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Linking forest diversity and tree health: preliminary insights from a large-scale survey in Italy

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Abstract

Forest health is currently assessed in Europe (ICP Forests monitoring program). Crown defoliation and dieback, tree mortality, and pathogenic damage are the main aspects considered in tree health assessment. The worsening of environmental conditions (i.e., increase of temperature and drought events) may cause large-spatial scale tree mortality and forest decline. However, the role of stand features, including tree species assemblage and diversity as factors that modify environmental impacts, is poorly considered. The present contribution reanalyses the historical dataset of crown conditions in Italian forests from 1997 to 2014 to identify ecological and structural factors that influence tree crown defoliation, highlighting in a special manner the role of tree diversity. The effects of tree diversity were explored using the entire data set through multivariate cluster analyses and on individual trees, analysing the influence of the neighbouring tree diversity and identity at the local (neighbour) level. Preliminary results suggest that each tree species shows a specific behaviour in relation to crown defoliation, and the distribution of crown defoliation across Italian forests reflects the distribution of the main forest types and their ecological equilibrium with the environment. The potentiality and the problems connected to the possible extension of this analysis at a more general level (European and North American) were discussed.

Keywords: Cluster analysis, Crown defoliation, Forest structure, ICP Forests, Neighbouring trees, Tree diversity, Tree identity

Highlights

- The role of tree diversity on crown defoliation was assessed in a large-scale monitoring network.
- No positive effect of tree diversity was found at a national scale.
- Each tree species showed its own 'signature' for crown defoliation.
- The level of crown defoliation at each plot depends on which species are present and their biotic and abiotic interactions.
- Crown defoliation of individual trees is more affected by identity than diversity of their neighbouring trees.

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Background

Health conditions of European forests are assessed since some decades in an extensive network (Level I) within the ICP Forests program (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests). This program was launched as part of CLRTAP (Convention on Long-Range Transboundary Air Pollution) implemented by EMEP (European Monitoring and Evaluation Programme) and directed by UNECE (United Nations Economic Commission for Europe) and was primarily devoted at assessing the effects of atmospheric deposition and air pollution on forests. Although focused on air pollution, new environmental challenges such as climate change and pathogenic invasions are included in recent years within the goals of the program (ICP Forests 2016). In this new perspective, the suitability of the tree health indicators currently adopted for terrestrial assessment (mainly crown defoliation, Ferretti and Fisher 2013) may be questioned (Bussotti and Pollastrini 2017a, b).

Correlations between tree crown defoliation and air pollution were rather weak and often non-significant at the European scale (Klap et al. 2000; Johnson and Jacob 2009), but these correlations may become stronger in analyses carried out at local and national level. A significant role of atmospheric deposition and ozone as factors involved in worsening crown conditions was proposed by Augustatis et al. (2007) in Lithuania, whereas ozone levels (but not fluxes) were connected to crown defoliation and discoloration in Southern France, Northern Italy (Sicard et al. 2016) and Romania (De Marco et al. 2017). Climatic fluctuation and extremes, however, have been recognized as the main powerful factors that drive spatial distribution and temporal changes of forest tree health, vitality, and productivity (Carnicer et al. 2011; Ferretti et al. 2014; de la Cruz et al. 2014; Bussotti et al. 2015; Popa et al. 2017).

Recently, increasing importance is attributed to the structure and composition of forest stands, especially to tree diversity, as factors influencing tree health and productivity. Experimental and observational pieces of evidence show that tree diversity has a positive effect on productivity (Baeten et al. 2013; Verheyen et al. 2016) and plays a stabilising role on annual growth rates of trees (Jucker et al. 2014). Upscaling experimental and observational results at a large-spatial scale presents difficulties because of the complexity and the variability of the biological and ecological interactions (Leuzinger et al. 2011). Forest inventories, however, can play a relevant role in the assessment of forest biodiversity (Corona et al. 2011) and its beneficial effects on tree growth and stand productivity (Ruiz-Benito et al. 2014; Watson et al. 2015; Liang et al. 2016). Interestingly, Ratcliffe et al. (2015) proved that tree diversity plays a more relevant role in enhancing growth in Mediterranean regions (water limited) than in continental forests.

The possible effects of tree diversity on defoliation are poorly investigated, until now. Eichhorn et al. (2005) identified tree diversity as a relevant factor that positively influences the crown conditions (i.e., reduced crown defoliation) at the stand level in oak-beech mixed forests in Germany. Species-specific effects of tree diversity on crown defoliation were also observed by Pollastrini et al. (2016) in observational plots in Tuscany (Italy).

It is generally accepted that tree diversity reduces the intensity of attacks by herbivorous insects (Jactel and Brockerhoff 2007; Guyot et al. 2015) through different mechanisms. According to the resource concentration hypothesis (Root 1973), the probability of a host plant to be located by insects decreases in mixed forests. Moreover, non-host plants could disrupt chemical or physical cues used by herbivores to locate a suitable host (Huber and Borden 2001; Castagneyrol et al. 2013). In addition, the natural enemy hypothesis (Root 1973; Russell 1989) suggests that more diverse plant assemblages provide natural enemies with more complementary resources and habitats, thereby promoting top-down regulation of herbivores (Guyot et al. 2015).

Pathogenic root fungi reduce their virulence in mixed forest stands or when the proportion of host trees is lower (Lindén and Vollbrecht 2002; Thor et al. 2005; Nguyen et al. 2016) because of the reduced plant-by-plant root connections in mixed stands. By contrast, airborne pathogens of mature trees may show different patterns. Mixed forests may provide more suitable microclimates for fungi (e.g., higher humidity) than monocultures (Lodge and Cantrell 1995; Jules et al. 2014). Furthermore, the predominance of generalist pathogen species that can spill over from one host tree to another could increase the inoculum and, consequently, the incidence of diseases in mixed stands (Maloney et al. 2005; Parker et al. 2015).

Several questions should be addressed for a proper analysis of forest health conditions in relation to tree diversity. The first need is to refine the choice of parameters to describe the actual physiological and health conditions of trees. The threshold of 25% (Michel and Seidling 2016) for defoliation was settled arbitrarily and does not have a true physiological meaning (Lorenz et al. 2007). Drobyshev et al. (2007) and Nakajima et al. (2011) suggested negative correlations between crown defoliation and tree growth, but this effect is not confirmed at wider scale. Searching for new defoliation thresholds, and for a combination of symptoms (Eichhorn et al. 2016) within synthetic indices (Pollastrini et al. 2015), as well as their subsequent validation as predictor of growth reduction and/or mortality, is an inescapable task. Secondly, the analysis of the complexity of interactions between tree species composition, forest structure, and ecological characteristics of the site claims for the adoption of the most advanced statistical tools (Green et al. 2005). Such analysis must take in account the adaptation and/or vulnerability of forest tree assemblages and their possible divergent responses to stress factors, such as extreme climate events (Carnicer et al. 2011). Tree functional traits should also be included as predictive factors (Bussotti 2008). Finally, there is the problem of capturing impacts unevenly distributed (e.g., at the edges of populations), and that may be not represented in a systematic monitoring network.

This study represents a first attempt to explore the role of tree diversity as a factor contributing to tree health through the ICP Forests (Level I) data at the national (Italian) level, highlighting the problems connected to this analysis and the needs for more effective tools of elaborations.

Methods

The level I network in Italy

In Italy, the Level I - ICP Forests monitoring network consisted of 245 plots (year 2014), with 4967 trees subdivided into 1310 conifers and 3656 broad-leaved trees. Ten to thirty trees per plot were assessed. The most frequent tree species are listed in Table 1. The plots are circular with a fixed 13 m radius, and the crown conditions of all trees with diameter at breast height (DBH) > 10 cm are assessed yearly. DBH is measured every 5 years. The field crews of the National Forest Service (formerly *Corpo Forestale dello Stato*, from 2017 *Carabinieri Forestali*) are trained before each field survey (Ferretti et al. 1999; Bussotti et al. 2009) using a national field manual (Bussotti et al. 2016; Gasparini et al. 2016). In this contribution, the crown condition was evaluated for the historical series from 1997 to 2014.

The main indicator of forest health in the ICP Forests program is the 'crown defoliation' (Eichhorn et al. 2016). It integrates intrinsic tree genetic variability, site effects, and external factors such as abiotic and biotic stresses. Crown defoliation expresses the percentage of lacking leaves with respect to an ideal healthy tree identified through photo guides (Müller and Stierlin 1990; Ferretti 1994) or local reference tree and it is measured by a proportional scale with 5% steps ranging from 0% (not defoliated) to 100% (dead tree). Alongside crown defoliation, damage symptoms (and, when evident, damaging agents) are currently assessed on leaves, branches, and trunk. The most widely used method to report the results is the percentage of trees for a sample population with crown defoliation higher than 25% (Michel and Seidling 2016), both at plot and national/European levels.

In the data set used for this study, the following variables are considered for each plot:

Table 1 Total number (Nr) and percentage (%) of tree with crown defoliation higher, respectively, than 25% and 60% (trees highly defoliated) for the most common tree species in Italy (survey 2014)

Tree species	Total Nr	Crown defoliation: % of trees with Def. > 25%	Crown defoliation: % of trees with Def. > 60%
Fagus sylvatica	1210	21.04	2.45
Quercus pubescens	819	35.52	2.91
Quercus cerris	697	24.39	4.66
Castanea sativa	550	61.78	15.06
Picea abies	501	21.57	4.01
Larix decidua	415	19.54	3.55
Pinus sylvestris	241	28.45	2.64

- Physical variables: geographical position (longitude and latitude) and altitude.
- Ecological variables: climatic parameters (rainfall and temperature data collected from www.worldclim.org); soil parameters (soil depth, pH, C/N ratio, Cationic Exchange Capacity - CEC, exchangeable elements collected within BioSoil project, www.forestry.gov.uk/fr/biosoil, and provided by the Italian National Forest Service).
- Vegetation variables: basal area per hectare, tree species composition and tree diversity. Tree diversity is calculated as taxonomic diversity (TD, Shannon and Simpson indices), functional diversity (FD) calculated as CWM (i.e., community weighted mean, Lavorel et al. 2008), and Rao's quadratic entropy (FD, Functional Divergence, see Rao 1982a, b). The functional traits considered to calculate FD in this study are specific leaf area (SLA), wood density (WD), seed mass (SM), leaf nitrogen content (N), and tree maximum height (H). The relative values of the functional traits for each species included in this study were derived from current public databases and literature (see Pollastrini et al. 2016; Liebergesell et al. 2016, with references and annexes therein).

Considering that the plots of the survey are distributed on a systematic network, some difficulties in this study are related to a non-balanced distribution at the national level of tree species, diversity levels, and ecological factors.

Statistical analyses

The dataset includes all the data collected in Italy from 1997 to 2014. The number of plots and trees, as well the protocols for the field surveys, varied during the years. The variable considered (crown defoliation), however was consistent during the whole assessment period. Dataset implementation and exploratory analyses were performed in R (R Core Team 2016). The general strategy for data processing consisted, as a first step, of reducing the extreme complexity and variability of the dataset by identifying more homogenous groups of plots. To achieve the expected result, two clustering techniques were used. A preliminary hierarchical clustering, considering all the explaining variables previously listed (physical, ecological, and vegetational), was carried out to assess the suitability of the dataset for clustering and explore the robustness of different metric and aggregation methods. This analysis was applied to define the number of clusters, and was performed using a complete aggregation method over the Euclidean distances. The variables connected to 'species' (a dummy variable, descriptive and without numerical significance) and 'crown defoliation' (which are the variables we aim to predict) were excluded from this analysis. For the final clustering, the data were standardised (average zero and standard deviation one), and the k-medoids method was applied and implemented in the 'pam()' function of the 'cluster' package in R (Maechler et al. 2016). This method can work with missing data, and it is more robust to noise and outliers. Therefore, it is more suitable for large and potentially problematic databases. The k-medoids algorithm was used with the same exclusion principles of the preliminary clustering and the same Euclidean metric, and was applied to define the composition of the clusters. Graphical plots of regression analysis between crown defoliation severity and tree diversity were obtained with ggplot2 (Wickham 2009) package, fitting a generalised additive model. For surrounding trees analysis, Shannon and Simpson diversity indices were calculated in the neighbourhood of target trees (*Picea abies* [L.] H. Karst as model tree species was selected). All trees closer than five meters from the target tree were included in its neighbourhood. The species and DBH of neighbouring trees were recorded.

Results

Descriptive statistics

The geographic national distribution of species and functional groups reflects the differences between climatic regions in Italy and their respective dominant

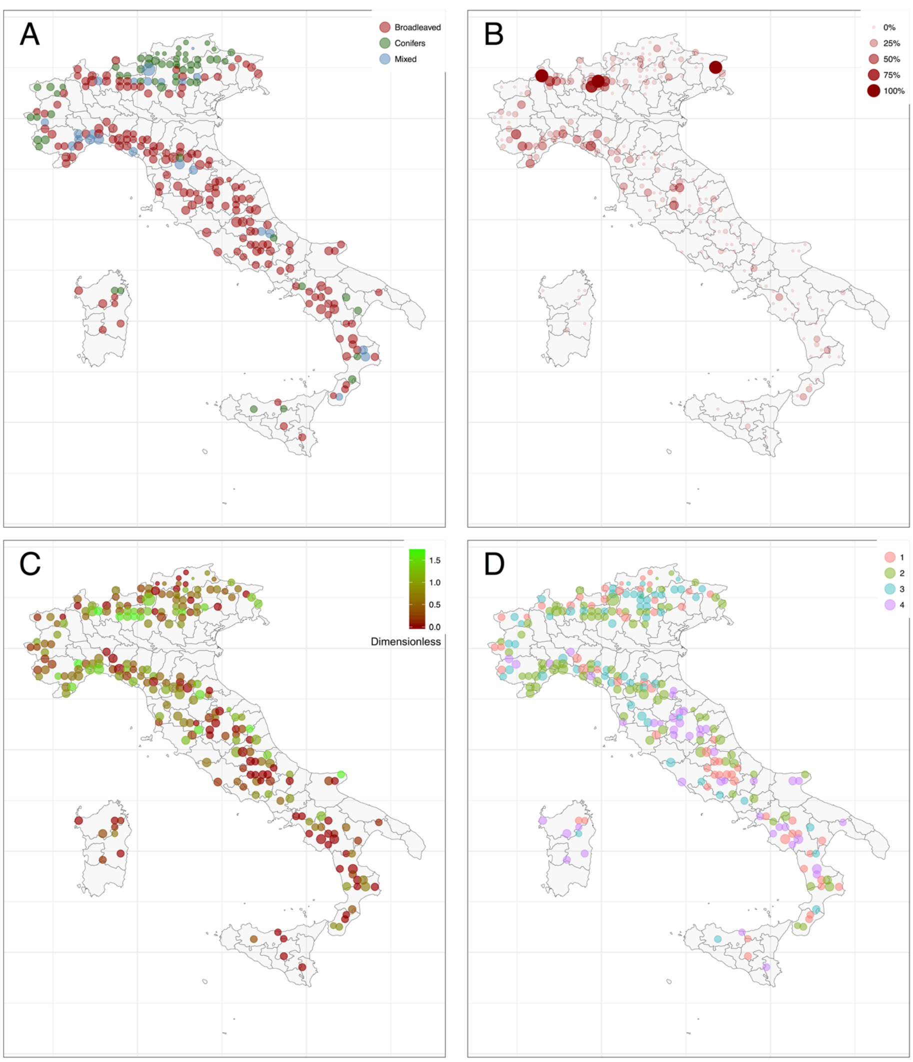

Fig. 1 **a**: Distribution of conifer, broadleaves and mixed forests of the Italian ICP Forests network (Level I). **b**: Distribution of the severity of defoliation per plot. Each point expresses the percentage of trees with defoliation > 60% at a given plot. **c**: Tree diversity (Shannon Index) for each plot, dimensionless. **d**: Distribution of the plots aggregated according to the results of the cluster analysis, taking in account physical, ecological and vegetational characteristics. The number in legend indicates the cluster (see Fig. 3)

forest types (Fig. 1a). Conifers (namely *P. abies*) are dominant at higher elevations (up to 1800 m a.s.l.) in the Alpine range (North Italy). Among broad-leaves, *Fagus sylvatica* L. is distributed at the intermediate elevation (800–1200 m a.s.l.) in the Alps, and it is the most common species at the high elevation (1000–1500 m a.s.l.) in the entire Apennine chain in Central and Southern Italy. At lower and intermediate elevations (up to 700–800 m a.s.l.), oak forests are dominant. Oak species include the deciduous species *Quercus cerris* L. and *Quercus pubescens* Willd. and the evergreen *Quercus ilex* L. Monospecific or few species-mixed forests are prevailing at high and low elevations, whereas the more diverse forests are distributed in the hills and in the intermediate mountain belt.

Crown defoliation rates are higher in broadleaved than in coniferous tree species (in the period 1997–2014, the mean percentage of trees with defoliation > 25% was 39.7 for broadleaves and 24.1 for conifers). The distribution of defoliation severity at the plot level (highly defoliated trees with defoliation > 60%) is shown in Fig. 1b and suggests the presence of a 'hot spot' in the north-western regions. The tree diversity (Shannon Index) for each plot is shown in Fig. 1c.

The temporal pattern of crown defoliation shows two distinct behaviours for conifers and broadleaves (Fig. 2):

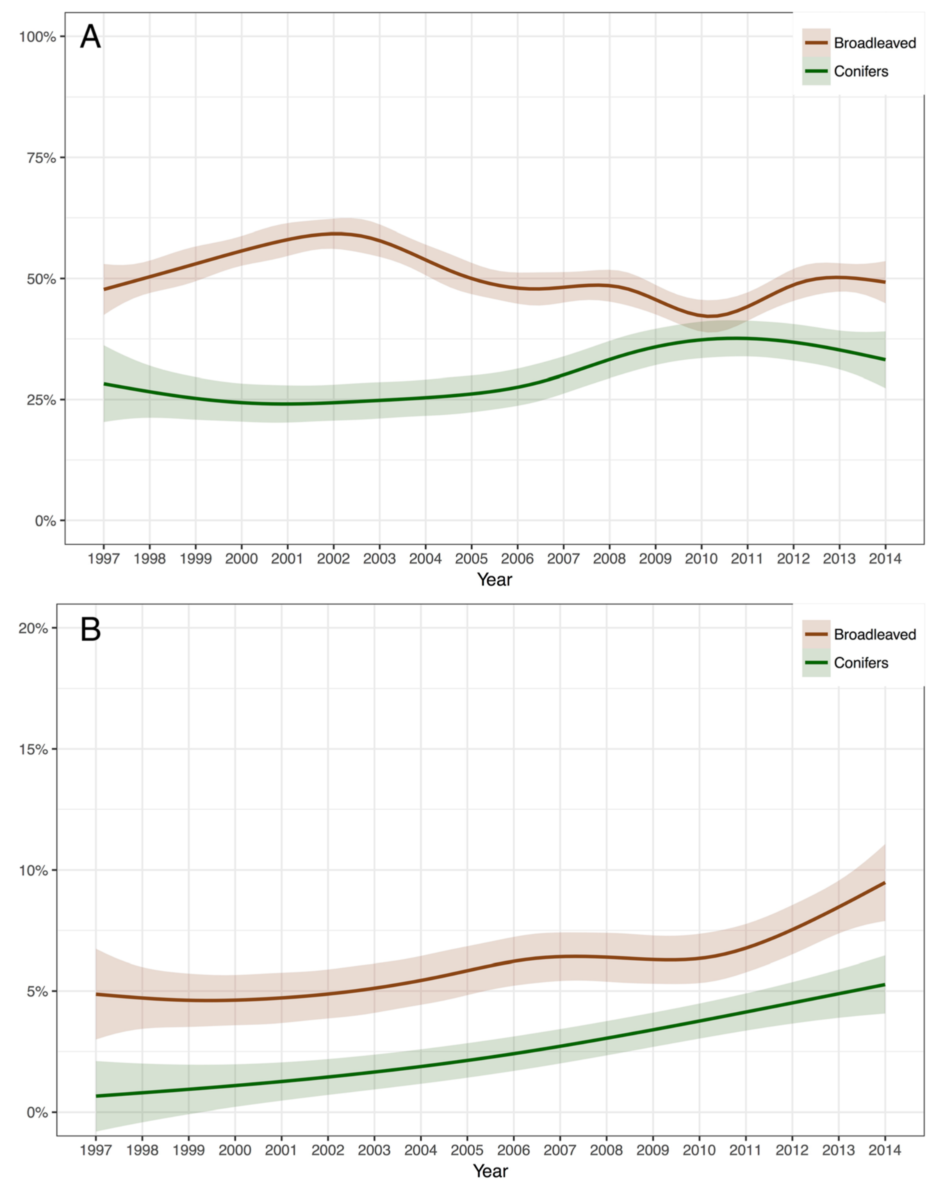

Fig. 2 Pattern of defoliation over time (expressed as percentage of defoliated trees during the period 1997–2014). **a**: Percentage of trees with defoliation > 25%. **b**: Percentage of trees with defoliation > 60%

between the years 1997 and 2000, the crown defoliation increased in broad-leaves and decreased in conifers. The opposite behaviour can be observed between 2001 and 2010. In both functional groups, the percent of highly defoliated trees (defoliation > 60%) increased over time (Fig. 2b).

Cluster analysis

The cluster analysis allowed to individuate four clusters of plots in relation to the set of geographical, ecological, and vegetational parameters described in Materials and Methods. Their geographic distribution is shown in Fig. 1d. The main characteristics of each cluster are shown in Fig. 3 alongside the severity of defoliation. Cluster 1 includes high-elevation forests, distributed across Italy, monospecific or with low diversity. Cluster 2 includes forests growing in the sub-mountain or in hilly areas, distributed in Northern and Central Italy, with high diversity. Cluster 3 includes sub-mountain forests, distributed especially in Central and North Italy, with low diversity. Cluster 4 includes low-elevation forests distributed in Central and Southern Italy, with low diversity. The most defoliated forests are included in clusters 2 and 4, whereas the less defoliated ones belong to clusters 1 and 3.

Effects of Neighbouring trees in state and change of crown defoliation

To examine the influence of tree species of surrounding trees and environmental factors on crown defoliation, the analysis at the neighbourhood level for *P. abies* growing in the Alpine belt in North Italy was carried out. This species was selected because it is one of the most numerous in survey at national level (Table 1). Its distribution is mostly limited in the Alpine belt (see Fig. 1a), so it is possible to consider relatively homogeneous the ecological conditions where it grows and, finally, because it is poorly affected by pests.

The crown defoliation of this species at a given year (2014) and the changes between the years 2006 and 2011 (when the highest increase of defoliation severity was recorded) are shown in relation to the local Shannon Index in Fig. 4a and b, respectively. Crown defoliation levels (Fig. 4a) and change in defoliation intensity (Fig. 4b) showed a slight but significant positive correlation with increasing local tree diversity.

Discussion

The spatial and temporal pattern of crown defoliation is the result of species-specific behaviours, especially for broad-leaf species that cover a wide range of climatic

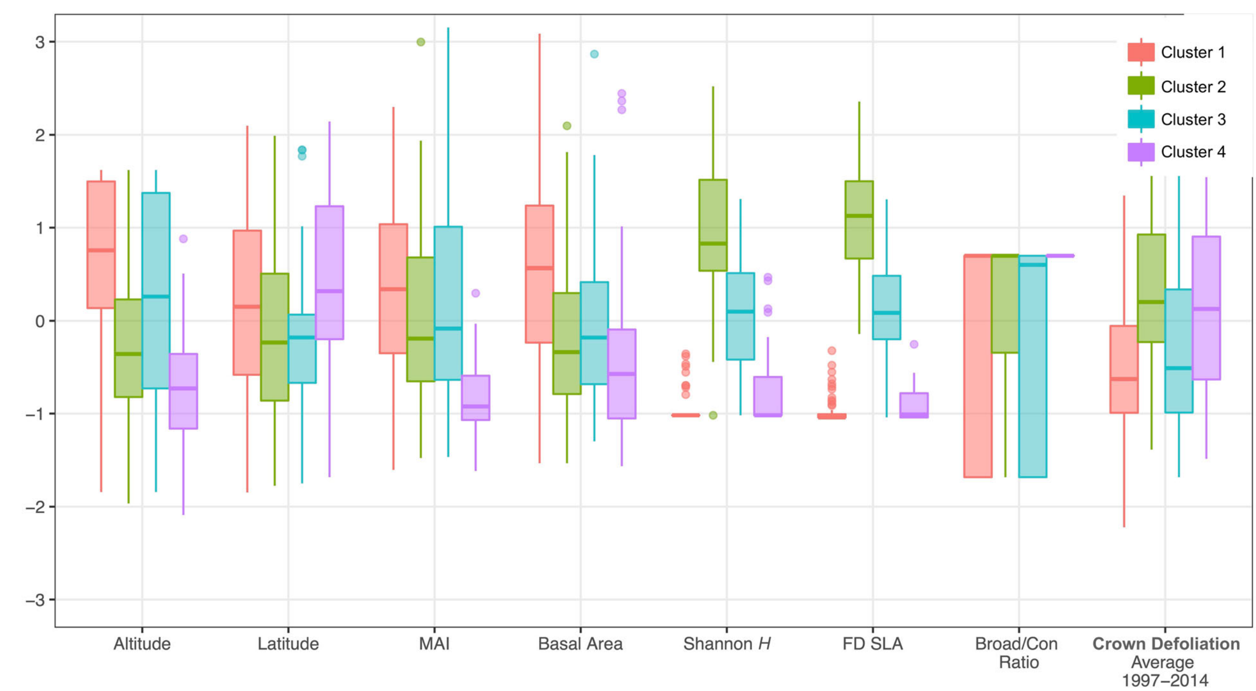

Fig. 3 Box and whisker plots of the main characteristics of the clusters of plots, identified by means of cluster analysis. The variable of plot included in the analysis were: altitude, latitude, MAI (Martonne Aridity Index*), basal area, Shannon Index, FD (Functional Divergence) for specific leaf area (SLA), percent of broadleaves (on basal area basis), and the severity of defoliation for each cluster. Mean value, quartiles, deviation standard and outliers are indicated. Data were normalized and standardized. *MAI = annual precipitation/(mean annual temperature + 10)

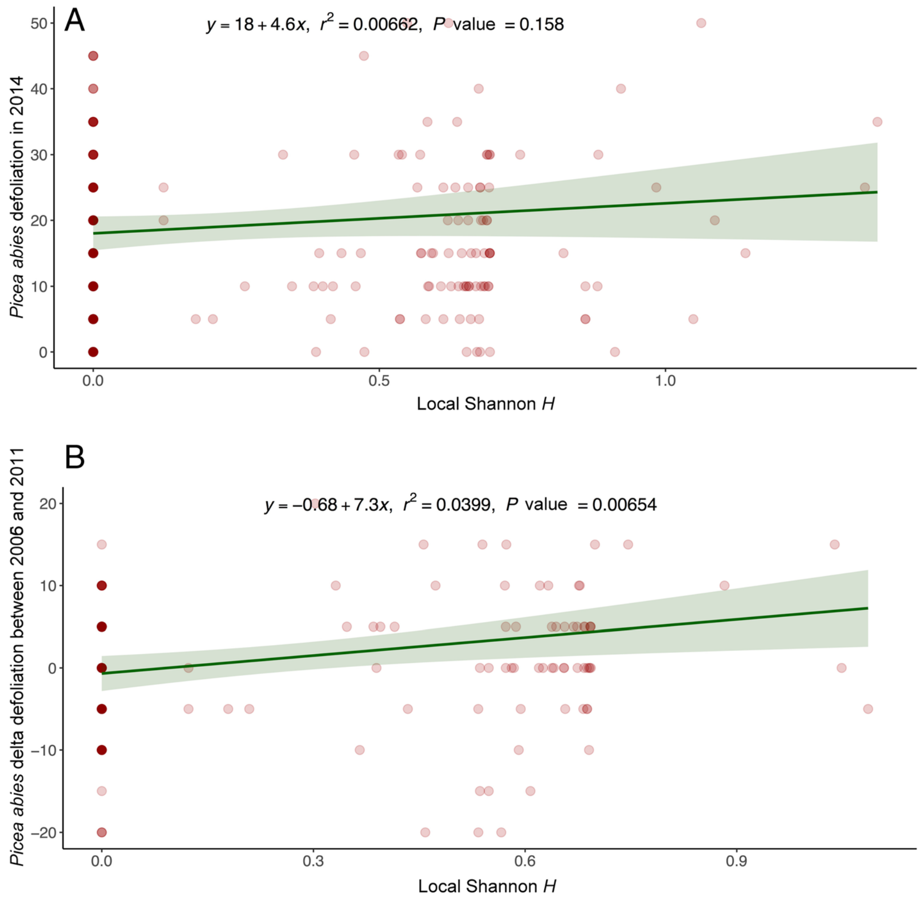

Fig. 4 Defoliation severity levels (year 2014, **a**) and changes (years 2006–2011, **b**) in *Picea abies* trees in relation to the local tree diversity (Shannon Index is calculated in an area with 5 m radius from the target tree)

and ecological conditions. *F. sylvatica*, *Quercus* sp.pl., and *Castanea sativa* Mill. showed different crown defoliation levels. Based on these considerations, the strong defoliation severity observed in north-western Italy is likely associated with the large presence of *C. sativa* and *Pinus sylvestris* L., which are the most defoliated species within broadleaves and conifers respectively. For several decades, *C. sativa* has been severely attacked by various pests and diseases (Battisti et al. 2014), whereas *P. sylvestris* populations are declining because of the worsening environmental conditions, with special reference to increasing drought in the southern Alpine belt (Dobbertin et al. 2007), including the regions of North-West Italy (Vacchiano et al. 2012). In these regions elevated levels of ozone pollution were also detected by Sicard et al. (2016).

While broadleaved tree species are widespread over the whole Italian territory and across different climatic regions, conifers are represented mostly by alpine species (*P. abies*, *Larix decidua* Mill., *P. sylvestris*) each of them occupying a specific ecological niche with a distribution limited to Northern Italy (Fig. 1a). The different distribution of the two functional groups (conifers and broadleaves), both spatial and ecological, may imply that they can be subject to different pressures and environmental drivers, thus explaining their different response trend through time. Specific environmental constraints in the Alpine and Mediterranean regions are reviewed by Therillat and Guisan, 2001; Bussotti et al. 2014; Ochoa-Hueso et al. 2017. Finally, the increase over time of the proportion of highly defoliated trees (> 60%) suggests that defoliation may not be reversible above this threshold.

Since each tree species has its own "signature" in terms of defoliation responses (see also ICP Forests 2016), the spatial distribution of stand-level defoliation is strictly related to the distribution of the most relevant species. European and Italian forests are largely composed by one or few dominant species (Inventario Nazionale Foreste e Serbatoi Forestali Carbonio, http://www.sian.it/inventarioforestale/; Eurostat http://ec.europa.eu/eurostat/statistics-explained/index.php/Archive:Forestry_statistics) because of past management and/or environmental constraints. Mixed forests are more likely to be found in transition zones, where some of the dominant species grow at their ecological limits and several secondary tree species also occur, as for example in central Italy (Carrari et al. 2016).

Results of the cluster analysis can be interpreted consistently with these considerations. The clusters that display the lower degree of defoliation (groups 1 and 3) include the stands at higher elevations in the Alps (with *P. abies*) and Apennine (with *F. sylvatica*). These species grow in their optimal climatic conditions and form climax forests, often monospecific or with low diversity (Nocentini 2009; Caudullo and De Rigo, 2016). *Picea abies* and *F. sylvatica* are, for conifers and broadleaves respectively, the species with lowest crown defoliation (Table 1). By contrast, in group 2, the forests with high diversity and crown defoliation are located mainly on hills and in the sub-montane belt of both the Alps and the Apennines, where a mixing of mountain conifers at their lower limit and broadleaf species at their upper limit occur at an intermediate altitude occurs. In this area, trees may be subject to variable and non-optimal ecological conditions (Vacchiano et al. 2012). Moreover, the forest vegetation of the subalpine belt includes species severely damaged by pests and fungi, such as *C. sativa* (Battisti et al. 2014). The elevated levels of crown defoliation in group 4 may be associated with the hotter and drier Mediterranean climate. Elevated levels of crown defoliation were in fact found on the Mediterranean region (ICP Forests 2016).

Our preliminary results concerning the role of the surrounding tree species on *P. abies* confirmed the findings previously reported. The positive correlation between crown defoliation and local Shannon Index may be connected to the fact that in its optimal habitat this species tends to form pure stands.

To refine the analyses, introducing variables that describe the structure of the forest is also necessary (Pommerening 2002, 2006; Motz et al. 2010). The interaction between forest structure and tree composition plays a key role in affecting growth and physiological responses of trees under stress (Ruiz-Benito et al. 2013; Madrigal-González et al. 2016; Grote et al. 2016). According to Fichtner et al. (2017), the interaction between diversity and structure produces different effects on acquisitive and conservative species, being the first ones favoured by low competition intensity and the latter in more competitive conditions.

Conclusions

The role of tree diversity as a predictive factor of crown defoliation and overall conditions, may differ in relation to the target species, the kind of mixture and the ecological features at each site, hence to the environmental context (Ratcliffe et al. 2017). In this perspective, it would be helpful to get a larger observational scale by expanding the study from national to the continental (European) level, and in connection to similar ones in North America (Randolph 2013). The ICP Forests programme can play a significant role for the comprehension of all the environmental and structural variables affecting tree health and growth, but some methods and criteria should be reconsidered to make that programme more suitable for the new challenges (Bussotti and Pollastrini 2017a). Among the questions to be addressed, the most relevant is connected probably to the different sampling designs adopted at the national level. According to Motz et al. (2010) the most adequate sampling method to assess tree diversity and related parameters consists in plots with fixed radius, but this design is not adopted everywhere (Ferretti and Fisher 2013). Moreover, the trees should be geo-referenced in the plot to apply the "neighbouring tree" approach, but not always these data are available. Finally, more convincing evidences concerning the actual reliability of tree defoliation as descriptor of overall physiological conditions and growth are necessary.

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Availability of data and materials

The dataset supporting the conclusions of this article is available on request on www.icp-forests.net.

Authors' contributions

FB and FS are responsible of the general idea and concepts concerning the role of diversity. FB wrote the first version of the text. MF managed the dataset. GI and FM are responsible for the statistical design and analyse. MP participated to the critical analysis of the text and discussion. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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