

**Global warming may reduce the effects of litter quality on
litter decomposition by aquatic microbes**

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Complete List of Authors:	Fernandes, Isabel; University of Minho, Department of Biology Pascoal, Claudia; University of Minho, Department of Biology Guimarães, Helena; University of Minho, Department of Biology Pinto, Rute; University of Minho, Department of Biology Sousa, Ines; University of Minho, Department of Mathematics Cássio, Fernanda; University of Minho, Department of Biology
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Review

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3 1 Title: Global warming may reduce the effects of litter quality on litter decomposition by
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9 4 Isabel Fernandes¹, Cláudia Pascoal¹, Helena Guimarães¹, Rute Pinto¹, Inês Sousa²,
10
11 5 Fernanda Cássio^{1*}
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13 6

14
15
16 7 ¹ Centre of Molecular and Environmental Biology (CBMA)

17
18 8 Department of Biology, University of Minho

19
20 9 Campus de Gualtar

21
22 10 4710-057 Braga

23
24 11 Portugal

25
26
27 12

28
29 13 ² Centre of Mathematics (CMAT)

30
31 14 Department of Mathematics and Applications, University of Minho

32
33 15 Campus de Gualtar

34
35 16 4710 - 057 Braga

36
37 17 Portugal

38
39
40 18

41
42 19 * Email: fcassio@bio.uminho.pt

43
44 20 Telephone: + 351 253604045

45
46 21 Fax: + 351 253 678980

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51 23 Running head title: Effects of temperature and litter quality on litter decomposition

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55 25 Keywords: leaf decomposition, litter quality, temperature, aquatic fungi, streams
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3 26 Summary
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- 5 27 1. We investigated the effects of riparian plant diversity (species number and identity)
6
7 28 and increased temperature on microbially mediated leaf decomposition by assessing
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9 29 leaf mass loss, fungal reproduction and diversity.
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11 30 2. Leaves of five riparian plant species were immersed in a stream to allow microbial
12
13 31 colonization, and then were exposed, alone or in all possible combinations, to 16 °C
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15 32 (ambient temperature) or to 24 °C to simulate a warming scenario.
16
17 33 3. Leaf species number did not significantly affect leaf mass loss. Consistently, we
18
19 34 found mostly additive effects of litter mixtures on leaf decomposition because
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21 35 decomposition of litter mixtures did not differ from that expected based on
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23 36 decomposition of single litter species.
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25 37 4. Leaf mass loss was affected by the identity of leaf mixtures (i.e. litter quality). This
26
27 38 was mainly explained by the negative correlation between leaf decomposition and
28
29 39 lignin concentration of leaves.
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31 40 5. The increase of temperature diminished the negative effects of lignin on microbially
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33 41 mediated leaf decomposition, suggesting that the predicted global warming may
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35 42 weaken the effects of litter quality on plant litter decomposition in streams.
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43 Introduction

44 Over the last decades, a massive extinction of species has been observed as a
45 consequence of the increasing human impacts on our planet (Chapin III *et al.*, 2000).
46 This has placed biodiversity under great focus as confirmed by the choice of 2010 as the
47 International Year of Biodiversity by the United Nations. Scenarios point to a
48 continuing decline of biodiversity during the 21st century, but there are also large
49 uncertainties in projections and opportunities to implement better policies (Pereira *et al.*,
50 2010) to revert this trend. The projections of climate change until the end of this century
51 point to an increase in air temperature between 1.8 °C and 4.0 °C (IPCC, 2007).
52 Consequently, changes of organism physiology, time of life cycle events and individual
53 species distribution are expected to occur (Parmesan & Yohe, 2003; Root *et al.*, 2003),
54 which may lead to species extinction due to direct physiological stress or to changes in
55 interspecific interactions (Hughes, 2000).

56 Forested streams are highly dependent on allochthonous material coming from riparian
57 vegetation that constitutes the main source of food and energy for aquatic biota
58 (Webster & Benfield, 1986). Microbial decomposers and invertebrate detritivores are
59 the main biotic players driving plant litter decomposition in streams (Gessner *et al.*,
60 2007). Among microbial decomposers, fungi, namely aquatic hyphomycetes, play a key
61 role at early stages of plant litter decomposition (Pascoal & Cássio, 2004; Gessner *et al.*,
62 2007) and enhance leaf nutritional value for invertebrate detritivores (Graça, 2001).

63 Environmental factors are known to influence the performance of aquatic decomposers
64 with impacts to plant litter decomposition. For instance, the increase in temperature by
65 5-10 °C stimulated fungal growth and reproduction, leading to a 2 times increase in
66 plant litter decomposition in stream microcosms (Dang *et al.*, 2009; Fernandes *et al.*,
67 2009; Ferreira & Chauvet, 2011b).

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3 68 Fungal diversity in streams was found to vary with riparian plant species richness and
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5 69 composition (Lecerf *et al.*, 2005; Laitung & Chauvet, 2005; Bärlocher & Graça, 2002;
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7 70 Lecerf & Chauvet, 2008a). Besides altering the diversity of aquatic biota, riparian plant
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9 71 diversity can also affect important ecosystem processes, like organic matter
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11 72 decomposition. A recent meta-analysis comprising 18 litter mixture experiments
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13 73 showed that decomposition of litter mixtures in streams is often non-additive, with 44%
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15 74 of litter mixtures decomposing faster than predicted from the sum of single litter species
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17 75 (synergistic effect) and 39% of litter mixtures decomposing slower than expected from
18
19 76 individual species performance (antagonistic effect) (Lecerf *et al.*, 2011). According to
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21 77 Gessner *et al.* (2010) litter mixture effects on decomposition can result from nutrient
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23 78 transfer from one litter type to another due to microbial activity, and passive transfer of
24
25 79 nutrients or inhibitory compounds via leaching and subsequent transport by water flow.
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27 80 However, the authors emphasize that water flow 1) continuously disrupts litter
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29 81 accumulation, creating strong physical barriers to fungal colonization of different litter
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31 82 species simultaneously; and 2) easily dilutes leached compounds before microbes can
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33 83 assimilate them, which may result in less probable positive or negative effects of litter
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35 84 mixture on decomposition. Actually, additive effects of litter diversity on
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37 85 decomposition were found in 17–33% of the studies in both terrestrial and aquatic
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39 86 ecosystems (Gartner & Cardon, 2004; Lecerf *et al.*, 2011). Besides, several studies
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41 87 suggested that composition of litter mixtures can play a more important role in
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43 88 controlling litter decomposition rates than the number of litter species *per se* (Swan &
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45 89 Palmer, 2004; Swan & Palmer, 2006; Lecerf *et al.*, 2007b; Ball *et al.*, 2008; Kominoski
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47 90 & Pringle, 2009). Thus, there is still uncertainty if riparian plant diversity loss can
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49 91 actually change ecosystem processes, like plant litter decomposition, and when it
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3 92 happens if it is more dependent on the number of litter species or on the identity of the
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5 93 constituting species.
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7 94 Moreover, the direction and magnitude of diversity effects on plant litter decomposition
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9 95 can change with the environmental context. Swan and Palmer (2004) observed changes
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11 96 from additive effects of litter mixtures on decomposition in autumn (lower temperature)
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13 97 to non-additive antagonistic effects in summer (higher temperature). Conversely, in a
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15 98 paired decomposition experiment, positive litter diversity effects were observed in a
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17 99 warmer stream in France, contrasting with the null effects in a cooler stream in Romania
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20 100 (Lecerf *et al.*, 2007b). So, it is still not clear how environmental factors, such as
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22 101 temperature, might influence plant diversity effects on litter decomposition in
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24 102 freshwaters.
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26
27 103 Our study aimed at assessing the interactive effects of resource availability (riparian
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29 104 plant diversity and identity) and temperature on plant litter decomposition by aquatic
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31 105 microbes. Specifically, we wanted to test if 1) increasing leaf diversity would result in
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33 106 non-additive effects, 2) an increase in temperature would lead to higher leaf
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35 107 decomposition, and 3) temperature would change the direction or magnitude of the
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37 108 observed diversity effects. To test these hypotheses, leaves from four common riparian
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39 109 plant species (alder, oak, grapevine and plane tree) in streams of Northwest Portugal
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41 110 were used either alone or in all possible combinations. Grapevine was chosen because it
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43 111 is commonly found as the only plant species bordering small streams in Northwest
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45 112 Portugal. Leaves were immersed in a stream to allow microbial colonization and, then,
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47 113 were exposed, in microcosms, to an ambient temperature of 16 °C or to 24 °C to
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49 114 simulate a warming scenario. The measured endpoints were leaf decomposition, fungal
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51 115 reproduction and diversity.
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54 116 Methods
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3 117 Stream site

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5 118 The Algeriz Stream is located in the Northwest Portugal (longitude: 41°35'24.56''N;
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7 119 latitude: 8°22'36.96''W). At the sampling site the stream was about 30 cm depth and 50
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9 120 cm wide and the bottom is composed of boulders, pebbles and sand. On 29th May 2008,
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11 121 physical-chemical parameters in the stream water were measured *in situ* (Multiline F/set
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13 122 3 no.400327, WTW, Weilheim, Germany) and showed that stream water had
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15 123 circumneutral pH (6.6), low conductivity (45 $\mu\text{S cm}^{-1}$) and high oxygen concentration
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17 124 (9.9 mg L^{-1}). At the same date, water samples were collected, filtered (filter paper,
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19 125 Macherey-Nagel, Düren, Germany), autoclaved (120°C, 20 min) and used in the
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21 126 microcosm experiment. Nutrient concentrations in stream water were measured and
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23 127 showed moderate nitrate (0.09 mg L^{-1} $\text{NO}_3\text{-N}$) and phosphate (0.03 mg L^{-1} $\text{PO}_4\text{-P}$)
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25 128 concentrations (HACH kit, program 355 and 480, respectively, HACH, Loveland, CO,
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27 129 USA).
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34 131 Microcosm experiment

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36 132 Leaves of alder (*Alnus glutinosa* (L.) Gaertn.), grapevine (*Vitis vinifera* L.), oak
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38 133 (*Quercus robur* L.) and plane tree (*Platanus* sp.) were collected just before abscission in
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40 134 autumn 2007 and air dried. Fine mesh bags (0.5 mm pore size, 16 x 12 cm) were used to
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42 135 avoid invertebrate colonization. Each bag was filled with 2.4 ± 0.2 g of each leaf type
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44 136 alone or in all possible combination of the four leaf species, keeping the proportion of
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46 137 leaf species in the mixtures (15 combinations; 3 replicates). On 29th May 2008, leaf
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48 138 bags were immersed in the Algeriz Stream to allow microbial colonization. After 7
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50 139 days, bags were retrieved and transported to the laboratory in a cool box. Leaves were
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52 140 washed with deionised water to remove sediments, and cut into 12-mm leaf disks.
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55 141 Twenty-four leaf disks were placed in 150 mL-Erlenmeyer flasks with 80 mL of
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3 142 sterilized stream water. One set of microcosms was placed at 16 °C (ambient
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5 143 temperature) and the other set at 24 °C (to simulate a warming scenario). Microcosms
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7 144 were kept on a shaker at 140 rpm during 21 days, and the stream water was changed
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9 145 every 7 days. After 14 days, stream water containing released fungal spores was mixed
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11 146 with formaldehyde (final concentration of 2%) to further assess fungal reproduction and
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13 147 diversity. At the end of the experiment, leaf disks from microcosms were used to assess
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15 148 leaf mass loss.
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21 150 Mass loss and nutrient concentration in leaves

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23 151 Leaf disks were freeze-dried (Christ alpha 2–4, B. Braun, Germany) and weighed
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25 152 (± 0.01 mg). Leaf mass loss was estimated by the difference between leaf mass at the
26
27 153 beginning and at the end of experiment. Initial leaf mass was estimated using sets of
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29 154 leaf disks from each treatment before microcosm assay.

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32 155 The concentration of nitrogen, phosphorus and lignin for each leaf type was calculated
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34 156 based on average values found in literature (Table 1). The nutrient concentration in leaf
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36 157 mixtures was estimated taking into account the initial percentage of each leaf type in the
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38 158 mixture.
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43 160 Fungal reproduction and diversity

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45 161 Fungal reproduction and diversity were assessed by counting and identifying spores
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47 162 released from decomposing leaves. Appropriate volumes of stream water containing
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49 163 suspended fungal spores were filtered (0.45- μ m pore size, Millipore, Billerica, MA,
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51 164 USA) and spores on filters were stained with 0.05% cotton blue. At least 300 spores per
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53 165 sample were counted and identified under a light microscope (Leica Biomed, Heerbrug,
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55 166 Switzerland) at 400x magnification.
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5 168 Statistical analyses
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7 169 A three-way nested ANOVA was used to test the effects of leaf diversity, leaf identity
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10 170 (nested in leaf diversity) and temperature on leaf decomposition, and fungal
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12 171 reproduction and diversity. To achieve normal distribution, percentage data of leaf
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14 172 decomposition was arcsine square root transformed and values of fungal reproduction
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16 173 were ln transformed (Zar, 1996). The expected leaf mass loss, fungal reproduction and
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18 174 diversity in litter mixtures were estimated as the sum of the component single litter
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20 175 species performances (weighted by their proportion in mixtures) (Bärlocher & Corkum,
21
22 176 2003; Duarte *et al.*, 2006). The differences between the observed and the expected
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24 177 performances were tested against the null hypothesis that the average difference
25
26 178 equalled 0 (t-test). ANOVAs and t-tests were done in Statistica 6.0 for Windows
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28 179 (Statsoft, Tulsa, OK, USA).
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30
31 180 Ordination of leaf species alone or in mixtures according to the initial litter
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33 181 concentration in nutrients (nitrogen, phosphorus and lignin) was done using Principal
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35 182 Component Analysis (PCA) (Legendre & Legendre, 1998). PCA was done in
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37 183 CANOCO 4.5 for Windows (Microcomputer Power, Ithaca, NY, USA).
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39 184 The percentage of leaf mass loss was used as response variable and modelled as a
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41 185 function of explanatory variables, namely temperature, lignin, nitrogen, phosphorous.
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43 186 Data were fitted to a model with multiple explanatory variables to adjust for possible
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45 187 interactions between them. We assumed that the proportion of leaf mass loss followed a
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47 188 Beta distribution, under the restriction that values range from 0 to 1. Therefore, a Beta
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49 189 regression model with a logit link function was used (Ferrari & Cribari-Neto, 2004).
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51 190 Beta regression was done in R 2.13.1 (<http://cran.r-project.org/>).
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192 Results

193 Fungal reproduction

194 After 14 days in microcosms, fungal sporulation rates were significantly affected by leaf
195 species number and identity, but not by temperature (3-way nested ANOVA, $p < 0.05$
196 and $p > 0.05$, respectively; Table 2; Figure 1a, b). In microcosms with single leaf
197 species, the lowest fungal sporulation rates were found on plane tree leaves at 16 °C
198 ($131 \text{ spores mg}^{-1} \text{ leaf dry mass d}^{-1}$) and on alder leaves at 24 °C ($59 \text{ spores mg}^{-1} \text{ leaf dry}$
199 mass d^{-1}) (Figure 1a), while the highest sporulation rates were found on grapevine
200 leaves (482 and $509 \text{ spores mg}^{-1} \text{ leaf dry mass d}^{-1}$ for 16 °C and 24 °C, respectively). At
201 both temperatures, sporulation rates in leaf mixtures did not differ or were lower than
202 that expected from the sum of fungal sporulation on individual leaf species (Figure 2a).
203 At 24 °C, the negative effect of leaf species number on fungal sporulation became less
204 pronounced as the number of leaf species increased.

205

206 Fungal diversity

207 Fungal diversity associated with decomposing leaves was affected by temperature, but
208 not by leaf species number or identity (3-way nested ANOVA, $p < 0.05$ and $p > 0.05$,
209 respectively; Table 2). In general, the increase in temperature led to a decrease in fungal
210 diversity (Figure 1c, d). At 16 °C, oak leaves had the lowest fungal diversity (7 species)
211 while alder leaves showed the highest diversity (12 species) (Figure 1c). At 24 °C, the
212 lowest fungal diversity was found on alder and plane tree leaves (ca. 8 species), and the
213 highest diversity was associated with grapevine leaves (10 species). At the lowest
214 temperature, fungal diversity in leaf mixtures did not differ from that expected based on
215 the weighted sum of fungal diversity on single leaf species (t-tests, $p > 0.05$; Figure 2b).

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3 216 At the highest temperature, the difference between observed and expected fungal
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5 217 diversity was negative for mixtures of 2 and 3 leaf species.
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9 219 Leaf decomposition

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11 220 After 21 days in microcosms, mass loss of single leaf species varied from 9.6 to 26.7%
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13 221 at 16 °C and from 17.4 to 50.4% at 24 °C for plane tree and alder, respectively (Fig. 1e).

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16 222 Leaf decomposition was significantly affected by species identity and temperature, but
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18 223 not by the number of leaf species (3-way nested ANOVA, $p < 0.05$ and $p > 0.05$,
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20 224 respectively; Table 2, Figure 1e, f). The increase in temperature led to an increase in
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22 225 leaf decomposition in microcosms containing single and multi-species (Figure 1e, f). In
23
24 226 multi-species microcosms, leaf mass loss did not differ from that expected from the
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26 227 weighted sum of mass loss of individual leaf species, except for mixtures with 3 leaf
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28 228 species at 16 °C that had lower values than expected (Figure 2c).
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34 230 Effect of litter quality on leaf decomposition

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36 231 The PCA ordination of initial nutrient concentrations in litter showed that the first PC
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38 232 axis explained 95% of the variance. Axis 1 mainly separated leaves of alder alone or in
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40 233 mixtures without plane tree from leaves of plane tree alone or in mixtures without alder.
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42 234 Nitrogen and phosphorus concentrations in the leaves were positively correlated with
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44 235 the first PC axis, while lignin was negatively correlated (Fig. 3).

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46 236 The estimated parameters of the Beta regression model adjusting leaf mass loss for leaf
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48 237 nutrient concentrations showed that lignin was the only variable that explained leaf
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50 238 decomposition (Table 3, Fig. 4, Beta regression, $p < 0.05$). The estimated parameters for
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52 239 the effect of nitrogen and phosphorus in percentage leaf mass loss were not significantly
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54 240 different from zero (Beta regression, $p = 0.502$ and 0.549 , respectively). Moreover, the
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3 241 effect of lignin on leaf mass loss varied with temperature. Although we found lower leaf
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5 242 decomposition at 16 °C than at 24 °C (Fig. 1), the negative effect of lignin on
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7 243 decomposition was higher at lower temperature (Table 3, Fig.4).
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11 245 Discussion

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13 246 We found mostly additive effects of litter diversity on leaf decomposition since
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15 247 decomposition of litter mixtures did not differ from that expected based on
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17 248 decomposition of single litter species. Even though meta-analyses from terrestrial and
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19 249 aquatic ecosystems have often pointed to non-additive effects, predominantly
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21 250 synergistic, of litter diversity on decomposition, additive effects have also been found in
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23 251 17–33% of the studies (Gartner & Cardon, 2004; Lecerf *et al.*, 2011). Some authors
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25 252 report larger effects of litter species diversity on decomposition in long term
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27 253 experiments due to increased complementarity effects over time (Srivastava *et al.*, 2009;
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29 254 Lecerf *et al.*, 2011). This may be true for litter decomposition which involves a
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31 255 succession in the dominance of litter consumers (fungi-detritivores-bacteria) that
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33 256 interact synergistically to fully exploit and decompose leaf litter (Lecerf *et al.*, 2011).
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37 257 In our study, fungal activity on leaves, measured as reproductive output, was affected by
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39 258 both leaf species number and identity. Aquatic fungi spread by producing conidia
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41 259 (Pascoal & Cássio, 2008), therefore if aquatic fungal reproductive activity is
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43 260 compromised by the lost of riparian plant species, we might face lower microbial
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45 261 diversity in streams. In our microcosm study, fungal diversity did not appear to be
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47 262 affected by changes in plant litter diversity, probably because experiment was
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49 263 conducted during a short period of time. However, impacts of riparian plant diversity on
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51 264 microbial diversity might be stronger at longer times. This can be of particular concern
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53 265 taking into account that predictions point to a loss of up to 32% of plant species in
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3 266 Europe until 2050 (Bakkenes *et al.*, 2002). Besides, there is evidence that environmental
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5 267 context, like differences in temperature/seasonality, may change the direction and
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7 268 magnitude of diversity effects (Swan & Palmer, 2004; Lecerf *et al.*, 2011). In the
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9 269 present study, the increase in temperature led to changes from additive to non-additive
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11 270 antagonistic effects of litter diversity on fungal reproduction and diversity. Moreover,
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13 271 increased temperature changes fungal community composition (Fernandes *et al.*, 2009;
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15 272 Dang *et al.*, 2009) and decreases fungal species diversity (this study; Bärlocher *et al.*,
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17 273 2008). Therefore, taking into account the predicted plant biodiversity loss (Bakkenes *et*
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19 274 *al.*, 2002) under the ongoing climate warming (IPCC, 2007), fungal diversity in streams
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21 275 can be seriously compromised in the future and this might affect leaf decomposition
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23 276 with consequences to freshwater ecosystem functioning.

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27 277 There is evidence that litter decomposition rates in streams are more controlled by plant
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29 278 litter composition than by the number of riparian plant species (Swan & Palmer, 2004;
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31 279 Swan & Palmer, 2006; Lecerf *et al.*, 2007b; Ball *et al.*, 2008; Kominoski & Pringle,
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33 280 2009). Our findings support this idea because leaf mass loss by microbial decomposers
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35 281 was affected by the identity of leaves in mixtures, but not by leaf species number.
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37 282 Lower leaf quality has been associated with lower leaf decomposition rates in
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39 283 freshwaters (Leroy & Marks, 2006; Lecerf & Chauvet, 2008b; Schindler & Gessner,
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41 284 2009; but see Kominoski *et al.*, 2009; Ferreira & Chauvet, 2011a). We confirmed that
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43 285 relationship because a decrease in leaf decomposition was observed with increasing
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45 286 concentration of lignin and decreasing concentrations of nitrogen and phosphorus in
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47 287 plant litter. Actually, the most diverse litter mixture tested in our study fell in a mean
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49 288 position in terms of litter quality and decomposition. This suggests that changes in
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51 289 process rates due to loss of riparian plant diversity can be of particular concern when the
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3 290 remaining species are of lower or higher quality than those of the original plant
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5 291 communities.

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7 292 To better discriminate if the effects of leaf species identity resulted from differences in
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9 293 litter quality, we modelled leaf decomposition in function of nitrogen, phosphorus and
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11 294 lignin concentrations. Nitrogen and phosphorus concentrations in the leaves were much
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14 295 less related to decomposition than lignin, which came out as the variable that best
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16 296 explained the process. Results agree with those that identified lignin as the key litter
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18 297 trait determining decomposition rate (Lecerf *et al.*, 2007a; Schindler & Gessner, 2009).
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21 298 However, the negative effects of lignin on leaf decomposition in our study seemed to be
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23 299 weakened by the increase of temperature, suggesting that the predicted climate warming
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25 300 (IPCC, 2007) might reduce the effects of litter quality on leaf decomposition. This
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27 301 might be counterbalanced by the increase in CO₂ concentration in atmosphere (IPCC,
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29 302 2007) that have been found to lower leaf quality by decreasing nitrogen and increasing
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31 303 lignin (Peñuelas & Matamala, 1990; Cotrufo, Ineson & Rowland, 1994; Tuchman *et al.*,
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33 304 2002). This can be of particular concern if riparian vegetation diversity loss occurs for
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35 305 higher quality plant species. Thus, further investigation on the combined effects of
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37 306 increasing CO₂ level, temperature and leaf litter diversity and quality on leaf
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39 307 decomposition are needed to better understand which factors are more critical for this
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42 308 process in streams.

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45 309 We found that increased temperature led to faster microbially mediated leaf
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47 310 decomposition, probably due to the increase of microbial metabolic rates (Brown *et al.*,
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49 311 2004). Enhanced activity of microbial decomposers with temperature has been observed
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51 312 in microcosms with either manipulated fungal assemblages (Dang *et al.*, 2009; Ferreira
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53 313 & Chauvet, 2011b) or natural fungal assemblages (Fernandes *et al.*, 2009), and in a
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55 314 recent global experiment in streams along a latitudinal temperature gradient (Boyero *et*

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3 315 *al.*, 2011). Therefore, the predicted climate warming will probably result in faster plant
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5 316 litter decomposition due to increased microbial activity.
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7 317 Altogether our results suggested that 1) plant litter quality seems to be more important
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9 318 than species number to litter decomposition; 2) a decrease of litter decomposition may
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11 319 occur if plant species loss result in lower quality plant species (i.e. higher lignin
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13 320 concentration); 3) the predicted global warming may lead to faster decomposition due to
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15 321 increased microbial activity; and 4) warming may also reduce the negative effects of
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17 322 lignin in microbially driven decomposition. Therefore, warming is expected to enhance
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19 323 microbial decomposition of plant litter even when species loss or replacement of
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21 324 riparian vegetation occurs towards poor quality litter. This may lead to a decrease in
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23 325 litter availability for stream biota with consequences for the functioning of freshwater
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25 326 ecosystems, if not counterbalanced by increased productivity of riparian forests.
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476 Table 1. Average initial concentrations of nitrogen, phosphorus and lignin (% leaf dry
 477 mass) in leaves of alder, grapevine, oak and plane tree.

Leaf species	Nitrogen	Phosphorus	Lignin	References
Alder	2.72 (1.9-3.46; n=17)	0.109 (0.034-0.187; n=14)	10.0 (3.9-19.0; n=13)	(Chauvet, 1987; Gessner & Chauvet, 1994; Canhoto & Graça, 1996; Sampaio, Cortes & Leão, 2001; Lecerf <i>et al.</i> , 2007b; Lecerf & Chauvet, 2008b; Schindler & Gessner, 2009)
Grapevine	1.63 (1.47-1.79; n=2)	0.11 (0.10-0.12; n=2)	11.9 (9.3-14.6; n=2)	(Nikolaidou <i>et al.</i> , 2010)
Oak	0.92 (0.71-1.14; n=6)	0.049 (0.042-0.055; n=3)	19.3 (17.8-20.8; n=2)	(Sampaio <i>et al.</i> , 2001; Schadler, Rottstock & Brandl, 2005; Lecerf <i>et al.</i> , 2007a; Lecerf <i>et al.</i> , 2007b)
Plane tree	0.66 (0.52-0.80; n=2)	0.049 (0.017-0.080; n=2)	32.5 (30.9-34.0; n=2)	(Gessner & Chauvet, 1994; Schindler & Gessner, 2009)

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 480 Table 2. Three-way nested ANOVAs on the effects of temperature, number of litter
 481 species and identity (nested in number of species) on leaf decomposition, fungal
 482 reproduction and diversity.

Parameter	Effect	SS	df	MS	F	p
Leaf decomposition	Temperature	2338.75	1	2338.75	31.073	<0.001
	Number of species	225.95	3	75.32	1.001	0.398
	Identity (Number of species)	2764.50	11	251.32	3.339	<0.001
	Temperature*Number of species	171.15	3	57.05	0.758	0.521
	Error	5343.86	71	75.27		
Fungal reproduction	Temperature	0.432	1	0.432	0.572	0.452
	Number of species	6.456	3	2.152	2.847	0.044
	Identity (Number of species)	19.484	11	1.771	2.343	0.016
	Temperature*Number of species	0.623	3	0.208	0.275	0.844
	Error	53.664	71	0.756		
Fungal diversity	Temperature	59.617	1	59.617	17.258	<0.001
	Number of species	11.358	3	3.786	1.096	0.357
	Identity (Number of species)	68.375	11	6.216	1.799	0.070
	Temperature*Number of species	15.625	3	5.208	1.508	0.220
	Error	245.264	71	3.454		

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3 484 Table 3. Estimated parameters of the Beta regression model applied to percentage leaf
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5 485 mass loss using nitrogen, phosphorus and lignin as explanatory variables. For lignin,
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7 486 separate effects at 16 °C and 24 °C were considered.
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	Estimate	Std. Error	z value	p (> z)
Nitrogen	0.182920	0.272485	0.671	0.502
Phosphorus	-3.535219	5.906652	-0.599	0.549
Lignin.16	-0.080330	0.008964	-8.961	< 2e-16
Lignin.24	-0.039178	0.007592	-5.160	2.46e-07

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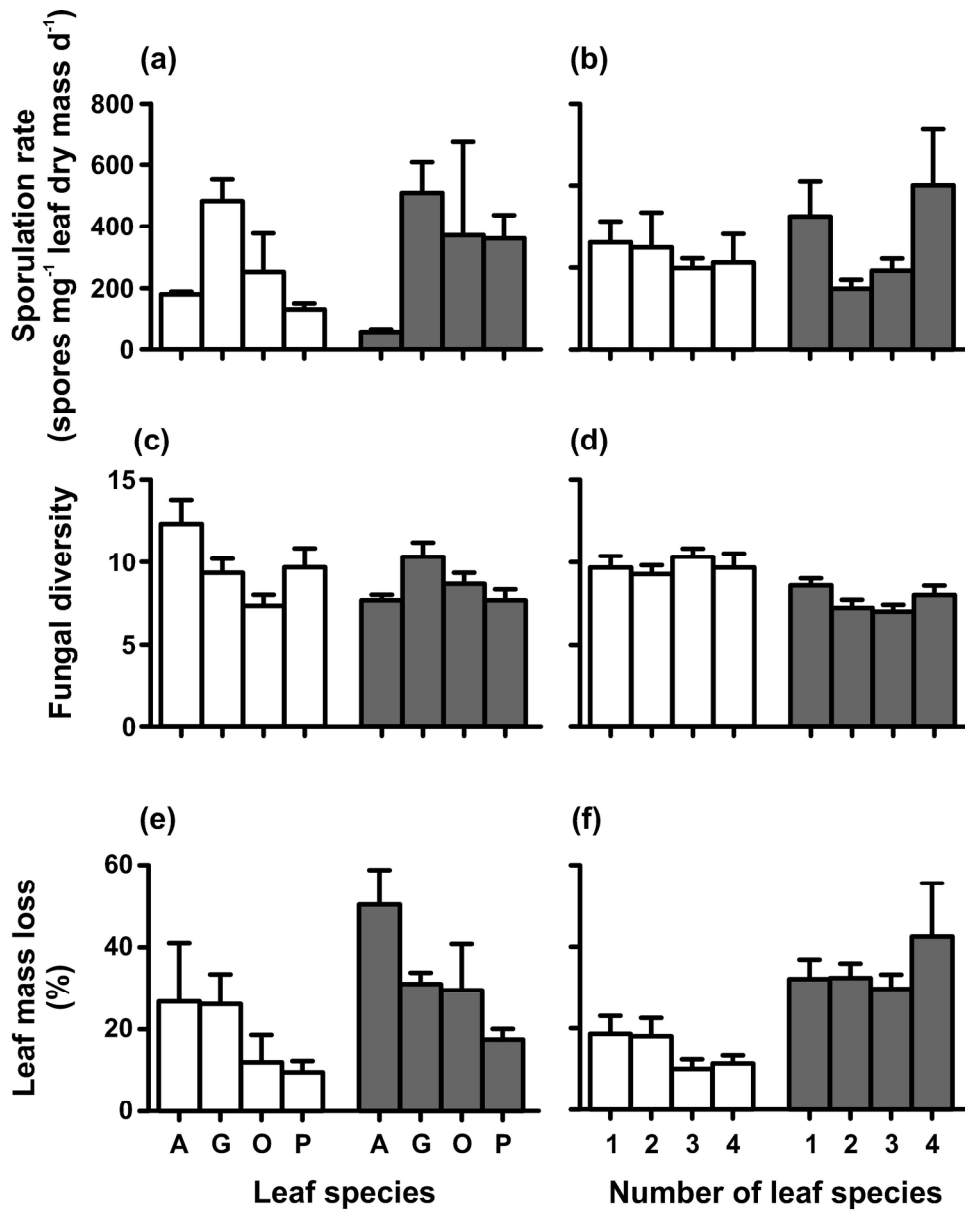
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4 489 Figure 1. Fungal sporulation rate (a, b), fungal diversity (c, d) and percentage of leaf
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6 490 mass loss (e, f) in treatments containing single leaf species (a, c, e) or in treatments
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8 491 containing the average of 1 to 4 leaf species (b, d, f) exposed to 16 °C (white bars) or 24
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10 492 °C (grey bars). Leaf mass loss was determined at the end of the experiment (21 days)
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12 493 and fungal sporulation and diversity was determined after 14 days in microcosms.
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14 494 M+SEM, n = 3. A, alder leaves; P, plane tree leaves; O, oak leaves; and G, grapevine
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16 495 leaves.
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22 497 Figure 2. Differences between observed and expected fungal sporulation rate (a) fungal
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24 498 diversity (b) and leaf mass loss (c) in mixtures with 2, 3 or 4 leaf species exposed to 16
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26 499 °C (white bars) or 24 °C (grey bars). Leaf mass loss was determined at the end of the
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28 500 experiment (21 days) and fungal sporulation and diversity was determined after 14 days
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30 501 in microcosms. M+SEM, n = 3. Differences were tested against zero by a t-test; *p <
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32 502 0.05.
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37 504 Figure 3. Principal component analysis of the initial nutrient concentrations (Lignin; N,
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39 505 nitrogen; and P, phosphorus) in single leaf species or in leaf mixtures. The direction of
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41 506 the arrow reflects the maximum change of each variable. A, alder leaves; P, plane tree
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43 507 leaves; O, oak leaves; and G, grapevine leaves.
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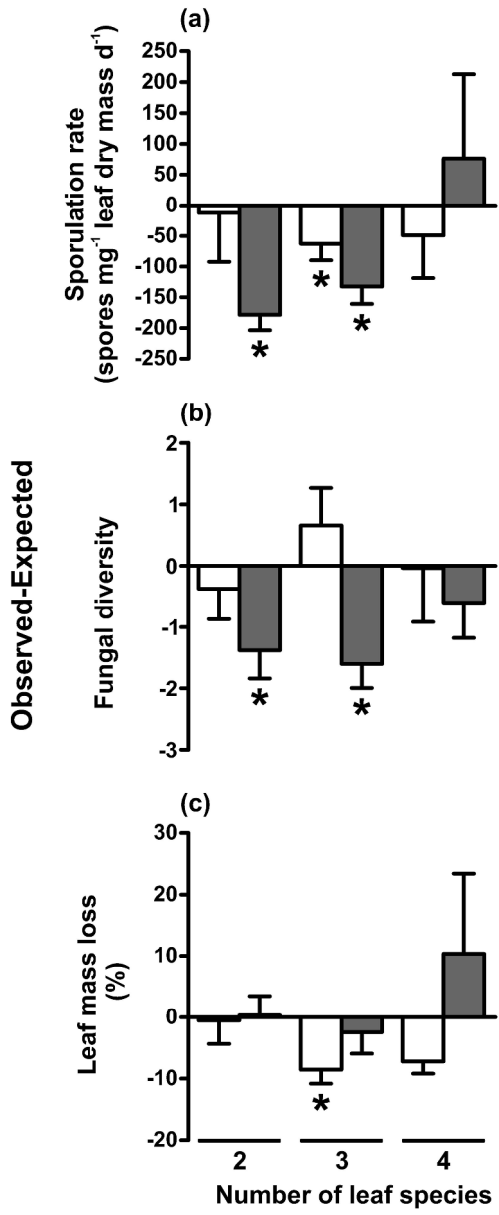
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48 509 Figure 4. Theoretical regression models of percentage leaf mass loss (full lines) as
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50 510 function of lignin concentration (%) at 16 °C and 24 °C. Symbols correspond to
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52 511 observed leaf mass loss values and dashed lines correspond to 95% confidence intervals
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54 512 of the estimated model.
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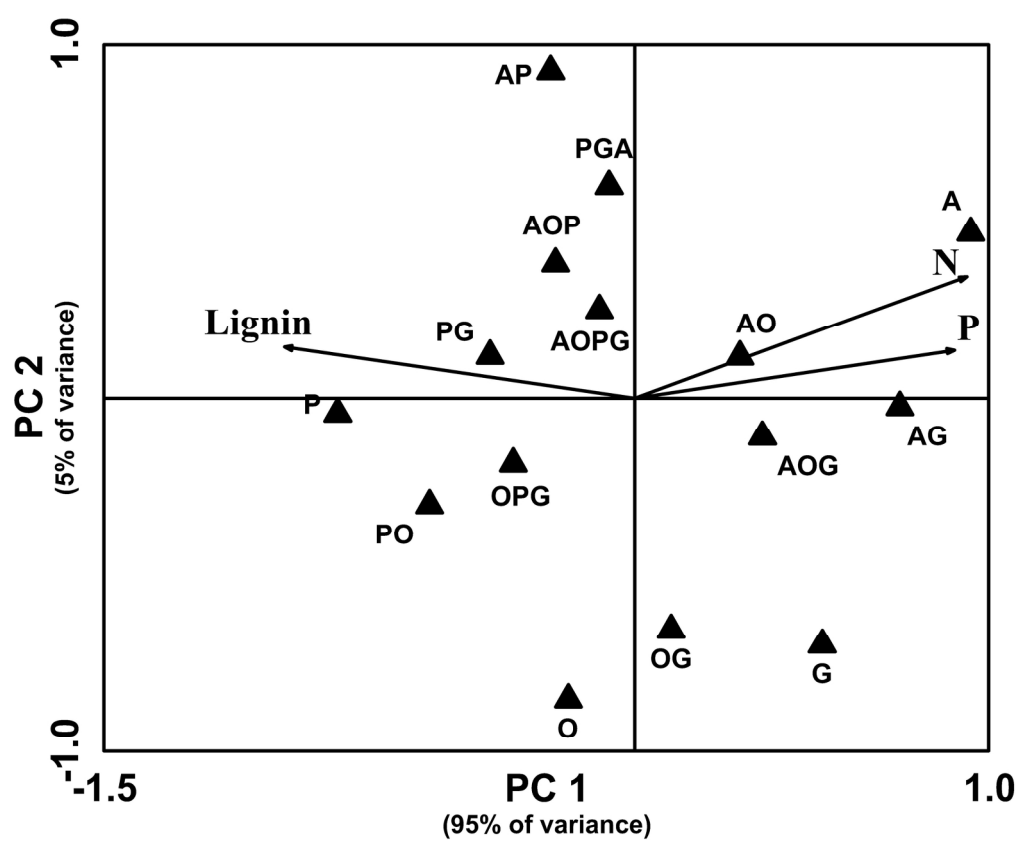
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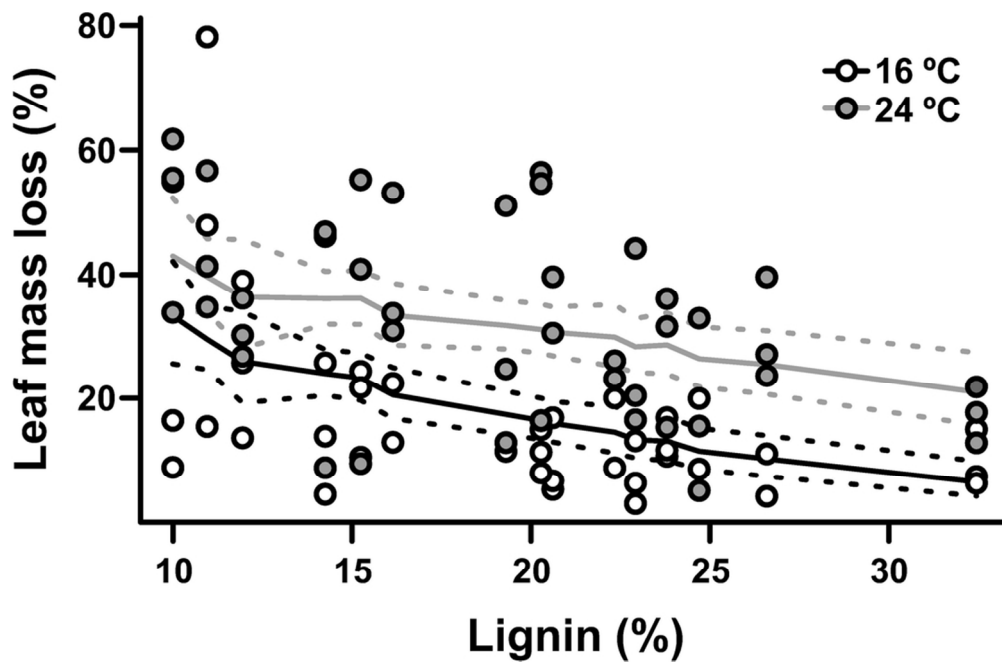


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