

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Litter quality, decomposition rates and saprotrophic mycoflora in *Fallopia japonica* (Houtt.) Ronse Decraene and in adjacent native grassland vegetation

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/132606> since 2016-06-23T14:58:19Z

Published version:

DOI:10.1016/j.actao.2013.03.010

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Litter quality, decomposition rates and saprotrophic mycoflora in *Fallopia japonica* (Houtt.) Ronse Decraene and in adjacent native grassland vegetation

T. Mincheva, E. Barni, G.C. Varese, G. Brusa, B. Cerabolini, C. Siniscalco
Acta Oecologica (2014) 54

DOI: 10.1016/j.actao.2013.03.010

The definitive version is available at:

<http://dx.doi.org/10.1016/j.actao.2013.03.010>

Litter quality, decomposition rates and saprotrophic mycoflora in *Fallopia japonica* (Houtt.) Ronse Decraene and in adjacent native grassland vegetation

T.Mincheva, E. Barni, G.C. Varese, G. Brusa, B. Cerabolini, C. Siniscalco

Abstract

Fallopia japonica succeeds in invading different ecosystems likely because of its huge biomass production. This biomass is characterized by low nutritional quality and low decomposition rates but knowledge on whether these features are correlated to microbial decomposers is still lacking. The aims of this work were: i) to determine litter decomposition rates of native grassland vegetation and *F. japonica* under different conditions in a year-round experiment; ii) to evaluate litter quality and/or site effect on the decomposition of the invader and native vegetation and iii) to characterize mycoflora isolated from *F. japonica* and native vegetation litter. The results showed that *F. japonica* litter decomposes 3–4 times slower than that of native grassland, mainly due to its low N content and consequently high C/N ratio both in leaves and stems. As decomposition proceeds C/N in *F. japonica* litter decreases to values approaching those of the grassland litter. Site had no effect on the decomposition rates of *F. japonica* and grassland litter. Total fungal load and composition differed between *F. japonica* and native litter, and also varied across sites. These results indicate that the successful invasive plant *F. japonica* affects the structure and functions of the invaded ecosystem through a huge production of low quality, slow-decomposing litter that selects saprotrophic fungi.

Keywords

C/N ratio; Ecosystem functioning; Litter decomposition; Plant invasion; Saprotrophic fungi

1. Introduction

The arrival of an alien plant species influences the composition of plant and soil communities and ecosystem processes (Wolfe and Klironomos, 2005). Changes in species composition and decreased biodiversity in the invaded community have been widely documented (Manchester and Bullock, 2000; Hejda et al., 2009 and references therein), but knowledge of impacts on soil fungi or fauna and ecosystem processes is still limited (Levine et al., 2003; Ehrenfeld, 2003).

Changes in biomass production and litter quality due to plant invasion have been shown to create feedbacks that either accelerate or slow nutrient cycling (Allison and Vitousek, 2004; Vanderhoeven et al., 2005; Angeloni et al., 2006). Functional leaf traits, carbon, nitrogen, and lignin content as well as soil characteristics and communities control the litter decomposition rates. At the same time litter decomposition influences soil nutrient availability and is therefore of primary importance for ecosystem functioning. Species that produce a large amount of biomass and form monospecific stands are expected to have a major impact on ecosystem functioning by altering litter production and quality.

Fallopia japonica is considered one of the most aggressive invaders in Europe, the United States and Canada, where it causes severe impacts on biodiversity (Beerling et al., 1994; Muller, 2004), and is one of the 100 worst invasive alien species in the world (Lowe et al., 2000). *F. japonica* is a herbaceous rhizomatous clonal geophyte that produces a huge amount of biomass (1.6–4.5 kg/m² dry biomass during the growing season, i.e. 1.8–5.7 times the biomass production of non invaded habitats (Beerling et al., 1994; Dassonville, 2008). High biomass production likely allows *F. japonica* to outcompete native resident species during the invasion process and is presumably fueled by large amounts of reserves stored in extensive rhizomes (15–20 m long and penetrating 2–3 m into the soil). Furthermore, a very wide and effective photosynthetic leaf system makes the plant extremely competitive with regard to the native herbaceous species. Because of these traits, this species forms very dense and almost monospecific stands after a few years from its arrival (Beerling et al., 1994; Aguilera et al., 2010) and even under harsh climates (Siniscalco et al., 2011). The latter is accompanied by noticeable litter accumulation at the soil surface.

The impacts of *F. japonica* on other plant species during the growing season have been largely studied. On the contrary its impacts on soil biota and processes are less known. *F. japonica* generally produces low-quality litter with a higher C/N ratio than the litter from native herbaceous species occurring in the invaded habitats (Maerz et al., 2005). In a 6-month experiment (Dassonville, 2008) high litter C/N ratio was considered the main reason of its very slow decomposition rate; *F. japonica* decomposed slower than native litter and its C/N ratio decreased during decomposition. Furthermore, *Fallopia* litter decomposition rates were found to be comparable to those of some native trees and shrubs (Braatne et al., 2007; Lecerf et al., 2007). Slow decomposition rates result in the accumulation of large, rough litter fragments and an increase in litter layer thickness (Maurel et al., 2010).

Another hypothesized reason for the slow decomposition rate in *F. japonica* is that it produces allelochemicals (Moravcová et al., 2011) as well as antimicrobial and antifungal molecules (Lecerf et al., 2007) that can negatively affect fungi. However, no data are available to our best knowledge on the fungi decomposing *F. japonica* litter. In this study we investigate i) the litter decomposition rates of *F. japonica* and native grassland species in a year-round experiment (i.e. a longer period than in previous studies); ii) the effects of litter type and site on decomposition rates; iii) the differences between mycoflora isolated from native vegetation and *F. japonica* litter.

2. Material and methods

2.1. Study site

The study site is situated near Meugliano, in the Piedmont Region, Northern Italy (45°28'52" N, 7°46'52" E), at 680 m a.s.l. The climate is temperate. Mean annual temperatures during the study period were 9.2 °C in 2010 and 10.9 °C in 2011. The total precipitations were 1847 mm in 2010 and 1511 mm in 2011, with peaks in spring and in autumn (ARPA Piemonte). The experiment site was located in an extensive plain where a grassland has partially been displaced by *F. japonica* over the last 30 years (personal communication from the landowner). The uninvaded grassland is mown twice yearly and is undisturbed by livestock. Two clearly recognizable adjacent plant communities were chosen for the experiment: i) a typical low-land grassland (*Arrhenatherion elatioris*) dominated by *D. glomerata*, *Festuca arundinacea*, *T. pratense*, *Taraxacum officinale* and *Achillea millefolium* (G stand); ii) a dense, nearly monospecific stand of *F. japonica* (F stand). Mean above-ground biomass

was 525 g/m² in the G stand and 3434 g/m² in the F stand, where *F. japonica* cover was above 98% (unpublished data collected by the authors at the beginning of the experiment).

Ten plots (0.5 × 0.5 m) were randomly placed: five in the G stand and five in the F stand. They were positioned at a minimum distance of 6–10 m from the border between stands in order to avoid any shading or underground effects of *F. japonica* on the native grassland (Aguilera et al., 2010). The two stands had similar topography and water availability and, presumably, similar soil conditions before the arrival of the invader. At the beginning of the experiment analyses were performed on soil samples collected at each stand (unpublished data). Some differences were observed in sand and silt content (76% and 22% in G, 89% and 10% in F respectively) as well as in total N content and C/N ratio (0.32% and 11.8 in G, 0.16% and 18.7 in F respectively).

Given the similarities in topography and the other soil characteristics for the two adjacent stands, it seems likely that the differences in soil properties are an effect of invasion rather than a cause, as shown by Barney et al. (2006). Therefore, we considered the plots located in the G and F stands as true replicates.

2.2. Litter sampling and analysis

Litter decomposition was evaluated through the litterbag method (Crossley and Hoglund, 1962). Litterbags of each litter type were placed under their own canopy and below the canopy of the other stand. Four litter types were collected in the study site in October 2010: 1) leaves (FL) and 2) pieces of stem (FS) of *F. japonica* from 10 stems in each plot of the F stand; 3) stems and leaves of native grassland species randomly mixed (GM) and 4) *D. glomerata* and *T. pratense* stems and leaves (DT) from the G stand. *D. glomerata* and *T. pratense* were selected as they were among the dominant species in the G stand, and because they were representative of the dominant functional types in the grassland (grasses and legumes). Senescent leaves and stems were collected just prior to abscission, but not from the ground, to avoid pre-existing infection by soil fungi. All the litter types were transported to the laboratory, oven-dried at 60 °C to constant weight and cut into approximately 5 cm² pieces. A subsample of each litter type was taken for chemical analyses. Total C and N contents were analysed through flash combustion using a CHN analyzer (NA-2000NProtein, Fisons Instruments S.p.A., Rodano, Italy).

The litterbags were prepared by placing 4 g of litter into 15 × 15 cm nylon bags (2 mm mesh). The bottom side was made of a double mesh layer to avoid material losses and the upper side of a single mesh layer to permit the access of soil mesofauna. A total of 240 bags were prepared, 60 bags for each litter type. Five plots were selected in each stand, six litterbags of each type were placed in each plot. All the litter lying on the soil surface in the selected plots was removed, the litterbags were placed at a depth of 5 cm and covered with soil. A year-round experiment was carried out beginning in December 2010. Samples were taken 2, 4, 6, 9, 10 and 12 months from the beginning of the experiment. One litterbag of each type was collected from each plot at each sampling date (total of 40 bags) and transported to the laboratory. The litter remaining in the bag was carefully brushed to remove soil particles, oven-dried at 60 °C, weighed and ground to a fine powder. C and N contents were analysed as explained above.

2.3. Fungi isolation and identification

Fungi were isolated from the litter remaining in the bags collected in April 2011. A modified washing method (Osono et al., 2004) was used to isolate the fungi from the decomposing litter. The litterbags

were collected, placed in sterile Ziplocked bags and transported to the laboratory. Soil particles were carefully removed from the litter under a sterile hood. A subsample of 1 g of fresh litter was used to isolate the fungi and another equivalent subsample was oven-dried to estimate the moisture content. The litter pieces were washed in a 0.02% Tween 80 solution, placed in a sterile tube and vortexed for 2 min, rinsed five times with sterile water and dried on sterile filter paper. The litter was then ground into a homogeneous mass with 1 ml of sterile water. The suspension was then serially diluted to 1:10000 and 1 ml of the final dilution was plated into 15 cm Petri dishes containing moulded LCA (Miura and Kudo, 1970). The dishes were not sealed to allow respiration. The plates were incubated at 25 °C in the dark and monitored daily for 30 days to allow the development of slow-growing colonies. The number of colony forming units (CFU) per g of dry weight of litter (CFU/g dwt) was estimated for both the total mycoflora and for each taxon or morphotype; the average and the standard deviation of the load values were calculated. For each fungal morphotype several strains were isolated in pure culture for taxonomic identification. The fungi were identified conventionally according to their macroscopic and microscopic features, at the genus level.

2.4. Statistical analyses

All statistical analyses were computed using R an open source version of S-Plus (R Development Core Team, 2011).

2.4.1. Litter decomposition

Mean remaining litter mass of the four litter types (DT, GM, FL and FS) at each decomposition stand (G and F) and sampling date was calculated. Decomposition curves were drawn and the k coefficient of decomposition was calculated on the log-transformed data using the model

$$M_t = M_0 e^{-kt},$$

where M_0 is the initial mass of litter in the litterbag (in g), M_t is the remaining mass at time t, and t is the time in years (Dassonville, 2008). Eight decomposition curves, one for each combination of litter type and decomposition stand, were subsequently derived from models calculated by means of linear regression. The eight model coefficients (k = decomposition rate) were then simultaneously compared following the method proposed by Gujarati (1970). This method consists in t-testing the interaction factors between time and, respectively, decomposition stand (G or F) and litter type (native, i.e. DT and GM, and *F. japonica*, i.e. FL and FS) in a linear model in which the stand and litter type are transformed into dummy variables and the log-transformed litter mass is the dependent variable.

A backward stepwise regression was also calculated to evaluate whether litter type, decomposition stand and time explain variation in log-transformed litter mass. Akaike's information criterion was used as the stopping rule (Sakamoto et al., 1986).

2.4.2. Litter quality

Differences in N percentages (arcsin transformed), ii) C percentages, and iii) C/N between litter types (DT, GM, FL and FS) before the beginning of the experiment were evaluated. Repeated-measures ANOVA was applied to test for differences in the above independent variables during the decomposition experiment (from the 2nd to the 9th month), considering the litter type and stand as fixed factors and time as covariate. Finally, differences in litter quality (i.e. same variables as

above) of *F. japonica* litter types (stems or leaves) and stand types at the end of the experiment were evaluated by means of two-way ANOVA. Differences in quality with native litter types were not evaluated at the end of the experiment because all litter from native grassland (i.e., DT and GM litter) was decomposed by that time.

2.4.3. Fungi

Only taxa with a percentage of occurrence higher than 10% in the 72 plates were considered in the analysis (*Cladosporium* sp., *Mucor* sp., *Phoma* sp., *Epicoccum* sp. and *Alternaria* sp.). Colony count data (CFU: colony forming units) were transformed by log transformation, $NCFU = \ln(CFU + 1)$. Two-way MANOVA was used to evaluate differences in fungal communities litter and stand types (see Hand and Taylor, 1987). Pillai–Bartlett statistics were used to test for statistical significance. Simpson's index was used as a measure of fungal diversity. Two-way ANOVA was used to test differences in the diversity index between litter type and stand.

3. Results

3.1. Litter decomposition rates

Stepwise regression illustrated that litter type significantly contributed to variation in the decomposition rates ($F_{3,232} = 113.2$; $p < 0.001$). Stand type had no significant effect (i.e. the factor was dropped in the stepwise regression). Time ($F_{1,232} = 854.0$; $p < 0.001$) and litter \times time ($F_{3,232} = 56.1$; $p < 0.001$) were also included in the final model ($r^2 = 0.861$; $F_{7,232} = 194.5$; $p < 0.001$).

Litter type was the main factor that controlled the decomposition rates (k). This was confirmed by the significance of the time \times litter type coefficients ($F_{1,234} = 154.2$; $p < 0.001$) in the model where the stand and litter type were transformed into dummy variables. No effect of decomposition stand (G, F) was detectable as the time \times stand coefficients were not significant ($F_{1,234} = 1.3$; $p = 0.261$).

The native plant litter decomposed faster than that of *F. japonica* (Table 1). Four months after the beginning of the experiment the remaining native plant litter was 26% (DT) and 39% (GM) respectively while the remaining litter from *F. japonica* leaves and stems was 76% and 77%, respectively (Fig. 1a, b). The remaining mass of both native litter types (DT and GM) was less than 13% at the ninth month and zero at the tenth month while about 40% of the initial mass of *F. japonica* leaves and stems was still present. Projections based on the k coefficient indicate that leaves and stems of *F. japonica* need approximately four years to decompose to 5% of their initial mass.

Table 1.

Decomposition rates (k) of the four litter types at each stand type in the litter bags and statistical significance for each combination of litter and stand: FL/F = *Fallopia* leaves in F (*Fallopia* stand); FL/G = *Fallopia* leaves in G (grassland stand); FS/F = *Fallopia* stems in F; FS/G = *Fallopia* stems in G; GM/F = grassland species mixture in F; GM/G = grassland species mixture in G; DT/F = *Dactylis* and *Trifolium* in F; DT/G = *Dactylis* and *Trifolium* in G. df num. = numerator degrees of freedom; df den. = denominator degrees of freedom.

Litter type	k (year ⁻¹)	S.E.	F	df den, df num.	p
GM/F	1.947	0.101	371.3	1, 28	<0.001
GM/G	1.960	0.130	221.7	1, 28	<0.001
DT/F	2.014	0.068	854.4	1, 28	<0.001
DT/G	1.945	0.086	508.1	1, 28	<0.001
FL/F	0.959	0.110	75.6	1, 33	<0.001
FL/G	1.102	0.179	38.0	1, 33	<0.001
FS/F	1.144	0.140	66.4	1, 33	<0.001
FS/G	1.196	0.135	78.4	1, 33	<0.001

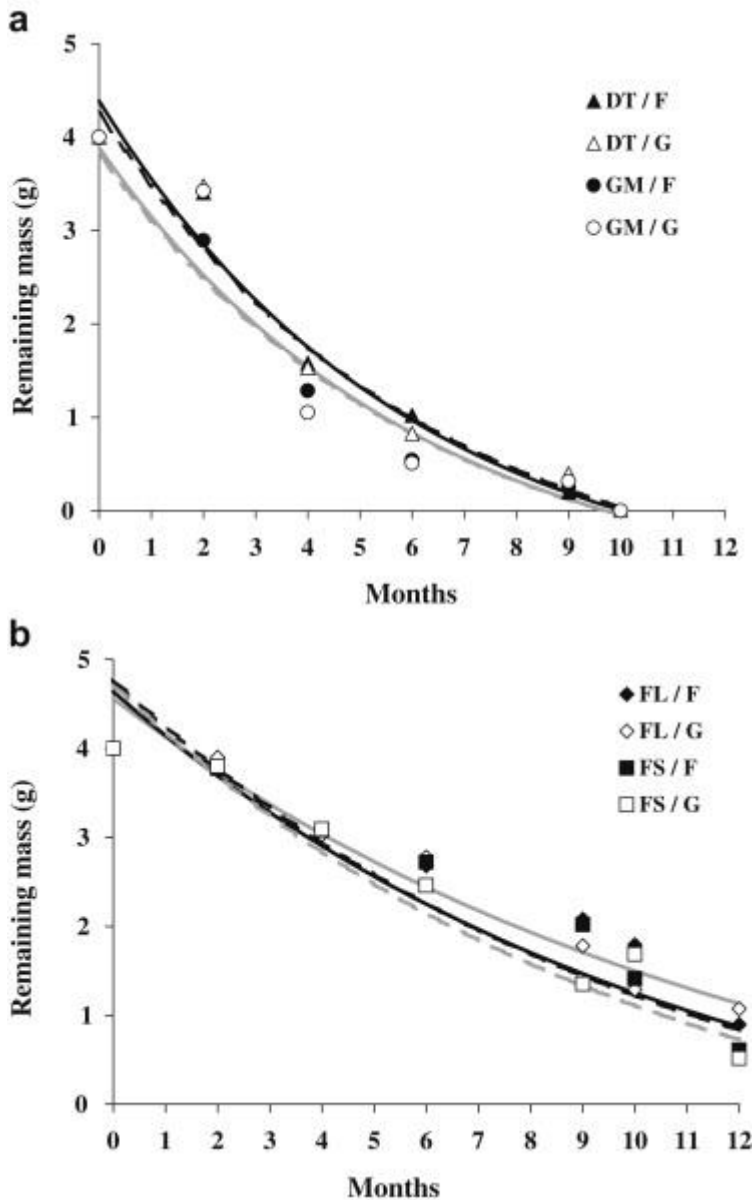


Fig. 1.

Remaining litter mass of (a) the grassland litters (GM = grassland species mixture, DT = *Dactylis* and *Trifolium* mixture) decomposing in two stands (F = *Fallopia* invaded stand; G = non invaded grassland stand (Continuous black line = DT/F; dotted black line = DT/G; continuous grey line = GM/F; dotted grey line = GM/G) and of (b) *F. japonica* litter types (FL

= *Fallopia* leaves, FS = *Fallopia* stems) decomposing in F and in G stands (Continuous black line = FL/F; dotted black line = FL/G; continuous grey line = FS/F; dotted grey line = FS/G).

3.2. Litter quality

Litter quality differed during the different phases of the experiment.

At the beginning of the experiment litter types differed in C content ($F_{3,16} = 46.0$; $p < 0.001$), N content ($F_{3,16} = 358.1$; $p < 0.001$) and C/N ratio ($F_{3,16} = 297.8$; $p < 0.001$). The N content in the native plant litters was 2.61% (GM) and 2.98% (DT) (Fig. 2a) leading to C/N ratios of 16.3 (GM) and 14.7 (DT) (Fig. 3a). *F. japonica* stems and leaves had lower N content (0.71% and 1.23% respectively, Fig. 2b) and, thus, a higher C/N ratio (64.8 and 38.9 respectively, Fig. 3b) than the native plant litter.

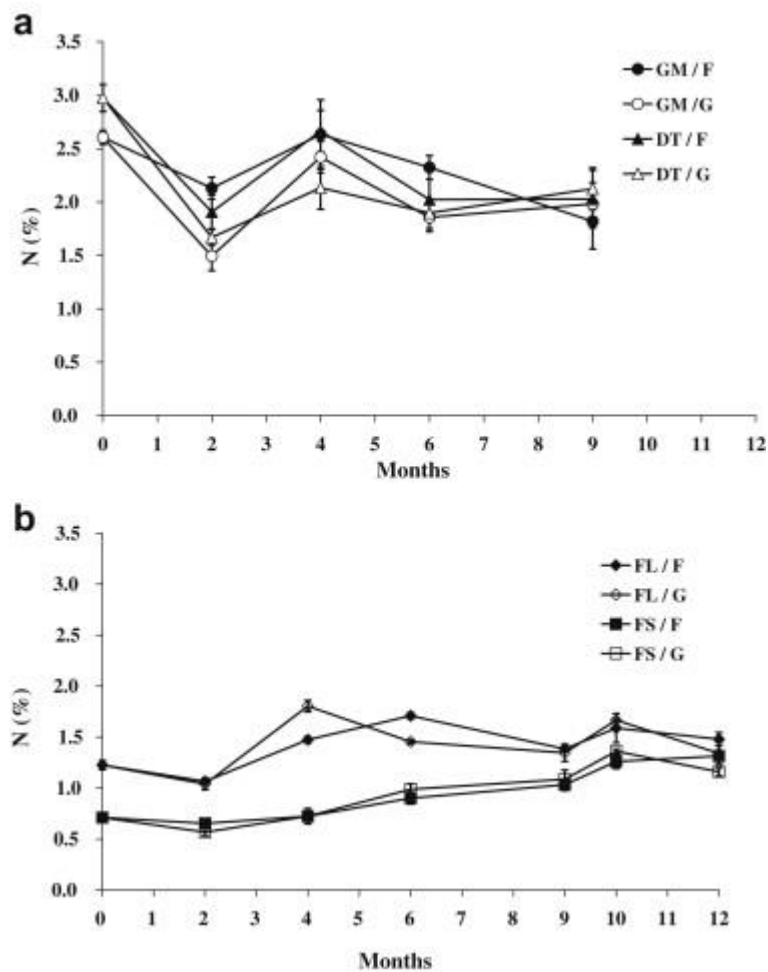


Fig. 2.

Dynamics of N (% mean \pm standard deviation) during decomposition of (a) grassland litters (GM = grassland species mixture, DT = *Dactylis* and *Trifolium* mixture) decomposing in two stands (F = *Fallopia* invaded stand; G = non invaded grassland stand) and of (b) *F. japonica* litter types (FL = *Fallopia* leaves, FS = *Fallopia* stems) decomposing in F and in G stands.

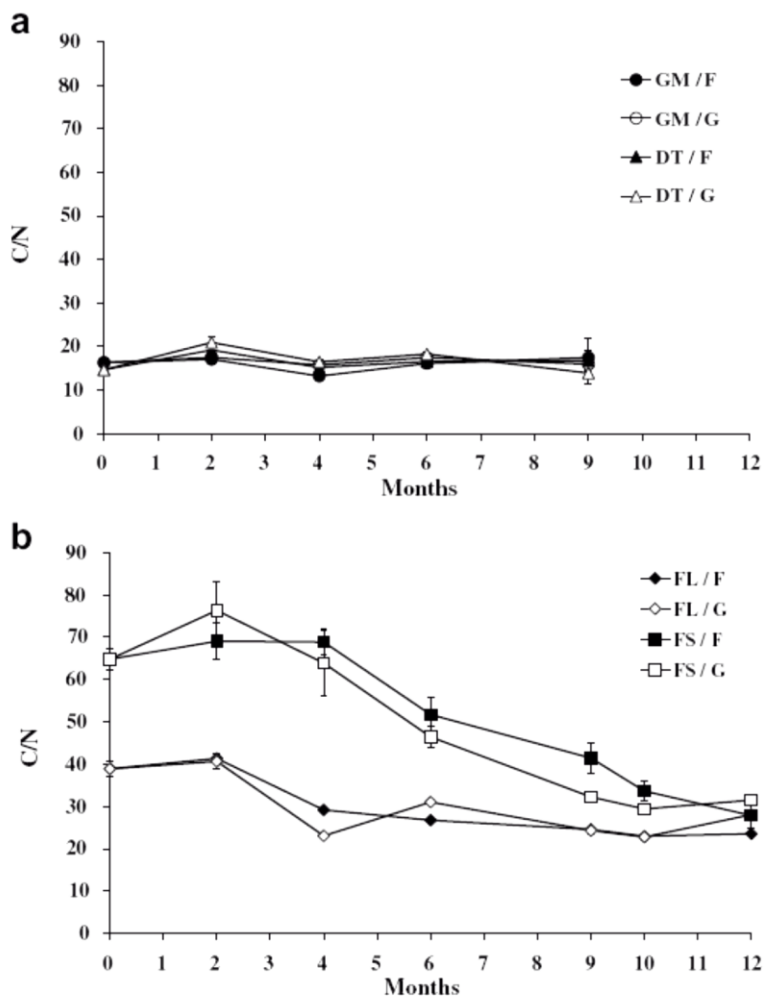


Fig. 3.

Dynamics of C/N (mean \pm standard deviation) during decomposition of (a) grassland litters (GM = grassland species mixture, DT = *Dactylis* and *Trifolium* mixture) decomposing in two stands (F = *Fallopia* invaded stand; G = non invaded grassland stand) and of (b) *F. japonica* litter types (FL = *Fallopia* leaves, FS = *Fallopia* stems) decomposing in F and in G stands.

The dynamics of the C content, N content and C/N ratio during the decomposition process differed between the litter types (Table 2). No significant effect of the decomposition stand nor interactive effects were detected except for the litter \times time interaction in the case of the C/N ratio. N content in the native plant litter did not follow a constant trend (Fig. 2a) and ranged from 1.5% (GM in the G stand, after two months) to 2.7% (DT in the F stand, after four months). A rapid initial decrease was observed followed by a comparable increase and a subsequent decreasing trend. Conversely, the C/N ratio remained constant within the 10–20 range as decomposition progressed (Fig. 3a).

Table 2.

Repeated measures ANOVA evaluating the effects of litter type, stand type and time on litter C (%), arcsin-transformed N content (%) and C/N during the decomposition experiment (2nd to 9th month).

Dependent variables	C content		N content		C/N ratio	
	df	F	p	df	F	p
Litter	3	40.48	<0.001	3	150.54	<0.001
Stand	1	9.37	0.003	1	5.13	0.025
Time	3	19.39	<0.001	3	16.42	<0.001
Litter × Stand	3	0.44	0.726	3	2.38	0.072
Litter × Time	9	2.82	0.042	9	3.06	0.030
Stand × Time	3	0.92	0.894	3	2.23	0.134
Litter × Stand × Time	9	2.18	0.435	9	0.94	0.426

N content in *F. japonica* litters slightly decreased initially but then underwent a general increase until the end of the experiment (Fig. 2b). The highest values were measured twelve months after the start of the experiment (1.3% in the leaves and 1.1% in the stems). On the other hand, the C/N ratio showed a general decrease after an initial stationary stage. C/N ratios in litter from *F. japonica* stems (FS) decreased continuously during the experiment while the C/N ratio of the leaf litter (FL) asymptotically approached the value of 20 (Fig. 3b). The quality of *F. japonica* litter did not differ between stand types at the end of the experiment (i.e. when litter from native plants was totally decomposed). In *F. japonica*, however, the litter from leaves and stems significantly differed in N content ($F_{1,13} = 12.9$; $p = 0.003$) and C/N ratio ($F_{1,13} = 43.5$; $p < 0.001$).

3.3. Saprotrophic fungi and their occurrence in decomposing litter

The total load (Fig. 4) and taxa distribution (Table 3) of the isolated fungi differed between the four litter types. Moreover, a stand effect was also observed on the load and distribution of the fungal taxa (Table 4).

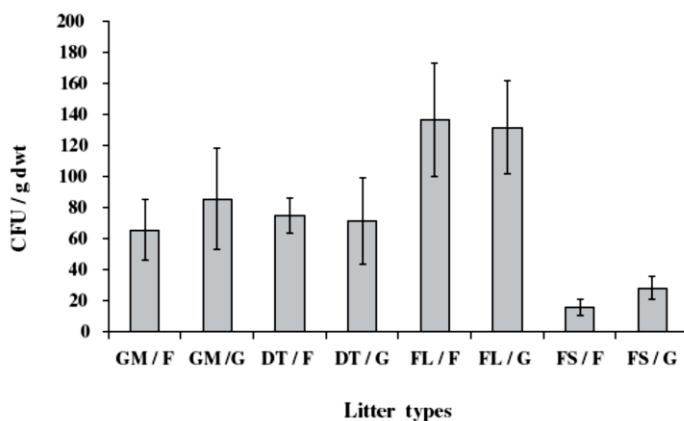


Fig. 4.

A total CFU/g dwt (mean \pm standard deviation) detected in grassland litters (GM = grassland species mixture, DT = *Dactylis* and *Trifolium* mixture) and in the *F. japonica* litters (FL = *Fallopia* leaves, FS = *Fallopia* stems) collected in grassland (G) and *F. japonica* (F) stands at the 4th month of decomposition experiment.

Table 3.

Fungal taxa isolated from the four litter types (FL = *Fallopia* leaves, FS = *Fallopia* stems, GM = grassland species mixture, DT = *Dactylis* and *Trifolium* mixture) decomposing in the two stands (F = *Fallopia* invaded stand; G = non invaded grassland stand). The load of each taxon is expressed as percentage of the total fungal load (CFU/g dwt). In brackets: number of exclusive fungal taxa. Standard errors for the Simpson's index ranged between 0.06 and 0.08.

Taxa (%)	Litter types							
	Invaded stand				Non-invaded stand			
	GM/F	DT/F	FL/F	FS/F	GM/G	DT/G	FL/G	FS/G
Alternaria	–	14.62	1.00	–	16.29	3.15	0.91	–
Cladosporium	35.54	45.13	6.29	17.80	67.64	57.49	8.84	4.27
Epicoccum	45.09	21.40	0.37	2.34	0.44	33.36	–	–
Mucor	16.75	17.16	0.97	1.00	9.57	3.46	–	0.94
Phoma	–	–	91.08	63.13	–	–	89.96	88.15
Other taxa	2.62	1.72	0.28	15.73	6.07	3.63	0.29	6.60
Simpson's index	0.56	0.51	0.79	0.56	0.62	0.68	0.82	0.80
Total n° taxa	7(2)	6(0)	7(0)	13(3)	9(0)	10(4)	5(0)	7(2)
Total CFU	6.6×10^5	7.5×10^5	1.4×10^6	1.6×10^5	8.6×10^5	7.2×10^5	1.3×10^6	2.8×10^5

Table 4.

Two-way MANOVA evaluating the effect of litter and stand type on the abundance of the fungal taxa with percentage of occurrence higher than 10% (*Cladosporium* sp., *Mucor* sp., *Phoma* sp., *Epicoccum* sp. and *Alternaria* sp.) in the four litter types. df num. = numerator degrees of freedom. df den. = denominator degrees of freedom.

	df num.	df den.	F	p
Litter	15	186	12.09	<0.001
Stand	5	60	6.04	<0.001
Litter × Stand	15	186	3.21	<0.001

The total fungal load was high in all the litter types decomposing in either stand and ranged from 1.6×10^5 CFU/g dwt for the FS litter in the F stand to 1.4×10^6 CFU/g dwt for the FL litter in the F stand (Table 3). The total load for GM and FS was higher in the G stand than in the F stand, while it was higher in the F stand for the litter types DT and FL (Table 3). Two exclusive fungal taxa (included in "Other taxa" in Table 3) were identified for the GM litter in the F stand (*Microdochium* sp. and *Trichoderma* sp.), 4 for the DT litter in the G stand (*Aspergillus* sp., *Myrothecium* sp., *Phaesosphaeria* sp., *Paecyllumyces* sp.), 1 for the FS litter in the G stand (*Absidia* sp.) and 2 for the FS litter in the F stand (*Mortierella* sp., *Rhizopus* sp.). *Paraconiothyrium* sp. was exclusively isolated from *F. japonica* stems decomposing under the G and F stands.

Cladosporium sp. was isolated from all the litter types but was more abundant in the litter types decomposing under the F stand. A specific association with certain litter types was observed for the other taxa. *Phoma* sp. was isolated in great abundance from *F. japonica* leaves (90% of the total CFU in FL/G and 91% in FL/F) and stems (78% of the total CFU in FS/G and 63% in FS/F) but was absent

in the native litter in both the F and G stands. *Mucor* sp. was one of the dominating taxa in the native litter types in both decomposition stands but was almost or completely absent in FL/G and occurred in very low percentages in FL/F, FS/G and FS/F. *Epicoccum* sp. was isolated from all the litter types in the G and F stands, except FL/G and FS/G. The same trend was observed for *Alternaria* sp., with the exception of GM/F, FS/G and FS/F.

Fungal diversity measured by means of the Simpson's index was affected by litter and stand but their interaction was not statistically significant (Table 5). The invaded stands showed a lower Simpson's index (0.61 on average) than the non-invaded stands (0.73), and, conversely, the *F. japonica* litters (0.74), and especially the *F. japonica* leaves (0.80), showed a higher index than the native litters (0.59) (Table 3).

Table 5.

Two-way ANOVA evaluating the effect of stand and litter type on the fungal diversity (Simpson's index).

	<i>df</i> num.	<i>df</i> den.	<i>F</i>	<i>p</i>
Litter	3	64	4.64	0.005
Stand	1	64	7.28	0.009
Litter × stand	3	64	1.17	0.327

4. Discussion and conclusions

Litter quality, decomposition rates, and dominant saprophytic mycoflora differed between *F. japonica* and the native grassland plants. The dominance of *F. japonica* thus alters the amount and dynamics of the organic matter in the invaded area and therefore affects the ecosystem functions. Since only one study site was considered, the experimental design could be criticized because of pseudoreplication (Hurlbert, 2004). However, efforts were deliberately concentrated on one study site in order to combine a litter decomposition experiment and microbiological analyses, the latter being laborious and time consuming (Barni and Siniscalco, 2000; Kourtev et al., 2002).

The modifications induced by *F. japonica* in the invaded ecosystems are mainly caused by its high biomass production, a trait that allows us to consider that this invasive plant is one of those that visually, structurally, and chemically transform ecosystems while reducing native plant biodiversity (Gerber et al., 2008; Aguilera et al., 2010). High above-ground biomass in *F. japonica* (6 times higher than that of the adjacent native vegetation at our study site) translates into high light interception. A high biomass production also leads to a high litter supply to the soil. A thick litter layer generally accumulates under *F. japonica* stands which suggests its low decomposability. Decomposition rates depend on litter quality and the decomposer community (Dassonville, 2008). In the present experiment the initial litter C and N contents and the C/N ratio differed remarkably between *F. japonica* and native species mixtures, and this resulted in different decomposition rates and litter quality dynamics. The litter from native species exhibited a relatively high N content and a C/N ratio similar to that of the soil of the study site (i.e. 10–20). C/N ratios lower than 20 generally lead to rapid decomposition (Lambers et al., 2008). On the contrary, the initial C/N ratio in *F. japonica* litter (either leaves or stems) was much higher than 20. Leaf litter decreased during the experiment approaching 20 by the end of the experiment. Differences in the C/N soil ratio between the G and the F stands can therefore be explained by the different litter qualities. The decomposition process

was slower in the *F. japonica* litter and almost one fourth of the initial mass remained at the end of the experiment. *F. japonica* decomposition accelerated right after an initial increase in the litter N concentration and a concomitant decrease in C/N ratio. Increases in N concentrations were observed in both *F. japonica* litter types during the first months of the experiment. These increases are likely a consequence of the microbial immobilization that occurs before the net mineralization. In fact, when the decomposing material is low in some nutrients microorganisms meet their nutritional demand by absorbing nutrients from the soil solution (i.e. immobilization; [Lambers et al., 2008](#)). As a consequence the C/N ratio of litter declines as microorganisms decompose the organic matter and net mineralization occurs when the litter C/N ratio approaches that of the decomposers (ca. 20, see [Gallardo and Merino, 1992](#)).

Litter type likely affects the mycoflora that occurs at a site, both in the soil and on senescent leaves and stems. Little attention has been paid to the occurrence, colonization and succession of fungi on the litter of invasive plants. The present study, to our knowledge, is the first study to isolate saprotrophic fungi from *F. japonica* litter in terrestrial ecosystems. Our results have shown that the total load of saprotrophic fungi isolated from litter depends more on the litter type than on the stand where the litter decomposes. Both native litter types, regardless of the decomposition stand, had a similar total fungal load. *F. japonica* leaves, either decomposing at the invaded or non-invaded stand, had significantly higher total loads than the native litter types. On the contrary, *F. japonica* stems showed the lowest total loads of the four litter types.

As far as fungal diversity is concerned, the high percentages of *Phoma* sp. in the *Fallopia* litter and a mix of other taxa in the grassland litter suggest that the *F. japonica* litter (and/or its living leaves and stems) are more selective for fungi than the grassland litter, as confirmed by Simpson's indices. In addition, it was found that both native and *F. japonica* litters were associated with a higher number of fungal taxa when they decomposed in their own decomposition stand. This finding suggests that *Epicoccum* species, for instance, were present in the soil of both decomposition stands, or were already present in the leaves as endophytes or epiphytes which are specifically associated with native species. *Phoma* sp., which was only isolated from *F. japonica* litter types irrespective of the decomposition stand, was probably associated with the litter of the invasive plant and was not able to associate with the native litter types. *Phoma* sp. has been detected on decomposing *Phragmites australis* ([Van Ryckegem and Verbeke, 2005](#)) and rice ([Pandey and Sinha, 2008](#)), both characterized by high C/N ratio litters, and as epiphyte and endophyte in the freshly fallen leaves of *Fagus crenata* ([Osono, 2002](#)). This suggests that *Phoma* sp. also occurs in standing but senescent shoots as well as in early decomposing leaves and stems. *Phoma* sp. is probably able to colonize and decompose low quality litters during the early decompositional stages while mycoflora occurring at later stages will be elucidated only with further studies in order to understand the role of the different fungal taxa.

Differences in the diversity of saprotrophic mycoflora that depend on the litter and on the stand type indicate that only a restricted group of fungi are able to decompose *F. japonica*, possibly because of its high C/N ratio and other chemical properties. Secondary metabolites, some of which have been listed in a recent work by [Fan et al. \(2010\)](#), may be involved in delayed litter decomposition because of their toxic effects on microorganisms and resistance to breakdown ([Lambers et al., 2008](#)). Studies on the allelopathy of *F. japonica* on other plant species have revealed a high capacity to depress seed germination and growth ([Csiszàr, 2009](#)), but to our knowledge, no studies exist on the allelopathic effect of the invader on fungal spore germination and growth.

The time *F. japonica* litter needed to decompose was longer than that of grassland plants. The decomposition rate of *F. japonica* was rather similar to that of riparian shrubs and trees that produce slowly decomposing leaf litter. The similarity of *F. japonica* litter quality and decomposition dynamics to some shrubs and trees rather than to forbs and grasses could be one of the reasons for the invasion success and notorious impacts of this species in grassland and other herbaceous communities. Soil legacies left by *F. japonica* via increased inputs of low quality litter and changes in saprotrophic mycoflora should be taken into account when assessing costs and benefits of eradication and environmental restoration.

Acknowledgements

We would like to thank Alessandra Luzzaro, University of Insubria, Luisella Celi and Stefania Sacconi, University of Turin, for their collaboration and technical assistance in utilizing the CHN Protein analyzer, as well as Samuele Voyron, University of Turin, for his assistance in fungal identification. We are grateful to Valeria Gosmar for her field and laboratory assistance.

4. Discussion and conclusions

Litter quality, decomposition rates, and dominant saprophytic mycoflora differed between *F. japonica* and the native grassland plants. The dominance of *F. japonica* thus alters the amount and dynamics of the organic matter in the invaded area and therefore affects the ecosystem functions. Since only one study site was considered, the experimental design could be criticized because of pseudoreplication (Hurlbert, 2004). However, efforts were deliberately concentrated on one study site in order to combine a litter decomposition experiment and microbiological analyses, the latter being laborious and time consuming (Barni and Siniscalco, 2000; Kourtev et al., 2002).

The modifications induced by *F. japonica* in the invaded ecosystems are mainly caused by its high biomass production, a trait that allows us to consider that this invasive plant is one of those that visually, structurally, and chemically transform ecosystems while reducing native plant biodiversity (Gerber et al., 2008; Aguilera et al., 2010). High above-ground biomass in *F. japonica* (6 times higher than that of the adjacent native vegetation at our study site) translates into high light interception. A high biomass production also leads to a high litter supply to the soil. A thick litter layer generally accumulates under *F. japonica* stands which suggests its low decomposability. Decomposition rates depend on litter quality and the decomposer community (Dassonville, 2008). In the present experiment the initial litter C and N contents and the C/N ratio differed remarkably between *F. japonica* and native species mixtures, and this resulted in different decomposition rates and litter quality dynamics. The litter from native species exhibited a relatively high N content and a C/N ratio similar to that of the soil of the study site (i.e. 10–20). C/N ratios lower than 20 generally lead to rapid decomposition (Lambers et al., 2008). On the contrary, the initial C/N ratio in *F. japonica* litter (either leaves or stems) was much higher than 20. Leaf litter decreased during the experiment approaching 20 by the end of the experiment. Differences in the C/N soil ratio between the G and the F stands can therefore be explained by the different litter qualities. The decomposition process was slower in the *F. japonica* litter and almost one fourth of the initial mass remained at the end of the experiment. *F. japonica* decomposition accelerated right after an initial increase in the litter N concentration and a concomitant decrease in C/N ratio. Increases in N concentrations were observed in both *F. japonica* litter types during the first months of the experiment. These increases are likely a consequence of the microbial immobilization that occurs before the net mineralization. In fact, when the decomposing material is low in some nutrients microorganisms meet their

nutritional demand by absorbing nutrients from the soil solution (i.e. immobilization; Lambers et al., 2008). As a consequence the C/N ratio of litter declines as microorganisms decompose the organic matter and net mineralization occurs when the litter C/N ratio approaches that of the decomposers (ca. 20, see Gallardo and Merino, 1992).

Litter type likely affects the mycoflora that occurs at a site, both in the soil and on senescent leaves and stems. Little attention has been paid to the occurrence, colonization and succession of fungi on the litter of invasive plants. The present study, to our knowledge, is the first study to isolate saprotrophic fungi from *F. japonica* litter in terrestrial ecosystems. Our results have shown that the total load of saprotrophic fungi isolated from litter depends more on the litter type than on the stand where the litter decomposes. Both native litter types, regardless of the decomposition stand, had a similar total fungal load. *F. japonica* leaves, either decomposing at the invaded or non-invaded stand, had significantly higher total loads than the native litter types. On the contrary, *F. japonica* stems showed the lowest total loads of the four litter types.

As far as fungal diversity is concerned, the high percentages of *Phoma* sp. in the *Fallopia* litter and a mix of other taxa in the grassland litter suggest that the *F. japonica* litter (and/or its living leaves and stems) are more selective for fungi than the grassland litter, as confirmed by Simpson's indices. In addition, it was found that both native and *F. japonica* litters were associated with a higher number of fungal taxa when they decomposed in their own decomposition stand. This finding suggests that *Epicoccum* species, for instance, were present in the soil of both decomposition stands, or were already present in the leaves as endophytes or epiphytes which are specifically associated with native species. *Phoma* sp., which was only isolated from *F. japonica* litter types irrespective of the decomposition stand, was probably associated with the litter of the invasive plant and was not able to associate with the native litter types. *Phoma* sp. has been detected on decomposing *Phragmites australis* (Van Ryckegem and Verbeken, 2005) and rice (Pandey and Sinha, 2008), both characterized by high C/N ratio litters, and as epiphyte and endophyte in the freshly fallen leaves of *Fagus crenata* (Osono, 2002). This suggests that *Phoma* sp. also occurs in standing but senescent shoots as well as in early decomposing leaves and stems. *Phoma* sp. is probably able to colonize and decompose low quality litters during the early decompositional stages while mycoflora occurring at later stages will be elucidated only with further studies in order to understand the role of the different fungal taxa.

Differences in the diversity of saprotrophic mycoflora that depend on the litter and on the stand type indicate that only a restricted group of fungi are able to decompose *F. japonica*, possibly because of its high C/N ratio and other chemical properties. Secondary metabolites, some of which have been listed in a recent work by Fan et al. (2010), may be involved in delayed litter decomposition because of their toxic effects on microorganisms and resistance to breakdown (Lambers et al., 2008). Studies on the allelopathy of *F. japonica* on other plant species have revealed a high capacity to depress seed germination and growth (Csizsàr, 2009), but to our knowledge, no studies exist on the allelopathic effect of the invader on fungal spore germination and growth.

The time *F. japonica* litter needed to decompose was longer than that of grassland plants. The decomposition rate of *F. japonica* was rather similar to that of riparian shrubs and trees that produce slowly decomposing leaf litter. The similarity of *F. japonica* litter quality and decomposition dynamics to some shrubs and trees rather than to forbs and grasses could be one of the reasons for the invasion success and notorious impacts of this species in grassland and other herbaceous communities. Soil legacies left by *F. japonica* via increased inputs of low quality litter and changes

in saprotrophic mycoflora should be taken into account when assessing costs and benefits of eradication and environmental restoration.

Acknowledgements

We would like to thank Alessandra Luzzaro, University of Insubria, Luisella Celi and Stefania Sacconi, University of Turin, for their collaboration and technical assistance in utilizing the CHN Protein analyzer, as well as Samuele Voyron, University of Turin, for his assistance in fungal identification. We are grateful to Valeria Gosmar for her field and laboratory assistance.

References

A.G. Aguilera, P. Alpert, J.S. Dukes, R. Harrington Impacts of the invasive plant *Fallopia japonica* (Houtt.) Ronse Decraene on plant communities and ecosystem processes *Biol. Inv.*, 12 (2010), pp. 1243–1252

S.D. Allison, P.M. Vitousek Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. *Oecologia*, 141 (2004), pp. 612–619

N.L. Angeloni, K.J. Jankowski, N.C. Tuchman, J.J. Kelly. The biology of an invasive cattail species (*Typha × glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland. *FEMS Microbiol. Lett.*, 263 (2006), pp. 86–92

ARPA Piemonte: <http://old.arpa.piemonte.it/annali/meteorologici>.

J.N. Barney, N. Tharayil, A. DiTommaso, et al. The biology of invasive alien plants in Canada. 5. *Polygonum cuspidatum* Sieb. & Zucc. [= *Fallopia japonica* (Houtt.) Ronse Decr.]. *Can. J. Plant Sci.*, 86 (3) (2006), pp. 887–905

E. Barni, C. Siniscalco. Vegetation dynamics and arbuscular mycorrhiza in old-field successions of the western Italian Alps. *Mycorrhiza*, 10 (2000), pp. 63–72

D. Beerling, J.P. Bailey, A. Conolly. Biological flora of the British Isles. *Fallopia japonica* (Houtt.) Ronse Decraene, (*Reynoutria japonica* Houtt.; *Polygonum cuspidatum* Sieb. & Zucc. *J. Ecol.*, 82 (1994), pp. 959–979

J.H. Braatne, S.M.P. Sullivan, E. Chamberlain. Leaf decomposition and stream macroinvertebrate colonisation of Japanese Knotweed, an Invasive Plant species. *Int. Rev. Hydrobiol.*, 92 (2007), pp. 656–665

A. Csiszàr. Allelopathic effects of invasive woody plant species in Hungary. *Acta Sil. Ling. Hung.*, 5 (2009), pp. 9–17

D.A. Crossley, M.P. Hoglund. A litter-bag method for the study of microarthropods inhabiting leaf litter. *Ecology*, 43 (1962), pp. 571–573

N. Dassonville. Impact des plantes exotiques envahissantes sur le fonctionnement des écosystèmes en Belgique. Thèse de doctorat Faculté des Sciences, Laboratoire de Génétique et Écologie Végétales, Université libre de Bruxelles, Belgique (2008)

- J.G. Ehrenfeld. Effects of exotic plant invasions on soil nutrient cycling processes
Ecosystems, 6 (2003), pp. 503–523
- P. Fan, K. Hostettmann, H. Lou. Allelochemicals of the invasive neophyte *Polygonum cuspidatum* Sieb. & Zucc. (Polygonaceae). *Chemoecology*, 20 (2010), pp. 223–227
- A. Gallardo, J. Merino. Nitrogen immobilization in leaf litter at two Mediterranean ecosystems of SW Spain. *Biogeochemistry*, 15 (1992), pp. 213–228
- E. Gerber, C. Krebs, C. Murrell, M. Moretti, R. Rocklin, U. Schaffner. Exotic invasive knotweeds (*Fallopia* spp.) negatively affect plant and invertebrate assemblages in European riparian habitats. *Biol. Conserv.*, 141 (2008), pp. 646–654
- D. Gujarati. Use of dummy variables in testing for equality between sets of coefficients in two linear regressions: a note. *Am. Stat.*, 24 (1970), pp. 18–22
- D.J. Hand, C.C. Taylor. *Multivariate Analysis of Variance and Repeated Measures: a Practical Approach for Behavioural Science*. Chapman and Hall, London (1987)
- M. Hejda, P. Pysek, V. Jarosyk. Impact of invasive plants on the species richness, diversity and composition of invaded communities. *J. Ecol.*, 97 (2009), pp. 393–403
- S.H. Hurlbert. On misinterpretation of pseudoreplication and related matters: a reply to Oksanen. *Oikos*, 104 (2004), pp. 591–597
- P.S. Kourtev, J.G. Ehrenfeld, M. Hägblom. Exotic plants alter the microbial community structure and function in the soil. *Ecology*, 83 (2002), pp. 3152–3166
- H. Lambers, F.S. Chapin, T.L. Pons. *Plant Physiological Ecology*. (Second ed.) Springer, New York (2008)
- A. Lecerf, D. Patfield, A. Boiche, M.P. Riipinen, E. Chauvet, M. Dobson. Stream ecosystems respond to riparian invasion by Japanese knotweed (*Fallopia japonica*). *Can. J. Fish. Aquat. Sci.*, 64 (2007), pp. 1273–1283
- J.M. Levine, M. Vilà, C.M. D'Antonio, J.S. Dukes, K. Grigulis, S. Lavorel. Mechanisms underlying the impacts of exotic plant invasions. *Proc. R. Soc. Lond. B*, 270 (2003), pp. 775–781
- S. Lowe, M. Browne, S. Boudjelas, M. De Poorter. *100 of the World's Worst Invasive Alien Species. A Selection from the Global Invasive Species Database*. ISSG, SSC and IUCN (2000)
www.issg.org/booklet.pdf
- J.C. Maerz, B. Blossey, V. Nuzzo. Green frogs show reduced foraging success in habitats invaded by *F. japonica*. *Biodivers. Conserv.*, 14 (2005), pp. 2901–2911
- S.J. Manchester, J.M. Bullock. The impacts of non-native species on UK biodiversity and the effectiveness of control. *J. Appl. Ecol.*, 37 (2000), pp. 845–864

- N. Maurel, S. Salmon, J. Ponge, N. Machon, J. Moret, A. Muratet. Does the invasive species *Reynoutria japonica* have an impact on soil and flora in urban wastelands? *Biol. Inv.*, 12 (2010), pp. 1709–1719
- K. Miura, M. Kudo. An agar medium for aquatic hyphomycetes. *Trans. Mycol. Soc. Jpn.*, 11 (1970), pp. 116–118
- L. Moravcová, P. Pyšek, V. Jarošík, P. Zákřavský. Potential phytotoxic and shading effects of invasive *Fallopia* (Polygonaceae) taxa on the germination of native dominant species. *NeoBiota*, 9 (2011), pp. 31–47
- S. Muller (Ed.), *Invasives en France*, Muséum d'Histoire Naturelle, Paris (2004)
- T. Osono. Phyllosphere fungi on leaf litter of *Fagus crenata*: occurrence, colonization, and succession. *Can. J. Bot.*, 80 (2002), pp. 460–469
- T. Osono, B.K. Bhatta, H. Takeda. Phyllosphere fungi on living and decomposing leaves of giant dogwood. *Mycoscience*, 45 (2004), pp. 35–41
- V. Pandey, A. Sinha. Mycoflora associated with decomposition of rice stubble mixed with soil. *J. Plant Protect. Res.*, 48 (2008), pp. 247–250
- R Development Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria (2011) <http://www.R-project.org/>
- Y. Sakamoto, M. Ishiguro, G. Kitagawa. *Akaike Information Criterion Statistics*. Reidel Publishing Company, Dordrecht/Tokyo (1986), p. 320
- C. Siniscalco, E. Barni, G. Bacaro. Alien species distribution along the elevation gradient in the western Italian Alps. *Plant Biosyst.*, 145 (2011), pp. 150–158
- S. Vanderhoeven, N. Dassonville, P. Meerts. Increased topsoil mineral nutrient concentrations under exotic invasive plants in Belgium. *Plant Soil*, 275 (2005), pp. 169–179
- G. Van Ryckegem, A. Verbeken. Fungal ecology and succession on *Phragmites australis* in a brackish tidal marsh. I. Leaf sheaths. *Fungal Divers.*, 19 (2005), pp. 157–187
- B.E. Wolfe, J.N. Klironomos. Breaking new ground: soil communities and exotic plant invasion. *Bioscience*, 55 (2005), pp. 477–487