Annals of Forest Science (2013) 70:571–578
DOI 10.1007/s13595-013-0297-5

ORIGINAL PAPER

Ozone fumigation of *Quercus ilex* L. slows down leaf litter decomposition with no detectable change in leaf composition

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Received: 2 May 2013 / Accepted: 21 May 2013 / Published online: 12 June 2013

Abstract
• ***Context*** The evaluation of changes in litter decomposition rate due to increasing trend in tropospheric ozone is an emerging field of investigation, providing relevant information on long-term forest ecosystem sustainability.
• ***Aims*** This research aims to clarify the effects of ozone exposure on *Quercus ilex* leaf chemical composition and decomposition slow down.
• ***Methods*** Young plants were fumigated in growth chambers at a cumulative dose of 17.15 ppm h. To assess the fumigation effectiveness, stomatal conductance and net photosynthesis were monitored. Leaves were analysed for C, N, S, Ca, Mg, K, Fe, Zn, Mn, total soluble sugars, starch, acid-detergent fibre (ADF), lignin and cellulose prior to the incubation in litter bags in mesocosms, and during decomposition along 395 days.
• ***Results*** Ozone-exposed leaves showed a significant reduction in net photosynthesis and stomatal conductance but did not differ from control leaves in all the chemical parameters analysed. Nevertheless, leaf decomposition rate was lower in treated leaves. The main differences between the models describing the mass loss in exposed and control leaves were played by ADF for exposed leaves and by lignin for control leaves, as well as by N, that showed a greater contribution in the model for the exposed leaves.
• ***Conclusion*** Ozone fumigation of *Q. ilex* results in leaf litter decomposition slowing down, mainly due to ADF joint dynamics with the other variables describing mass decay, even if no detectable changes in initial leaf composition occur.

Keywords Decomposition · Leaf chemistry · Litter bags and mesocosms · Mediterranean area · Holm oak · Tropospheric ozone

1 Introduction

The evaluation of the effects of tropospheric ozone (O_3) on forest long-term sustainability in the Mediterranean area is an emerging field of investigation. Mediterranean area, indeed, characterised by high summer temperature and solar radiation, is subjected to high annual average background O_3 concentration, ranging approximately between 20 and 45 ppb (Paoletti 2006). The vegetation community of the Mediterranean area, mainly represented by maquis flora, presents many ozone-tolerant species, particularly sclerophylls. *Quercus ilex* L., a xerophile oak widely distributed in the Mediterranean basin, is surely one of the most important and widespread elements of the plant communities of this geographical region. Like many sclerophylls, it is an ozone-tolerant species (Manes et al. 1998; Paoletti 2006) and, in addition, it is the most tolerant species of its genus in Italy (Calatayud et al. 2011). Many relevant physiological processes of *Q. ilex*, indeed, are not influenced up to high ozone concentrations (Manes et al. 1998; Loreto et al. 2004; Vitale et al. 2008; Bussotti et al. 2011). The high tolerance of *Q. ilex* to ozone may be likely due to either morpho-

Handling Editor: Erwin Dreyer

Contribution of the co-authors Daniela Baldantoni and Alessandro Bellino performed the analyses and wrote the paper.
Fausto Manes and Anna Alfani designed the experiment, coordinated and supervised the work.

Electronic supplementary material The online version of this article (doi:10.1007/s13595-013-0297-5) contains supplementary material, which is available to authorized users.

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functional adaptations related to sclerophylly (Manes et al. 1998) or to chemical responses, such as the synthesis of antioxidant compounds (Loreto et al. 2004), which may affect leaf decomposition. This in turn may influence both the energy flow and the nutrient cycling dynamics in ecosystems (Lindroth et al. 2001), playing a key role in long-term forest ecosystem sustainability (Talhelm et al. 2012).

Studies on the effects of tropospheric ozone on quality and decomposition rate of leaves have generally been carried out on few herbaceous (Kim et al. 1998; Booker et al. 2005; Williamson et al. 2010) and tree (Parsons et al. 2008; Pritsch et al. 2008; Liu et al. 2009) species, but the published results are often in disagreement.

In a recent study, we demonstrated that the exposure of *Q. ilex* plants in open-top chambers (OTCs) to ozone concentrations typical of the Mediterranean area slows down the leaf decomposition rate in respect to unexposed plants (Baldantoni et al. 2011). However, we were not able to establish to which changes in leaf quality this effect was related. With the aim to clarify which chemical alterations in *Q. ilex* leaves could be involved in decomposition slowing down, we exposed young plants to ozone fumigation in growth chambers and analysed C, N, S, Ca, Mg, K, Fe, Zn, Mn, total soluble sugar, starch, acid-detergent fibre, lignin and cellulose leaf concentrations. These parameters were measured prior to incubation of litter bags in mesocosms and during the decomposition in the subsequent 13 months.

2 Materials and methods

2.1 Plant species

Q. ilex L. (holm oak) is a xerophile evergreen oak widely distributed in the Mediterranean area from the sea level to the submontane vegetation belt. It is a dominant element of the temperate belt of the maquis flora and shows fine adaptations to thermo-xerophily, like sclerophylly and a thick layer of trichomes on leaf abaxial side. As many other sclerophylls, it is considered an ozone-tolerant species (Paoletti 2006), due to the high threshold of toxicity of this pollutant on many physiological processes (Manes et al. 1998; Vitale et al. 2008; Bussotti et al. 2011).

2.2 Ozone fumigation

Forty plants in pots, 3-year-old, from Castelporziano Estate (41°45′N, 12°26′E) near Rome (Italy), were kept for 13 days in two growth chambers (Labco, CT15) at controlled conditions, in order to reach acclimation. The controlled conditions (15-h photoperiod, 25–18 °C day–night temperature, 65 % relative humidity, 400 μmol m^{-2} s^{-1}) were chosen considering the typical values measured in ilex woods during summer season (Gratani 1997). In one growth chamber, the 20 randomly chosen plants were subjected, under the same conditions, to acute ozone exposure (350±10 ppb for 7 h a day) for a total of 7 days (4 days of ozone exposure—4 days of no exposure—3 days of ozone exposure) reaching a cumulative dose of 17.15 ppm h. The exposure treatment was chosen accordingly to Manes et al. (1998) who demonstrated that net photosynthesis, transpiration, chlorophyll fluorescence and peroxidase activity in *Q. ilex* leaves were not influenced until a cumulative O_3 concentration of 7.2 ppm h. The remaining 20 plants, in the other growth chamber, were kept as controls. Ozone was produced by a UV lamp (Helios Italquarz) in a controlled oxygen flow, and its concentration in the chamber was continuously monitored by a photometric UV analyzer (Dasibi, 1108-RS). Net photosynthesis and stomatal conductance, measured with a CIRAS-1 (PP Systems), were used as indicators of the effectiveness of ozone treatment. The measurements were carried out on both ozone treated (T) and control (C) leaves in the day before starting of the first fumigation and at 5, 8, 12 and 14 days after.

2.3 Litter bag and mesocosm preparations

Since young *Q. ilex* plants were grown in pots and too few leaves were naturally shed after exposure in growth chambers, 1-year-old leaves were collected from the plants and used to study the decomposition, as often carried out in studies on holm oak leaf litter decomposition (Cotrufo et al. 1995; Sadaka-Laulan and Ponge 2000). Healthy leaves were collected at the 14th day after the start of the first fumigation, excised at the petioles, cleaned by paper tissues to remove small parasites and pooled according to ozone exposure. Dry mass on oven-dried (75 °C) leaves was obtained, and leaf surface area was measured by a Portable Area Meter (Li-COR, LI-3000C). Measurements were carried out on ten leaves from exposed and ten from unexposed plants. All the other leaves were air-dried until constant weight and placed (about 1 g) in terylene bags with 1-mm mesh in order to allow migration of small detritivores, minimizing the loss of small litter particles.

Twelve mesocosms were performed in plastic containers (32×65×12 cm) by using clods of soil (0–10 cm depth) collected, preserving the litter above, in sites with steep slopes and extensive limestone outcrops of a holm oak forest in southern Italy (Mt. Acero; 41°15′N, 14°29′E, 650–700 m a.s.l.). The mesocosms (each of them containing ten litter bags) were kept for 395 days in a dark room (although with a few light spots) at controlled conditions (22–25 °C, 65–85 % relative humidity), and were irrigated once a week with distilled water.

Three subsamples of the employed soil (sieved at 2-mm mesh and oven-dried at 75 °C to constant weight) were analysed for pH and for concentrations of organic C, total C, N, S and total and available Ca, Mg, K, Fe, Zn, Mn (Online Resource 1). In particular, pH was measured by potentiometric method (1.0:2.5 *w*/*w*=soil/distilled water; Hanna, HI 4212), total C, N and S concentrations, as well as organic C after carbonates dissolution with HCl 10 %, were measured by a CHNS-O Analyzer (Thermo, Flash EA 1112), total and available Ca, Mg, K, Fe, Zn, Mn concentrations were measured by flame atomic absorption spectrometry (PerkinElmer, AAnalyst 100). Measurements of total concentrations were made on soil grounded to a fine powder in a planetary ball mill (Retsch, PM4) with agate mortars and digested with HF (50 %) and HNO_3 (65 %) at a ratio of 1:2 (*v*/*v*) in a micro-wave oven (Milestone, Ethos). To obtain the Ca, Mg and K available fractions, as well as the Fe, Zn and Mn available fractions, solutions of $BaCl_2$ and TEA at pH 8.1 and of EDTA and $CH_3CO_2NH_4$ at pH 4.6 were respectively used.

2.4 Decomposition rate and leaf chemical parameters

After 32, 61, 157, 244, 333 and 395 days from the start of incubation, ten litter bags per treatment were randomly sampled from the mesocosms to monitor mass decay and changes in leaf chemical composition. At each sampling, leaves from each litter bag were carefully cleaned to remove exogenous bodies, dried to a constant weight at 75 °C and ground to fine powder samples by a planetary ball mill (Retsch, PM4).

Before the incubation and at each sampling, measurements of C, N, S, Ca, Mg, K, Fe, Zn, Mn, total soluble sugars, starch, acid-detergent fibre (ADF), lignin and cellulose concentrations were carried out. Element concentrations were quantified as described for soil total concentrations. Total soluble sugars and starch were extracted and quantified following the method of Chow and Landhäusser (2004). ADF, lignin and cellulose concentrations were determined as reported in Fioretto et al. (2005). In the text *cellulose* always refers to *cellulose plus ashes*, the ashes amounting to less than 10 %.

2.5 Data processing

To evaluate the differences between the treated and control leaves in their net photosynthesis and stomatal conductance, a two-way multivariate analysis of variance (MANOVA; α=0.05; n=237) was performed using the treatment and the time as fixed factors, followed by two-way ANOVA with the same model for each response variable, according to Rencher (2002). Canonical generalized discriminant analysis (α=0.05; n=237) was then performed to evaluate the relative contribution of the two physiological processes in determining the differences between T and C leaves.

Due to the high dimensionality of the data set, the overall difference in the initial physical and chemical characteristics between treated and control leaves was assayed by permutational one-way MANOVA with the treatment as the fixed effect (α=0.05; n=10), using the Bray–Curtis dissimilarity metric. The Student *t* test or the Wilcoxon rank sum test (α=0.05; n=10) was then performed to evaluate the difference between T and C leaves in the initial value of each parameter analysed.

At each sampling, the litter remaining mass, C, N, S, Ca, Mg, K, Fe, Zn, Mn, total soluble sugars, starch, ADF, lignin and cellulose concentrations were expressed as percentages of the initial values (P_{ij}), according to the equation:

$$P_{ij} = \frac{C_{ij} \times W_i}{C_{0j} \times W_0} \times 100$$

where C_{0j} is the initial concentration of the *j*th parameter analysed, W_0 the initial litter weight, C_{ij} the concentration of the *j*th parameter at the *i*th sampling and W_i the litter weight at the *i*th sampling.

The mass decay was modelled according to an exponential-linear composition of the form:

$$M_t = M_0 + Ae^{-kt} + Bt$$

where M_t is the leaf mass at time *t* (days), M_0 is the initial leaf mass, *A* and *B* are the slopes of the exponential and the linear terms, respectively, and *k* is the daily decomposition constant. The model was fitted separately on the data from the treated and control leaves, and the significance of the differences (α=0.05) between the two models was evaluated by a log-likelihood ratio test.

The importance of C, N, S, Ca, Mg, K, Fe, Zn, Mn, total soluble sugars, starch, ADF, lignin and cellulose (expressed as percentage of the initial value) and C/N ratio of the leaves in determining the remaining mass was evaluated by Least Angle Regression (LAR), separately for ozone treated (T) and control (C) leaves (each with n=30). Best models were selected according to a cross-validation criterion.

The significance of the overall difference between the treated and the control leaves considering all the parameters analysed was evaluated by a two-way MANOVA (α=0.05; n=60) on the logarithmic transformed data set with the treatment and time as fixed effects. The significance of the differences in each chemical parameter between T and C leaves was then evaluated by two-way ANOVA with the same model. The significance of the differences in the remaining mass between T and C leaves was evaluated by multiple-way analysis of covariance (ANCOVA; α=0.05; n=60) with the treatment and the time as fixed factors and the variables selected by the LAR as covariates. The significance of the

expected mass decay slowing down in T leaves in respect to C leaves at the end of the observations was evaluated by a one-tailed Student *t* test (α=0.05; n=10).

Normality of the residuals, heteroschedasticity and linearity of the models were assessed for α=0.05 by the Kolmogorov–Smirnov, the Breusch–Pagan and the rainbow tests, respectively. All the analyses were performed with the R 2.12 programming environment, using the functions of the "lmtest", "nortest", "lars", "minpack.lm" and "stats" packages.

3 Results

Leaves of ozone treated plants showed a significant (Pillai's trace=0.193, P<0.001) reduction in net photosynthesis and stomatal conductance along the time in respect to those of control plants (Fig. 1). Photosynthesis was more affected by the ozone treatment than stomatal conductance (canonical structure, 0.9999 and 0.8843, respectively; P<0.001 for both the parameters). No visible injury was detected on the leaves belonging to the two treatments, and no significant difference was found in the initial physical and chemical characteristics (Table 1) between the ozone exposed and control leaves.

The exponential-linear models used to describe the mass decay during the 395 days of the study accounted for a great proportion of the total variance (R^2=0.9376 and R^2=0.9466, respectively, for treated and control leaves). Mass decay reached the asymptotic part of the curves after 157 days for both the treatments, when T and C leaves lost about 37 and 39 % of their initial weight, respectively. The model for the ozone treated leaves had a lower decay constant than that for control leaves (0.0189 and 0.0243 $k\,day^{-1}$, respectively) and, in addition, a higher slope of the linear term (Fig. 2); the two models differed (P<0.001) according to the log-likelihood ratio test. Significant differences in the mass decay related to the treatment (F=20.22, P<0.001) were also highlighted by the multiple-way ANCOVA, which, moreover, pointed out an effect of the interaction of the treatment with the ADF (F=4.37, P<0.05). At the end of the observations, higher remaining mass values were found in the treated in respect to the control leaves (t=2.63, P<0.05).

Carbon, potassium, zinc and manganese decreased along the time: C and K with exponential dynamics and Zn and Mn with linear dynamics. Iron concentrations ranged around the initial values, whereas nitrogen, sulphur, calcium and magnesium increased along the time (Fig. 3), the last three with close dynamics. C/N ratio (Table 2), total soluble sugars, starch, ADF, lignin and cellulose decreased along the time (Fig. 4). Total soluble sugars within 31 days were lost by about 90 % of the initial values; cellulose and starch showed similar dynamics, characterised by an early (31 days) decrease, a subsequent (61 days) increase and a late decay (Fig. 4).

Among the measured parameters, LAR selected, in order of importance, C, ADF, N, K, total soluble sugars and Fe for T leaves and C, K, lignin, total soluble sugars and N, for C leaves to model the remaining mass (Table 3). The main differences between the two models were accounted by the selection of ADF in that for T leaves and, conversely, by the selection of lignin in that for C leaves and by the greater coefficient for N in the model for T leaves (Table 3).

The two-way MANOVA highlighted significant differences between treated and control leaves along the time

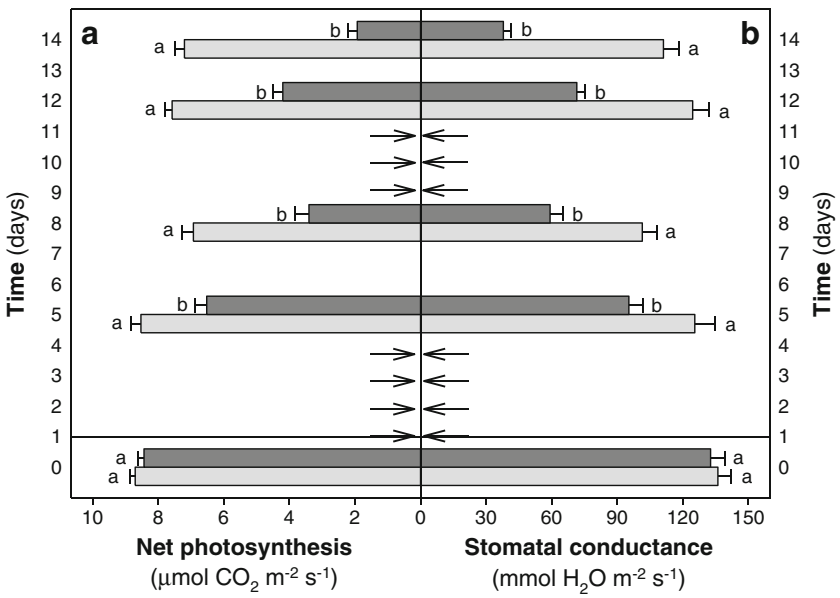

Fig. 1 Net photosynthesis (**a**) and stomatal conductance (**b**) of treated (*dark grey*) and control (*light grey*) leaves of *Q. ilex*, measured before the start of the first ozone fumigation and at 5, 8, 12 and 14 days after. Days in which fumigation occurred are indicated with *arrows*; *error bars* represent standard error of the means. *Different letters* indicate significant differences (P<0.001) between the treatments

Table 1 Initial physical and chemical characteristics of *Q. ilex* leaves from control (C) and ozone treated (T) plants

	C	T
Dry mass (mg/leaf)	135.08±12.99	171.25±25.12
Leaf area (cm^2/leaf)	8.23±0.75	9.33±1.32
Specific leaf mass (mg/cm^2)	16.80±3.16	18.48±5.33
C (% d.w.)	47.81±0.06	47.63±0.24
N (% d.w.)	1.59±0.01	1.63±0.01
S (% d.w.)	0.08±0.01	0.09±0.01
Ca (mg g^{-1} d.w.)	4.89±0.24	5.26±0.23
Mg (mg g^{-1} d.w.)	1.66±0.05	1.68±0.04
K (mg g^{-1} d.w.)	6.84±0.13	7.19±0.16
Fe (mg g^{-1} d.w.)	0.26±0.01	0.29±0.01
Zn (mg g^{-1} d.w.)	0.04±0.01	0.05±0.01
Mn (mg g^{-1} d.w.)	0.15±0.01	0.13±0.01
Total soluble sugars (mg g^{-1} d.w.)	172.92±4.57	160.27±2.82
Starch (mg g^{-1} d.w.)	17.02±1.30	17.24±2.07
ADF (% d.w.)	64.35±0.71	64.65±0.46
Lignin (% d.w.)	31.18±1.46	33.02±0.82
Cellulose (% d.w.)	33.17±1.92	31.63±1.08

Mean values±standard errors of the means of ten (dry mass, leaf area, specific leaf mass) or five (C, N, S, Ca, Mg, K, Fe, Zn, Mn, total soluble sugars, starch, ADF, lignin, cellulose) replicates are reported

(Roy's largest root=14.333 and $P<0.001$ for the treatment effect, Pillai's trace=3.483 and $P<0.001$ for the time effect, Pillai's trace=1.830 and $P<0.05$ for the interaction term). Significant differences along the time between the ozone treated and control leaves were highlighted by the two-way ANOVA models uniquely for C ($F=91.68$, $P<0.001$) and Mn ($F=5.54$, $P<0.05$).

4 Discussion

Ozone fumigation at a cumulative dose of 17.15 ppm h, as expected basing on a broad literature (Wittig et al. 2007; Vitale et al. 2008), drastically affected both net photosynthesis and stomatal conductance of *Q. ilex* leaves, which, at the end of the treatment, were reduced, in respect to the controls, by about the 73.3 and 65.8 %, respectively. Nevertheless, such physiological alterations did not resolve into physical or chemical differences between the ozone treated and the control leaves: no visible injuries were detected, and no significant difference was found in weight, size and specific mass, as well as in the initial concentrations of all the chemical parameters analysed. Despite the similar initial characteristics of the leaves, a significant slowing down of mass decay was observed in the ozone treated leaves, indicating negative effects of plant exposure to O_3 on leaf decomposition, as also described for some grass (Kim et al. 1998; Booker et al. 2005) and tree (Parsons et al. 2008; Liu et al. 2009; Baldantoni et al. 2011) species. The daily decomposition constant of the treated leaves was reduced, indeed, of 22.2 % in respect to that of control leaves, determining, at the end of the observations, a significant difference of 6.6 % in the mass loss between the treated and the control leaves. These findings are similar to our previous observations about the effects of tropospheric ozone on the chemical composition of *Q. ilex* leaves and their decomposition rate (Baldantoni et al. 2011). The exposure of the plants at concentrations typical of the Mediterranean summer climate in OTCs, indeed, determined a reduction in the decay constant of 16 % and, after 6 months of decomposition, a difference of about 5 % in the mass loss between the leaves from exposed and unexposed plants (Baldantoni et al. 2011). Astonishingly, the acute ozone fumigation at unnatural high concentrations, that drastically compromised important physiological processes of the treated plants, determined similar effects on the decomposition rate to those determined by the tropospheric ozone concentrations commonly experienced by the vegetation communities of the Mediterranean area. Since data from Baldantoni et al. (2011) were obtained in different climatic and edaphic conditions, the results from these two experiments are not fully comparable. The similar slowing down of mass decay, however, indicates that the effects of O_3 on the decomposition rate are not proportional to the dose. We thus hypothesize the presence of a threshold effect at low O_3 concentrations involving chemical and/or physical alterations which modify the mass loss dynamics.

Among the chemical parameters analysed, Mn and C were the unique elements to show different dynamics between the treated and control leaves, with slower element losses in the ozone exposed leaves. Even if Mn plays a key role in regulating lignin decomposition, being the manganese peroxidase the most important and widespread ligninolitic enzyme (Hofrichter 2002), we did not find any

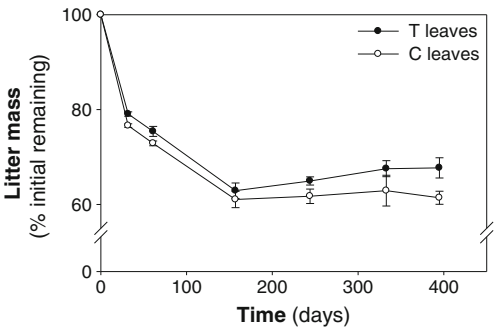

Fig. 2 Remaining mass, as percent of the initial value, of *Q. ilex* leaves from ozone fumigated (T) and control (C) plants, during decomposition. *Vertical bars* represent standard error of the means

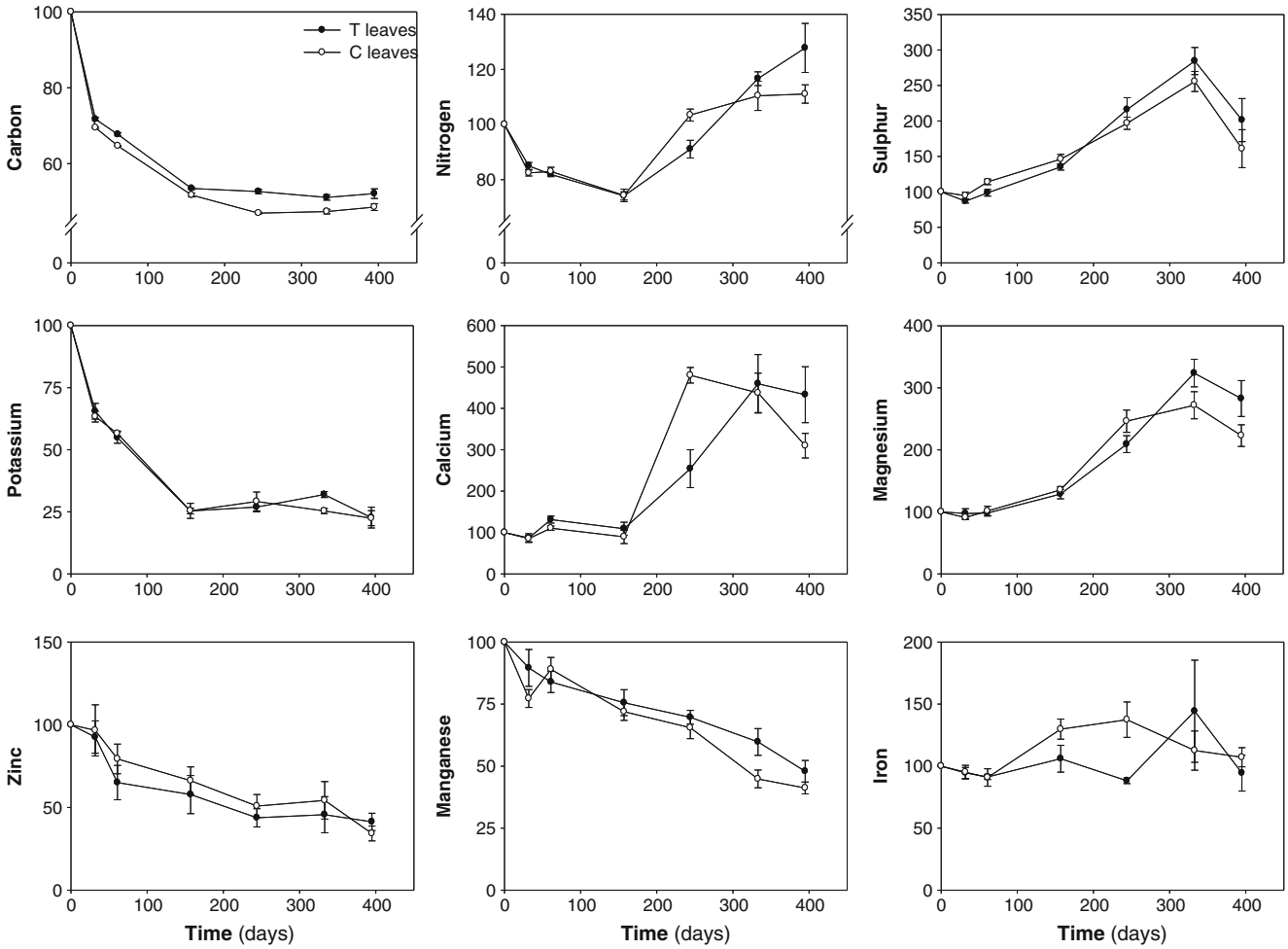

Fig. 3 Dynamics of C, N, S, K, Ca, Mg, Zn, Mn and Fe, as percent of the initial value, in *Q. ilex* leaves from ozone fumigated (T) and control (C) plants, during decomposition. *Vertical bars* represent standard error of the means

difference in lignin decay between ozone exposed and control leaves. It is possible that N could have concealed the effect of Mn on lignin decomposition increasing the variance in the data. N, indeed, could have affected lignin decomposition, hampering it by inhibiting ligninase synthesis (Berg and McClaugherty 2008) or hiding it by favouring the formation of recalcitrant compounds (Piccolo et al. 1999). The lack of predictive value of Mn in the LAR models indicates that this element is not related to the differences observed in mass loss between the treated and control leaves. The causes of the mass decay slow down due to ozone exposure have then to be searched among the carbon compounds. The LAR models that describe the mass loss dynamics for the treated and control leaves differ not only in the selected predictors but also in their relative contribution. Different interactions among the predictors are thus at the basis of the observed differences in mass decay between the ozone exposed and unexposed leaves. However, since ADF was selected in the LAR models only for treated leaves, with the second highest coefficient, and is the only parameter that significantly affects the mass loss through the interaction with the treatment (as highlighted by the multiple-way ANCOVA) it is possible to conclude that ADF plays a primary role in determining the observed differences in the leaf decomposition rate. Nonetheless, it

Table 2 Values of C/N ratio in *Q. ilex* leaves from control (C) and ozone treated (T) plants during decomposition

Incubation days	C	T
0	30.03	29.19
32	25.31	24.64
61	23.41	24.12
157	20.91	21.05
244	13.63	16.86
333	12.89	12.78
395	13.13	11.91

Mean values of five replicates are reported

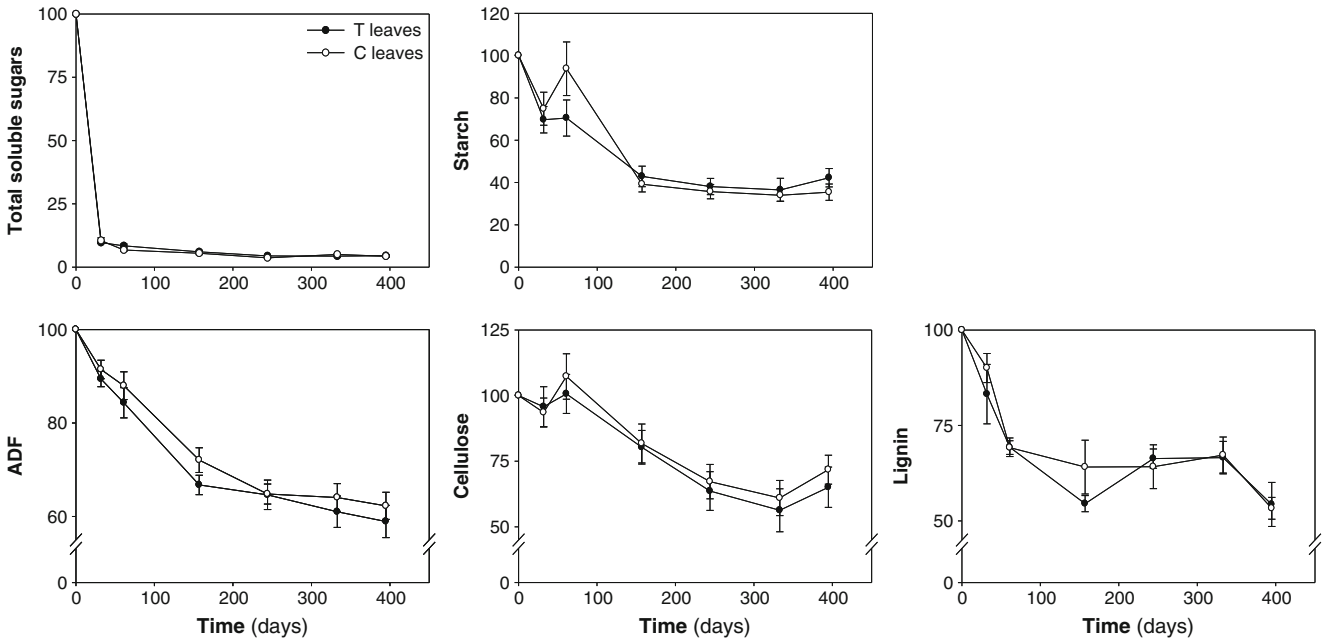

Fig. 4 Dynamics of total soluble sugars, starch, ADF, lignin and cellulose, as percent of the initial value, in *Q. ilex* leaves from ozone fumigated (T) and control (C) plants, during decomposition. *Vertical bars* represent standard error of the means

has to be pointed out that the effects of the ADF are likely realized through some interactions with the other predictors and not by simple variations in its concentration. ADF represents a heterogeneous group of recalcitrant compounds, and it is known that variations in either their concentrations and composition may affect leaf decomposition rate, as sometimes reported for the influence of fibres (Booker et al. 2005)

Table 3 Estimated values of the predictors in the LAR models of the litter remaining mass for the ozone treated (T) and control (C) leaves

	C	T
C (% initial)	0.5214	0.3903
N (% initial)	0.0248	0.1050
C/N	n.s.	n.s.
S (% initial)	n.s.	n.s.
Ca (% initial)	n.s.	n.s.
Mg (% initial)	n.s.	n.s.
K (% initial)	0.0585	0.0559
Fe (% initial)	n.s.	0.0064
Zn (% initial)	n.s.	n.s.
Mn (% initial)	n.s.	n.s.
Total soluble sugars (% initial)	0.0362	0.0464
Starch (% initial)	n.s.	n.s.
ADF (% initial)	n.s.	0.1746
Lignin (% initial)	0.0408	n.s.
Cellulose (% initial)	n.s.	n.s.

n.s. parameter not selected by the LAR analysis

on decomposition slow down linked to ozone exposure. Other causes of the observed differences in mass and carbon loss could not be ruled out, such as alterations in some other secondary metabolites (see for example Loreto et al. 2004, for holm oak and Liu et al. 2005, for trembling aspen and paper birch) or cuticular waxes (Barnes et al. 1988; Karnosky et al. 1999). Elevated ozone concentrations may indeed trigger a biochemical defence or damage response that elevates synthesis of secondary compounds and could accelerate structural degradation of cuticular waxes. Our previous study (Baldantoni et al. 2011) also suggested an effect of peroxidation by ozone of lipids and aliphatic compounds on decomposition slow down of *Q. ilex* leaves. All these alterations may turn into a different substrate availability and different microbial colonization (Kim et al. 1998; Liu et al. 2009; Lindroth 2010).

The marked increases over the initial values of N, S, Mg and Ca, especially in treated leaves, could be attributed to the immobilization of these elements in the microbial biomass and thus suggest an important colonization (Karnosky et al. 1999; Fioretto et al. 2005) that could explain the late weigh take-up of the treated leaves. The increases of starch and cellulose after 2 months of decomposition, on the other hand, could be attributable only to algal colonization, probably due to the nitrogen release in the early decomposition stages.

Funding This work was funded by the Ministero dell'Ambiente e Tutela del Territorio and the University of Salerno (Italy).

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