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D6.2-Cross-RI service to contrast climate change (elevated N, CO_2, O_3) impacts on Italian terrestrial ecosystems, with focus on: grain quality, sanitary risk and crop selection; O_3 and N deposition critical levels.

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1. INTRODUCTION: ITINERIS – WP 6.3 HARMONIZATION AND INTEGRATION OF FREE-AIR MANIPULATIVE PLATFORMS DATA IN TERRESTRIAL ECOSYSTEMS

Deliverable D6.2 is part of the work carried out within WP6.3, regarding the *“Harmonization and integration of free-air manipulative platforms data in terrestrial ecosystems”*.

The main goal of WP6.3 is to integrate and harmonize the Italian free-air manipulative platform data of terrestrial ecosystems to improve FAIR access to their data and services.

The ANAEE (Analysis and Experimentation on Ecosystems) Research Infrastructure consists of long-term, field-scale experimental manipulation platforms located across various terrestrial ecosystems and climatic conditions to have a comprehensive understanding of the fate of forest ecosystems and crops due to elevated Carbon dioxide, Tropospheric Ozone and Nitrogen deposition in a long-term perspective. Data integration, harmonization and data sharing are prerequisites to exploit data potential from the different manipulative experiments for contrasting the effects of climate change drivers (i.e., elevated nitrogen, CO_2 and ozone), developing realistic risk assessments and models on the responses of terrestrial ecosystems to global changes by overcoming the limits of having standalone experimental platforms.

During the first year, two technicians, one PhD and one technologist, were hired and the digitalization of the three ANAEE platforms was discussed with WP 2 and WP 8 team members. A metadata base was developed; acquisition and installation of the scientific instrumentation and technological equipment at each site/platform started and it will be completed in the second year; the third year will be spent for implementing the platform set up, full operation and transfer of data.

2. STATE OF ART OF THE EFFECT OF CLIMATE CHANGE DRIVERS

2.1 The effects of Nitrogen deposition on forest ecosystems

Human activity has critically altered the nitrogen cycle during the last decades, thus resulting in increased nitrogen deposition in all ecosystems. The production of fertilizer and fossil fuel combustions have increased the amount of N reactive compounds such as NO_X and NH_3 to double compared to preindustrial levels, with unclear short- and long-term effects on forest ecosystems. In nitrogen-limited environments such as temperate forests, an increased nitrogen input stimulates tree growth, the rate of litter decomposition and nutrient turnover, while in non-limited environments nitrogen addition can lead to severe consequences causing ecosystem nitrogen saturation, which leads to ecosystem degradation. The first symptoms of nitrogen saturation imply elevated nitrogen leaching and soil N_2O emissions; resulting in increased nitrification rate, soil acidification, nutrient losses and a general decrease in forest productivity and biodiversity with nitrophilous species being advantaged over the others. At the foliar level, symptoms such as tip necrosis and chlorosis correlate to increased N input which in turn decreases the availability of Ca^{2+} and Mg^{2+}, thus affecting the key component of photosynthetic pigments and photosynthetic processes. This may induce growth reduction and increase plant sensitivity to other stress and/or disturbances (i.e., pests, drought). There is an urgent need for characterizing N species-specific critical levels to forecast future forest ecosystem scenarios.

2.2 The effects of Carbon dioxide on crop production

The rising CO_2 concentrations in the atmosphere caused by fossil fuel combustion are considered the major drivers of rising temperatures and climate change. Also, the higher CO_2 concentrations directly influence plant growth and physiology, posing relevant concerns related to agricultural crop production, food security, food safety and sanitary risk. In elevated CO_2 environments, plants improve their photosynthetic activity and reduce their stomatal conductivity, enhancing water use efficiency, which helps to overcome drought conditions. As a result, elevated CO_2 concentrations enhance above and below-ground biomass, as well as harvestable yield of crops. Despite the beneficial effect on crop biomass, several studies highlighted the uncertainties of elevated CO_2 concentration on grain quality and nutritional properties. In elevated CO_2 concentrations plants showed reduced nitrogen content, which affects protein concentrations, and also decreased mineral content negatively affecting grain quality. Further, the relationship between elevated CO_2 and other climate change effects such as increased temperatures, and extreme events such as heat waves, drought and changes in precipitation patterns cause relevant concerns related to agricultural crop production, and the matter needs further investigation.

2.3 The effects of Tropospheric ozone on plants

Tropospheric ozone (O_3) is an air pollutant and one of the major greenhouse gases. This highly reactive and oxidative gas occurs naturally in the environment. However, it has increased in the troposphere since the industrial revolution, resulting from photochemical reactions of its precursors such as methane, carbon monoxide, volatile organic compounds and nitrogen oxides. Although a reduction in tropospheric O_3 peaks was obtained in some areas of the globe, the high O_3 concentrations remain a global relevant concern.

The O_3 negatively affects plant biochemistry and physiology, damaging cells and tissues. Once it enters the stomata, O_3 and its oxidative derivatives (ROS) provoke a cascade of effects that finally can lead to impairment of plant functioning. O_3 can significantly alter biochemical pathways such as photosynthesis and carbon assimilation, cause dysfunctions in stomatal control, produce signs of foliar injury and accelerate leaf senescence. Relevant concerns about plant functioning and ecosystem services such as crop production in response to O_3 pollution persist, and investigations are needed considering other co-occurring stressors related to climate change, and the future scenario that forecasts rising concentrations of O_3 in the troposphere. The O_3 species-specific critical level needs to be characterized for counteracting and mitigating the adverse effect of this air pollutant on plants.

2.4 The effect of climate change on Italian cropping system

The Italian cropping system is particularly vulnerable to the impacts of climate change, which are especially pronounced in the Mediterranean region. The observed effects such as rising temperatures, erratic precipitation patterns, pest proliferation, and water scarcity, pose significant threats to both the quantity and quality of agricultural production. These challenges place the agricultural sector and the Italian food systems at serious risk. The anticipated decline in agricultural productivity, exacerbated by farmers' difficulties in adapting to and mitigating the impacts of climate change underscores the urgent need for long-term studies. Such studies are crucial for a comprehensive understanding of the complex interactions related to climate change and for the development of effective adaptation strategies for the Italian cropping system. The implementation of these strategies, supported by robust policy frameworks and technological innovation, is essential to safeguard Italy's agricultural heritage and to ensure food security in the face of a changing climate.

3. EXISTING OPEN-AIR TERRESTRIAL PLATFORMS OF ANAEE-ITALY

The Institute of Research for Terrestrial Ecosystems at the National Research Council (hereafter, IRET - CNR) of Sesto Fiorentino (FI) coordinates AnaEE open-air terrestrial platforms and develops vast scientific experience on how climate change drivers impact plants, crops and forest ecosystems. The acknowledgeable expertise in field manipulative experiments where higher concentrations of the major climate change drivers (i.e., N, CO2, O3) are simulated in various types of ecosystems and climatic conditions with sophisticated experimental designs, long-term data measurements, high geographic distribution and resolution and several plant species involved made IRET-CNR a leader in this field of study and a unique institution for conducting cross RIs research for counteracting climate change effects.

A description of the research platforms coordinated by IRET – CNR follows.

3.1 The Italian Nitrogen Deposition Network (INDN) - FOR2N

The Italian Nitrogen Deposition Network (INDN) developed an ANAEE Research platform named FOR2N, that consists of long-term manipulative experiments in forest sites located across various terrestrial ecosystems and climatic conditions, that simulate elevated nitrogen deposition. The FOR2N platforms can highlight the existing uncertainties related to the potential long-term nitrogen cumulative effects on forests exposed to elevated atmospheric nitrogen loads, considering the slow processes involved in forest ecosystems. Further, the manipulative experimental platforms evaluate the role of the canopy layer in intercepting and absorbing nitrogen by applying it above the canopy and not only to the forest floor. The role of the canopy was often neglected, but its integration into the manipulative experiments can lead to more realistic future scenarios about the long-term nitrogen deposition effects on forest ecosystems.

The network was developed in 2015 as a part of AnaEE RIs under the coordination of IRET-CNR to study the effect of increased nitrogen deposition on forest ecosystems' capacity to act as C-sink and to assess N deposition critical level for trees and forests ecosystems by field scale manipulative approach. The sites cover different climatic conditions and forest types and provide novel information on how forest ecosystems respond to the increased N deposition. In all sites, N salts diluted in H_2O was applied to forests below the canopy layer and above the canopy layer to simulate the increased nitrogen depositions forecasted for future decades. The treatments are replicated and compared against natural nitrogen deposition intra-site, but comprehensive inter-site comparisons are still lacking.

Monticolo

The experimental site is managed by the Free University of Bolzano-Bozen coordinated by IRET-CNR, Sesto Fiorentino. The location is in a sessile oak forest (*Quercus petraea* (Matt.) Liebl) near Monticolo, Province of Bolzano, Italy (46°255’35″ N; 11◦17́55″ E, 550 m elevation). The climate is temperate submontane with mean annual temperature of 11.4°C and mean precipitation of 824 mm y^{-1}. The stand grows on a shallow Cambisol with a pH value of 5.5, over a quartz porphyritic bedrock. The experimental design consists of 9 circular plots (12 m radius), where the three treatments (e.g., unfertilized control plots, N fertilization to the ground below canopies, N fertilization above the canopies) have been replicated three times. N-addition is being provided with 20 kg N $ha^{-1}y^{-1}$ through 5 monthly applications (each adding 4 kg N ha^{-1}). A meteorological station was installed in the site and soil temperature and water content are also recorded. Dendrometric bands were mounted on 10% of the trees until 2018, from 2018 onwards every tree inside the plots was endowed with dendrometers. Each year the leaves were collected to analyze the total C, the total N, the leaf area and the dry weight to calculate the specific leaf area. Also, C and N content were analyzed in the litter components (i.e., branches, leaves, fruits) and the soil. N bulk depositions (throughfall and control) and stemflow N flux are analyzed annually with ion-exchange resins.

Cembra

The experimental site is managed by the Edmund Mach Foundation coordinated by IRET-CNR, Sesto Fiorentino. It is located in Val di Cembra 46°12'9"N, 11°12'35"E, at 1270 m a.s.l.in a temperate mountain forest beech forest (*Fagus sylvatica* L.). The stand grows on an acidic brown earth soil, over a porphyry (Rhyolite) bedrock. The mean annual temperature is 11.0 °C and the mean precipitation is 1053 mm yr^{-1}. An eddy covariance flux tower and a PhenoCam are installed in the proximity of the site, and soil water content is also monitored. The experimental design is the same as the one in Monticolo. Tree talkers were mounted on the trees since 2019 and stem increments are recorded annually. Total C and N are analyzed in the soil, in the leaves and the litter. N bulk depositions (throughfall and control) and stemflow N flux are analyzed annually with ion-exchange resins.

Cansiglio

The study site is located in the Cansiglio forest 46° 3′ 19” N 12° 22′ 51″ E at 1100 m a.s.l. It is managed by the Alma Mater Studiorum, University of Bologna and coordinated by IRET-CNR, Sesto Fiorentino. The site is characterized by the presence of an oceanic climate. The forest grows on a Calcareous pedogenetic substrate (Haplic Luvisol). The meteorological variables, forest growth rates, atmospheric N deposition data, and leaf and soil biochemical characteristics are monitored in the proximity to permanent Forest Level 2 monitoring plots since 1996. The experimental design includes four treatments: unfertilized control plots, N fertilization to the ground below canopies with 30 kg N ha^{-1} yr^{-1}, N fertilization to the ground below canopies with 60 kg N ha^{-1} yr-1, N fertilization above the canopies 30 kg N ha^{-1} yr^{-1}, in three replicated plots (30 x 30 m). The following parameters are analyzed: tree growth through dendrometric bands, leaf macro and micronutrient, total C and N in litter and soil, and litter weight.

Collelongo

The study site is managed by the Institute of Bioeconomy (IBE) of the National Research Council, Sesto Fiorentino and coordinated by coordinated by IRET-CNR, Sesto Fiorentino. The site is located in Central Italian Apennines, Collelongo 41° 50′ 59" N 13° 35′ 6″ E at 1560 m a.s.l on a calcareous substrate with stones and clay components (Humic Alisols). The climate is Mediterranean with mild summer drought. Also in this case, the meteorological variables, forest growth rates, atmospheric N deposition data, and leaf and soil biochemical characteristics are monitored in the proximity to permanent Forest Level 2 monitoring plots since 1996. The experimental design consists of three treatments: unfertilized control plots, N fertilization to the ground below canopies 30 kg N ha^{-1} yr^{-1}, N fertilization to the ground below canopies 60 kg N ha^{-1} yr^{-1}, in three replicated plots (30 x 30 m). Consistently with the site in Cansiglio, the same parameters are recorded.

3.2 Durum FACE - Free-Air CO_2 Enrichment

The Research Infrastructure FACE (Free Air CO_2 Enrichment) exposes crops to elevated atmospheric CO_2 concentrations in an open-air site and is considered the best chance to study the effects of CO_2 without altering the microclimate and with minimal interference with the natural environment. This technology allows the investigation of rising CO_2 on plant growth, unravelling possible future crop production under climate change scenarios. In the spring of 2025 the Research Centre for Genomics and Bioinformatics (CREA) and the Institute of BioEconomy (IBE) of the National Research Council, Sesto Fiorentino, will complete the platform renovation in Fiorenzuola d'Arda (PC) coordinated by IRET-CNR, Sesto Fiorentino. The biomass, the physiological traits and other specific productive and qualitative traits of Durum wheat (*Triticum durum* Desf.) will be evaluated when subjected to elevated CO_2 concentrations in comparison with current CO_2 concentrations (control plots). The meteorological data will be recorded by in situ- meteorological stations, also soil biophysical parameters will be recorded. The experimental design will include a rainfall exclusion experiment to investigate the interaction between elevated CO_2 and water availability in durum wheat.

3.3 FO3X - Ozone FACE - Free air-controlled exposure

FO3X (Free air O_3 eXposure) is a new-generation facility that best simulates the effects of elevated O_3 concentrations on plants without altering field conditions, to assess present and future O_3 risk for plants. The facility is coordinated by IRET – CNR, a world leader in ozone and vegetation studies and located in Sesto Fiorentino (FI) 43°48′59″N, 11°12′01″E, 55 m a.s.l. on a Endogleyi Vertic Cambisols in a hot summer Mediterranean Climate.

The FO3X exposes plants continuously at three levels of O_3 concentrations with three replicates for a total of nine plots measuring 5 × 5 × 2 m (L × W × H). The design allows to evaluate the effects of O_3 on plants alone and in concomitance with other stressors, such as soil water stress, nutrient alterations, salinity, heavy metal presence etc. The effects of O_3 on plants are evaluated considering many plant attributes, including growth, root characteristics, gas exchanges, emission of biogenic volatile organic compounds, nutrient content, and antioxidants. The sophisticated FO3X is the only facility simulating the effects of elevated concentrations of O_3 on plants in concomitance with other stressors in a Mediterranean Climate, thus representing a fundamental facility to develop appropriate plant risk assessment against future global changes.

3.4 CIRCULAR - Inter-University Centre for Agroecosystems Research

The Circular research platform is coordinated by IRET – CNR and involves several research teams from the University of Florence, the University of Catania, the University of Padua, the University of Perugia, the University of Sassari and the University of Turin. The network allows the establishment of long-term experiments of the most important Italian cropping systems over a wide range of Mediterranean environments, which can then be integrated or made available for modelling and remote sensing applications., thus having a prominent role in the understanding of climate change impacts and climate change mitigation on the agricultural systems. The platform offers the chance to investigate several relevant agronomic questions and assess the reliability of various cropping systems in Mediterranean environments with a specific focus on food quality, food production and food security. The data collected in the long-term experiments will be used to calibrate, validate and apply crop growth models capable of assessing the best strategies for adapting to and mitigating climate change. The platform is under renovation processes that will be completed by the spring of 2025.

Within CIRCULAR, the productive traits of various crops such as wheat, maize, sugar beets, legumes, sunflower, horticultural crops, lucerne, and sorghum, will be assessed under manipulated abiotic conditions (e.g., water regime, nutrient availability, soil characteristics) and agricultural management practices (e.g., fertilizer application) to name a few, to best simulated future condition under a changing climate. The platform provides a unique opportunity for a comprehensive evaluation of Italian cropping systems under climate change scenarios. Furthermore, it is poised to generate critical knowledge for developing effective mitigation and adaptation strategies aimed at sustainable agricultural management. These strategies will focus on counteracting the adverse effects of climate change, ensuring the maintenance of food production with a particular emphasis on food quality and security.

4. DATA COLLECTED IN THE NETWORK, SCIENTIFIC PRODUCTION AND PURCHASE OF INSTRUMENTS AND TECHNOLOGIES

The IRET – CNR, as leader coordinator for the research platforms is fundamental to ensure the harmonization, standardization and sharing of data collected at each platform. Each platform collected the data independently, and there is no uniformity nor homogeneity of the data type and format to date. During the first two years of the ITINERIS project, IRET – CNR scheduled several meetings with the network members to establish newly acquired equipment protocols and to proceed with setting up instruments and technologies. The coordination activity of IRET – CNR was fundamental to discuss the aspects relevant to WP6.3 namely harmonization and standardization of data collection through the purchase of new instruments, and the establishment of common protocols on data collection regarding old and newly acquired data.

A metadata base responding to FAIR principles was developed for the research platforms. Quality and rich metadata are essential for managing large numbers of datasets and conducting appropriate data selection enhancing accessibility, interoperability and data reuse. The metadata base contains relevant qualitative and quantitative information about the data describing experiments, site conditions, sensors, techniques and methodologies used to monitor biological, physical and chemical attributes and it is the first concrete action for conducting outcomes and processes harmonization and standardization of the ITINERIS Research Infrastructures.

Finally, IRET – CNR focused on data acquisition and defined shared strategies to facilitate the process of data harmonization and sharing as well as data availability over time. We established specific agreements and adopted shared policies regarding the acquisition of past and future data collected taking into consideration specific needs and complexity of each site and the WP6.3 requirements of improving FAIR access to the data and services.

The ITINERIS project aimed at purchasing scientific instrumentation and technological equipment to improve research activities and data collection and harmonization at each ANAEE site. Specific instrumentations are necessary to respond to the manipulative experiment peculiarities and the plant species subjected to the experiments. At present, we have completed the purchase of several instruments whose temporary installation at each platform is taking place. A number of them remain in the purchasing process. The research platforms implementation with new instrumentations and similar policies regarding data acquisition and sharing on dedicated informatics infrastructures are prerequisites to guarantee the accomplishment of WP6.3. Data acquisition from the free-air manipulative platform data of terrestrial ecosystems started, and it will be performed and implemented during the following months and years of the project.

The following is a comprehensive summary of purchased instrumentations and equipment and the relative data acquisition and scientific outcomes produced from the beginning of the ITINERIS project for each free-air manipulative platform in terrestrial ecosystems, enabling the implementation, harmonization, and standardization of procedures and data collection across the sites.

4.1 FOR2N

The nitrogen deposition network benefitted from the purchase of several instruments and technologies whose employment is fundamental for implementing the research infrastructure and promoting research innovation, data harmonization and collection.

The available datasets from the FOR2N network will consist of environmental data such as the daily hour average of air temperature, air relative humidity, precipitation, soil water content and soil temperature obtained through the eddy covariance tower and/or in situ weather stations. Additionally, the datasets will be enriched with several types of data whose presence will provide further information and insights about the N cycle in forest ecosystems with particular attention to the role of tree canopies that recently emerged as an essential layer involved in the N cycle. Further interest is dedicated to the relationship between increased N deposition and greenhouse gas emission production as well as tree physiology to identify possible species-specific N deposition critical levels.

Specifically, the purchase of diffusive samplers for monitoring background dry nitrogen deposition (NO_2 and NH_4), and chambers and gas analyzers to monitor simultaneous and continuous soil emissions of concentrations of ammonia, methane, carbon dioxide and NOx, will provide the datasets with relevant data about site N dry deposition estimation and will reveal whether the N addition will increase soil greenhouse gases emission by positively influencing the micro bacterial soil community. Also, tree responses will be investigated through the collection of visible-near-infrared and fluorescence hyperspectral images, and tree talker devices installed on the target trees will record hourly dendroecological data such as sap flow, stem humidity, canopy light transmission, tree trunk radial growth and tree trunk axis movement to identify possible signals related to the increased N deposition.

Scientific production

The outcomes obtained from the beginning of the ITINERIS project highlight the importance of the FOR2N research platform for a better understanding of the nitrogen cycle in forest ecosystems that have direct implications on forest carbon uptake capacity, hence for climate change mitigation, as well as for forest management and timber production.

The FOR2N platform has the peculiarity of delivering nitrogen not only to the forest floor but also above the canopy layer to assess the role of the tree canopy in intercepting, transforming, and assimilating atmospheric nitrogen inputs. Recent findings obtained from the FOR2N research platform recognize the prominent role of the canopy in intercepting considerable amounts of nitrogen from atmospheric deposition (Da Ros et al., 2023; Guerrieri et al., 2024). The microbial communities of nitrifiers that live in tree canopies cover a fundamental step in the nitrogen cycle by converting ammonium to nitrate, likely increasing the long-term nitrogen retention in forest ecosystems (Da Ros et al., 2023). The microbiota on the phyllosphere directly impacts the nitrogen available for the soil by reducing the NH_4^+ and increasing soluble NO_3^-, an activity that was ignored and underrecognized to date but that is essential for a deeper mechanistic understanding of the nitrogen cycle in forest ecosystems (Guerrieri et al., 2024). Further, a need to rethink the current models used to estimate nitrogen deposition effects on forest ecosystems emerged (Guerrieri et al., 2024). Indeed, most manipulative experiments deliver nitrogen only to the forest floor without accounting for the role of the canopy, introducing biases in the understanding of forest responses to increase nitrogen availability (Da Ros et al., 2023). Nitrogen addition improves carbon sequestration capacity in nitrogen-limited environments such as temperate forests. Minikaev et al. (2024) evaluated the tree ring properties, namely ring width and ring density of trees subjected to the experimental design in the FOR2N platform. The results highlighted species-specific altered wood densities of trees subjected to the more realistic nitrogen deposition approach (i.e., above canopy fertilization), with potential relevant implications for forest management and timber production in a future scenario where increased nitrogen input in forests is foresees. Given the tree's long life and delayed responses to several environmental stresses and changes, the studies suggested that more time and further investigations are needed for a thorough assessment of forest ecosystem response to nitrogen addition and that the decadal scale will enlighten more clearly tree and forest ecosystem responses to nitrogen addition, possibly identifying a species-specific critical level and ecosystem critical nitrogen load beyond which processes and plant community composition altered.

The unique experimental design of the FOR2N research platform at the Italian level plays a prominent role in investigating the nitrogen cycle in forest ecosystems. The results obtained with a more realistic nitrogen deposition simulation showed that the FOR2N research platform actively contributes to nitrogen limitation policy formulation (Da Ros et al., 2023; Guerrieri et al., 2024).

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4.2 Durum FACE

The FACE (Free air-controlled exposure) facility is planned to be installed at the beginning of the vegetative season, in 2025.

The manipulative experiment will be conducted under constant monitoring of several environmental parameters such as air temperature, air relative humidity, precipitation, soil temperature, solar radiation, soil water content and soil biophysical parameters by in situ weather station. To evaluate the effect of elevated CO_2 on *Triticum durum* grain quality, morphological and productive traits such as average plant height, ear density, and other specific physiological and nutritional plant traits will be recorded. Also, the project will evaluate the crops under the effect of increased CO_2 in concomitance with another stressor, such as scarcity in water availability to provide a realistic projection dealing with food production and security for the near future. Also, a particular aspect crucially explored in the manipulative experiment will be the crop selection in light of the sanitary risk.

Scientific production

The research platform will be renovated with a new FACE technology and installed in the spring of 2025. An overview of the investigations of the main relevant and recent findings on the CO_2 effects on *T. durum* follows.

Triticum durum is the primary ingredient for manifold traditional foods such as pasta and bread and plays an essential role in the Mediterranean diet.

Several studies highlighted that elevated CO_2 concentrations decrease the grain's nutritional properties. This effect is attributed to an increased carbohydrate production that diluted the content per unit weight of essential minerals such as zinc, iron, and magnesium (Beleggia et al., 2018) and protein (Fernando et al., 2012). Also, elevated CO_2 concentrations impact gluten content (Fares et al., 2016) and changes in starch granule size and distribution (Wei et al., 2024) with unclear implications for celiac and gluten-related diseases and direct impacts on pasta, bread elasticity and texture and on cooking properties, posing at risk the quality of traditional products whose large part of Italian economy rely on.

Recent research revealed the effects that elevated CO_2 concentrations have on sanitary risk. Bencze et al. (2017) showed that elevated CO_2 could enhance the fungal activity of *Fusarium* species that leads to yield loss and mycotoxin contamination. *Fusarium* species produce a mycotoxin found in high content in the grain subjected to elevated CO_2 concentrations by the authors. These mycotoxins are harmful contaminants and pose serious health risks to durum wheat product consumption.

The Durum FACE plays an essential role as an Italian and International research platform where simulated elevated CO_2 concentration in combination with water scarcity will provide an excellent setup to fill research gaps and contribute to the current research lines, namely food security and sanitary risk, that have as main objectives the maintaining of the nutritional properties of the grain and the avoidance of fungal contaminations.

References:

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4.3 FO3X

The ozone free air-controlled exposure facility was equipped with several new instruments and technologies that provide important additional data sources for infrastructure and experimental condition implementation to respond adequately to current research gaps. The environmental data available are hourly averaged records of air temperature, relative humidity, precipitation, soil water content, soil temperature, solar radiation and wind speed obtained through meteorological stations in situ. Further, air pollutant hourly averaged concentrations of NOx, O_3, CO and PM2.5 are obtained through the purchased AIRQino device, O_3 and NOx monitors.

Plant productivity as well as LAI, will be assessed through instruments such as drone technologies and LAI meter. From the elemental point of view, the validation will be carried out by spectrophotometer and ICP-OES.

The purchase of Li-Cor6800 and porometer are used to estimate evapotranspiration. These two instruments, in combination with the zero-air generator (ZAG), are used to assess the stomatal ozone flux that constitutes the basis to calculate the species-specific flux-based critical level (4% biomass reduction). This parameter is used to quantify species or vegetation risk to be negatively affected by ozone, and it is fundamental to scientists and policymakers to formulate detailed risk assessments of the impacts of ozone.

In the context of ameliorating the leaf ozone-induced symptoms assessment, which presents several uncertainties given the high variability in the ozone-induced leaf symptoms also among individuals of the same species, data on ozone-induced leaf damage will be primarily assessed with a visual inspection, will be validated with tissue processing and the creation of glass slides, which will allow the validation of leaf ozone symptoms under the microscope at high resolutions.

Finally, given that gas pollutant removal constitutes one of the most relevant ecosystem services provided by urban trees and shrubs, to increase the information on the urban tree/shrub species mitigation effect against the following air pollutants CO_2, O_3, NO2 and PM software and related apps will be developed.

Scientific production

During ITINERIS, the data collected in the FO3X - Ozone FACE - Free air-controlled exposure produced relevant and interesting outcomes for ameliorating our understanding of the effect of ozone pollution on plant physiology and the manifold implications related to it.

The ozone FACE facility allows to explore and characterize a wide range of species responses subjected to ozone in concomitance with multiple stressors. The multidisciplinary approaches are often adopted as the best choice for a thorough comprehension of plant and ecosystem responses to ozone.

A consistent part of the research is about Indexes and methodologies formulations for more accurate and early detection of ozone effects on plants.

In 2023, Manzini et al. formulated a new Index named LIF (Leaf Index Flux). Considering that damages caused by ozone are related to species-specific stomatal ozone flux rather than ozone atmospheric concentrations and that leaf structural traits such as thickness improve ozone resistance by exposing minor mesophyll surface area to ozone, the LIF combines the stomatal ozone flux and leaf mass per area (Manzini et al., 2023). The Index was tested on several species (*Arbutus unedo* L., *Phillyrea angustifolia* L., *Pinus pinea* L., *Sorbus aucuparia* L., *Alnus glutinosa* L., *Vaccinium myrtillus* L., *Populus maximowiczii* Henry X *P.* × *berolinensis* Dippel, and *Populus x euramericana* I-214), it correlates confidently with tree ozone-induced visible foliar injuries, therefore it is considered an interesting and promising new proxy for evaluating ozone damages on forest species, although the fundamental parameters to formulate this Index are not readily available for several species (Manzini et al., 2023). In Meschini et al., 2023, the deeper analyses on DNA integrity after exposing the moderately ozone-sensitive *Arbutus unedo* and the hybrid highly ozone-sensitive *Populus maximowiczii* Henry × *berolinensis* Dippel, reveal that DNA and ecophysiological damages were detected in the highly-sensitive species, while the DNA was damaged prior that ecophysiological symptoms occur in the moderately sensitive species (Meschini et al., 2023) open the possibility to predict ozone-induced damage detection. In the study of Moura et al., 2023, the authors successfully employed the colour composition methodology for ozone-induced foliar injury assessments on three years of ozone exposure for *Vitis vinifera* L. Cabernet Sauvignon variety. The powerful tool developed by the researchers uses image analysis to identify, analyze and classify the colour composition of symptoms associated with ozone-visible foliar injuries. The colour pattern was associated with the leaf's physiological status and utilized as a diagnostic tool for the assessment, identification, and quantification of ozone foliar injuries in manipulative experiments (Moura et al., 2023).

Regarding the valuable crop *V. vinifera* “Cabernet sauvignon”, the three-year ozone exposure study highlighted another important adverse effect of ozone: the cumulative effect. The third year *V. vinifera* showed minor bunches and fruits with decreased soluble sugar content (Moura et a., 2023). Those berries, interestingly, were disliked by local magpies compared to the *V. vinifera* berries non-exposed to the fumigation with ozone, highlighting the potential cascade effects of high ozone concentrations in natural ecosystems (Viviano et al., 2023). In the deep and rigorous study of Hoshika et al., (2023) the researchers characterized two common Mediterranean tree species responses to ozone, *Pinus pinea* and *Pinus halepensis*. The two pine species showed differential responses to ozone in light of their differences in phenological development, leaf gas exchange, ozone damage repair and detoxification abilities (Hoshika et al., 2023).

The species-specific ozone responses evidenced the importance of characterizing numerous plants and trees to increase knowledge about their ecophysiological responses for enhancing ozone risk assessment relevant to the agricultural sector and Mediterranean natural ecosystem preservation. Finally, the possibility of predicting the ozone-induced damage, or detecting it at the very early stages using several types of methodologies, produced valuable insights for developing ozone risk assessment and for improving management and mitigation strategies regarding natural ecosystems and agriculture.

Ozone response is species-specific. In the years of the project, the following plants were characterized and evaluated under ozone pollution:

- ·*Pinus pinea*, *Pinus halepensis* (Hoshika et al., 2023);

- *Arbutus unedo* L., *Phillyrea angustifolia* L., and *Pinus pinea* L., *Sorbus aucuparia* L., *Alnus glutinosa* (L.) Gaertn., *Vaccinium myrtillus* L., *Populus maximowiczii* Henry; X P.× *berolinensis* Dippel, and *Populus x euramericana* I-214 (Manzini et al., 2023; Meschini et al., 2023)
- *Vitis vinifera* L. cv "Cabernet Sauvignon" (Moura et al., 2023, Viviano et al., 2023)

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4.4 CIRCULAR

The sites belonging to the Circular research platform are in the renovation process and they are planned to be installed in the spring of 2025.

The manipulative experiments will be carried out with continuous monitoring of various environmental factors, including air temperature, relative humidity, precipitation, soil temperature, solar radiation, soil water content, and soil biophysical properties, using an on-site weather station. To assess the various impacts of climate change on the crops, the key morphological and productive traits of the targeted species as well as specific physiological and nutritional characteristics will be recorded. Additionally, the research platform characteristics allow to examine the effects of combined stressors, like water scarcity and altered soil characteristics (e.g. salinity), to provide a realistic projection of future food production and security. An important aspect that will be evaluated is crop selection, particularly considering potential health risks.

Scientific production

The Circular research platform will be installed in the spring of 2025. A resume of the main recent outcomes on the effects of climate change on the Italian cropping system is provided.

Agriculture in the Mediterranean region, including Italy, is particularly vulnerable to the adverse effects of climate change (Wiebe et al., 2015). The increasing intensity of extreme weather events, coupled with the unpredictability of future climate conditions, is likely to place considerable strain on crop productivity and quality. These effects will vary depending on the specific characteristics of the crops and local growing conditions (Pulighe et al., 2024). Understanding how climate change affects agricultural yields and income is critical for formulating effective mitigation and adaptation strategies. Extreme weather not only results in financial losses for farmers but also disrupts the broader agri-food chain (Reidsma et al., 2010).

The rising temperatures and the increasing frequency of heat waves, occurring especially during summer, can have complex and unpredictable consequences for crop production, necessitating adaptive management practices. For example, while higher temperatures threaten the productivity of various crops, such as maize (Brás et al., 2021) and grapes (Moriondo et al., 2011) biophysical modelling and field experiments indicate that certain vegetables, like tomatoes and lettuce, may maintain yield stability although their quality might decline (Di Bene et al., 2022).

Also, high temperature produces critical and differential modifications to the crop life cycle depending on crop species. Studies have shown that grapevines, for instance, may experience a shortened growth cycle (Moriondo et al., 2011), while certain horticultural crops could see longer vegetative periods, possibly increasing annual yields (Bisbis et al., 2018). While some regions may become less suitable for specific crops, others might become more favorable, such as higher altitudes becoming suitable for premium wine production (Moriondo et al., 2011). Projections consistently indicate temperature increases, while precipitation changes remain highly uncertain and location-dependent. Perennial crops are particularly vulnerable due to their prolonged exposure to varying weather conditions, and the lack of robust models for predicting yields further complicates impact assessments (Winkler et al., 2013).

The establishment of an adequate long-term research platform for a better simulation and assessment of climate change's impact on the Italian cropping system is crucial to developing mitigation and adaptation strategies for sustainable agriculture under a changing climate. To protect Italy's agricultural heritage and ensure food security it is essential to implement agricultural management strategies supported by robust policy frameworks and technological innovations (Reidsma et al., 2010).

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5. CROSS-RI SERVICE TO CONTRAST CLIMATE CHANGE

The integrated research from the ITINERIS project underscores the importance of cross-RI collaboration in understanding and mitigating the main climate change-related driver impacts. The long-term studies and the innovative methodologies developed inside the AnaEE research platforms provide unique insights into how environmental factors affect ecosystems and agriculture, significantly advancing our understanding of the contemporary and future challenges related to global changes.

The data and services produced in the research platforms constitute the groundwork for effective cross RIs climate change mitigation strategies and for developing policy formulation to counteract the adverse effects of climate change-related drivers focusing on pollutant critical levels, preserving species and natural ecosystems, concerning food security and sanitary risk.

The AnaEE data, services and research products are available in the ITINERIS HUB, a unique repository of the Italian Research Infrastructures in the environmental scientific domain responding to the FAIR principles of Findability, Accessibility, Interoperability and Reusability. Each research platform team member is responsible for uploading the data, which are freely accessible to anyone. Researchers, stakeholders, managers, politicians and citizens can access the HUB, download the data of interest and combine several research platform data, thus having a service cross-RI information to contrast climate change related-drivers.

In particular, to facilitate and assist the various research groups in data management and to meeting the FAIR principles, the designed IT architecture is based on the Data Services (sometimes described as Data-as-a-Service) paradigm. Data services generally refer to small, independent, and loosely coupled functions that enhance, organize, share, or calculate information collected and saved in data storage volumes. Data services amplify traditional data by improving its resiliency, availability, and validity, as well as adding characteristics to data that it doesn't already have natively, like metadata. Data service architectures can involve multiple kinds of data and application services working together to achieve a goal, such as in ANAEE Data-as-a-Service (DaaS) Research Infrastructure architecture. The ANAEE Daas infrastructure is self-contained units of software functions that give data characteristics it doesn't already have. ANAEE Daas can make data more available, resilient, and comprehensible, which makes data more useful to users and programs. More generally, ANAEE Daas implemented functions turn inputs into outputs. The inputs are varied sets of raw data (e.g., data that hasn't been processed for a specific purpose) configured in its native format and saved in physical, virtual, or cloud-based storage volumes. The outputs are usually: Organizational, Transferable, and Procedural. The first feature concerns the consolidation, management, batching, and structure of data, usually pulled from structured (databases), semi-structured (data warehouses), or unstructured (data lakes) sources. The transferable is related to the movement of data from their place of origin across a network to an end point, like an application or platform. Finally, the procedural is about the processing of data, usually as part of data modeling, analytics, or artificial intelligence/machine learning (AI/ML) software.

Regarding the accessibility and interoperability aspects of the FAIR principles, these are ensured by the implementation of two common architectural patterns for building APIs, like GraphQL and REST. Both GraphQL and REST are popular API architecture styles that enable the exchange of data between different services or applications in a client-server model. REST and GraphQL allow you to create, modify, update, and delete data on a separate application, service, or module via API. GraphQL and REST are two distinct approaches to designing API for exchanging data over the internet. REST enables client applications to exchange data with a server using HTTP verbs, which is the standard communication protocol of the internet. On the other hand, GraphQL is an API query language that defines specifications of how a client application should request data from a remote server.

Specifically, GraphQL is a query language and server-side runtime for application programming interfaces (APIs) that prioritizes giving clients exactly the data they request and no more. GraphQL is designed to make APIs fast, flexible, and developer friendly. It can even be deployed within an integrated development environment (IDE) known as GraphiQL. As an alternative to REST, GraphQL lets developers construct requests that pull data from multiple data sources in a single API call.

A REST API (also known as RESTful API) is an application programming interface (API or web API) that conforms to the constraints of REST architectural style and allows for interaction with RESTful web services. REST stands for representational state transfer and was created by computer scientist Roy Fielding. An API is a set of definitions and protocols for building and integrating application software. It's sometimes referred to as a contract between an information provider and an information user establishing the content required from the consumer (the call) and the content required by the producer (the response). In other words, if you want to interact with a computer or system to retrieve information or perform a function, an API helps you communicate what you want to that system so it can understand and fulfill the request.