Deliverable "Evaluation of impacts of *Xylosandrus* spp. and project activities on main ecosystem processes"

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SUMMARY

To quantify the impact of tree mortality on different ecosystem processes/services, twelve monitoring plots have been set in the "Quarto freddo" forest sector of "Mount Circeo" and in "Mount Peretto", both in the Circeo National Park (Italy), representing two different forest structures: high-forest and open forest-shrubland, both composed by *Quercus ilex* tree and other Mediterranean woody shrubs

The ecosystem processes monitored were:

- plant mortality in the forest plots
- tree litterfall, one of the ways for carbon and nutrients to be recycled in the soil system, resulting from annual leaf production and duration
- soil CO2 effluxes resulting from soil respiration, in proximity of alive and dead trees
- direct impact of *Xylosandrus* spp. on *Quercus ilex* crowns

The evergreen mixed forest of the north side of Mount Circeo - "Quarto freddo" (dominated by *Q. ilex*), managed in the past as coppice, is today under transition. The tree mortality significantly impacts the carbon and nutrient input to the soil system, in absence of compensation processes generated by a multi-specific forest canopy. Furthermore, tree mortality significantly impacts the soil respiration and the related emission of CO2 in the atmosphere, only in absence of compensation processes generated by a multi-specific forest structure.

Xylosandrus spp. produces tunnels on small twigs of the upper canopy of *Q. ilex* trees, which can consequently desiccate causing a reduction of the active foliar biomass in the crown, with negative effects on the productivity of the tree.

The reduction of *Xylosandrus* spp population and/or an improvement of the resilience of the forest ecosystems of Mount Circeo, should be considered in the new forest management planning.

PREFACE

To quantify the impact of tree mortality on different ecosystem processes/services, monitoring plots have been set in the "Quarto freddo" forest sector of "Mount Circeo" (Italy) and in "Mount Peretto". The plots are located at different altitude, in the areas identified as attacked by *Xylosandrus* spp. beetles in the 2016. In both sites a pair of impacted or not impacted plots where selected.

In each plot, an analysis of the forest structure and the mortality of woody plants was carried out. Hence, some primary processes related to the carbon cycle were monitored for two consecutive years, and finally, the role of *Xylosandrus* spp. on observed mortality was estimated.

1. THE MONITORING PLOTS

The monitoring plots have been set on Spring 2019 in the two areas of Mount Circeo (Quarto freddo and Peretto) representing two different forest structure: high-forest and open forest-shurbland, both composed by *Quercus ilex* tree and other Mediterranean woody shrubs.

Figure 1: Quercus ilex high-forest in the Quarto freddo area (left) and mixed tree-shrubland ecosystem in the Peretto area (right)

Quarto Freddo

Six experimental plots were established in this area. Plot position was determined analysing the satellite images of the area. In the images taken in 2016, an extensive crown browning associated to the *Xylosandrus* outbreak was detected and mapped. In the satellite images of the year 2017, desiccated tree crowns were identified inside the areas showing crown browning in the previous years.

Figure 2: Centroid of the browned crowns (yellow markers) detected on July 2017, overlapping the browned areas detected on September 2016 (red contours) in the north side of Monte Circeo

Desiccated trees were identified by a field survey in spring 2019 and selected as centroid of three monitoring plots representing the area impacted by the mortality (Dieback plots). Three additional plots were set in areas where both satellite images analysis and field survey did not show evidence of browned crowns (Healthy plots). Each circular plot has a diameter of 20 meters. All the border trees located out of the plots, have been marked (as normally in use for forest management).

Peretto

Also, in this area six monitoring plots were established, using as centroid a mature *Quercus ilex* tree surrounded by several woody shrubs. Each plot (diameter 4-5 m) corresponds to the projected area of the tree crown. Similarly to Quarto Freddo, three Dieback plots (including a Quercus ilex tree in declining stage with partially desiccated crown) and three Healthy plots (including an healthy Quercus ilex tree) were set.

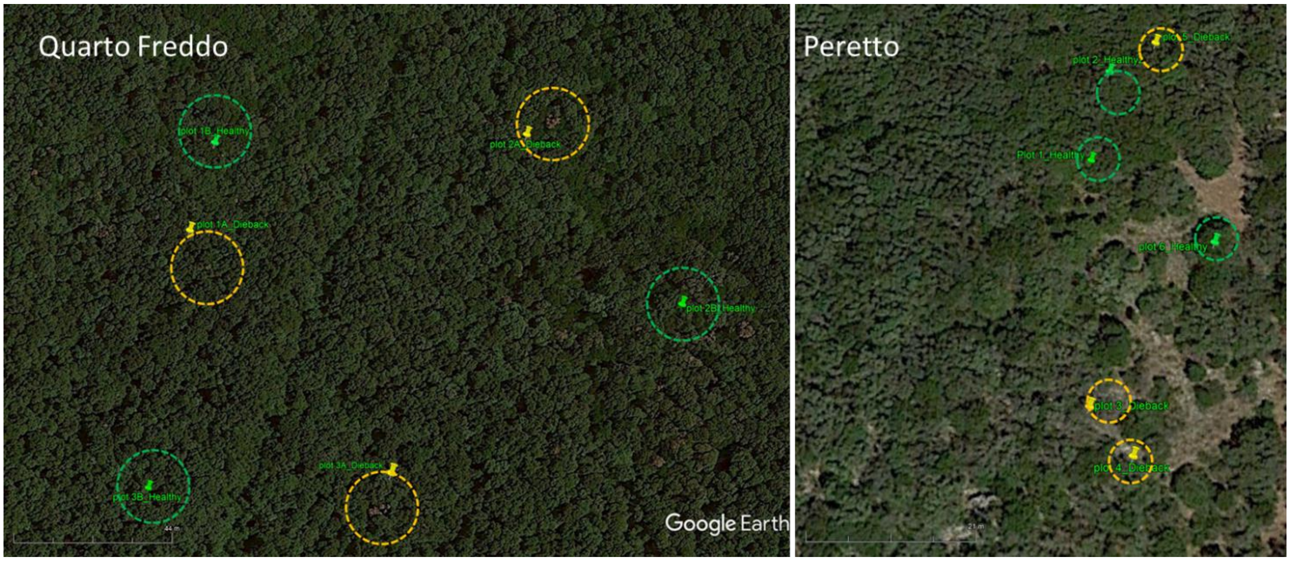

Figure 3: Distribution of the experimental plots in the two areas of Monte Circeo

2. THE FOREST SURVEY

In each forest plot a preliminary survey was realised to define the forest structure and to analyse the plant mortality.

For each woody plants were recorded: species, stem dimension (DBH) stage of dieback (see figure 4), coppice shoot or standard, diffuse epicormic shoots, subcortical fungi stroma.

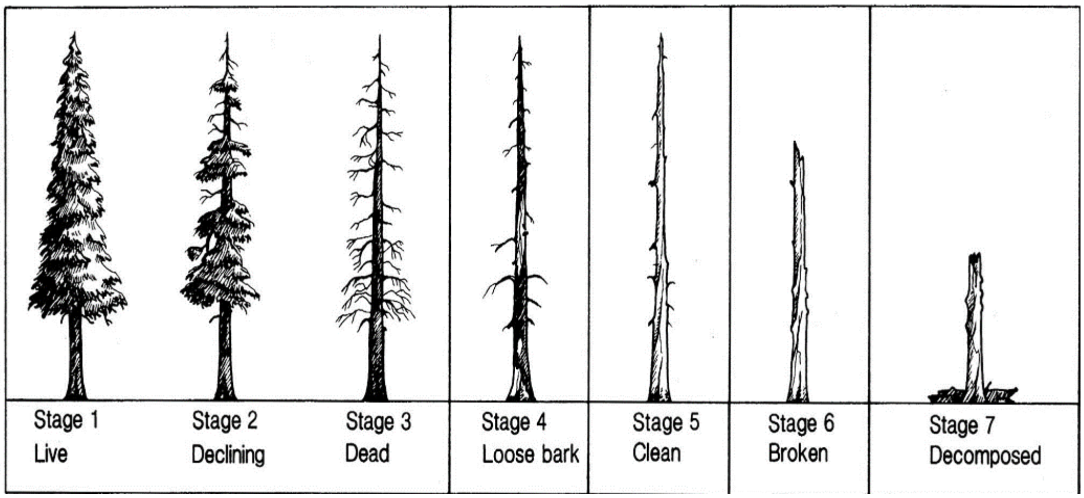

Figure 4: Visual classification of the tree stages decay

Forest structure

The forest has the typical structure of an old coppice not managed anymore (actual rotation time about 2 times the normal). Dieback plots are characterised by a lower frequency (n. of stems) of *Phyllirea latifolia*, counterbalanced by a relatively higher frequency of *Fraxinus ornus* and *Arbutus unedo*. The frequency of *Quercus ilex* (n. of stems) was practically the same in the two groups (about 40%). The total number of "standing" stems was slightly higher in the dieback plots.

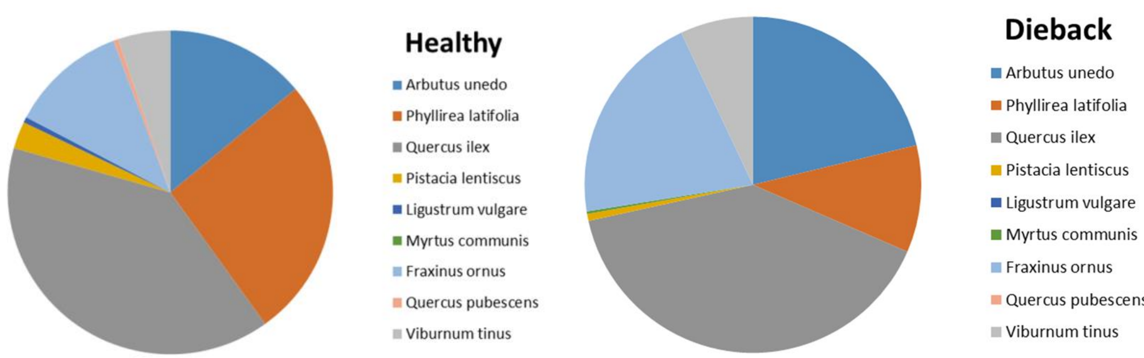

Figure 5: Forest diversity expressed by the frequency of species stems, in both plot types

Using the basal area as a proxy of the forest stock, the contribution of the different species to the stand productivity can be analysed. *Quercus ilex* is the main component of the forest stock, representing on average the 70% of the basal area (consequently dominating the wood volume and biomass). The reduction of *Phyllirea latifolia* in the dieback plots, is stronger when calculated on the basal area. The relative increases of both *Fraxinus ornus* and *Arbutus unedo* are confirmed. As for the stem density, the basal area was slightly higher in the dieback plots.

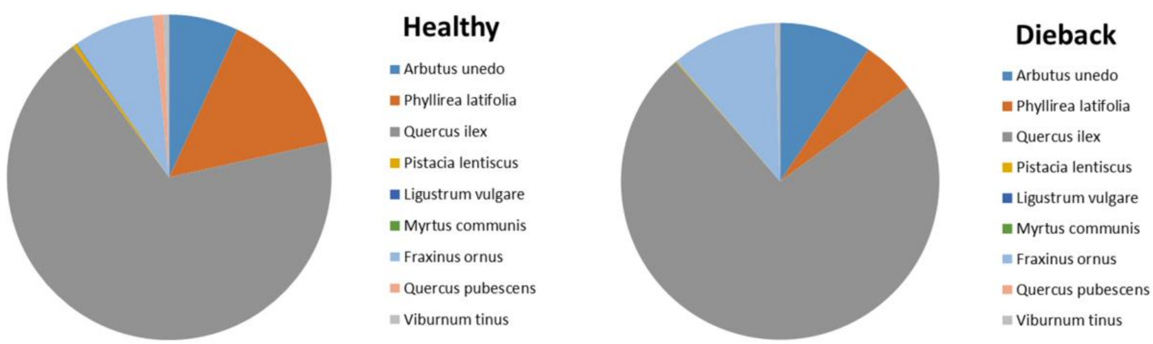

Figure 6: Forest stock composition expressed by the specific basal area fractions, in both plot types

Tree mortality

The mortality of the standing trees is quite high in both plots categories, but higher in the dieback plots (38% and 46% in the healthy and dieback plots, respectively).

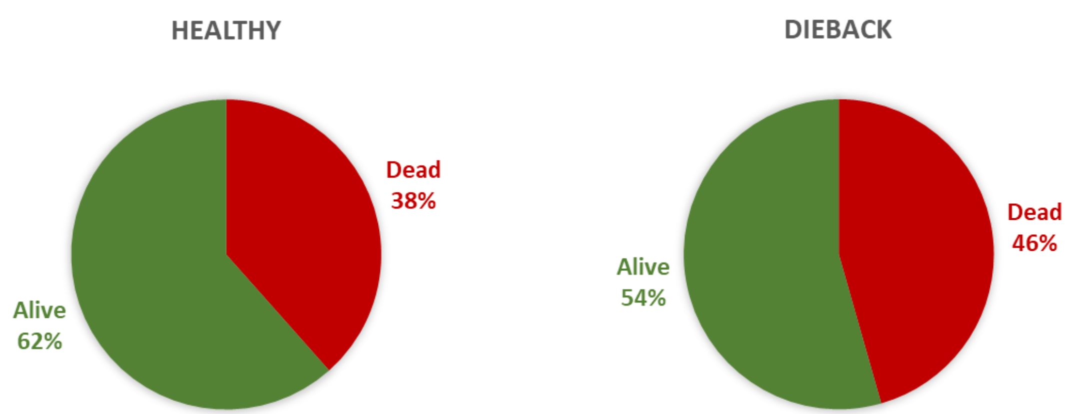

Figure 7: Overall tree mortality in both plot types

The stand is characterized by high frequencies of standing stems in the smaller diameter classes and the mortality is concentrated on the first two diameter classes, as typically occurs during the development of even-aged forest stand, in both categories of plots.

On the other hand, a slight increase of mortality is evident in the dieback plots in the middle and larger diameter classes.

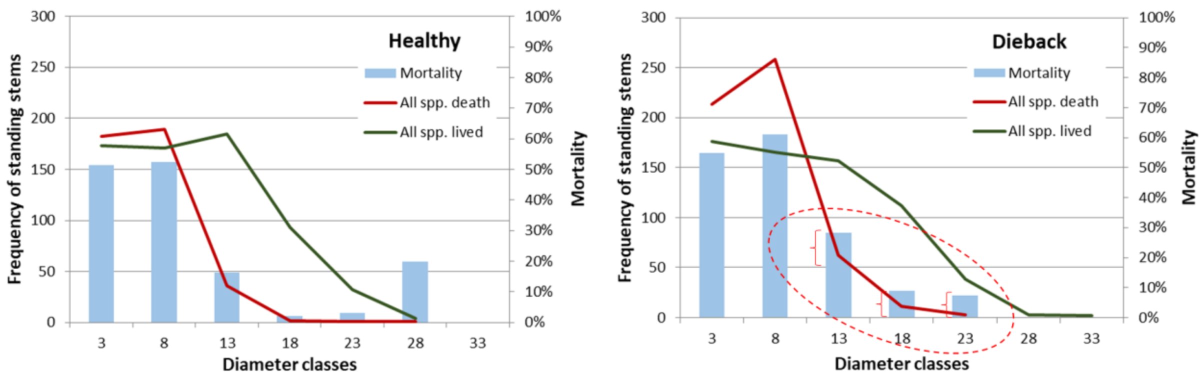

Figure 8: Frequency distributions of stem diameters and associated mortality, in both plot types

The mortality differs significantly among the species, according to the different causing factors. The *Arbutus unedo* suffering the light competition caused by the old coppice habit of the forest, practically disappeared in both plots. Differently, *Phyllirea latifolia* and *Quercus ilex* suffering from a more complex stress related mortality, showed higher rates of mortality in the dieback plots. The mortality is also affecting *Fraxinus ornus* trees, but at a lower level (about 30%). The evergreen shrubs/small trees of the understory (*Pistacia lentiscus, Viburnum tinus, Ligustrum vulgare*) are mainly not affected by mortality.

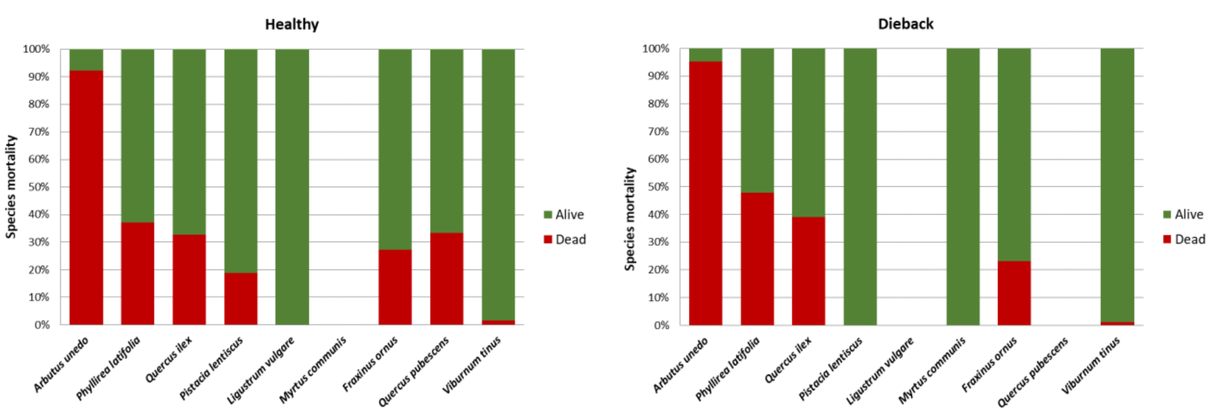

Figure 9: Specific tree mortality, in both plot types

Using the tree decay classification, it is possible to deduce the temporal dynamics of mortality. In fact, each decay class is separated by the time interval necessary for the degradation of the different woody tissues.

Table 1: Frequency distribution in decay classes of trees, in both plots

Distribution in classes of vitality								
	Q. ilex		Phyllirea		Fraxinus		Arbutus	
Classes	Dieback	Healthy	Dieback	Healthy	Dieback	Healthy	Dieback	Healthy
Alive	8%	22%	14%	38%	**46%**	**61%**	2%	4%
Declining	**53%**	**45%**	**38%**	**25%**	31%	12%	3%	4%
Dead	13%	11%	5%	7%	2%	7%	10%	15%
Loose bark	10%	7%	7%	10%	9%	8%	18%	28%
Clean	2%	2%	4%	5%	2%	4%	21%	14%
Broken	6%	4%	9%	2%	4%	2%	11%	4%
Decomposed	9%	9%	**23%**	**13%**	5%	7%	**36%**	**32%**
Total	100%	100%	100%	100%	100%	100%	100%	100%

Analysing the distribution in the class of vitality-decay of the main woody species, we observed that:

- *Q. Ilex* is mainly in the declining stage as consequence of a relatively recent dieback;
- *Fraxinus ornus* is also recently declining but at lower rate;
- *Arbutus unedo* is at the end of a decay process started long time ago, and now is sporadic in the forest
- *Phyllirea spp. shows two peaks as consequence of an older and a new recent dieback*

Overall, declining trees are more frequent in the Dieback plots.

In summary

The evergreen mixed forest of the north side of Mount Circeo (dominated by Q. *ilex*), managed in the past as coppice, is today under transition. The original mixture of species is changing mainly because the suppression of A. *unedo* trees by the internal competition for light, but also as consequence of relatively recent new stress pressures.

The additional pressure created by the diffusion of *Xylosandrus* species, could increase the vulnerability of this forest to recurrent intense drought stresses reducing its resilience and possibly changing the structure to a new status.

3. TREE CANOPY PRODUCTIVITY AND LITTER PRODUCTION

According to the annual leaf productivity and leaf duration, the rate of litterfall represent one of the ways for carbon and nutrients to be recycled in the soil system.

To monitor this process, 30 littertraps (60 cm diameter, 0.31 m^2) were installed in both experimental areas of the Monte Circeo. In the Quarto Freddo area, four littertraps were installed in each plot on 11 June 2019, placed at the four corners of a square (side =7 m) centred in the plot centre (distance littertrap-plot centre = 5m) (tot = 24 traps). In the Peretto area one littertrap was placed below each monitored tree (tot = 6 littertraps). The litterfall was periodically collected and dried at room temperature, sorted in leaves, woody organs, reproductive parts and residual fraction (other) and weighted.

In both monitored areas the litterfall was mainly composed by leaves, woody organs and residual fraction, with a minor contribution of the reproductive parts (Figure 10). To be noted that the residual fraction, representing about one third of the total litter biomass, was mainly composed by the droppings of *Lymantria* sp. caterpillars. Two *Lymantria* spp. outbreaks occurred in fact during the study period (spring 2019 and spring 2020), leading to an almost complete defoliation of Quercus ilex trees.

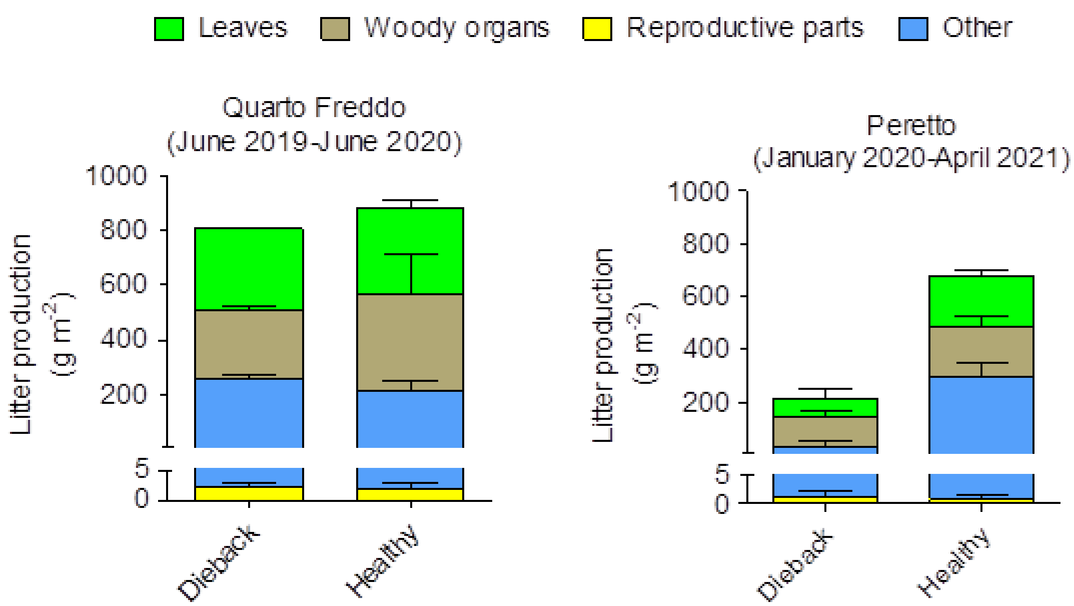

Figure 10: Annual litter collected in both areas per category

In Quarto Freddo area the amount and the composition of litter was similar in Healthy and Dieback plots, as a consequence of the multi-specific composition of the forest canopy. Differently, in the Peretto area, the amount of litter collected under Healthy trees was much higher (about three times) than under Dieback trees. The different litter productivity was mainly related to the leaf fraction (2.7 times higher in Healthy plots, $p=0.05$) and residual fraction (9.5 times higher in Healthy plots, $p<0.01$), according to the different productivity of the two categories of tree crowns.

Over the two years monitoring period, the cumulative amount of litterfall in the forest of Quarto freddo was similar in both plot types, showing a limited impact of tree mortality in the mixed dense forest. On the opposite, at the limit of the forest (Peretto) where the main trees are spaced and the canopy closure is limited by a lower tree density caused by drought and a low soil fertility, any reduction of *Q. ilex* tree productivity (dieback) is immediately coupled to a low carbon and nutrient input to the soil system (Figure 11).

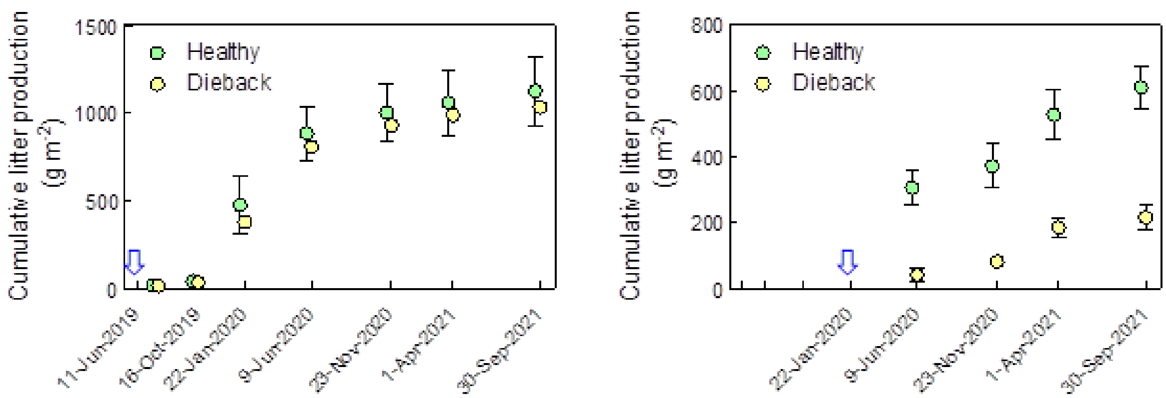

Figure 11: Cumulative litter production during the study period in Quarto freddo (left) and Peretto (right). The arrows indicate the beginning of litterfall collection.

In summary

Tree mortality significantly impacts the carbon and nutrient input to the soil system, in absence of compensation processes generated by a multi-specific forest canopy. In other words, drought prone tree communities (Peretto) are more vulnerable to the dieback caused by additional biotic factors, which in turn accelerates the soil degradation thus the ecosystem productivity.

4. SOIL CO_2 EFFLUXES

The soil CO_2 efflux produced by the soil respiration processes is an essential component of the biosphere – atmosphere interactions and of the climate system. The input of organic carbon to the soil system by litterfall, root mortality and root exudates, is used by a wide community of degrading organisms releasing as end-product of their metabolism gaseous CO_2. The instantaneous rate of this flux is proportional to the abiotic drivers of the soil microbial activity, while the overall emission is related to the amount of carbon inputs and nutrient availability.

To assess the impact of tree status (alive or dead) on soil respiration, soil CO_2 effluxes were measured in both monitoring areas, in proximity of alive or dead trees selected as reference points. In the Quarto Freddo area, 12 trees have been selected in each plot as reference points for the measurements. The trees were selected to represent the dominant species (*Quercus ilex* and *Fraxinus ornus*) and the plant status (alive or dead). Furthermore, in proximity of each tree soil CO_2 effluxes were measured several times, on two different paired points (Figure 12). Similarly, in the Peretto area the six monitored trees have been used as reference point for the measurements, but with 3 replicates per tree.

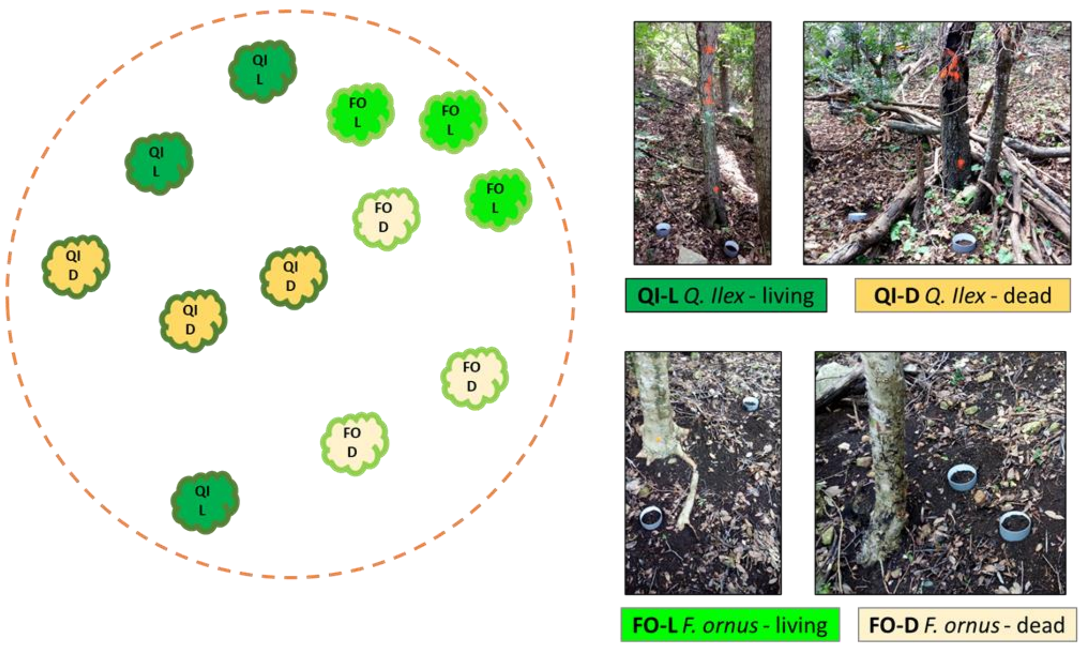

Figure 12: Scheme of a monitoring plot at Quarto Freddo and images of soil collars placed in proximity of *Quercus ilex* and *Fraxinus ornus* trees.

Soil CO_2 effluxes were measured using the Licor 8100 instrument (Licor Biosciences, Inc., Lincoln, NE, USA) equipped with an 8100–102 chamber. During measurement the soil chamber was deployed on PVC collars (diameter =10 cm, height 5 cm) inserted in the soil for 1-2 cm, about one hour before the measurement. The first campaign of measurements has been done on 23 July 2019 in the Quarto Freddo area and on 16 October 2019 in the Peretto area. The positions of the measurement points were recorded in the first campaign, measuring the distance from the tree stem and taking a picture of the area with collars. In the following measurement campaigns, collars were placed approximately in the same positions. Soil CO_2 effluxes were measured four times in the Quarto Freddo area, three times in the Peretto area. For each measurement date, 144 soil respiration measures were performed in the Quarto freddo area (2 measure x 12 trees x 6 plots) and 18 in the Peretto area (3 measure x 6 plots). Just after each measurement, soil temperatures at 10 cm depth were also measured and recorded.

Repeated Analysis of Variance was applied to estimate, for each species separately, the effect of the plant status on soil respiration and soil temperature over the entire study period. On each sampling date, the plant status effect on soil respiration was evaluated by unpaired, two-tailed *t*-test. At Quarto Freddo area, the data collected in Healthy and Dieback plots were analysed together. The sample units for statistical analysis were the single tree (n=18 for *Quercus ilex* and *Fraxinus ornus* in the Quarto Freddo area, and n=3 for for *Quercus ilex* in the Peretto area). The soil CO_2 efflux associated to each tree, for each date, was the average of 2-3 sampling points.

Impact of tree mortality on soil CO_2 effluxes in the forest of Quarto freddo (M. Circeo) and in the open forest-shrubland of Peretto

The seasonal variability of the soil respiration follows the seasonal changes of soil temperature, with a summer limitation caused by the limiting soil water content. Minimum fluxes have been measured in January, in both sites (about 2 μmol CO_2 m^{-2} s^{-1} in Quarto freddo and about 5 in Peretto), while the maximum in October and June-July (Figure 13).

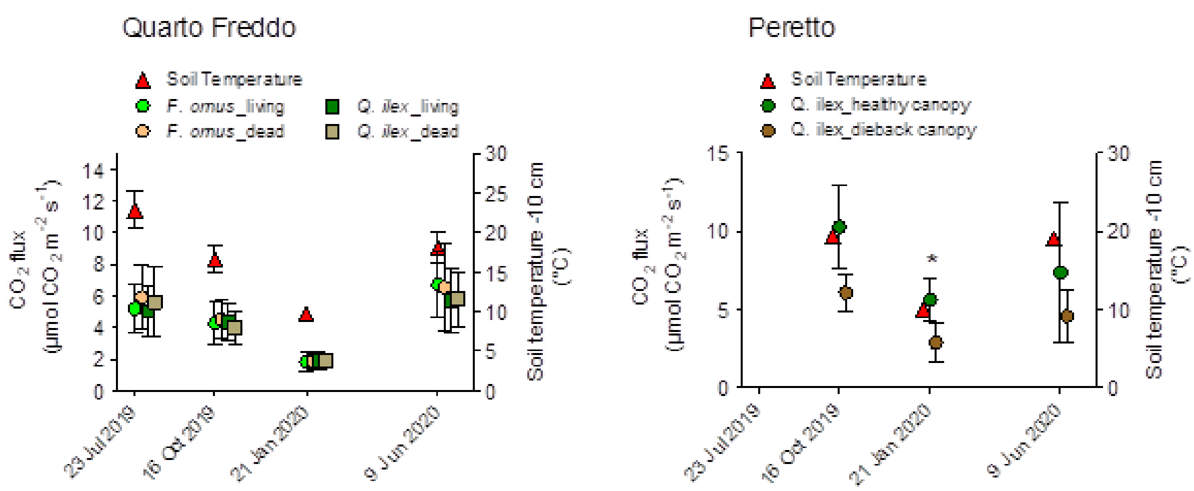

Figure 13: Soil CO_2 effluxes and soil temperatures measured during the monitoring period in both areas.

The tree mortality was not affecting the soil respiration in the forest of Quarto freddo, while significant reductions were observed below the dieback trees of Peretto. More specifically, in the Quarto freddo forest no significant effects of plant status on soil CO_2 effluxes were found for both *Quercus ilex* and *Fraxinus ornus* (p=0.85 and p=0.61, respectively), differently, in the Peretto area, these effluxes resulted lower (-42% on average) under the Quercus ilex trees with desiccated canopy (p=0.10), with a significant effect recorded in January 2020.

In summary

Tree mortality significantly impacts the soil respiration and the related emission of CO_2 in the atmosphere, only in absence of compensation processes generated by a multi specific forest structure. In other words, in open forest the soil processes are strictly dependent to the tree productivity.

5. DIRECT ASSESSMENT OF THE DAMAGES PRODUCED BY *XYLOSANDRUS* SPP ON *QUERCUS ILEX* CROWNS

On 23 November 2020 a branch sampling campaign was carried out in the Quarto Freddo monitoring plots, to assess the damage caused by *Xylosandrus* spp. on *Quercus ilex* trees. In each of the six plots previously set in this area, two *Q. ilex* trees have been selected according to two categories: Healthy (no significant canopy desiccation) and Declining (presence of canopy desiccation). For each tree, a canopy branch representative of the top crown has been collected, using a pruner mounted on a telescopic rod.

The sampled branches (six branches from Healthy trees and six branches from Declining trees, Ø 15-23 mm) were inspected to identify the insect entrance holes. The diameters and the status (living or dead) of each twig/branch with holes were recorded. Twigs were further inspected by UNITUS entomologists to exclude holes not related to *Xylosandrus* spp. and the *Xylosandrus* spp. holes not developing a gallery inside the twig/branch. Finally, for each sampled branch, the biomass of living and dead parts and of leaves has been also determined (Figure 14).

Figure 14: Analysis of the branch sampled for the determination of the crown damage caused by *Xylosandrus* spp.

Diffusion of the *Xylosandrus* spp. galleries on top crown branches

Most of the galleries were found in small twigs (Ø 3-11 mm), but also bigger branches (Ø 15-23 mm) were attacked. On the other hand, twigs mortality was associated only to the smaller diameters (3 – 9 mm) even if not all twigs with galleries were dead at the time of sampling. Furthermore, the abundance of twigs with *Xylosandrus* spp. galleries was higher (p=0.08) in the branches collected on declining trees (Figure 15).

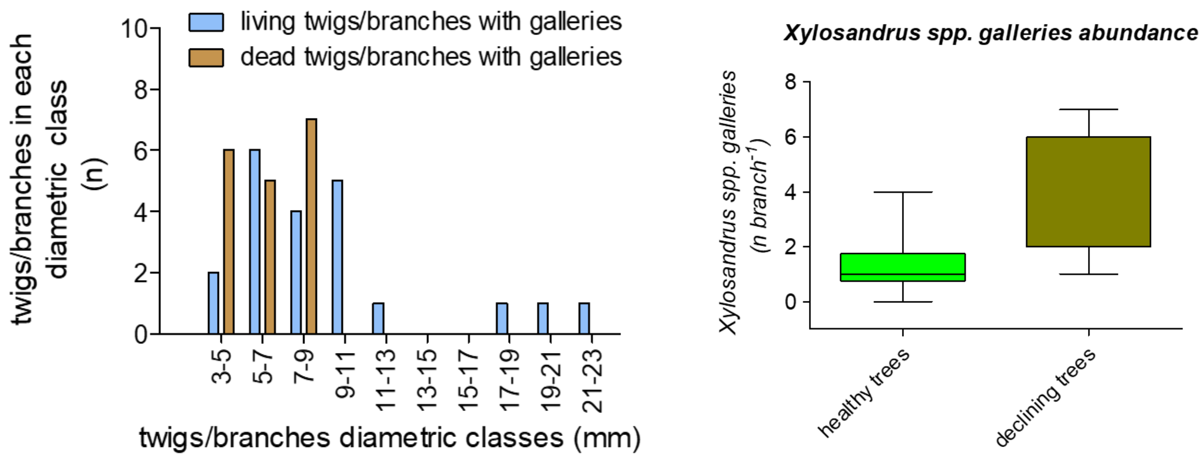

Figure 15: (left) frequency distribution of twigs/branches diameters with *Xylosandrus* spp. galleries. Branches sampled from both Healthy and Declining trees are included in this analysis; (right) number of *Xylosandrus* spp. galleries per branch in Healthy and Declining trees.

Impact of *Xylosandrus* spp. on Q. ilex crowns

The branches collected on Healthy and Declining trees had a similar living wood biomass (p=0.64), while dead wood biomass was higher in the branches belonging to the Declining trees (p=0.05). The impact of *Xylosandrus* on desiccation of these branches was evaluated identifying the dead wood associated to the galleries of these insects. This analysis showed that 36% of the dead wood biomass in branches of Declining trees was associated with *Xylosandrus* spp. galleries, supporting the hypothesis of a desiccation caused by the insect galleries (directly or by associated fungi). The direct effect of the twig's mortality is a reduction of leaf biomass (55%, p=0.02) on branches of Declining trees (Figure 16).

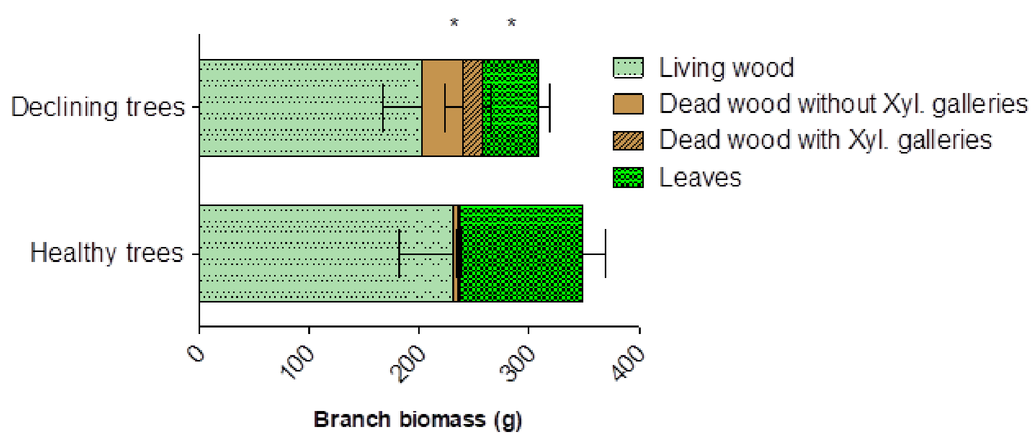

Figure 16: Distribution of biomass in the different compartments of branches sampled in Healthy and Declining *Quercus ilex* trees.

To evaluate the leaf biomass lost due to the twig's desiccation associated to the *Xylosandrus* spp. galleries, the leaf biomass present on those twigs before the desiccation was estimated using allometric relationships, correlating the branch and twig diameters with leaf biomass in healthy crowns (Figure 17).

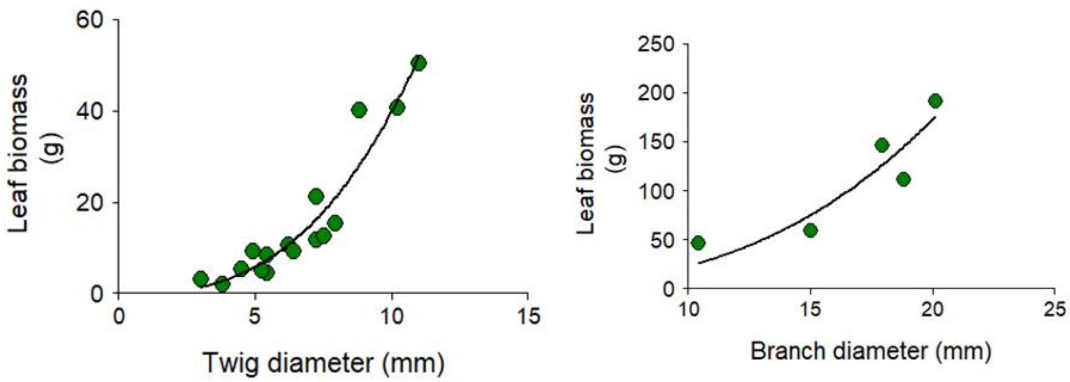

Figure 17: Allometric relationships (power functions) relating the twig/branch diameter and the twig/branch leaf biomass in healthy *Q. ilex* crowns.

The lost leaf biomass associated to the *Xylosandrus* spp. galleries (1.1 g branch^{-1} in Healthy trees and 17.1 g branch^{-1} in Declining trees) accounts for about 2% of the total leaf biomass in Healthy trees, and for about 32% of the total leaf biomass in Declining trees (p=0.05) (Figure 18).

Figure 18: Estimated leaf biomass loss associated to twigs/branches desiccated by the *Xylosandrus* spp. galleries

In summary

Xylosandrus spp. produces tunnels on small twigs of the upper canopy of *Q. ilex* trees, which can consequently desiccate causing a reduction of the active foliar biomass in the crown, with negative effects on the productivity of the tree.

6. CONCLUSIONS

The analysis of the ecosystem functions in Mount Circeo was carried out in two different forest ecosystems: mixed evergreen high forest dominated by Quercus ilex (Quarto freddo) and open forest-shrubland where Q. ilex trees are isolated (Peretto).

The evergreen mixed forest of the north side of Mount Circeo (dominated by *Q. ilex*), managed in the past as coppice, is today under transition. The original mixture of species is changing mainly because the suppression of *A. unedo* trees by the internal competition for light, but also as consequence of relatively recent new stress pressures. The additional pressure created by the diffusion of *Xylosandrus* species, could increase the vulnerability of this forest to recurrent intense drought stresses reducing its resilience and possibly changing the structure to a new status.

The tree mortality significantly impacts the carbon and nutrient input to the soil system, in absence of compensation processes generated by a multi-specific forest canopy. In other words, drought prone tree communities (Peretto) are more vulnerable to the dieback caused by additional biotic factors, which in turn accelerate the soil degradation thus the ecosystem productivity.

The tree mortality significantly impacts the soil respiration and the related emission of CO_2 in the atmosphere, only in absence of compensation processes generated by a multi-specific forest structure. In other words, in open forest the soil processes are strictly dependent to the tree productivity.

Xylosandrus spp. produces tunnels on small twigs of the upper canopy of *Q. ilex* trees, which can consequently desiccate causing a reduction of the active foliar biomass in the crown, with negative effects on the productivity of the tree.

The reduction of *Xylosandrus* spp population and/or an improvement of the resilience of the forest ecosystems of Mount Circeo, should be considered in the new forest management planning.