Physiological significance of forest tree defoliation: Results from a survey in a mixed forest in Tuscany (central Italy)

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Abstract: A survey of tree crown defoliation and leaf physiological traits (chlorophyll a fluorescence, nitrogen content, and stable carbon isotope composition) was carried out in the thermophilous deciduous forests in Tuscany (central Italy). In contrast to large scale surveys, where variation in defoliation can be associated with the change in environmental conditions, in a limited homogenous area the defoliation of co-existing tree species may have different significance and depends on the interaction between the characteristics of each individual species with biotic stress and environmental conditions. The survey included measurements of structural and vegetational characteristics of the forest stands, such as Leaf Area Index (LAI), basal area and tree diversity, which is expressed as the Shannon diversity index. The five tree species studied (Castanea sativa, Quercus cerris, Quercus ilex, Quercus petraea and Ostrya carpinifolia) showed species-specific crown conditions and physiological features relative to stand structure and diversity. The shape of the crowns and their area (LAI) affected forest defoliation. Tree diversity reduced defoliation in C. sativa, which was the tree species most affected by defoliation, and likewise for Q.ilex. Chlorophyll a fluorescence parameters showed lower photosynthetic efficiency in defoliated C. sativa, O. carpinifolia and Q. petraea trees. Similarly, foliar nitrogen content decreased in defoliated C. sativa and O. carpinifolia trees, whereas δ13C was higher in defoliated C. sativa. These responses may be related to the health status of C. sativa, since it was subjected to pathogen damages and insect attacks. In contrast, the mast year in O. carpinifolia may have diverted the nutrient resources from leaves to fruits, and consequently explaining the physiological effects on the tree crown. These results suggest that the combined analysis of defoliation with foliar features and stand characteristics can provide insights into tree health and vitality.

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2   **mixed forest in Tuscany (central Italy)**

3

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28

29   **Highlights**

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31   • Crown condition and leaf physiological traits were assessed in mixed forests

32   • Tree species and Leaf Area Index were the most important predictors of defoliation

33   • Tree diversity reduced the defoliation in chestnut and in holm oak

34   • Defoliation has different physiological significance in forest tree species

35   • Crown assessment is more informative when defoliation and leaf traits are combined

36

Summary

A survey of tree crown defoliation and leaf physiological traits (chlorophyll *a* fluorescence, nitrogen content, and stable carbon isotope composition) was carried out in the thermophilous deciduous forests in Tuscany (central Italy). In contrast to large scale surveys, where variation in defoliation can be associated with the change in environmental conditions, in a limited homogenous area the defoliation of co-existing tree species may have different significance and depends on the interaction between the characteristics of each individual species with biotic stress and environmental conditions. The survey included measurements of structural and vegetational characteristics of the forest stands, such as Leaf Area Index (LAI), basal area and tree diversity, which is expressed as the Shannon diversity index. The five tree species studied (*Castanea sativa, Quercus cerris, Quercus ilex, Quercus petraea* and *Ostrya carpinifolia*) showed species-specific crown conditions and physiological features relative to stand structure and diversity. The shape of the crowns and their area (LAI) affected forest defoliation. Tree diversity reduced defoliation in *C. sativa*, which was the tree species most affected by defoliation, and likewise for *Q.ilex*. Chlorophyll *a* fluorescence parameters showed lower photosynthetic efficiency in defoliated *C. sativa*, *O. carpinifolia* and *Q. petraea* trees. Similarly, foliar nitrogen content decreased in defoliated *C. sativa* and *O. carpinifolia* trees, whereas δ^{13}C was higher in defoliated *C. sativa*. These responses may be related to the health status of *C. sativa*, since it was subjected to pathogen damages and insect attacks. In contrast, the mast year in *O. carpinifolia* may have diverted the nutrient resources from leaves to fruits, and consequently explaining the physiological effects on the tree crown. These results suggest that the combined analysis of defoliation with foliar features and stand characteristics can provide insights into tree health and vitality.

Keywords: crown condition, defoliation, foliar analysis, FunDivEUROPE, mixed forests, tree diversity

Abbreviations List:

BA = Basal Area (m^2 ha^{-1});

CC = crown compression;

ChlF = Chlorophyll *a* fluorescence;

C/N = Carbon/ Nitrogen ratio;

F_0: minimum (basal) fluorescence in dark adapted samples;

F_M: maximal fluorescence in dark adapted samples;

F_V: total variable fluorescence (F_M-F_0);

$F_V/F_M = \varphi_{P0} = TR_0/ABS = [F_M$-$F_0]/F_M$: maximum quantum yield of PSII photochemistry of a dark adapted sample;

LAI = Leaf Area Index (m^2 m^{-2});

LI = Light Interception Index;

M_0: initial normalized slope of the fluorescence transient;

OJIP: labels of the different time-steps of the fluorescence transient;

PI_{ABS}: Performance Index on absorption basis. Index for energy conservation of photons absorbed by PSII, through the electron transport chain to the reduction of the electron acceptors in the intersystem between PSII and PSI;

PI_{TOT}: Performance Index total. Potential capacity for energy conservation until the reduction of the final acceptors beyond the PSI;

PSI: photosystem I;

PSII: photosystem II;

V_I: relative variable fluorescence at I step (30 ms);

V_J: relative variable fluorescence at the J step (2 ms);

$\delta^{13}C$: carbon isotope composition (‰);

$\Delta V_{I-P} = 1 - V_I =$ I-P phase: relative contribution of the I-P phase to the fluorescence transient OJIP (it is regarded as a measure for the efficiency of the electron flux through PSI to reduce the final acceptors of the electron transport chain);

$\Psi_{Eo} = 1 - V_J = J$step $= ET_0/TR_0$: probability of an electron to move from reduced Q_A, the secondary PSII electron acceptor, into the electron transport chain.

4

94   **1. Introduction**

95   Tree crown defoliation is the main parameter adopted in surveys (International Co-operative

96   Programme on Assessment and Monitoring of Air Pollution Effects on Forests, ICP Forests

97   programme, www.icp-forests.net) to assess the health of European forests (Eichhorn et al., 2010).

98   Defoliation is a raw visual indicator of the relative amount of foliage on the tree crown compared to

99   a reference standard tree, and is assessed visually by trained field teams (Ferretti et al., 1999).

100   Defoliation is an unspecific parameter integrating intrinsic tree genetic variability, site effects (soil

101   fertility, climatic features, structure and composition of a forest stand), and external factors such as

102   abiotic and biotic stresses. Consequently, defoliation (as assessed according to the ICP Forests

103   criteria) is not necessarily equivalent to damage and can be considered indicative of the plastic

104   equilibrium of a tree in a given environment.

105   Crown defoliation has been assessed extensively in many European countries since the

106   1980s, and the trends recorded are assumed to correlate with the effects of environmental stress,

107   such as air pollution and climate change (Van Leeuwen et al., 2000; Meining and Fischer, 2011;

108   Bussotti et al., 2014, 2015). Recent papers examining these historical trends found relationships

109   between the increasing defoliation levels and the change of climatic conditions, with special

110   reference to drought and heat waves (Seidling, 2007; Carnicer et al., 2011; De la Cruz et al., 2014).

111   To increase the effectiveness of the surveys, and to evaluate the overall conditions of the trees,

112   visual assessment of defoliation and crown status could be combined with the analysis of the

113   functional traits most likely linked to the responses of trees to environmental stress (Bussotti and

114   Pollastrini, 2015).

115   The physiological consequences of defoliation have not been thoroughly explored.

116   Defoliation implies reduction of the leaf area, light absorbing area and the whole tree

117   photosynthetic apparatus, and is commonly assumed that defoliated trees have reduced growth. This

118   assumption, though supported by observational evidence (Augustatis and Bytnerowicz, 2008), does

119   not take into account the so-called *compensatory photosynthesis*, i.e. the capacity to compensate the

loss of leaves with higher photosynthetic rates in the remaining foliage (Nowak and Caldwell, 1984; Desotgiu et al., 2012a). Eyles et al. (2011) observed compensatory photosynthesis in aphid-defoliated *Pinus radiata* D.Don, and attributed this effect to the enhanced exploitation of sunlight by leaves in the inner layers of the crown. It is likely that there is a threshold of defoliation whereby the remaining foliage is no longer able to restore the full photosynthetic activity. The altered light regime within a thinned crown affects photosynthetic function (Lavigne et al., 2001; Turnbull et al., 2007) and leaf nitrogen content that is directly related to the protein composition of the photosynthetic apparatus (Ellsworth and Reich, 1995; Wright et al., 2004). Moreover, foliar transpiration can be either enhanced or suppressed by the altered microclimate inside the canopy (Quentin et al., 2011). It is reasonable to assume that the altered physiological functions in defoliated trees may be reflected by an array of leaf features detectable with foliar analysis (Bussotti and Pollastrini, 2015).

Among stand features, tree species composition and diversity are thought to be important. Mixed forests are assumed to be more productive (Jucker et al., 2014) and more resilient to environmental stress (Grossiord et al., 2014a, b) than monospecific ones that result from positive interactions among tree species and the ability to exploit resources more efficiently (Bengtsson et al., 2000; Balvanera et al., 2006; Knoke et al., 2008). Eichhorn et al. (2005) identified tree diversity as a relevant factor that positively influences the crown conditions (i.e. reduced defoliation) at the stand level in mixed oak – beech forests in Germany.

The present research was part of a project on the functional significance of forest biodiversity in Europe (FunDivEUROPE, Baeten et al., 2013), and was carried out in mixed broadleaved forests in central Italy (Tuscany). Defoliation and crown conditions, assessed according to the guidelines of the ICP Forests manual (Eichhorn et al., 2010), were studied in the context of stand characteristics and foliar features. Stand characteristics provide information on the possible detrimental (or beneficial) effects of forest structure and composition (basal area, leaf area index, tree species mixture) on crown condition. Foliar features are relevant to investigation of the

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cause and/or consequences of defoliation on tree health and relative physiological functions. In contrast to large scale surveys, where variation in defoliation can be associated with the change in environmental conditions, for example drought or elevation gradients (Michel and Seidling, 2014) in a local homogenous area, with uniform climatic and soil conditions, defoliation may have contrasting significance to different tree species and depends on the interaction between the characteristics of each individual species with biotic stress and environmental conditions.

Within the hypothesis that defoliation may have different ecological and physiological meanings in tree species sharing the same environment, the present survey is aimed at exploring the effectiveness of comprehensive foliar analysis, combined with the structure and composition of the forest stands, to analyze species-specific responses connected to defoliation.

2. Materials and Methods

2.1.Site description

This study was carried out in the Italian forests (Tuscany, Colline Metallifere) of the exploratory platform of the FunDivEUROPE project (www.fundiveurope.eu, Baeten et al., 2013). The study design of the survey in Tuscany has been described by Bussotti et al. (2012). The sites were located at 43.27° N, 11.26° E, mainly at 350-450 m asl (for detailed characteristics of the plots see Table S1). The mean annual precipitation in the plots is 733 ± 42 mm and the mean annual temperature is 13.35 ± 0.38 °C (data from WorldClim-Global Climate Data, www.worldclim.org, with spatial resolution of 1x1 km). The bedrock is predominantly siliceous (sandstones and various conglomerates) and the soil is Cambisol (FAO classification), with a mean soil depth of 68 cm. Almost all of the plots have northern exposure and mean slope <50%. Thirty-six plots (30x30 m) with five focal tree species (*Quercus ilex* L.; *Quercus cerris* L.; *Quercus petraea* (Matt.) Liebl.; *Ostrya carpinifolia* Scop.; and *Castanea sativa* Mill.) were selected. Forest stands are around 50 -70 years old and originate from old coppices (the cutting of the stumps was suspended after the World War II). The trees of *C. sativa* are 60 year-old-stumps sprouted from the oldest trees cultivated in

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172   the past for fruits and then abandoned. At present, the forests considered in this study are public and

173   managed as natural reserves.

174   In this survey thirty-two plots were used (four plots were discarded because data were biased by

175   uncontrolled conditions). The plots had different levels of tree species diversity, ranging from

176   monocultures to a maximum of four species. The level of tree diversity was calculated as the

177   Shannon diversity index (Staddon et al., 1997; Spellerberg and Fedor, 2003), taking into account

178   tree basal area, for each plot.

179

180   *2.2. Leaf Area Index and Light Interception Index*

181 Canopy closure of the forest stands was assessed by means of Leaf Area Index (total one-side area

182 of leaf tissue per unit ground surface area, $m^2 m^{-2}$, Watson, 1947). Five measurements of Leaf Area

183 Index (LAI) in each plot were carried out at two time points, either early in the morning (shortly

184 before sunrise) or late in the evening (shortly after sunset) in order to work in the presence of

185 diffuse solar radiation and thus reduce the effect of scattered blue light in the canopy. LAI

186 measurements were carried out in early September 2012, before the beginning of leaf shedding,

187 using a Plant Canopy Analyzer LAI-2000 (LI-Cor Inc., Nebraska). With the LAI-2000, the incident

188 light above the canopy and the light transmission below the canopy were measured using one sensor

189 with five fisheye light sensors (lenses), with central zenith angle of 7°, 23°, 38°, 53° and 68° (LAI-

190 2000 manual, Li-Cor 1991). The protocol used in each plot consisted of five measurements within

191 the plots (light transmission below the canopy), and five measurements outside the forest (as proxy

192 of the light incidence above the canopy), in an open space that was in close proximity of the

193 sampled plots. LAI data were processed using Li-Cor's FV2200 software (LI-COR Biogeosciences,

194 Inc. 2010). The light transmittance measurements of the fifth ring were removed to minimize the

195 boundary effects on LAI. The LAI value per plot was the mean value of the five measurements for

196 each plot.

197   Furthermore, the capacity of trees to intercept light was determined. The light interception

198   index (LI, King et al., 2005) was calculated for each tree, according to Jucker et al. (2015):

199                                $$LI = CPA \times CI^2$$

200   where CPA is the crown projected area of each tree (in m^2, calculated using the crown radius

201   measurements taken in the field), and CI is the crown illumination index, which scores each tree on

202   a scale of 1 to 5 based on exposure to direct sunlight (Clark and Clark, 1992).

203

204   *2.3.Crown condition assessment*

205   In each plot, between six and 12 dominant trees were selected. Six trees were selected in

206   monocultures, and three trees per focal species in mixture plots. The trees were randomly selected

207   among the trees with the largest diameter breast height. Defoliation and damage symptoms on

208   leaves and branches were assessed for each selected tree, on the visible portion of the crown, in

209   June 2012 following the guidelines of the ICP Forests (Eichhorn et al., 2010). Defoliation was

210   evaluated according to a proportion scale in 5% intervals (0= not defoliated tree; 5; 10; 15 ...

211   100%= dead tree), by comparing the sampled tree with a photographic standard ("photoguide"

212   method, Müller and Stierlin, 1990; Ferretti, 1994). Defoliation was defined as leaf loss (fallen and

213   undeveloped leaves, dieback of parts of the crown, as well the loss of foliar surface as a

214   consequence, e.g. of herbivores and/or by hail) compared to a reference tree, regardless of the cause

215   of foliage loss. Damage on leaves and branches due to biotic and abiotic factors were evaluated

216   from the ground, with binoculars, in the visible part of the crown, according to a scale with 5%

217   intervals from 0 to 100%. Causes of the damage symptoms (meteorological, mechanical, leaf

218   senescence, biotic attacks, etc.) were determined when visually recognizable. The interaction with

219   the neighbouring trees ("crown compression") was also assessed on an ordinal scale (0= crown

220   completely free in all four sides; 1= one side of the crown compressed by neighbouring trees; 2=

221   two sides of the crown compressed; 3= three sides of the crown compressed; and 4= all four sides

222   of the crown compressed).

2.4.Leaf sampling

From each selected tree, branches with leaves attached were sampled in two parts of the crown, the

highest, southern exposed part, and the lower third of the same side. The sampling was done in June

2012, with the assistance of tree climbers and extension loppers. Fully developed current-year

leaves were collected both from deciduous and evergreen species.

2.5.Chlorophyll a fluorescence analysis

Measurements of chlorophyll *a* fluorescence (ChlF) were done, after four to five hours of dark

adaptation of the sample, on 16 leaves for each tree. The dark adaptation of leaves was carried out

in hermetic black plastic bags immediately after sampling, and stored in a dark room at ambient

temperature. Leaves were humidified to avoid de-hydration. A long dark adaptation period was

necessary to reduce both dynamic and chronic leaf photoinhibition (Werner et al., 2002; Desotgiu et

al., 2012b; 2013) and allow leaves to return to standard conditions. Measurements were done with a

HandyPEA fluorimeter (Hansatech Instruments Ltd., Petney, Norfolk, UK). Plotted on a

logarithmic time scale, the fluorescence induction transient shows polyphasic behaviour. The

different time-steps of this polyphasic transient are labelled as: O (20-50 µs), J (2 ms), I (30 ms) and

P (peak). The latter indicates the highest fluorescence intensity (F_M), when saturating light is used.

Generally, F_M is reached around 0.8 s. For reviews of the theoretical background of ChlF

parameters obtained from the ChlF induction curve (fast kinetics) of dark-adapted leaves, see

Strasser et al. (2000, 2004, 2010) and Kalaji et al. (2014). ChlF parameters used in this study,

calculated on the basis of JIP-test (Strasser et al., 2004), were: F_V/F_M, Ψ_{Eo}, ΔV_{I-P} and Performance

Indices (PI_{ABS} and PI_{TOT}) (see Abbreviation list). PI_{ABS} combines three parameters related to the

photosynthetic activity: (1) the density of reaction centres; (2) the quantum yield of primary

photochemistry; (3) and the ability to feed electrons into the electron chain between PSII and PSI.

PI_{TOT} considers also the efficiency by which an electron can move from the reduced intersystem

electron acceptors to the PSI end electron acceptors.

249   *2.6.Chemical analyses: total foliar nitrogen and carbon content; carbon isotope composition*

250   Twenty fully expanded leaves were sampled from each sampled tree for the analysis of carbon

251   isotope composition (δ^{13}C, ‰), total carbon (C, %) and nitrogen (N, %) contents. Foliar samples

252   were dried at 60°C for 48 h, after which the foliar samples from the same species in the same plot

253   were pooled together and finely grounded.

254   For the analysis of δ^{13}C about 1.0 mg of the dried powdered material from each sample was

255   placed into tin capsules. The analyses were performed by the Technical Platform of Functional

256   Ecology at the INRA Forest Ecology and Ecophysiology Unit (Champenaux, France), with an

257   isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). The isotopic

258   composition of the sample was reported in delta notation (δ^{13}C) relative to Vienna Pee Dee

259   Belemnite standard.

260   For the determination of the N and C content, 2.5 mg of the dried sample was used. All

261   samples were analysed by Near Infra Red Spectroscopy (NIRS), as described by Niederberger et al.

262   (2015). For this purpose, a subset of the samples was analysed for N and C with a flash CHN

263   Elemental Analyser (Flash EA1112 Series, ThermoFinnigan, Milan, Italy) to calibrate the NIRS

264   spectra. The spectra were averaged from five replicates taken with a Fourier Transform Mid- and

265   Near Infrared combination instrument (Tensor 37, Brukeroptics, Ettlingen, Germany). Each single

266   spectrum was a mean of 32 individual scans over the range of 12000 to 4000 cm^{-1} wave numbers

267   with a resolution of 8 cm^{-1}. The choice of samples for calibration was done for each component

268   separately and carried out with an automatic function in the OPUS spectroscopy software (version

269   6.5, Brukeroptics Ettlingen, Germany) after a first prediction with an existing model. A second

270   subset of the samples was analysed with the same equipment to validate the calibration. Calibration

271   was performed with cross validation with one leave-out sample. The statistical parameters r^2

272   (coefficient of determination), RMSECV (root mean square error of cross validation) for calibration

273   or RMSEP (root mean square error of prediction) for validation and RPD (ratio of standard error of

274   prediction to standard deviation) were used for the evaluation of prediction quality. Leaf N and C

275 content at the species level for each plot was calculated as average value of three or six trees per

276 species sampled in the plot.

277

278 *2.7.Symptoms of parasitic attacks on leaves*

279 A number of fresh leaves were selected to assess the presence of fungi (25-60 leaves per branch;

280 50-100 leaves per tree) and insects symptoms (30 leaves per branch). Damage symptoms on leaves

281 that were not visible during crown evaluations were assessed. Fungal symptoms were classified into

282 two categories: powdery mildew and leaf spots. The percentage of leaves with the presence of either

283 type of symptom was counted. After fungal assessment, the leaves were frozen at -18°C until the

284 assessment of the endophagous insects. Four insect guilds were considered for all tree species:

285 miners, gallers, rollers and tiers. As the sap-feeder species *Trioza remota* Foerster was the only one

286 easily detectable on deciduous oak leaves (nymphs stay on the underside of the leaves), there was a

287 fifth insect guild for *Q. cerris* and *Q. petraea*. A mite guild was also assessed for *Q. ilex*. The

288 number of leaves with at least one of the six guilds was counted. Insect damage at the tree level was

289 then aggregated by calculating the percentage of leaves with at least one damage present.

290 Ectophagous insects were not included in the analysis since the loss of foliar surface was already

291 accounted in the assessment of defoliation, according to the definition of Eichhorn et al. (2010).

292 Data of fungi and insect damage are reported as percent of damaged leaves (separately for fungi and

293 insects) relative to the total number of assessed leaves (including both damaged and healthy leaves).

294

295 2.8.*Data analysis*

296 To test the univariate correlations of defoliation with the foliar and stand structure parameters on

297 the whole sample (i.e. all tree species considered together) and for each species individually, the

298 Spearman rank correlation test was applied. Significant differences between tree species for each

299 parameter were assessed with a two-sample Kolmogorov-Smirnov test (for independent samples).

300 Linear mixed models were used to determine the importance of stand structural parameters,

301   diversity and crown properties of trees as predictors of tree defoliation. A number of alternative

302   mixed effects models of defoliation were fitted and compared using Aikake Information Criteria

303   (AIC). Models included different combinations of predictors: plot basal area (BA), Leaf Area Index

304   (LAI), Shannon diversity index, crown compression and light interception index were treated as

305   fixed effects, and tree and plot were treated as random effects in the model. We performed two sets

306   of models: (1) linear mixed models for the whole dataset (i.e. all tree species together). In this case

307   the variable 'species' was included in the models as a fixed-effect variable, and (2) linear mixed

308   models for each tree species separately to examine species-specific responses to defoliation. Before

309   running the models, the correlation between the predictor variables was checked to avoid

310   autocorrelation between them. The assumptions of normality and homogeneity of variance of

311   defoliation were checked. All analyses were implemented in R (3.1.2; R Core Team 2014). For the

312   linear mixed models, the package lmerTest (Kuznetsova et al., 2014) was used.

313

314   **3. Results**

315 The results of the survey carried out in Tuscany considered 244 trees in 32 plots (Table 1). The

316 variability of each foliar and crown trait assessed, both between trees in the same plot and between

317 the plots, is provided in Table 2. The traits estimated by visual assessment (crown defoliation,

318 damage to leaves and branches, insect and pathogen attacks) showed high variability among the

319 trees and the plots. Photosynthetic performance indices also had high coefficient of variability (CV).

320 The lowest CV values were in $Fv/F_{M,}$ C content and $\delta^{13}C$.

321   The *C. sativa* monocultures had the highest BA and the lowest LAI compared to the other

322 species, and *Q. ilex* monocultures had the highest LAI (Table 1). Crown condition features for each

323 species, as visually assessed from the ground, are reported in Table 3. *C. sativa* showed the highest

324 levels of defoliation and damage to leaves and branches, followed by *O. carpinifolia*. Oak species

325 had the lowest defoliation levels and crown damage. Defoliation was significantly correlated, in

326 many species, to the presence of dead or dying branches (*C. sativa*: $r^2 = 0.53$; *O. carpinifolia*: $r^2 =$

13

327   0.22; *Q. cerris*: $r^2 = 0.21$; all species: $r^2 = 0.53$). On *C. sativa*, crown dieback was related to past

328   attacks by the fungus *Cryphonectria parasitica* (Murr.) Barr. and oomycete *Phytophthora*

329   *cambivora* (Petri) Buisman. More recently, the new agent causing defoliation and damage to leaves

330   was attributed to the Asian wasp *Dryocosmus kuriphilus* Yasumatsu that produces galls on buds

331   (and subsequent desiccation of branches) and leaves. The causes of branch dieback in oaks were not

332   easily identifiable in the field, but were most likely caused by insects, e.g. *Coroebus florentinus*

333   (Herbst), and by the opportunistic fungus *Biscogniauxia mediterranea* (De Not.) Kunze that acts

334   synergistically with drought. Climatic agents (drought and high summer temperatures) probably

335   induced early senescence and loss of leaves in *O. carpinifolia*.

336   The effects of the stand structure and tree composition as predictors of tree defoliation were

337   examined using of univariate correlations (Table 4) and linear mixed models (Tables 5 and 6).

338   Species and LAI were the most important predictors for defoliation in the whole sample (Table 5),

339   whereas specific factors were important in each individual tree species. LAI was negatively

340   correlated with defoliation in *C. sativa*, *O. carpinifolia* and *Q. cerris*. Since LAI was positively

341   correlated with plot BA ($r^2 = 0.21$), we expected to observe an inverse correlation between BA and

342   defoliation. That was indeed observed for *O. carpinifolia*, *Q. cerris and Q.ilex*, whereas for *C.*

343   *sativa*, the opposite trend was noted. Tree diversity (Shannon diversity index) showed significant

344   negative correlation with defoliation in *C. sativa* and *Q. ilex*. Light interception index (LI) and

345   crown compression (CC) exerted significant effects on defoliation in the whole sample and in some

346   species, but in opposite directions: LI was negatively correlated with defoliation, and CC was

347   positively correlated with tree defoliation (Table 4).

348   Physiological features were measured on detached leaves. The highest foliar concentration

349   of N was detected in *C. sativa* and the lowest in the sclerophyllous evergreen species *Q. ilex* (Table

350   3). The highest $\delta^{13}C$ values were observed in *Q. petraea* and the lowest in *O. carpinifolia* (Table 3).

351   For ChlF parameters (Table 3), Fv/F_M and Ψ_{Eo} were not significantly different among species,

352   whereas the lowest values of $\Delta V_{I\text{-}P}$ and PI_{TOT} were observed both in *O. carpinifolia* and *Q. ilex*. The

univariate correlations between defoliation and leaf features are shown in Table 4. C/N ratio was positively correlated to defoliation in many species, and negatively in the whole sample. ChlF parameters were negatively correlated with defoliation in *C. sativa* (Fv/F$_M$ and PI$_{ABS}$), *O. carpinifolia* and *Q. petraea* (Ψ_{Eo}, ΔV_{I-P}, PI$_{ABS}$ and PI$_{TOT}$), but positively in *Q. ilex*. δ^{13}C was positively correlated with defoliation in the whole sample and in *C. sativa*. Insect and pathogen damage (assessed on detached leaves) were negatively correlated with defoliation in *Q. cerris* and *O. carpinifolia,* respectively. Pathogen damage was negatively correlated with defoliation in the whole sample.

4. Discussion

In the present survey, the main patterns of defoliation were associated with parasitic attacks (*C. sativa*) or abiotic agents (*O. carpinifolia*). Stand factors could enhance or suppress tree crown defoliation. The closure of the canopies, expressed with high LAI, was the main stand factor associated with reduced defoliation. The positive correlation between crown compression and defoliation in some species seemed to suggest an opposite trend, i.e. enhanced defoliation. This apparent contradiction can be explained by a negative effect of the aboveground competition between crowns, resulting in mechanical abrasions (Hajek et al., 2015). In *C. sativa*, the positive correlation between basal area and defoliation was explained by the high defoliation rates of old large chestnut trees under parasitic attacks.

Tree diversity reduced tree defoliation in *C. sativa* and *Q. ilex*. In the studied forests, *C. sativa* was severely affected by the Asian gall wasp *D. kuriphilus*, which constitutes a tremendous threat for tree health and fruit production (Quacchia et al., 2008; Panzavolta et al., 2012; Battisti et al., 2014). Tree diversity is expected to reduce the intensity of insect herbivore attacks (Jactel and Brockerhoff, 2007). Several hypotheses have been proposed to explain this associational resistance effect of diverse plant communities (Tahvanainen and Root, 1972). According to the "resource concentration hypothesis" (Root, 1973), the probability of a host plant to be located by insects

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379   decreases in plurispecific systems. Non-host plants could disrupt chemical or physical cues used by

380   herbivores to locate a suitable host (Huber and Borden, 2001; Castagneyrol et al., 2013). In addition,

381   the "natural enemy hypothesis" (Root 1973; Russell 1989) suggests that richer plant assemblages

382   provide natural enemies with more complementary resources and habitats, thus promoting top-down

383   regulation of herbivores. Tree species richness *per se*, however, has no effect on the probability of

384   attack by pests. The infestation rate is also strongly dependent on plot composition (Castagneyrol et

385   al., 2014) and species-specific interactions. Guyot et al. (2015) found the decrease of *D. kuriphilus*

386   attacks on chestnut tree crowns in more diverse forests. The authors suggested that it is connected

387   with the presence of oak species, housing cynipid galls and associated parasitoids (Aebi et al., 2006,

388   2007, Panzavolta et al., 2013, Quacchia et al., 2013). At opposite, in *Q. ilex*, the negative correlation

389   between defoliation and the Shannon diversity index was probably due to the smaller size of this

390   species in comparison with the tallest deciduous oaks, and as a result, *Q. ilex,* a sciaphilous species,

391   benefits from the shading effect of dominant canopies.

392   Foliar damage assessed from the ground (ICP Forests protocol) and on detached leaves gave

393   contrasting information. Ground assessment provides a general overview and allows the

394   identification of the most relevant foliar attacks affecting the status of the whole crown. Fungal and

395   insect damages assessed directly from leaves on hand may, however, be indicative of a demography

396   in equilibrium with the crown status (Leather, 2005), although these parameters showed high

397   variability (Table 2).

398   Among the physiological traits assessed, the C/N ratio was related to defoliation in many

399   species. For each species considered individually, the C/N ratio was positively associated with

400   defoliation (reduction of N with increasing defoliation), the opposite was observed in the whole

401   sample. This apparent contradiction can be explained by the result of the combination of species-

402   specific behaviors, with the most N-rich species (*C. sativa*) being also the most defoliated. In *O.*

403   *carpinifolia*, we found increasing C/N ratio associated with decreasing levels of photosynthetic

404   efficiency in defoliated trees. Nikiforou and Manetas (2011) found that the decrease of ΔV_{I-P} may

405   be indicative of low nitrogen foliar concentrations. In *O. carpinifolia*, defoliation was probably

406   related to intense fructification and seed production (masting) that occurred during the sampling

407   period. Masting is a well know phenomenon that has a relevant role in forest ecology (Kelly and

408   Sork, 2002; Packham and Hilton, 2002), whereby the recurrence of mast year may be indicative of

409   altered environmental conditions (Jonard, 2009). In *O. carpinifolia*, this behavior was already

410   observed in previous surveys in Tuscany (Bussotti, data not published). Inverse relationships

411   between fructification and defoliation was found on *Fagus sylvatica* L. (beech) in central Europe by

412   Eichhorn et al. (2005) as a consequence of nutrient allocation strategies (carbon and nitrogen were

413   diverted from leaves to increase its availability for fructification, Jonard et al., 2009). Thomas et al.

414   (2002) demonstrated that unbalanced nutritional status (in this case decreased K/N and P/N ratios,

415   and decreased production of allochemicals caused by high nitrogen deposition) was responsible of

416   poor crown conditions in European species of deciduous oak (*Quercus robur* L. and *Q. petraea*),

417   and pathogenic attacks were favoured.

418   Defoliation in *C. sativa* was correlated to the capacity to trap the solar energy (Fv/F_M and

419   light interception index). A reduction of the photosynthetic efficiency, measured with the JIP-test,

420   was also observed by Ugolini et al. (2014) in *C. sativa* leaves attacked by *D. kuriphilus*. According

421   to the authors, the quantum yield efficiency (Fv/F_M) was not negatively affected by the presence of

422   galls, but Ψ_{Eo} was affected. Deciduous oaks had low levels of defoliation, almost always falling

423   below the value of 25%, which is the threshold that defines "healthy" trees (Eichhorn et al., 2010).

424   This narrow range of defoliation impeded the effective analysis of the relationships with ChlF

425   parameters, although contrasting patterns were detected on *Q. petraea* and *Q. ilex*. The positive

426   correlation between defoliation and ChlF parameters in *Q. ilex* can be attributed to better

427   exploitation of sunlight in lighter crowns, as explained by the enhanced electron transport (Bussotti,

428   2004).

429   Higher values of $\delta^{13}C$ (less negative values) in defoliated trees revealed a potential relation

430   between defoliation and drought stress. High $\delta^{13}C$ content in plant tissues indicates decreased leaf

431   internal CO_2 concentration that can be caused by a decrease in stomatal conductance induced, for

432   example, by drought (Francey and Farquhar, 1982). In the present research, we found a positive

433   correlation between $\delta^{13}C$ and defoliation in the whole sample, and among individual tree of *C.*

434   *sativa*. The loss of foliage mass may cause higher irradiation levels and a drier microclimate in the

435   crown, which may induce stomatal closure. As far as *C. sativa* is concerned, the relatively high

436   values of $\delta^{13}C$ may be a result of parasitic attacks on the functionality of roots (*P. cambivora*) and

437   xylem (*C. parasitica*), and by the leaf damage (*D. kuriphilus*).

438   There are many papers about the relationship between crown defoliation and drought, where

439   water shortage is a causal factor, both in space (increasing defoliation in drier sites, Zierl, 2002) and

440   in time (increasing defoliation after drier years, Solberg, 2004; Ferretti et al., 2014). In previous

441   research carried out in Italian beech forests, Bussotti et al. (2005) found that the most defoliated

442   trees, growing in the southernmost drought-prone sites, had smaller and thicker leaves, and higher

443   C/N ratio, than trees growing in mesic sites. Drought is expected to play an increasing role in

444   Mediterranean forests in light of climate change (Galiano et al. 2012; Bussotti et al., 2014, 2015).

445   The present paper supports the idea that the use of physiological indicators in forest surveys may be

446   relevant to validate the responses observed in controlled conditions and field studies realized "ad

447   hoc" over a larger scale (Iovi et al., 2009; Galiano et al., 2012; Moreno-Gutiérrez et al., 2012;

448   Granda et al., 2014).

449

5. Conclusions

451   The results presented here suggest that defoliation is not an unequivocal phenomenon, but may have

452   different causes and physiological significance in different tree species. Defoliation in *C. sativa* was

453   associated with insect and pathogen attacks, and subsequently resulted in increased exposure to

454   drought. In *O. carpinifolia*, the effect of fruiting with the diversion of N from leaves to fruit was

455   found. In these two species, defoliation was accompanied by the decrease of photosynthetic

456   efficiency, as observed by JIP-test parameters. The opposite pattern observed in *Q. ilex* (higher

457 photosynthetic efficiency in more defoliated trees) maybe related to specific physiological strategies

458 to exploit sunlight.

459   This "leaf trait analysis" approach can be applied both at local and large scale for purposes

460 of forest management and policy. Locally it can be used to individuate the most appropriate

461 structural characteristics of the forests to enhance the physiological efficiency of the different tree

462 species. Moreover, through surveys repeated over time, it would be possible to assess the responses

463 of trees to sylvicultural practices. At a large scale, this analysis can contribute to strengthening the

464 informative potential of the ongoing routine monitoring activities (e.g., ICP Forests) providing

465 insights about the ecological equilibrium of different tree species in a changing environment. In this

466 perspective the leaf traits analysis can provide basic knowledge to support the elaboration of

467 adaptive strategies.

468   The main limitation of this study relies in the difficult of representing, with a consistent

469 number of replicates (within and among forest stands), all the possible combination of tree species

470 mixture whilst avoiding bias deriving from the variability of environmental factors (e.g., bedrock

471 and exposure). Such limitations are due to the characteristics of landscape fragmentation and the

472 difficulty of sampling leave in high forests. A large scale survey with a consistent number of forest

473 stands may provide more robust data evidencing general tendencies and avoiding interference from

474 local factors. The analysis of the variability of the foliar features assessed in this study (Table 2) can

475 help to select the more robust and reliable parameters to design an effective cost-benefit surveys

476 according to the criteria of ecological monitoring (Elzinga et al., 2001).

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References

492 Aebi, A., Schönrogge, K., Melika, G., Alma, A., Bosio, G., Quacchia, A., Picciau, L., Abe, Y.,

493   Moriya, S., Yara, K., Seljak, G., Stone, G.N., 2006. Parasitoid recruitment to the globally

494   invasive chestnut gall wasp *Dryocosmus kuriphilus*. In: Ozaki, K., Yukawa, J., Ohgushi, T.,

495   Price, P. (Eds), Galling arthropods and their associates. Springer-Verlag, Tokyo. pp 103-121.

496 Aebi, A., Schönrogge, K., Melika, G., Quacchia, A., Alma, A., Stone, G.N. 2007. Native and

497   introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*.

498   EPPO Bulletin 37:166-171.

499 Augustatis, A., Bytnerowicz, A., 2008. Contribution of ambient ozone to Scots pine defoliation and

500   reduced growth in the Central European forests: A Lithuanian case study. Environ. Pollut.

501   155, 436-445.

502 Baeten, L., Verheyen, K., Wirth, C., Bruelheide, H., Bussotti, F., Finér, L., Jaroszewicz, B., Selvi,

503   F., Valladares, F., Allan, E. et al., 2013. A novel comparative research platform designed to

504   determine the functional significance of tree species diversity in European forests. Persp. Pl.

505   Ecol. Evolut. Syst. 15, 281-291.

506 Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.-S., Nakashizuka, T., Raffaelli, D., Schmid, B.,

507   2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and

508   services. Ecol. Let. 9, 1146–1156.

509 Battisti, A., Benvegnù, I., Colombari, F., Haack, R.A., 2014. Invasion by the chestnut gall wasp in

510   Italy causes significant yield loss in *Castanea sativa* nut production. Agr. For. Entom. 16,

511   75–79.

512 Bengtsson, J., Nilsson, S.G., Franc, A., Menozzi, P., 2000. Biodiversity, disturbances, ecosystem

513   function and management of European forests. For. Ecol. Manag. 132, 39-50.

514 Bonan, G.B., 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of

515   forests. Science 320,1444–1449.

516 Bussotti, F., 2004. Assessment of stress conditions in *Quercus ilex* L. leaves by O-J-I-P chlorophyll
517   *a* fluorescence analysis. Plant Biosys. 138, 101-109.

518 Bussotti, F., Pancrazi, M., Matteucci, G., Gerosa, G., 2005. Leaf morphology and chemistry in
519   *Fagus sylvatica* L. (beech) trees as affected by site factors and ozone: results from
520   CONECOFOR permanent monitoring plots in Italy. Tree Physiol. 25*,* 211-219.

521 Bussotti, F., Pollastrini, M., 2015. Evaluation of leaf features in forest trees: methods, techniques,
522   obtainable information and limits. Ecol. Ind. 52, 219-230.

523 Bussotti, F., Coppi, A., Pollastrini, M., Feducci, M., Baeten, L. , Scherer-Lorenzen, M., Verheyen,
524   K., Selvi, F., 2012. Le aree forestali italiane di FunDiv Europe: un nuovo progetto FP7 sul
525   significato funzionale della biodiversità forestale in Europa (The Italian forest sites of
526   FunDivEurope: a new FP7 project on the Functional Significance of Forest Biodiversity in
527   Europe). Forest@ 9, 251-259.

528 Bussotti, F., Ferrini, F., Pollastrini, M., Fini, A., 2014.The challenge of Mediterranean
529   sclerophyllous vegetation under climate change: from acclimation to adaptation. Environ.
530   Exp. Bot. 103, 80-98.

531 Bussotti, F., Pollastrini, M., Holland, V., Brüggemann, W., 2015. Functional traits and adaptive
532   capacity of European forests to climate change. Environ. Exp. Bot. 111, 91-113.

533 Carnicer, J., Coll, M., Ninyerola, M., Pons, X., Sánchez, G., Peñuelas, J., 2011. Widespread crown
534   condition decline, food web disruption, and amplified tree mortality with increased climate
535   change-type drought. Proc. Nat. Acad. Sci. U.S.A. (PNAS) 108, 1474-1478.

536 Castagneyrol, B., Giffard, B., Péré, C., Jactel, H., 2013. Plant apparency, an overlooked driver of
537   associational resistance to insect herbivoy. J. Ecol. 101, 418-429.

538 Castagneyrol, B., Régolini, M., Jactel, H., 2014. Tree species composition rather than diversity
539   triggers associational resistance to the pine processionary moth. Bas. Appl. Ecol. 15, 516-
540   523.

541   Clark, D.A., Clark, D.B., 1992. Life history diversity of canopy and emergent trees in a neotropical

542   rain forest. Ecol. Monogr. 62, 315-344.

543   De la Cruz, A., Gil, P.M., Férnandez-Cancio, A., Minaya, M., Navarro-Cerrillo, R.M., Sánchez-

544   Salguero, R., Grau, J.M., 2014. Defoliation triggered by climate induced effects in Spanish

545   ICP Forests monitoring plots. For. Ecol. Manag. 331, 245-255.

546   Desotgiu, R., Cascio, C., Pollastrini, M., Gerosa, G., Marzuoli, R., Bussotti, F., 2012a. Chlorophyll

547   a fluorescence analysis along a vertical gradient of the crown in a poplar (Oxford clone)

548   subjected to ozone and water stress. Tree Physiol. 32, 976–986.

549   Desotgiu, R., Cascio, C., Pollastrini, M., Gerosa. G., Marzuoli, R., Bussotti, F., 2012b. Short and

550   long term photosynthetic adjustments in sun and shade leaves of *Fagus sylvatica* L.,

551   investigated with the fluorescence transient (FT) analysis. Plant Biosys. 146 (Supp. 1), 206-

552   216.

553   Desotgiu, R., Cascio, C., Pollastrini, M., Gerosa, G., Marzuoli, R., Bussotti, F., 2013. Responses to

554   ozone on *Populus* "Oxford" clone in an open top chamber experiment assessed before

555   sunrise and in full sunlight. Photosynthetica, 51 (2): 267-280.

556   Eichhorn, J., Icke, R., Isenberg, A., Paar, U., Schönfelder, E., 2005. Temporal development of

557   crown condition of beech and oak as a response variable for integrated evaluations. Eur J

558   Forest Res 124, 335–347.

559   Eichhorn, J., Roskams, P., Ferretti, M., Mues, V., Szepesi, A., Durrant, D., 2010. Visual

560   Assessment of Crown Condition and Damaging Agents. Manual Part IV. In: Manual on

561   methods and criteria for harmonized sampling, assessment, monitoring and analysis of the

562   effects of air pollution on forests. UNECE ICP Forests Programme Co-ordinating Centre,

563   Hamburg. http://www.icp-forests.org/pdf/FINAL_Crown.pdf

564   Ellsworth, D.S., Reich, P.B., 1995. Canopy structure and vertical patterns of photosynthesis and

565   related leaf traits in a deciduous forest. Oecologia 96, 169-178

566 Elzinga, C.L., Salzer, D.W., Willoughby, J.W., Gibbs, J.P., 2001. Monitoring plant and animal
567   populations. Blackwell Science, Malden.

568 Eyles, A., Smith, D., Pinkard, E.A., Smith, I., Corkrey, R., Elms, S., Beadle, C., Mohammed, C.,
569   2011. Photosynthetic responses of field-grown *Pinus radiata* trees to artificial and aphid-
570   induced defoliation. Tree Physiol. 31, 592-603.

571 Ferretti, M. (Ed.), 1994. Mediterranean Forest Trees. A Guide for Crown Assessment. CEC –
572   UN/ECE, Brussels, Geneva.

573 Ferretti, M., Bussotti, F., Cenni, E., Cozzi, A., 1999. Implementation of Quality Assurance
574   procedures in the Italian programs of forest condition monitoring. Wat. Air Soil Pollut. 116,
575   371-376.

576 Ferretti, M., Nicolas, M., Bacaro, G., Brunialti, G., Calderisi, M., Croisé, L., Frati, L., Lanier, M.,
577   Maccherini, S., Santi, E., Ulrich, E., 2014. Plot-scale modeling to detect size, extent, and
578   correlates of changes in tree defoliation in French high forests. For. Ecol. Manag. 311, 56-69.

579 Francey, J.R., Farquahar, G.D., 1982. An explanation of $^{13}C/^{12}C$ in tree rings. Nature 297, 28-31.

580 Galiano, L., Martínez-Vilalta, J., Sabaté, S., Lloret, F., 2012. Determinants of drought effect on
581   crown condition and their relationship with depletion of carbon reserves in a Mediterranean
582   holm oak forest. Tree Physiology 32, 478-489.

583 Granda, E., Scoffoni, C., Rubio-Casal, A.E., Sack, L., Valladares, F., 2014. Leaf and stem
584   physiological responses to summer and winter extremes of woody species across temperate
585   ecosystems. Oikos 123, 1281–1290.

586 Grossiord, C., Granier, A., Ratcliffe, S., Bouriaud, O., Bruelheide, H., Chećko, E., Forrester, D.I.,
587   Dawud, S.M., Finér, L., Pollastrini, M., Scherer-Lorenzen, M., Valladares, F., Bonal, D.,
588   Gessler, A., 2014a. Tree diversity does not always improve resistance of forest ecosystems
589   to drought. Proc. Nat. Acad. Sci. U.S.A. (PNAS) 111, 14812–14815.

590 Grossiord, C., Gessler, A., Granier, A., Pollastrini, M., Bussotti, F., Bonal, D., 2014b. Interspecific
591   competition influences the response of oak transpiration to increasing drought stress in a
592   mixed Mediterranean forest. Forest Ecol. Manag. 318, 54-61.

593 Guyot, V., Castagneyrol, B., Vialatte, A., Deconchat, M., Selvi, F., Bussotti F., Jactel, H. 2015.
594   Tree diversity limits the impact of an invasive forest pest. PLOS ONE
595   Doi:10.1371/journal.pone.0136469 .

596 Hajek, P., Seidel, D., Leuschner, C., 2015. Mechanical abrasion, and not competition for light, is
597   the dominant canopy interaction in a temperate mixed forest. For. Ecol. Manag. 348, 108–
598   116

599 Huber, D.P.W., Borden, J.H., 2001. Angiosperm bark volatiles disrupt response of douglas-fir
600   beetle, *Dendroctonus pseudotugae*, to attractant-baited traps. J.Chem. Ecol. 27, 217-233.

601 Jactel, H., Brockerhoff, E.G., 2007. Tree diversity reduces herbivory by forest insects. Ecol. Let. 10,
602   835–848

603 Jonard, M., André, F., Dambrine, E., Ponette, Q., Ulrich, E., 2009. Temporal trends in the foliar
604   nutritional status of the French, Walloon and Luxembourg broad-leaved plots of forest
605   monitoring. Ann. For. Sci. 66, p. 412.

606 Jucker, T., Bouriaud, O., Avacaritei, D., Coomes, D., 2014. Stabilizing effects of diversity on
607   aboveground wood production in forest ecosystems: linking patterns and processes. Ecol.
608   Let. 17, 1560-1569

609 Jucker, T., Bouriaud, O., Coomes, D.A., 2015. Crown plasticity enables trees to optimize canopy
610   packing in mixed-species forests. Funct. Ecol. In press, doi: 10.1111/1365-2435.12428

611 Kalaji, H.M., Schansker, G., Ladle, R.J., Goltsev, V., Bosa, K., Allakhverdiev, S.I., Brestic, M.,
612   Bussotti, F., Calatayud, A., Dąbrowski, P., et al., 2014. Frequently asked questions about in
613   vivo chlorophyll fluorescence: pratical issues. Photos. Res. 122, 121–158.

614 Kelly, D., Sork, V.L., 2002. Mast seeding in perennial plants: Why, How, Where? Ann. Rev. Ecol.
615   Sys. 33, 427-447.

616 King, D.A., Davies, S.J., Nur Supardi, M.N., Tan, S., 2005. Tree growth is related to light
617   intercetption and wood density in two mixed dipterocarp forests of Malaysia. Funct. Ecol.
618   19, 445-453

619 Knoke, T., Ammer, C., Stimm, B., Mosandl, R., 2008. Admixing broadleaved to coniferous tree
620   species: a review on yield, ecological stability and economics. Eur. J. For. Res. 127, 89–101.

621 Kuznetsova, A., Brockhoff, P.B., Christensen, R., 2014. Test in Linear Mixed Effects Models
622   (package lmerTest).

623 Iovi, K., Kolovou, C., Kyparissis, A., 2009. An ecophysiological approach of hydraulic
624   performance for nine Mediterranean species. Tree Physiol. 29, 889-900.

625 Lavigne, M.B., Little, C.H.A., Major, J.E., 2001. Increasing the sink:source balance enhances
626   photosynthetic rate of 1-year-old balsam fir foliage by increasing allocation of mineral
627   nutrients. Tree Physiol. 21, 417–426.

628 Leather, S., 2005 (Ed.). Insect sapling in forest ecosystems. Blackwell Publishing, Oxford UK.

629 Li-Cor, 1991. LAI-2000 Plant Canopy Analyzer Operating Manual. Lincoln, NE, USA.

630 Meining, S., Fischer, R., 2011. Tree crown condition and damage causes. In: Fischer, R., Lorenz, M.
631   (Eds.), Forest Condition in Europe. 2011 Technical Report of ICP Forests and FutMon.
632   Work report of the Institute for World Forestry 2011/1. Hamburg.

633 Michel, A., Seidling, W. (eds), 2014. Forest Condition in Europe: 2014 Technical Report of ICP
634   Forests. Report under the UNECE Convention on Long-Range Transboundary Air Pollution
635   (CLRTAP). Vienna: BFW Austrian Research Centre for Forests. BFW-Dokumentation
636   18/2014.

637 Moreno-Gutiérrez, C., Dawson, T.E., Nicolás, E., Querejeta, J.I., 2012. Isotopes reveal contrasting
638   water use strategies among coexisting plant species in a Mediterranean ecosystem. New
639   Phytologist 196, 489-496.

640 Müller, E., Stierlin, H.R., 1990. Tree Crown Photos. Sanasilva. Swiss Federal Institute for Forest
641   Snow and Landscape Research, Birmensdorf, Switzerland.

642 Niederberger, J., Todt, B., Boča, A., Ritschke A., Kohler, M., Kühn, P, Bauhus, J. Use of near-
643   infrared spectroscopy to assess phosphorus fractions of different plant availability in forest
644   soils. Geobiosciences 12, 3415–3428.

645 Nikiforou, C., Manetas, Y., 2011. Inherent nitrogen deficiency in *Pistacia lentiscus* preferentially
646   affects photosystem I: a seasonal field study. Funct. Pl. Biol 38, 848–855

647 Nowak, R.S., Caldwell, M.M., 1984. A test of compensatory photosynthesis in the field:
648   Implications for herbivory tolerance. Oecologia 3, 311-318.

649 Ogaya, R., Peñuelas, J., Asensio, D., Lluisà, J., 2011. Chlorophyll fluorescence responses to
650   temperature and water availability in two co-dominant Mediterranean shrub and tree species
651   in a long-term field experiment simulating climate change. Environmental and Experimental
652   Botany 73, 89-93.

653 Packham, J.R., Hilton, G.M., 2002. Inter- and intra-site variation in the fruiting of common beech
654   (*Fagus sylvatica* L.) in England over a twenty two year period (1980-2001). Arbor. J. 26, 1-
655   22.

656 Panzavolta, T., Bracalini, M., Croci, F., Campani, C., Bartoletti, T., Miniati, G., Benedettelli, S.,
657   Tiberi, R., 2012. Asia chestnut gall wasp in Tuscany: gall characteristics, egg distribution
658   and chestnut cultivar susceptibility. Agric. For. Entom. 14, 139-145.

659 Panzavolta, T., Bernardo, U., Bracalini, M., Cascone, P., Croci, F., Gebiola, M., Iodice, L., Tiberi,
660   R., Guerrieri, E., 2013. Native parasitoids associated with *Dryocosmus kuriphilus* in
661   Tuscany, Italy. Bull. Insect. 66,195-201

662 Pollastrini, M., Holland, V., Brüggemann, W., Koricheva, J., Jussila, I., Scherer-Lorenzen, M.,
663   Berger, S., Bussotti, F., 2014a. Interactions and competition processes among tree species in
664   young experimental mixed forests, assessed with chlorophyll fluorescence and leaf
665   morphology. Plant Biol. 16, 323–331.

666 Pollastrini, M., Desotgiu, R., Camin, F., Ziller, L., Gerosa, G., Marzuoli, R., Bussotti, F., 2014b.

667   Severe drought events increase the sensitivity to ozone on poplar clones. Env. Exp. Bot. 100,

668   94-104.

669 Quacchia, A., Moriya, S., Bosio, G., Scapin, I., Alma, A., 2008. Rearing, release and settlement

670   prospect in Italy of Torymus sinensis, the biological control agent of the chestnut gall wasp

671   *Dryocosmus kuriphilus*. BioControl 53, 829–839.

672 Quacchia, A., Ferracini, C., Nicholls, J.A., Pia.zza, E, Saladini, M.A., Tota, F., Melika, G., Alma, A.

673   2013. Chalcid parasitoid community asociated with the invading pest *Dryocosmus*

674   *kuriphilus* in north-western Italy. Insect Cons. Div. 6, 114-123

675 Quentin, A.G., O'Grady, A.P., Beadle, C.L., Worledge, D., Pinkard, E.A., 2011. Responses of

676   transpiration and canopy conductance to partial defoliation of *Eucalyptus globulus* trees.

677   Agr. For. Met. 151, 356-364.

678 R Core Team, 2014. R: A language and environment for statistical computing. R Foundation for

679   Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

680 Root, RB., 1973. Organization of a plant-arthropod association in simple and diverse habitats: the

681   fauna of collards (*Brassica oleracea*). Ecol. Mon. 43, 95-124

682 Russell, E.P., 1989. Enemies hypothesis: a review of the effect of vegetational diversity on

683   predatory insects and parasitoids. Environ. Entom. 18, 590-599

684 Seidling, W., 2007. Signals of summer drought in crown condition data from the German Level I

685   network. Eur. .J For. Res. 126, 529–544.

686 Solberg, S., 2004. Summer drought: a driver for crown condition and mortality of Norway spruce in

687   Norway. For. Path. 34, 93-104.

688 Spellerberg, I.F., Fedor, P.J., 2003. A tribute to Claude Shannon (1916-2001) and a plea for more

689   rigorous use of species richness, species diversity and the 'Shannon-Wiener' Index. Glob.

690   Ecol. Biog. 12, 177-19.

691   Staddon, W.J., Duchesne, L.C., Trevors, J.T., 1997. Microbial diversity and community structure of

692   postdisturbance forest soils as determinate by sole-carbon-source utilization patterns.

693   Microbiol. Ecol. 34, 125-130.

694   Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to

695   characterize and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P.

696   (Eds.). Probing photosynthesis: mechanisms, regulation and adaptation. Taylor & Francis

697   London. pp. 445-483.

698   Strasser, R.J., Tsimilli-Michael, M., Srivastava, A., 2004. Analysis of the fluorescence transient. In:

699   Chlorophyll fluorescence: a Signature of Photosynthesis. Advances in Photosynthesis and

700   Respiration Series. Papageorgiou, G.C., Govindjee (Eds.), Springer Dordrecht, The

701   Netherland, pp. 321-362.

702   Strasser, R.J., Tsimilli-Michael, M., Qiang, S., Goltsev, V., 2010. Simultaneous in vivo recording of

703   prompt and delayed fluorescence and 820-nm reflection changes during drying and after

704   rehydration of the resurrection plant *Haberlea rhodopensis*. Biochim. Biophys. Acta 1797,

705   1313-1326.

706   Tahvanainen, J.O., Root, R. B., 1972. The influence of vegetational diversity on the population

707   ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae).

708   Oecologia 10, 321-346.

709   Thomas, F.M., Blank, R., Hartmann, G., 2002. Abiotic and biotic factors and their interactions as

710   causes of oak decline in Central Europe. For. Path. 32, 277-307.

711   Turnbull, T.L., M.A. Adams and C.R. Warren. 2007. Increased photosynthesis following partial

712   defoliation of field-grown *Eucalyptus globulus* seedlings is not caused by increased leaf

713   nitrogen. Tree Physiol. 27, 1481–1492.

714   Ugolini, F., Massetti, L., Pedrazzoli, F., Tognetti, R., Vecchione, A., Zulini L., Maresi G., 2014.

715   Ecophysiological responses and vulnerability to other pathologies in European chestnut

716   coppices, heavily infested by the Asian chestnut gall wasp. For. Ecol. Mana. 314, 38-48.

717   Van Leeuwen, E.P., Hendriks, K.C.M.A., Klap, J., De Vries, W., De Jon,g E., Erisman, J.W., 2000.

718   Effects of environmental stress on forest crown condition in Europe. Part II: Estimation of

719   stress induced by meteorology and air pollutants. Wat. Air. Soil Pollut. 119, 335–362.

720   Watson, D.J, 1947. Comparative physiological studies in the growth of field crops. I. Variation in

721   net assimilation rate and leaf area between species and varieties, and within and between

722   years. Ann. Bot. 11, 41-76

723   Werner, C., Correia, O., Beyschlag, W., 2002. Characteristic patterns of chronic and dynamic

724   photoinhibition of different functional groups in a Mediterranean ecosystem. Functional

725   Plant Biol. 29, 909-1011.

726   Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z. et al., 2004. The worldwide leaf

727   economics spectrum. Nature 428, 821-827

728   Zierl, B., 2004. A simulation study to analyse the relations between crown condition and drought in

729   Switzerland. For. Ecol. Manage. 188, 25-38.

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732 **Table 1** Number of plots and trees sampled in the study. For Basal Area (BA, m^2 ha^{-1}) and Leaf Area Index
733 (LAI, m^2 m^{-2}), mean and standard deviation for each species and mixture level are indicated.
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Species	Mixture level	No. Plots	No. Trees	BA M	±sd	LAI M	±sd
Castanea sativa	1-sp	2	11	29.33	±0.49	2.95	±0.13
	2-sp	3	9	29.07	±3.54	4.13	±0.12
	3-sp	5	15	26.01	±1.66	3.24	±0.70
	4-sp	4	12	27.95	±5.15	4.24	±0.54
Ostrya carpinifolia	1-sp	2	11	23.72	±2.51	3.49	±1.69
	2-sp	3	8	22.80	±1.84	4.09	±0.61
	3-sp	4	12	26.45	±4.67	4.16	±0.65
	4-sp	5	13	28.10	±3.84	3.77	±0.54
Quercus cerris	1-sp	1	12	28.30	±0.29	3.48	±0.04
	2-sp	3	9	30.24	±6.42	5.25	±1.15
	3-sp	5	14	26.83	±4.06	3.72	±1.00
	4-sp	6	18	27.65	±5.24	4.13	±0.58
Quercus ilex	1-sp	2	12	28.48	±5.60	4.54	±0.67
	2-sp	4	12	28.46	±7.74	4.77	±1.32
	3-sp	5	13	27.84	±3.62	4.06	±0.65
	4-sp	7	19	27.89	±5.10	4.05	±0.60
Quercus petraea	1-sp	2	12	28.85	±0.97	3.76	±0.28
	2-sp	1	3	21.85	±0.00	4.91	±0.00
	3-sp	5	15	25.72	±2.27	3.47	±0.84
	4-sp	5	14	26.03	±2.92	3.82	±0.49
All species	1-sp	10	58	27.77	±3.38	3.65	±0.93
	2-sp	7	41	27.39	±6.09	4.61	±1.01
	3-sp	8	69	26.53	±3.34	3.70	±0.83
	4-sp	7	76	27.53	±4.55	4.00	±0.57

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736 **Table 2** Variability (coefficient of variability, CV) of the tree and foliar parameters among trees in the same plot and between plots. For foliar nitrogen content
737 (N), carbon content (C) and carbon isotope composition (Leaf δ^{13}C), the variability is indicated between plots because these parameters were measured at the tree
738 species level in each plot.
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| | Castanea sativa | | Ostrya carpinifolia | | Quercus cerris | | Quercus ilex | | Quercus petraea | |
| | CV (%) | | CV (%) | | CV (%) | | CV (%) | | CV (%) | |
	tree	plot	tree	plot	tree	plot	tree	plot	tree	plot
Tree characteristics										
Defoliation	29.36	46.06	29.47	36.65	38.33	53.63	76.56	111.13	42.02	54.49
Damage to leaves	57.00	64.36	43.89	63.82	93.38	81.33	141.29	165.92	74.47	59.20
Damage to branches	38.21	65.60	52.72	59.12	66.91	63.28	121.45	111.95	77.31	55.85
Foliar characteristics										
Insect damage	20.68	19.53	75.13	55.62	35.08	27.84	31.98	22.59	47.54	32.95
Pathogen damage	61.81	224.32	71.69	249.72	83.75	134.85	44.32	43.63	115.30	96.29
Nitrogen (N %)		8.23		10.26		6.71		8.31		6.49
Carbon (C %)		1.52		1.59		1.42		1.19		1.79
Leaf δ^{13}C (‰)		-1.49		-2.37		-1.51		-2.32		-2.37
C/N		9.37		9.06		5.92		9.02		5.54
Fv/F$_M$	1.30	1.19	0.99	1.61	1.84	1.78	1.82	1.83	1.19	1.97
Ψ_{Eo}	5.07	4.70	3.23	8.33	6.47	9.11	5.59	7.75	4.07	8.48
ΔV_{I-P}	8.53	10.49	9.52	21.41	8.65	15.27	11.17	15.80	8.12	12.67
PI$_{ABS}$	17.84	14.54	11.34	24.39	22.31	25.07	24.27	41.40	15.02	26.22
PI$_{TOT}$	19.92	25.10	19.83	43.99	22.90	34.03	25.47	45.00	19.83	33.53

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748 **Table 3** Mean (± standard deviation) of the crown and leaf parameters assessed for each tree species in the whole sample (i.e. all tree species together). The data
749 are pooled for all plots (monospecific and mixed plots). Letters indicate the significant differences of the parameters among the species (Kolmogorov-Smirnov
750 test for independent samples). Descriptions of each parameter can be found in the Materials and Methods. See Abbreviation list.
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	Castanea sativa			*Ostrya carpinifolia*			*Quercus cerris*			*Quercus ilex*			*Quercus petraea*		
	Mean	±sd		Mean	±sd		Mean	±sd		Mean	±sd		Mean	±sd	
Crown parameters:															
Defoliation (%)	36.90	±16.99	a	17.44	±6.39	b	12.16	±6.51	b	6.51	±7.23	c	12.76	±6.95	b
Damage to leaves (%)	23.02	±14.81	a	10.77	±6.87	b	2.97	±2.41	b	0.93	±1.53	c	4.29	±2.54	b
Damage to branches (%)	32.57	±21.36	a	13.77	±8.14	b	7.08	±4.48	b	1.94	±2.17	c	7.24	±4.04	b
Foliar parameters:															
Insect damage (%)	45.26	±14.23	a	3.25	±2.71	c	33.10	±13.97	b	38.72	±15.44	b	36.46	±20.10	b
Pathogen damage (%)	17.73	±0.19	a,b	24.68	±0.26	a,c	4.96	±0.06	c	41.57	±0.14	b	8.39	±0.07	c
Nitrogen (N %)	2.62	±0.21	a	2.36	±0.26	b	2.26	±0.14	c	1.28	±0.12	d	2.27	±0.16	c
Carbon (C %)	48.93	±0.81	b	49.22	±0.90	c	50.22	±0.66	a,b	50.37	±0.56	a	49.70	±0.73	b,c
Leaf δ^{13}C (‰)	-27.06	±0.38	c	-28.26	±0.64	c	-27.54	±0.39	a	-27.79	±0.67	b	-27.04	±0.64	b
C/N	18.83	±1.74	d	21.06	±2.08	c	22.35	±1.25	b	39.58	±3.86	a	22.04	±1.62	b
Fv/F_M	0.79	±0.01	a	0.79	±0.02	a	0.78	±0.02	a	0.78	±0.02	a	0.79	±0.02	a
Ψ_{Eo}	0.60	±0.04	a	0.58	±0.05	a	0.57	±0.06	a	0.58	±0.06	a	0.59	±0.05	a
ΔV_{I-P}	0.28	±0.04	a	0.20	±0.04	c	0.31	±0.06	a	0.26	±0.05	b	0.29	±0.04	a,b
PI_{ABS}	36.19	±7.96	a	25.93	±7.00	b	31.38	±10.39	a	34.24	±17.44	a	34.30	±10.99	a
PI_{TOT}	33.75	±10.77	a	13.78	±6.25	c	39.83	±15.83	a	27.67	±14.35	b	35.44	±14.91	a

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Table 4 Correlations (Spearmann rank correlation) between defoliation and stand parameters and leaf traits in the whole sample (i.e. all tree species together) and in each tree species. Significant correlation coefficients ($p< 0.05$) are in bold.

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	All species	C. sativa	O. carpinifolia	Q. cerris	Q. ilex	Q. petraea
Stand and tree characteristics						
Tree Diversity (Shannon Index)	-0.107	**-0.325**	0.083	0.074	**-0.350**	-0.203
Plot basal Area (BA)	-0.010	**0.427**	-0.178	**-0.436**	-0.119	0.058
Leaf Area Index (LAI)	-0.333	**-0.401**	**-0.586**	**-0.397**	0.037	-0.257
Crown compression (CC)	0.051	**0.313**	**0.381**	0.114	-0.01	-0.165
Light Interception (LI)	**-0.365**	-0.187	-0.119	-0.075	-0.198	-0.110
Foliar characteristics						
Leaf δ^{13}C	**0.175**	**0.426**	0.074	-0.072	0.168	0.157
C/N	**-0.572**	**0.517**	**0.330**	0.206	**0.534**	0.224
F_v/F_M	**0.155**	**-0.332**	-0.244	0.036	0.224	-0.274
Ψ_{Eo}	0.088	-0.172	**-0.328**	0.005	**0.428**	**-0.492**
ΔV_{I-P}	0.044	-0.057	**-0.378**	-0.007	**0.349**	**-0.401**
PI_{ABS}	0.123	**-0.402**	**-0.403**	-0.048	**0.409**	**-0.413**
PI_{TOT}	0.085	-0.208	**-0.452**	-0.081	**0.493**	**-0.437**
Insect damage	0.005	0.056	0.084	**-0.315**	-0.035	-0.278
Pathogen damage	**-0.240**	-0.109	**-0.395**	0.057	0.160	-0.185

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Table 5 Degrees of freedom (df), F and p-value from the linear mixed models used to test the fixed effects of stand parameters (plot basal area, Shannon diversity index and LAI), tree species, crown characteristics of trees (light interception index, crown compression) and the effects of fungal attacks on tree defoliation in the whole sample (all tree species together). The significant effect ($p<0.05$) of the predictor on the variability of defoliation is noted in bold.

Predictor	df	F	p-value
Plot basal area	1	0.080	0.780
Shannon diversity index	1	0.205	0.655
LAI	1	5.047	**0.034**
Light interception index	1	0.000	0.993
Crown compression	1	1.210	0.273
Species	4	33.741	**0.000**
Pathogen damage	1	3.750	0.054

Table 6 Degree of freedom (df), F and p-values from the linear mixed models used to test the fixed effects of stand parameters (plot basal area, Shannon diversity index, LAI), and crown characteristics of trees (light interception index, crown compression) on defoliation in each tree species. The models did not include the correlated predictors (plot basal area and Leaf Area Index in the model for *Q. petraea* and *Q. ilex*).

Predictor	*Castanea sativa*			*Ostrya carpinifolia*			*Quercus cerris*			*Quercus petraea*			*Quercus ilex*		
	df	F	p value	df	F	p value	df	F	p value	df	F	p value	df	F	p value
Plot basal area	1	7.338	**0.01**	1	0.038	0.846	1	1.513	0.24				1	0.023	0.88
Shannon diversity index	1	1.716	0.197	1	0.033	0.857	1	0.056	0.816	1	0.159	0.7	1	1.736	0.207
Light interception index	1	3.975	0.053	1	1.669	0.204	1	2.614	0.112	1	0.306	0.583	1	1.316	0.257
Crown compression	1	2.93	0.095	1	0.879	0.355	1	10.846	**0.002**	1	0.06	0.807	1	0.645	0.426
Leaf Area Index							1	1.504	0.241	1	4.354	0.063			

Highlights

- Crown condition and leaf physiological traits were assessed in mixed forests
- Tree species and Leaf Area Index were the most important predictors of defoliation
- Tree diversity reduced the defoliation in chestnut and in holm oak
- Defoliation has different physiological significance in forest tree species
- Crown assessment is more informative when defoliation and leaf traits are combined

FORECO15463R2

Reviewers' Comments
Authors responses

Reviewer #1:
The authors followed the main recommendations of the first review. From my view, the value of the paper increased significantly. I recommend accepting the paper for printing after some minor changes. One point for further considerations and improvement: Some phrases and statements of the paper are not precise enough. Some examples:
Introduction gives the scientific environment of the study and questions that might be still open. However, it should be stated even more clearly what is the task of the study and what can be achieved by the scientific concept. It seems important to specify what can be achieved and what is out of reach by methodological limitations and difficulties of the study.

The limitation are discussed in Conclusions. In Introduction some statements are changed:
"The present research was part of a project on the functional significance of forest biodiversity in Europe (FunDivEUROPE, Baeten et al., 2013), and was carried out in mixed broadleaved forests in central Italy (Tuscany). Defoliation and crown conditions, assessed according to the guidelines of the ICP Forests manual (Eichhorn et al., 2010), were studied in the context of stand characteristics and foliar features. Stand characteristics provide information on the possible detrimental (or beneficial) effects of forest structure and composition (basal area, leaf area index, tree species mixture) on crown condition. Foliar features are relevant to investigation of the cause and/or consequences of defoliation on tree health and relative physiological functions. In contrast to large scale surveys, where variation in defoliation can be associated with the change in environmental conditions, for example drought or elevation gradients (Michel and Seidling, 2014) in a local homogenous area, with uniform climatic and soil conditions, defoliation may have contrasting significance to different tree species and depends on the interaction between the characteristics of each individual species with biotic stress and environmental conditions. Within the hypothesis that defoliation may have different ecological and physiological meanings in tree species sharing the same environment, the present survey is aimed at exploring the effectiveness of comprehensive foliar analysis, combined with the structure and composition of the forest stands, to analyze species-specific responses connected to defoliation." [line 139-155]

I appreciate to combine aspects of site, stand, LAI, defoliation, C/N, Carbon isotopes and ChlF as well as insects and fungi in the given study. However, my concern is: The main questions raised are quite general. Is the paper able to give answers to general questions such as (Summary 41) "to detect the main causes of tree defoliation in forest tree species".... E. g. water use and drought are not at all included in the concept.

The main question is the different responses of tree species sharing the same environment. In Summary the statement was changed:
"In contrast to large scale surveys, where variation in defoliation can be associated with the change in environmental conditions, in a limited homogenous area the defoliation of co-existing tree species may have different significance and depends on the interaction between the characteristics of each individual species with biotic stress and environmental conditions". [line 40-44]

Another example: the authors relate the results to forest management. However, is there a real good argumentation how to use the results for practical forestry? Under which conditions show the results a general applicability in practical forestry?

Forest management issues are not included now in Summary and Introduction. In Conclusions the statements were changed:

"This "leaf trait analysis" approach can be applied both at local and large scale for purposes of forest management and policy. Locally it can be used to individuate the most appropriate structural characteristics of the forests to enhance the physiological efficiency of the different tree species. Moreover, through surveys repeated over time, it would be possible to assess the responses of trees to sylvicultural practices. At a large scale, this analysis can contribute to strengthening the informative potential of the ongoing routine monitoring activities (e.g., ICP Forests) providing insights about the ecological equilibrium of different tree species in a changing environment. In this perspective the leaf traits analysis can provide basic knowledge to support the elaboration of adaptive strategies." [line 459-467]

Is the investigation and inclusion ChlF sufficient to state: "the present paper demonstrated the feasibility of the use of physiological indicators in forest surveys?
This statement is deleted. It is now added a discussion about the limits and difficult of the survey. [line 468-476]

Discussion tries to explain major results quite completely. However, it is not adequately stated, which contradictions might be given by restrictions of the data base or by the complexity of the concept. Questions such as: What might be a needed next step to clarify complex results. Which consequences can be derived from the experimental set up (samples, repetitions). Which limitations are given by statistical methods?
In conclusions the statements were changed:
"The main limitation of this study relies in the difficult of representing, with a consistent number of replicates (within and among forest stands), all the possible combination of tree species mixture whilst avoiding bias deriving from the variability of environmental factors (e.g., bedrock and exposure). Such limitations are due to the characteristics of landscape fragmentation and the difficulty of sampling leave in high forests. A large scale survey with a consistent number of forest stands may provide more robust data evidencing general tendencies and avoiding interference from local factors. The analysis of the variability of the foliar features assessed in this study (Table 2) can help to select the more robust and reliable parameters to design an effective cost-benefit surveys according to the criteria of ecological monitoring (Elzinga et al., 2001)." [line 468-476]

Therefore, I would prefer to be more precise what the authors really intend to explain. This is mainly valid in the chapter introduction (questions) and discussion/summary. In my opinion, this would be a further step of improvement of the overall value of the paper.

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Pollastrini M., Feducci M., Bonal D., Fotelli M., Gessler A., Grossiord C., Guyot V., Jactel H., Nguyen D., Radoglou K., Bussotti F., '**Physiological significance of forest tree defoliation: results from a survey in a mixed forest in Tuscany (central Italy)**'

Dear Editor,

We herewith enclose a new revised version of the manuscript and a detailed list of responses to each point raised by Reviewers.

We thank the two anonymous Referees for their constructive comments and suggestions, which has helped us to improve the paper.

Waiting for your kind consideration,

Yours Sincerely,

Martina Pollastrini