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Temperature sensitivity and decomposition rate of 101 leaf litter types from Mediterranean ecosystems

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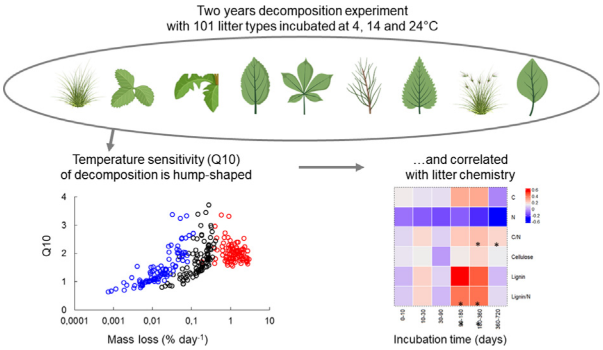
HIGHLIGHTS

- Litter N, lignin, C/N, and lignin/N correlates negatively with early-stage decomposition rate.
- Late-stage decomposition rate correlated negatively with N and positively with C/N and lignin/N.
- Temperature sensitivity shows unexpected hump-shaped relationship with decay rate.
- Results suggest caution when using certain chemistry ratios to predict decomposition rate.

GRAPHICAL ABSTRACT



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ABSTRACT

Litter decomposition is a fundamental process, and the number of published studies has steadily increased in recent decades. However, few experiments have systematically compared a large number of litter types and evaluated their temperature sensitivity. We conducted a two-year experiment on the decomposition of litter bags containing 101 leaf litter sampled in Mediterranean ecosystems and incubated under laboratory conditions at 4 °C, 14 °C, and 24 °C. Litter was chemically characterized and analysed for carbon (C), nitrogen (N), cellulose and lignin concentration, C/N, and lignin/N ratios, which serve as predictors of decomposition rate. The sensitivity of litter decay rate to temperature was evaluated using Q_{10}. Leaf litter chemistry varied widely in nitrogen content (range 0.52–6.80 %), lignin content (range 1.53–49.31 %), C/N ratio (range 5.21–77.78), and lignin/N ratio (range 0.34–34.90). Litter decomposition rate was negatively related to initial lignin concentration, lignin/N ratio, and C/N ratio, but only in the early stage. In the late stages of decomposition, litter decay rate was negatively correlated with initial N concentration but positively correlated with C/N and lignin/N ratios. Temperature sensitivity was negatively correlated with N concentration and positively correlated with lignin and lignin/N ratio. It is noteworthy that, contrary to expectations, temperature sensitivity exhibited a hump-shaped relationship with decay rate. N, C/N, and lignin/N ratios should be used with caution because their predictive power is reversed with respect to decomposition rate during the decomposition process. In addition, the new finding that temperature sensitivity has a hump-shaped relationship with decomposition rate deserves further confirmation and could be considered in ecosystem-level organic C modeling.

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1. Introduction

Litter decomposition is a fundamental process in which organic material is broken down into simpler organic and inorganic compounds by a complex microbiome consisting mainly of bacteria, fungi, and detritivores such as earthworms and arthropods (Gergócs and Hufnagel, 2016; Baldrian et al., 2023). During the decomposition process, complex macromolecules such as proteins, cellulose, and lignin are gradually hydrolyzed, oxidized, and finally mineralized into simpler compounds such as carbon dioxide, water, and inorganic nutrients such as nitrogen, phosphorus, calcium, potassium, and microelements. Overall, plant litter decomposition plays a critical role in the global carbon cycle by regulating the exchange of organic carbon between the soil and the atmosphere. In fact, decomposition rate and dynamics control the timing and amount of carbon stored in the soil. The balance between carbon storage and release during litter decomposition is influenced by abiotic and biotic factors, including the chemical composition of the litter, the local microclimate, and the microbiota that carries out the decomposition process (Berg and McClaugherty, 2014).

At the local scale, i.e., within an ecosystem, the chemical properties of litter, along with the composition of the microbiota, are the most important factors in controlling the fate of organic carbon (Cornwell et al., 2008). However, at both regional and global scales, the role of climate, i.e., temperature and moisture, is becoming increasingly important (Aerts, 1997). In general, the influence of temperature on litter decomposition is fairly well established (e.g., Meentemeyer, 1978; Hobbie, 1996), with decomposition rates increasing progressively with temperature. Because temperature affects the activity of microorganisms involved in decomposition, the response of the overall process follows the Arrhenius equation, which states that the rate of biological activity increases exponentially with temperature (Sierra, 2012). However, the temperature sensitivity of litter decomposition remains an open issue as conflicting results have been reported (Giardina and Ryan, 2000). Theoretical considerations and empirical evidence suggest that temperature sensitivity increases with substrate recalcitrance (Davidson and Janssens, 2006; Hartley and Ineson, 2008). In other words, litter with rapid mass loss is expected to increase its decay rate with increasing temperature less than slowly decomposing materials such as roots, woody debris, or stabilized organic matter (Bonanomi et al. 2021a). More generally, climate change is expected to have significant impacts on litter decomposition as rising temperatures increase the rate of decomposition and lead to more rapid release of soil organic carbon from ecosystems. To predict the response of litter decomposition to climate change, detailed knowledge of the response of litter with different traits to temperature is needed.

Plant functional type and leaf traits can significantly influence litter decomposition rates (Allison, 2012) and therefore play an important role in regulating the global carbon cycle. Different functional plant types, i.e., grasses, sedges, forbs, vines, shrubs, and trees, have different litter properties and decomposition rates due to their differences in leaf morphology and chemistry (Cornwell et al., 2008). For example, plant tissue with high lignin content decomposes more slowly than herbaceous species with low lignin but high nitrogen content. Similarly, nitrogen-fixing species that have high nitrogen concentrations typically decompose faster than similar species that have not mastered this symbiosis (Incerti et al., 2018). Overall, leaves with a high carbon-to-nitrogen ratio (C/N) and a high lignin-to-nitrogen ratio (lignin/N) generally decompose more slowly than plant tissues with opposite characteristics (Taylor et al., 1989). From a microbial perspective, nitrogen availability limits decomposition when the litter C/N ratio is above a value of ~30–35 (Hodge et al., 2000; Berglund & Ågren, 2012). For plant tissues with very low initial N content, such as those found in coarse roots, woody debris, and some leaf litter, microbial activity is severely limited by nitrogen availability, and degradation of cellulose and lignin proceeds slowly in the absence of exogenous nitrogen sources (Bonanomi et al., 2017). In particular, leaf traits show great variation not only between ecosystems but also within ecosystems in terms of resource acquisition strategies, tissue longevity, and adaptation to abiotic stresses (Cornwell et al., 2008). The importance of plant functional type and leaf traits in litter decomposition has implications for carbon and nutrient cycling in ecosystems and for understanding ecosystem responses to environmental changes such as climate change and land use. Therefore, incorporating variation in plant functional types and litter traits that occur at different spatial scales would improve our ability to predict ecosystem responses to global environmental change.

To account for the variability in leaf and litter traits observed in different ecosystems and to predict decomposition rates, studies should include a large number of species to provide a broad data coverage. Accordingly, Cornelissen (1996) compared litter decomposition of 125 British vascular plants. Later, Cornwell et al. (2008) used a meta-analytic approach to collect data from 66 decomposition experiments involving 818 species. More recently, Jo et al. (2016) conducted a decomposition experiment with 42 native and 36 exotic species from deciduous forests in the eastern United States. Most decomposition studies in Mediterranean ecosystems have used very few species at a time, with a few exceptions. For example, Kazakou et al. (2006) used 12 herbaceous species from old fields in France. De la Riva et al. (2019) studied leaf traits and decay rates of 38 species from southern Spain, while Incerti et al. (2018) compared decay rates of 8 invasive and 24 autochthonous species from grasslands, shrublands, and forests in fire-prone ecosystems in southern Italy. Although the number of published studies on litter decomposition has steadily increased in recent decades, including for Mediterranean ecosystems, there have been no systematic experiments comparing a large number of litter species and evaluating their temperature sensitivity. In this context, the aim of this work was to compare the chemical properties, decomposition rate and temperature sensitivity measured by the Q_{10} method of 101 Mediterranean species. We carried out a two-year (720 days) decomposition experiment with litterbags incubated at 4 °C, 14 °C, and 24 °C to evaluate the temperature sensitivity of decomposition. Undecomposed leaf litter was characterized for carbon, nitrogen, lignin, cellulose, C/N and lignin/N ratios. The specific aims were to (i) describe the characteristics of leaf litter in relation to plant functional types based on chemical traits, (ii) evaluate the rate of decomposition of 101 leaf litter, (iii) demonstrate the relationships between the rate of decomposition and the chemical traits, and (iv) investigate the degree of temperature sensitivity of leaf litter and explore its correlation across different ranges of leaf chemical traits. Based on the above considerations, the following hypotheses were tested: (1) litter decay rate varies with plant functional type and is negatively correlated with lignin concentration but positively correlated with nitrogen concentration; (2) temperature sensitivity is negatively correlated with litter decay rate.

2. Materials and methods

2.1. Leaf litter collection

One hundred and one species common throughout the Mediterranean region were selected, belonging to different plant functional types (Xiao et al., 2021; Supplementary Table S1, Fig. S1). Plants identification was based to Pignatti (1982). The nomenclature follows the World Flora Online (WFO, 2021). Families are organized based on APG IV (Chase et al., 2016) for angiosperms. The species list includes both invasive and native plants, including twenty-two deciduous woody (*Acer cappadocicum* subsp. *lobelii, Broussonetia papyrifera, Castanea sativa, Celtis australis, Crataegus monogyna, Euphorbia dendroides, Fagus sylvatica, Ficus carica, Fraxinus ornus, Ilex aquifolium, Ostrya carpinifolia, Populus alba, Populus nigra, Pyrus communis* subsp. *pyraster, Quercus cerris, Quercus pubescens, Rosa canina, Salix alba, Salix purpurea, Sorbus aria, Tilia cordata,* and *Ulmus minor*), four coniferous woody (*Juniperus communis, Pinus halepensis, Pinus mugo,* and *Pinus nigra* subsp. *nigra*), nine evergreen woody (*Arbutus unedo*, *Cistus monspeliensis, Erica arborea, Eucalyptus camaldulensis, Laurus nobilis, Myrtus communis, Pistacia lentiscus, Quercus ilex,* and *Salvia rosmarinus*), ten nitrogen-fixing woody (*Acacia longifolia., Alnus glutinosa, Amorpha fruticosa, Ceratonia siliqua, Hippocrepis emerus, Cytisus scoparius, Genista aetnensis, Laburnum anagyroides, Robinia pseudoacacia,* and *Spartium junceum*), thirty two forbs (*Acanthus mollis, Alcea rosea, Amaranthus retroflexus, Arctium lappa, Arum* *italicum, Asphodelus ramosus, Borago officinalis, Chenopodium album, Erigeron canadensis, Dittrichia viscosa, Eryngium amethystinum, Euphorbia characias, Foeniculum vulgare, Galium aparine, Mentha aquatica, Oxalis pes-caprae, Papaver rhoeas, Parietaria diffusa, Plantago lanceolata, Plantago serraria, Ricinus communis, Rumex acetosa, Sambucus ebulus, Smyrnium olusatrum, Solanum nigrum, Taraxacum campylodes, Tradescantia fluminensis, Tussilago farfara, Typha latifolia, Urtica dioica, Verbascum thapsus,* and *Xanthium italicum*), four nitrogen-fixing forbs and herbs (*Hedysarum coronarium, Medicago sativa, Bituminaria bituminosa,* and *Trifolium repens*), eight grasses (*Aegilops geniculata, Ampelodesmos mauritanica, Arundo donax, Avena fatua, Hyparrhenia hirta, Dactylis glomerata, Heteropogon contortus,* and *Sorghum halepense*), four sedges (*Carex pendula*, *Eleocharis palustris*, *Juncus effusus*, and *Scirpoides holoschoenus*), four vines (*Clematis vitalba*, *Hedera helix*, *Ipomoea purpurea*, and *Rubus ulmifolius*), and four ferns (*Asplenium adiantum-nigrum*, *Equisetum telmateja*, *Polystichum aculeatum*, and *Pteridium aquilinum*). The selected plant species, representing 47 families and 10 functional groups, cover a wide range of leaf litter chemical traits.

Leaf litter samples were collected across woodlands, shrublands, grasslands, and agricultural ecosystems in the Campania region (southern Italy). For trees and shrubs, freshly fallen leaves were collected from adult plants ($N = 10$) randomly selected. For annual and perennial herbs, litter was collected at the end of the growing season from a large number of individuals (usually $N > 100$), until we reach the required amount of litter. After collection, leaves were placed in paper bags and air dried in a ventilated chamber at 25 °C for 20 days until they reached a constant weight. Leaf litter was then stored in paper bags at room temperature.

2.2. Decomposition experiment

Under field conditions, decomposition of organic matter is controlled by litter chemistry, temperature, and water availability. Here, we decomposed leaf litter under laboratory conditions to focus on the interactive role of temperate and litter chemistry under constant water availability. To investigate the relationship between temperature sensitivity and litter chemistry, we decomposed the 101 litter species at three temperatures, 4, 14, and 24 °C. The chosen values represent the mean temperatures in winter, spring/autumn, and summer at many locations in the Mediterranean region.

The experiment was conducted using the litter bag decomposition method (Berg and McClaugherty, 2014). The litter bags were square with 10 × 10 cm sides and a mesh size of 1 mm. The litter bags were filled with 2 g of dry litter for each of the 101 species. The litter bags were then placed under laboratory conditions over plastic trays (30 × 20 × 15 cm) filled with soil collected from the site where a large number of the species were collected and where they naturally occur (Cicerale 40°19′41.71″ N; 15°07′29.68″ E, 182 m asl). The soil is loamy-sandy with a neutral pH (7.06), with 26.30 g kg^{-1} organic carbon and 3.07 g kg^{-1} total nitrogen. Complete soil chemical properties are listed in Table S2. The plastic trays were in growth chambers (2.5 m^3 volume, temperature maintenance accuracy of $+/-$ 0.5 °C) at three temperatures, i.e., 4, 14, and 24 °C. The trays were manually irrigated with distilled water every three days to maintain the soil at field capacity and avoid waterlogging and drought. The field capacity of the soil was equivalent to 18 % of the volume as previously determined (Bonanomi et al. 2021a).

The experimental design included 101 litter species incubated at three temperatures with five replicates for each species. For each experimental factor, six retrieval dates were scheduled since the beginning of the experiment, i.e., 10, 30, 90, 180, 360, and 720 days. A total of 9090 litter bags were prepared (101 litter types × 3 temperatures × 6 retrieval dates × 5 replicates). At each time point, the removed litter bags were dried at 25 °C in a ventilated oven until a constant weight was reached. The organic material was then carefully cleaned of soil residues and other extraneous materials, and weighed to the nearest 0.001 g.

2.3. Initial litter chemistry

Dry, freshly fallen leaf litter was ground in a mortar to a fine powder that fit through a 1 mm sieve. Chemical determination was performed on the fine powder fraction in triplicate. Total organic C and N were determined by flash combustion of 5 mg samples using a C and N elemental analyzer (Flash EA2000 ThermoFisher). Proximate lignin and cellulose were determined following Gessner (2005). Briefly, cellulose was determined as the hydrolyzable fraction after sulfuric acid digestion, i.e., mass loss after application of 72 % H_2SO_4 for 3 h. After this step, the lignin content was determined as the non-hydrolyzable fraction, i.e., as the mass loss of the fraction derived from sulfuric acid digestion after annealing at 500 °C. The C, N, cellulose and lignin content are expressed as ash-free dry mass. The C/N and lignin/N ratios were calculated for all litter types.

2.4. Data analysis and visualization

We used general linear models (GLMs) to evaluate the main and interactive effects of leaf litter species (101 species), incubation temperatures (4, 14, and 24 °C), and decomposition times treated as continuous covariate on remaining mass. The fraction (%) of mass remaining was log-transformed before applying GLM and Tukey HSD post-hoc tests. Two GLMs were applied for both single plant species and for plant functional types. Differences in initial litter chemistry were assessed using the ANOVA test, and the means were pairwise separated using the post hoc Tukey test, and the corresponding jitter boxplots were generated using the ggplot2 package in R.

The decay rate of litter in the different decomposition phases (i.e., 0–10, 11–30, 31–90, 91–180, 181–360, 361–720 days) was calculated by dividing the lost mass by the number of days in the interval considered (Berg, 2000). The relationship between litter decomposition rate at different stages of decomposition and initial chemical characteristics (i.e. C, N, cellulose and lignin concentration, and C/N and lignin/N ratios) was calculated using Pearson's correlation, with significance evaluated at $P < 0.05$.

The temperature sensitivity of leaf litter to decomposition was calculated using the Q_{10} index (Fung et al., 1987), i.e., which evaluates the change in the rate of a process, in this case litter mass loss, with a temperature change of 10 °C, as follows:

$$Q10 = \left(\frac{K_{24^{\circ}C}}{K_{4^{\circ}C}}\right)^{\frac{10}{24^{\circ}C\ -\ 4^{\circ}C}}$$

Where $K_{24^{\circ}\text{C}}$ and $K_{4^{\circ}\text{C}}$ are the decay constant calculated with data obtained with litter incubation at 24 and 4 °C, respectively.

The Q_{10} index was calculated for each species and functional group across various decomposition phases, namely 0–10, 11–30, 31–90, 91–180, 181–360, 361–720 days, in order to assess its correlation with litter chemical traits. Subsequently, the relationship between litter mass loss and Q_{10} for all species and functional groups encompassing the entire decomposition period of 720 days was investigated. STATISTICA 13.3 (StatSoft Inc., USA) and R (version 4.2.2, R Core Team, 2020) software were used for statistical analyses and data visualization.

3. Results

3.1. Leaf litter chemical traits

The chemistry of undecomposed leaf litter varied dramatically among species and functional groups (Supplementary Table S3). N content ranged from 6.80 % in *Medicago sativa* to 0.52 % in *A. mauritanicus*, with the highest values in nitrogen-fixing forbs, forbs, and vines and the lowest in woody conifers and ferns (Fig. 1). In contrast, lignin content was very high in ferns, sedges, and woody conifers, very low in nitrogen-fixing forbs, forbs, and vines, and intermediate in the other functional groups. Lignin content ranged from 49.31 % in the fern *P. aculeatum* to 1.53 % in the forb *A. italicum*. Cellulose content varied from 31.30 % in *L. nobilis* to 4.95 % in *A. mollis*. In terms of functional group, cellulose content was highest in grasses, sedges, and ferns and lowest in woody evergreens. In terms of carbon content, little difference was found between functional groups and plant species. Instead, C/N and lignin/N ratios varied greatly

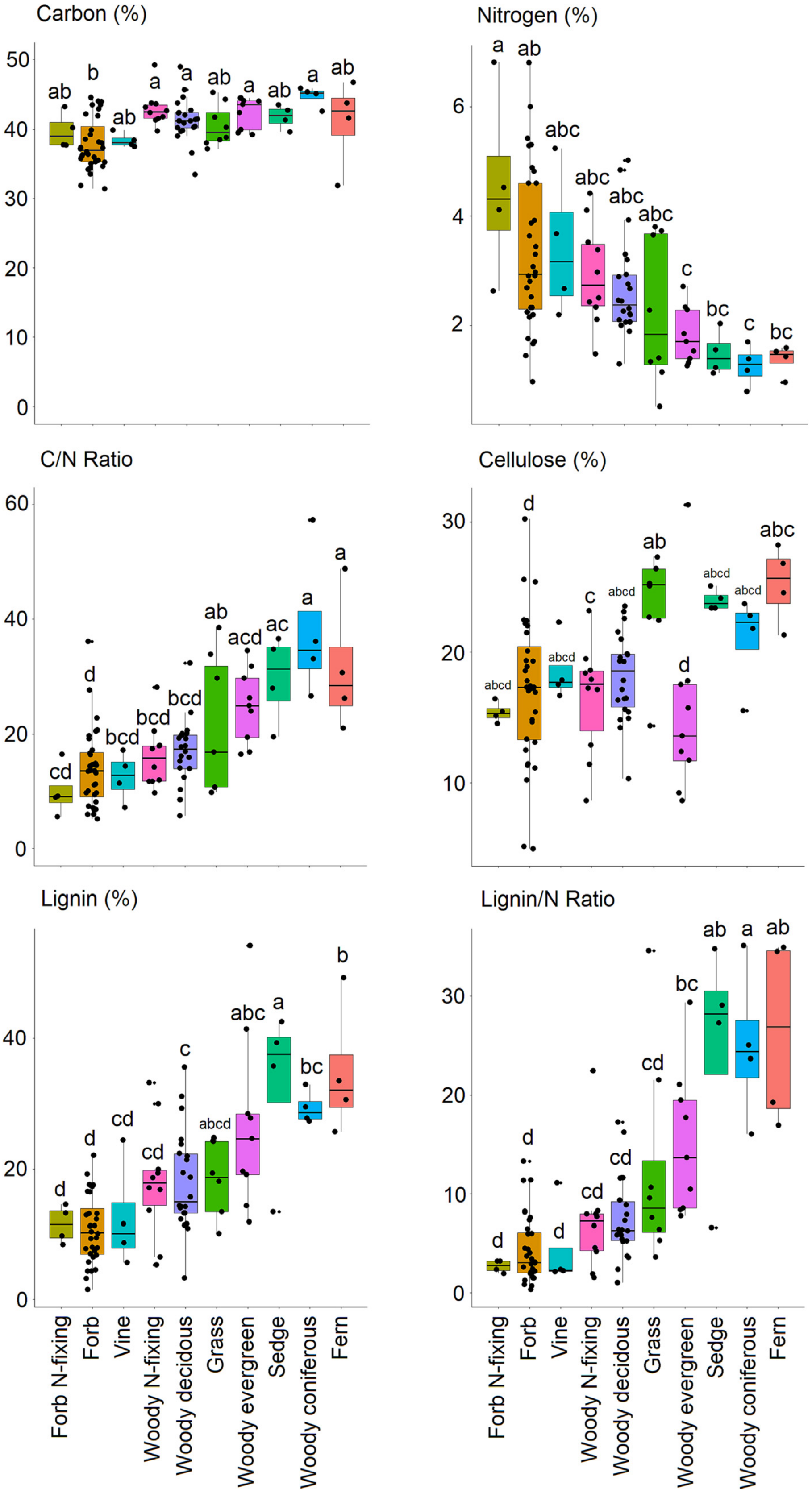

Fig. 1. Litter chemical traits of leaf litter of 10 functional types. Different letters indicate statistically significant differences ($P < 0.05$), raw data of chemical traits for each species are reported in Supplementary Table S3.

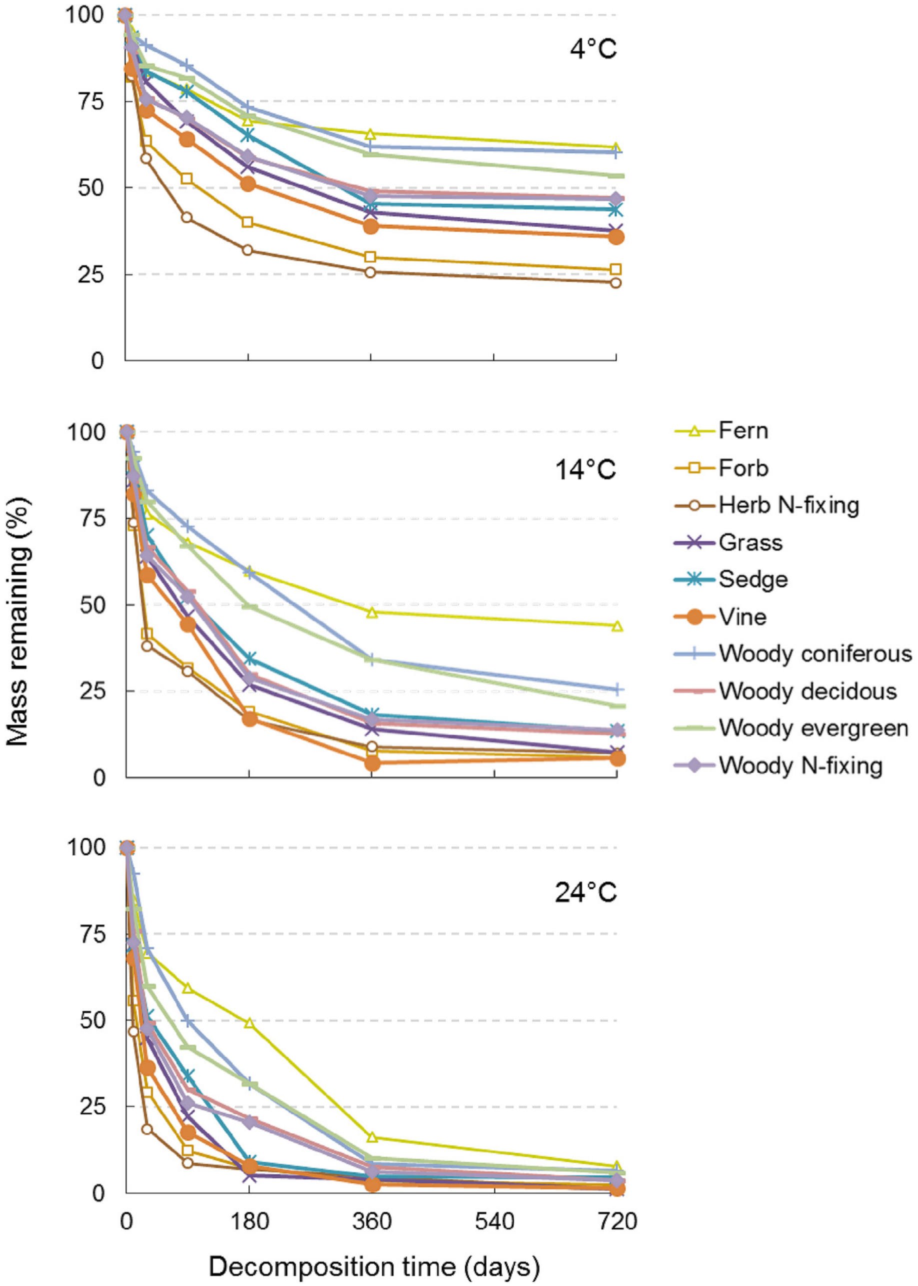

Fig. 2. Mass remaining (%) for leaf litter of ten plant functional types incubated at three temperatures as a function of time. Values are average within plant functional types; associated statistics are reported in Table S4. The mass remaining recorded for the 101 plant species are reported in Supplementary and Table S6.

among species and functional groups. For both ratios, nitrogen-fixing forbs, forbs, and vines had the lowest values, while ferns, sedges, woody conifers, and grasses had very high values. Specifically, C/N ratios ranged from 77.78 for the grass *A. mauritanicus* to 5.21 for the forb *S. nigrum*. For the lignin/N ratio, the values ranged from 34.90 in the fern *P. aquilinum* to 0.34 in *A. italicum*.

3.2. Leaf litter decomposition

Leaf litter mass remaining largely varied among species and functional groups being significantly affected by the litter types, temperature of incubation, and incubation time (Table S4, S5). Specifically, the remaining mass varied largely among the plant functional types with the following ranking: fern > woody coniferous > woody evergreen > woody deciduous = woody N-fixing > sedge > grass > vine > forb > nitrogen-fixing herb and forbs (Fig. 2). Notably, rank remained almost unchanged at 4 and 14 °C incubation, while at 24 °C rank was maintained after 180 and 360 days of incubation, but after 720 days most litter species were covered to very low levels of remaining mass (< 10 %). At the species level, the magnitude of litter type on remaining mass was outstanding. For example, *A. italicum* and *P. bituminosa* lost 96.73 % and 82.06 % of the original mass, respectively, after only 30 days of incubation at 24 °C, whereas *J. communis* and *P. aculeatum* lost only 23.63 % and 7.33 % of the initial mass, respectively, under the same conditions (Fig. 3). It is noteworthy that some species lost a considerable part of the initial mass in the first 10 days of incubation, up to 73.46 % in *A. italicum*, indicating an important contribution of leaching. In general, decomposition in rapidly decomposing species such as *A. italicum*, the nitrogen-fixing herb *P. bituminous*, and the vine *H. helix* follows a classic exponential negative curve (Fig. 3). Other species, including nitrogen-fixing woody such as *S. junceum*, deciduous shrubs such as *A. cappadocicum*, and grasses such as *C. irsutum*, follow the exponential negative curve of decomposition, but with a generally slower rate of

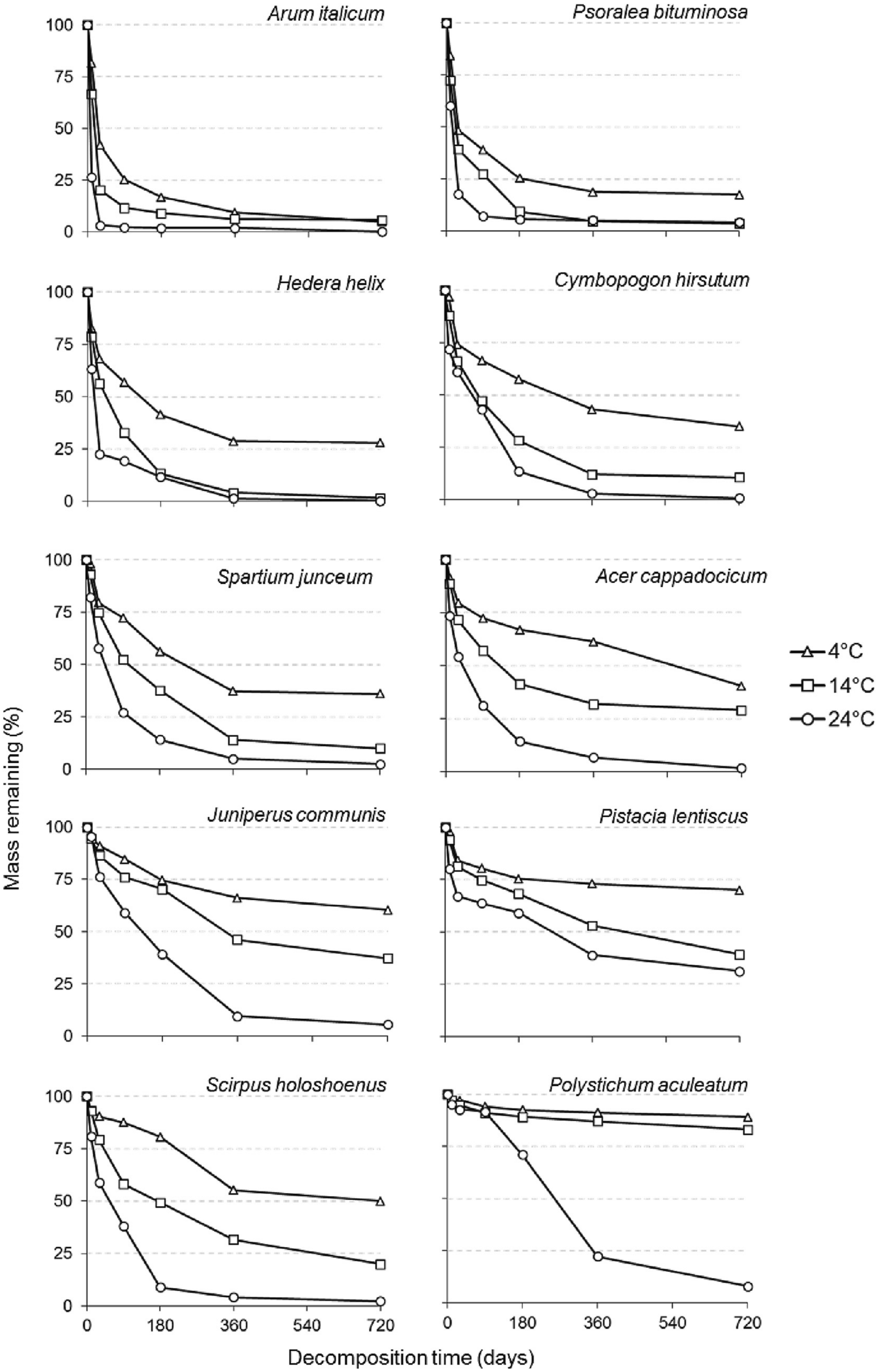

Fig. 3. Mass remaining for leaf litter incubated at three temperatures for two years of ten species representative of the plant functional type: *Arum italicum* – forb; *Bituminaria bituminosa* – herb N-fixing; *Hedera helis* – vine; *Cymbopogon hirsutum* – grass; *Spartium junceum* – woody N-fixing; *Acer cappadocicum* subsp. *lobelii*– woody deciduous; *Juniperus communis* – woody coniferous; *Pistacia lentiscus* – woody evergreen; *Scirpus holoschoenus* – sedge; *Polystichum aculeatum* – fern. Statistics associated with mass remaining are reported in Supplementary Table S4, S5. The mass remaining recorded for the 101 plant species are reported in Supplementary and Table S6.

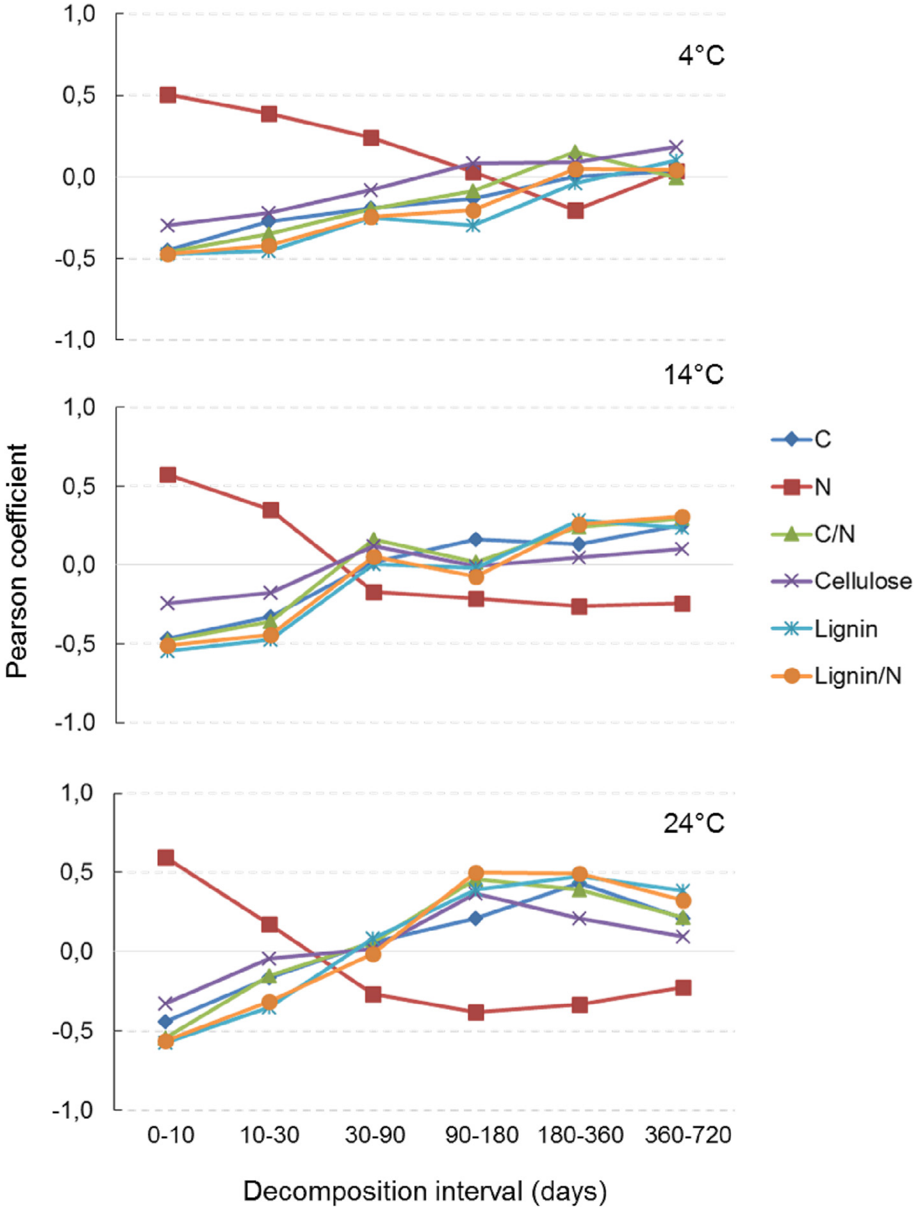

Fig. 4. Correlation (Pearson's r coefficient) between litter chemical traits (i.e., C, N, cellulose, and lignin concentration, C/N and lignin/N ratios) with mass loss of 101 litter types decomposed for two years at three incubation temperatures. For each temperature, correlations are calculated for mass loss recorded in different incubation intervals (i.e., 0–10, 10–30, 30–90, 90–180, 180–360, and 360–720 days).

decomposition in the early stages. The evergreen shrub *P. lentiscus* follows a two-stage decomposition pattern with rapid mass loss in the first 90 days, followed by a long period of slow decomposition, especially when incubated at 4 and 14 °C. Finally, at 4 and 14 °C, the extremely low decomposing fern *P. aculeatum* showed very limited mass loss throughout the incubation period, 11.04 % and 17.83 %, respectively, after two years. In contrast, at 24 °C, *P. aculeatum* follows a three-stage decomposition pattern with very low mass loss in the first 90 days, followed by a rapid mass decline in the following years, with a final stage of slow decomposition until reaching a total mass loss >80 %.

3.3. Linking decay rate with leaf litter traits

The correlation between litter chemical composition and mass loss showed clear and similar trends during the decomposition phases at 4, 14, and 24 °C (Fig. 4). The correlation between litter mass loss and initial N content changed from positive in the very early phase to non-significant at 4 °C and 14 °C and even negative in the middle and later phases when incubated at 24 °C. For all other variables, i.e., cellulose, C, C/N, and lignin/N ratios, the opposite trend was observed, with the correlation, which was positive at the early stage, shifting to non-significant at the later stages of decomposition and even positive in some cases. Specifically, lignin, lignin/N, and C/N ratio show the strongest negative correlation in the early stage at all incubation temperatures, but the shift during decomposition becomes non-significant at 4 and 14 °C, while significant positive correlations were observed in the intermediate and later stages of decomposition when the material was incubated at 24 °C. Finally, cellulose content showed a weak negative correlation with mass loss at the initial stage of decomposition and became non-significant at the later stages at all incubation temperatures.

3.4. Temperature sensitivity of leaf litter decomposition

Mass loss increased significantly with temperature in all plant functional types, with Q_{10} values highest in ferns (2.07), lowest in vines (1.05), and intermediate in the other groups (Fig. 5a). Q_{10} was negatively

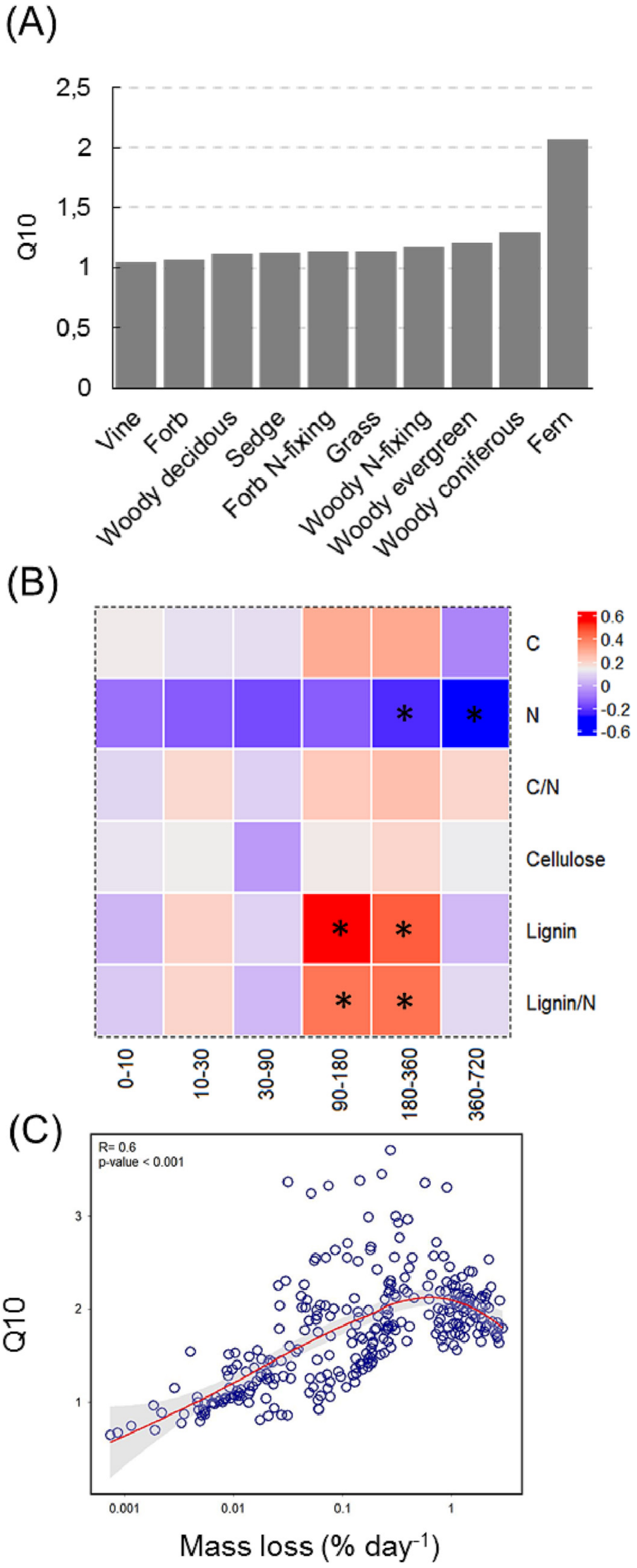

Fig. 5. Temperature sensitivity of leaf litter decomposition. (A) Q_{10} calculated for leaf litter that belong to different functional groups; (B) Heat-map reporting the correlations (Pearson's r) between litter chemical traits and Q_{10} for leaf litter (asterisks indicate statistically significant correlation values, negative or positive; * $P < 0.01$); and (C), relationship between Q_{10} and decay rate of leaf litter calculated as mass loss per day.

correlated with N content at late stages of decomposition (180 to 720 days after incubation), but not with cellulose and C/N ratios (Fig. 5b). On the other hand, a strong negative correlation was found between lignin and lignin/N ratio and Q_{10}, but only in the middle stages of decomposition, i.e., between 90 and 360 days after incubation. Overall, Q_{10} of leaf litter recorded a unimodal, hump-shaped relationship with mass loss (Fig. 5c). Notably, the relationship was asymmetrical with the lowest Q_{10} values found for litter with extremely low mass loss.

4. Discussion

4.1. Litter decomposition and functional plant types: the role of chemical traits

Our experiment, based on one hundred and one species, showed the great variability in the chemical properties of litter and decomposition at the regional level in the Mediterranean region. The chemistry of leaf litter varied greatly in terms of nitrogen content (range 0.52–6.80 %), lignin content (range 1.53–49.31 %), C/N ratio (range 5.21–77.78) and lignin/N ratio (range 0.34–34.90), with cascading effects on decomposition rate and organic carbon storage. According to previous studies, decomposition of leaf litter was positively correlated with initial nitrogen content but negatively correlated with lignin content, lignin/N ratio, and C/N ratio. These results essentially confirm what was demonstrated several decades ago, both with respect to the retarding effect of lignin (Meentemeyer, 1978) and the stimulation of nitrogen decomposition (Taylor et al., 1989) in the early phase of decomposition. Recently, however, the use of the C/N ratio has been criticized in terms of its ability to predict soil ecosystem functions, including the decomposition rate of plant residues (Cartenì et al., 2018). Indeed, the C/N ratio does not define the chemical quality of organic carbon and, therefore, is not able to distinguish between material with similar ratios but composed of different carbons that may be high (e.g., woody debris, large roots, charred residues) or low (e.g., insect fecal matter, microbial remains, fungal hyphae, etc.). In the last decade, numerous studies have shown that C/N and lignin/N ratios are weakly correlated with the decomposition rate of root tissues, especially first- and second-order roots (Goebel et al., 2011; Beidler and Pritchard, 2017; Sun et al., 2018). Furthermore, similar relationships have been observed in studies focusing on leaves within tropical ecosystems (Hättenschwiler et al., 2011) and Mediterranean ecosystems (Bonanomi et al., 2013). In this context, our results help to explain the contradictory results in the literature. First, the positive correlation with the decomposition rate for nitrogen is limited to the early stages and then gradually decreases and even becomes negative in the late stages of the process. Accordingly, previous studies found that external supply of inorganic nitrogen, as well as nitrogen transfer from other litter types or soil, can promote decomposition rates in the early stages but inhibit mass loss of lignin and recalcitrant substrates (Craine et al., 2007; Berg and McClaugherty, 2014). This dual and opposing effect of nitrogen on litter and organic matter decomposition is well established, with a stimulatory effect in the early stages (up to ~40 % of mass loss) and a limiting effect as litter approaches its limits (Berg and Matzner, 1997; Berg, 2018). Looking at the fundamentals of decomposition processes, a large amount of N can support intense microbial activity in the initial stages of decomposition. This activity can rapidly consume readily degradable organic compounds, resulting in rapid mass loss. On the other hand, nitrogen may have an inhibitory effect on the decomposition of persistent organic carbon sources, which is explained by the microbial mining hypothesis. This hypothesis states that the energy required to decompose stabilized organic carbon to obtain nitrogen becomes less valuable to microorganisms seeking such nutrients (Craine et al., 2007). The meta-analysis by Knorr et al. (2005) also supports this inhibitory effect of nitrogen, especially in lignin-rich substrates. Moreover, there is evidence that nitrogen may limit litter decomposition in the late stages by complexing with organic compounds or suppressing the production of ligninolytic enzymes (Berg and Matzner, 1997; Hobbie et al., 2012).

Lignin content, C/N and lignin/N ratios showed an opposite tendency of correlation with decay rate compared to nitrogen. Initially, there was a strong and negative correlation between these factors and decay rate. However, as the decay process progressed, the correlations became non-statistically significant and, in some cases, even positive. Typically, in the initial phase of litter decomposition, low N concentrations and high lignin content may be nitrogen limiting for microbes. Substrates with C/N ratios >30 are likely nitrogen limited (Taylor et al., 1989). Our results support the prevailing view that C/N and lignin/N ratios primarily control litter decomposition in the early stages. In contrast, the positive correlation between the initial C/N and lignin/N ratios and the decomposition rate measured in the late stages could be explained by the observation that lignin-rich litter decomposes slowly in the early stages and has not reached its limit even after 360 days (Berg, 2018). Consequently, after more than a year, these materials are still in an active decomposition phase and losing weight, in contrast to the N-rich and lignin-poor materials that have already reached their threshold. Further studies are needed to investigate the mechanisms, the associated microbiome, and the link between lignin and nitrogen in controlling decomposition, also in conjunction with increasing temperatures. In general, our study highlights the limitations of using C/N and lignin/N ratios to predict litter decomposition rates. In fact, these indices correlate negatively with mass loss only at the initial stages of decomposition, but as the materials approach their limit, the predictive power reverses with the same C/N and lignin/N ratios correlating positively with decomposition rate.

4.2. Temperature sensitivity of decomposing leaf litter

Temperature sensitivity of organic matter is a controversial topic, and theoretical models supported by some empirical evidence suggest that temperature sensitivity increases with substrate recalcitrance (Fierer et al., 2005; Hartley and Ineson, 2008; Conant et al., 2008; Bonanomi et al. 2021a). Contrary to the predictions of theoretical models, our findings reveal a non-linear relationship between temperature sensitivity and litter decomposition. Specifically, we observed a hump-shaped pattern, with lower Q_{10} values found for litter with fast mass loss but also for litter having extremely low decay rate. In other words, biologically stabilized and already decomposed material that has reached or is about to reach its limit value (Berg, 2018) appears to be far less sensitive to an increase in temperature than leaf litter in middle stages of decomposition having intermediate mass loss rate. Overall, our data based on 909 observations (101 litter types at three temperatures and in three decay stages) show that temperature sensitivity is unimodal and hump-shaped, with low sensitivity of both rapidly decomposing fresh litter and stabilized and highly recalcitrant materials. Decomposition is a temperature-dependent process, and the theory of thermodynamics predicts that organic material with high recalcitrancy, which normally resists decomposition, is sensitive to a rise in temperature (Bosatta and Ågren, 1999). Indeed, recalcitrant organic substrates would require a higher activation energy and consequently be more sensitive to temperature than labile, rapidly degradable organic material (Davidson and Janssens, 2006). However, we found that very recalcitrant material reaching its degradation threshold (Berg, 2018) is nearly insensitive to temperature, suggesting that the activation energy above a certain threshold is so high that the process is minimally affected by temperature, at least in the rage testes (4–24 °C). Our data also suggest that this threshold is quantifiable at a decomposition rate of <0.01 % of the mass lost per day, relative to the initial mass.

Regarding the chemistry of litter, our data provide useful information for understanding the complex interaction between temperature and decomposition. During the first 90 days of decomposition, no parameters were significantly correlated with Q_{10}, suggesting that for fresh or partially decomposed litter, chemical properties are not relevant. In fact, these materials exhibit rapid decomposition rates, always exceeding 0.1 % of the original mass loss per day. As decomposition progressed, we found weak but increasing negative correlations between Q_{10} and initial N concentration. A previous study reported that the temperature sensitivity of recalcitrant substrates such as woody debris and root tissue can be masked by N limitation (Bonanomi et al., 2017). Here, we also found the opposite, where materials with high N content were less temperature sensitive late in the process. This is likely due to their very low decomposition rate due to the possible inhibitory effect of this nutrient in the late stages of decomposition, as described above.

Recently, Bonanomi et al. (2021) reported a positive correlation between Q_{10} of leaf and root litter and lignin content. Here, we confirmed and refined these results for a wide range of leaf litter where Q_{10} was positively correlated with lignin content, but only in the intermediate decomposition stages. We also speculate on a possible correlation of this result: low lignin material decomposes rapidly, reaching its threshold in <180 days, and is initially insensitive to temperature. Conversely, lignin-rich litter decomposes relatively slowly at the beginning and have not yet reached their threshold value in the intermediate phases (180–360 days), and in this phase they have the higher Q_{10} value. Finally, in the late stages of the process, all materials, including the lignin- rich ones, approach their threshold value and become insensitive to temperature and exhibit very low Q_{10}. Further studies are needed to confirm these results with other organic materials, such as root and wood residues and organic soil material, and to explore the possible underlying mechanisms.

5. Conclusions

Based on our analysis of one hundred and one species, our experiment reveals a significant degree of variability in the chemical properties of litter and decomposition at the regional scale in the Mediterranean region. Our findings demonstrate that leaf litter exhibits considerable variation in N content, lignin content, C/N ratio, and lignin/N ratio, which can significantly affect the rate of decomposition and organic carbon storage. While prior research has shown that decomposition of leaf litter is negatively correlated with lignin content, lignin/N ratio, and C/N ratio in the early decomposition stages, our study found the opposite to be true in the later stages of decomposition. Additionally, our results suggest that N concentration is positively correlated with decomposition rate, but only in the early stages of decomposition. Our study emphasizes the limitations of using C/N and lignin/N ratios to predict litter decomposition rates. While previous research has indicated that these indices are negatively correlated with mass loss, this relationship only holds in the early stages of decomposition, with the opposite being true as the materials approach their limit. Furthermore, we found that temperature sensitivity, as measured by Q_{10}, exhibits a hump-shaped relationship with decomposition rate, differently to what was expected. Notably, our research presents a new finding that warrants consideration and future inclusion in modeling efforts aimed at simulating organic carbon dynamics at the ecosystem level.

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Giuliano Bonanomi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Supervision. **Riccardo Motti:** Conceptualization, Validation, Resources, Writing – review & editing. **Anna De Marco:** Methodology, Validation, Investigation, Writing – review & editing. **Mohamed Idbella:** Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization.

Data availability

I have uploaded Table S6 (Excel table) that included the used data

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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