moved away from the system by surface sliding and leaching in the deep layers.

### **2.3.2 Visibility**

#### **Introduction**

Visibility is defined by the WMO as the greatest distance at which a black object of suitable dimensions located on the ground can be clearly seen against the horizon during daylight (Manara et al. 2019). Meteorological conditions (especially relative humidity) and the emission of pollutants (in particular, gases

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



and particulate matter) can affect visibility, as light is absorbed and scattered by the particles dispersed in the atmosphere. Resulting haze, fog, plumes and smog can impair visual range and its traits. In simple words, visibility refers to the transparency of air and offers the most instinctive measure of air quality (Hyslop 2009). When referred to natural protected landscapes, visibility can be also considered an aesthetical value and an ecosystem service, impacting on the recreational potential of natural areas.

In the United States, national parks have been monitoring air quality since the late 1980s to better understand how air pollution affects what we can see.

In 1985, the IMPROVE program (Interagency Monitoring of Protected Visual Impairment) was started, as a cooperative effort to document long term trends in visibility: the US Environmental Protection Agency (EPA), the US National Park Service (NPS) and Forest Service (FS), together with other national environmental agencies, implemented an extensive long term monitoring program to establish visibility conditions, track the changes and determine causal mechanism for the visibility impairment in the National Parks and Wilderness Areas.

Visibility monitoring, according to the IMPROVE protocol, has an “aerosol component” regarding the analysis of air composition and quality, an “optical component” concerning physical properties of the atmosphere like extinction, scattering and absorption coefficients and a “scenery component” concerning scene characteristics like colour, texture, contrast of the view. All components are relevant to LIFE MODERn(NEC), as the project is going to use specific indicators to measure the impacts of air pollution on ecosystems.

Visibility monitoring can be also integrated with different indicators, like atmospheric depositions chemistry and lichens biodiversity. Moreover, being able to make air pollution measurable, visibility is useful to explain air pollution to citizens and the general public and raise their awareness about the topic.

Here we consider and briefly review a selection of publications and scientific articles concerning visibility. Being a new indicator for Europe, emerging literature is mainly from the United States, with one report by EPA (publication n.1), one book (publication n.2) and three scientific papers (publications 3, 4 and 5), one of which from an Italian working group.

### Results and discussion

EPA (1999) and Malm (1999) can be considered primary publications concerning visibility monitoring. The EPA “Visibility Monitoring Guidance” (EPA 1999) is a comprehensive reference about visibility monitoring, including a legislative background in U.S.A., definitions and theory, a summary of visibility monitoring goals and objectives, guidance for site selection, description of methods, protocols and equipment, a classification of air impairments.

Concerning visibility goals, the first objective of the guidelines of this monitoring activity is “... the prevention of any future, and the remedying of any existing, impairment of visibility in mandatory Class I Federal areas which impairment results from man-made air pollution”, where Class I Federal areas, also specified by the US Clean Air Act, are mainly national parks and national wilderness areas. Thus, visibility becomes a protected resource among the various ecosystem services and it is clear that, in order to protect visibility, scientists have to monitor air quality.

Air quality is considered under several aspects in Malm (1999), where the author investigates the electromagnetic nature of light, the interaction of light with suspended particles in the atmosphere, transport and transformation of particles, visibility metrics and a comparison among them, including (i)

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Visual Range (VR) = the greatest distance that an observer can see a black object viewed against the horizon sky; (ii) Light extinction ( $b_{\text{ext}}$ ) = the attenuation of light due to scattering and absorption as it passes through a medium; (iii) Deciview (DV) = unitless metric of haze, proportional to the logarithm of  $b_{\text{ext}}$ ; this measure overcomes the limitation that  $B_{\text{ext}}$  is not linearly related to one's perception of haze, it means that a change is one DV is perceived to be the same on clear and hazy days.

The author also presents the expected visibility natural conditions and the current conditions and trends in the U.S.A. at the time of the publication (late 1990s)

Moving to scientific papers, Malm et al. (1993) investigates the major visibility-reducing aerosol species, sulfates, nitrates, organic, light-absorbing carbon and wind blown dust as well as light scattering and extinction in the period 1988 - 1991, that is the first three years of the IMPROVE program, at a network of 36 sites mostly located in the western US. Sulfates and organics are responsible for most of the extinction throughout the U.S., while in southern California nitrates are dominant. In eastern U.S. sulfates contribute to about two thirds of the extinction. In most cases, concentrations and extinction are higher during summer.

Hyslop (2009) provides a further contribution in the study and understanding of visibility, also enriched by a socio-philosophical point of view. In fact, the author investigates human aesthetic appreciation of nature and perception of air pollution, considering the dominance of sight in the human nervous system and the fact that 25-35 % of our brain is dedicated to the processing of visual inputs.

In Manara et al. (2019), the dataset is composed of daily visibility records from 97 Italian Air Force stations in the period between 1951 and 2017. Visibility is measured with a subjective method (an operator scanning the horizon for pre-determined objects, see reference) and the metric used is linear (like VR). This dataset is used to calculate regional average visibility for 5 Italian climatic regions. Although no protected areas and environmental value is considered, the lowest values were found in the Po Valley. The time threshold for visibility increase-decrease is 1980.

The present literature selection suggests the high relevance of the measuring of visibility in Italy, especially for the purposes of LIFE MODERN(NEC) although the size of our national territory, biodiversity levels and the transition among the various ecosystems poses challenges to the scientific assessment of visibility related to air quality.

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## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### 3. Collection of time series of data

The aim of this activity is to gather available data time-series at national level of pollutants emissions on one side and of ecological responses on the other side, in order to assess the current status of the impacts of atmospheric pollution on forests and freshwater ecosystems and evaluate the possibility to design a baseline.

Data sets have been provided mainly by the project beneficiaries, being in charge of the assessment and management of the main monitoring programmes in the context of forest and freshwater ecosystems in Italy. The data time-series of the last 20 years (2000-2020) has been considered, with from 6 to 31 sites monitored per year. In particular:

- Forest sites: data were mainly obtained by the ICP Forests network (CON.ECO.FOR.), the LIFE project Smart4Action (2014-2018), the LIFE MOTTLES (2016-2020), and the existing monitoring sites of the NEC Italy Network (2019-2020).
- Freshwater sites: data were gathered mainly from the ICP Waters programme.
- Air pollutants: data on air pollutants, both emissions and depositions, were obtained by the ICP Forests network (CON.ECO.FOR.), the EMEP models and by the network of automated gauges of the Italian Environmental Protective Agency (ISPRA).

Collected data on ecosystems responses have been organized into two folders, forests and freshwaters. Worksheets have been organized in Excel and/or CSV format.

Please refer to the following section (see § 4) to obtain detailed information on the data characterization (see in particular § 4.1: Tables 4.1 - 4.3 ), their structure, time series and gaps (see also Annex A1.1).

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



## 4. Data processing and synthesis of the state of the art

The main objective of this activity is to perform a data screening to discriminate the impacts of air pollution on reactive components of ecosystems from those pressures related to different environmental factors (i.e., forest management, climate change).

The dataset collected during the second phase of the Action (see § 3) have been refined and processed to further improve their suitability for the data analyses.

As expected by the project, a multivariate approach was adopted with a double in-depth analysis: i) descriptive multivariate statistics for each indicator; and ii) a comprehensive integrated elaboration by means of multiple linear regression models.

Please refer to the Annex A1.1 for detailed information on the procedures and results.

### 4.1 Data characterization

A thorough screening of the set of data was performed to explore their completeness and to obtain a clear framework of their distribution both from the spatial (number of represented sites) and the temporal (number of yearly surveys) point of view.

Please refer to the Annex A1.1 for detailed information on the procedures and results.

#### 4.1.1 Environmental pollution data (data from 2000 to 2020)

The following datasets were considered:

- EMEP models based on the concentrations of 6 air pollutants ( $PM_{10}$ ,  $NO_x$ , O<sub>3</sub>, RDN,  $SO_4$ ,  $SO_2$ ). The concentration values modelled in the cell to which they belong (about 10x10 km) have been attributed to each forest or freshwater site.
- EMEP models based on the depositions of 3 air pollutants (O<sub>3</sub>, RDN,  $SO_2$ ) and precipitations. The deposition values modelled in the cell to which they belong (about 10x10km) have been attributed to each forest or freshwater site.
- Air pollution data deriving from the Italian level network of automatic gauges ( $C_6H_6$ , CO,  $NO_2$ ,  $NO_x$ , O<sub>3</sub>,  $PM_{10}$ ,  $PM_{2.5}$ ,  $SO_2$ ). This dataset includes the series of data (years 2013-2020) measured by approx. 200 sampling sites at the national level. The values deriving from the nearest automatic gauge (range from 3 to 50 km) were attributed to each forest site, by preferring the selection of the automatic gauges positioned in rural or remote sampling sites. The free cartographic software Quantum GIS 2.18.22 was used for all the analyses. This dataset was available only for forest sites since freshwater sites are too far from the network of automatic gauges.
- Atmospheric depositions measured in the forest sites belonging to CONECOFOR network (both in open field and under the canopy).
- Ozone concentrations measured in the forest sites belonging to the NEC Italy network.

Table 4.1 reports a summary of the available dataset for environmental pollution data, with a detail for each indicator of: number of variables/parameters, number of sites, number of years, data completeness and the responsible beneficiary collecting or providing the data.

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



A total of 3 indicators have been obtained for forest and freshwater sites, with variables ranging 4-13, number of sites 6-31 (for modelled data: 10 freshwater sites and 31 forests), number of years 3-21. For each relative combination of sites x years, the missing data are very few, with values of data completeness ranging from 87.4 to 100%.

Please refer to the Annex A1.1 for detailed information on the procedures and results.

*Table 4.1 – Summary of the available dataset for environmental pollution data.*

Indicator	Detail of the measured or sampled parameters	N. variables	N. sites	N. years	Data completeness (%)	Responsible Beneficiary
EMEP models, concentrations (2000-2019)	Modelled values of air pollutants concentrations at the forest and freshwater sites: PM <sub>10</sub> , NO <sub>x</sub> , OXN, RDN, SO <sub>4</sub> , SO <sub>2</sub>	6	10-31	20	100	TerraData, CNR
EMEP models, depositions (2000-2019)	Modelled values of air pollutants depositions at the forest and freshwater sites: OXN, RDN, SO <sub>2</sub> , prec.	4	10-31	20	100	TerraData, CNR
Air pollution concentrations measured by the ARPA-ISPRA automatic gauges network (2013-2020)	Air pollutants concentrations measured by the automatic gauges and attributed to each forest site: C <sub>6</sub> H <sub>6</sub> , CO, NO <sub>2</sub> , NO <sub>x</sub> , O <sub>3</sub> , PM <sub>10</sub> , PM <sub>2.5</sub> , SO <sub>2</sub>	12	31	8	87.4	ENEA, ISPRA
Atmospheric depositions	Pollutants measured in the waters collected in the plot (throughfall) and in the open field	13	26	21	97.2	CUFAA, CNR (IRSA)
Ozone concentrations	Ozone concentrations measured in forest sites	5	6	3	96	CNR (IRET)

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



### 4.1.2 Forests (data from 2000 to 2020)

The map of Figure 4.1 shows the distribution of the forest sites in Italy (CONECOFOR Level II sites). Annex A1.1. provides also an interactive map where it is possible to obtain the following general information for each site: plot number, plot code, locality, prov, zone, E (UTMWGS84), N (UTMWGS84), altitude, main tree species, forest type, forest management.

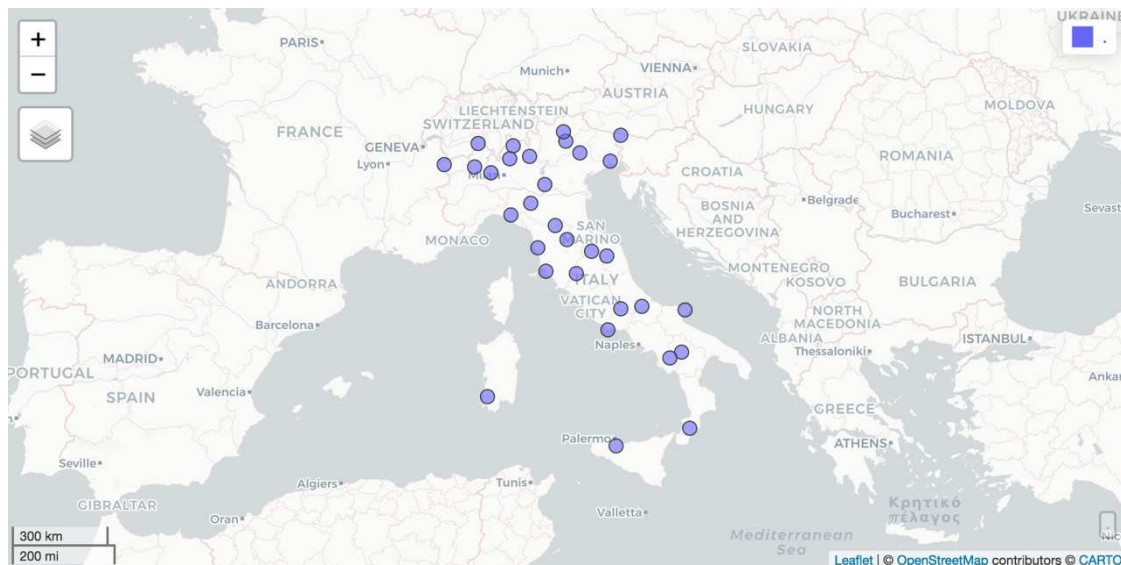


Figure 4.1 – Map with the distribution of CONECOFOR Level II sites.

Table 4.2 reports a summary of the available dataset for forests, with a detail for each indicator of: number of variables/parameters, number of sites, number of years, data completeness and the responsible beneficiary collecting or providing the data.

A total of 8 datasets are represented in forest sites, with variables ranging 3-24, number of sites 6-31, number of years 4-21. For each relative combination of sites x years, the missing data are very few, with values of data completeness ranging from 86.1 to 100%.

Please refer to the Annex A1.1 for detailed information on the procedures and results.

# LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Table 4.2 – Summary of the available dataset for forest sites.

Indicator	Detail of the measured or sampled parameters	N. variables	N. sites	N. years	Data completeness (%)	Responsible Beneficiary
Foliar analysis	Element concentration in the leaves	6	31	10	95	CNR (IRET)
Soil solution	pH, nitrates, sulphates	6	12	21	88.4	CUFAA, UNIFI (DST)
Ground vegetation	List of species and diversity	6	31	18	100	UNICAM
Epiphytic lichens	List of species and diversity	6	16	21	86.5	TerraData
Crown condition	Crown defoliation and damages by pests	11	31	20	89.5	CUFAA, UNIFI (DAGRI)
Forest growth	Volume and its increase, carbon content	3	31	5	98.2	CREA
Ozone injuries	Symptoms on plants: % of symptomatic species; % of symptomatic leaves	3	6	4	100	CNR (IRET)
Meteorological data	Main climatic parameters within forest sites	24	22	21	86.1	CREA

### 4.1.3 Freshwaters (data from 2000 to 2020)

The map of Figure 4.2 shows the distribution of the freshwater sites in Italy (ICP Waters sites). Annex A1.1. provides also an interactive map where it is possible to obtain the following general information for each site: plot code, plot name, locality, E (UTMWGS84), N (UTMWGS84), Altitude.

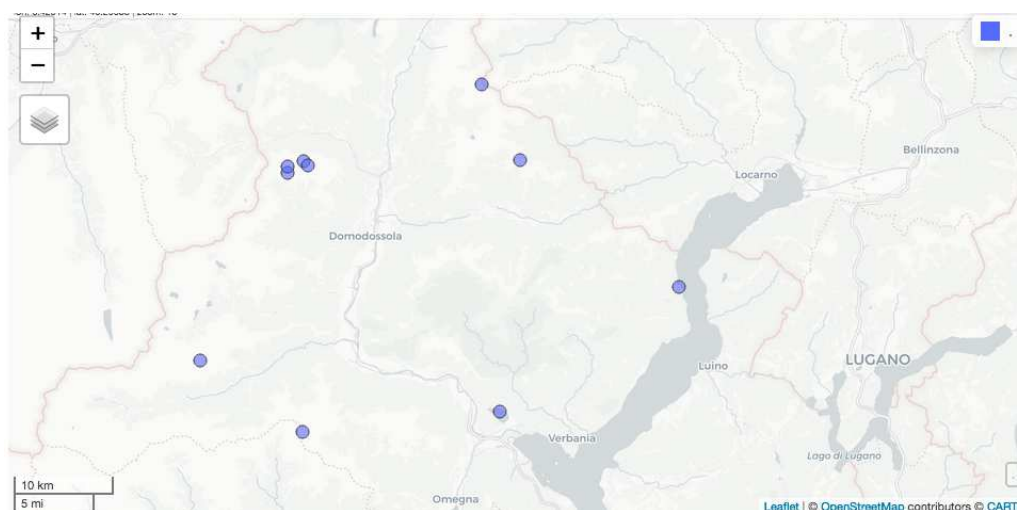


Figure 4.2 – Map with the distribution of the freshwater sites.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Table 4.3 reports a summary of the available dataset for freshwater sites, with a detail for each indicator of: number of variables/parameters, number of sites, number of years, data completeness, and the responsible beneficiary collecting or providing the data. A total of 3 indicators is represented in freshwater sites, with variables ranging 5-22, number of sites 6-8, number of years 2-7. For each relative combination of sites x years, the missing data are very few, with values of data completeness ranging from 88.2 to 100%.

Please refer to the Annex A1.1 for detailed information on the procedures and results.

*Table 4.3 – Summary of the available dataset for freshwater sites.*

Indicator	Detail of the measured or sampled parameters	N. variables	N. sites	N. years	Data completeness (%)	Responsible Beneficiary
Water chemistry	Chemical data required by the ICP Waters Manual	22	6	7	88.2	CNR (IRSA)
Diversity of diatoms	List of species and diversity	12	8	2	100	
Diversity of macroinvertebrates	List of species and diversity	5	6	2	100	

## 4.2 Conceptual scheme

Figure 4.3 reports a simplified conceptual scheme grouping the variables enlisted in the previous paragraphs into two main categories:

- i) **drivers**, that is the predictive variables which can influence the ecological indicators considered in the project.
- ii) **ecosystem responses**, that is all the environmental variables (ecological indicators) which can be affected by the environmental predictors. It is possible to hypothesize potential relationships between drivers and ecosystem responses, with a positive or negative effect to be assessed by further data processing. This is only a simplified scheme since it is possible that correlative relationships exist also among the indicators grouped within the ecosystem responses or within the drivers. For example, crown conditions may affect light availability for plants and epiphytic lichens, with an influence in their species composition, or water chemistry may influence macroinvertebrate communities.

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Deliverable, action A1



### Predictive variables (drivers)

Air pollutants concentrations  
(EMEP models)

Air pollutants depositions  
(EMEP models)

Air pollutants  
(ISPRA automatic gauges)

Ozone concentrations  
(MOTTLES)

Atmospheric depositions

Meteorological data



### Indicators (ecosystem responses)

Foliar analysis

Soil solution

Ground vegetation

Epiphytic lichens

Ozone symptoms

Crown condition

Forest growth

Water chemistry

Diversity of diatoms

Diversity of  
macroinvertebrates

Figure 4.3 – Simplified conceptual scheme grouping the variables in drivers and ecosystem responses.

### 4.3 Selection of the most suitable data processing procedure

Starting from the type, structure and completeness of the available data, the following univariate and multivariate analyses were adopted.

- i) Considering each indicator individually (both driver and response), we studied the temporal trends of each variable, we obtained the correlation matrix between couples of variables, and we performed a Principal Component Analysis (PCA). In particular, PCA was used as explorative unsupervised multivariate analysis to study the relationships among variables within each indicator.
- ii) Once this first exploratory survey was carried out, multiple linear regression models (MLRM) were adopted to obtain an overall picture of the relationships among drivers and ecosystem responses. In particular, Generalized Linear Models (GLM) were adopted to explore the relationship among the response and predictive variables in order to find out the best predictors explaining its variability. We have used a combined approach of multiple linear regression models to study the multicollinearity among variables, and GLM to explain response variable distribution in relation to predictive variables.

### 4.4 Results of the univariate and multivariate analysis for each indicator

This section reports a detailed analysis of the distribution of each indicator belonging to the three groups: air pollutants, forests and freshwaters. In particular, the following results are reported for each indicator, deriving from univariate and multivariate analyses:

- exploratory charts with the temporal distribution of each variable, to provide useful information for identifying any trend along the explored data series.

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- Pie charts. They have been used for variables with a categorization in score values (percentage levels of defoliation and ozone symptoms).
- Correlation matrix between the variables for each forest and freshwater site. The variables were correlated in pairs in order to identify positive or negative relationships. This preliminary study on the correlation among variables is important for the further regressive model settings to avoid multicollinearity and to select only those variables not too correlated with the others.
- Principal Component Analysis (PCA) results: score plots of the PCA ordination were provided based on several categorical variables (year, site, altitude, tree species, forest type and forest management). For quantitative variables, the length of overlaid vectors is proportional to Pearson  $R^2$  with the axis.

All the analyses have been carried out by means of the software R (R Core Team 2021).

Please refer to the Annex A1.1 for detailed information on the procedures and results.

The main results are commented in the following paragraphs.



## 4.4.1 Air pollutants

**Modelled air pollutants concentrations in forests.** In the considered time span (2000-2019), a general decrease of the modelled concentrations (EMEP models) of the air pollutants is observed, even if it is less evident for oxidized nitrogen (OXN) and reduced nitrogen (RDN). These results are confirmed by the PCA, where positive values of PC1 show clear temporal and altitudinal increasing gradients, in relation to a general decrease of air pollutants, which are mainly correlated to negative values of PC1 (Figure 4.4).

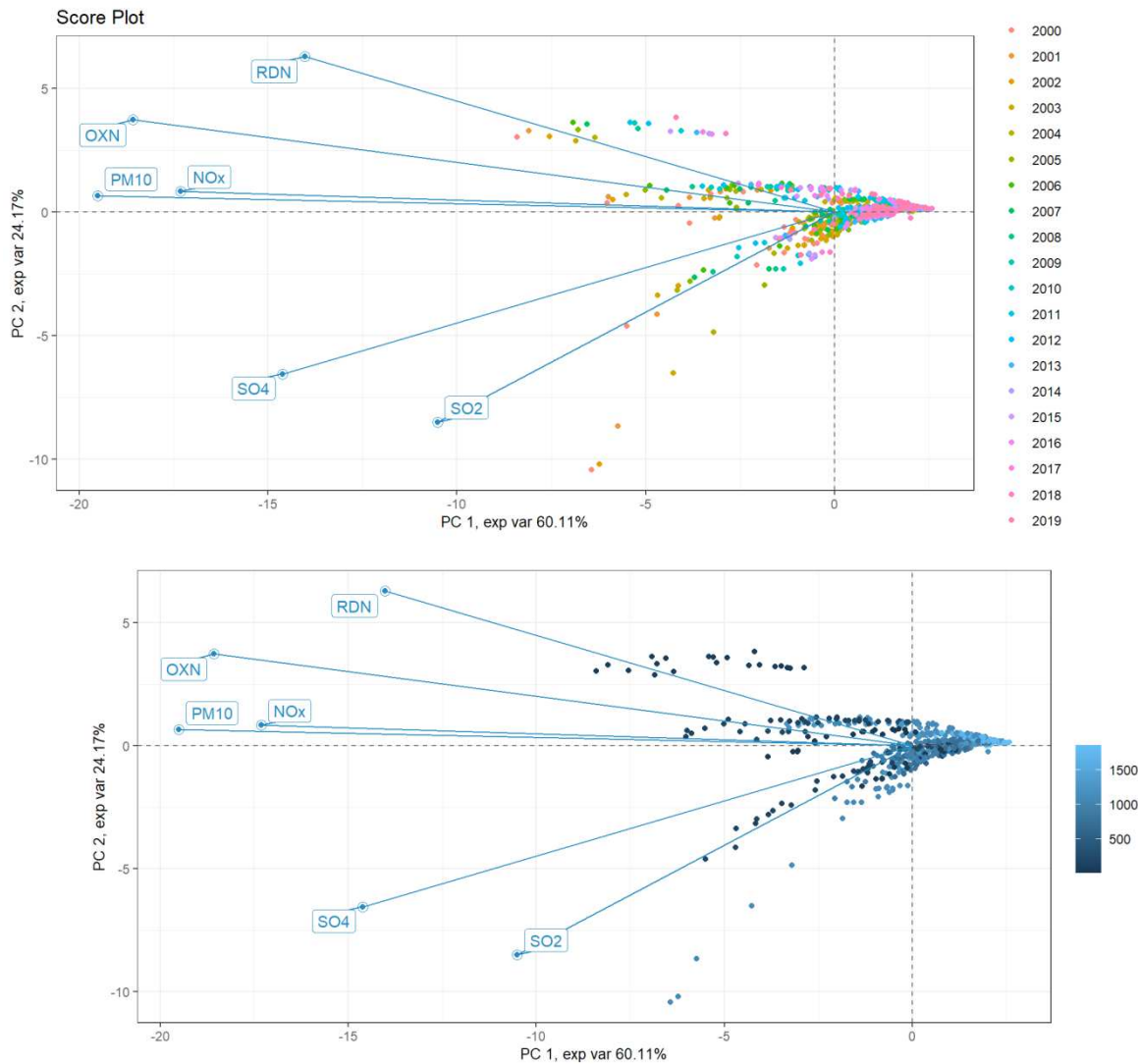


Figure 4.4 – Score plots of the PCA ordination of modelled concentrations of air pollutants (EMEP models) in correspondence of the forest sites categorized by year (above) and altitude (below).

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**Modelled air pollutants concentrations in freshwaters.** Similar results were obtained for freshwater sites, even if with less evident trends. PCA results show a decreasing temporal and altitudinal gradient along positive values of PC1, in relation to an increasing gradient of air pollutant concentrations (Figure 4.5).

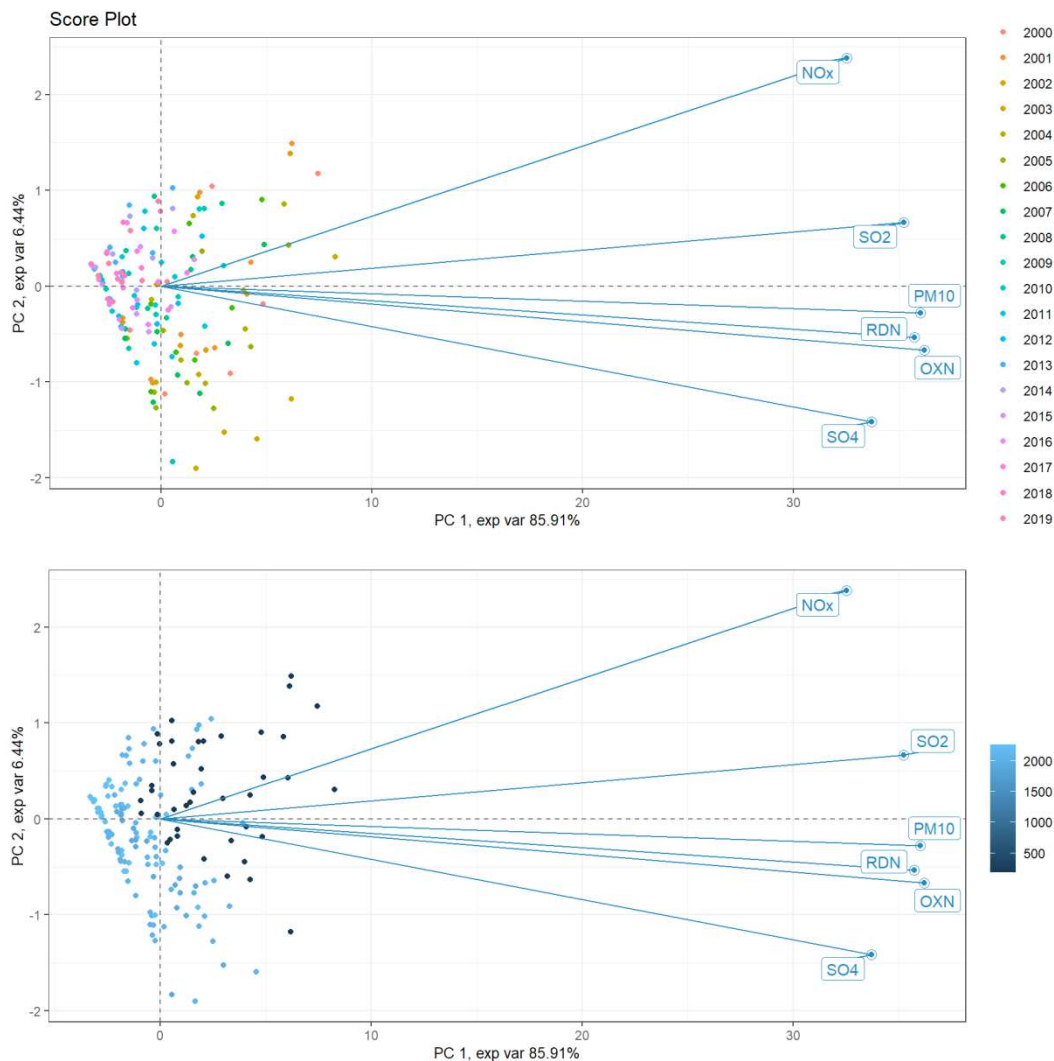


Figure 4.5 – Score plots of the PCA ordination of modeled concentrations of air pollutants (EMEP models) in correspondence of the freshwater sites categorized by year (above) and altitude (below).

**Modelled air pollutants depositions in forests.** In the considered time span (2000-2019), a general decreasing of the modelled depositions (EMEP models) of the air pollutants is observed only for SO<sub>x</sub> in the first decade of the period, while a trend is not evident for oxidized nitrogen (OXN) and reduced nitrogen (RDN). As regards the results of the PCA, PC1 explains the variability due to the altitudinal gradient, with the highest depositions values at lower altitudes. The second principal component explains the variability in the modelled precipitation values, with *Quercus cerris* and *Larix decidua* sites showing respectively the lowest and the highest rainfall values (Figure 4.6).

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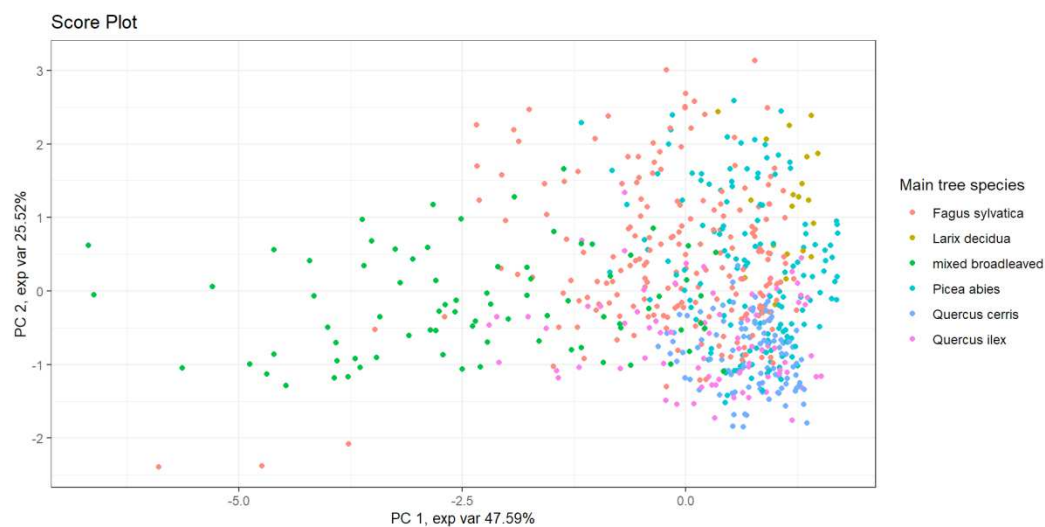
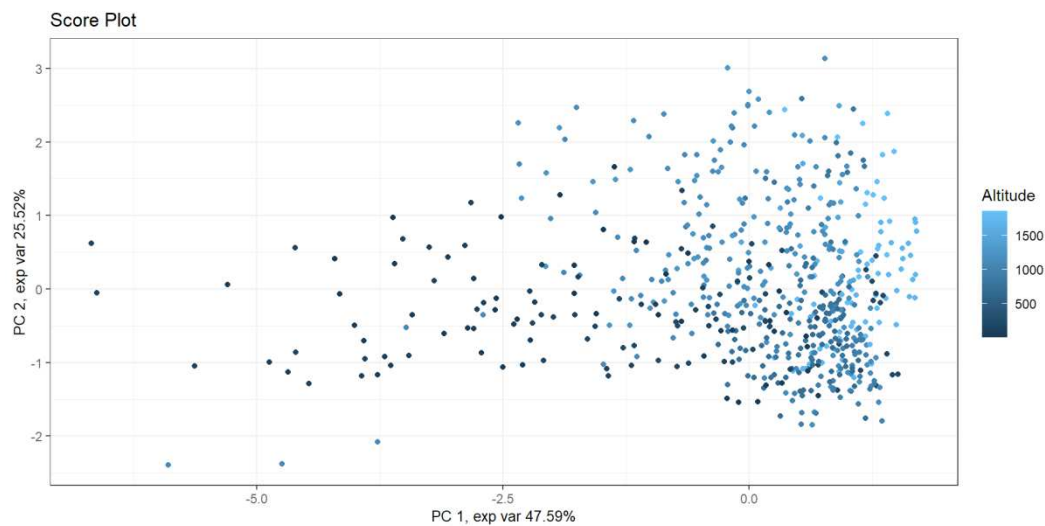
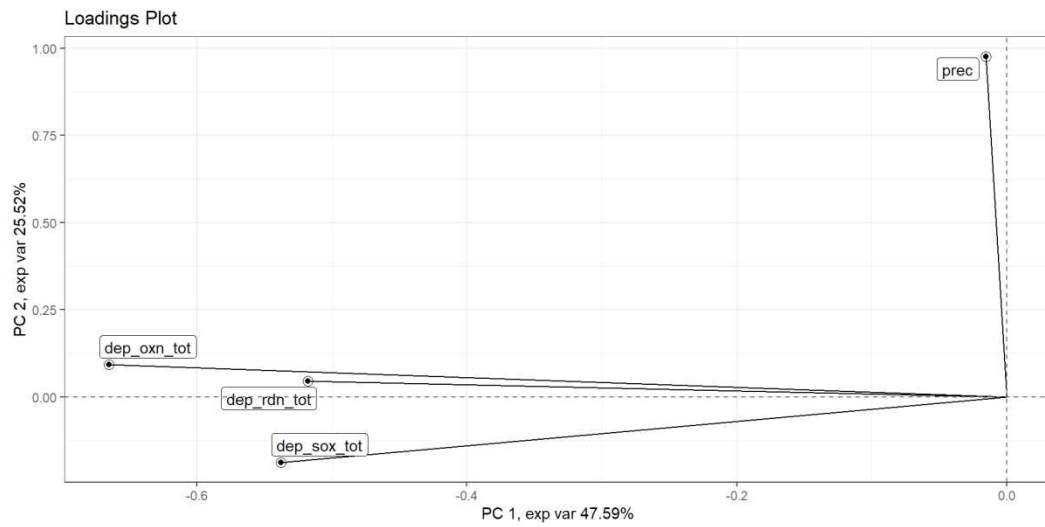


Figure 4.5 – Loadings (above) and score plots of the PCA ordination of modeled depositions of air pollutants (EMEP models) in correspondence of the forest sites categorized by altitude (middle) and main tree species (below).

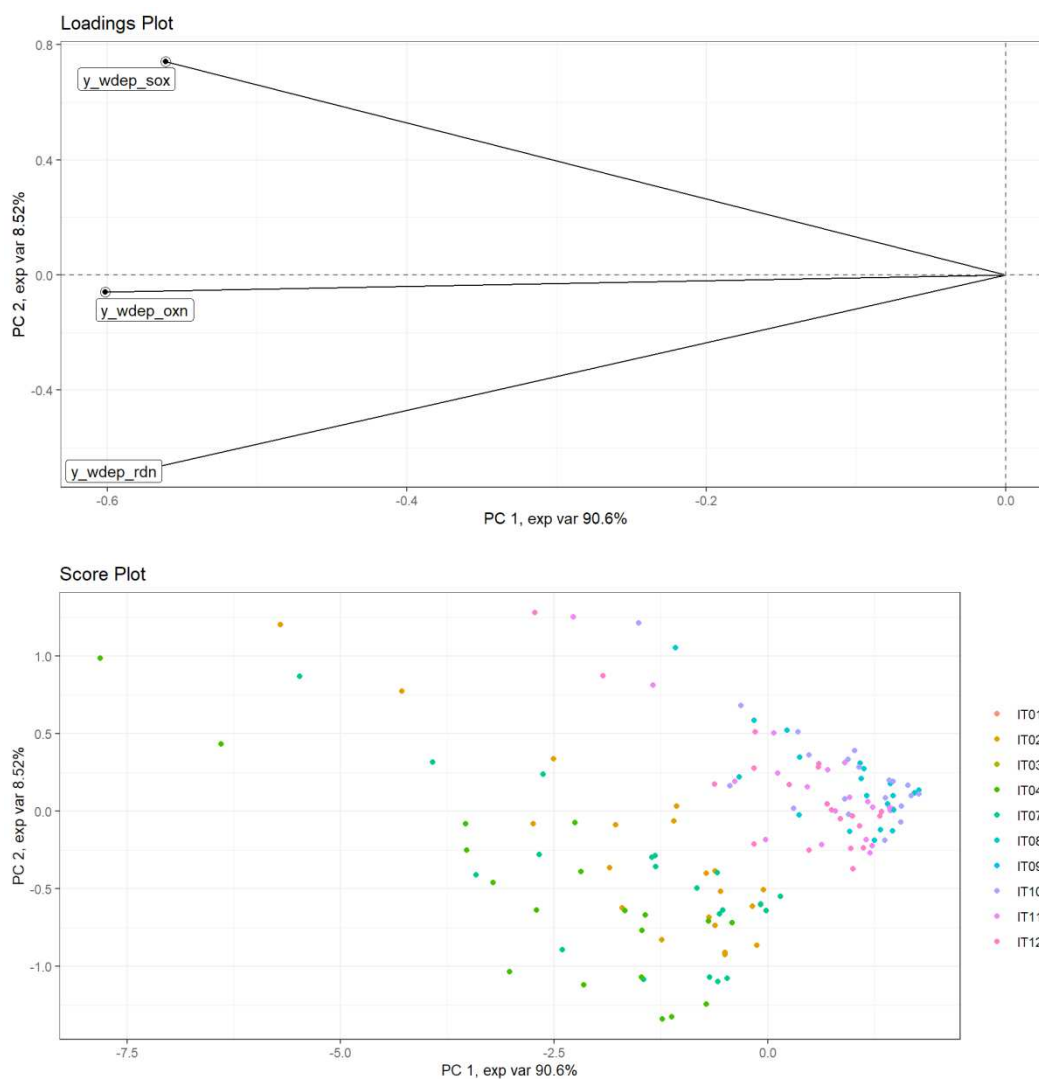
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**Modelled air pollutants depositions in freshwaters.** In the considered time span (2000-2019), a general decrease of the modelled depositions (EMEP models) of the air pollutants is observed, even if it is less evident for reduced nitrogen (RDN). The PC1 of the PCA explains most of the variability (90.6%), showing an altitudinal gradient where the sites at the lowest altitudes are characterized by the highest deposition values (Figure 4.6).



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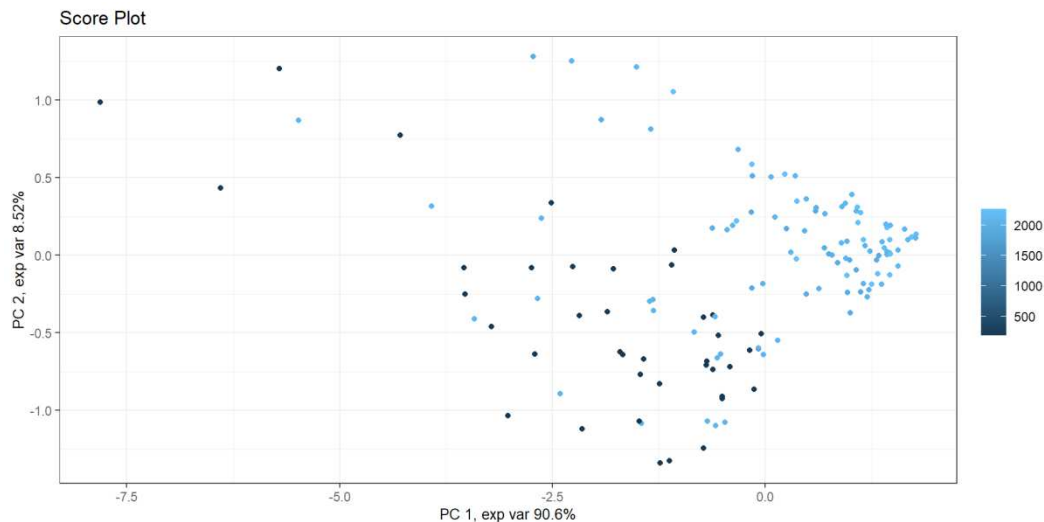
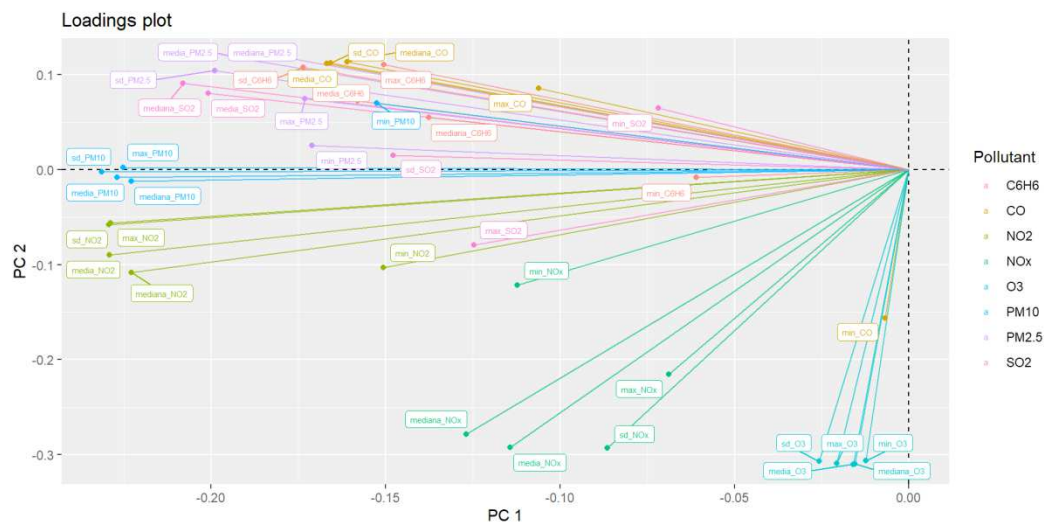


Figure 4.6 – Loadings (above) and score plots of the PCA ordination of modelled depositions of air pollutants (EMEP models) in correspondence of the freshwater sites categorized by site code (middle) and altitude (below).

**Air quality automatic gauges network.** In the considered time span (2013-2020), a clear temporal trend in air pollutants is not evident. The PCA explains the variability in terms of forest site: the yearly-observations relating to the same plot are grouped together, and no evident trends in main tree species or altitudes are observed (Figure 4.7).



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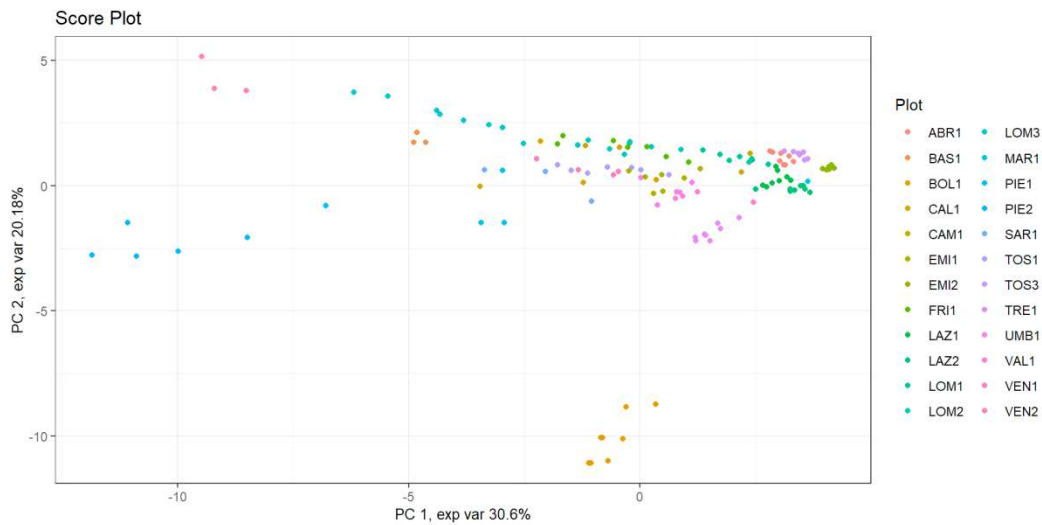
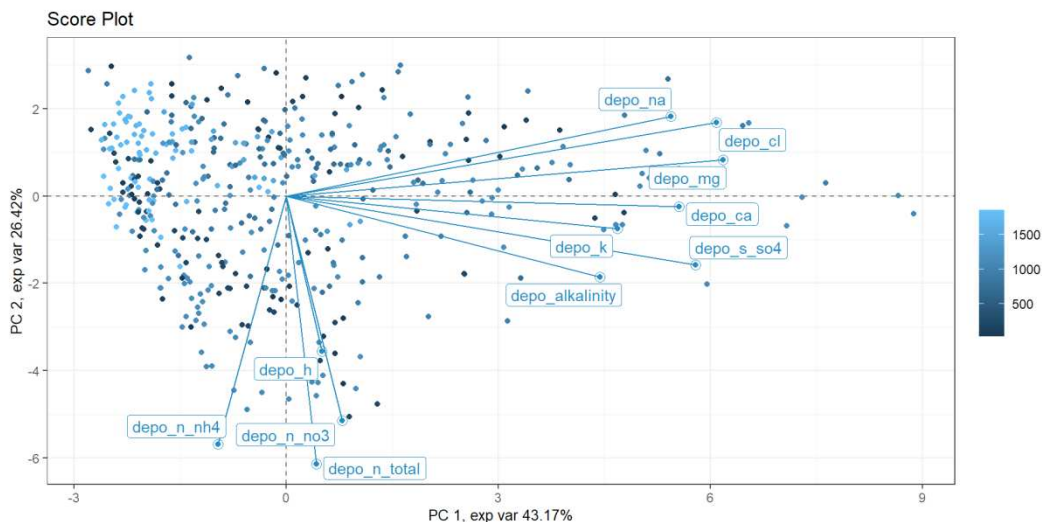


Figure 4.7 – Loadings (above) and score plots of the PCA ordination of air pollutants measured by the automatic gauges nearest to each of the forest sites categorized by site code (below).

## Measured atmospheric depositions in forests.

Both ‘Throughfall’ (‘Sottochioma’ in the dataset) and ‘Open field’ data have been considered for data processing. For each forest site boxplots were obtained (see Annex A1.1). In the considered time span (2000-2019), a general trend of the measured atmospheric depositions is not evident.

A first PCA was performed using both the datasets (Throughfall and Open field data). Two uncorrelated groups of variables can be distinguished along PC1 (main elements, SO<sub>4</sub> and alkalinity) and PC2 (nitrogen depositions). PC1 shows an altitudinal gradient, with the sites at the highest altitudes characterized by the lowest values of the first group of variables. PC2 explains the variability due to the distribution of the main tree species, with *Picea abies* and *Quercus cerris* showing the lowest values of nitrogen depositions (Figure 4.8, above and middle). PC3, explaining only the 9.1% of the variability, showing that the mixed broadleaved forests are characterized by the highest values of NH<sub>4</sub> depositions (Figure 4.8, below). Similar results were obtained from the PCA elaborated separately for Throughfall and Open field data (see Annex A1.1).



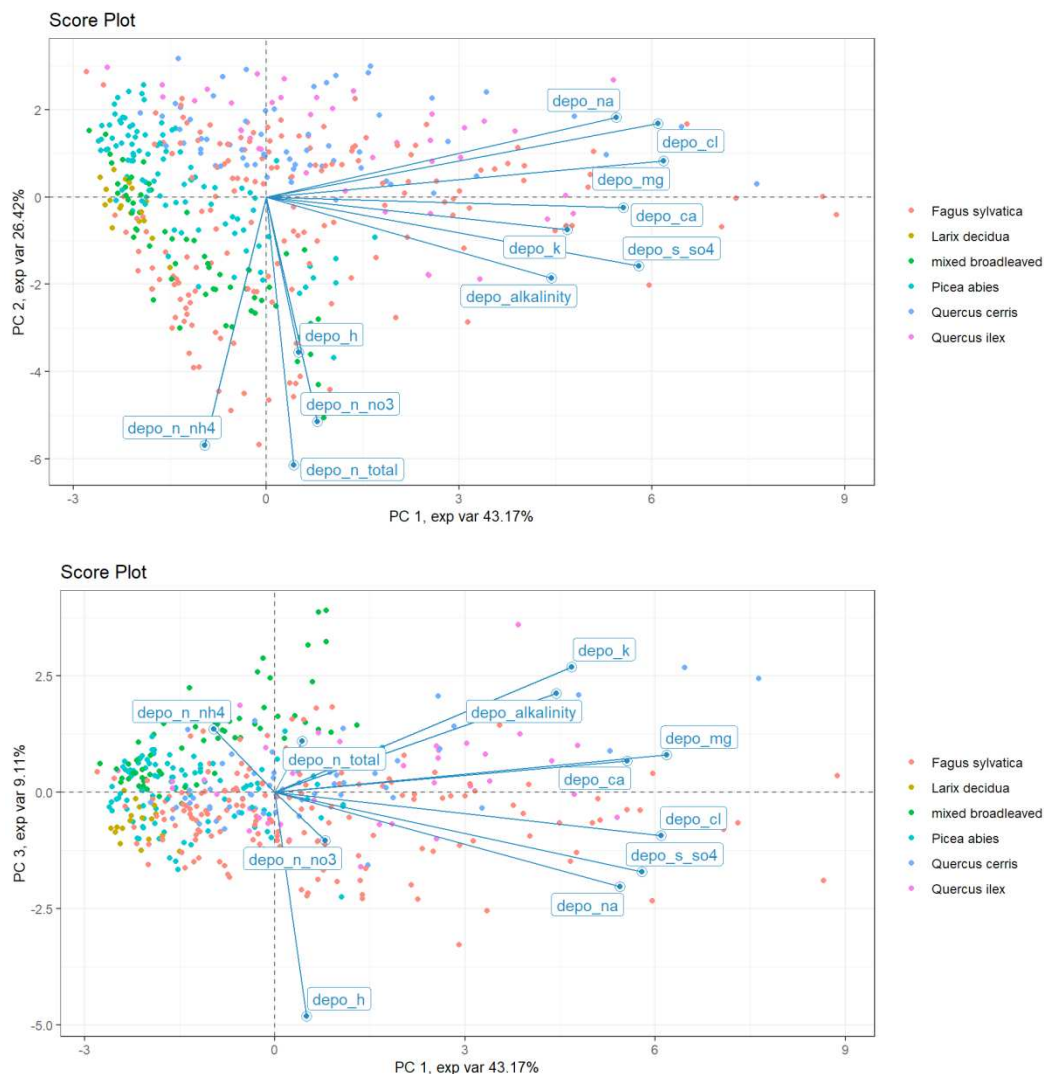


Figure 4.8 – Score plots of the PCA ordination of the measured atmospheric depositions in the forest sites (including both throughfall and open field data) categorized by altitude (above, PC1 vs PC2) and main tree species (middle, PC1 vs PC2; below, PC1 vs PC3).

**Measured ozone concentrations in forests.** As for the measured ozone concentrations, three yearly surveys (2018-2020) for 6 forest sites of the Italian NEC network are available. Although it is a very short period, the 2020 survey shows a decrease in the values of  $O_3$  (both annual and during the growing season) and AOT40 in all the plots (Figure 4.9), while the POD values do not show obvious trends.

The PC1 of the PCA clearly distinguishes POD1 and POD2 from the other variables, while the PC2 explains the variability between plots. In particular, mixed broadleaves and Turkey oaks, which are distributed at lower altitudes, are linked to positive values of PC2, showing the lowest values of  $O_3$  (Figure 4.10).

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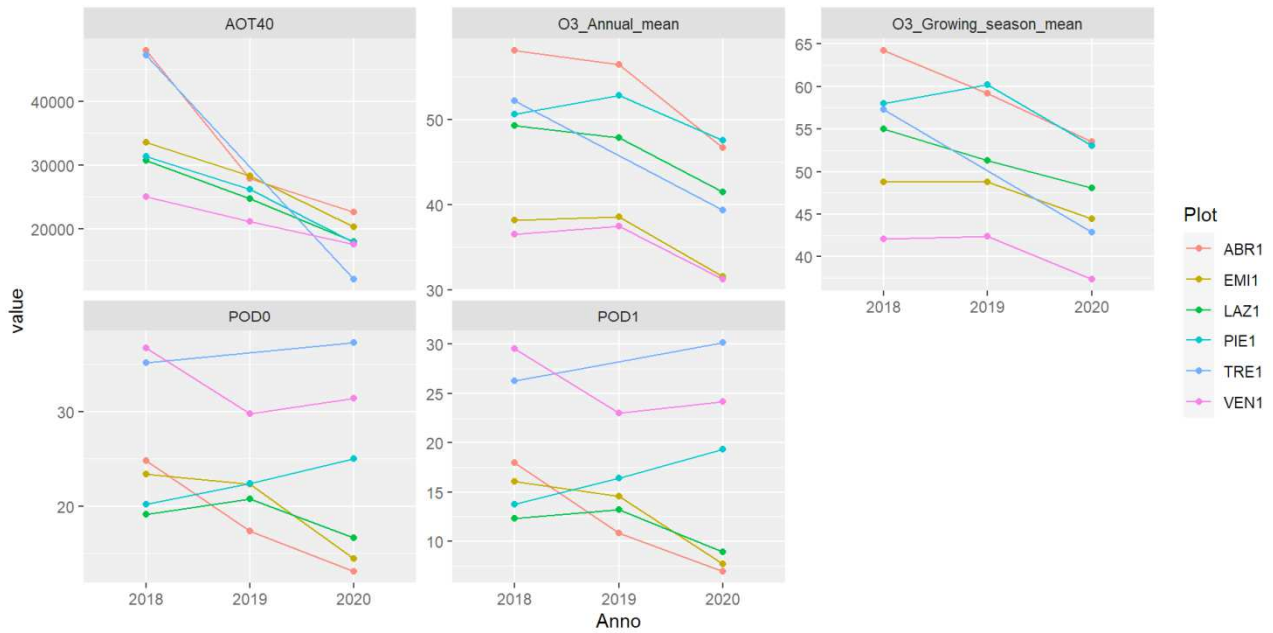
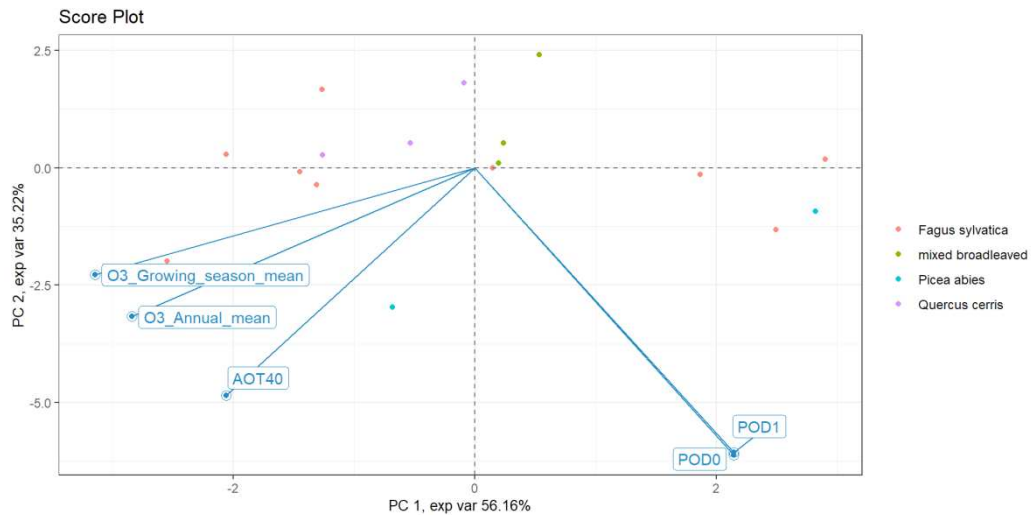


Figure 4.9 – Temporal trends in ozone concentrations measured in the forest sites.



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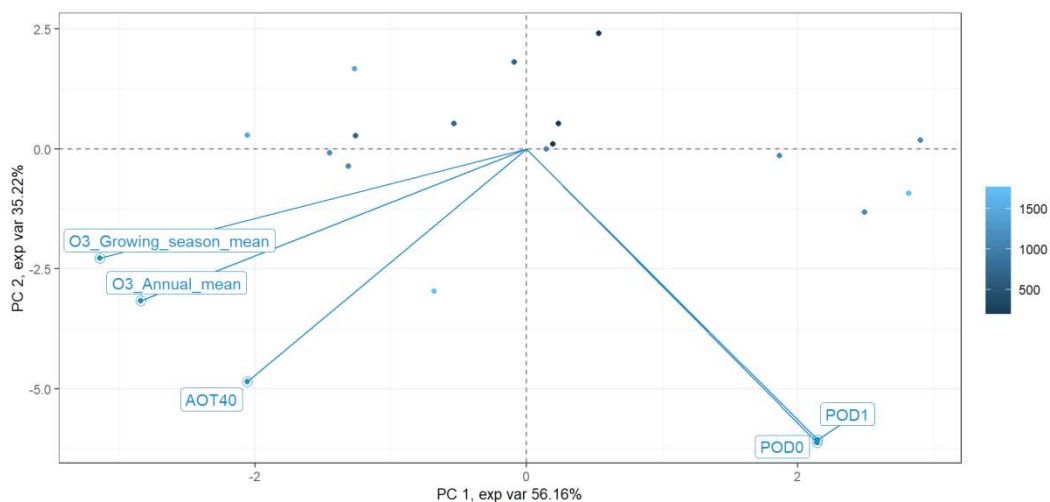
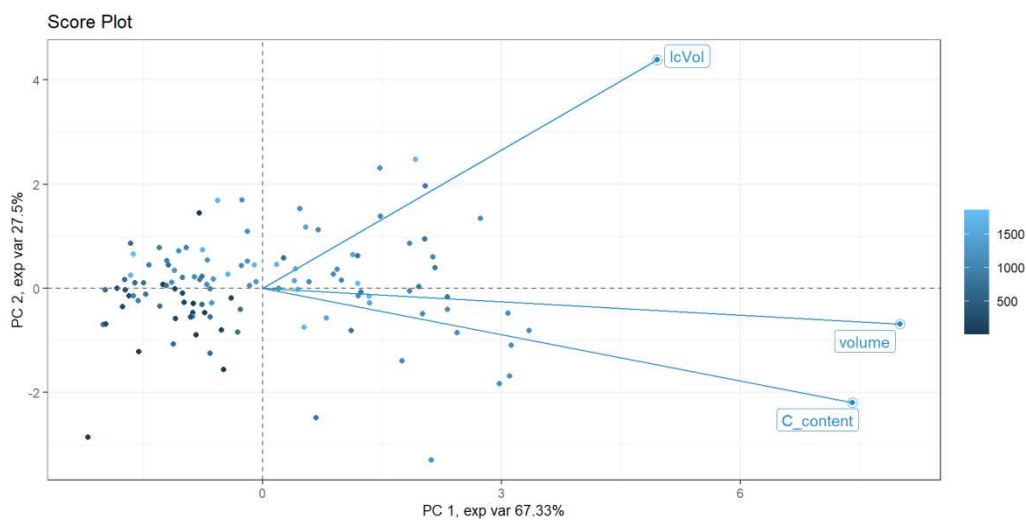


Figure 4.10 – Score plots of the PCA ordination of the ozone concentrations measured in the forest sites categorized by main tree species (above) and altitude (below).

## 4.4.2 Forests

**Forest growth.** Positive values of PC1 show an increasing gradient of IcVol, Vol and Carbon content, in relation to an increasing altitudinal gradient, corresponding to *Fagus sylvatica* and *Picea abies* forest sites, managed as high forests (Figure 4.11).



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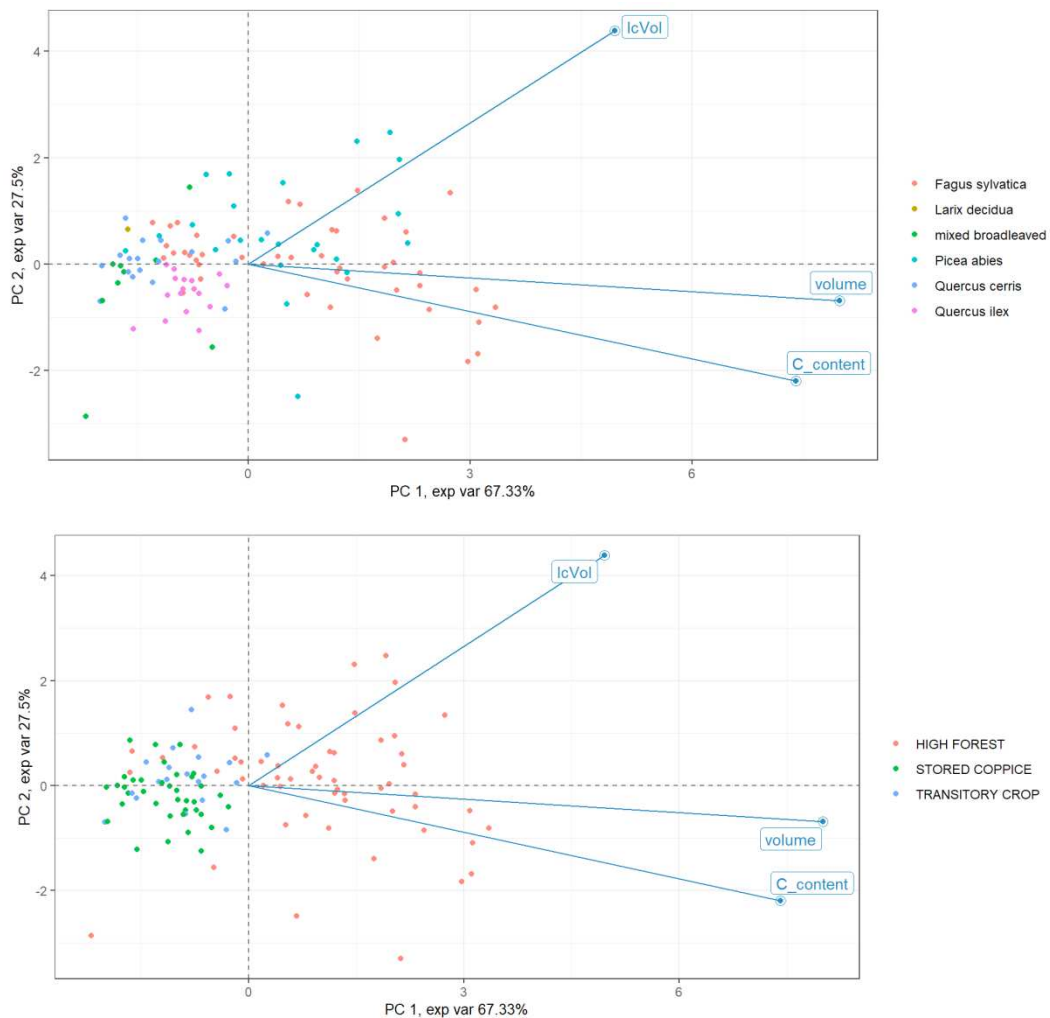


Figure 4.11 – Score plots of the PCA ordination of forest growth variables categorized by altitude (above), tree species (middle) and forest management (below).

**Foliar analysis.** In general, there is no temporal trend in the values of the elements measured in the leaves. However, the surveys of 2013 and 2017 differ from the other years. In particular, some plots of 2017 show higher values of Mg and Ca and some plots of 2013 show higher values of P and S (Figure 4.12). Further, an increasing gradient of all the parameters is observed for negative values of PC1 in relation to most of *Fagus sylvatica* and *Quercus cerris* forest sites, while *Picea abies* and *Quercus ilex* are distributed for positive values of the axes. Stored coppices show low values of S, P, N, K and high values of Mg (Figure 4.12).

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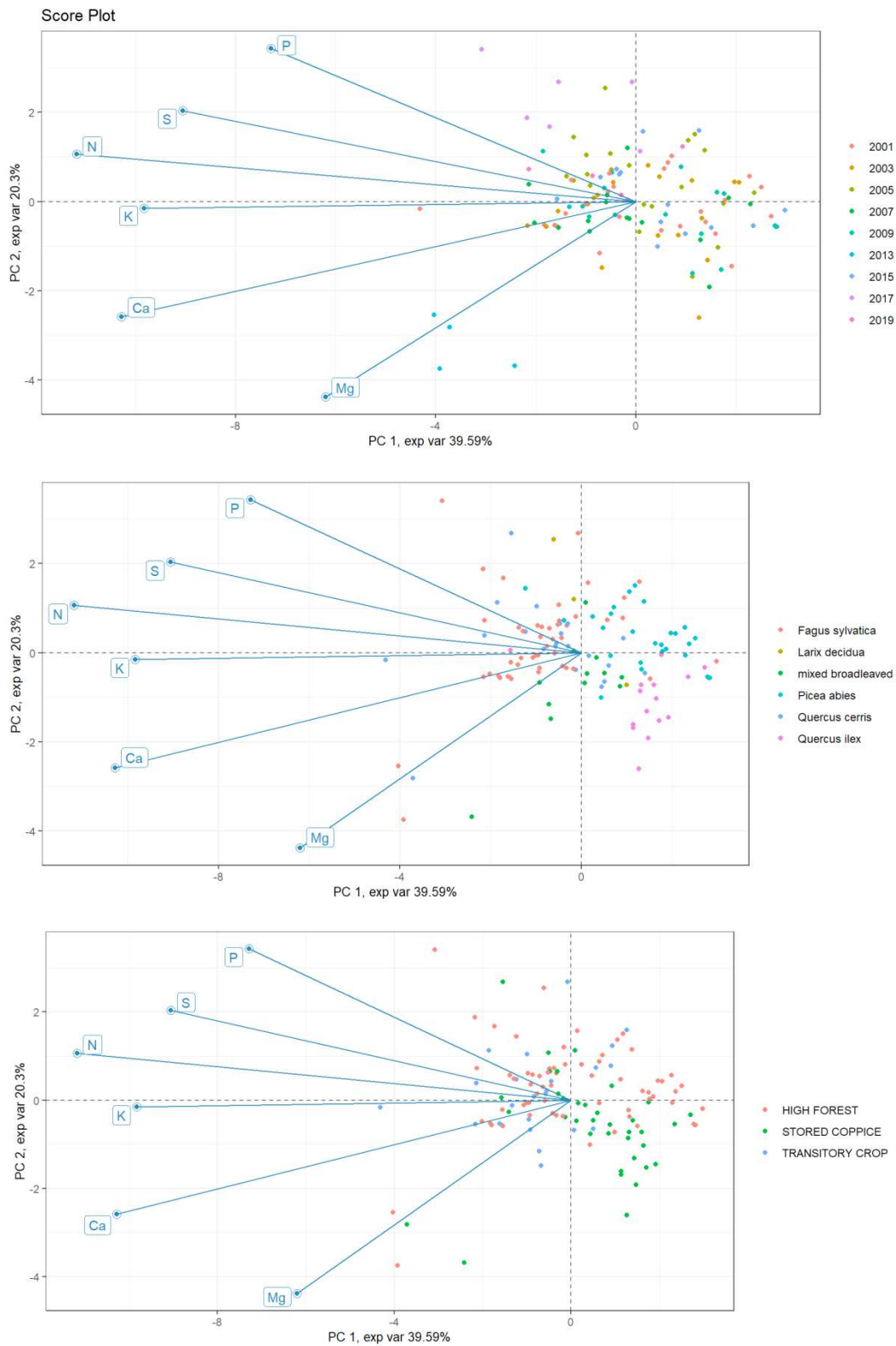


Figure 4.12 – Score plots of the PCA ordination of foliar analysis variables categorized by year (above), tree species (middle) and forest management (below).

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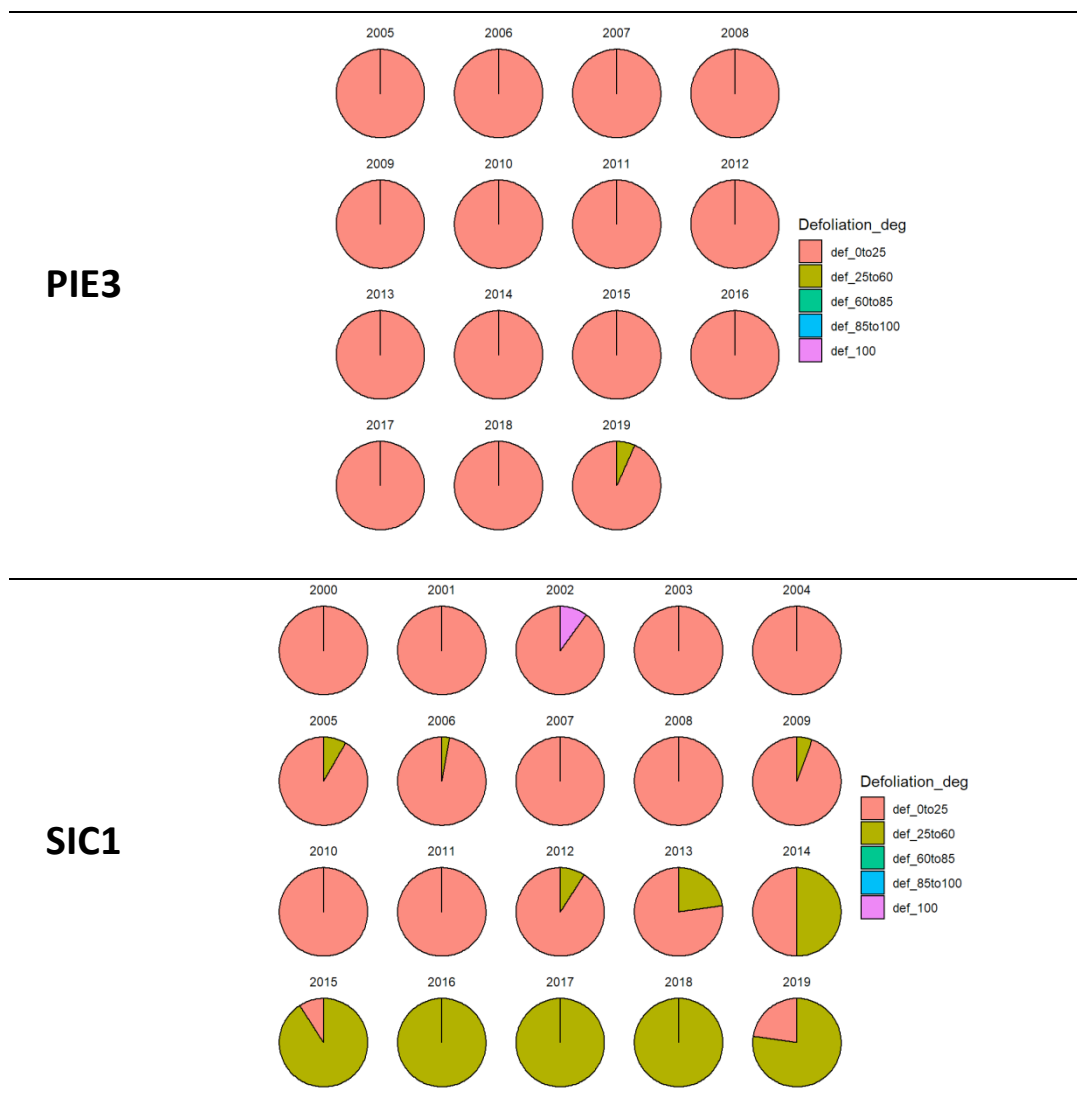
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**Crown condition and damaging agents.** Overall, a clear temporal trend in the variables related to the crown condition is not evident. However, by analyzing the single plots, in some cases we can observe quite marked differences in the distribution of defoliation classes during the considered time span. Figure 4.13 shows three examples of plots with contrasting trends: in the PIE3 plot no defoliation has been observed over the years; the SIC1 plot, on the other hand, shows a clear temporal trend, with an increase in defoliation values starting from 2013; for the EMI1 plot all defoliation classes are represented over the years, in some cases with a high incidence of completely defoliated trees.

The PCA does not show any evident gradient, with the exception of the discrimination, along the PC1, between the conifer and broadleaves plots. The formers are distributed by negative values of the axis, showing the lowest defoliation and damage values (Figure 4.14).



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## EMI1

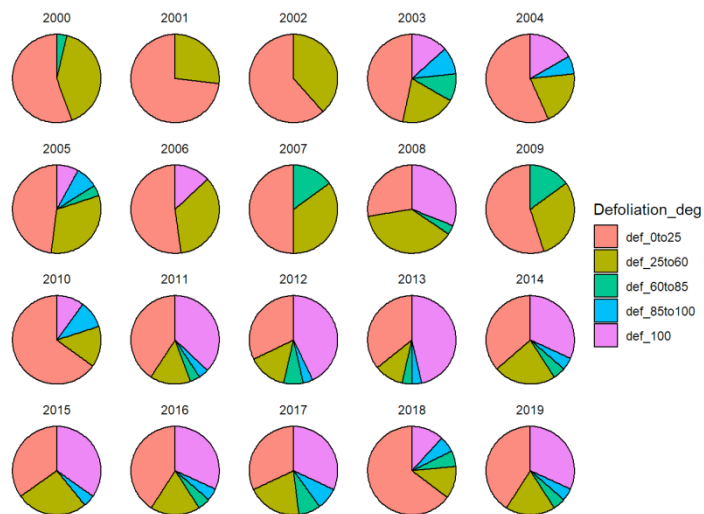


Figure 4.13 – Pie-charts showing the distribution of the percentage defoliation classes in the considered period.

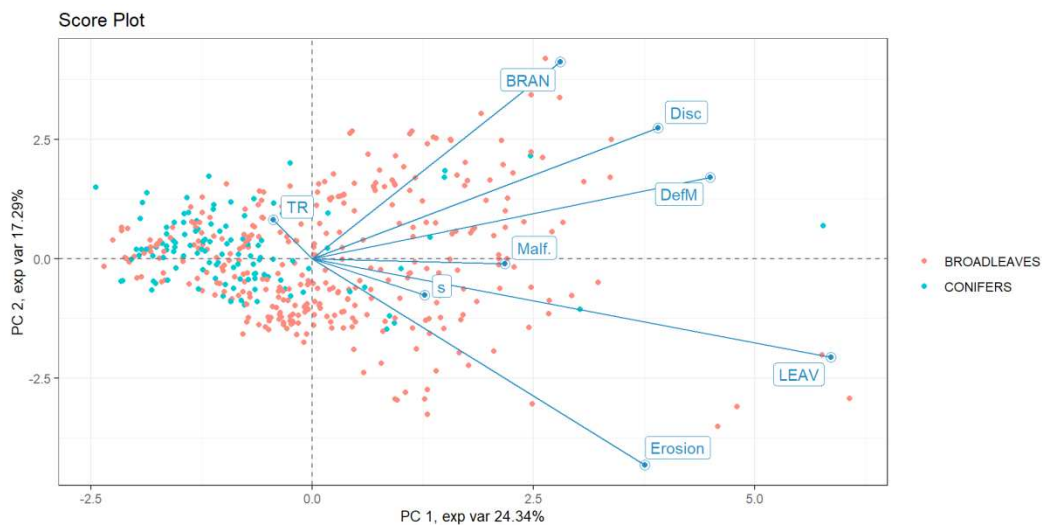


Figure 4.14 – Score plot of the PCA ordination of crown condition variables categorized by forest type.

**Epiphytic lichens.** For this indicator we have data for three years (2005, 2019 and 2020) and for the last 2 years only for the 6 forest sites of the NEC Italy network. With the available data it is not possible to observe particular trends in the considered diversity variables.

PCA mainly explains a variability related to the tree species, with *Quercus cerris* and *Fagus sylvatica* showing higher values of biodiversity, distributed by negative PC1 values. On the contrary, *Quercus ilex* and mixed broadleaves show the lowest values of species richness (Figure 4.15).

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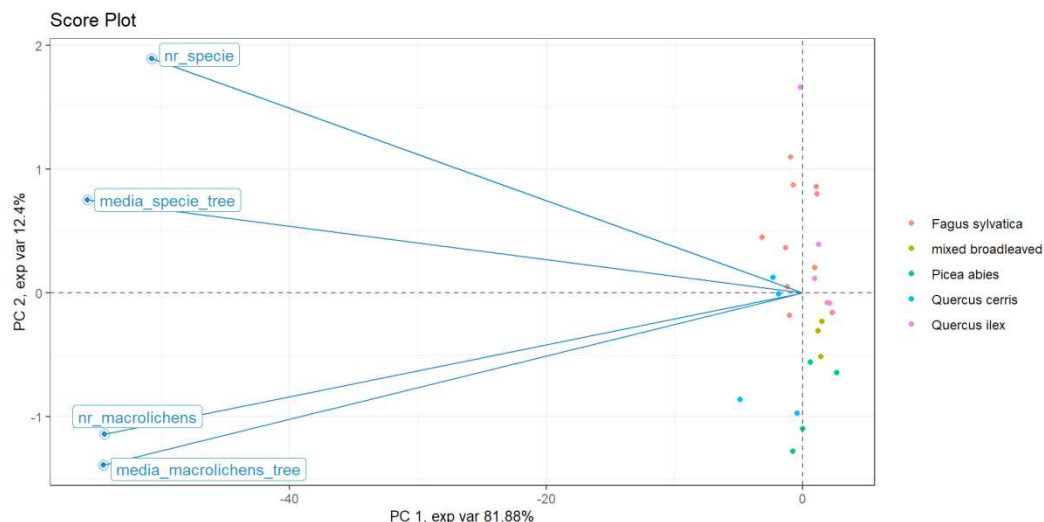


Figure 4.15 – Score plot of the PCA ordination of epiphytic lichens variables categorized by main tree species.

**Ground vegetation.** The results of the univariate analysis do not show specific temporal trends, with the exception of the years 2013 and 2014, when an increase in species density and a decrease in other biodiversity parameters are observed (Figure 4.16). The PCA explains the variability between forest sites in terms of main tree species. In particular, the *Fagus sylvatica* and *Quercus cerris* plots show, respectively, the lowest and the highest species richness. Conifers are characterized by higher moss layer cover and species density, unlike *Quercus ilex* forests, which are distributed by negative PC1 values (Figure 4.17).

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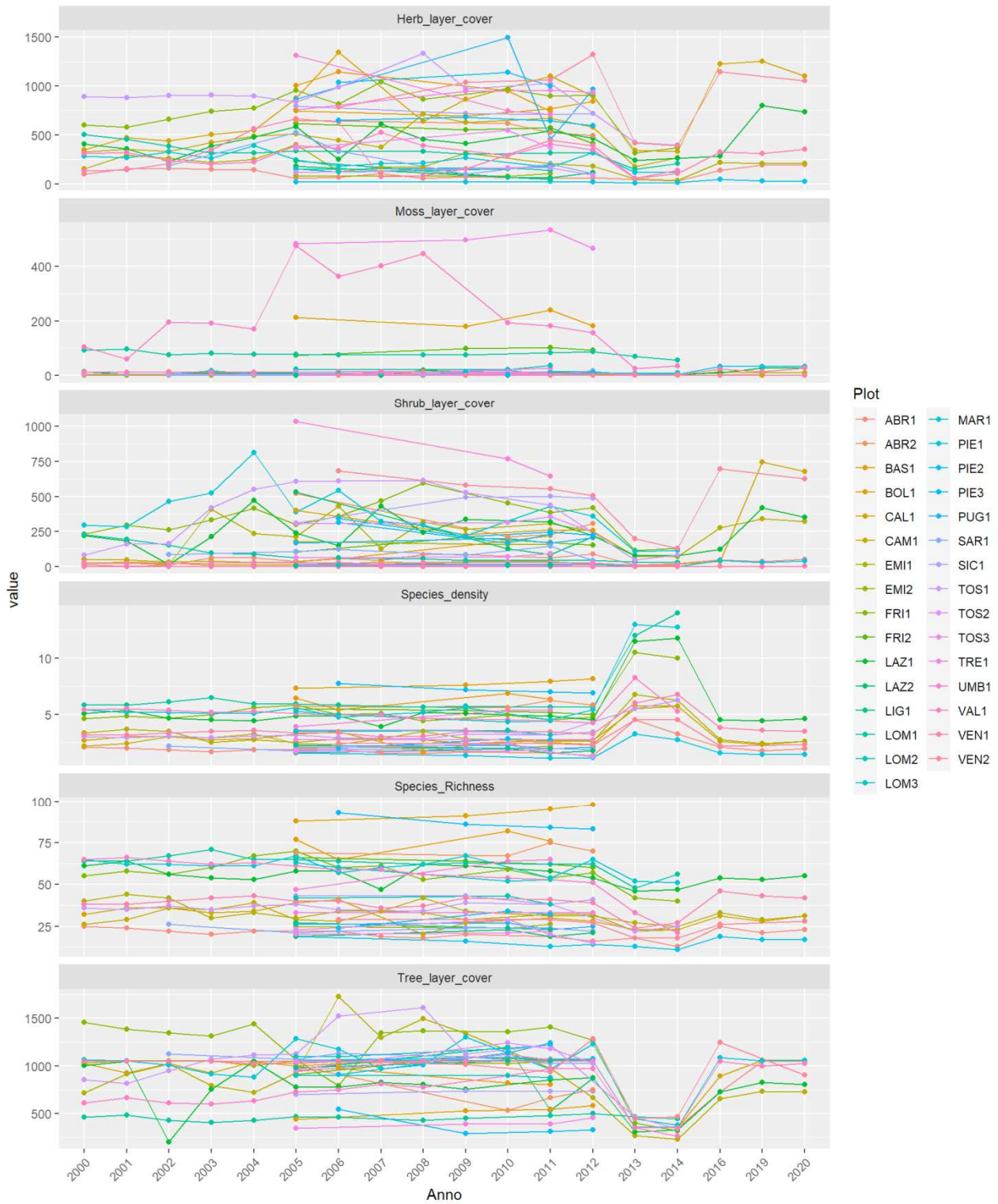


Figure 4.16 – Temporal trends in ground vegetation variables measured in the forest sites.

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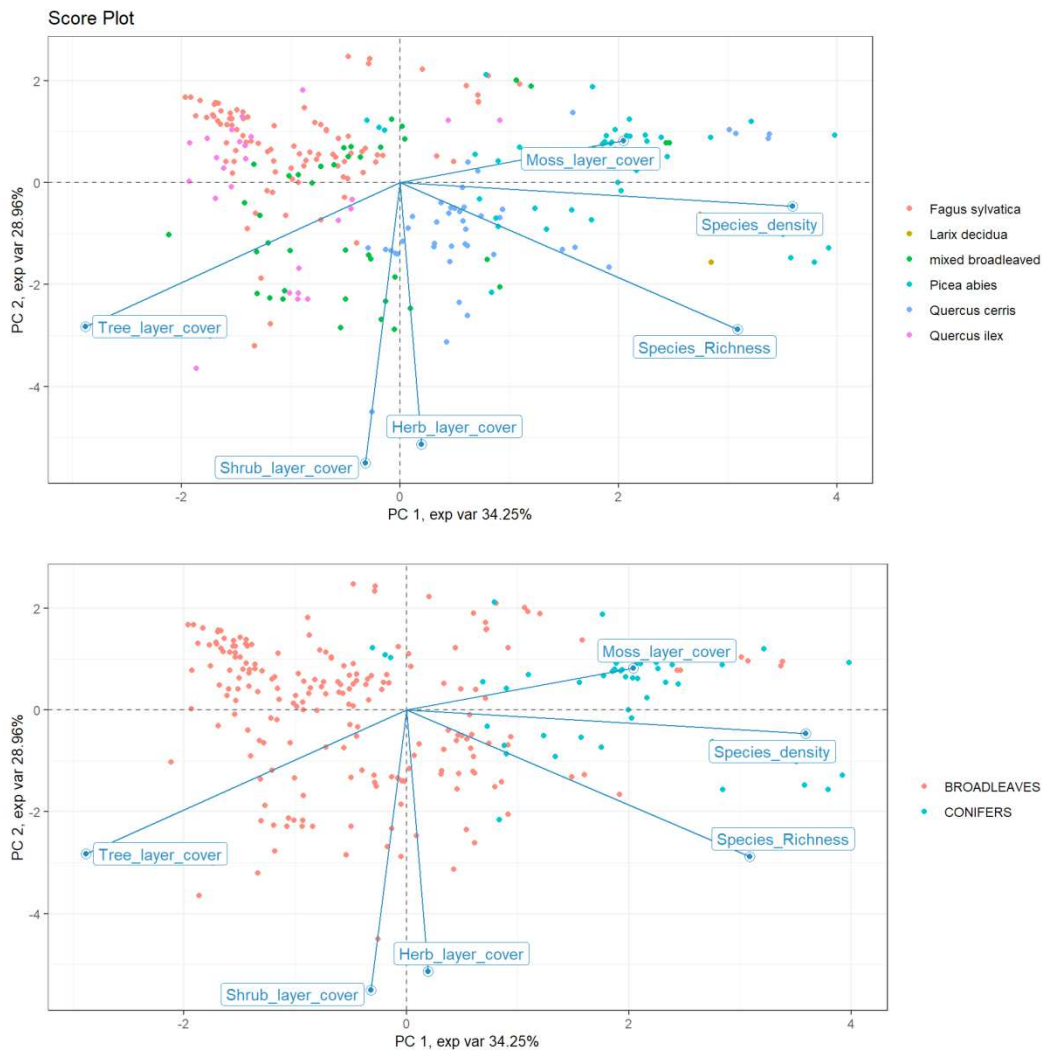


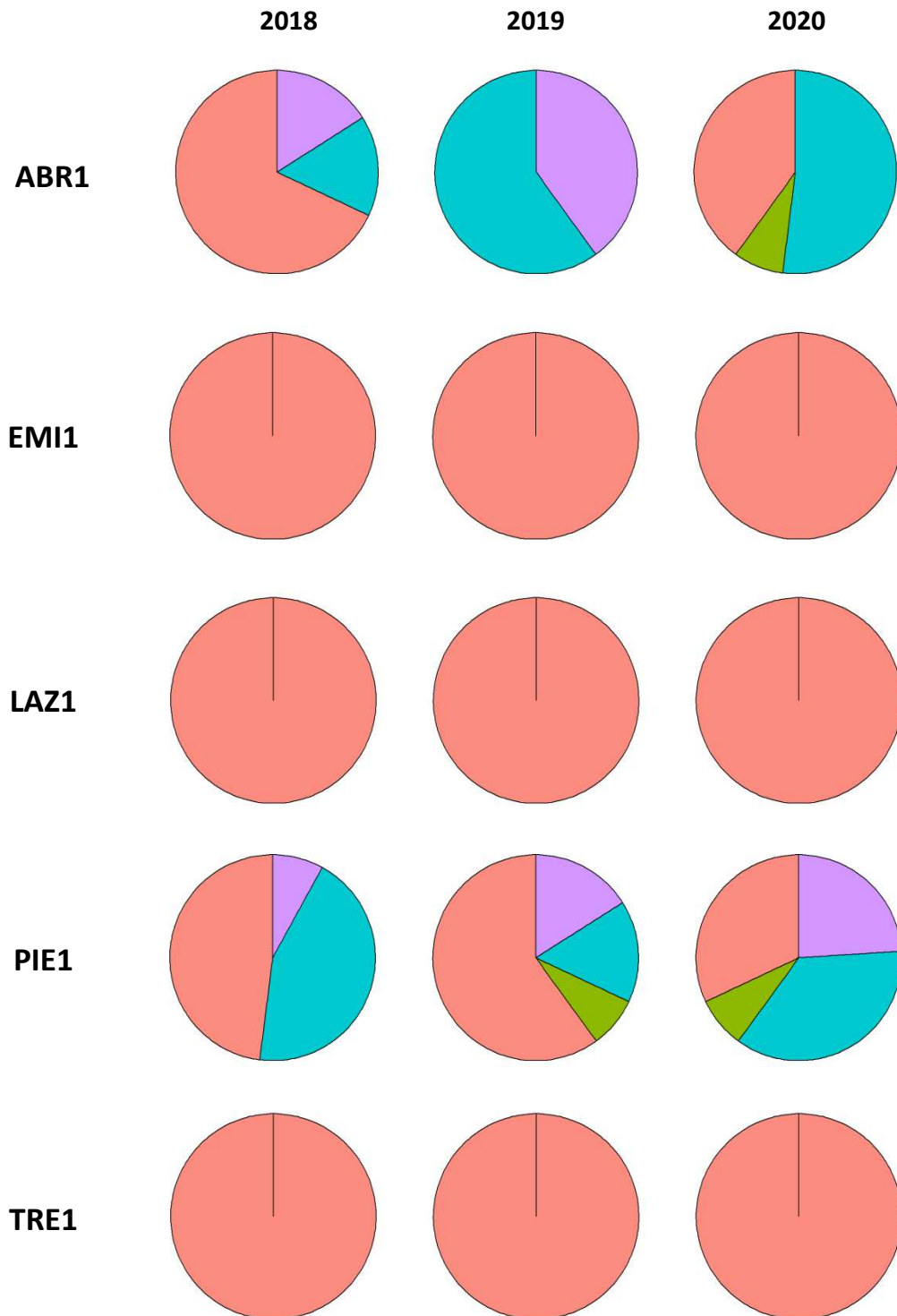
Figure 4.17 – Score plots of the PCA ordination of ground vegetation variables categorized by main tree species (above) and forest type (below).

**Ozone injuries.** The pie charts show the percentage distribution of leaves per branch in the four classes of ozone injury over the three years of investigation (% of symptomatic leaves, scores from 0 to 3). The ABR1, PIE1 and VEN1 sites show the highest incidence of ozone damage, while the other three sites are characterized by class 0 (Figure 4.18).

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VEN1

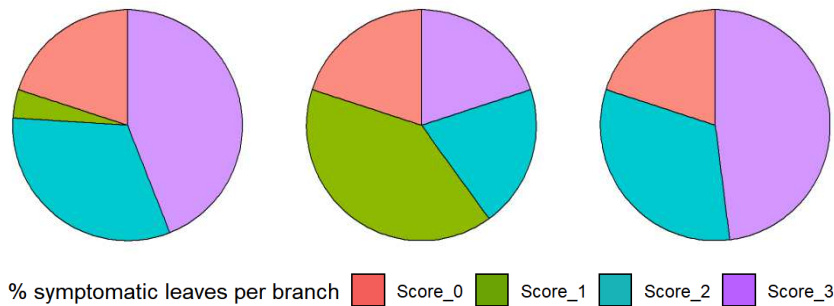
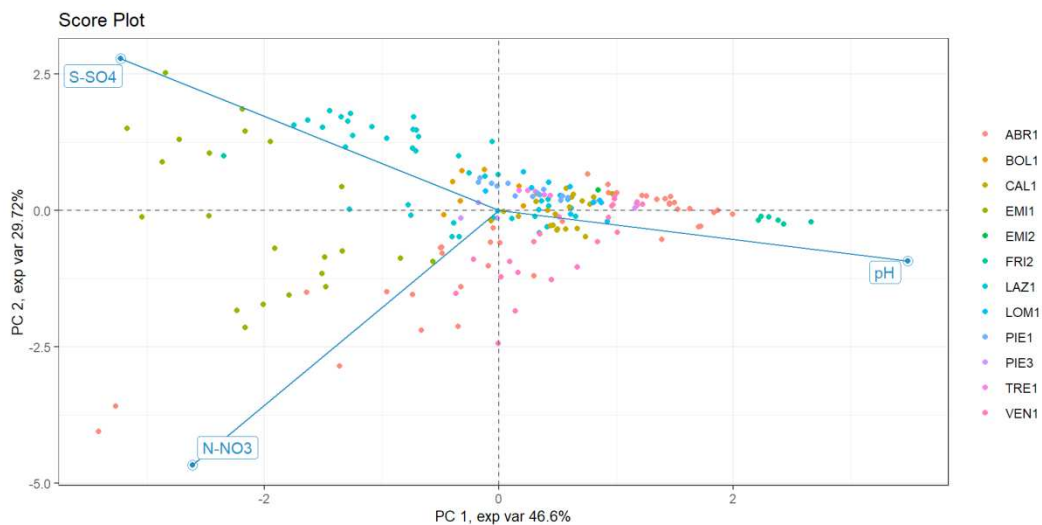


Figure 4.18 – Pie-charts showing the percentage distribution of leaves per branch in the four classes of ozone injury over the three years of investigation in the six forest sites.

**Soil solution.** A clear temporal trend in the variables related to soil solution is not evident. The PCA explains the variability related to the different forest sites. LAZ1 *Quercus cerris* plot and EMI1 mixed broadleaves plot, both managed as coppice forests, are characterized by the highest values of S-SO<sub>4</sub> and low pH values. EMI2 shows an opposite distribution with the highest pH values, and *Picea abies* plots have the lowest N-NO<sub>3</sub> values (Figure 4.19).



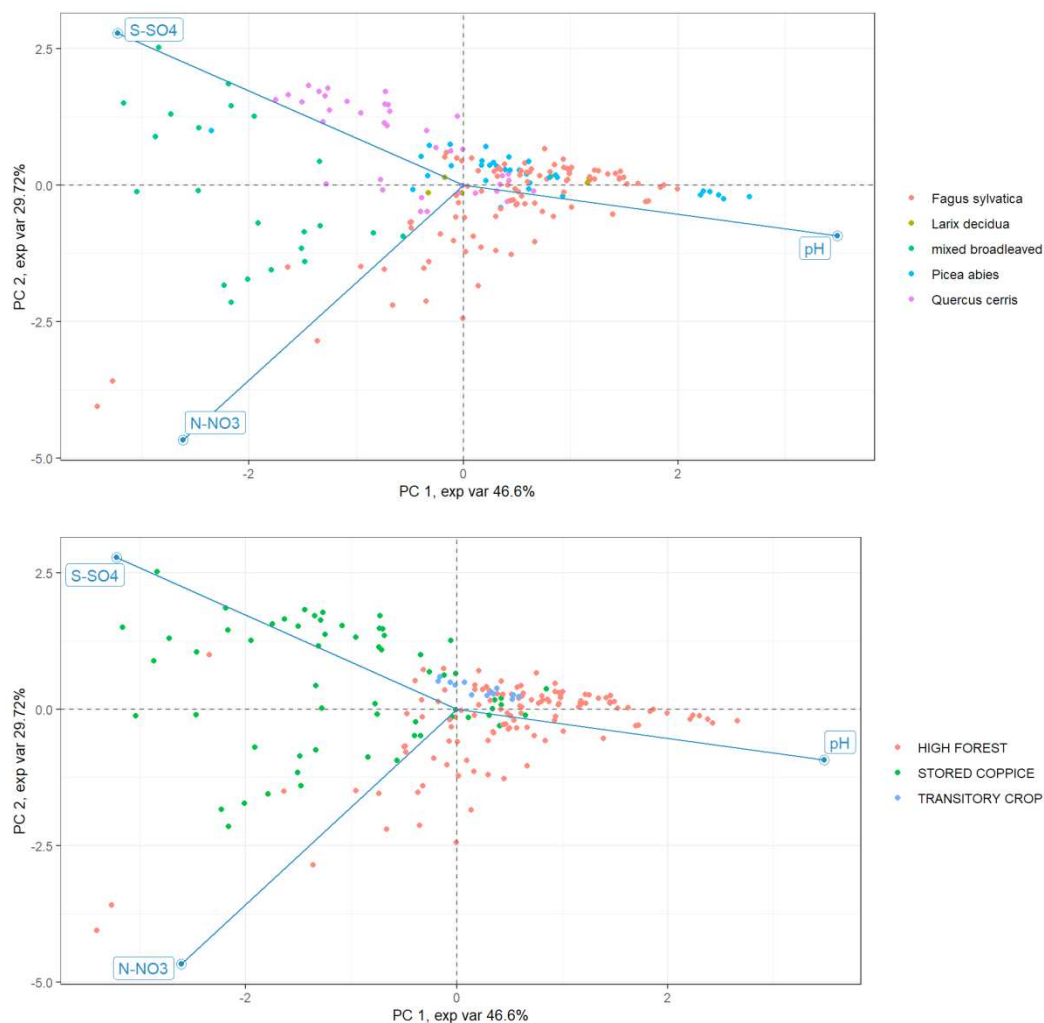


Figure 4.19 – Score plots of the PCA ordination of the soil solution variables categorized by plot (above), main tree species (middle) and forest management (below).

**Meteorological data.** Ten variables of main interest have been identified (Figure 4.20). Some of them show several missing values, especially in some plots (PIE2, PIE3, TRE1, VAL1, VEN1) and mainly concern values of wind speed, ground temperature and solar radiation (Figure 4.20). To manage these missing values, and to perform multivariate analysis (PCA), two approaches were adopted, by obtaining two different subsets:

- (i) all observations having at least one missing value in any variable were deleted (subset with 125 observations);
- (ii) variables with missing values >20% were deleted, and then the observations still having missing values were deleted (subset with 293 remaining observations).

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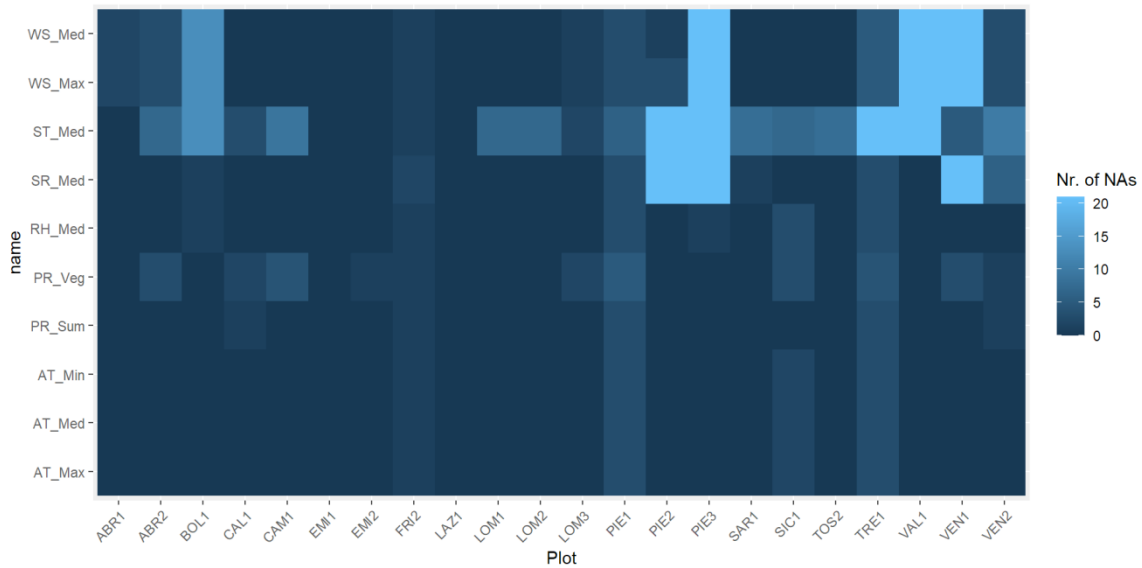
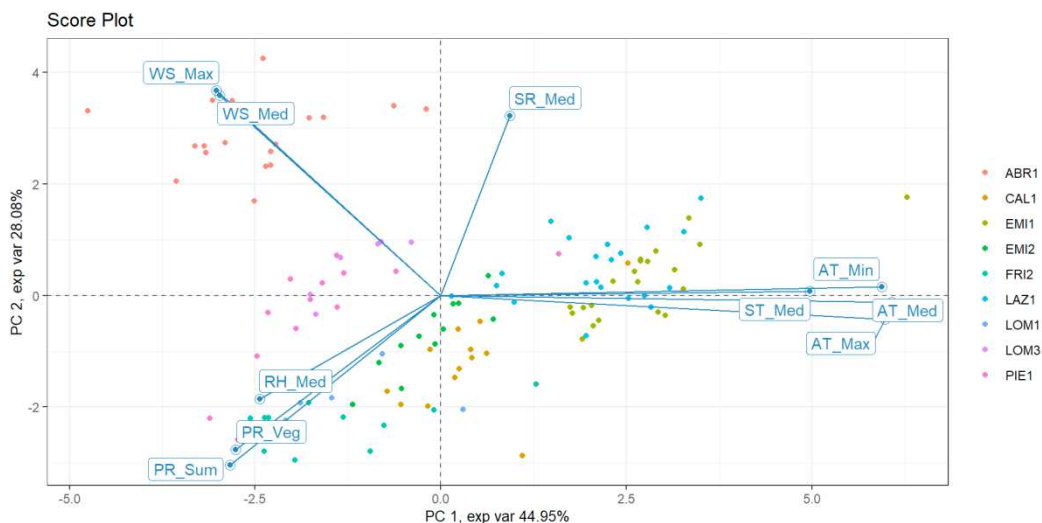


Figure 4.20 – Schematic graph showing the completeness of meteorological data collected in the forest sites.

**PCA results from approach (i).** The ordination identifies 4 well-separated groups of highly correlated variables (Figure 4.21): 1) temperature parameters show an increasing gradient along PC1 in relation to stored coppices of mixed broadleaved and *Quercus cerris* forests, at the lowest altitudes; 2) precipitation and humidity increase for negative values of PC1 and PC2, in relation to *Picea abies* sites; 3) wind parameters increase for negative values of PC1 and positive values of PC2, in relation to *Fagus sylvatica* forests situated at higher altitudes (ABR1); 4) an increasing trend of solar radiation on the opposite of precipitation variables.



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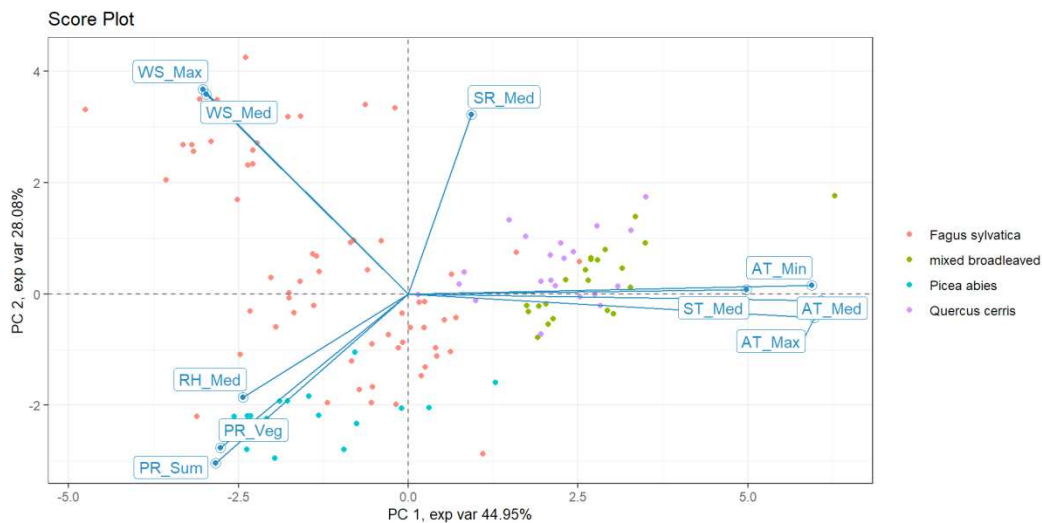
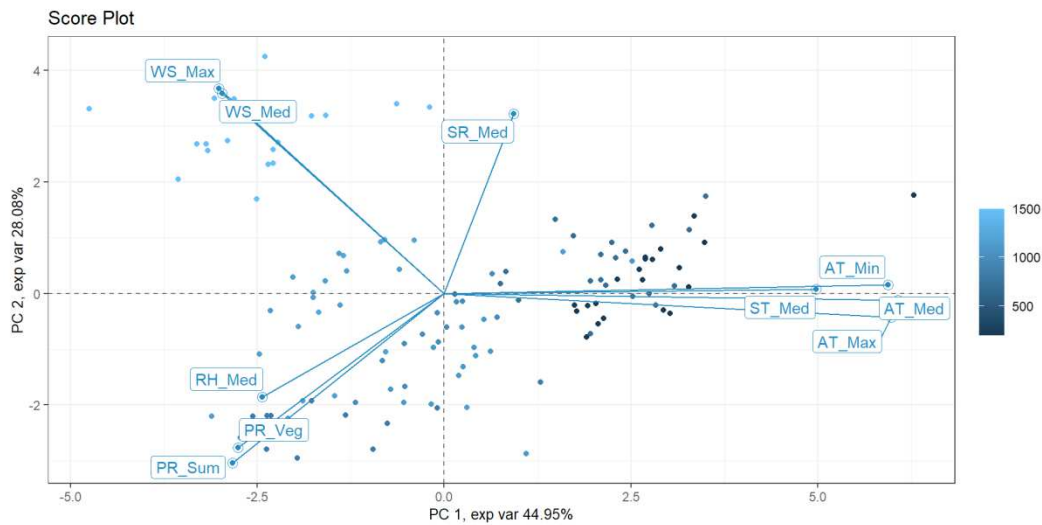


Figure 4.21 – Score plots of the PCA ordination of the meteorological data in the forest sites categorized by site code (above), altitude (middle) and main tree species (below). Dataset: all observations that have at least one missing value in any variable are deleted (125 remaining observations).

**PCA results from approach (ii).** With this solution, wind and solar radiation parameters and soil temperature were deleted. The PCA explains the variability between forest sites in terms of altitude and main tree species (Figure 4.22). In particular, the mixed broadleaved, *Quercus cerris* and *Quercus ilex* plots, with the lowest altitudes, show the highest values of temperature (positive values of PC1), while the conifers (*Larix decidua* and *Picea abies*), at higher altitudes, have an opposite trend. The negative values of PC2 identify an increasing gradient in precipitation and humidity parameters.

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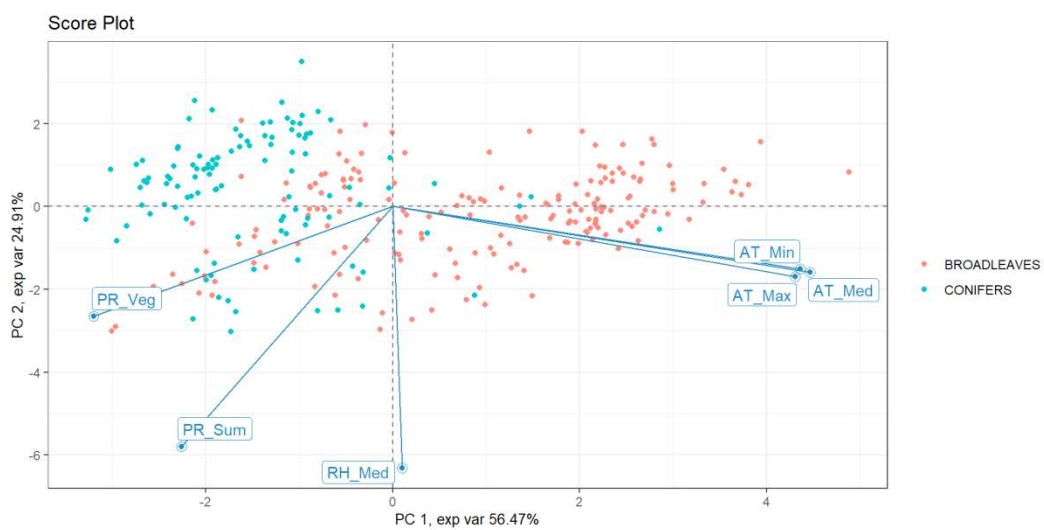
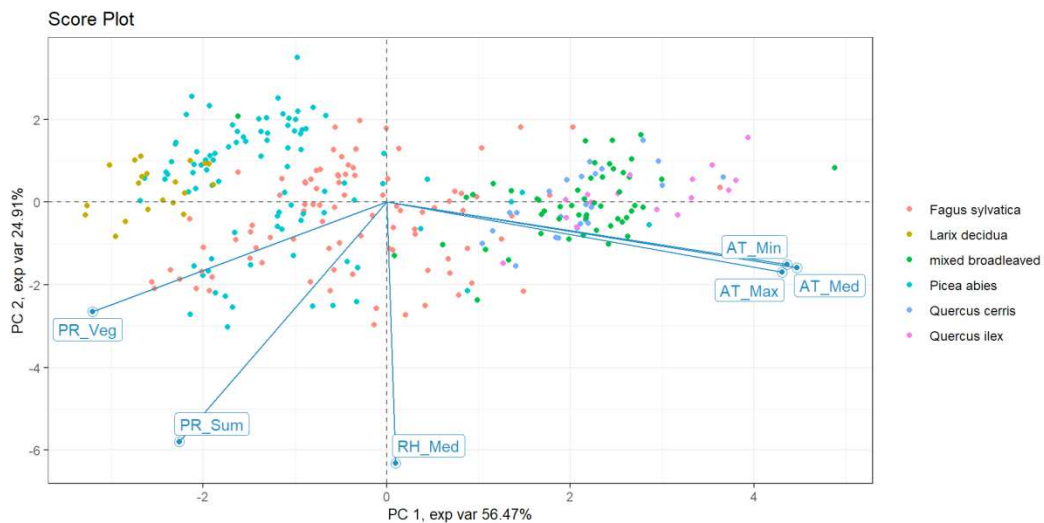
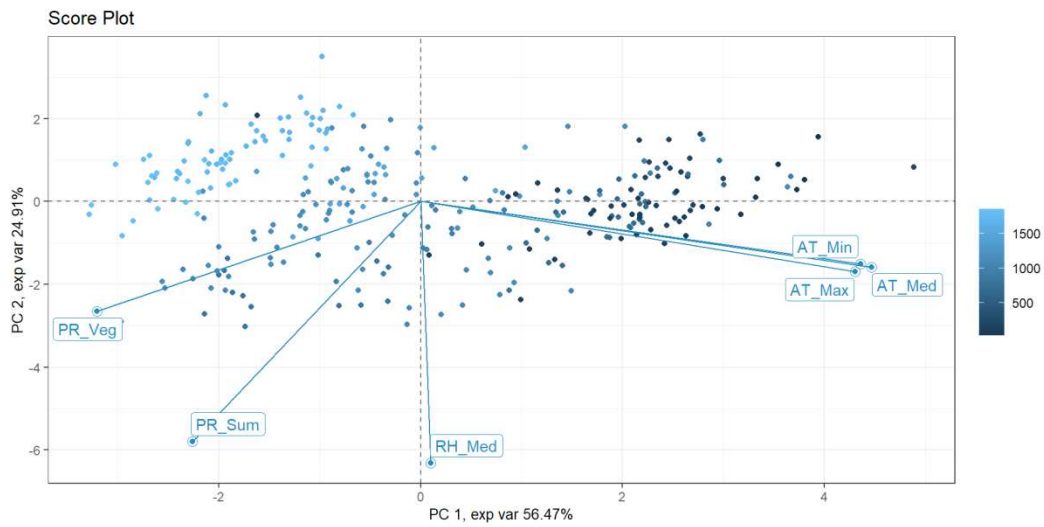


Figure 4.22 – Score plots of the PCA ordination of the meteorological data in the forest sites categorized by altitude (above), main tree species (middle) and forest type (below). Dataset: variables with missing values >20% are deleted, and then the observations still having missing values are deleted (293 remaining observations).



**4.4.3 Freshwaters**

**Water chemistry.** The PCA shows a decreasing altitudinal gradient along positive values of PC1, with lakes at lower altitudes showing higher values for Ca, K, Mg, NO<sub>3</sub>-N and ALK (Figure 4.23). Nitrate in particular shows a decreasing pattern in relation to both latitude and altitude, being the main emission sources of N oxides located south of the study sites (Rogora et al.,2016).

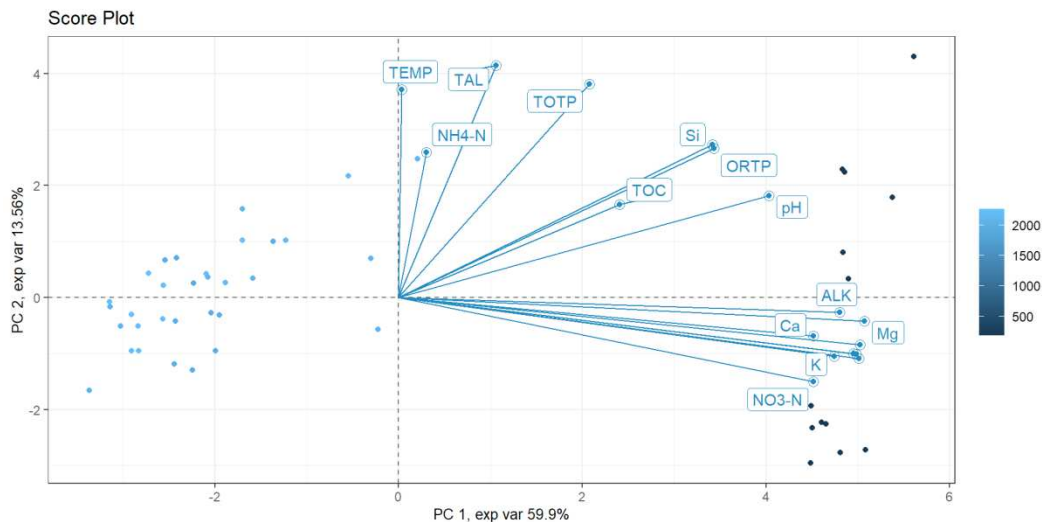
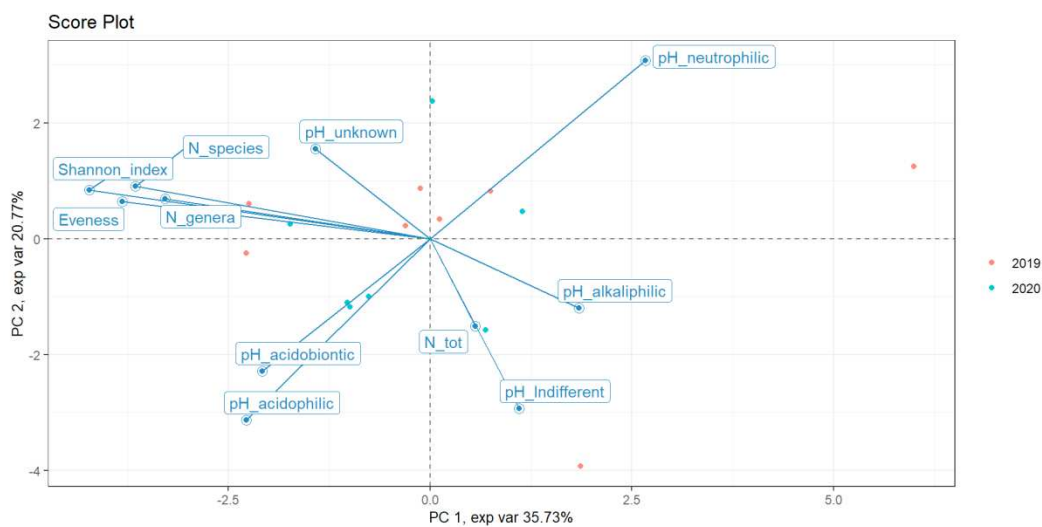


Figure 4.23 – Score plot of the PCA ordination of the concentrations of water chemistry parameters (collected according to ICP Waters) in correspondence of the freshwater sites categorized by year.

**Diversity of diatoms and macroinvertebrates.** For these indicators we have data only for two years (2019 and 2020). With the available data it is not possible to observe temporal trends in the considered indicators (diversity indexes and pH preference), and this is confirmed by the multivariate analysis both for diatom (Figure 4.24) and macroinvertebrate diversity (Figure 4.25).



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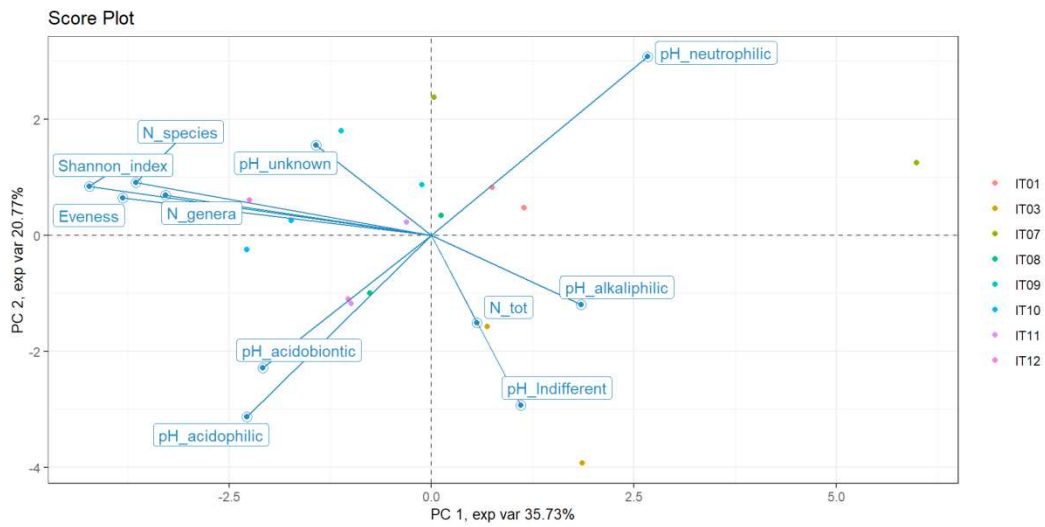
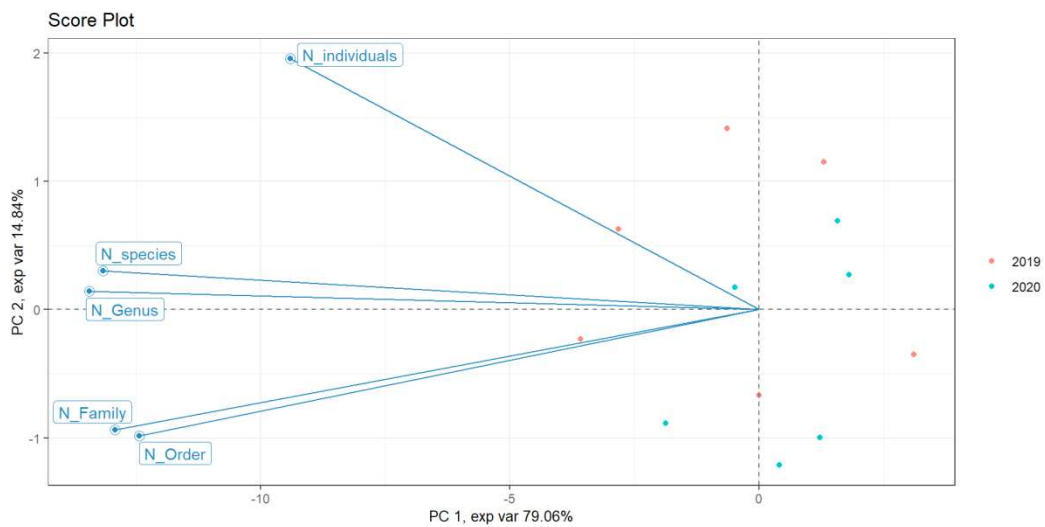


Figure 4.24 – Score plot of the PCA ordination of diversity indexes and pH preference for diatoms s in correspondence of the freshwater sites categorized by year (above) and site code (below).



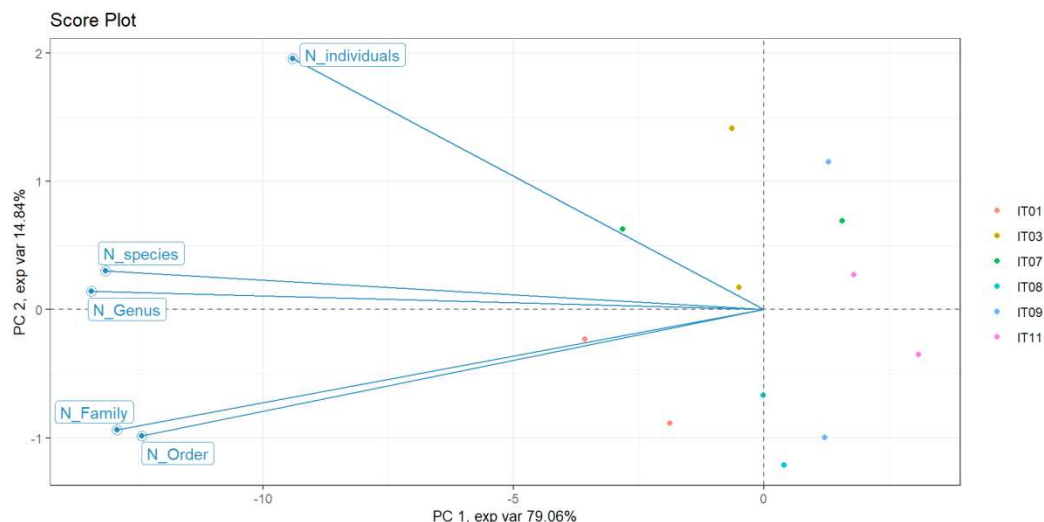


Figure 4.25 – Score plot of the PCA ordination of the diversity of macroinvertebrates in correspondence of the freshwater sites categorized by year (above) and site code (below).

#### 4.5 Results of the multiple linear regression models (MLRM)

The following steps have been undertaken to obtain the final predictive models, both for forest and for freshwater sites:

- Data completeness of the whole integrated dataset was explored, searching for the best selection of indicators per time series.
- Response variables were self-scaled.
- A preliminary study on the correlation among variables has been carried out, in order to avoid multicollinearity and to select only those variables not too correlated with the others: i) A PCA on responses and drivers together was performed; ii) A PCA with only drivers was performed to select the most explanatory variables to use as representatives of groups of auto-correlated variables.
- A type II MANOVA test (Pillai test statistic) was carried out to find the most significant predictive variables.
- The best regression model was fitted.

Please refer to the Annex A1.1 for detailed information on the procedures and results.

##### 4.5.1 Forests

To select the best drivers to include in the models, an analysis of the correlation structure among them and a PCA were performed (Figure 4.26), showing a very strong correlation among several variables, grouping them into two main clusters. The first one is very correlated with the negative values of PC1, and it includes SO<sub>4</sub> concentrations (EMEP models), and the atmospheric depositions (both throughfall and open field) related to alkalinity, SO<sub>4</sub> and K. The second group of variables, very correlated with positive values of PC2, includes the atmospheric depositions of nitrogen compounds (N total, NH<sub>4</sub>, NO<sub>3</sub>), and the modelled concentrations of reduced (RDN) and oxidised nitrogen (OXN). Further, the modelled concentrations of PM10 and NO<sub>x</sub> are positively correlated with temperatures and negatively correlated with the

# LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



precipitations in the vegetative period (PR\_Veg), while Average Relative Humidity (RH\_Med) is not correlated with the other variables.

Since the number of drivers is very high compared to the number of available observations, and due to their strong reciprocal correlations, the above results suggest selecting only these four ‘representative’ predictors to fit the models:

- Average Relative Humidity (RH\_Med).
- Precipitations in the vegetative period (PR\_Veg).
- Modelled total deposition of reduced nitrogen (dep\_rdn\_tot). This driver represents all the variables included in the second cluster of the PCA (positive values of PC2).
- Measured deposition of K in open field (of\_depo\_k). It represents all the variables included in the first cluster of the PCA (negative values of PC1).

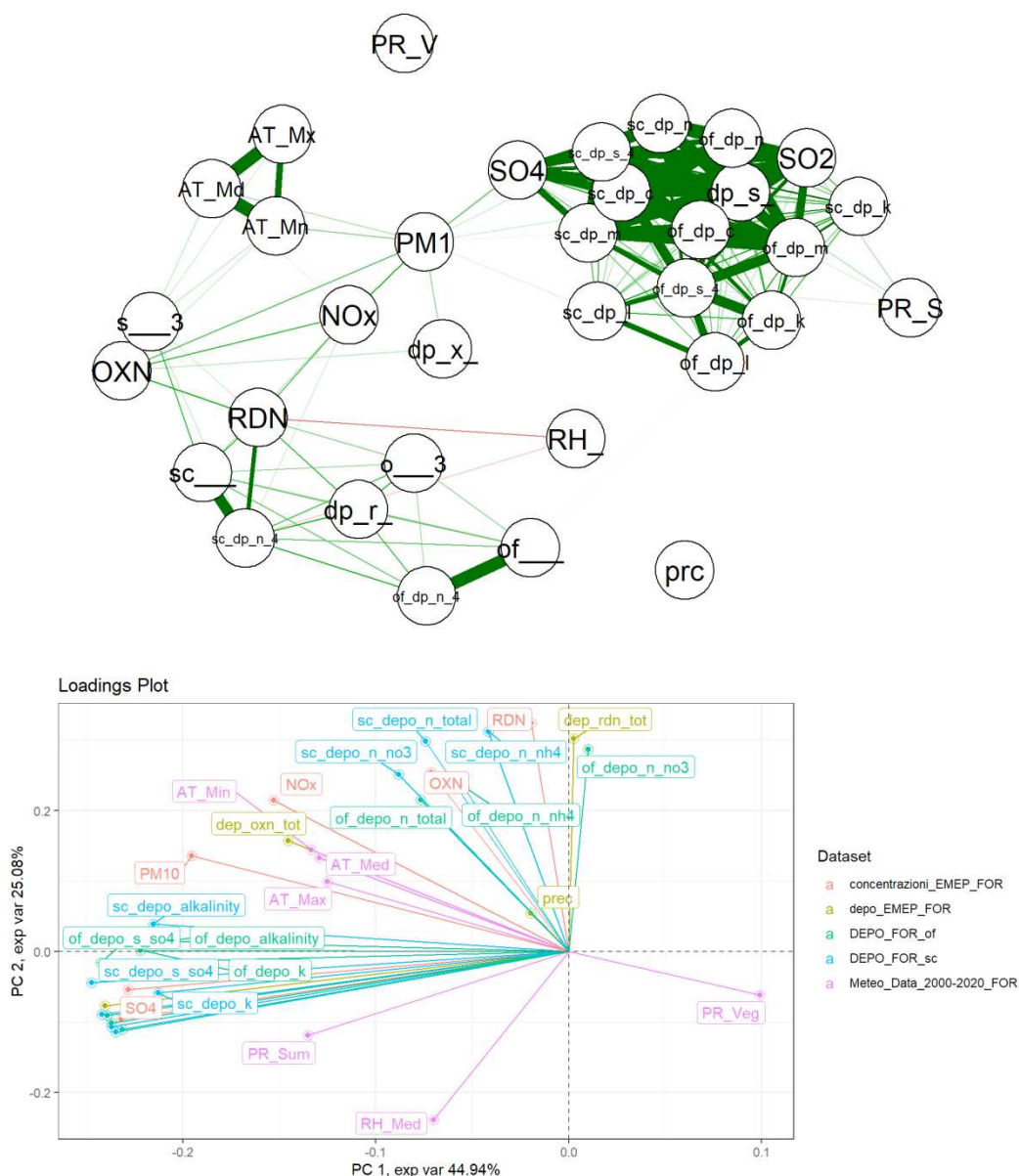


Figure 4.26 – Above: correlation structure among drivers. Positive correlations are reported in green, while negative correlations are in red. The line thickness is proportional to the correlation values. Below: Loading plot of the PCA ordination of the drivers measured or modelled in correspondence of the forest sites.

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



One multivariate regression model was fitted for each of the following indicators: i) foliar analysis; ii) crown condition and damaging agents; iii) ground vegetation; iv) soil solution. These are the indicators showing the most complete datasets both in terms of time series and from the spatial point of view.

**Foliar analysis.** Table 4.4 reports the results of the multiple linear regression models describing the effects of the selected drivers on foliar element content (see also Annex A1.1 for detailed results). The models are not significant for any of the response variables ( $p > 0.05$ ), even if the precipitations in the vegetative period are negatively correlated with K and Mg foliar content.

*Table 4.4 – Multiple Linear Regression Models describing the effects of the selected predictive variables on Foliar analysis variables. Estimates values are reported together with statistically significant  $p$  values (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Last column shows the summary statistics of each model (156 df), with Multiple R2 (Mult R2), Adjusted R2 (Adj R2), F-statistic (on 4 and 17 df),  $p$  value.*

Response variables	Intercept	Relative humidity (average)	Precipitation in the vegetative period	Total deposition of reduced nitrogen	Deposition of K, open field	Summary statistics of each model
Nitrogen (N)	26.4	-0.07	-0.01	0.25	0.17	Mult R2: 0.304; Adj R2: 0.140 F: 1.85; $p > 0.05$
Calcium (Ca)	1.42	0.14	-0.01	0.02	0.06	Mult R2: 0.238; Adj R2: 0.059 F: 1.33; $p > 0.05$
Magnesium (Mg)	5.70	-0.04	<b>-0.004*</b>	-0.01	-0.002	Mult R2: 0.389; Adj R2: 0.245 F: 2.71; $p > 0.05$
Potassium (K)	4.29	0.12	<b>-0.01*</b>	-0.06	-0.26	Mult R2: 0.352; Adj R2: 0.199 F: 2.31; $p > 0.05$

**Crown condition and damaging agents.** Table 4.5 reports the results of the multiple linear regression models describing the effects of the selected drivers on the parameters related to crown condition (see also Annex A1.1 for detailed results). The models are significant for defoliation and the damage to branches ( $p < 0.05$ ), with the latter showing an opposite trend compared to relative humidity ( $p < 0.01$ ). Precipitations in the vegetative period have an influence on the damage to tree trunks ( $p < 0.05$ ).

# LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Table 4.5 – Multiple Linear Regression Models describing the effects of the selected predictive variables on crown condition and damaging agents variables. Estimates values are reported together with statistically significant *p* values (\* *p*<0.05; \*\* *p*<0.01; \*\*\* *p*<0.001). Last column shows the summary statistics of each model (156 df), with Multiple R<sup>2</sup>(Mult R<sup>2</sup>), Adjusted R<sup>2</sup> (Adj R<sup>2</sup>), F-statistic (on 4 and 17 df), *p* value.

Response variables	Intercept	Relative humidity (average)	Precipitation in the vegetative period	Total deposition of reduced nitrogen	Deposition of K, open field	Summary statistics of each model
Average defoliation (DefM)	78.1	-0.75	-0.02	0.72	-0.02	Mult R <sup>2</sup> : 0.435; Adj R <sup>2</sup> : 0.302 F: 3.27; <i>p</i> <0.05
Affected part, leaves/needles (LEAV)	33.6	-0.24	-0.01	-0.36	0.60	Mult R <sup>2</sup> : 0.160; Adj R <sup>2</sup> : -0.037 F: 0.81; <i>p</i> >0.05
Affected part, branches, shoots, buds and fruits (BRAN)	155.9*	-1.75**	0.01	-0.66	-0.26	Mult R <sup>2</sup> : 0.416; Adj R <sup>2</sup> : 0.278 F: 3.02; <i>p</i> <0.05
Affected part, stem and collar (TR)	1.87	-0.02	0.004*	-0.06	-0.0003	Mult R <sup>2</sup> : 0.363; Adj R <sup>2</sup> : 0.214 F: 2.43; <i>p</i> >0.05
Erosion	0.81	-0.002	-0.001	-0.004	0.05	Mult R <sup>2</sup> : 0.260; Adj R <sup>2</sup> : 0.085 F: 1.49; <i>p</i> >0.05
Discolouration (Disc)	1.14	-0.01	0.0002	-0.004	0.005	Mult R <sup>2</sup> : 0.208; Adj R <sup>2</sup> : 0.021 F: 1.12; <i>p</i> >0.05
Malf	0.27	0.002	0.000	0.007	0.002	Mult R <sup>2</sup> : 0.093; Adj R <sup>2</sup> : -0.121 F: 0.44; <i>p</i> >0.05

**Ground vegetation.** Table 4.6 reports the results of the multiple linear regression models describing the effects of the selected drivers on the biodiversity of the ground vegetation (see also Annex A1.1 for detailed results). Only the model for herbaceous layer cover is significant (*p*<0.01), with the deposition of nitrogen compounds negatively affecting this parameter (*p*<0.01) and the other depositions showing a positive influence (*p*<0.01).

## LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Table 4.6 – Multiple Linear Regression Models describing the effects of the selected predictive variables on ground vegetation variables. Estimates values are reported together with statistically significant *p* values (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Last column shows the summary statistics of each model (156 df), with Multiple R2 (Mult R2), Adjusted R2 (Adj R2), F-statistic (on 4 and 17 df), *p* value.

Response variables	Intercept	Relative humidity (average)	Precipitation in the vegetative period	Total deposition of reduced nitrogen	Deposition of K, open field	Summary statistics of each model
Species richness	49.6	-0.16	0.05	-0.63	-0.44	Mult R2: 0.187; Adj R2: -0.005 F: 0.98; $p > 0.05$
Species density	8.04	-0.04	-0.0007	-0.06	-0.11	Mult R2: 0.067; Adj R2: -0.153 F: 0.30; $p > 0.05$
Tree layer cover	-899.3	17.8	0.01	22.1	22.7	Mult R2: 0.321; Adj R2: 0.161 F: 2.01; $p > 0.05$
Shrub layer cover	307.3	-1.24	-0.04	-5.35	-10.4	Mult R2: 0.172; Adj R2: -0.023 F: 0.88; $p > 0.05$
Herbaceous layer cover	1611.5	-15.3	0.75	<b>-37.5**</b>	<b>53.9**</b>	Mult R2: 0.524; Adj R2: 0.412 F: 4.67; <b><math>p &lt; 0.01</math></b>
Moss layer cover	526.1	-6.16	0.35	-7.69	0.71	Mult R2: 0.205; Adj R2: 0.018 F: 1.11; $p > 0.05$

**Soil solution.** Table 4.7 reports the results of the multiple linear regression models describing the effects of the selected drivers on the soil solution variables (see also Annex A1.1 for detailed results). Only the model for nitrogen as nitrate (N-NO<sub>3</sub>) is significant ( $p < 0.01$ ), with relative humidity showing a negative effect on this parameter ( $p < 0.05$ ). Further, the precipitations in the vegetative period have a negative influence on sulphur as sulphate ( $p < 0.05$ ).

# LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Table 4.7 – Multiple Linear Regression Models describing the effects of the selected predictive variables on soil solution variables. Estimates values are reported together with statistically significant *p* values (\* *p*<0.05; \*\* *p*<0.01; \*\*\* *p*<0.001). Last column shows the summary statistics of each model (156 df), with Multiple R<sup>2</sup>(Mult R<sup>2</sup>), Adjusted R<sup>2</sup> (Adj R<sup>2</sup>), F-statistic (on 4 and 17 df), *p* value.

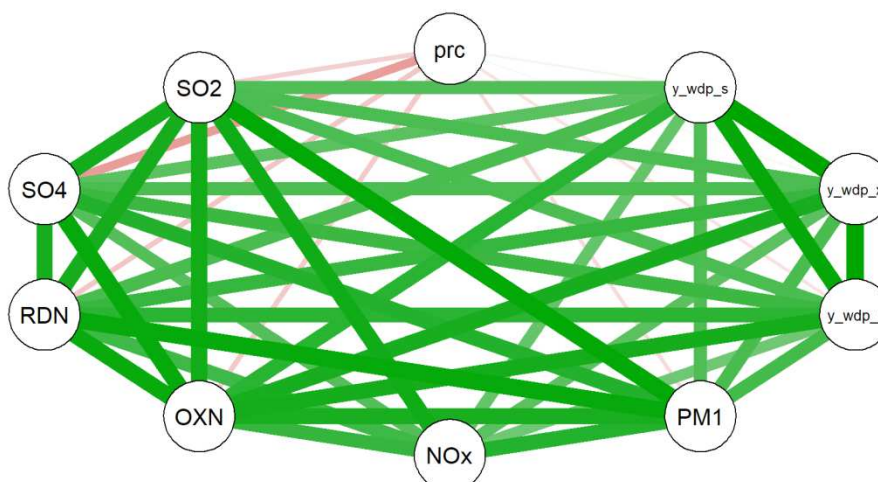
Response variables	Intercept	Relative humidity (average)	Precipitation in the vegetative period	Total deposition of reduced nitrogen	Deposition of K, open field	Summary statistics of each model
pH deepest	3.19	0.03	0.001	0.006	0.03	Mult R <sup>2</sup> : 0.138; Adj R <sup>2</sup> : -0.065 F: 0.68; <i>p</i> >0.05
Nitrogen as nitrate (N-NO <sub>3</sub> deepest)	<b>4.60*</b>	<b>-0.06*</b>	-0.0006	0.03	0.02	Mult R <sup>2</sup> : 0.564; Adj R <sup>2</sup> : 0.461 F: 5.49; <b><i>p</i>&lt;0.01</b>
Sulphur as sulphate (S-SO <sub>4</sub> deepest)	8.14	-0.07	<b>-0.005*</b>	-0.0002	-0.08	Mult R <sup>2</sup> : 0.361; Adj R <sup>2</sup> : 0.211 F: 2.40; <i>p</i> >0.05

## 4.5.2 Freshwaters

To select the best drivers to include in the models, an analysis of the correlation structure among them and a PCA was performed (Figure 4.27), showing a strong auto-correlation among the variables (positively correlated to PC1), with the exception of the precipitations (positively correlated to PC2).

Since the number of drivers is very high compared to the number of available observations, and due to their strong reciprocal correlations, the above results suggest selecting only these two ‘representative’ predictors to fit the models:

- Modelled precipitations (prec).
- Modelled total deposition of oxidised nitrogen (OXN). This driver represents all the other variables, positively correlated with PC1 of the PCA.



# LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1

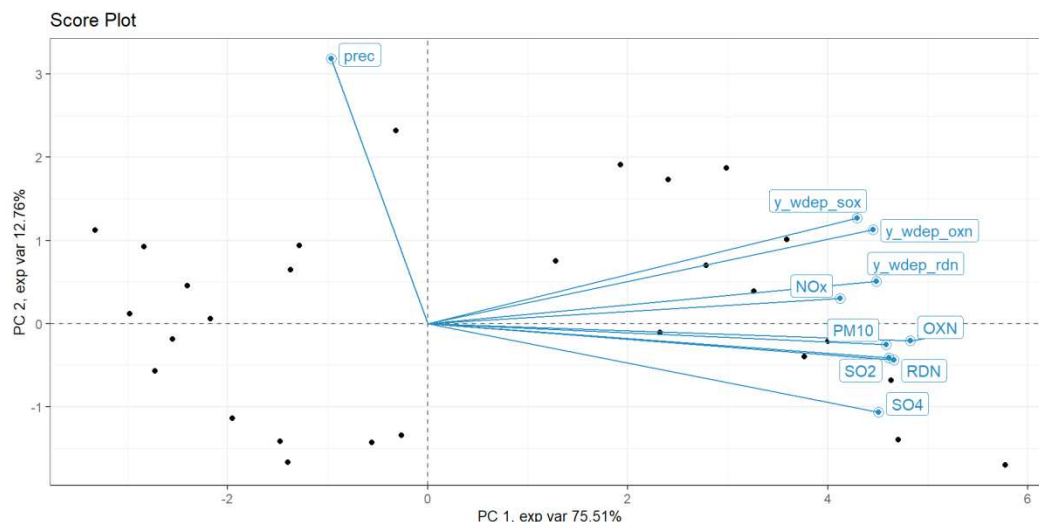


Figure 4.27 – Above: correlation structure among drivers. Positive correlations are reported in green, while negative correlations are in red. The line thickness is proportional to the correlation values. Below: Score plot of the PCA ordination of the drivers modelled in correspondence of the freshwater sites.

Table 4.8 reports the results of the multiple linear regression models describing the effects of the selected drivers on the water chemistry variables (see also Annex A1.1 for detailed results). The models are significant for most of the response variables ( $p < 0.05$ ), except for ammonium as nitrogen ( $\text{NH}_4\text{-N}$ ), total phosphorus (TTP), and total aluminium (TAL). Both the precipitation and the depositions/concentrations of nitrogen and sulphate compounds, and PM10 show a positive influence on water chemistry parameters.

# LIFE MODERN (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Table 4.8 – Multiple Linear Regression Models describing the effects of the selected predictive variables on water chemistry variables. Estimates values are reported together with statistically significant *p* values (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Last column shows the summary statistics of each model (156 df), with Multiple R2 (Mult R2), Adjusted R2 (Adj R2), F-statistic (on 2 and 33 df), *p* value.

Response variables	Intercept	Precipitation	Concentration of oxidized nitrogen	Summary statistics of each model
Alkalinity (ALK)	-305.7***	0.10***	734.0***	Mult R2: 0.878; Adj R2: 0.871 F: 119; $p < 0.001$
Sulphate (SULF)	-4.53***	0.001**	14.4***	Mult R2: 0.877; Adj R2: 0.870 F: 118.1; $p < 0.001$
Nitrate as nitrogen (NO3-N)	-192.6	0.06	1267.9** *	Mult R2: 0.681; Adj R2: 0.662 F: 35.2; $p < 0.001$
Chloride (Cl)	-2.09***	0.0005**	5.39***	Mult R2: 0.822; Adj R2: 0.811 F: 76.3; $p < 0.001$
Total Organic Carbon (TOC)	-0.27	0.0003*	1.21**	Mult R2: 0.258; Adj R2: 0.213 F: 5.75; $p < 0.01$
pH	5.41***	0.0003*	2.41***	Mult R2: 0.577; Adj R2: 0.551 F: 22.5; $p < 0.001$
Calcium (Ca)	-4.73***	0.002***	12.3***	Mult R2: 0.780; Adj R2: 0.766 F: 58.3; $p < 0.001$
Magnesium (Mg)	-1.54***	0.0004**	4.19***	Mult R2: 0.861; Adj R2: 0.853 F: 102.5; $p < 0.001$
Sodium (Na)	-2.14***	0.0006**	6.16***	Mult R2: 0.831; Adj R2: 0.821 F: 81.1; $p < 0.001$
Potassium (K)	-0.37	0.0001	1.76***	Mult R2: 0.693; Adj R2: 0.674 F: 37.2; $p < 0.001$
Ammonium as nitrogen (NH4-N)	14.4	-0.001	-2.36	Mult R2: 0.002; Adj R2: -0.058 F: 0.04; $p > 0.05$
Specific conductivity at 25°C (K25)	-4.63***	0.001***	13.8***	Mult R2: 0.892; Adj R2: 0.885 F: 135.9; $p < 0.001$
Total Phosphorus (TOTP)	1.06	0.0007	5.91*	Mult R2: 0.157; Adj R2: 0.105 F: 3.06; $p > 0.05$

# LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



Water temperature (Temp)	4.33	<b>0.003*</b>	-1.67	Mult R2: 0.183; Adj R2: 0.133 F: 3.69; <b>p&lt;0.05</b>
Total nitrogen (TOTN)	-182.2	0.09	<b>1371.3**</b> *	Mult R2: 0.695; Adj R2: 0.677 F: 37.6; <b>p&lt;0.001</b>
Soluble reactive phosphate (ORTP)	0.07	0.0004	<b>3.82***</b>	Mult R2: 0.329; Adj R2: 0.288 F: 8.08; <b>p&lt;0.01</b>
Silicium (Si)	-1.30	0.0005	<b>4.69***</b>	Mult R2: 0.367; Adj R2: 0.328 F: 9.55; <b>p&lt;0.001</b>
Total aluminium (TAL)	1.93	0.003	9.63	Mult R2: 0.054; Adj R2: -0.004 F: 0.93; <b>p&gt;0.05</b>



## 5. Conclusion

In the last 20 years (time-span 2000-2020) a general decrease in the modelled concentrations of the main air pollutants was observed in correspondence to the selected forest and freshwater sites, that is less evident for nitrogen compounds. Similarly, modelled depositions showed a general decreasing trend is evident for sulphur and nitrogen oxides, both in our forest and freshwater sites, while for reduced nitrogen a decreasing trend is less evident (Fig. 5.1).

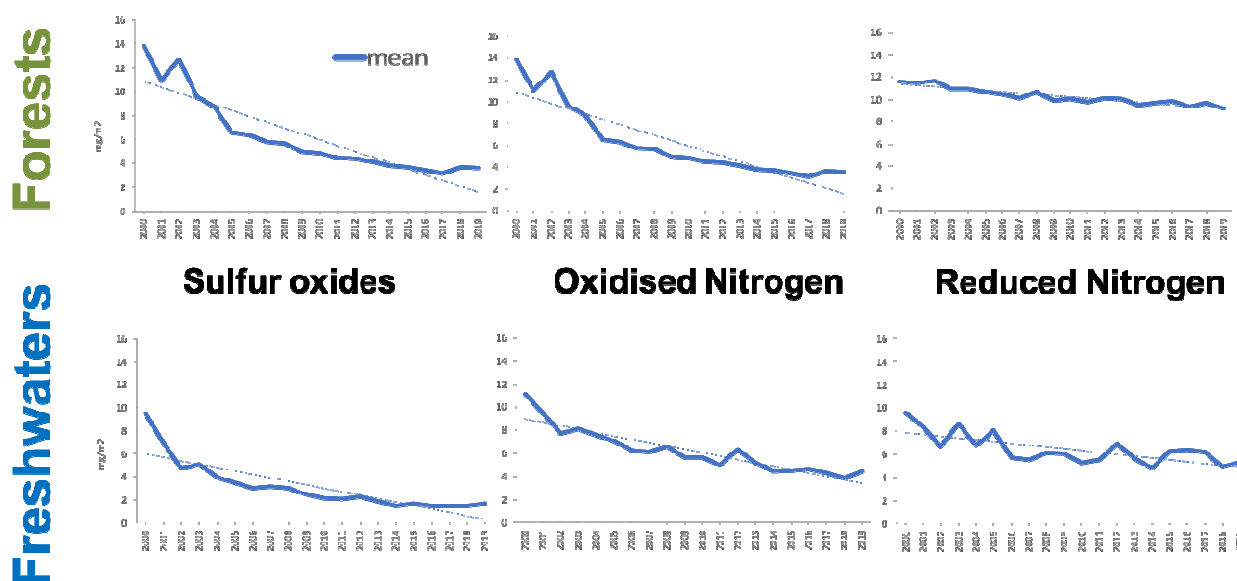


Figure 5.1 – Trend of the modelled depositions (EMEP models: sulphur oxides, oxidised and reduced nitrogen) obtained for the sites of the project (both forest and freshwater sites) in the last 20 years.

Further, both concentrations and depositions show a clear correlation with altitude, with the sites at higher altitudes showing the lower air pollution levels, and this is true both for forest and freshwater sites.

By analysing the single indicators, both for forests and for freshwaters, a clear trend in ecosystem responses is not evident, which seem to be more influenced by site-specific features, such as altitude (mainly freshwater sites, this variable covariates with precipitations and, indirectly, with air pollutants) or the proximity to atmospheric emissions (for example the plots situated in the surroundings of the Po Plain).

The predictive models provide an overview that can be summarised as follows:

- Forest ecosystems. Only some response variables belonging to three indicators are influenced by the considered drivers (crown condition, soil solution, and ground vegetation). Damage to branches and  $\text{NO}_3$  concentrations in the soil solution are negatively affected by relative humidity. The herbaceous layer cover is negatively affected by nitrogen depositions and positively influenced by the depositions of  $\text{SO}_4$ , K and alkalinity.

## LIFE MODERn (NEC)

LIFE20 GIE/IT/000091

Delivarable, action A1



- Freshwater ecosystems. Most of the water chemistry variables are positively influenced by depositions, both nitrogen and sulphur compounds, and by precipitation.

The fact that only a few of the indicators/response variables significantly respond to the air pollution drivers, can be explained on the basis of the following general considerations:

- i) probably our indicators have slower response times compared to the speed of changes recorded in the levels of air pollutants during this period;
- ii) the variables considered for each indicator are not sufficiently explanatory of the drivers, thus highlighting the need to develop new indices starting from these variables or to identify new more suitable indicators;
- iii) we performed the current data processing without separating the forest types, with a potential confounding effect on possible trends.

The results of this Action will be extremely important to obtain a baseline for Actions B.1 (selection of the new monitoring sites), B.2 (definition of a new set of indicators) and B.3 (implementation and testing; point 3).

### Additional analyses

On the basis of the considerations reported above and of the suggestions of the scientific partners of the project, a second round of data processing has been performed to explore the following issues:

- i) chemical variables were log-transformed rather than autoscaled and descriptive multivariate statistics (PCA) and models (MLRM) have been performed.
- ii) the potential effect of forest types in the results. For each indicator, models were elaborated separately for each forest type. However, we must consider that, by separating the data by forest type, the sample size of the single datasets is reduced, with an effect on the quality of the models.

The results are not far from those obtained in the first exploratory analysis, but they could be useful for Action B.3. Detailed results are reported in Annex A1.2.