REGULAR ARTICLE

# Differences in nutrients, organic components and decomposition pattern of *Phillyrea angustifolia* leaf litter across a low maquis

**A. De Marco · R. Spaccini · A. Virzo De Santo**

## Abstract

*Aims* In Mediterranean ecosystems fire and drought are two natural disturbances that affect plant traits and generate a highly heterogeneous landscape. The objective of this research was to analyze leaf chemistry, one important driver of carbon and nutrients cycles, and decomposition of *Phillyrea angustifolia* L. in a landscape affected by recurrent fire disturbance.
*Methods* We investigated leaf litter collected in a low-maquis at sites burned by a wildfire 10 years before the current study, at sites burned by an experimental fire 1 year before and at sites located at the edge low-maquis/gaps.
*Results* The three litters differed in chemical composition, mass loss and dynamics of lignin/AUR (Acid-Unhydrolizable-Residue), nitrogen and manganese. During the early decomposition phase lignin/AUR increased in litters from sites burned 1 year before and from sites located at the edge low-maquis/gaps and decreased in litter from sites burned 10 years before. Nitrogen and manganese were immobilized in litter during the early and the middle phase of decomposition. $^{13}$C NMR spectroscopy revealed that all the litters were rich in Alkyl-C, the fraction including waxes and cutin that are effective barriers to water loss from the leaves. Litter from sites burned 1 year before had the highest hydrophobicity and aromaticity indexes and the highest alkyl ratio, and decomposed at the lowest rate.
*Conclusions* Differences of leaf chemical composition within the same plant species across an uneven landscape affect decomposition. The traditional approach and $^{13}$C NMR spectroscopy complementarily explain decomposition pattern and give a comprehensive view of the individual drivers of litter decomposition.

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## Introduction

In Mediterranean ecosystems, typical forests and maquis are characterized by thick-leaved evergreen trees and shrubs adapted to seasonal drought. The high content of structural compounds of sclerophyllous leaves is very effective at facing drought but slows down decomposition (Cornwell et al. 2008). Moreover, schlerophylls growing on nutrient-poor soil, before a leaf dies, retrieve

nutrients in the biomass (Fioretto et al. 2003) and release a low-nutrient leaf litter that decomposes slowly. Both, structural compounds, particularly lignin, and nutrient content are rate regulating factors of leaf litter decomposition (Berg and McClaugherty 2014). Litter decomposition is a fundamental process in ecosystem functioning as it regulates the cycle of matter, $CO_2$ release into the atmosphere, carbon sequestration in the soil and nutrients mineralization. In Mediterranean ecosystems frequent wildfires contribute to nutrient mineralization, returning to the soil nutrients immobilized in the aboveground plant biomass (Arno et al. 1995). The increase of available nutrients in burned soils may enhance nutrient content of the leaves in plant re-sprout; this in turn positively affects the rate of photosynthesis (Manes et al. 2002) and promotes a vigorous growth of re-sprouts and a greater input of organic matter into the soil (Capogna et al. 2009).

In a previous study (De Marco et al. 2005) performed in the Mediterranean low-maquis of Castel Volturno Nature Reserve (CE-Italy), where *Phillyrea angustifolia* L. is a dominant species, we assessed that soil total and available nutrient contents increased after experimental fires. Leaf litter of *Ph. angustifolia* collected from plant re-sprout after fire had higher concentration of nutrients (De Marco et al. 2004). Wildfires are frequent in the Reserve especially in summer and the site is a mosaic of plant patches of high maquis, low maquis, and gaps that represent different stages of the fire-induced secondary ecological succession in the Mediterranean region. According to Rutigliano et al. (2004), wind disturbance controls the successional development of low maquis into high maquis. Indeed high wind speeds affect gas exchanges, reduce photosynthesis's rate (Nobel 1981) and, consequently, the input of organic matter to the soil (Fierro et al. 2007). The latter effect, together with soil erosion due to wind, prevents further development of soil and, consequently, of plant community.

We addressed the importance of accounting for the heterogeneity of leaf chemistry across the maquis's landscape and its impact on litter decomposition. Replicate spots for collection and for incubation of leaf litter at sites burned since different time lapse may well represent the differences in leaf chemistry across the uneven landscape structure caused by fire disturbance. To this goal in the current work we compare three independent sets of data assessing chemical composition and decomposition of *Ph. angustifolia* leaf litter collected in the Mediterranean low maquis of Castel

Volturno Nature Reserve (De Marco et al. 2004, 2008; Maisto et al. 2011). The three sets of data were collected within the same area burned by a large wildfire in 1990. The first set of data (WF10) was collected 10 years after the wildfire. The second set of data (EF1) refers to leaf litter collected from shrub regrowth 1 year after an experimental fire. The third set of data (EMG) uses leaf litter at the edge between low maquis and gaps. Gaps, created by fire, are dominated by herbs and bryophytes. Studies comparing litter decomposition and soil organic matter stability have demonstrated that herbs are more decomposable than shrub leaves and that organic matter of gap soil is less stable than that of maquis soils (Rutigliano et al. 2001; De Marco et al. 2008). Shrubs of *Ph. angustifolia* at the edge with gaps are exposed to more severe moisture and temperature conditions that may shape leaf chemical composition and affect decomposition.

To achieve the objective of this study we analyze the three sets of data for chemical composition of newly shed leaf litters and the changes with litter decomposition, over at least one year and until two years. We present results of traditional analyses as well as ones using $^{13}$C NMR spectroscopy for determining organic compounds in decomposing litter. To our knowledge there is no study dealing with organic components and their changes during decomposition of Mediterranean sclerophylls assessed by $^{13}$C NMR spectroscopy.

As for traditional analysis we focus on lignin (determined as acid-unhydrolizable residue: lignin/AUR), nitrogen (N) and manganese (Mn) contents, that have been recognized as main factors controlling litter decomposition (Berg and McClaugherty 2014). Lignin has a rate-reducing influence on litter decomposition both in the early and later stages, in particular, when associated with high N content, because of the formation of new and stable complexes (Berg and Ekbohm 1991; Côuteaux et al. 1995). High nitrogen content enhances the growth of microorganisms that degrade labile compounds in the early decay phase, but repress the formation of lignolytic enzymes (Keyser et al. 1978). The availability of Mn limits lignin degradation in needle litter of Norway spruce (Berg 2000), in leaf litter of common oak (Davey et al. 2007) and in several pine needle litters (Berg et al. 2007; Virzo De Santo et al. 2009; Berg et al. 2010). Moreover the most efficient degraders of lignin are white-rot fungi that produce manganese peroxidase able to degrade lignin in vitro (Hofrichter, 2002).

To explore the composition in organic constituents and the changes with litter decay we analyze through ^{13}C NMR spectroscopy samples from the three sets of data. [13]C Nuclear Magnetic Resonance (NMR) is a powerful technique for nondestructive study of complex organic matrices, such as plant litter, as well as decomposition of organic inputs into the soil of natural ecosystems (Preston et al. 2009a; De Marco et al. 2012; Wang et al. 2017; Bento et al. 2020). The analysis of leaf litter using ^{13}C NMR allows a new view on plant litter chemistry during decomposition giving specific information on the types of chemical bonds present in a sample, and the degradation of the different chemical components as litter decomposes (Preston et al. 1997, 2009b; Ono et al. 2009, 2011, 2013; De Marco et al. 2012; Preston and Trofymov 2015; Kögel-Knabner 2017). We focus on 6 main chemical shift regions, representative of the major types of carbon functional groups (C-bonds): Alkyl-C, Methoxyl-C, O-Alkyl-C, Aryl-C, Phenol-C, and Carboxyl-C. Furthermore we analyze in details two small bands, one between 29 and 33 ppm and the other around 50 ppm, related respectively to cutin and suberin and to alkaloids, that are key components of sclerophyllous leaves.

We hypothesize that there may be differences of leaf chemical composition within the same plant species across an uneven landscape and that the differences will affect decomposition. To this purpose we assess (1) through a traditional approach, leaf litter quality and changes with decomposition of commonly recognized drivers of litter decomposition as N, Mn, and lignin/AUR, (2) through ^{13}C NMR spectroscopy, leaf litter quality in terms of organic components and their decay trajectories. Thus this study will provide useful information about the complementarity of the two approaches to explain the decomposition pattern and to get a more comprehensive view of the individual drivers of litter decomposition.

## Methods

### Study site

Leaf litter chemistry and decomposition were investigated in the low maquis of the Castel Volturno Nature Reserve, southern Italy (40° 57' N, 13° 33'E) characterized by a dense cover of evergreen sclerophyllous-shrubs in which *Ph. angustifolia* L. (Oleaceae), *Pistacia*

*lentiscus* L. (Anacardiaceae) and *Quercus ilex* L. (Fagaceae) are dominant species. The Nature Reserve is a flat, narrow costal area on stabilized dunes of alluvial deposits and loose siliceous-calcareous sands of the late Quaternary. The slightly alkaline, deep (> 150 cm), well-drained soil is a Calcaric Arenosol according to the FAO system of soil classification (FAO 1998). The area has a typical Mediterranean climate with dry summers and rainy autumns and winters. Based on data (years 1999–2007) from the nearest Meteorological Station Giugliano- Lago Patria (40° 57' N, 14° 04'E, 26 m a.s.l.) mean annual rainfall was 756 mm year$^{-1}$; mean annual temperature was 16 °C; the warmest month was August (24.4 °C) and the coldest was January (8.7 °C). The dominant winds blow from west and southwest.

In a low maquis area burned by a large wildfire in 1990, 6 paired plots, each 50 m$^2$ in size, were selected (Fig. 1). At three plots leaf litter of *Ph. angustifolia* was collected 10 years after the wildfire (WF10) and used to assess leaf chemistry and its changes with decomposition following litter bags incubation in the same plots. In the other three plots an experimental fire of low severity (fuel: above ground biomass set to 2 kg m$^{-2}$) was ignited 10 years after the wildfire (details in De Marco et al. 2005). The shrubs of *Ph. angustifolia* burned only aboveground and quickly-recovered. After 1 year new growth was higher than one metre. Litter from new growth shrubs (EF1) was collected and used as representative of fire effect on litter chemistry and decomposition in the short term. The third set of data refer to *Ph. angustifolia* litter collected 16 years after the 1990 wildfire, at the edge maquis/gap (EMG) of 13 gaps, nearby the WF10 and the EF1 plots, located from the sand dune inward along the path of the dominant West to East winds (Fig. 1). Gaps make up to about 20 % of the low Macchia area at the Castel Volturno Nature Reserve and their fraction may vary in space and time depending on fire disturbance and/or successional development to low maquis. The plant community in the gaps consists mainly of small annual species (legumes, grasses and forbs) that germinate in November and die out before the onset of summer drought.

### Litter collection, incubation and decomposition

Leaf litter of *Ph. angustifolia* was collected at the above described sites WF10, EF1 and EMG. Senesced leaves were collected during the period of maximum litter fall
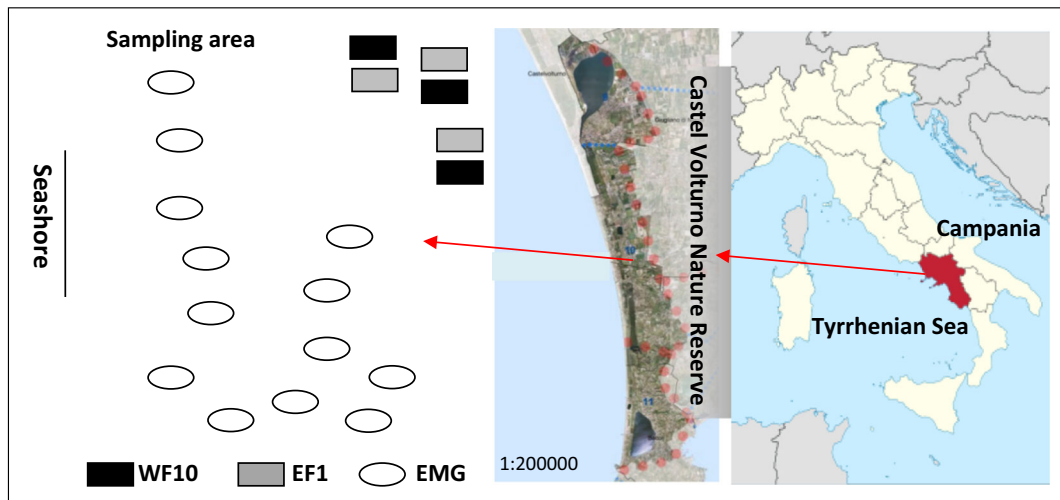
**Fig. 1** Location of the " Castel Volturno" Nature Reserve and schematic diagram of sampling area within the low maquis

(May to July) using 25 litter traps (1.5 mm mesh screen with 1.4 m$^2$ collecting surface) suspended under the canopy of the shrub.

Immediately after collection, litter was air dried at room temperature and stored until further use. Three subsamples of litter were dried (75 °C) to a constant mass and used to calculate the correction factor for converting air dried mass to water-free dry mass. Three sub-samples were dried to a constant mass at 75 °C (48 h) and then combusted at 550 °C (2 h) to get the ash content.

Litter bags made of terylene net (1 mm mesh) filled with a known amount of litter were placed on the litter layer under the shrubs of *Ph. angustifolia* and fastened to the ground by 10–15 cm long pegs (fiberglass, PVC coated) through a 1-cm wide edge on the bags. Litter bags WF10 and EF1 had the size 15 × 12.5 cm and contained each about 2 g of litter; the bags containing EMG had the size 15 × 25 cm and contained each about 6 g of litter. The total number of litter bags for WF10 and EF1 was 81 (3 plots x 9 field replicates in each plot, x 3 sampling times). The total number of litter bags for EMG was 52 (13 spots x 2 field replicates, x 2 sampling times).

Litter bags WF10 were incubated on December 21, 2000; litter bags EF1 were incubated on July 26, 2002; litter bags EMG were incubated on October 10, 2006. Litter bags were retrieved after about 100 (early phase), 400 (middle phase) and 800 (late phase) days, transported directly to the laboratory and processed within 48 h. The litter remaining in each bag was gently brushed to remove adhering soil debris and used for

determination of mass loss as percent of the initial mass, and litter chemistry. All litters were followed until a maximum of c. 60 % accumulated mass loss.

Soil sampling and analyses

Soil samples (0–5 cm depth) were collected under *Ph. angustifolia* shrubs at all experimental sites just before litter incubation, and sieved through a 2 mm mesh. The pH was measured in distilled water (1.0/2.5, soil/water) via the electrometric method. Organic matter content was evaluated by loss-on-ignition at 550 °C on soil samples dried at 105 °C. Soil samples were ground into a fine powder with an agate pocket (Fristch pulverisette 00.502) to determine C, N and Mn contents. Carbon and N content were measured by a C, N, S analyser (Thermo Finnigan Flash EA 1112). To determine Mn concentration, soil samples were digested in a Milestone (mls 1200) Microwave Laboratory System with a mixture of hydrofluoric and nitric acid (HF 50 % v/v: HNO$_3$ 65 % v/v = 1:2). Mn concentration of digested samples was measured by atomic absorption spectrometry via flame (AAS; SpectrAA 20 Varian) using standard solutions (STD Analyticals, Carlo Erba), diluted in the same acid matrix.

Litter proximate analyses

Three subsamples from each type of newly shed litter and for each type of decomposing litter sampled from each plot/spot at each retrieving date were used to determine lignin, C, N and Mn contents.

Litter samples were ground into a fine powder with an agate pocket (Fristch pulverisette 00.502).

Lignin was determined by a two phases procedure according to Goering and Van Soest (1970), and Kirk and Obst (1988). It has to be pointed out the procedure gives the fraction Acid Unhydrolizable Residue (AUR) which includes native lignin and lignin-like substances formed during decomposition (Osono 2007), as well as products originating from cutin, surface waxes, and condensed tannins (Preston et al. 2009b).

Carbon and N content were measured by a C, N, S analyzer (Thermo Finnigan Flash EA 1112). To determine the concentration of Mn the samples were digested in a Milestone (mls 1200) Microwave Laboratory System with a mixture of hydrofluoric and nitric acid (HF 50 % v/v: $HNO_3$ 65 % v/v = 1:2). Mn concentration of digested samples was measured by atomic absorption spectrometry via flame (AAS; SpectrAA 20 Varian) using standard solutions (STD Analyticals, Carlo Erba), diluted in the same acid matrix.

The changes of C, N, Mn and Lignin/AUR during decay are reported as per cent of the initial mass versus decomposition days.

## Litter ^{13}C CPMAS NMR spectroscopy

Fine-powdered subsamples from newly shed and decomposing litters were used for ^{13}C CPMAS NMR spectroscopy. Separately for WF10, EF1 and EMG litters and for each sampling date a composite sample was achieved by mixing together subsamples of all the replicated samples. The use of composite samples highlights the difference between types of litter accounting for the variability within each type of litter. The composite samples were analyzed by solid-state NMR spectroscopy (^{13}C CPMAS NMR) on a Bruker AV300 Spectrometer (Bruker Instrumental Inc, Billerica, MA, USA) equipped with a 4 mm wide-bore MAS (magic angle spinning) probe. NMR spectra of decomposing litters were obtained by applying the following parameters: 13,000 Hz of rotor spin rate; 2 s of recycle time; 1 min of contact time; 30 min of acquisition time; 4000 scans. Samples were packed in 4 mm zirconium rotors with Kel-F caps (Wilmad/Lab Glass, Buena, NJ, USA). The cross-polarization pulse sequence was applied with a composite shaped "ramp" pulse on 1 H channel in order to account for the inhomogeneity of Hartmann-Hann condition at high rotor spin frequency. The

Fourier transform was performed with 4k data point and an exponential apodization of 50 Hz of line broadening.

For the interpretation of solid state ^{13}C NMR spectra, the different signals are conventionally grouped into 6 main chemical shift regions, representative of the major types of carbon functional groups: Alkyl-C: 0–45 ppm; Methoxyl-C: 45-60ppm; O-Alkyl-C: 60–110 ppm; Aryl-C: 110–145 ppm; Phenol-C: 145–160 ppm, and Carboxyl-C: 190 − 160 ppm. The area of each of the regions was determined by integration (MestreNova 6.2.0 software, Mestre-lab Research, 2010), and expressed as percentage of the total area. Similarly the area of the region 29–34 ppm and that of the region 45–49 ppm were calculated.

The molecular features of litters at different decay stages are summarized by the calculation of dimensionless indices from the relative distribution of specific functional groups. The biochemical stability and the extent of decomposition may be inferred by the following parameters (De Marco et al. 2012; Bento et al. 2020): the aromaticity index (Ar/OA), is the ratio of peak area in the 110–160 ppm interval over that in the 60–110 ppm interval, i.e. (Phenol-C + Aryl-C)-to-O-Alkyl-C; the hydrophobicity index (HF-to-HI) relates the signal intensity of apolar alkyl and aromatic C components (0–60 ppm + 110–160 ppm) over those of bio-labile hydrophilic C molecules (60–110 ppm + 160–190 ppm), i.e. (Alkyl-C + Phenol-C + Aryl-C)-to-(O-Alkyl-C + Carboxyl-C); finally the Alkyl ratio (A-to-OA) compares the C distribution in the 0–45 ppm over that found in the 60–110 interval, i.e. Alkyl-C-to- O-Alkyl-C. The additional ratio Methoxyl-C-to-Phenol-C (MC/PC), involving the signal intensity in the 45–60 ppm interval over that in the 140–160 ppm interval, is used to better discriminate between signals owing to lignin and other phenolic moieties (lower MC/PC) with respect to the prevalent inclusion of peptidic moieties (larger MC/PC) in the 45–60 ppm range (De Marco et al. 2012; de Aquino et al. 2019).

The mass of each C fraction per gram of litter was calculated from peak integrations of NMR spectra and the total C per gram of litter (relative spectral area of each C bond x C content in one gram of litter/100). The changes of each C fraction during decay are reported as per cent of the initial mass versus decomposition days.

Statistics

The normality of the data distribution was assessed by the Shapiro–Wilks test. The significance of differences for the measured variables was tested by One Way ANOVA, followed by the Student Newman Keuls Test (Systat_SigmaPlot_12.5, software, Jandel Scientific, San Jose, CA).

Spearman's correlations were performed (Systat_SigmaPlot_12.5, software, Jandel Scientific, San Jose, CA) to evaluate the relationships between chemical variables, C-bonds, Temperature (T) and Precipitation (P). Chemical variables of newly shed leaf litter were correlated to annual mean T and to P of the year before litter collection. Chemical variables of decomposing leaf litter at different decomposition phase were correlated to mean T and to P of the period.

The decay constant (k) in a given period was calculated according to Olson (1963) using the equation $\ln(M_t/M_0) = -kt$ where $M_t$ is mass at time t and $M_0$ the initial mass. We applied this equation to calculate rates of C mass loss, and mass loss of organic constituents (C-bonds) during the early, the middle, and the late decomposition phases.

To compare litters across the decomposition process for amount of the organic components, as C-bonds, we used the most basic measure of variation, i.e. the range of variation (VR) as the difference between the smallest and the largest item in the set of the three litters. To account for the differences in concentration of the litter components we normalized VR dividing the range of variation by the mean of the three items (MI) and multiplying per 100 (VR = Variation Range / MI x 100).

Principal component analysis (PCA) was performed on the nutrient and lignin/AUR contents, and the C bonds across the decomposition period. PCA data were processed using package Syn-tax 2000 (Podani 1993).

## Results

Main soil characteristics

Soil collected at the sites WF10 and EF1 had similar OM, N and Mn contents (Table 1). Soil collected at the sites EMG was significantly poorer in OM and Mn, but was significantly richer in N compared to the other two soils (Table 1).

Litter decomposition and proximate analyses

Newly shed litters WF10, EF1 and EMG had different chemical composition and litter EMG was the poorest in lignin/AUR, N and Mn (Table 2).

Decomposition was slowest in litter EF1 and fastest in litter EMG (Figs. 2 and 3). The remaining C mass was 86 %, 76 and 68 % of the initial C mass for litter EF1, litter WF10 and litter EMG, respectively, after about 100 days, and lowered to 75 %, 57 and 50 % of the initial C mass after about 400 days since the start of incubation. After two years of field incubation the remaining C for litter EF1 and WF10, was respectively 58 and 28 % of the initial mass. Climatic conditions were different across the field incubation of the three litters. The early decomposition period for litter WF10 was 2 times more rainy and had a lower mean temperature than that of litters EF1 and EMG (Table 3). The decay rate was lowest in the middle period that was much more rainy for litter EMG (Fig. 3). Precipitation during the late decomposition period was high for both litters WF10 and EF1 with a similar recorded temperature (Table 3). No significant correlation was found between remaining C with either precipitation or temperature along the decay periods (Table 4).

Newly shed litter EF1 compared to the other two litters had larger initial N and Mn concentrations (Table 2). During the first 400 days of decomposition, N content showed an almost steady level in litter EF1 and a slight increase in litter WF10 and thereafter decreased in both litters (Fig. 2). Conversely, litter EMG doubled the N content during 400 days of incubation (Fig. 1). In all three litters the relative Mn amounts increased during decomposition with a raising rate as larger as lower the initial concentration (Table 2; Fig. 2). The remaining Mn was positively correlated to the remaining N and both nutrients were negatively correlated to the remaining C (Table 4).

In newly shed litter WF10, that had the highest lignin/AUR concentration, this recalcitrant component decreased since the early decay phase with a final halving of the initial content (Table 2; Fig. 2). In contrast litters EF1 and EMG showed a significant accumulation (+ 25 %) of lignin/AUR fraction in the first 3 months followed by a loss of lignin/AUR till the end of incubation, thereby retaining about 100 and 75 % of original amounts for EMG and EF1 respectively (Fig. 2).

**Table 1** Main chemical characteristics of soil (0–5 cm depth) collected under *Phillyrea angustifolia* L. in the low maquis of the "Castel Volturno" Nature Reserve

| Soil | pH | OM (% d.w.) | N (% d.w.) | C/N | Mn (mg/g) |
|------|-----|-------------|------------|-----|-----------|
| WF10 | 7.85±0.03[a] | 13.66±0.97[a] | 0.23±0.02[a] | 19.80±2.94[a] | 1.12±0.04[a] |
| EF1 | 7.50±0.10[b] | 12.22±0.75[a] | 0.18±0.03[a] | 24.18±1.16[a] | 1.00±0.02[a] |
| EMG | 7.85±0.04[a] | 7.18±0.22 [b] | 0.35±0.02 [b] | 11.72±0.78 [b] | 0.16±0.01[b] |

Soil was collected immediately before incubation of litter at sites burned by a wildfire since 10 years (WF10), at sites burned by an experimental fire since 1 year (EF1) and at sites located at the edge low-maquis/gaps (EMG). Values are means (WF10 and EF1: n = 9; EMG: n = 13) ± standard errors. Different letters show statistically significant differences between the three litters (P < 0.05, One Way ANOVA)

## ^{13}C NMR spectra and changes in amount of C-bond fractions during litter decay

The NMR spectra of newly shed and decomposed litters are shown in Fig. 3 while the relative contribution of specific functional groups to the total area of the spectrum are presented in Table 5.

In the alkyl-C interval (0–45 ppm) the shoulder marked by peaks at 17 and 19 ppm indicated the presence of either methyl groups in cyclic components, such as sterols, and of methyl substituents in hemicelluloses and glycosidic group of flavonoids (Martínez-Richa and Joseph-Nathan 2003; De Marco et al. 2012). The sharp bands in the range 29–34 that make up 1/4 - to 1/3 of total Alkyl-C, are related to the large abundance of bulk methylenes (CH$_2$) segments of different lipid molecules such as phospholipid fatty acid, waxes, and biopolyesters (cutin and suberin) components, discriminated to each other by a specific molecular rigidity and biochemical stability (Lorenz et al. 2000; Wang et al. 2017). These signals associated to less decomposable components of forest litters are correlated with the SOC accumulation in both organic and mineral horizons (Lorenz et al. 2007; Wang et al. 2019). The less intense

signals around 37–39 ppm, are mainly attributable to the inclusion of tertiary (CH) and quaternary (C-R) carbons in assembled rings of flavonoids and lignans (DellaGreca et al. 2002, 2011).

In the Methoxyl-C interval (45–60 ppm) the isolated small peak centered at 48 ppm (Fig. 4) may be associated with C-N bonds of alkaloid compounds which are typical intermediates and final products of iridoids biosynthetic pathway in Oleaceae species (Jensen et al. 2002; Torres et al. 2012). The signal at 56 ppm subtends the possible overlap of various functional groups pertaining to both methoxyl substituent on the aromatic rings of guaiacyl and syringyl units in lignin and lignan components, and to the C-N bonds of peptidic moieties (DellaGreca et al. 2011; De Marco et al. 2012).

The different resonances in the O-alkyl-C region (60–110 ppm) represent the C nuclei of carbohydrate units in oligo- and poly-saccharide chains mostly due to cellulose and hemicelluloses (De Marco et al. 2012; Bento et al. 2020). In addition to the monomers of polysaccharidic chains, the O-alkyl regions of NMR spectra of *Phillyrea* litters, may also include a contribution from the specific components of flavonoid/lignan structures, characterized by specific resonances at 60–70 and 95–100 ppm (DellaGreca et al. 2007; Wolniak et al. 2008).

**Table 2** Chemical composition of leaf litters of *Phillyrea angustifolia* L. collected in the low maquis of the "Castel Volturno" Nature Reserve at sites burned by a wildfire since10 years (WF10), at sites burned by an experimental fire since 1 year (EF1) and at sites located at the edge low-maquis/gaps (EMG)

| Leaf litter | Lignin/AUR (%) | N (mg/g) | C/N | Lignin/N | Mn (mg/g) |
|-------------|----------------|----------|-----|----------|-----------|
| WF10 | 27.49±0.22[a] | 6.90±0.51[a] | 70.70±2.20[a] | 39.84 | 0.050±0.030[a] |
| EF1 | 20.40±0.32[b] | 7.25±0.27[a] | 84.84±10.48[a] | 28.14 | 0.070±0.003[b] |
| EMG | 13.30±0.02[c] | 4.30±0.02[b] | 124.31±9.22[b] | 30.93 | 0.023±0.0005[c] |

Values are means (WF10 and EF1: n = 9; EMG: n = 13) ± standard errors. Different letters show statistically significant differences between the three litters (P < 0.05, One Way ANOVA)
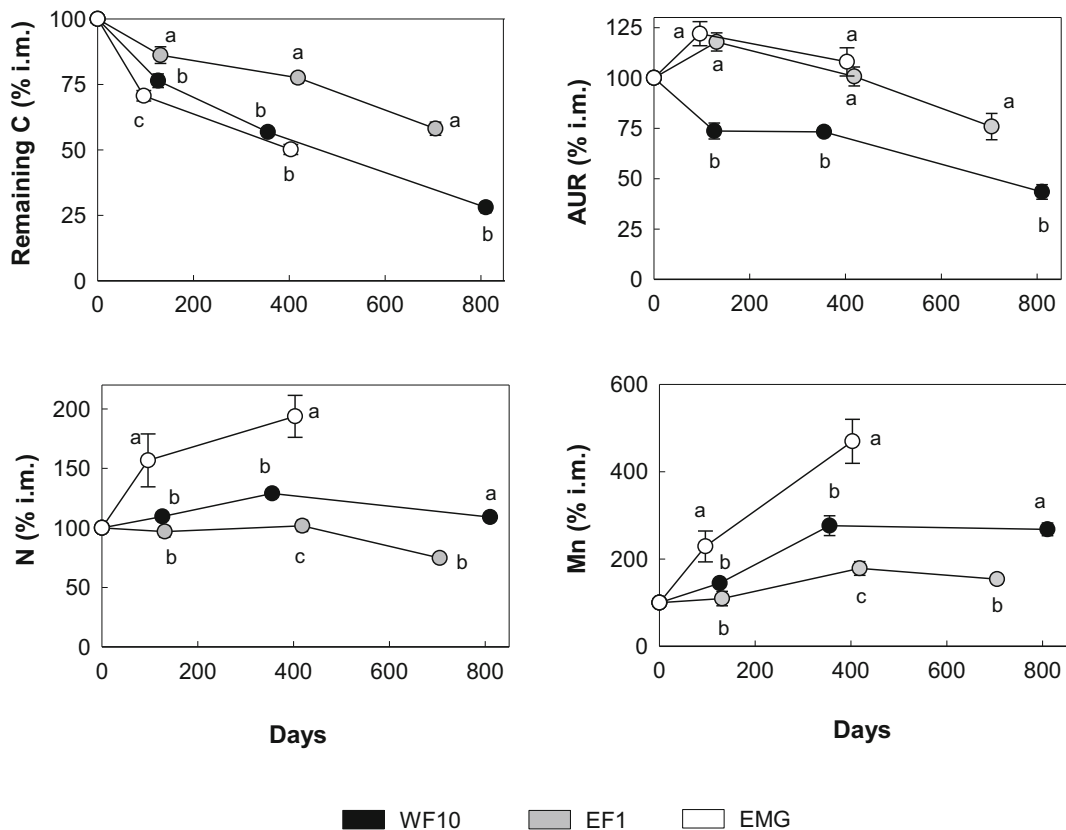
**Fig. 2** Remaining C, and changes in amount of AUR, N and Mn, as percent of the initial mass, in leaf litters of *Phillyrea angustifolia* L. during decomposition. Leaf litters were collected in summer in the low maquis of the " Castel Volturno" Nature Reserve at sites burned by a wildfire since10 years (WF10), at sites burned by an experimental fire since1 year (EF1) and at sites burned since 16 years located at the edge low-maquis/gaps (EMG). Different letters indicate significant differences between the three litters for each decomposition period (P < 0.05, One Way ANOVA)

The broad bands around 110 and 130 ppm may be mainly related to unsubstituted and C-substituted phenyl carbon of lignin monomers, as well as to typical aromatic moieties of *Phillyrea* tissues such as lignans and flavonoids (Wolniak et al. 2008). This assignment is claimed also for the subsequent NMR region extended in the 145–160 ppm range which may include the O-bearing carbons 3, 4, and 5 in the aromatic ring of lignin fraction, together with the O-aryl- functions of lignans
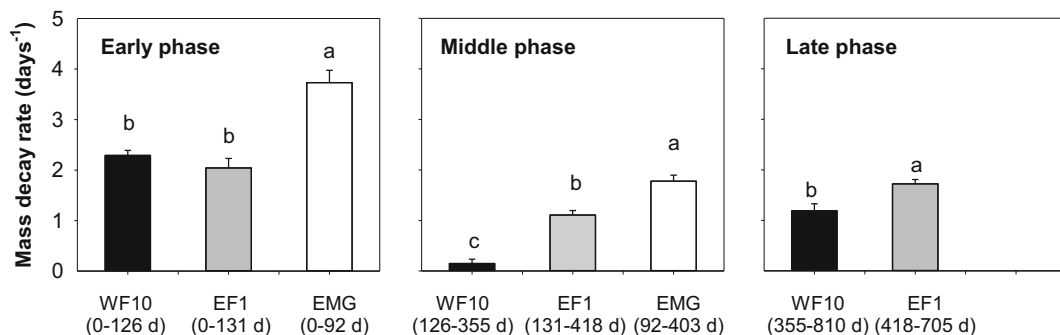


**Fig. 3** Decay rates of *Phillyrea angustifolia* L. litters WF10, EF1 and EMG. Rates were calculated using the constant-fractional-rate model (Olson 1963) for the early, the middle and the late phase of decomposition. In parentheses number of days for each decomposition phase

**Table 3** Mean temperature and precipitation over the year before litter collection and over each of three decomposition periods for litters WF10, EF1 and EMG

| Litter | Year before litter collection | | Early phase | | | Middle phase | | | Late phase | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | T (°C) | P (mm) | Days | T (°C) | P (mm) | Days | T (°C) | P (mm) | Days | T (°C) | P (mm) |
| WF10 | 15.98 | 879 | 0-126 | 10.87 | 424 | 126–355 | 19.52 | 280 | 355–810 | 14.88 | 495 |
| EF1 | 16.67 | 1175 | 0-131 | 19.45 | 199 | 131–418 | 16.48 | 182 | 418–705 | 13.30 | 622 |
| EMG | 16.17 | 745 | 0–92 | 13.40 | 221 | 92–403 | 15.00 | 257 | -- | -- | -- |

and flavonoids (Wolniak et al. 2008; Cao et al. 2010; DellaGreca et al. 2011).

The signals in the carboxylic region (160–190 ppm) indicated the overlapping of different carboxyl groups related to aliphatic acids, amide groups in amino acid moieties and acetyl substituent in carbohydrates components of hemicelluloses and flavonoids.

The relative C distribution (%) of signal area over chemical shift regions in newly shed litters indicated an overall composition dominated by the polysaccharides and alkyl components (which global amount represented the 77 %, 73 and 75 % of total spectral area respectively in the litters WF10, EF1 and EMG (Table 5). Litter organic composition is represented by the dimensionless structural index derived from the relative distribution of molecular components. The overall lower values of hydrophobic and aromaticity indexes combined with the alkyl ratio (Table 5) underlined the larger prevalence of bio-decomposable components in newly shed litters WF10 and EMG compared to newly shed litter EF1. With respect to aromatic compounds the trend observed for Methoxyl-C-to-Phenol-C in the newly shed litters (Table 5) suggested the occurrence of a

strict correlation of O-substituted aryl carbons with the corresponding methoxyl groups in lignin and phenolic derivatives (de Aquino et al. 2019). The even maintenance of low values (< 2) shown by the newly shed *Phillyrea* litters collected at different sites confirmed the significant contribution of flavonoids and lignan components to the phenolic region of NMR spectra (De Marco et al. 2012).

During the decay process of the three litters, all C-bond fractions decreased in amount (Fig. 5). The decay rate for all C bonds was highest in the first 100 days (Fig. 6). Among the three litters, litter EMG showed, up to 400 days, the highest decay rates for all C-bonds (Fig. 6). The remaining fractions of all C bonds were not correlated to Precipitation. Remaining fractions of Carboxyl-C and Phenol-C were positively and significantly correlated to Temperature (Table 4). MC-to-PC was negatively correlated with Temperature (Table 4). The remaining fractions of all C bonds were negatively correlated to N and Mn. HF-to-HI and A-to-OA were positively correlated to remaining Mn (Table 4).

Compared to litter EF1, litters WF10 and EMG showed a different decay behavior, characterized by a

**Table 4** P values (Spearman method) for correlations between litter chemical variables, temperature (T) and precipitation (P)

| | AUR | N | Mn | T | P |
|---|---|---|---|---|---|
| AUR | -- | 0.188 | **0.050** | 0.946 | -0.149 |
| N | 0.188 | -- | **0.004** | -0.595 | -0.188 |
| C | −0.149 | **-0.010** | **-0.002*10$^{-4}$** | 0.296 | 0.109 |
| Carboxyl-C | −0.433 | **-0.021** | **-0.010** | **0.047** | 0.257 |
| Phenol-C | -0.467 | **-0.013** | **-0.010** | **0.029** | 0.353 |
| Aryl-C | −0.384 | **-0.015** | **-0.005** | 0.065 | 0.168 |
| O-alkyl-C | -0.124 | **-0.002*10$^{-4}$** | **-0.002*10$^{-4}$** | 0.575 | 0.168 |
| Methoxyl-C | -0.270 | **-0.010** | **-0.002*10$^{-4}$** | 0.178 | 0.141 |
| Alkyl-C | −0.210 | **-0.003** | **-0.002*10$^{-4}$** | 0.270 | 0.159 |
| MC-to-PC | - 0.734 | 0.310 | 0.520 | **-0.007** | 0.324 |
| HF-to-HI | 0.124 | 0.199 | **0.006** | 0.538 | -0.296 |
| A-to-OA | 0.060 | 0.082 | **0.003** | 0.557 | -0.149 |

Significant correlations in bold

**Table 5** Fractions of specific C bonds for newly shed litters and decomposed litters collected/incubated at sites burned by an experimental fire since 1 year (EF1) and at sites located at the edge low-maquis/gaps (EMG), at sites burned by a wildfire since 10 years (WF10). Data are percent of total area for $^{13}$C CPMAS NMR spectra (in brackets: chemical shift ranges in ppm). Ratios between specific spectral ranges: MC (Methoxyl-C)-to-PC (Phenol-C); Aromaticity index (Phenol-C + Aryl-C)-to-(O-Alkyl-C); Hydrophobicity index (HF-to-HI) i.e. (Aryl-C + Phenol-C + Alkyl-C)-to-(Carbonyl-C + O-Alkyl-C); and (Alkyl-C)-to-(O-Alkyl-C) (A-to-OA). Signals around 29–34 ppm within the Alkyl-C, and around 45–49 ppm within Methoxyl-C are reported in brackets

| Years | Decay days | Carboxyl-C (190–160) | Phenol-C (160–145) | Aryl-C (145–110) | O-alkyl-C (110–60) | Methoxyl-C (60–45) | Alkyl-C (45–0) | MC-to-PC | (PC+ArC) to OAC | HF-to-HI | A-to-OA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Newly shed litter** | | | | | | | | | | | |
| WF10 | 0 | 4.39 | 3.29 | 9.57 | 48.57 | 4.96 (0.88) | 29.22 (6.71) | 1.51 | 0.27 | 0.80 | 0.60 |
| EF1 | 0 | 5.82 | 4.42 | 11.69 | 41.30 | 5.70 (1.57) | 31.06 (6.69) | 1.29 | 0.39 | 1.00 | 0.75 |
| EMG | 0 | 4.79 | 4.50 | 9.77 | 48.39 | 5.17 (1.10) | 27.36 (6.13) | 1.49 | 0.30 | 0.78 | 0.56 |
| **Early phase** | | | | | | | | | | | |
| WF10 | 126 | 4.74 | 3.55 | 9.62 | 43.84 | 5.40 (1.42) | 32.84 (9.86) | 1.52 | 0.30 | 0.95 | 0.75 |
| EF1 | 131 | 5.97 | 4.30 | 11.64 | 39.88 | 5.73 (1.55) | 32.48 (7.70) | 1.33 | 0.40 | 1.06 | 0.81 |
| EMG | 92 | 4.47 | 3.53 | 9.30 | 45.39 | 5.05 (1.25) | 32.25 (6.93) | 1.43 | 0.28 | 0.90 | 0.71 |
| **Middle phase** | | | | | | | | | | | |
| WF10 | 355 | 5.07 | 4.01 | 10.70 | 42.49 | 5.48 (1.57) | 32.25 (8.42) | 1.37 | 0.35 | 0.99 | 0.76 |
| EF1 | 418 | 5.32 | 4.26 | 11.02 | 41.48 | 5.27 (1.92) | 32.64 (7.19) | 1.24 | 0.37 | 1.02 | 0.79 |
| EMG | 403 | 4.96 | 3.87 | 11.06 | 42.56 | 5.85 (1.24) | 31.70 (6.10) | 1.51 | 0.35 | 0.98 | 0.75 |
| **Late phase** | | | | | | | | | | | |
| WF10 | 810 | 4.97 | 3.53 | 11.04 | 40.33 | 6.32 (1.14) | 33.81 (9.05) | 1.79 | 0.36 | 1.07 | 0.84 |
| EF1 | 705 | 4.73 | 3.83 | 11.35 | 42.13 | 5.77 (1.70) | 32.20 (7.99) | 1.51 | 0.36 | 1.01 | 0.76 |

Data are percent of total area for $^{13}$C CPMAS NMR spectra (in brackets: chemical shift ranges in ppm). Ratios between specific spectral ranges: MC (Methoxyl-C)-to-PC (Phenol-C); Aromaticity index (Phenol-C + Aryl-C)-to-(O-Alkyl-C); Hydrophobicity index (HF-to-HI) i.e. (Aryl-C + Phenol-C + Alkyl-C)-to-(Carbonyl-C + O-Alkyl-C); and (Alkyl-C)-to-(O-Alkyl-C) (A-to-OA). Signals around 29–34 ppm within the Alkyl-C, and around 45–49 ppm within Methoxyl-C are reported in brackets
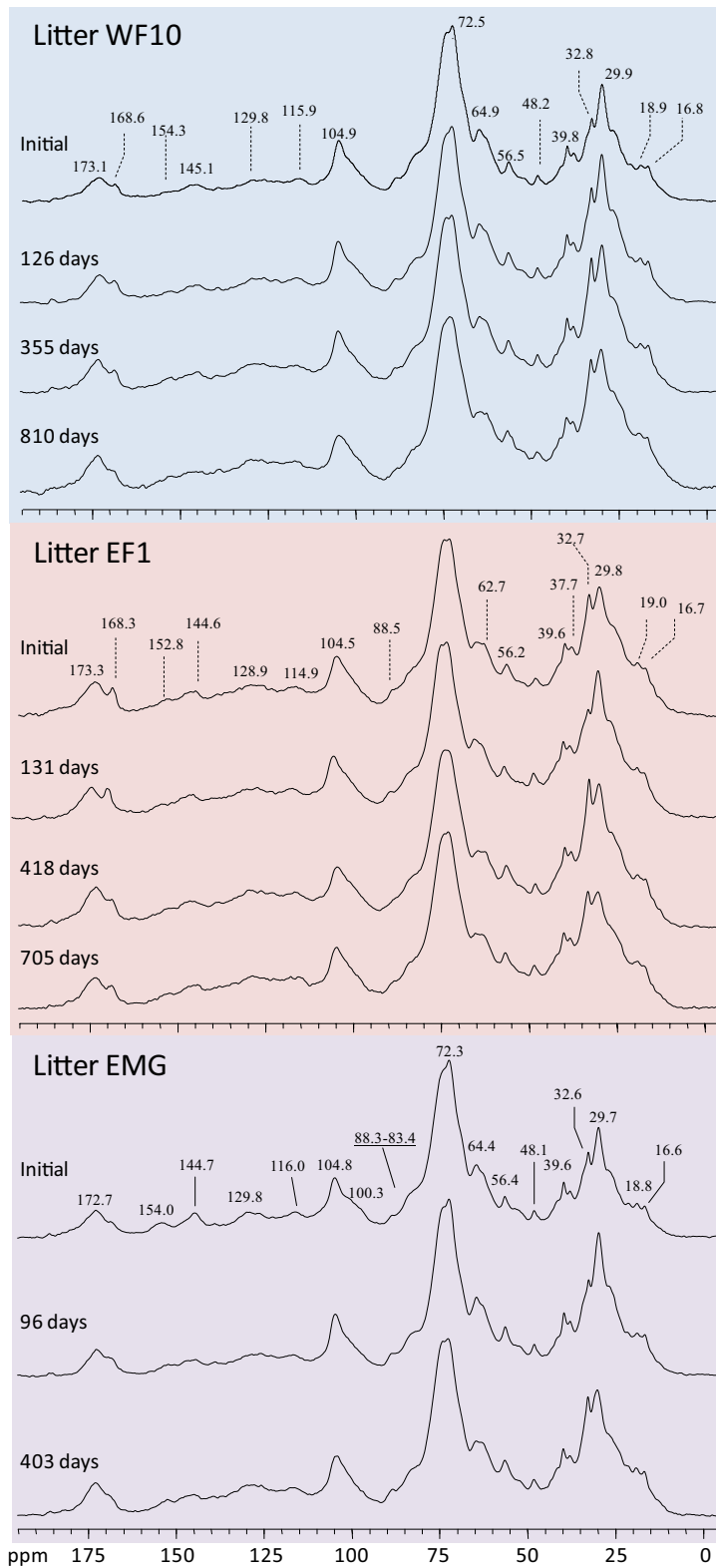
**Fig. 4** Solid-state $^{13}$C CPMAS NMR spectra for newly shed and decomposing leaf litters of *Phillyrea angustifolia* L. Number of days for each phase and each litter are reported on the links. Numbers on the spectra identify signals from specific Carbon bond
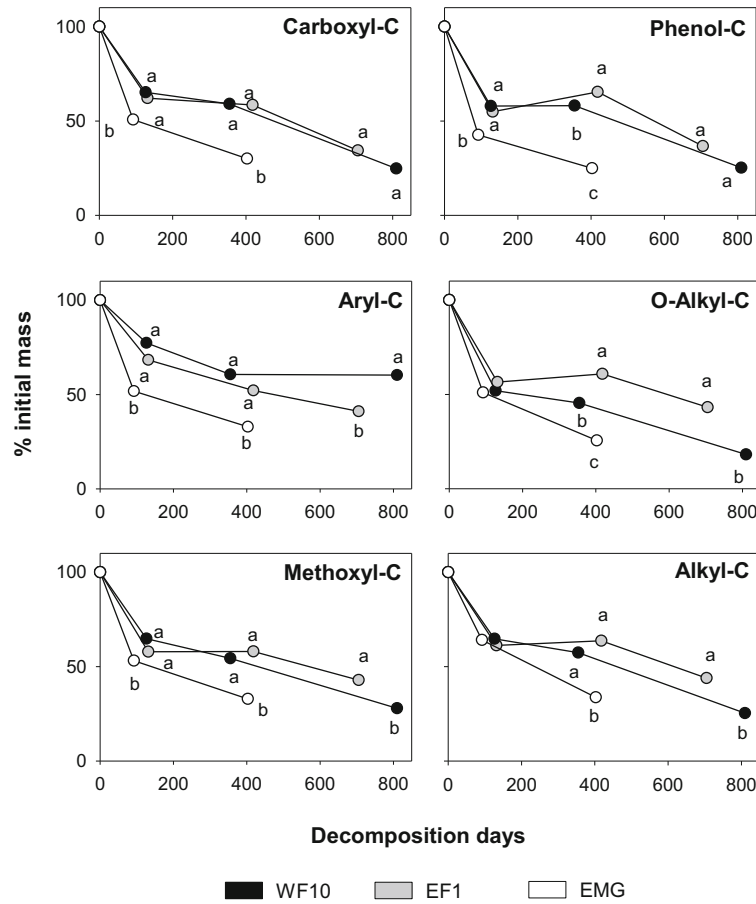
Fig. 5 Remaining mass, as percent of the initial mass, of six C-bond fractions in decomposing leaf litters of *Phillyrea angustifolia* L. Different letters indicate significant differences between the three litters for each decomposition period (P < 0.05, One Way ANOVA)
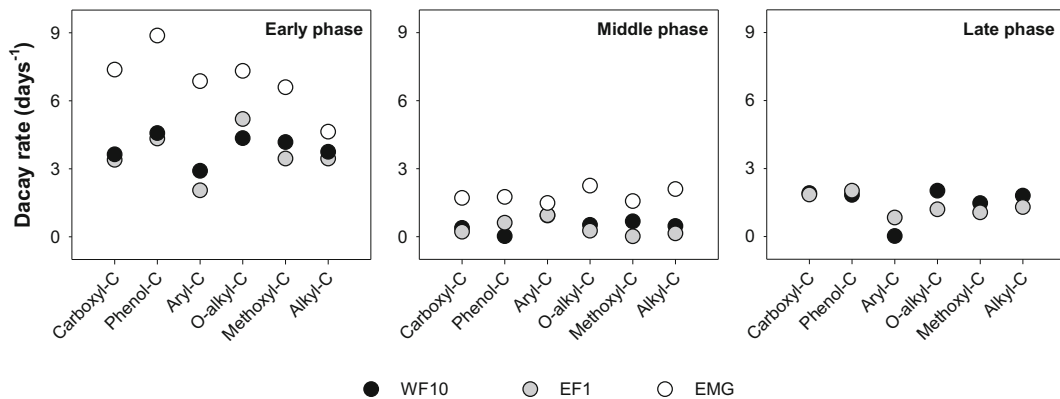


Fig. 6 Decay rates of six C-bond fractions in *Phillyrea angustifolia* L. litters. Rates were calculated using the constant-fractional-rate model (Olson 1963) for the early, the middle and the late phase of decomposition. Number of days in each period and each litter are reported in Fig. 2

degradation of O-alkyl fraction and a corresponding relative larger abundance of alkyl and aromatic components toward the end of the incubation, while a less modified molecular composition was found for litter EF1 all over the incubation period (Fig. 4; Table 5). The selective preservation of apolar recalcitrant compounds produced a progressive increase of the three indices of decomposition for litters WF10 and EMG which final values evened up those of litter EF1 (Table 5). The shared increase observed at the end of incubation for the MC/PC ratio (Table 5) may be related to either the slight decrease of O-aryl bonds (145–160 ppm) in decomposable lignin components with a possible concomitant incorporation in the 45–60 ppm NMR region, of slowly decomposing C-N bearing moieties (Lorenz et al. 2000) as also indicated by the data of N concentration (Fig. 2).

The normalized variation range (VR) values indicate that newly shed litters WF10, EF1 and EMG mostly differ for Phenol-C, Carboxyl-C and Aryl-C contents, whereas the differences in O-Alkyl-C, Methoxyl-C and Alkyl-C are smaller (Fig. 7). Differences in organic components decrease during decomposition and the three litters approach a more uniform organic composition as indicated by the low VR for all C-bonds in the middle and in the late phase compared to the early phase and the newly shed leaf litter (Fig. 7).

Principal Component analysis

Figure 8 shows the PCA of main variables that describe chemical composition of newly shed and decomposing leaf litter of *Ph. angustifolia*. Nutrients (N and Mn) and lignin /AUR contents, and the six C bond fractions, defined by $^{13}$CNMR spectra, for the three litters WF10, EF1 and EMG across the whole decomposition period have been used. The results of the PCA highlight a positive loading on PC1 (that explains 84 % of the total variance) of C content and C-bond fractions and a negative loading of lignin/AUR, N, and Mn contents. The investigated litters were clearly separated in the PC space and in particular, along the first axis, newly shed litter and litter in a gradual sequence of decomposition were clustered in the negative direction (Fig. 8). The difference among sites for litter chemistry at each decomposition phase separates WF10, EF1 and EMG along the second axis that explains about 8 % of the total variance.
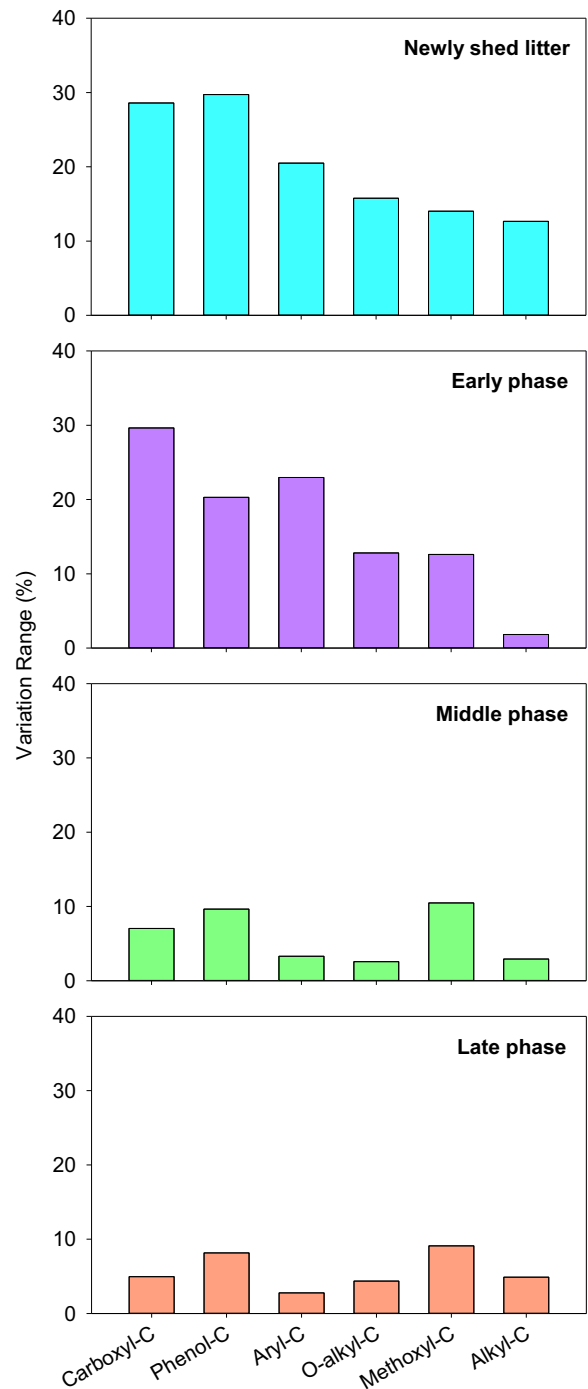


**Fig. 7** Variation range of organic components, as C-bond fractions, in newly shed litter and in decomposing litters of *Phillyrea angustifolia* L. To account for the differences in concentration of the litter components we normalized VR dividing the range of variation by the mean of the three items (MI) and multiplying per 100 (VR = Variation Range / MI x 100)
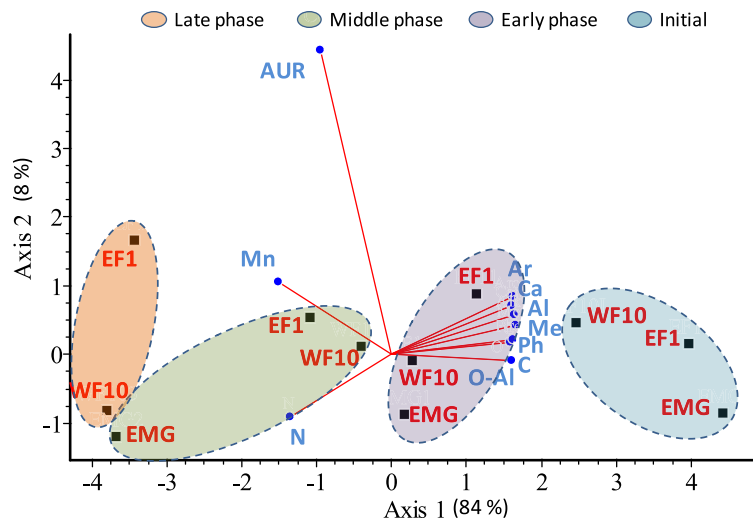
**Fig. 8** PCA of variables related to decomposition of *Phillyrea angustifolia* leaf litter. Nutrients (N and Mn) and lignin /AUR contents, remaining carbon (C) and the six C bond fractions (Al: Alkyl-C; Me: Methoxyl-C; O-Al: O-Alkyl-C; Ar: Aryl-C; Ph: Phenol-C; Ca: Carboxyl-C), defined by $^{13}$C NMR spectra, for the three litters WF10, EF1 and EMG, have been used. Initial litter (i.e. before incubation); decaying litter at three time intervals since the incubation in situ: after about 100, 400 and 800 days

## Discussion

General pattern of decomposition and regulating factors

Initial traits largely differ in the three litters not only for nutrients but also for organic components (Tables 2 and 5; Fig. 7) and the patterns of decomposition are expected to be different. According to Berg and McClaugherty (2014) the general model of decomposition is related to the concentration of specific litter components that affect the early phase and the late phase. Litter decomposition in the early phase is dominated by nutrients; late phase starts with the decrease of lignin AUR and is lignin-dominated (Berg and McClaugherty 2014). It has been demonstrated (Berg and Staaf 1981; Berg and Matzner 1997) that the decomposition pattern of boreal forest litter in the late stage follows other rules than those for the early stage.

Litter EMG was the poorest in N and Mn among the three *Ph. angustifolia* litters. Therefore, based on the rate regulating factors of mass loss in the early phase, we expected the slowest decomposition for litter EMG that in contrast had the fastest decomposition in the early phase as well as in the following middle phase. Thus decomposition rates seem to be not affected by nutrients concentration in a way that fit to what could have been expected based on the bulk of decomposition literature derived from research primarily directed toward boreal and temperate forest systems.

Moreover in *Ph. angustifolia* (Fig. 3) the separation between the nutrient regulated phase and the AUR/ lignin dominated phase is not clear-cut as the changes in AUR/lignin with decomposition show a different pattern in the three litters. AUR/lignin decomposition (Fig. 2) starts soon in litter WF10 that has a higher initial AUR/lignin content (27 %), while it starts after 3–4 months in litters EF1 and EMG (initial lignin content 20 and 13 % respectively), apparently when lignin AUR has reached a concentration above 20 %. The decay of lignin since the start of the early phase of litter decomposition has been reported to occur in litters of other shrubs of the Mediterranean maquis by Fioretto et al. (2005).

The differences in organic components, determined as C bonds by $^{13}$C NMR spectroscopy, are consistent with the pattern of decomposition of the three litters (Figs. 2 and 5). Indeed the overall lower values of hydrophobic and aromaticity indexes combined with the alkyl ratio (Table 5) underline the larger prevalence of bio-decomposable components in newly shed litters EMG and WF10 that decompose more fast compared to litter EF1.

Most interestingly the variation range of organic components changes across the decomposition process and in the most advanced decay phases (Fig. 7) within each C-bond the three litters become fairly uniform.

The results of the Principal Component Analysis (PCA) reveal that most of the total variance is due to

the shifts in litter chemical composition throughout the decomposition phases. Still part of the total variance is due to the differences among the three litters WF10, EF1 and EMG i.e. to the differences in chemical composition within the same plant species across an uneven landscape.

There was any significant correlation of chemical composition of newly shed litter and decomposing litter with Temperature and Precipitation during each decomposition phase (Table 4). However it cannot be excluded that the differences in decay rates among the three litters are at least in part due to temperature and precipitation. The lowest decay rates were those of litter WF10 during the middle phase of decomposition when average temperature was the highest measured over the whole study period (19.5 °C) and the accumulated precipitation were relatively low (280 mm). The late decomposition phase was the most rainy for litter WF10 (accumulated precipitation 495 mm) and litter EF1 (accumulated precipitation 622 mm) and likely favored rates of decomposition even higher than in the middle phase.

## Changes in organic components during litter decomposition

In decaying litters of *Ph. angustifolia* all C fractions are gradually decomposed and the changes in amount of $^{13}$C NMR fractions follow consistent trajectories with % C remaining (Figs. 2 and 5). All the C bond fractions, show a steep decrease in amount (as percent of the initial mass) during the first 100 days and continue to decrease more slowly thereafter (Fig. 5). The lower decay constant of most fractions (Fig. 6) in litters WF10 and EF1 compared to litter EMG confirms a less stable molecular composition of litter EMG (Figs. 4, 5 and 6; Table 4). The highest observed decay rates are those of Phenol-C followed by those of Carboxyl-, O-Alkyl- and Aryl-C in the early decomposition phase of litter EMG (Fig. 6). The lowest decay rate are those of Carboxyl-, Phenol- and Aryl-C of litters WF10 and EF1 during the middle phase (Fig. 6). In litter EMG all C fractions in the middle phase continue to decompose more slowly than in the early phase, still they reach final masses as low as those of litters WF10 and EF1 after 800 days (Fig. 5). Litter WF10 and litter EF1, notwithstanding the initial differences, after 400 days have similar organic composition (Table 5) and continue to decompose at similar rates (Fig. 6).

Alkyl-C have overall low decay rates (Fig. 6). The Alkyl fraction in leaf litter of *Ph. angustifolia*, is the second most abundant C fraction (about 27–34 % of total carbon), after O-Alkyl-C (40–49 % of total carbon), and includes bulk methylenes ($CH_2$) segments of different lipid molecules of wax and cutin components. Waxes and cutin form barriers to water loss that protect leaf surfaces thus being important adaptive traits in a system like that of Mediterranean maquis prone to seasonal drought, and what is more, hardened by the poor water retention of the sandy soil. Litter EF1 produced by plant re-sprout after fire, and thus originating from leaves developed in a climatically even more severe environment, such as that of an open canopy, is the richest in Alkyl-C.

A general intrinsic biochemical stability and slow decomposability of the three leaf litter of *Ph. angustifolia*, is outlined by the indices of decomposition comparable to those of stabilized litters and humified biomasses (De Marco et al. 2012; Wang et al. 2017; Bento et al. 2020). During decay of *Ph. angustifolia* litters, a substantial increase of 35 and 25 % in hydrophobic character occurs in litters WF10 and EMG, respectively, which final values almost equalize the larger initial hydrophobicity of the most stable litter EF1 (Table 5). The A/OA and PC + Ar C/OA ratios suggest a combined contribution of alkyl and aromatic components, to the spectroscopic hydrophobic properties of *Ph. angustifolia* litter. Although a direct correlation of $^{13}$C NMR parameters with decomposition processes and their extensive application to different organic materials might be questioned (Khalaf et al. 2014), the application of hydrophobic index (HF/HI), alkyl and aromatic ratios (A/OA and PC + ArC/OA), are reliable probing tools to elucidate different reactivity and recalcitrance of decomposing organic materials such as composts, litter and soil OM (De Marco et al. 2012; Wang et al. 2017; de Aquino et al. 2019; Bento et al. 2020). The preservation of aliphatic molecules is conceived among the underpinning mechanisms in the biochemical stabilization of organic debris in forest ecosystems (Wang et al. 2017; Piccolo et al. 2018). Along with an effective preservation of Alkyl-C, a specific dynamic may be highlighted for the three litters in NMR spectral region of lipid molecules. The limited variation observed for the resonances at 37/39 indicated a steady maintenance of the cyclic compounds in the assembled ring of lignans and flavonoids (Wang et al. 2019). Furthermore, the differential

temporal variation found for the $CH_2$ functions in methylenic chains, with the relative final increase of the signals at 33 ppm (Fig. 4) is probably related to the selective degradation of, both, bio-labile free lipids of wax and phospholipid-derived fatty acids compared to cutin and suberin biopolyesters (Wang et al. 2017).

Besides the cyclic aliphatic components, also the relative amount of Aryl-C and Phenol-C signals (110–160 ppm), revealed a significant preservation of various aromatic structural units of *Ph. angustifolia* litter over the whole decay period. The slight increase of the Methoxyl-C-to-Phenol-C structural index during the decay of the three litters (Table 5), may have raised from a partial preferential decomposition of phenolic components within less condensed units of lignin fragments associated to cellulose and hemicellulose of plant cell walls (De Marco et al. 2012), coupled with the incorporation in the stabilizing organic materials of less decomposable nitrogen derivatives (Lorenz et al. 2000). This finding is also supported by the preservation during the decomposition stages of the peak at 48 ppm associated to the C-N bond in alkaloid compounds (Fig. 4; Table 5).

Finally, the progressive disappearance of the signals at 168 ppm during litter decomposition (Fig. 4) may be associated to loss of acetyl substituent of carbohydrate components in hemicelluloses and flavonoid units. The concomitant steady maintenance, over the decay period, of the global area related to carboxylic groups (190 − 160 ppm) further confirms the lower oxidation and the biochemical stability trend of organic components in decaying *Ph. angustifolia* litters (Table 5).

Litters WF10, EF1 and EMG at the start of decomposition show for all organic components relatively higher variation ranges than at the end of the decomposition period when a more uniform chemical composition occurs (Fig. 7). Methoxyl C are the less variable components across the whole decomposition period, even less variable than Alkyl-C. On the other hand the large differences for Carboxyl-C, Phenol-C and Aryl-C between newly shed litters decrease with decomposition. This suggest that the between litters differences may be due to a labile fraction of such components that decomposes fast in the first months and/or is possibly lost by leaching. According to Hernes et al. (Hernes et al. 2001) leaching is an important pathway for loss of phenolics from the leaves.

Changes in nutrients and lignin/AUR

The decay of organic components over the whole study period is not accompanied by the nutrient release although litter EF1 shows a slight release of N and Mn during the late decay period. Immobilization of nutrient (i.e. the incorporation of exogenous nutrients into organic matter) in decaying litter is a mechanism to improve N and Mn conservation in a system like Mediterranean maquis, that is poor in nutrients. This reveals an important feature of the nutrient conservation strategy for *Ph. angustifolia* to live in coastal sandy soils with low nutrient availability. The highest increase of N during decay in litter EMG that had the lowest initial N concentration (Fig. 2) is consistent with the findings of Berg and Staaf (1981) and Hobbie and Vitousek (2000). A possible condensation reaction in which tannin plays a role may be involved in nitrogen immobilization (Hernes et al. 2001). Nitrogen accumulation in litter EMG is likely enhanced by the high N level in the soil that may mitigate limitation posed by litter chemical composition (Berg and Ekbohm 1983; Sjögersten and Wookey 2004). Although this soil, in a site burned 16 years before the current study, is poor in organic matter, a dense legume cover is likely responsible of the high N content. According to De Marco et al. (2008) in the N-limited maquis environment, the abundance of legumes, that increases with the amount of precipitation, is responsible for marked differences between years in nitrogen input to the soil.

The three litters had different initial concentrations of lignin/AUR. Only in litter WF10, the absolute amount of lignin/AUR decreases with decomposition, while it increases in the early decomposition period in litter EF1 and EMG and thereafter slowly decreases (Fig. 2). The increase in lignin/AUR with litter decomposition is well-known. It has been demonstrated, using specific chemical methods that true lignin is generally not preserved with decomposition (Johansson et al. 1986; Grandy et al. 2007). Several studies (Johansson et al. 1986; Zech et al. 1987; Almendros et al. 2000; Preston et al. 1997, 2006, 2009a) have demonstrated that throughout the decomposition process, AUR integrates the least soluble and most hydrolysis-resistant organic structures, being an operational fraction derived from lignin, cutin, waxes and condensed tannins in varying proportions. In this study, as the whole range of C bonds, thus including lignin moieties, loses carbon since the start of decomposition, the increase in AUR during

the early period in litters EF1 and EMG cannot be accounted for by the increase in lignin. Rather the increase of resistant structures derived from lignin, tannins, and cutin may collectively account for increasing AUR. The higher initial AUR in litter WF10 might be attributable to the contribution of lignin to high initial contents of condensed tannins and or waxes and cutin. However AUR was correlated to any C bonds nor to decay indexes or total C (Table 4).

The steep increase of lignin AUR in the early phase is accompanied in EMG by the steep increase of N. It has been demonstrated that in decomposing Scots pine litter (Berg and Theander 1984) about a third of the litter's total N may be bound in the AUR and that N incorporated in the AUR gives new and more recalcitrant compounds that influence its decomposition rate (Nommik and Vahtras 1982; Knicker 2004). This is possibly the reason for the sharp drop of litter EMG decay rate in the middle phase compared to the early phase.

## Conclusions

The differing leaf chemistry of *Ph. angustifolia* affects litter decomposition across the landscape. Spatial changes in litter chemistry occurring between areas that had burned since different time span and the presence of gaps generated by fire, show that fire disturbance can impact on litter decomposition on the short-, the medium- and the long term.

The dual approach to determine litter chemistry, allows a better understanding of the individual drivers of litter decomposition.

Nutrient analyses do not explain the between litters difference in decomposition pattern but highlight the ability of decaying litter of *Ph. angustifolia* to immobilize N and Mn. Litter chemistry, analyzed through solid state [13] C NMR, reveals the changes in organic composition of litters collected after different time span since fire disturbance.

The differences in organic components between litters account for different decay rates and likely for different fluxes of Carbon into the soil. All the three investigated litters are rich in Alkyl-C, that is the fraction including waxes and cutin, structural components of the leaves that form barriers to water loss and therefore are important adaptive characteristics in the droughty maquis environment. It is meaningful that litter EF1, originated from leaves developed under the more severe environmental conditions after a fire, was richer in more stable C bond fractions.

The results of proximate analyses for lignin/AUR and the data from [13] C NMR spectroscopy for C bonds reflecting true lignin moieties, as Methoxyl-C and Phenol-C, were inconsistent. Although a relation between lignin/AUR and C bonds reflecting true lignin moieties does not exist, lignin/AUR is largely recognized as a regulating factor that negatively affects decay rate. In *Ph. angustifolia* the litter with the lowest AUR content was the litter with highest decomposition rate.

**Author contributions** A. DM. and A.VD. conceived and designed the experiments; R.S. performed the [13] C NMR measurements and analyzed the data; A.DM. performed field work and contributed to proximate analyses and statistical analyses; A.VD. contributed support and advice in the development of research; A.VD, A.DM and R.S. wrote the paper and all authors contributed to manuscript revision.

**Data availability** Data available on request.

**Declarations**

**Conflicts of interest/Competing interests** The authors declare no conflict of interest.

**Code availability** Not applicable.

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

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