1 **Leaf litter traits of invasive species slow down decomposition**

2 **compared to Spanish natives: a broad phylogenetic comparison**

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Abstract

Leaf traits related to the performance of invasive alien species can influence nutrient cycling through litter decomposition. However, there is no consensus yet about whether there are consistent differences in functional leaf traits between invasive and native species that also manifest themselves through their afterlife effects on litter decomposition. When addressing this question it is important to avoid confounding effects of other plant traits related to early phylogenetic divergences and to understand the mechanism underlying the observed results to predict which invasive species will exert larger effects on nutrient cycling. We compared initial leaf litter traits, and their effect on decomposability as tested in standardised incubations, in 19 invasive-native pairs of co-familial species from Spain. They included twelve woody and seven herbaceous alien species representative of the Spanish invasive flora. The predictive power of leaf litter decomposition rates followed the order: growth form > family > status (invasive vs. native)>leaf type. Within species pairs litter decomposition tended to be slower and more dependent on nitrogen and phosphorus in invaders than in natives. This difference was likely driven by the higher lignin content of invader leaves. Although our study has the limitation of not representing the natural conditions from each invaded community, it suggests a potential slowing down of the nutrient cycle at ecosystem scale upon invasion.

1   Introduction

2   Litter decomposition is a key process in nutrient cycling (Swift et al. 1979; Hobbie

3   1992; Sayer 2006). Rates of litter decomposition can greatly influence nutrient

4   availability and thereby other ecosystem processes (Vitousek and Howarth 1991). At

5   community level, decomposition rates are controlled by species or functional type

6   composition (Hättenschwiler and Vitousek 2000; Garnier et al. 2004; Quested et al.

7   2005). Therefore, the introduction or invasion of alien species into new ecosystems can

8   cause profound changes in community structure and function (Mack et al. 2000; Kolar

9   and Lodge 2001), including changes in litter decomposition and nutrient cycling

10   (Allison and Vitousek 2004; Ashton et al. 2005). To determine the direction of these

11   changes, several studies have compared litter decomposition between invasive alien

12   species and natives. However, results greatly differ between studies, taxa and regions

13   (Ehrenfeld 2003) and no generalization (a frequent objective in the study of biological

14   invasion) has arisen so far. Faster litter decomposition from invasive alien species

15   compared to native litter has been reported when invaders were nitrogen-fixers and

16   native species were not (Liao et al. 2008), when nutrient concentration in the exotic

17   litter was higher than the native one (Allison and Vitousek 2004) and when specific leaf

18   area (SLA) of invaders was higher than that of native species (Castro-Díez et al. 2009;

19   Kueffer et al. 2008). By contrast, slower leaf litter decomposition of invasive alien

20   species has been found when this litter had higher polyphenolic content (Knight et al.

21   2007), higher lignin content or higher C/N ratio than native litter (Drenovsky and Batten

22   2007).

23   Differences between studies may be partly due to over-representation of

24   particular regions (e.g. Hawaii islands, Allison and Vitousek 2004) or functional types

25   (e.g. at least 1/3 of the articles involve nitrogen fixers, Levine et al. 2003), and they

1 have generally included only a few species pairs, not accounting for phylogenetic and

2 life history differences. Comparing large numbers of invasive and native species by

3 means of phylogenetic independent contrasts (PIC´s) is necessary to unveil general

4 trends avoiding confounding effects of phylogeny.

5   As important as avoiding confounding effects, is to understand the mechanisms

6 underlying the direction of the change upon invasion. This will potentially help to

7 predict which invasive alien species are likely to exert larger effects on nutrient cycling

8 and which communities are likely to suffer larger impacts (Levine et al. 2003). Invasive

9 species possessing leaf litter traits similar to natives will be functionally redundant in

10 the nutrient cycling mechanisms and no changes may be expected. By contrast, invasive

11 species with leaf litter traits largely different from those of natives, may impact the

12 nutrient cycling. Thus, the direction and magnitude of the change will depend on the

13 trait difference between both groups (Dukes and Mooney 2004; Strauss et al. 2006).

14 Under mediterranean soil conditions, where the availability of nitrogen and phosphorus

15 is low (Gallardo and Parama, 2007), we may predict that leaf litter from invasive alien

16 species with traits that retard decomposition will inhibit and slow down nutrient cycling,

17 while those promoting decomposition will have no effect (because of microrganism

18 immobilisation in cases of low nutrient soil concentration) or will slightly speed up

19 nutrient cycling (Knorr et al. 2005).

20   Taking this less used approach into account, we have the additional opportunity

21 to link ecosystem impacts to functional strategies of invasive species, since many leaf

22 litter traits are directly related to plant performance strategies such us competitive

23 ability (e.g. variation in nitrogen content), defence against herbivore (e.g. variation in

24 polyphenolic compounds) or resource use efficiency (e.g. variation in lignin content).

25 Thus, while many studies have tried to link species traits with invasiveness, the

1 importance of studying these traits is more related to differences in ecosystem impacts

2 than on invasiveness.

3   The objective of the present study was to compare leaf litter decomposability

4 between invasive alien and native species from Mediterranean ecosystems in Spain by

5 means of an experimental design using phylogenetically independent contrasts

6 (Armstrong and Westoby 1993). We addressed the following questions: i) Do leaf litter

7 properties of invasive alien species differ consistently from those of native ones? ii)

8 Does leaf litter of invasive alien species decompose faster than that of native species?

9 iii) Do initial leaf litter traits influence the decomposition process in the same way in

10 invasive and native species? To address these questions, a set of 19 invasive-native co-

11 familial pairs were selected and subjected to an experimental standardised screening for

12 leaf litter decomposability together with physical-chemical analyses.

13

Methods

Species selection

16 Our species set comprised introduced species that are clearly invasive (Pyšek et al.

17 2004), local dominants in some native ecosystems (Valéry et al. 2008) and with

18 potential impact in the invaded ecosystems (Richardson et al. 2000). Species compiled

19 by The Atlas of the Invasive Alien Species in Spain (Sanz Elorza et al. 2004) matched

20 our criteria of invasive species definition. Thus, it was used to select the invasive alien

21 species and the localities where the senesced leaf material was collected, ranging from

22 central to eastern Spain (Table 1). The final selection of invasive species was done on

23 the basis of leaf litter availability and aimed to represent of a broad diversity of growth

24 forms (annual and biannual herbs, lianas, shrubs and trees), invaded habitats

25 (woodlands, shrublands, grasslands, and riparian areas), and different life histories.

1   Nomenclature followed the Iberian Flora (Castroviejo 1986-2008). Each of the selected

2   species was paired with one closely related native species based on two criteria: (1)

3   within each pair, the native species had to belong to the same family as the invasive

4   species and (2) the two species had to belong to the same growth form (i.e., herbaceous

5   vs. woody species).

6   The selection of phylogenetically independent invasive-native pairs for

7   methodological reasons may induce biases in the ecological meaning of our results. To

8   minimize this problem, we conducted a screening to select native species with a

9   common occurrence throughout Spain and especially in the eastern part, which hosts the

10   highest diversity and abundance of invasive alien species (Gasso et al. 2009). Native

11   species with small distribution range, rare or endangered were excluded. Additionally,

12   we discarded those native species occurring in habitats different from those where alien

13   species invade or with occurrence in successional community stages different from

14   natives. The extended Herbarium database at Complutense University of Madrid

15   (MACB, founded 1968) was used to select, within pairs, native species recorded in the

16   same localities or next to where the alien species pair invade. This procedure has

17   permitted to do not lose the potential ecological meaning of our results in the whole

18   range of the invasive species, except for the case of *Ailanthus altissima* vs. *Cneorum*

19   *tricoccon* (Table 1), which only has ecological sense in the Balearic Islands and north of

20   Cataluña.

21

22   *Collection of leaf litter*

23   Recently senesced, undecomposed and herbivore-damage free leaf litter of invasive

24   alien species and their native relatives were collected following the criteria of

25   Cornelissen (1996). Leaf litter from deciduous woody species was collected throughout

1 the autumn of 2005, while leaf litter of evergreen woody and herbaceous species was

2 collected in the spring of 2006, according to their natural leaf senescence period. Ten

3 mature individuals for each species of the same population at full sun-exposure (in the

4 case of trees) were preferentially chosen for senesced leaf collection. Large variation in

5 the proportion of petioles to lamina was found between species (6.7±5.3%), but petioles

6 from all species were discarded to asses the decomposition ability of leaf litter, since the

7 variability within pairs was low (1.2±0.4%). Approximately 40 g of senesced leaves per

8 species were obtained after pooling the leaves from all individuals. They were

9 transported to the laboratory, air-dried at 20-25ºC and stored in open paper bags until

10 further analysis.

11

12 *Litter decomposition experiment set up*

13 For each species, four subsamples of 1.0±0.1g air-dried senesced leaves were used to

14 calculate their initial water content by weighing them again after three days in the oven

15 at 60ºC. Sixteen samples of air-dried leaves were extracted from each species pool,

16 weighed (1.0±0.1g) and corrected for water content. Then each of these samples was

17 sealed into a 1 mm-mesh nylon bag to allow free movement of macro- and meso-

18 decomposers into the bags (Cornelissen 1996). Large leaf size variation between species

19 lead to variation in bag size in order to spread out whole leaves in one single layer, since

20 surface access to soil decomposers is a determinant of decomposition rate (Cornelissen

21 1996). Litter bags were buried randomly in a purpose-built litter decomposition bed (7m

22 x 3.5m) at Alcalá University Botanical Garden (Madrid province, Central Spain). This

23 bed consisted of a thoroughly mixed 5 cm litter layer collected from a nearby mature

24 *Quercus* spp. forest. We selected this material trying to create an incubation matrix of

25 natural non-invaded forest communities representative of Spain with close-to-natural

1 microbial and soil fauna activity. Additionally, we covered the bed with a single layer

2 of senesced *Platanus hispanica* leaves. The decomposition bed was displayed below a

3 shade frame covered by a neutral shade mesh, which removed 30 % of the solar

4 radiation, to avoid rapid water loss, especially in summer. On 23 December 2005, the

5 litter bags of the autumn-deciduous invasive-native pairs (pair number 1, 4, 8 and 18 in

6 Table 1) were placed in the litter decomposition bed at 2 cm depth in the matrix, while

7 the rest of the fifteen pairs (where at least one of the species pair shed its leaves in

8 spring) were placed there on 22 June 2006. Two harvests of eight litterbags per species

9 were collected, the first after 147 and 143 days of incubation (for the December and

10 June pairs respectively), and the final after 289 and 231 days. First harvest date was

11 chosen by expert criteria to select a steady-state of mean litter decomposition around

12 50% of mass loss considering all species, while final harvest date was chosen when the

13 species with faster litter decomposition (*Colutea arborescens, Anagyris foetida*) were

14 close to a full decomposition. Eight additional litterbags of *Acer campestre, Lonicera*

15 *japonica* and *Ulmus pumila* were placed in each of the two periods of our experiment to

16 assess the effect of starting date on the decomposition rate. At each harvest, the material

17 remaining in each litterbag was carefully brushed clean, dried at 60ºC during 3 days,

18 and weighted to calculate the percentage of litter mass loss. For contamination by dust

19 particles see below.

20   At the beginning of the experiment, three 2.5 x 2.5 cm anion and two 2.5 x 2.5

21 cation exchange resin membranes (types I-100 and I-200, Electropure excellion-inc.,

22 Laguna Hills, California) fixed to a plastic label were introduced into the soil at three

23 equidistant points of the litter bed at 4 cm depth, and left for 15 days to evaluate

24 inorganic nitrogen and phosphorus availability (Abrams and Jarrell 1992; Subler et al.

25 1995). Resin membranes had been previously conditioned in the lab by immersing them

1 in demineralised water at 82-90ºC for 48 hours. Ions were extracted from the resins

2 using 20-25 ml KCl and ammonia, nitrate and phosphate contents were assessed

3 following Sims et al. (1995). Finally, local air temperature, available photon flux

4 density (PPFD) and soil moisture within the first 15 cm soil depth were registered every

5 15 minutes during the litter decomposition experiment with a data logger (HOBO model

6 H08-006-04, Onset, Pocasset, MA) and measured by external sensors (Li-Cor, Lincoln,

7 NB, USA; ThetaProbe sensors (Delta-T Devices, Cambridge, U.K)). The litter

8 decomposition bed was occasionally watered during dry periods, when soil moisture

9 dropped below 60% gravimetric water content (GWC).

10

11 *Measurements*

12 The initial area of the senesced leaves was measured in eight subsamples of eight leaves

13 each per species (Delta-T leaf area meter device, Cambridge), then oven-dried at 60ºC

14 during 3 days and weighed to calculate their specific leaf area (SLA=leaf area/leaf dry

15 mass). The remaining leaf litter, after removing samples for litter decomposition and

16 SLA assessment, was ground in a single pool per species, using a Cullati mill (1mm

17 particle size). Chemical analyses to determine litter leaf traits were conducted at the

18 laboratory of the Dept. of Systems Ecology, VU University, Amsterdam, The

19 Netherlands. Total initial concentrations of organic carbon, nitrogen, phosphorus,

20 calcium, potassium, magnesium and pH were calculated from a set of four ground

21 subsamples per species, while initial lignin and cellulose contents were assessed in one

22 ground sample per species. Carbon and nitrogen were also measured in four ground

23 samples of the final harvest. Finally, in four subsamples per species, ash content was

24 measured (mass loss on ignition, 575ºC) to correct litter sample dry mass for possible

25 sand or clay contamination. Since some plants can contain significant amounts of silica

1 and other minerals in their tissues (Epstein 1994), we only corrected for ash fractions

2 greater than 2-3% for species where we observed or suspected contamination by dust.

3   Total carbon and nitrogen were determined by dry combustion with a NA1500

4 series II elemental analyzer (Carlo Erba, Rodana, Italy). After digestion (of ground leaf

5 material) in a 1:4 mixture of 37% (v/v) HCl and 65% (v/v) HNO_3 (Sneller et al. 1999)

6 phosphorus was measured colorimetrically, calcium and magnesium were measured

7 with AAS and potassium with AES (both: Perkin Elmer 1100, Waltham, MA USA). pH

8 was measured using a narrow (5 mm diameter) SenTix Mic electrode connected to an

9 Inolab Level 2 pH meter (both: WTW, Weilheim, Germany). Initial litter lignin and

10 cellulose concentration was determined following Poorter and Villar (1997): in short,

11 after several extraction steps to ensure that only cellulose and lignin made up the

12 composition of the residue of the sample, the C and N concentrations of this residue

13 were used to calculate the lignin concentration, based upon the difference in carbon

14 content between cellulose and lignin.

15

16 *Statistics*

17 We applied Statistica 6.1 (StatSoft, Inc) and R package (library "stats") for all analyses

18 below. Percentage of litter mass loss at the first and final harvests (with initial mass as

19 the reference for both harvests) was arcsine-square-root(x/100)-transformed to approach

20 normal distributions and homogeneity of variance (Quested et al. 2003). Given that

21 some families were over-represented in our dataset (e.g. Fabaceae) (Table 1) and that

22 the 19 species pairs were not randomly selected, a Mantel test was conducted to test

23 whether differences in litter decomposition within pairs were influenced by pair

24 selection. The phylogenetic distance from one species to another between all the species

25 pairs was calculated through the first common ancestor to both species using the plant

1 phylogenetic supertree described by Soltis et al. (2000) and modifications by Bremer et

2 al. (2003).

3   Pairwise Student-t tests compared initial physical-chemical leaf litter traits and

4 percentage of litter mass loss across all pairs of invasive alien species and native co-

5 familial counterpart, at the first and final harvests respectively. The effect of the date of

6 incubation start (autumn or spring) on the percentage of litter mass loss was assessed for

7 the three control species (*Acer campestre, Lonicera japonica* and *Ulmus pumila*) by a

8 two-way ANOVA using time and species as main independent factors.

9   In order to assess the relative importance of different predictors for the litter

10 mass loss, a four-way ANOVA was conducted taking as the main independent variables

11 Growth form (GF), Family (F) nested in GF -because none of the families comprised

12 both woody and herbaceous species- Leaf Type (LT- deciduous or evergreen) and Plant

13 Status (invasive (I) or native (N)). Additionally, multiple regression models were

14 conducted to evaluate which senesced leaf traits better predicted the percentage of litter

15 mass loss in each species group (invasive alien and native species) at each harvest.

16 Pearson correlations were conducted to check for co-linearity between predictors.

17 Predictors not or poorly correlated were chosen ($r<0.10$), while among those highly

18 correlated ($r>0.70$) only one predictor with high ecological meaningful was selected

19 (Fowler and Cohen, 1992). Therefore, initial lignin concentration was discarded because

20 it was highly correlated with the initial carbon concentration in both species sets

21 (invasive species $r>0.80$ and native species $r>0.75$). Different models were fitted

22 selecting different sets of predictive variables by the approach based on information

23 theory (Akaike information criteria, AIC) (Burnham and Anderson, 2002). AIC was

24 calculated for each model and used to choose the best-fitted. A value of difference in

25 AIC (ΔAIC) higher than 10 represents a poor fitted model compared to the best model,

1   whereas a value of less than 2 indicates that both models are equivalent (Burnham and

2   Anderson, 2002).

3

4   **Results**

5   *Litter bed characteristics*

6   The most abundant form of available mineral nitrogen in the litter decomposition bed

7   was NO_3^--N (1.82 µg $m^{-2}day^{-1}$), two times more than NH_4^+-N (0.86 µg $m^{-2}day^{-1}$). The

8   availability of PO_4^--P was 0.72 µg $m^{-2}day^{-1}$, and thus the N/P ratio of the available pool

9   was N/P= 1.9. Mean (±standard error) air temperature, soil GWC and daily PPFD

10   during the first incubation period for deciduous autumn pairs (see Methods) were

11   10.21(±7.06) ℃, 68.74(±3.46) % and 24 mol m^{-2} day^{-1} respectively, whereas for the

12   final incubation period with the rest of the pairs these means were 16.84(±9.77) ℃,

13   65.12(±5.73) % and 32 mol m^{-2} day^{-1} respectively.

14

15   *Leaf litter traits, decomposition rates and decomposition predictors*

16   SLA did not differ between invasive alien and native species (Table 2). Initial calcium

17   and lignin concentration were significantly higher in invasive species. Total carbon

18   concentration was marginally higher in invasive species. Cumulative mass loss at the

19   final harvest was significantly higher in invasive species of a pair than in their co-

20   familial native species, whereas a similar trend was not significant at the first harvest

21   (Table 2). Mean values of all measured traits for each species are shown in the

22   Electronic supplementary material (Table S1). Mantel test R (% Mass loss - Phylogeny

23   =1.34, p=0.529), showed that differences in % litter mass loss between invasive and

24   native species were not influenced by phylogenetic structure of invasive-native pair

25   selection. In addition, two-way ANOVA showed that the effects of the date of

12

1   incubation start (as determined by starting date) were comparable because significant

2   differences were only found for species ($F_{2,90}$=41.68, p<0.001), but not for time period

3   ($F_{1,90}$=0.09, p=0.769) or the time period by species interaction ($F_{2,90}$=1.66, p=0.206)

4   Four-way ANOVA showed that growth form was the most important categorical

5   factor explaining the variation of final litter mass loss across the whole data set, mass

6   loss being faster in herbaceous than in woody species. Family was the second best

7   predictor, followed by plant status (invasive-native), native species decomposes faster

8   than invasive species, and finally leaf type, deciduous decomposing faster than

9   evergreen species. No significant effect was found in the interaction between plant

10   status and the other categorical factors (Fig. 1).

11

12   *Leaf litter decomposition processes*

13   Total initial concentrations of C, N, P, cations (sum of Ca + K + Mg), pH, cellulose and

14   SLA were the independent predictors selected to assess the relative importance of leaf

15   litter traits in litter decomposition of both harvests and for the invasive and native

16   species groups separately (Table 3). Initial lignin was excluded due to its high

17   correlation with initial C concentration (see above, Methods). Multiple-regression

18   models revealed that the predictors were different between invasive and native species

19   and between harvests (Table 3). Nitrogen was related positively to litter decomposition

20   in all cases and its relative importance increased at the final harvest for invasive species.

21   Phosphorus was the most important positive predictor of % litter mass loss, but only for

22   invasive species at the final harvest. Carbon and thus lignin were negatively related to

23   litter mass loss in native species across the whole incubation period, while only in the

24   first harvest in invasive species (Table 3). pH was a positive predictor of native litter

25   decomposition rate at the final harvest and of invasive species only at the first harvest.

1   A positive relation between decomposition rate and SLA only emerged at final harvest

2   for both groups of species. Finally, cellulose was slightly negatively related to

3   decomposition rate in native species at both harvests (Table 3).

4   The goodness-of-fit of the models (r^2) was higher for native than for invasive

5   species. Regression models generally predicted litter decomposition rate better at the

6   final than at the first harvest for invasive species (Table 3).

7

8 **Discussion**

9 *Leaf litter characteristics and mass loss rates*

10   Leaf litter of alien species invading Mediterranean ecosystems in Spain tended to

11   decompose slightly more slowly than that of the co-familial native species. This can be

12   due to large initial lignin content, which was significantly higher in invasive alien

13   species across invasive-native species pairs. This well-studied relationship (Swift et al.

14   1979, Cadisch and Giller 1997) was recently confirmed within multiple sites worldwide

15   (Cornwell et al. 2008). Invasive alien species combine higher lignin with higher calcium

16   initial content (Table 2). Calcium plays an important role in lignin synthesis and a

17   deficit of this macroelement in soils limits lignification of plant tissues (Lautner et al.

18   2007). Indeed, the soils of the locations where leaf litter was collected (Table 1) are

19   characterised by high availability of calcium (Rubio and Escudero 2005), apparently not

20   limiting tissue lignification of invasive alien species. Although plant species trade off

21   plant growth for tissue lignification (Villar et al. 2006), the elevated carbon assimilation

22   capacity for growth commonly described in invasive species (Pysek and Richardson

23   2007) and the higher levels of litter lignin found in our study, may reflect a carbon

24   availability enough to maximise both strategies. This overall carbon strategy apparently

25   improves the growth rate and protection of leaf tissues which leads to a higher nutrient

1   use efficiency, another strategy also reported in invasive alien species (Pysek and

2   Richardson 2007).

3   Our results do not match those from previous studies comparing leaf litter

4   properties of invasive and native species, as they showed that, in general, invasive alien

5   species tended to exhibit higher specific leaf area and higher nutrient concentrations

6   (see Ehrenfeld (2003) for a detailed description). However, these differences seem to be

7   biased by the selection of species and regions (mostly N_2 fixing invasive species in

8   oligotrophic soils) (Levine et al. 2003; Yelenik et al. 2007).

9

10 *Predictors of litter decomposition rate*

11 Nitrogen content in litter was related to litter decomposition in all species groups and in

12 both harvests (Table 3). This agrees with the well known fact that high nitrogen content

13 in litter and in soil promotes decomposition (Ehrenfeld 2003, Cornwell et al. 2008).

14 However, its relative importance was higher in invasive alien species in the final harvest

15 (Table 3), which may be attributed to their higher lignin content. Lignin, which forms

16 complex associations to litter proteins, inhibits the action of decomposing organisms

17 and delays the decomposition processes during the first months (Gallardo and Merino

18 1993). Thus, nitrogen, unlike lignin, becomes an important predictor later on, when soil

19 organism activity overcome the lignin barrier complex and starts to release nitrogen

20 from litter proteins (Gallardo and Merino 1992). In addition, phosphorus content in leaf

21 litter exhibited a great positive influence on the decomposition of invasive alien litter,

22 but not on native litter (Table 3). Both results are in concordance with Bubb et al.

23 (1998) and Xuluc-Tolosa et al. (2003), who demonstrated that litter of high lignin

24 content needs high nitrogen content in leaves and soil for its decomposition, and that

25 this process becomes phosphorus dependent. Consequently it may become important as

1   a limiting factor for the decomposition of the lignin-rich litter of invasive alien species

2   when lignin degradation by micro-organism takes off. Perhaps the lower lignin content

3   of the litter of the native species makes them less dependent on phosphorus and on

4   nitrogen (Table 3) (Knorr et al. 2005).

5   Overall, leaf traits predicted litter decomposition rate better for native than for

6   exotic species, especially at the first harvest. This suggests that other unquantified litter

7   traits of exotic species might initially exert a tighter control on the decomposition

8   process than the measured traits. For instance, high levels of relatively mobile

9   polyphenolic compounds have been commonly reported in invasive species (Ehrenfeld

10   2003; Knight et al. 2007). These carbon-based defences may be useful under the full-

11   sun habitat conditions of the early community succession stages, where aliens mainly

12   invade because they provide protection from UV light, and defence against generalist

13   herbivores, but not against the specialist ones (See references in Marko et al. 2008).

14   Such ecological strategy of invasive species remains in the senesced leaves, influencing

15   the initial processes of litter decomposition (Hättenschwiler and Vitousek 2000).

16

17   *Consequences of invasions for nutrient cycling*

18   Lignin is an immobile carbon-based substance that influences nutrient cycles at

19   ecosystem scale slowing down litter decomposition (Swift et al. 1979, Cornwell et al.

20   2008). Contrary to most other studies on litter decomposition rates by aliens versus

21   natives, our results have demonstrated that invasive alien species produce less

22   decomposable leaf litter compared to co-familial native Mediterranean plants, probably

23   due to their higher lignin content. Our results therefore suggest that the soil nutrient

24   turnover, and thereby nutrient availability, will not be enhanced upon invasion in

25   Spanish Mediterranean ecosystems; actually it might even be slightly inhibited.

1   However, our experiment has the limitation of not representing natural soil and litter

2   layer conditions in the habitats where invasive species are becoming abundant. In

3   addition, our decomposition bed litter matrix was composed of species (*Quercus* ssp.)

4   that generate low decomposition rates (Gallardo and Merino 1993) possibly selecting

5   organism adapted to decompose lignin-rich litter but reducing the soil fauna and

6   microbial diversity (Gallardo and Merino 1992). Thus, changes in decomposer

7   community, soil conditions, or both caused by invasion processes may feed back or

8   moderate the decomposition patterns reported here. For instance, although litter from

9   invasive *Acacia* species is hard to decompose (see "Electronic supplementary material",

10   Table S1; Yelenik et al. 2007) their nitrogen-fixing strategy highly augments nitrogen

11   soil availability and microbial soil activity, accelerating litter decomposition upon

12   invasion (Yelenik et al. 2007). High soil nitrogen and phosphorus availability by natural

13   or human-mediated actions may lead to the same results (Knorr et al. 2005). However,

14   Spanish Mediterranean soils are in general characterised by their low nitrogen and

15   phosphorus content (Gallardo and Parama 2007) and their low microbial activity and

16   biomass (Gallardo and Merino 1992). Accordingly, a progressive increment of invasive

17   plant species characterised by their lignin-rich litter and without a significant increment

18   in nitrogen and phosphorus soil availability, would cause a slow down of nutrient

19   cycling by reducing the litter quality and the abundance and diversity of decomposer

20   communities (Gallardo and Merino 1992; Chigineva et al. 2009). In this sense, Cotrufo

21   et al. (1998) demonstrated that litter of impoverished quality due to an increment in

22   lignin content and lignin:N ratio was avoided by soil fauna in a food choice experiment.

23   Our results also suggest that under the scenario of a progressive displacement of

24   native species by invasive ones, altering the spectra of growth forms in an ecosystem,

25   invokes the highest relative impact on the litter decomposition rate (the relative impact

depending on whether the native community is completely displaced or invasive and native species coexist) (Fig. 1). Consequently, changes in community structure from herbaceous to less decomposable invasive woody species, which have been reported for different Mediterranean-type ecosystems worldwide (Di Tomaso 1998, Bousquet-Melou et al. 2005) could augment this negative feedback on litter decomposition. Our data also highlights the importance of Family and, to a lesser extent, Leaf type on the litter decomposition rate (Fig. 1). The impoverishment of taxonomic diversity in native woody communities due to the introduction of forestry evergreen exotic trees, belonging to a few selected taxonomic groups (e.g. *Pinus* -Pinaceae- and *Eucalyptus* -Myrtaceae-) (see "Electronic supplementary material", Table S1 for mean mass loss values), could also augment this negative feedback. There may be worse consequences for nutrient cycling of the invasive plant species establishment when both effects occur at the same time, as it has been reported in the Cape Region of South Africa (Manders and Richardson 1992).

In summary, invasive species have the potential to slow down nutrient cycling in Spanish Mediterranean ecosystems through slower litter decomposition. The high lignin content that drives this change is a reflection of the features displayed by alien species to become invasive. The degree of the impact will be the highest when invasive species had higher lignin content than natives, which involves a few genera of woody evergreen alien species.

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Figure Legend

Figure 1 Four-way analysis of variance assigning the relative importance of family, growth form (woody/herbaceous), leaf type (evergreen deciduous) and status (invasive/native) on percentage mass of leaf litter, based on the 19 invasive-native co-familial pairs (see Table 1). Mass loss % was arcsine (square-root(x/100)) transformed prior to analysis. Family, as a predictor, was nested in Growth Form because there was no family with both woody and herbaceous species. Explained/Residual variance corresponds with the F values of the ANOVA.

Table 1 Invasive-native phylogenetic pairs, invaded-type ecological community and locations selected. W: Woody species, H herbaceous species, E: Evergreen, D: Deciduous.

Family	Invasive species	Native species	Ecological community	W/H	E/D	Spatial location, Spanish Province
Aceraceae	*Acer negundo*	*Acer campestre*	Woodland	W	D	40º 30´ N, 3º 20´ W, Madrid
Anacardiaceae	*Schinus molle*	*Pistacia terebinthus*	Shrubland	W	E/D	39º 10´ N, 0º 16´ W, Valencia/ 40º 34´ N, 3º 09´ W, Guadalajara
Asteraceae	*Achillea filipendula*	*Achillea millefolium*	Grassland	H	D	40º 30´ N, 3º 20´ W, Madrid
Caprifoliaceae	*Lonicera japonica*	*Lonicera etrusca*	Shrubland & Riparian areas	H	D	40º 30´ N, 3º 20´ W, Madrid
Amaranthaceae	*Atriplex semibaccata*	*Atriplex halimus*	Shrubland	H	E	38º 14´ N 0º 32´ W, Alicante/ 40º 30´ N, 3º 20´ W, Madrid
Convolvulaceae	*Ipomoea indica*	*Calystegia sepium*	Riparian areas	H	D	38º 24´ N 0º 26´ W, Alicante
Elaeagnaceae/ Rhamnaceae	*Elaeagnus angustifolia*	*Rhamnus alaternus*	Shrubland	W	D/E	40º 30´ N, 3º 20´ W, Madrid
Fabaceae	*Gleditsia triacanthos*	*Colutea arborescens*	Shrubland	W	D	40º 34´ N, 3º 09´ W, Guadalajara
Fabaceae	*Sophora japonica*	*Ceratonia siliqua*	Woodland	W	E	40º 30´ N, 3º 20´ W, Madrid/ 38º 33´ N 0º 31´ W, Alicante
Fabaceae	*Robinia pseudoacacia*	*Coronilla glauca*	Shrubland	W	D/E	40º 30´ N, 3º 20´ W, Madrid
Fabaceae	*Acacia saligna*	*Anagyris foetida*	Shrubland	W	E/D	38º 31´ N 0º 10´ W, Alicante/ 39º 09´ N 0º 21´ W, Valencia
Myrtaceae	*Eucalyptus globulus*	*Myrtus communis*	Shrubland	W	E	40º 30´ N, 3º 20´ W, Madrid
Onagraceae	*Oenothera biennis*	*Epilobium hirsutum*	Grasslands & Riparian areas	H	D	39º 06´ N 0º 23´ W, Valencia/ 40º 30´ N, 3º 20´ W, Madrid
Pinaceae	*Pinus radiata*	*Pinus pinaster*	Woodland	W	E	40º 30´ N, 3º 20´ W, Madrid
Simaroubaceae/ Cneoraceae	*Ailanthus altissima*	*Cneorum tricoccom*	Shrubland	W	D/E	40º 30´ N, 3º 20´ W, Madrid
Solanaceae	*Nicotiana glauca*	*Lycium intricatum*	Shrubland	H	E	38º 05´ N 0º 40´ W, Alicante
Solanaceae	*Solanum bonaeriense*	*Solanum nigrum*	Shrubland &			39º 33´ N 2º 42´ W, Mallorca/

Table 2 Leaf litter trait comparison using a pairwise-T test based on the 19 invasive-native co-familial pairs. Mean values ± SE are shown. An asterisk on p-values means that differences were found between pairs. Each pair was considered as a replicate.

Leaf litter traits	Invasive	Native	t-value	P
% Litter Mass First Harvest	0.36±0.01	0.42±0.01	-1.84	0.11
% Litter Mass Final Harvest	0.52±0.02	0.60±0.02	-2.02	0.049*
Initial Nitrogen (mg/g)	1.36±0.01	1.38±0.01	-0.13	0.90
Initial Carbon (mg/g)	51.87±1.98	50.98±2.10	1.79	0.091
Initial C:N	50.25±2.67	47.19±2.36	0.47	0.65
Final Nitrogen (mg/g)	1.95± 0.39	2.04± 0.63	-0.52	0.62
Final Carbon (mg/g)	52.68± 1.68	52.00±1.15	0.95	0.21
Final C:N	35.36±3.04	32.77±3.12	0.63	0.55
Initial Phosphorus (mg/g)	0.13±0.08	0.16±0.07	-1.03	0.31
Initial Calcium (mg/g)	4.10±0.03	3.21± 0.02	2.17	0.041*
Initial Potassium (mg/g)	1.37±0.01	1.34±0.01	0.08	0.95
Initial Magnesium (mg/g)	0.72±0.05	0.72±0.04	-0.01	0.99
Total Initial Cations (mg/g)	5.86±0.05	5.27±0.05	1.48	0.16
pH	5.89±1.13	5.55±0.61	1.55	0.25
Initial Lignin (mg/g)	139.39±2.92	95.19±1.53	2.61	0.029*
Initial Cellulose (mg/g)	166.10±1.92	170.39±2.04	-0.32	0.68
SLA (m2/kg)	9.87±3.54	9.32±2.92	-0.65	0.52

Table 3 Multiple-regression models for invasive and native species at first and final harvest. Dependent variable was the decomposition rate within harvest and independent predictors were selected among initial leaf traits. Empty cells indicate that the leaf trait was not included in the model. Models are ranked in each case by AIC from best to worst-fitting model, and only the models with ΔAIC<2 are presented. For each variable entering the model the standardized regression coefficient is shown to evaluate its relative importance. An asterisk indicate that the variable is significant in the fitted model at p<0.05.

Group	C	N	P	Cations	pH	SLA	Cellulose	AIC	Δ AIC	r²
Invasive first harvest	-0.08*	0.07*						-62.028	0.0	0.21
	-0.08*	0.06*			0.12*			-61.434	0.6	0.22
	-0.09*				0.13*			-60.652	1.4	0.18
	-0.08*	0.07*					-0.01	-60.405	1.6	0.19
	-0.08*	0.06*		0.01				-60.211	1.8	0.20
	-0.09*	0.07*				0.04		-60.208	1.8	0.17
Native first harvest	-0.23*	0.12*		0.02*	0.04	0.05	-0.06*	-118.058	0.0	0.73
	-0.22*	0.11*		0.02*	0.05		-0.06*	-117.004	1.1	0.74
Invasive final harvest	-0.06	0.33*	0.65*	0.03*		0.09		-56.183	0	0.52
	-0.06	0.33*	0.66*	0.04*	-0.02	0.11*		-56.031	0.2	0.52
	-0.06	0.32*	0.66*	0.03*		0.01	0.03	-55.165	1	0.52
	-0.06	0.33*	0.67*	0.04*	-0.02	0.11*	0.03	-54.655	1.5	0.51
	-0.07	0.35*		0.04*	-0.02	0.11*		-54.574	1.6	0.47
Native final harvest	-0.23*	0.11*		0.02*	0.18*	0.14*	-0.10*	-75.564	0.0	0.69
	-0.23*	0.12*	-0.12	0.02*	0.19*	0.15*	-0.11*	-73.856	1.7	0.70

Predictor variable codes are: C: Carbon (%), N: Nitrogen (%), P: Phosphorus (%), Cations: (Magnesium (%) + Calcium (%) + Potassium (%)) in litter before incubation. SLA: Specific Leaf Area. Initial lignin concentration is included in Carbon.
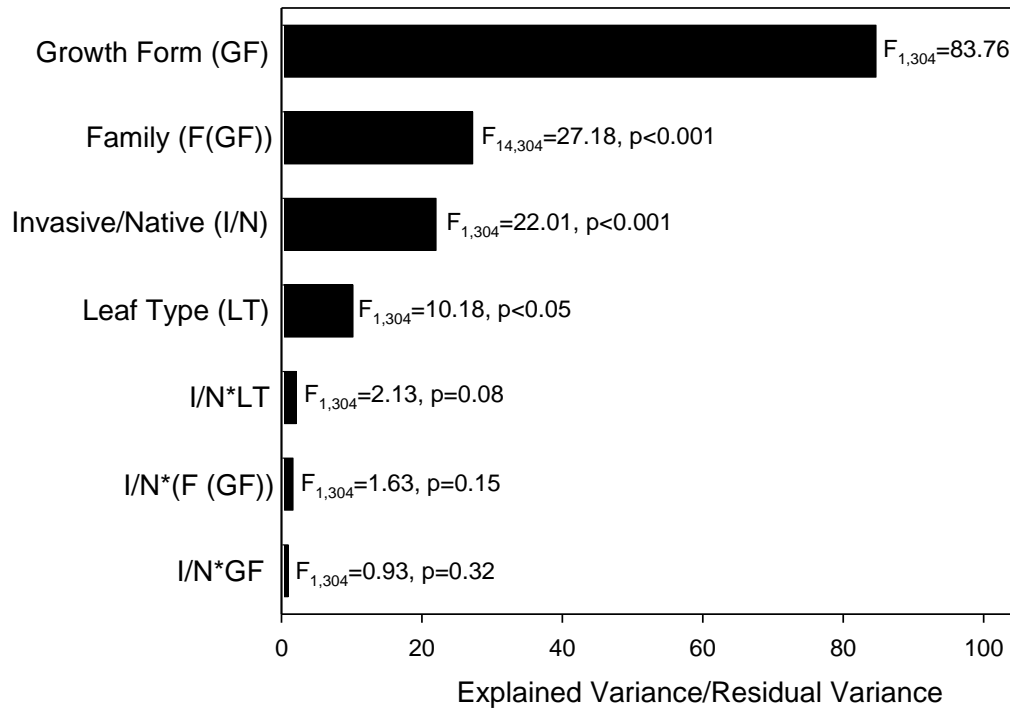
Figure 1