



# Dose-dependent positive-to-negative shift of litter effects on seedling growth: a modelling study on 35 plant litter types

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## Abstract

Modelling the inter-relationships between litter accumulation and plant–soil feedback is a major challenge to predict natural and agricultural ecosystem dynamics. At increasing levels of undecomposed plant litter, seedling growth tends to show a multi-faceted response trend, characterised by a peak of positive stimulation at lower dosage followed by inhibition at higher dosage. In this study, a new logistic model was developed to describe such trend and disentangle substrate-specific positive and negative effects of plant litter. The model was tested on 35 litter types applied to the common phytometer *Lepidium sativum*; all litter types were collected in Mediterranean shrublands of Campania region (southern Italy). Model fitting resulted to be generally higher relative to the widely used linear log response model, although in only half of the cases it also gave more parsimonious results in terms of minimising information loss. Positive and negative effects of plant litter resulted to be uncorrelated, showing that the overall trend is probably the result of the combined action of separate factors rather than the effect of a single factor behaving differently at the different doses. The results of this work provide new tools to finely tune the optimal doses in experiments on hormesis and litter phytotoxicity, through the identification of the most suited doses to centre the range of nearly linear response to litter concentration. A wide screening is also presented on the phytotoxicity profiles of a number of spontaneous plant species widely distributed in the Mediterranean area.

**Keywords** Allelopathy · Autotoxicity · Dose–response · Facilitation · Hormesis · Logistic model · Non-linear modelling

## Introduction

Dead leaves in the soil litter layer can widely affect plant growth, with magnitude and direction of such effects ranging between positive stimulation and negative, inhibitory effects (Rice 1984; Facelli and Pickett 1991; Xiong and Nilsson 1999; van der Putten et al. 1997; Blum et al. 1999; Bonanomi et al. 2006; Campanella et al. 2019). Negative effects of undecomposed litter have been reported either towards heterospecific (allelopathy) or towards conspecific organisms (self-inhibition or autotoxicity). The former,

allelopathy, has been widely studied, not only in relation to interactions in agricultural systems (Kruse et al. 2000; Cheng and Cheng 2015) but also in a wider ecological context (Kruse et al. 2000; Meiners et al. 2012), particularly for its possible relation with alien species invasion (Callaway and Haschehoug 2000; Ridenour and Callaway 2001). On the other side, autotoxicity deserves a higher recognition in community ecology for its importance in pattern formation, species coexistence, and successional dynamics (Singh et al. 1999; Vincenot et al. 2017; Rietkerk 2022). Recently, self-inhibition has been recognised as a major underestimated factor involved in several biological processes (Mazzoleni et al. 2015a, 2015b; Chiusano et al. 2021; Lanzotti et al. 2022), induced to a great extent by the effects of extracellular DNA (exDNA) released by undecomposed residuals in soil environment (Carteni et al. 2016).

Research on negative effects of undecomposed litter has been a rapidly developing field during recent years, providing new perspectives in theoretical and applied biology (Bonanomi et al. 2005a; Dorrepaal et al. 2007; Lopez-Iglesias et al. 2014; Meiners 2014; Bonanomi 2021). Initially,

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negative plant–soil feedback was mostly attributed to secondary allelopathy, i.e. the negative effect on plant growth determined by the release of toxic metabolites produced as a defence against herbivory (Wink 2003; Bonanomi et al. 2005b; Mazzoleni et al. 2007, 2010). According to this hypothesis, toxic metabolites, which in green leaves are compartmentalised in cellular structures, are gradually released by dead leaves into the soil as far as microbial decomposition proceeds, giving rise to inhibitory syndromes by the so-called secondary allelopathic factors (Rice 1984). More recently, however, the hypothesis that these effects could also depend on the toxicity of extracellular DNA released through the cell lysis, whose effects would be quite more accentuated with self DNA compared to heterologous DNA, was affirmed and supported by a wide series of experiments spanning in different fields of biology (Mazzoleni et al. 2015a, 2015b; Carteni et al. 2016; Monticcolo et al. 2020; Bonanomi et al. 2022; Germoglio et al. 2022; Palomba et al. 2022).

Beside negative effects, plant litter is also known to stimulate plant growth (Facelli and Pickett 1991; Xiong and Nilsson 1999), with positive plant–soil feedbacks mostly depending on nutrients release and on the improvement of soil moisture or to biostimulator effect (Bonanomi et al. 2021). This concurrence of positive and negative interactions may partly mask their separate contribution to the overall net effect, as the extent to which these two factors are represented may vary depending on several elements, such as the sensitivity of the species tested, its phenological stage, the associated microbiota, and the degree of disturbance and/or stress the plant is undergoing. A crucial point to clarify to fully understand the response of plants exposed to decaying materials is to disentangle as much as possible these two contrasting contributions of opposite sign and trying to provide a separate estimation of their effect sizes as independent processes.

Different litters can be compared experimentally for both their positive and inhibiting effects by submitting the same indicator plants (phytometers) to standard concentrations of the litters tested. As toxicity levels can span over extremely different scales from plant to plant, the choice of the appropriate concentration for such a comparative experiment is crucial for the success of the experiment itself. In general, when wide-ranging screenings have to be performed, the prevailing trend in toxicological research is to compare response curves along logarithmic concentration gradients of the toxic agent rather than comparing responses to a given concentration level of that agent, which makes crucial to previously define the expected response pattern in order to adequately model and compare the phytometers responses. Previous research on the dose–response pattern of plants growing under different concentrations of undecomposed litter suggests that positive effects of litter are mostly detectable at very low concentration

levels, whilst negative effects tend to be found at increasing substrate concentrations (Zucconi 1994; Li et al. 2021). This model corresponds to a more general pattern, described in other field of biology as “hormesis” (Calabrese and Baldwin 2003; Costantini et al. 2010, 2012; Calabrese 2014). The analysis of hormetic phenomena also poses the relevant question of verifying whether the positive effects at lower doses are inherently linked to the toxicity level expressed at higher dosage or are just the result of separate processes whose combined effect determines the overall pattern.

The mathematical formalisation and the experimental testing of this kind of dose–response pattern has been extensively investigated (Brain and Cousens 1989; An et al. 1993; Liu et al. 2003, 2011; Tang et al. 2019). The approach common in toxicology of linearizing the response curves against the logarithm of the dose, as well as any simple logistic or other monotonic model, has relevant intrinsic limits, as being unsuited to finely tune the peak of positive performance, the shift towards a negative trend, and its final asymptotic stage. The most appropriate model for describing hormetic dose–response trends and disentangling their opposite components is still unresolved (Brain and Cousens 1989; An et al. 1993; An 2005; Liu et al. 2011; Tang et al. 2019).

In this work, following an approach already proposed by Liu et al. (2011), we tried to define a suitable non-linear model for plant response to litter concentration by combining two independent logistic models, the first one accounting for positive and the second one for negative effects of litter on plant growth. Trying to refine the model proposed by Liu et al. (2011), we reformulated the logistic equations in order to directly relate the equations parameters to the biological variables implied in the overall phenomenon, so that the results of model fitting could be better used for further comparative analysis. Finally, we tested this model using 35 litter types that span a wide range of litter chemical traits against *Lepidium sativum* used as phytometer. The aims of the work were as follows: i. to compare the overall fitting of this model with the corresponding fitting of the log-dose–response regression on a reasonable number of litter types; ii. to disentangle positive and negative effects of plant litters and provide a separate estimate of their relative contribution to phytometer performance; and iii. to evaluate whether negative and positive effects of plant litter behave as intercorrelated patterns depending on the same causal factors or as uncorrelated effects just occurring simultaneously.

## Materials and methods

### Experimental trial

An experimental dataset was obtained by analysing the response of the phytometer *Lepidium sativum* to 35 litters

of wild plant species. The green foliage of 30 species was collected in the spring–summer period in Mediterranean shrubland areas of Campania region (southern Italy). For some of these species, in addition to the green foliage, also senescent and partially decomposed leaves were tested, in the hypothesis that higher positive and lower negative effects should be found with the progressive litter decomposition, and specifically to test the model under a variable range of positive-to-negative combinations. In particular, for grass species not provided with abscission layer, leaves were collected either green, senescent (yellowish standing leaves), or decomposed (brownish standing necromass not yet touching ground level). Thus, the total number of tested litter types was 35, whose species, types, and labels are reported in Table 1.

The collected foliage was dried in an oven at 50° C till constant weight was reached and then pulverised at < 2-mm fragments using a steel blade mini blender. The material thus obtained was stored at room temperature in closed plastic containers. After completing the pilot experiments, the main trial was conducted. Non-perforated cylindrical pots were filled with 50 g of a substrate consisting of washed and sieved sand mixed with variable quantities of pulverised litter, at 6 different litter concentrations (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4% m/m, respectively). Seeds of *L. sativum*, a fast germinating species highly sensitive to phytotoxins (Gehring et al. 2003), were buried in this substrate at a depth of 5 mm. The material was kept damp for the entire duration of the experiment, and placed in a growth chamber with alternating light and temperature conditions, with 12/12 h of light/dark and a corresponding 20°/15° C thermoperiod. Controls were set-up in pots containing 50 g of inert substrate (sand) without any addition of litter, kept under the same conditions of litter-treated replicates. The test was replicated on 10 seeds per treatment. The number of emerging seedlings was counted on a daily basis, noting all mortality events. After 8 days from sowing, all emerged seedlings were sampled to measure the maximum root and shoot lengths. Subsequently, the plant material was oven-dried till reaching a constant weight, for subsequent determination of the overall shoot and root dry weights. Raw data are available in ESM-1A, with a visual representation of the experimental design in ESM-1B.

## The logistic model

The model proposed in this work was conceived to account for both positive and negative effect components and to disentangle as much as possible these two concurrent effects. The two opposite trends were treated as independent effects and merged in one single equation. So, in the most general approach, the model can be represented as follows:

**Table 1** Tested litter species/type with labels used in this paper

Species	Litter type	Label
<i>Acanthus mollis</i>	Green	Aca-g
<i>Acanthus mollis</i>	Senescent	Aca-s
<i>Ampelodesmos mauritanica</i>	Green	Amp-g
<i>Ampelodesmos mauritanica</i>	Senescent	Amp-s
<i>Arbutus unedo</i>	Green	Arb
<i>Broussonetia papyrifera</i>	Green	Bro
<i>Castanea sativa</i>	Green	Cas
<i>Celtis australis</i>	Green	Cel
<i>Cistus monspeliensis</i>	Green	Cis
<i>Coronilla emerus</i>	Green	Cor
<i>Dactylis glomerata</i>	Decomposed	Dac-d
<i>Dactylis glomerata</i>	Green	Dac-g
<i>Dactylis glomerata</i>	Senescent	Dac-s
<i>Daphne gnidium</i>	Green	Dap
<i>Eucalyptus globulus</i>	Senescent	Euc
<i>Fagus sylvatica</i>	Green	Fag
<i>Festuca drymeia</i>	Green	Fes-g
<i>Festuca drymeia</i>	Senescent	Fes-s
<i>Fraxinus ornus</i>	Green	Fra
<i>Hedera helix</i>	Green	Hed
<i>Juniperus communis</i>	Green	Jun
<i>Myrtus communis</i>	Green	Myr
<i>Nerium oleander</i>	Green	Ner
<i>Phillyrea angustifolia</i>	Green	Phy
<i>Picea abies</i>	Green	Pic
<i>Pinus pinea</i>	Green	Pin
<i>Pistacia lentiscus</i>	Green	Pis
<i>Populus alba</i>	Green	Pop
<i>Quercus ilex</i>	Green	Qui
<i>Quercus pubescens</i>	Green	Qup
<i>Robinia pseudoacacia</i>	Green	Rob
<i>Salix alba</i>	Green	Sal
<i>Teucrium chamaedrys</i>	Green	Tec
<i>Teucrium polium</i>	Green	Tep
<i>Viburnum tinus</i>	Green	Vib

sen: standing senescent leaves; dec: fully decomposed

$$P(x) = \Phi[P_0, F(x), I(x)], \quad (1)$$

where  $P(x)$  represents the performance of the plant as a function of the concentration  $x$  of the litter,  $P_0$  is the value expressed by the control replicates in absence of litter,  $F(x)$  is the relative increase of performance depending on litter, and  $I(x)$  is a negative component accounting for the relative inhibition induced by litter toxicity. In our approach, the overall performance was supposed to depend on the plant potential in absence of litter effect,  $P_0$ , corrected by a factor including both positive and negative litter effect, here treated as two independent functions of litter concentration. The

overall performance as a function of litter dose was then first modelled as follows:

$$P(x) = P_0 \cdot [1 + F(x) + I(x)]. \quad (2)$$

Our first experimental observations allowed us to hypothesise that both positive and negative effects, if separated, could fit quite well a logistic type trend, characterised by an exponential increase (or decrease) in the first sections of the curve, tending to be linear around the inflection point and then becoming asymptotic in the final saturation zone. So, we chose to follow an approach already proposed by Liu et al. (2011), using two separate logistic curves to model the two contrasting effects as separated processes. Differing from Liu's approach, we reformulated the proposed equation to obtain explicit parameters more closely linked to litter effects on plant performance.

As the first observed effect of litter was the increase of plant performance at the lower litter doses, we first defined a logistic equation for positive relative effects as:

$$F(x) = \frac{F_{\max} - 1}{1 + \left(\frac{F_{50}}{x}\right)^\alpha}, \quad (3)$$

where  $F_{\max}$  represents the highest increased percent performance relative to control depending on facilitative process (with  $F_{\max} \geq 1$ ),  $F_{50}$  represents the dose that induces a 50% performance increase, relative to maximum performance, and  $\alpha$  is an arbitrary positive coefficient linked to the slope of the curve at the inflection point. This sigmoidal trend is consistent, for instance, with a positive response to the supply of a limiting nutritional factor, characterised by a starting exponential increase of plant performance followed by an asymptotic trend at saturation.

Assuming that  $F_{\max}$  is the highest percent performance relative to control, we then defined the maximum percent inhibition as follows:

$$I_{\max} = \frac{O_{\min} - F_{\max}}{F_{\max}}, \quad (4)$$

where  $O_{\min}$  (with  $0 \leq O_{\min} \leq F_{\max}$ ) represents the overall resulting asymptotic value of the plant performance under the combined action of the facilitative and the inhibiting processes, expressed as a fraction of control value. Note that, with the given definitions, it results that  $-1 \leq I_{\max} \leq 0$ , pointing out  $I_{\max}$  as a non-positive factor expressing the percent inhibiting power of a given litter, ranging from the total inhibition ( $I_{\max} = -1$ ) to the null effect ( $I_{\max} = 0$ ).

With these premises, the negative logistic model can be described as follows:

$$I(x) = F_{\max} \cdot \frac{I_{\max}}{1 + \left(\frac{O_{50}}{x}\right)^\alpha} = \frac{O_{\min} - F_{\max}}{1 + \left(\frac{O_{50}}{x}\right)^\alpha}, \quad (5)$$

where  $O_{50}$  is the dose inducing a performance decrease equal to 50% of the difference between the theoretical maximum and the minimum overall performance. This last parameter is quite relevant, as it allows to predict the average dose at which the negative response of the phytometer tends to be linear to the litter concentration, allowing to optimise the dosage for any factorial experiment in which a linearity of the response is expected. The negative model presented in Eq. (5) is also consistent with the effect of a toxic factor able to inactivate one or more functions of the phytometer, producing a progressive decrease of plant performance as litter concentration increases, till reaching a final, residual plant performance once the given functions has been totally deactivated.

By combining the three previous equations, we finally obtained the overall model:

$$P(x) = P_0 \left[ 1 + \frac{F_{\max} - 1}{1 + \left(\frac{F_{50}}{x}\right)^\alpha} + \frac{O_{\min} - F_{\max}}{1 + \left(\frac{O_{50}}{x}\right)^\alpha} \right]. \quad (6)$$

The symbols used in this model are synthesised in Table 2.

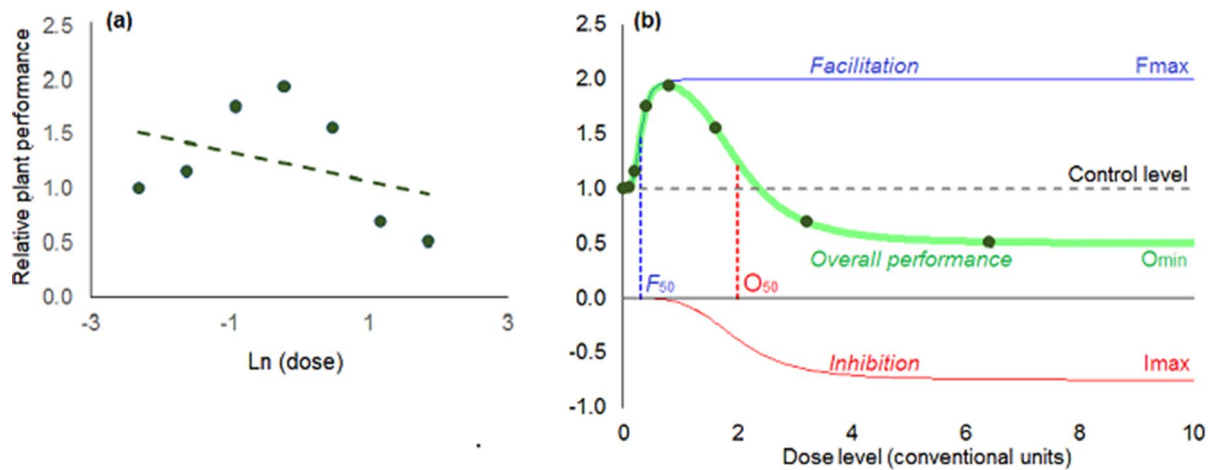
Once the reference control value ( $P_0$ ) is experimentally determined, the model depends on 5 parameters. By modulating opportunely these parameters, it can fit a wide range of experimental datasets, characterised by either a classical monotonic logistic pattern or by hormetic behaviour (Fig. 1). Moreover, by analysing the function properties, the first derivative can be calculated to determine curve slope in any point and peak (if any) coordinates (ESM-1C).

## Data analysis

Maximum root length was the main parameter used to test the logistic model, given the higher plasticity shown by this attribute in response to the treatments. Data were first normalised by dividing each single measure for the average values found on control replicates of the same block. Variance analysis with two main factors (litter species and doses) was used to determine the significance of main effects and interactions. A cluster analysis and a principal coordinate analysis were then conducted with the package Syntax per Windows (Podani 1994), to classify the response patterns in a limited number of general types. To this aim, a matrix was built with litter types as the objects and the average performances of the phytometer as the variables. Euclidean distance and further neighbours were used as measure of dissimilarity and agglomeration criterion, respectively.

**Table 2** Symbols used in the logistic model, with corresponding attributes

Symbol	Description	Unit	Range limits
$P(x)$	The overall performance as a function of the doses ( $x$ ) of litter applied	Depending on the variable tested (e.g. g for biomass and cm per root length)	Depending on the variable tested, in the most general cases: $P(x) \geq 0$
$x$	Independent variable of the model, representing the litter dose in the growth substrate	In this experiment: % (m/m) (g of litter per 100 g of total substrate)	$x \geq 0$
$P_0$	Control value obtained in absence of litter, i.e. when $x = 0$	Depending on the variable tested (e.g. g for biomass and cm per root length)	Depending on the variable tested, in the most general case: $P_0 \geq 0$
$F_{max}$	The maximum potential relative increase of phytometer performance due to litter type in absence of any negative effect	Adimensional	$F_{max} \geq 1$ In the fitting procedure, a conventional limit was imposed so that $F_{max}$ never exceed 125% of the highest measured datum
$O_{min}$	The final asymptotic value of the model, representing the overall combined result of positive and negative effects of litter	Adimensional	$0 \leq O_{min} \leq F_{max}$ In the fitting procedure, a conventional limit was imposed so that $O_{min}$ never be lower of 75% of the lowest measured datum
$F_{50}$	Dose at which 50% of max potential facilitation by litter is reached	The same unit used for the independent variable $x$ In this experiment: % (m/m)	$0 < F_{50} < O_{50}$ In the fitting procedure, both $F_{50}$ and $O_{50}$ conventionally limited to the range of the tested doses ( $0 < F_{50} < 6.4$ , in this experiment)
$O_{50}$	Dose at which the intermediate point between $F_{max}$ and $O_{min}$ is reached	The same unit used for the independent variable $x$ In this experiment: % (m/m)	$O_{50} > F_{50}$ In the fitting procedure, both $F_{50}$ and $O_{50}$ conventionally limited to the range of the tested doses ( $0 < O_{50} < 6.4$ , in this experiment)
$\alpha$ (alpha)	A positive coefficient linked to the slope of the curve at the inflections points. The highest its value, the sharper the curve	Adimensional	$\alpha > 0$ In the fitting procedure, a further limit was imposed not to obtain biologically meaningless very sharp curves, conventionally set as $\alpha < 8$



**Fig. 1** Comparison of the two modelling approaches. Two alternative models are interpolated to a conventional dataset (green filled circles) showing growth stimulation at low litter concentration and increasing toxicity at higher litter concentration. **a** This dataset is poorly fitted by a classical linear log(dose) interpolation (dotted line), given the non-monotonic pattern shown by the dataset. **b** The logistic model shows a better interpolation to the same dataset. The overall performance of the interpolated model (green line) is the resulting effect of a positive logistic response, starting to effect at very low dosage (blue line)

The average values obtained at the end of the experiment for each species at the different litter doses were then introduced as input data for modelling. In the first step of the procedure of model fitting, excel macro tools were implemented and used to explore the datasets. This first step of the analysis allowed us to obtain a starting set of parameters with a sufficiently good result in terms of low residuals. Then, the best fitting was obtained by entering the data, the equation, and the starting parameters in the iterative model fitting procedure of the package SPSS per windows. Sequential quadratic programming with bootstrap estimation of standard error was the algorithm used, with sum of squared residuals as the loss function (i.e. the function that was minimised through the sequential procedure). This procedure allows to determine the best values of the equation parameters to give the highest possible fitting inside the limits previously set for the range of variation of the parameters. In particular, in order to avoid the system to give output models too distant in their predictions from the measured data, limits were imposed to the range of variation of the equation parameters so that  $F_{\max}$  could not exceed 125% of the highest recorded datum,  $O_{\min}$  could not be lower than 75% of the lowest recorded datum,  $F_{50}$  and  $O_{50}$  should both stay into the range of the tested doses ( $0 \div 6.4\%$ ) and  $\alpha$  should not exceed a conventional limit value fixed at 8. The iterative procedure so described also provides a bootstrap estimate of parameters' standard error, basing on the number of iteration needed to converge towards the final output; moreover, it provides an estimate of the goodness of fitting as

and an inhibitory effect acting at relatively higher dosage (red line).  $F_{\max}$  is the highest possible relative increase relative to control value if inhibition was not present. Similarly,  $I_{\max}$  is the highest relative decrease that the given factor can induce on plant growth.  $O_{\min}$  is the value of plant performance expected in the final asymptotic sector, resulting from the combined stimulative and inhibitory actions.  $F_{50}$  and  $O_{50}$  represent the doses at which 50% stimulation or inhibition are found, respectively

$$R^2 = 1 - (\text{residual sum of squares})/(\text{corrected sum of squares}).$$

The coefficients of determination so obtained were finally compared with the corresponding  $R^2$  values obtained by the use of a linear regression on the logarithm of the dose, in order to evaluate to what extent the proposed model could improve the fitting obtained with a traditional linear-logarithmic procedure. Of course, as parameters estimates in non-linear model cannot be used in the same way as in a linear regression analysis, this comparison is to be intended as a qualitative rather than a quantitative assessment of the best fitting between the two procedures.

In a further step, a comparison between the two models was attempted, based not only on the goodness of fitting, but also on the level of parsimony in minimising information loss (Akaike 1973). Specifically, the difference between the respective sums of the residual squares of the two models was weighted by the number of explanatory parameters of the two models, according to the formula:

$$AIC = n \cdot \ln(SSr) + 2K, \quad (7)$$

where AIC is an estimate of Akaike's information criterion based on least squares method,  $n$  is the sample size,  $SSr$  is the residual sum of squares of each of the two models, and  $K$  is the number of predicting parameters of each of the two models. This function was calculated for the two models in each fitting and then the difference between the two values

was kept as the indicator of the most parsimonious model as for minimising information loss.

## Results

### Overall results of the experimental trial

Overall, when all litter types were analysed together for their effects on the phytometer, the higher levels of litter concentration induced a considerable reduction of plant performance, although this effect varied greatly from species to species. In the global average, increasing litter concentrations induced a progressive reduction of germinating seeds per pot, till reaching 56% of control values at the higher dose (Fig. 2a), a reduction in mean root length (till 45% of control) (Fig. 2b), and a slight progressive decrease of stem length (up to 74% of control value) (Fig. 2c).

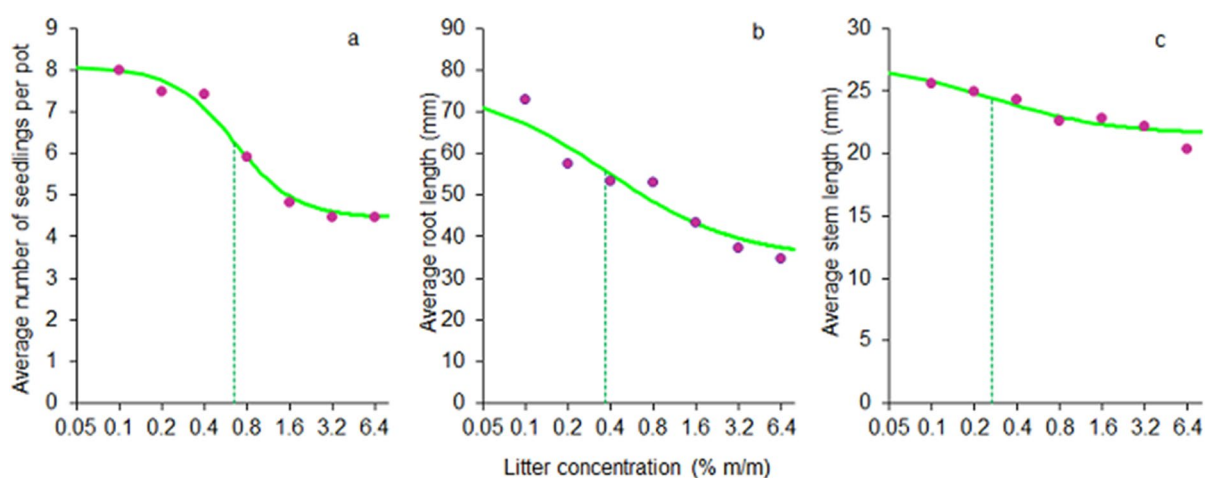
Whilst the overall trends only accounted for the toxic effects of litters on plant growth, when analysing the details referred to the single litter types, several of the tested litter types revealed the ability to produce stimulation effects at lower concentration. Referring to root length data, a great variability of response was found, going from dramatically toxic to positive response of the phytometer. In particular, at least 16 litter species out of 35 resulted able to induce a positive response at lower concentration, whose effect size were higher in senescent foliage, till reaching values up to 300% relative to control values in the case of highly decomposed grass litter.

A good synthesis of the different responses recorded was obtained with multivariate analysis techniques. Cluster analysis applied to root length of phytometers revealed

the presence of 3 main clusters and several sub-clusters of toxicity profile (Fig. 3a).

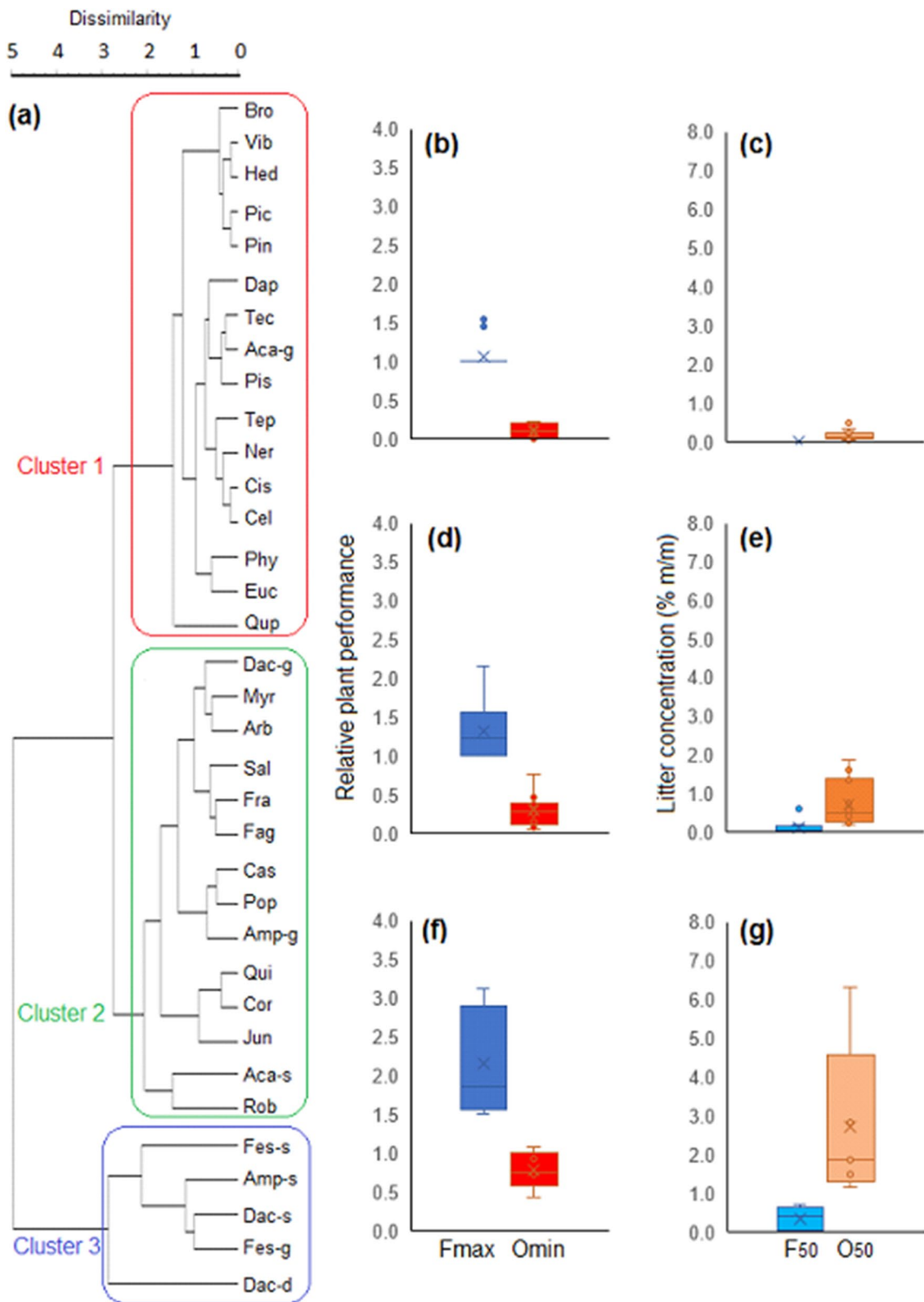
The highest inhibition was found in cluster 1 (Fig. 3b and 3c), with only two litter types showing some stimulative effect at low dosage and with an overall very strong inhibition at higher doses ( $O_{\min} = 0.1$ ,  $O_{50} = 0.2\%$  m/m, in the averages). In the second cluster, a light stimulation was found at very low doses (average  $F_{\max} = 1.3$ ; average  $F_{50} = 0.1\%$  m/m; Fig. 3d and 3e), with considerable inhibition at relatively low doses (average  $O_{\min} = 0.3$ ; average  $O_{50} = 0.7\%$  m/m). Cluster 3 mainly included grass litters, some of which were collected in an advanced stage of decomposition. Litter effects on phytometer root growth were significantly stimulative at low doses (average  $F_{\max}$ : 2.2; Fig. 3f and 3g) and just slightly inhibitory at higher doses (average  $O_{\min}$ : 0.8). In this cluster, 50% of max stimulation was reached with an average  $F_{50}$  dose of 0.3% (m/m), whilst 50% of overall inhibition was given at the average  $O_{50}$  dose of 2.7% (m/m).

Principal coordinate analysis (Fig. 4) revealed the presence of a principal axis explaining about 76% of the overall variability, mainly related to decreasing litter toxicity and increasing stimulating effect, with a relatively lower importance of axis 2 (10%) and 3 (5%). Litter species giving the highest values of stimulation were plotted on the right quadrant, with one only litter species showing no inhibition at all, namely decomposed *Dactylis glomerata*, scattered in a relatively isolated position. The most strongly inhibiting litters were plotted on the left quadrant, whilst litters showing slight stimulation at low doses and slight inhibition at higher doses were found in the central position.



**Fig. 2** Overall trends of phytometers growth in response to all 35 litter types plotted together, at increasing litter concentration (% mass/mass). Plant response was measured in terms of average number of seedlings per pot (a), average max root length (b), and average stem

length (c). Vertical dotted lines point to the litter concentration levels that determine a performance decrease equal to 50% of the difference between control value and the minimum overall performance. Note the logarithmic scale on x-axes





**Fig. 3** Results of a cluster analysis applied to phytometers final root length in response to 35 litter species  $\times$  7 litter concentration doses. **a** Dendrogram showing three main cluster (1, 2, and 3). **b, d, f** Boxplots showing the overall distribution of the values assumed by 2-model parameters ( $F_{max}$ —the maximum relative increase of phytometer performance—and  $O_{min}$ —the final asymptotic value of the model) amongst the species belonging to each of the three main clusters (**b** cluster 1; **d** cluster 2; **f** cluster 3). **c, e, g**— Boxplots showing the overall distribution of the values assumed by 2-model parameters ( $F_{50}$  and  $O_{50}$ ) amongst the species belonging to each of the three main clusters (**c**: cluster 1; **e**: cluster 2; **g**: cluster 3)

### Model fitting

The comparison between the two tested models (logistic vs log-dose) showed that adjusted  $R^2$  values of the non-linear logistic models were generally higher relative to the corresponding  $R^2$  values of ln dose regressions, with the exception of just few cases (Table 3).

In only 5 cases out of 35,  $R^2$  values of log response–dose models were higher than the corresponding adjusted  $R^2$  values of logistic models, but these gaps were quite low, with differences ranging from 0.01 to 0.08. These few cases were mostly linked to curve responses lacking any stimulative effect and showing a relatively linear response in the decreasing sector of the curve. Curve fitting values were overall equivalent or slightly favourable to logistic model in about 11 cases, with relative advantage of logistic-adjusted  $R^2$  values not exceeding 0.07 compared to log response model. In all the other cases (19 litter types) curve fitting was considerably higher with the logistic interpolation than with log response model, with differences reaching very high values when a stimulative effect was found at intermediate litter concentration, as in the case of *Dactylis glomerata* litter collected at intermediate decomposition level; in presence of oscillating patterns in the final sector, as in the case of *Teucrium polium*; or with a relevant horizontal trend in the final asymptotic trait, as for *Juniperus communis*.

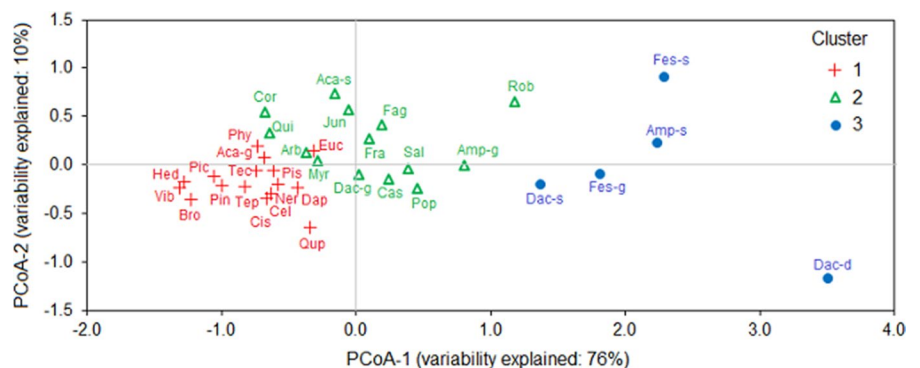
However, when the two models were compared in deep by also taking in account an estimate of the Akaike’s information criterion (AIC), in only half of the cases the best fittings

obtained with the logistic models did also correspond to a more parsimonious result in terms of minimising the information loss (Table 4). These cases were mostly corresponding to datasets, including a positive stimulation, although in some cases also purely inhibitive patterns very well fitted by the logistic model got a better result in terms of Akaike’s criterion compared to log-linear model.

For a wider and more detailed comparison between logistic and log response model fitting, the whole repertory of figures is shown in the supplementary material (ESM-1D, logistic curves, and ESM-1E, log regression lines). A quite interesting example of how decomposition time can affect the relative suitability of the two models is given by the analysis of phytometer response to *Dactylis glomerata* litter at different stages of decomposition (Fig. 5). Similarly to the other tested grasses (reported in supplementary materials ESM-1D and 1E), green leaves of *Dactylis* showed a certain degree of toxicity, although not very strong (Fig. 5a). This toxicity tended to decrease, and some stimulation at low dosage appeared, when the same litter was tested at intermediate decomposition time (Fig. 5b), whilst strong stimulation occurred when litters were collected in an advanced stage of decomposition (Fig. 5c). When these three response patterns were analysed with the two alternative models, the adjusted  $R^2$  values of logistic fitting always exceeded  $R^2$  values of the corresponding log response models (Fig. 5d, 5e, and 5f), but the relative advantage of the logistic model reached the highest level with the highly non-monotonic response to partly decomposed litter (Fig. 5b vs 5e), whilst the two models tended to show closer fitting values when phytometer response was either monotonically decreasing (Fig. 5a vs 5d) or mainly increasing (Fig. 5c vs 5f) along the axis of litter concentration.

When looking for correlation between stimulating and inhibiting effects amongst litters, maximum performance values ( $F_{max}$ ) resulted widely uncorrelated to the corresponding values of maximum inhibition ( $I_{max}$ ) ( $R^2 < 0,014$ ). Of course, only the litters producing a stimulation effects ( $F_{max} > 1$ ) were included in this analysis, as in absence of

**Fig. 4** Principal coordinate analysis applied to phytometers final root length in response to 35 litter species  $\times$  7 litter concentration doses. Different markers were used for species belonging to each of the three clusters found with cluster analysis: red cross: cluster 1; green triangle: cluster 2; blue circle: cluster 3



**Table 3** Main parameters of the logistic models applied to the 35 tested litter types, with R<sup>2</sup> values of the corresponding log response models, for comparison

Litter label	Fmax		Omin		F50		O50		α (alpha)		Logistic		LnDose	
	Value	S Err	Value	S Err	Value	S Err	Value	S Err	Value	S Err	Adj R <sup>2</sup>	Loss f	R <sup>2</sup>	Sig
Aca-g	1.00	-	0.00	0.05	-	-	0.35	0.08	0.96	0.20	0.91	0.05	0.94	0.000
Aca-s	1.89	0.14	0.15	0.16	0.19	0.11	0.40	0.34	8.00	0.51	0.87	0.29	0.66	0.026
Amp-g	1.57	0.19	0.27	0.44	0.60	0.63	1.88	1.07	1.58	2.93	0.71	0.18	0.60	0.041
Amp-s	1.63	0.10	1.08	0.11	0.00	0.00	1.88	0.61	5.38	1.90	0.74	0.13	0.52	0.067
Arb	1.00	-	0.29	0.06	-	-	0.26	0.19	1.83	3.13	0.82	0.08	0.69	0.021
Bro	1.00	-	0.00	0.00	-	-	0.03	0.03	0.64	0.20	0.82	0.02	0.89	0.001
Cas	1.00	-	0.37	0.06	-	-	1.43	0.69	8.00	2.61	0.49	0.23	0.42	0.114
Cel	1.00	-	0.19	0.08	-	-	0.06	0.06	0.58	2.06	0.76	0.02	0.75	0.012
Cis	1.00	-	0.21	0.05	-	-	0.03	0.09	0.52	1.08	0.66	0.02	0.63	0.034
Cor	1.22	0.15	0.07	0.03	0.00	0.02	0.29	0.08	4.54	1.28	0.99	0.01	0.77	0.009
Dac-d	3.12	0.52	0.75	2.59	0.57	0.44	6.33	1.42	1.47	2.67	0.87	0.21	0.47	0.088
Dac-g	1.00	-	0.07	0.12	-	-	1.61	1.32	0.89	1.56	0.52	0.29	0.47	0.089
Dac-s	1.51	0.21	0.43	0.70	0.40	0.17	2.83	0.66	4.81	1.42	0.98	0.02	0.23	0.278
Dap	1.00	-	0.00	0.11	-	-	0.34	0.39	0.39	1.99	0.28	0.29	0.28	0.223
Euc	1.55	0.14	0.16	0.19	0.00	0.99	0.16	0.21	1.00	2.64	0.72	0.25	0.71	0.180
Fag	1.37	0.18	0.38	0.14	0.00	0.35	0.33	0.39	1.31	3.15	0.78	0.17	0.72	0.016
Fes-g	2.68	0.29	0.72	0.13	0.73	0.92	1.48	0.91	8.00	1.98	0.75	0.42	0.19	0.327
Fes-s	1.86	0.29	0.93	0.50	0.05	0.08	1.15	0.80	8.00	0.76	0.47	1.57	0.18	0.344
Fra	1.19	0.09	0.17	0.05	0.02	0.09	0.73	0.16	1.20	0.14	1.00	0.00	0.99	0.000
Hed	1.00	-	0.02	0.01	-	-	0.11	0.03	2.61	0.94	0.99	0.00	0.60	0.040
Jun	2.17	0.06	0.38	0.04	0.09	0.05	0.17	0.12	8.00	0.96	0.98	0.03	0.58	0.047
Myr	1.00	-	0.11	0.09	-	-	0.56	0.34	0.93	2.22	0.77	0.12	0.77	0.009
Ner	1.00	-	0.08	0.07	-	-	0.11	0.11	0.40	0.61	0.63	0.05	0.63	0.034
Phy	1.46	0.13	0.20	0.10	0.00	1.03	0.12	0.08	6.81	2.60	0.85	0.14	0.51	0.043
Pic	1.00	-	0.03	0.06	-	-	0.12	0.03	1.24	1.02	0.91	0.02	0.81	0.005
Pin	1.00	-	0.11	0.04	-	-	0.09	0.02	1.48	0.47	0.93	0.01	0.77	0.010
Pis	1.00	-	0.11	0.04	-	-	0.24	0.09	0.94	1.95	0.94	0.02	0.89	0.001
Pop	1.00	0.09	0.49	0.09	0.00	0.90	1.35	0.70	8.00	2.87	0.65	0.13	0.36	0.155
Qui	1.25	0.10	0.12	0.03	0.00	0.03	0.22	0.11	2.16	1.49	0.99	0.01	0.81	0.006
Qup	1.00	-	0.21	0.07	-	-	0.50	0.22	0.78	1.11	0.80	0.04	0.81	0.015
Rob	1.58	0.21	0.76	0.12	0.08	0.06	0.56	0.53	8.00	0.80	0.82	0.22	0.64	0.031
Sal	1.41	0.18	0.46	0.19	0.00	0.47	0.21	1.15	0.65	2.09	0.64	0.11	0.65	0.028
Tec	1.00	-	0.00	0.06	-	-	0.21	0.08	0.67	0.98	0.84	0.05	0.86	0.003
Tep	1.00	-	0.23	0.02	-	-	0.11	0.04	4.07	2.00	0.93	0.01	0.29	0.213
Vib	1.00	-	0.00	0.00	-	-	0.09	0.04	1.61	1.16	0.99	0.00	0.71	0.017

Value: a parameter estimate obtained with the iterative procedure described in methods; S err: a bootstrap estimate of parameters' standard error (see methods); Loss f: value assumed by the loss function used to evaluate the significance of the fitting procedure. Sig: significance of the log-dose regressions. Also refer to Tables 1 and 2 for details about model parameters and litter labels, respectively

such positive effects  $F_{\max}$  values were constantly equal to 1 by definition, making correlation analysis unsuitable.

## Discussion

The non-linear model tested in this work showed a very good capacity to fit dose–response trends resulting from a wide range of litter types and experimental conditions. In general terms, when compared to the log-dose model, the non-linear model better described the response of the phytometer to increasing concentrations of the different litters tested: this was verified not only in terms of higher determination coefficients, but specifically in its ability to identify any hormetic peak and to estimate its approximate size and dose range. In at least half of the cases tested, the proposed model was the best performing according to Akaike's information criterion (AIC) values, an approach recommended in studies specifically approaching hormesis (Kim and Sanders 2017). The 5-parameter model has a precise physical/biological meaning and takes species-specific values that can be easily determined experimentally. Compared with simple models with fewer parameters, the 5-parameter model has a better fit with the added value of potential predictive and applied purposes. However, there were limitations to the predictivity of the non-linear model. The five species for which the log-linear model provided a better fit and the other cases in which the residual squares of the logistic model did not compensate for the added complexity (according to AIC values), both provide some conflicting results. In general, the logistic model performed less well in some cases where there was a pure inhibitive effect, whilst it definitely outperformed the log-linear model where hormetic estimation was present. A further reason for the limits of the results in terms of parsimony may be found in the limited sample size per litter, an inherent gap in studies aimed to get broad screenings rather than a high-detail analysis on a single litter type; further experiments, more focused on individual litter types, could provide larger datasets to refine the result also in terms of AIC.

A relevant advantage deriving from the adoption of such a model is related to the possibility of obtaining an indirect estimate of key parameters of plant–litter interaction, such as the maximum stimulating potential or the highest inhibiting power. Both these variables can have relevant application in research as well as in ecosystem and agroecosystem management, giving the opportunity, for instance, to better design mulching systems for weed control and/or for plant growth stimulation. Moreover, compared to previous works proposing a similar approach to modelling hormesis through a double logistic function (Liu et al. 2011), the model presented in this work was expressly structured to allow the identification of other key parameters that are essential for

designing experiments on litter toxicity and hormesis. In particular, identifying the dose able to induce 50% inhibition ( $O_{50}$ ) is crucial to estimate the dose range at which phytometer response tends to be linear, allowing to design at that dose any specific tests in which linearity of response is required. By applying mathematical analysis to the fitted model, we were able to determine the most probable dose at which the hormetic peak, if any, was found, another crucial parameter to design experiments expressly aimed at studying this phenomenon (ESM-1F).

When stimulation was detected,  $F_{50}$  values resulted overall low, although they were expressed in a relatively variable range of doses. In particular,  $F_{50}$  tended to be reached at relatively higher dosage when the litter had gone through previous decomposition process. Considering the very low value of  $F_{50}$  found in some litter type, we cannot exclude that stimulation could have also been detected in some of the other litter types, if still lower doses were applied. In general, any experiment expressly addressing hormesis would take benefit from a preliminary screening of the tested material to finely tune the most suited range to detect the searched effect.  $O_{50}$  values, on the contrary, were found in relatively higher ranges of concentration level, evidencing the variable level of toxicity expressed by the different litter types.

The logistic model presented here also allowed us to provide a separate estimate of the two opposing effects, which is not easily practicable through purely experimental methodologies, given the difficulty of separating the two phenomena. The lack of correlation found between the intensity of positive and negative effects of litter ultimately suggests that these two effects could depend on separate processes. It must be noted that we did not consider, with this dataset, the possibility to selectively reduce negative effects of litter using activated carbon as a mean to sequester organic molecules. Indeed, the use of activated carbon in allelopathic studies has been widely discussed in literature (Ridenour and Callaway 2001; Inderjit and Callaway 2003), and there is not a full consensus on considering it as a merely detoxifying agent (Lau et al. 2008; Weißhuhn and Prati 2009; Wurst et al. 2010). In our prior pilot experiments (data not shown), we found some relevant interaction between activated carbon and plant growth, both in absence of litter and with low litter doses, with a potential impact on the detection of the hormetic peak. Similarly, experimental strategies could be designed to selectively inactivate the stimulating effects, for instance, by putting all phytometers in a non-limiting condition as for nutrient uptake, i.e. with appropriate nutrient supply. However, also in this case, the results could be somehow biased by an indirect inactivation of any allelopathic process specifically linked to nutritional stress (Qasem and Hill 1989). Although addressing this topic was not amongst the aims of the present study, further research

**Table 4** Comparison between the two models log-linear and logistic in terms of least squares, integrated with an estimate of Akaike's information criterion

Litter species and type	SSr LogLin	SSr Logistic	AIC LogLin	AIC Logistic
<i>Acanthus mollis</i> green	<b>0.033</b>	0.048	– 19.953	– 11.254
<i>Acanthus mollis</i> senescent	0.765	<b>0.286</b>	<b>2.125</b>	1.242
<i>Ampelodesmos mauritanica</i> green	0.248	<b>0.179</b>	– 5.761	– 2.029
<i>Ampelodesmos mauritanica</i> senescent	0.245	<b>0.132</b>	– 5.853	– 4.175
<i>Arbutus unedo</i>	0.132	<b>0.077</b>	– 10.155	– 7.957
<i>Broussonetia papyrifera</i>	<b>0.009</b>	0.016	– 28.655	– 18.874
<i>Castanea sativa</i>	0.266	<b>0.233</b>	– 5.276	– 0.192
<i>Celtis australis</i>	0.024	<b>0.022</b>	– 22.186	– 16.688
<i>Cistus monspeliensis</i>	0.021	<b>0.019</b>	– 23.048	– 17.722
<i>Coronilla emerus</i>	0.339	<b>0.010</b>	– 3.563	– 22.459
<i>Dactylis glomerata</i> decomposed	0.869	<b>0.211</b>	3.016	– 0.907
<i>Dactylis glomerata</i> green	0.317	<b>0.288</b>	– 4.036	1.286
<i>Dactylis glomerata</i> senescent	0.626	<b>0.015</b>	0.723	– 19.316
<i>Daphne gnidium</i>	0.291	<b>0.291</b>	– 4.635	1.355
<i>Eucalyptus globulus</i> senescent	0.252	<b>0.246</b>	– 5.661	0.195
<i>Fagus sylvatica</i>	0.210	<b>0.165</b>	– 6.914	– 2.615
<i>Festuca drymeia</i> green	1.336	<b>0.417</b>	6.030	<b>3.885</b>
<i>Festuca drymeia</i> senescent	2.432	<b>1.571</b>	<b>10.222</b>	13.160
<i>Fraxinus ornus</i>	0.009	<b>0.001</b>	– 29.270	– 36.082
<i>Hedera helix</i>	0.107	<b>0.002</b>	– 11.660	– 34.242
<i>Juniperus communis</i>	0.670	<b>0.025</b>	1.192	– 15.819
<i>Myrtus communis</i>	0.670	<b>0.025</b>	1.192	– 15.819
<i>Nerium oleander</i>	<b>0.048</b>	0.048	– 17.268	– 11.260
<i>Phillyrea angustifolia</i>	0.381	<b>0.139</b>	– 2.748	– 3.799
<i>Picea abies</i>	0.050	<b>0.024</b>	– 16.958	– 16.073
<i>Pinus pinea</i>	0.037	<b>0.011</b>	– 19.170	– 21.886
<i>Pistacia lentiscus</i>	0.032	<b>0.018</b>	– 20.025	– 18.146
<i>Populus alba</i>	0.239	<b>0.132</b>	– 6.010	– 4.184
<i>Quercus ilex</i>	0.161	<b>0.006</b>	– 8.787	– 26.318
<i>Quercus pubescens</i>	0.393	<b>0.036</b>	– 2.530	– 13.190
<i>Robinia pseudoacacia</i>	0.429	<b>0.215</b>	– 1.930	– 0.759
<i>Salix alba</i>	<b>0.111</b>	0.113	– 11.389	– 5.241
<i>Teucrium chamaedrys</i>	<b>0.042</b>	0.046	– 18.202	– 11.506
<i>Teucrium polium</i>	0.125	<b>0.013</b>	– 10.537	– 20.634
<i>Viburnum tinus</i>	0.052	<b>0.002</b>	– 16.699	– 33.528

The bold fonts point to the lower value in each comparison (best model)

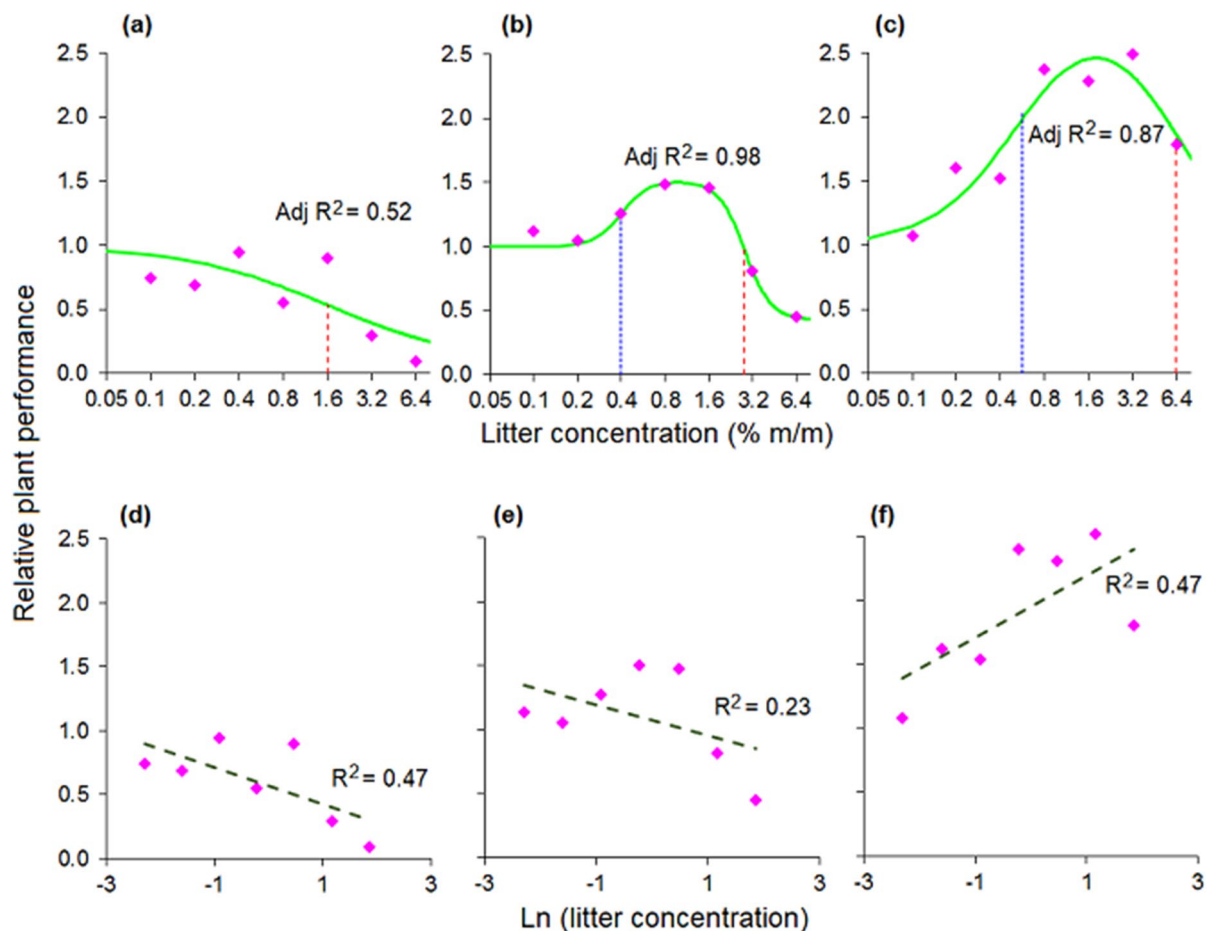
SSr residual sum of squares, LogLin log-linear model, Logistic logistic model, AIC estimate of Akaike's information criterion based on least squares (see methods for details)

could be developed to finely focus on the experimental isolation of the two distinct effects.

Allelopathic effects tended to disappear with decomposition time, being replaced by strong stimulation when the leaves were fully decomposed. Interestingly, this phenomenon was quite evident in the foliage of *Dactylis glomerata* collected as standing biomass and necromass on the intact canopies of the grass. The tendency of grasses to keep the standing necromass, determined by the rarity of abscission layers in this group of plants, has been related

to the enhanced flammability of grassland communities (Mutch 1970) or to a possible defence against grazing by large herbivores (Mingo and Oesterheld 2009). Further research could be developed to explore whether decomposing potentially toxic leaves at canopy level, rather than releasing them through abscission, could also reduce to some extent the toxicity to root apparatus in presence of decaying material.

Ultimately, this research also provided interesting information on the allelopathic potential of a relevant number



**Fig. 5** Example of phytometers responses to increasing litter concentrations (% mass/mass), with litter of *Dactylis glomerata* at three different decomposition stages, analysed with either a logistic (a–c) or a linear log response model (d–f). Litter decomposition levels are represented as follows: a, d— green litter (standing biomass); b, e senescent litter (standing necromass collected on senescent leaf apices); and c, f highly decomposed litter (dead decomposed leaves col-

lected at the base of plant canopy). Relative plant performance were expressed as final phytometer root lengths standardised by control values. In diagrams a, b, and c, blue-dotted lines represent the doses at which 50% of maximum stimulation ( $F_{50}$ ) was found, whilst red-dashed lines represent the doses corresponding to 50% inhibition ( $O_{50}$ ). Note the logarithmic scale of the x-axes of diagrams a, b and c

of tested species. Allelopathic effects of green biomass had already been widely documented for several woody species, such as *Eucalyptus globulus* (Chandra Babu and Kandrasamy 1997), *Pinus halepensis* and *Quercus coccifera* (Alrababah et al. 2009), and *Pinus pinea* (Valera-Burgos et al. 2012), and could also play a relevant role in alien plant invasion dynamics (Li et al. 2016; Warren et al. 2017; Cheng et al. 2021). In general, a great attention should be given to allelopathic effects when designing sustainable agro-ecosystems, where the combined short-term weed inhibition and long-term fertilisation induced by plant litter could be applied to design effective mulching systems (Kamara et al. 2000; Caamal-Maldonado et al. 2001; Hou et al. 2021). Also

in agroforestry systems, knowledge about plant allelopathic potential can help to select trees and crops that do not negatively interfere between them (Alrababah et al. 2009) or in forest restoration, where the allelopathic potential of selected native species have been studied to prevent invasion by alien species (Cummings et al. 2012). The results of this work, where 35 litter types were screened and modelled, mostly deriving from spontaneous species for which not much data was available about their toxicity profile, also adds to the general knowledge in the field of allelopathy in Mediterranean ecosystems. The application of these processes in the management of agro-ecosystems and natural environments will be important.

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**Authors' contribution** All authors identified the scientific question and defined the line of investigation. AM conceived the details of the research, carried out the experimental work, and drafted the first copy of the manuscript, which was then revised and rewritten with contributions from all authors. FG contributed particularly to the review of the mathematical aspects of the model, GB to the ecological implications of the experimental results, and GI to the statistical approach adopted. SM contributed to all of the above aspects and coordinated all phases of the research.

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**Availability of data and materials** The datasets generated and analysed during the current study are included in this published article, in the attached online supplementary information. They can also be obtained from the corresponding author on reasonable request.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

**Consent for publication** Not applicable.

**Ethical approval** Not applicable.

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