



Comparative analysis of leaf-litter decomposition from the native *Pouteria salicifolia* and the exotic invasive *Ligustrum lucidum* in a lowland stream (Buenos Aires, Argentina)

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With 6 figures and 2 tables

Abstract: We compared loss in leaf mass and chemical changes (concentrations of carbon, nitrogen, phosphorous, acid detergent fiber, lignin and phenolic compounds) during the decay of submersed leaf-litter of the native *Pouteria salicifolia* and the exotic invasive *Ligustrum lucidum*, in a lowland stream of Buenos Aires (Argentina). Leaf input of *L. lucidum* was greater than that of *P. salicifolia*. In addition, leaves of *L. lucidum* decomposed more rapidly than those of *P. salicifolia*, which appear to be related to leaf chemistry, i.e. initial content of carbon, phosphorous, acid detergent fiber, lignin and phenolic compounds and the greatest microbial colonization. Differences in leaf chemistry and in the contribution of each plant species to litter changed the quality and quantity of organic matter entering the stream, and therefore influenced differently the process of decomposition. Consequently, we expect that these changes might alter nutrient cycles and food web dynamics.

Key words: decomposition, exotic tree, food web dynamics, invasion, native tree.

Introduction

Organic matter is the main source of matter and energy for saprotrophic microbiota (Wallace et al. 1999) in aquatic food webs (Gulis et al. 2009). Many lotic environments depend on allochthonous inputs, especially from the leaves of riparian trees (Cummins 1974, Wallace et al. 1997). Consequently, leaf decomposition is considered a fundamental process in nutrient cycles of

forested streams, particularly in small streams where the amount of solar energy available for primary producers is limited (Webster et al. 1995, Graça & Canhoto 2006).

In general, lotic systems crossing the Pampean area (Buenos Aires, Argentina) are characterized by the absence of riparian forests in the upper portions of the catchment basins and the presence of abundant submerged and floating vegetation, which are their ma-

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major sources of matter and energy (Tangorra 2005). Las Cañas is a small stream located in the Natural Reserve “Selva Marginal Punta Lara” which is unique among Pampean streams because its banks are surrounded by a sub-climax forest dominated by an assemblage of the natives *Ocotea acutifolia* (Ness) Mez (Lauraceae), *Allophylus edulis* (St. Hil.) Radlk. (Sapindaceae), *Sebastiania brasiliensis* Spreng. (Euphorbiaceae) and *Pouteria salicifolia* (Spreng.) Radlk. (Sapotaceae) (Cabrera & Dawson 1944).

Some Pampean streams like Las Cañas are subjected to invasion of non-native species (Gantes et al. 2011), such as the exotic *Ligustrum lucidum* Ait. (Oleaceae), commonly known as glossy privet. This plant, which is a widespread invasive species, has become well established in many riparian forests of SE and central Argentina and continues to spread into new ecosystems (Montaldo 1993, Aragón & Groom 2003, Hoyos et al. 2010, Gavier-Pizarro et al. 2012).

Invasion by exotic trees into riparian areas has the potential to impact aquatic systems and food web dynamics. Some studies have shown that invasive exotic species produce litter with higher rates of decay than the native species (Ehrenfeld 2003). Dascanio et al. (1994) compared the rates of decay of *L. lucidum* litter with those of native species (mainly composed of *P. salicifolia*, *O. acutifolia* and *Lonchocarpus nitidus*) on the forest floor of the Natural Reserve of Punta Lara and found that the leaves of *L. lucidum* decomposed faster than the native species. These differences can be mainly attributed to leaf chemistry (Melillo et al. 1984, Webster & Benfield 1986, Boulton & Boon 1991, Gessner & Chauvet 1994), which is considered to be an important trait that can alter nutrient cycles in an ecosystem (Ehrenfeld 2003). Globally, invasive species are a major driver of change in ecosystems, yet understanding their impacts remains a critical challenge.

It is not known how changes in the quality and quantity of leaf inputs, which are a consequence of replacement of native by exotic species, will affect microbial colonization and consequently nutrient cycling in aquatic systems of invaded areas. Therefore, it is ecologically important to understand and compare the dynamics of decomposition and leaf chemistry of both native and non-native species, in order to predict the potential effects of invasion on food web dynamics.

In general, we expect that changes in the quality and quantity of leaf litter entering the stream as a consequence of invasion by exotic species, would have a significant effect on the structure and activity of the

microbiota and would subsequently affect ecological services. In particular, we expect that the exotic *L. lucidum* would decompose at a faster rate than the native *P. salicifolia* and that this is due to different leaf chemistry. Consequently, we estimated and compared the contribution to litter inputs, decay rates and leaf chemistry of submerged leaves of *Ligustrum lucidum* and *Pouteria salicifolia* in Las Cañas stream.

Material and methods

Study area

The study was conducted at Las Cañas stream (34° 47' 58.5" S –57° 57' 19.3" W; 34° 47' 29.3" S –57° 59' 49.2" W) which is a 600 m long stream (Table 1) located in the Natural Reserve “Selva Marginal Punta Lara”, on the northeastern side of Ensenada and Berazategui districts, Buenos Aires province, Argentina.

The stream runs through a riverine marginal forest of native species (*Blephalocalix tweedii* (Hook et Arn) Berg., *O. acutifolia*, and *P. salicifolia*), which represent 82 % of the forest biomass and the remaining 18 % of biomass belongs to the exotic *L. lucidum* (Cabrera & Dawson 1944, Cabrera 1960, Dascanio et al. 1994). Both *L. lucidum* and *P. salicifolia* are evergreen species that were selected due to their high frequency and abundance in the study area (Dascanio & Ricci 1988), particularly along the stream banks (personal observation).

Sampling

Senescent leaves (just before abscission) from several individuals of *L. lucidum* and *P. salicifolia* growing along stream banks were collected on August 26, 2007. The leaves were air-dried in the laboratory for 96 h at room temperature (Moreira 2006). The litterbag technique (Bocock & Gilbert 1957) was

Table 1. Range (average $\pm$ SD) of the environmental parameters of Las Cañas stream (data from Marano et al. 2011). SD: Standard deviation; DO: Dissolved oxygen; BOD₅: Biological oxygen demand; COD: Chemical oxygen demand; SRP: Soluble reactive phosphorous.

Parameter	Range
Width (m)	6.82 ($\pm$ 1.71) – 9.57 ($\pm$ 1.77)
Depth (m)	0.34 ($\pm$ 0.16) – 0.88 ($\pm$ 0.27)
Flow (m s ⁻¹)	0.02 ($\pm$ 0.01) – 0.05 ($\pm$ 0.01)
Temperature (°C)	9.17 ($\pm$ 0.41) – 21.67 ($\pm$ 1.63)
pH (range)	6.70–8.31
DO (mg L ⁻¹)	2.87 ($\pm$ 1.39) – 5.20 ($\pm$ 0.12)
BOD ₅ (mg L ⁻¹)	2.17 ($\pm$ 0.75) – 9.67 ($\pm$ 1.75)
COD (mg L ⁻¹)	22.5 ($\pm$ 1.05) – 49.83 ($\pm$ 1.33)
Sulfate (mg L ⁻¹)	0.41 ($\pm$ 0.28) – 19.08 ($\pm$ 0.24)
Nitrate (mg L ⁻¹)	0.05 ($\pm$ 0.01) – 0.62 ($\pm$ 0.17)
Nitrite (mg L ⁻¹)	0.01 ($\pm$ 0.003) – 0.10 ($\pm$ 0.01)
Ammonium (mg L ⁻¹)	0.004 ($\pm$ 0.07) – 0.66 ($\pm$ 0.12)
SRP (mg L ⁻¹)	0.17 ($\pm$ 0.04) – 0.57 ($\pm$ 0.01)

employed using 25 × 20 cm, 1-mm mesh plastic bags, each one containing 15 g of *L. lucidum* or 10 g of *P. salicifolia* leaves (fresh mass). Three bags of each plant species were placed onto 45 × 9 × 20 cm expanded polystyrene supporting structures and distributed in the stream on August 30 following a randomized block design (Zar 1996). Three bags of each species were retrieved at 5, 10, 14, 20, 30, 42, 57, 72, 90, and 120 days for *L. lucidum* and 5, 14, 30, 42, 72, 120, 180, 240, and 270 days for *P. salicifolia* and transported to the laboratory in a coolbox. The times of submersion were defined according to a previous preliminary experiment conducted in 2006. In order to assess the contribution of *L. lucidum* and *P. salicifolia* to litter inputs, in June and October 2006, we collected three samples of floating organic matter at six sampling sites equidistantly distributed along the stream from headwaters to where the stream discharges into the Río de La Plata river. Samples from each site were homogenized and weighed in the laboratory (fresh mass). The relative contribution of each species to the total sampled biomass was calculated as percentage.

Laboratory analysis

Litterbags were processed within 1 h after collection. The material in each bag was placed in a plastic 1-mm mesh sieve, washed repeatedly with sterile deionized water to remove undesirable sediments and, whenever possible (according to the consistency of the leaves), carefully cleaned with a wet brush. The material was then oven-dried (80 °C) until constant mass (Mettler Toledo PB 302, Max: 310 g, Min: 0.2 g).

Initial oven-dry mass (T_0 : leaves before submersion) was determined for three subsamples per species in order to estimate the dry mass at the beginning of the experiment (initial dry mass). After drying, leaves were pulverized with a coffee blender. Samples of pulverized leaves were analyzed individually for the concentrations of phosphorous (P), nitrogen (N), carbon (C), phenolic compounds, acid detergent fiber (ADF) and acid detergent lignin (ADL). Chemical analyses were performed for all samples collected after variable times of submersion until day 90 for *L. lucidum* and day 180 for *P. salicifolia* due to limitations for obtaining the required amount of material (minimum required of ca. 5 g). The concentrations of P, N and C were analyzed by wet digestion with a mixture of hot (235 °C) nitric: perchloric acid (ratio 2:1) under reflux for 3 h (Johnson & Ulrich 1959). Determinations were made according to EPA Standard 200.7, with an Atomic Emission Spectrometer for Inductively Coupled Plasma (ICP-AES, Shimadzu Model 1000 Sequential III, with conventional nebulization). The ADF and ADL were determined according to the methods described by Van Soest et al. (1991) and Komarek (1993), using the filter bag technique with a computer model ANKOM® AK 8/ Model 05 and 200, respectively (ANKOM Tech Corp., Fairport, New York). The material was pre-treated with an acid diluted solution of detergent, followed by acetone extraction in order to remove the fraction of acid detergent cell wall (ADCW). This fraction consists of labile components, including soluble carbohydrates, soluble proteins, organic acids, hemicellulose and other soluble organic materials. The remaining fraction, which corresponds to lignocellulosic residues, was hydrolyzed by treatment with 72 % sulfuric acid to obtain the lignin fraction and heated at 500 °C for 4 h in order to correct the ash content (ANKOM Technology 2005).

Extraction of free phenolic compounds was performed following the protocol of Slinkard & Singleton (1977) and Osono

& Takeda (2001). Leaves were pulverized in a mortar with the addition of a volume of 50 % methanol. The mixture was incubated for 1 h at 75 °C under stirring conditions and then centrifuged. Determinations were made according to the Folin-Ciocalteu technique and expressed as equivalents of tannic acid (Saparrat et al. 2010).

Statistical analysis

Decomposition rates

Decay rates (k) were estimated by calculating the percentage of dry mass remaining after each time of submersion using an exponential decay model (Olson 1963, Petersen & Cummins 1974):

$$M_t = M_0 \cdot e^{-kt}$$

where M_0 is the initial dry mass, M_t is the dry mass remaining at the time of submersion t and k is the decay rate.

To test for significance between the slopes (decay rates) [dependent variable] we used a one-way analysis of covariance (ANCOVA) considering the time as a covariant (Zar 1996). F-test for r^2 was used to compare regression equations (Sokal & Rohlf 1981).

Leaf chemistry

The concentrations of each chemical compound in the leaves of both species (dependent variables) at T_0 were compared using a one-way analysis of variance (ANOVA) followed by Tukey's test (Zar 1996).

Results

Contribution of *L. lucidum* and *P. salicifolia* to litter inputs

The contribution of *L. lucidum* to total sampled biomass varied from 23 to 98 % depending on the site and sampling date, and was always greater than the contribution of *P. salicifolia*, which ranged from 0 to 5 % (Fig. 1).

Decay rates

The percentage of dry mass remaining at each time of submersion is shown in Fig. 2. The annual decay rate of the leaves of *L. lucidum* was significantly greater than of *P. salicifolia* (k : 4.094 year⁻¹ and k : 0.934 year⁻¹, respectively; $p < 0.01$). The daily decay rate was k : 0.0112 d⁻¹ for *L. lucidum* and k : 0.0026 d⁻¹ for *P. salicifolia*. Regression slopes were significantly different ($p < 0.01$; F : 63.76).

Leaf chemistry

Initial concentrations of most compounds were higher for *P. salicifolia* ($p < 0.01$), except for C:N and C:L (carbon:lignin) ratios that were higher in *L. lucidum*.

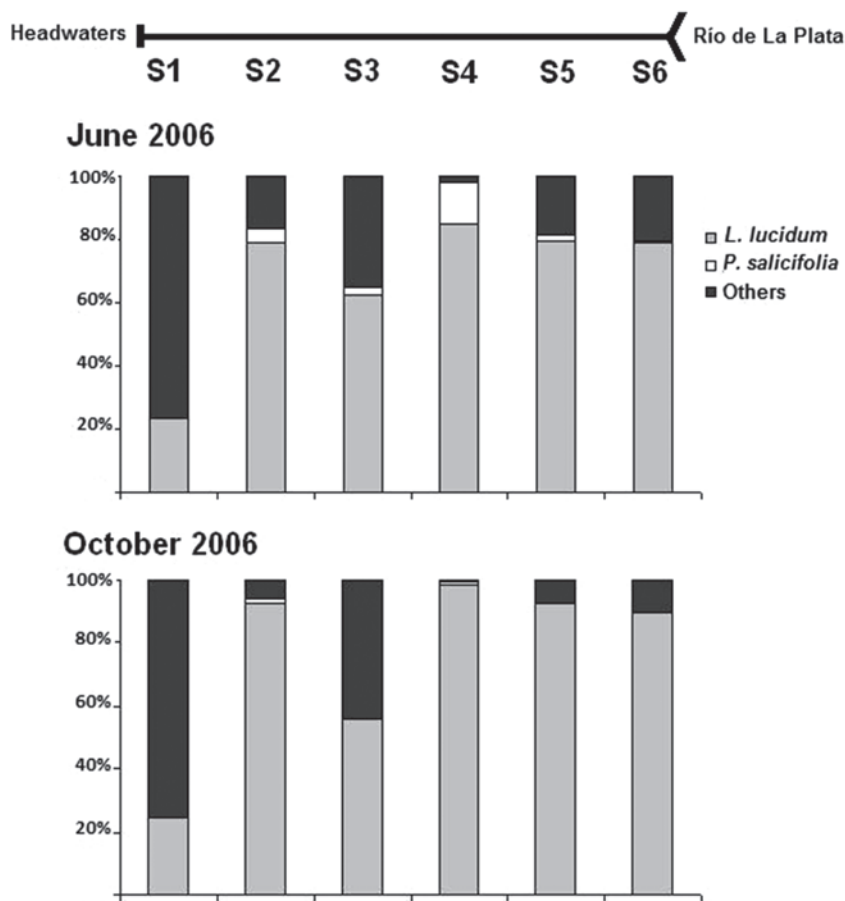


Fig. 1. Relative contribution of *Ligustrum lucidum* and *Pouteria salicifolia* to total organic matter biomass (fresh mass of leaves and fruits) at Las Cañas stream. S1–S6: stream sampling sites.

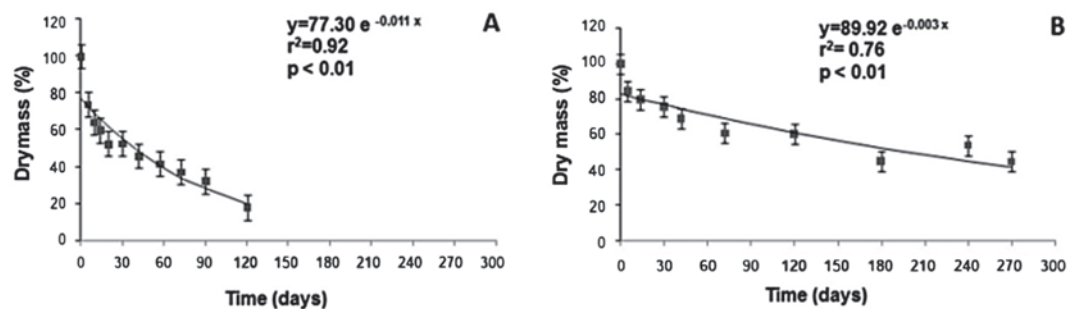


Fig. 2. Dry mass remaining (%) at each time of submersion for *Ligustrum lucidum* (A) and *Pouteria salicifolia* (B). Error bars: SE.

Although N level, C:N, C:P and N:P ratios were similar for both species ($p > 0.01$; Table 2), C:L and L:N ratios differed ($p < 0.01$). The concentration of P showed similar patterns in both species with a sudden decrease until day 14 for *L. lucidum* and day 42 for *P. salicifolia*, followed by an increase. The concentrations of C remained approximately constant throughout the decomposition period in both species. The concentrations of N, ADF and L tended to increase, whereas

phenolic compounds content rapidly decreased until day 15 (Fig. 3).

The C:N and C:L ratios decreased throughout the decomposition period. Similar patterns in the C:P and N:P ratios were observed in both species, with an increase until day 20 in *L. lucidum* and day 40 in *P. salicifolia* (Fig. 4). The remaining percentage of each chemical compound at each time of submersion is shown in Fig. 5.

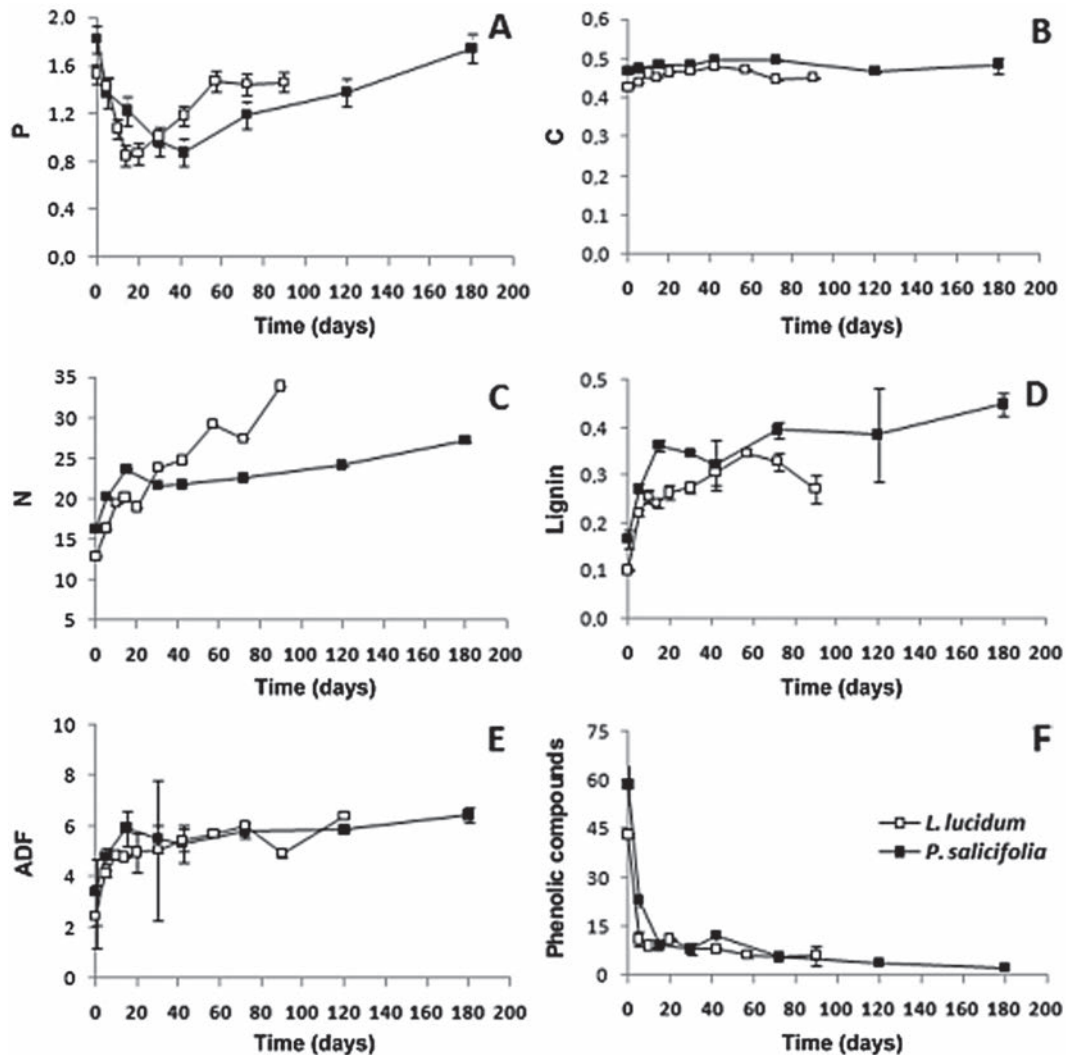


Fig. 3. Concentration of chemical compounds at each time of submersion of *Ligustrum lucidum* and *Pouteria salicifolia*. **A.** Phosphorous (P), **B.** Carbon (C), **C.** Nitrogen (N), **D.** Lignin, **E.** Acid detergent fiber (ADF) and **F.** Phenolic compounds. A, C, E and F (mg g^{-1}); B and D (g g^{-1}). Error bars: SD.

Table 2. Initial concentration of chemical compounds in leaves of *Ligustrum lucidum* and *Pouteria salicifolia* before submersion. Different letters indicate significant differences ($p < 0.01$). Phosphorous (P), Carbon (C), Nitrogen (N), Lignin (L), Acid detergent fiber (ADF).

	<i>Ligustrum lucidum</i>	<i>Pouteria salicifolia</i>
P* (%)	0.15 (± 0.006) b	0.18 (± 0.000) a
C* (%)	42.74 (± 0.35) b	46.82 (± 0.50) a
N* (%)	1.29 (± 0.08) a	1.63 (± 0.32) a
L (%)	10.16 (± 0.09) b	16.71 (± 1.90) a
ADF (%)	27.63 (± 5.12) b	34.03 (± 3.93) a
Phenols (%)	4.31 (± 0.05) b	5.85 (± 0.04) a
C:N	33.26 (± 2.35) a	28.80 (± 5.52) a
C:P	278.36 (± 8.87) a	257.15 (± 2.68) a
N:P	8.37 (± 0.35) a	8.93 (± 1.77) a
C:L	4.21 (± 0.29) b	2.80 (± 0.003) a
L:N	7.90 (0.89) b	10.28 (0.56) a

* Total available concentration

Discussion

It is noteworthy that the exotic *Ligustrum lucidum* contributed in a greater proportion to litter entering the stream than *Pouteria salicifolia*, which might be related to the high biomass and density of this species in the studied area. The most important and potential effects of invasion by *L. lucidum* in aquatic food webs, as compared with *P. salicifolia* are shown in Fig. 6. The high input of allochthonous material, in this case mostly from leaves of *L. lucidum*, appeared to have a twofold effect on the stream dynamics. On one hand, it contributed largely to the pool of substrates available for microbial colonization. On the other, it could be in part responsible for the low dissolved oxygen concen-

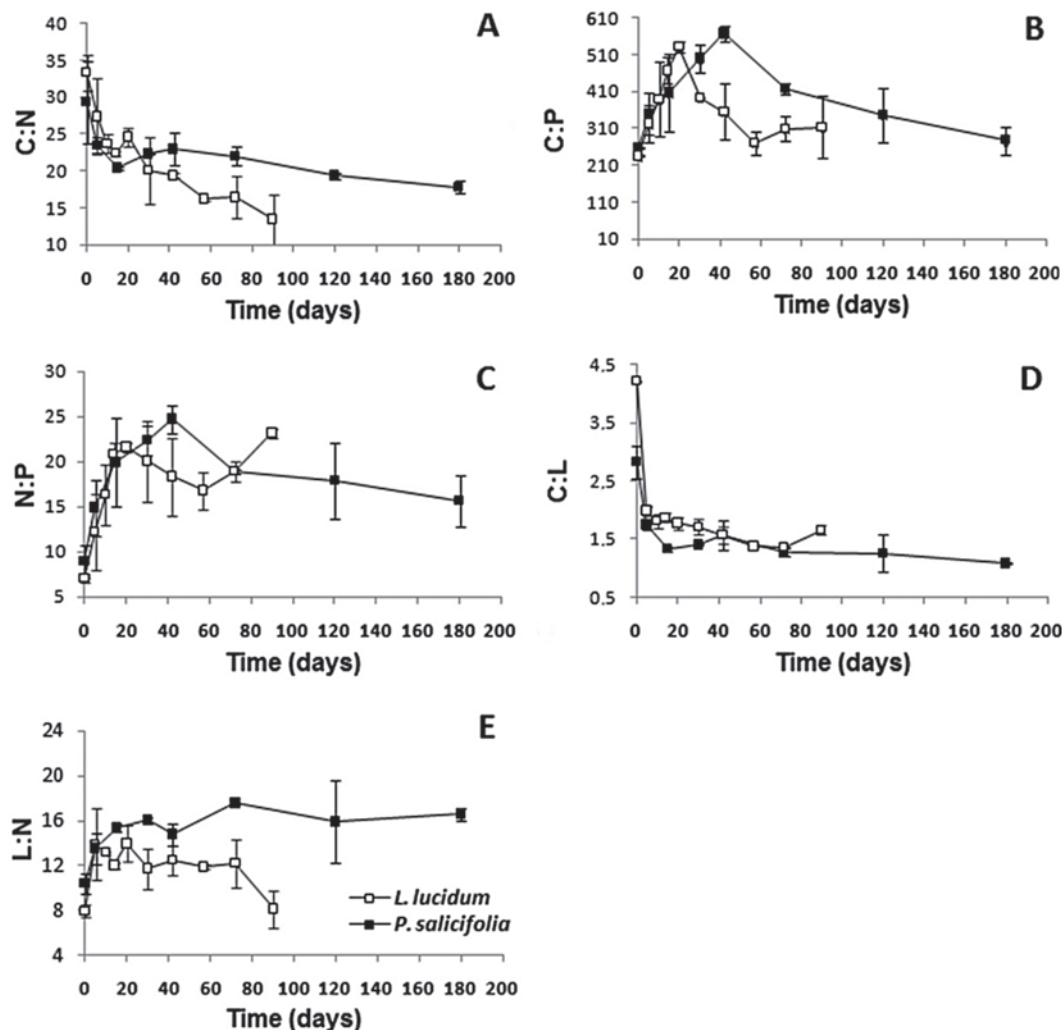


Fig. 4. Time course of C:N (A), C:P (B), N:P (C), C:L (D) and L:N (E) after each period of submersion of the leaves of *Ligustrum lucidum* and *Pouteria salicifolia*. Error bars: SD. Nitrogen (N), Carbon (C), Phosphorous (P), Lignin (L).

trations recorded in the stream, which in turn might affect the microbial activity.

The concentrations of lignin and phenolic compounds were higher in *P. salicifolia* than in *L. lucidum*. It is well-known that leaf chemistry may influence the colonization and activity of decomposers, especially of fungi (Robinson & Gessner 2000). Lignin is one of the most recalcitrant compounds in plant tissues and is highly resistant to microbial degradation (Gallardo & Merino 1993). Also, high concentrations of lignin and free phenolic compounds (e.g. tannins) might limit the availability of C (Gessner & Chauvet 1994) and restrict the accessibility to polysaccharides that are rapidly assimilated with the consequent inhibition of the degradation of these compounds (Bärlocher & Oertli 1978, Gessner & Chauvet 1994). These differences in the chemical content together with differences in the

fiber levels of leaf tissues and the presence of other inhibitors for microbial growth, might have affected the microbial activity differently in both leaves (Webster & Benfield 1986). Bertucci et al. (2009) documented the presence of metabolites with inhibitory activity against anamorphic fungi in *P. salicifolia*. Therefore, leaves of *L. lucidum* proved to be a substrate of better quality for decomposers than leaves of *P. salicifolia*.

Changes in the stream fungal assemblages due to invasion of exotic species in comparison with native riparian vegetation have been previously reported in the literature (Bärlocher & Graça 2002, Laitung & Chauvet 2005). Our previous study demonstrated that the composition of the fungal assemblages did not change significantly, but a higher frequency and abundance of zoospore fungi and straminipiles was recorded colonizing the leaves of *L. lucidum* (Marano et

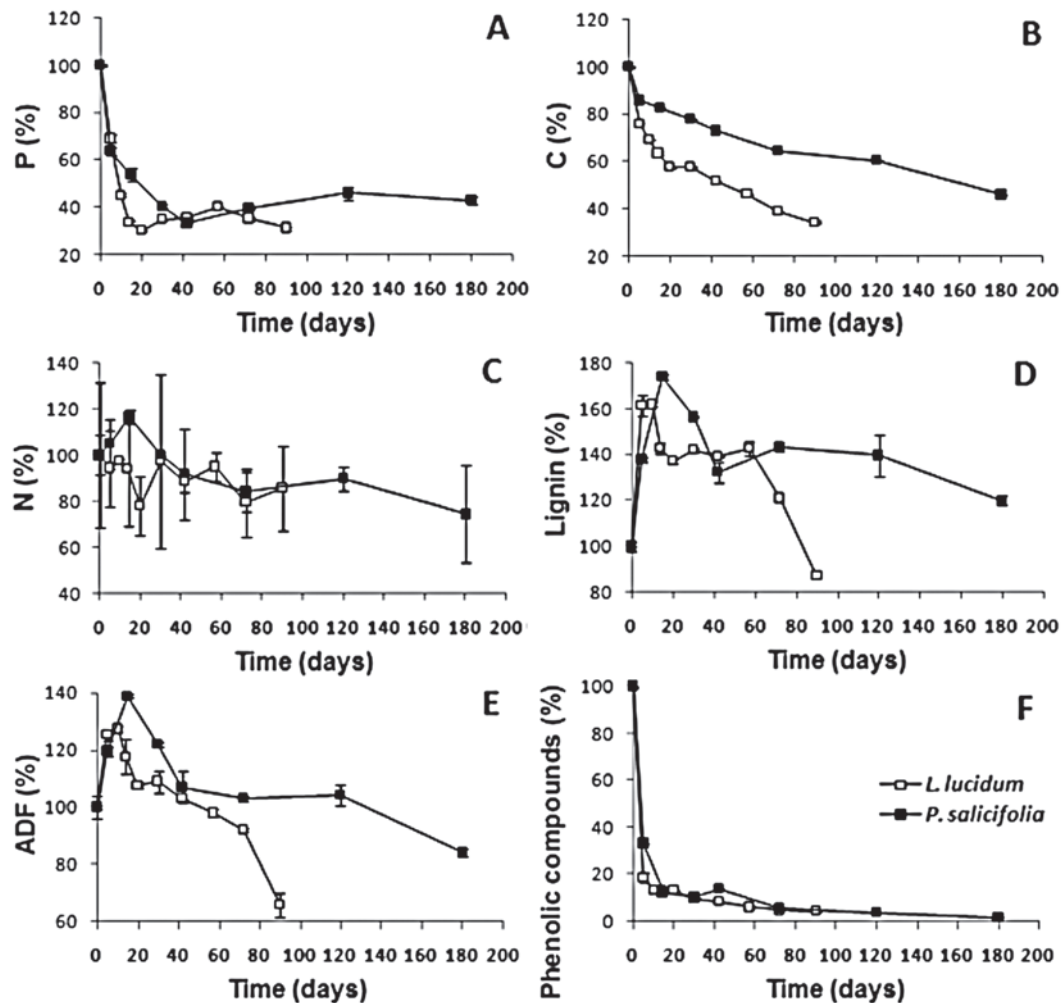


Fig. 5. Time course of chemical compounds in the leaves of *Ligustrum lucidum* and *Pouteria salicifolia* remaining after each period of submersion. **A.** Phosphorous (P), **B.** Carbon (C), **C.** Nitrogen (N), **D.** Lignin, **E.** Acid detergent fiber (ADF) and **F.** Phenolic compounds. Error bars: SD.

al. 2011). This could be due to the fact that zoospore fungi and straminipiles are able to select appropriate substrates, due to the mobility and chemotaxis of their zoospores (Dick 1976, Mitchell & Deacon 1986) that enable them to selectively accumulate at or avoid a substrate through the direction of swimming (Carlile 1993). The presence of a thick cuticle in *P. salicifolia* (Monteiro et al. 2007) might have also contributed to the lower colonization of zoospore fungi and straminipiles as compared to *L. lucidum* (Marano et al. 2011), since the cuticle of several plants proved to be determinants of low fungal colonization (Bärlocher et al. 1978). As a consequence of lower fungal colonization, the palatability of the leaves to detritivorous invertebrates can be reduced (Cameron & LaPoint 1978).

Differences in litter decay rates between exotic and native plant species are therefore indirectly depend-

ent on initial litter quality (Webster & Benfield 1986, Meier & Bowman 2008). The higher initial content of P, C, ADF and lignin, and consequently the lower microbial colonization, appeared to be responsible for the slow decomposition rates in the native *P. salicifolia*. In our study, differential levels of lignin and therefore N:L in the litter proved to be good indicators of decay rate in *P. salicifolia*. Similarly, Saparrat et al. (2008) found slower decomposition rates of the litter of *Scutia buxifolia* in a terrestrial forest as compared to *Celtis tala*, which can be attributed to higher levels of lignin in the former litter. These differences can affect stream nutrient dynamics and determine whether some nutrients are limiting for the growth of algae and aquatic plants (bottom-up control). Concentrations of all nutrients in the stream were moderately high, which can be related in part to the high litter input

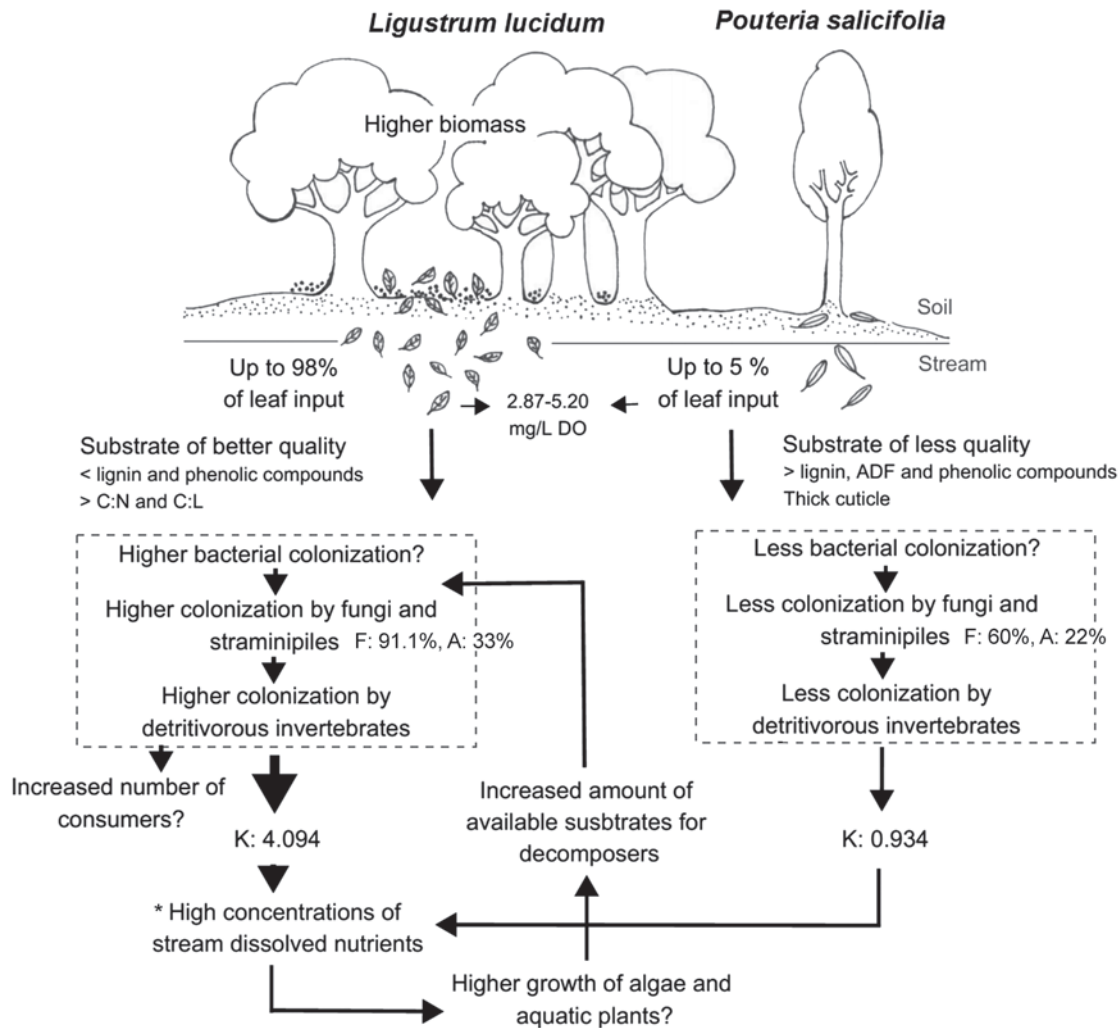


Fig. 6. Simplified diagram showing the most important actual and potential (?) effects of the invasion of *Ligustrum lucidum* in aquatic food webs, as compared with the native *Pouteria salicifolia*. References: k is expressed in year^{-1} , DO: dissolved oxygen, F: frequency, A: abundance (data from Marano et al. 2011); *see Table 1

mostly provided by *L. lucidum* and its rapid decomposition. In this sense, detritus or extracellular exudates from aquatic plants and algae (autochthonous organic matter) can also contribute to the pool of substrates available for microbes.

Time-evolution of compounds transformation

Submersed leaves of both species showed a rapid loss in mass between days 5 to 14, probably due to the loss of soluble and labile materials, particularly P, and the use of assimilable organic compounds by microbial metabolism (leaching phase), as previously reported (Petersen & Cummins 1974). The microbial colonization phase appeared to occur earlier than described by Petersen & Cummins in their study (1974). From day 5, N concentrations in both leaves increased as loss in

mass decreased, which can be attributed to microbial growth (Kaushik & Hynes 1968, Kaushik & Hynes 1971). Recently, published data showed that early-to-intermediate stages of leaf decomposition had high species richness, abundance and diversity of fungi and straminipiles (Marano et al. 2011).

Subsequently, the rate of loss in mass was slower, because the remaining materials of cell walls (structural polymers such as cellulose and hemicellulose) are chemically and physically associated with lignin forming lignocellulose, a very resistant matrix that results in the cellulose and hemicellulose being less accessible to extracellular enzymes of microorganisms (Kirk et al. 1977, Sarkanen & Ludwig 1971, Martinez et al. 2005). In general, hemicellulose and cellulose gradually decrease in proportion to loss in mass whereas the lignin content increases, which can

be because this polymer is highly recalcitrant towards microbial attack (Triska et al. 1975, Suberkropp et al. 1976, Polunin 1982, Rosset et al. 1982, Martínez et al. 2005). In the present study, the ADF and lignin tended to increase while free phenolic compounds tended to decrease with dry mass loss in both species. In this sense, the increase in lignin content observed in the present study might be attributed to the formation of lignin artifacts (Suberkropp et al. 1976) and/or to relative enrichment in lignin of the leaves due to the reduction of other polymers such as cellulose and hemicellulose. Even though the C:P ratio and the content of P have also been considered of importance in predicting decomposition dynamics (Schlesinger & Hasey 1981, Kwabiah et al. 2001), no net change in the P content of the leaves was found in our study. Furthermore, the remaining content of P and C did not show any particular pattern of change during decomposition.

Conclusions

Undoubtedly the exotic *L. lucidum* contributed greatly to the allochthonous pool of organic matter and changed the quality of leaf litter entering the stream, providing a high-quality substrate for microbial colonization, especially for fungi. Consequently, the structure of heterotrophic microbial assemblages might change, with inhibition and/or stimulation of certain taxonomic groups. Likewise, the lower colonization of zoospore fungi and straminipiles in *P. salicifolia* found in a previous study (Marano et al. 2011) can be related to the presence of a high concentration of recalcitrant compounds with inhibitory effects, i.e. physical-chemical association of lignin and phenolic compounds to the cellulose and hemicellulose of the cell walls, which makes C less accessible to microbial extracellular enzymes. Consequently, faster decomposition rates of the non-native species as compared to the native species was observed, which can be attributed to initial lower concentrations of P, C, ADF, lignin and phenolic compounds.

Our results indicated that changes in the quantity and quality of litter inputs, due to exotic plant invasions, can alter the structure of microbial assemblages and possibly the functioning of the aquatic ecosystem as a whole, as previously reported (Liao et al. 2008, Ehrenfeld 2010). As a consequence of higher decay rates of leaf inputs entering the stream, stimulation of nutrient cycling and increased nutrient availability would be expected, with possible cascade effects in food webs and other ecosystem processes. Under-

standing bottom-up and top-down mechanisms of control might help to elucidate the effects of invasion of exotic species on the dynamics of nutrient cycles. It is also important to comprehensively investigate the differences in morphological, functional and phenological traits between coexisting invasive and native plant species in order to help fully understand the ecological impacts of invasion. Further research aimed at assessing other effects of invasion by *L. lucidum* are needed, in order to provide a comprehensive view of the consequences to aquatic food webs.

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