The effect of pesticides and alien invasive species on soil biota and litter decompositon rates in a Mediterrean-climate ecosystem of Western Australia

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Abstract. Ecosystems are complex systems and changing one of their components can alter their whole functioning. Decomposition and biodiversity are two factors that play a role in this stability, and it is vital to study how these two factors are interrelated and how other factors, whether of human origin or not, can affect them. This study has tested different hypotheses regarding the effects of pesticides and invasive species on the biodiversity of the soil fauna and litter decomposition rate. Decomposition was measured using the litterbags technique. Our results indicate that pesticides had a negative effect on decomposition whereas invasive species increased decomposition rate. At the same time, the diversity of the soil biota was unaffected by either factor. These results allow us to better understand the response of important ecosystem functions to human-induced alterations, in order to mitigate harmful effects or restore them wherever necessary.

Keywords: *Australia, litter decomposition, soil fauna diversity, invasive species, herbicide.*

Resum. Els ecosistemes són sistemes complexos i el canvi d'un dels seus components en pot alterar tot el seu funcionament. La descomposició i la biodiversitat són dos factors que juguen un paper molt important en aquesta estabilitat, i és vital estudiar com aquests dos factors estan relacionats entre si, i com altres factors, ja siguin d'origen humà o no, els poden afectar. Aquest estudi ha posat a prova diverses hipòtesis sobre els efectes dels pesticides i les espècies invasores sobre la biodiversitat de la fauna del sòl i taxa de descomposició de la fullaraca. La descomposició es va mesurar utilitzant la tècnica de bosses amb fullaraca. Els nostres resultats indiquen que els pesticides han tingut un efecte negatiu en la descomposició, mentre que les espècies invasives augmenten la seva velocitat de descomposició. Alhora, la diversitat de la biota del sòl no va ser afectat per cap dels factors. Aquests resultants ens permeten entendre millor les respostes de les funcions dels ecosistemes en alteracions on l'home hi ha estat involucrat, permeten una mitigació dels efectes danyosos o una millor restauració pel medi quan sigui necessari.

Paraules clau: *Austràlia, descomposició de la fullaraca, diversitat de la fauna del sòl, espècies invasores, herbicides.*

Resumen. Los ecosistemas son sistemas complejos y el cambio de uno de sus componentes puede alterar todo su funcionamiento. La descomposición y la biodiversidad son dos factores que juegan un papel en esta estabilidad, y es vital estudiar cómo estos dos factores están relacionados entre sí, y como otros factores, ya sean de origen humano o no, puedan afectar. Este estudio ha puesto a prueba diversas hipótesis sobre los efectos de los pesticidas y las especies invasoras sobre la biodiversidad de la fauna del suelo y la tasa de descomposición de la hojarasca. La descomposición se midió utilizando la técnica de bolsas de hojarasca. Nuestros resultados indican que los pesticidas han tenido un efecto negativo en la descomposición, mientras que las especies invasivas aumentan la velocidad de descomposición. Al mismo tiempo, la diversidad de la biota del suelo no fue afectada por ninguno de los factores. Estos resultantes nos permiten entender mejor las respuestas de las funciones de los ecosistemas en alteraciones donde el hombre ha estado involucrado, permitiendo una mitigación de los efectos dañinos o una mejor restauración del medio cuando sea necesario.

Palabras clave: *Australia, descomposición de la hojarasca, diversidad de la fauna del suelo, especies invasoras, herbicidas.*

1. Introduction

Plants need substances such minerals, carbon dioxide and salts to develop and grow, and to sustain the productivity and energy flow of ecosystems. Most of these substances come from the soil, and they would be limited if nutrient cycle and decomposition would not exist (Chapin, Matson and Mooney, 2002). Litter decomposition is vital to nutrient cycling and the productivity of forests (Didham, 1998) and is an important component of the global carbon budget (Aerts, 1997). Ecosystem function depends on many components, and changing one of them can alter the whole community. Nutrient cycle and decomposition are so important to maintain its stability, for permeating the recirculation of nutrients. In terrestrial ecosystems, litterfall represents the primary pathway for nutrient return to the soil (Karberg, Scott and Giardina, 2008), so it is really important to study how different factors, natural or human, direct or indirect, can alter it, to have the right tools and better manage natural ecosystems to prevent their destruction or restore the functionality of those that have been already modify by the human activities.

Litter decomposition depends on different factors; the most important are soil temperature and moisture content and litter quality. Depending on them, the faster or slower will be the breakdown of organic matter, if the conditions are good enough to allow insects, worms, bacteria and fungi, to carry out the decomposition. It is increasingly being recognized that faunal community structure, especially the influence of earthworms, are another important factor determining decomposition rates and interacting with temperature and moisture conditions (Bohlen et al., 1997; Dechaine *et* al., 2005).

Different types of leaves may constitute very different substrates for the microbial decomposers, and depending on its chemical composition, the leave breakdown will be faster or slower (Karberg, Scott and Giardina, 2008). Also many studies have shown that there are differences among species, due to differences in the chemical composition of their litter (Adams and Angradi, 1996; Cornelissen, 1996).

Humans with their activities have modified the natural development of nutrient cycles and decomposition. The growth in fossil fuels use during the last centuries has released large quantities of carbon, nitrogen and sulphur oxides to the atmosphere and has increased their inputs to ecosystems. Altering the natural background rate of nitrogen inputs may increase plant production enough to affect the global carbon cycle (Chapin, Matson and Mooney, 2002). Changes in nutrient cycling and decomposition affect the interactions between ecosystems, the carbon cycle and the climate of our planet. Plant species and soil types will be altered as a consequence of new temperature and moisture regimes above-and belowground, which will interact with the effects of carbon enrichment and changes in nutrient availability (Anderson, 1996).

Litter decomposition has an important role on the global carbon and nitrogen cycles, and climate change may alter both cycles as a consequence of changing temperature and decomposition rates. In addition, litter quality can be directly or indirectly influenced by climate change and the complexity of the feedbacks involved makes it difficult to predict how ecosystems are going to respond (Harte and Shaw, 1995).

Pesticides have been used since Egyptians to prevent production losses (due to the presence of competing plants or the presence of animals that can eat the harvest) and curing or preventing any diseases of plants and animals (fungicides, bactericides, etc.). Humans have abused of the pesticides and have brought to the surface the problems that they can cause: health problems to humans and life forms in general and ecological problems to the environment (Aktar, Sengupta and Chowdhury 2009). Pesticides can have direct and indirect benefits (Aktar, Sengupta and Chowdhury 2009), but also has been showed the evidence that some of these chemical compounds pose a potential risk to life forms and may have unexpected effects on the environment (Forget, 1993), such as the alteration of the nitrogen cycle.

Also, the movement of people around the world has brought species from one side of the Earth to the other. This event, either voluntarily or involuntarily, has led to changes in the diversity of organisms in ecosystems or has affected the relationships between plant or animal species and the relation of them with the environment. In addition, the presence of a new species in an ecosystem may affect its global stability. Also the replacement of a native for an invasive species has important consequences for conservation and human utilization of ecosystem services.

Exotic plants have potential impacts on nutrient cycling processes including carbon, nitrogen, water and other cycles in ecosystems. The available data suggest that invasive plant species frequently increase biomass, net primary production, N availability, alter N fixation rates and the litter produced has higher decomposition rates compared to co-occurring natives. But there are also studies where no difference between exotics and native species was found, or other cases where a given species has different effects at different sites, depending on the composition of the invaded community and/or depending on other environmental factors such as soil type, that may determine the direction and magnitude of ecosystem-level impacts (Ehrenfeld, 2003).

When a new species is introduced in a community it will have different effects on the nutrient or other ecological cycles depending on its differences from the constellation of traits present within the existing plant community. A large number of studies have reported that the soil properties change as a consequence of the introduction of new traits and new functional groups in a community (Gill and Burke, 1999). However, other studies have found no such effects (McCarrron and Scott, 2001) and the effect of exotic plant invasions on ecosystem and soil properties remains controversial (Ehrenfeld and Scott, 2001).

The main objective of this study was to assess the effect of herbicides and alien species on litter decomposition rates and the diversity of soil biota. This was explored at two different sites: Ridgefied and Nalya Reserve. Different factors and treatments were tested, including litter type (native or invasive plants), use of herbicides, mesh size of litterbags and community type (native or invasive). Specifically, we tested the following hypotheses:

(1) Decomposition rate would be higher for invasive litter because invasive species often have higher concentrations of leaf nitrogen (Vitousek *et* al., 1987; Vitousek and Walker, 1989; Witkowski, 1991; Baruch and Coldstein, 1999; Nagel and Griffin, 2001).

(2) Higher decomposition rate would be associated with higher soil biodiversity and herbicides would reduce both decomposition and soil biodiversity.

(3) Large mesh will favour decomposition because it will make it easier for the soil fauna to get inside the bag to breakdown the litter.

2. Materials and methods

2.1. Study sites and experimental design

The project has been carried out in two different areas, Nalya Reserve and Ridgefield. The two sites are located in the same region, just separated by 30Km one from the other. Both areas are found in a Mediterranean-climate region, were the precipitation is dominant in winter with some occasional heavy falls in summer. However, both locations have different background. Nalya (32^{o}22'S 117^{o}12'E) is a natural reserve, where no human activities have been carried out for a long period of time and Ridgefield (32^{o}29'S 116^{o}58'E) was used for cropping and grazing, but nowadays is used by the University of Western Australia as a research site. Soil composition is slightly different at both sides where in Ridgefield has a bit more of nitrogen and phosphorous due to prior use of fertilizers in the area for cropping, and the prior grazing activities.

In both regions four blocks were delimited, each with two plots corresponding to the main treatments, each plot was 11.5m x 11.5m. Blocks in Nalya Reserve were named N01, N02, N03 and N04 and the treatment corresponded to the type of plant communities in the plot: native (N) or invasive (I). In Ridgefield, plots were named R08, R13, R63 and R70, and the treatment corresponded to the use or not of herbicide[1] to prevent the growth up of non-native weed cover: herbicide (H) or control (C).

Different size of mesh was used to optimize or minimize the access of different soil organisms into the bags and the two mesh sizes used were a large one of 7 mm and a small one of 1 mm. The size of the bags was 15 x 15cm and they contained 12 grams of dried native or invasive leaves. These two different types of bags, native litterbags and invasive litterbags, where made with three different species of plants. Native litterbags were formed by 6g of *Acacia acuminate*, 4 g of *Eucalyptus loxophleba* and 2 g of *Banksia sessilis.* Invasive litterbags were constituted by 6g of *Avena spp*., 4g of *Erodium spp*. and 2g of *Arctotheca calendula*. All leaves where previously dried before preparing the litterbags in the oven at 40^{o}C during a week.

2.2. Placement and harvest litterbags

Litterbags were placed in the soil ground randomly in two different moments, first one was the 4th and 6th of November 2011, when 64 litterbags were placed in Nalya Reserve and Ridgefield (8 for each combination of treatments) and the second one was the 8th of February of 2012 where 32 litterbags were placed in both study sites (4 for each combination of treatments). All bags were tagged with a unique identification number. Litterbags were subjected in the soil

[1] Herbicides (active ingredients haloxyfop and glyphosate) were applied to early winter to remove the winter-active non-native species found at the site, but native species maybe were unintentionally removed with this treatment.

floor with the help of two small pieces of metal tied together with a twine, and after their placement, they were not touched until their harvest date.

Removal of the bags was done at two times, the first harvest was done the 8th of February of 2012, the same day that we placed 32 new litter bags in both study sites, and the second harvest was done the 11th of May of 2012, so that in each site we had 64 bags that had been 3 months in the field and 32 that had been in the field for a period of 6 months.

2.3. Measuring invertebrate biodiversity

After harvesting the litterbags, all invertebrates were taken off of the bags using the Tullgren Funnels technique. The bags were left inside the funnels during three days, and after this period of time specimen jars with 70% of ethanol solution were closed and tagged. Then the specimen jars were brought to the laboratory. Invertebrates found where classified by their order. In this study, we used the Shannon index to calculate the diversity of the soil fauna at the order level (e.g. Coleoptera, Aranae, Diptera, Hemiptera, etc.) using *Microsoft Office Excel*.

2.4. Calculating the litter decomposition rate

After invertebrates were taken off from the litterbags, the bags were introduced in the oven with care during two days at 40^{o}C to dry their content. After this period of time passed litterbags were weighted. Once we had all data, we estimated the decomposition rate with the following regression approach, assuming an exponential decay of litter weight:

$$B = B_o \cdot e^{-k \cdot t}$$

Where B is the final litter weight, B_o the initial litter weight, t is the time that the litterbag has spent in the field and k is the decomposition rate constant.

2.5. Statistical methods

We used mixed linear models to study the effect of the different treatments on litter decomposition rate and diversity index. A different model was fitted to each site. For Nalya our model had four fixed factors: community type (Native or Invasive), leaf type (Native or Invasive), litterbag mesh size (Large or Small) and the time each litterbag had spent in the field (3 or 6 months). For Ridgefield the four fixed factors were: herbicide use (Herbicide applied or Control), leaf type (Native or Invasive), litterbag mesh size (Large or Small) and the time each litterbag had spent in the field (3 or 6 months). In both cases block was considered a random factor. Additional mixed linear models were fitted to test whether differences in decomposition rate associated to the main treatments (ecosystem type in Nalya and herbicide use in Ridgefield) were mediated by differences in biodiversity. All response variables were normally distributed and the residuals of the mixed models showed no obvious pattern. All statistical analyses were conducted using the R package interfaced with the software *Deducer*.

3. Results

In both study sites, Nalya Reserve and Rigdefield, decomposition rates were influenced by the time that litterbags spent in the field, indicating that litter decay was not perfectly exponential or, more likely, that the particular time period that each sample spent in the field influenced its decomposition rate.

Also small mesh size and native leaf type were associated to lower decomposition rates. This latter variable was the one with a stronger effect on decomposition. Finally, the herbicide treatment resulted also in lower decomposition rates, although the effect was much lower than that of leaf type (Table 2).

	Value	Std. Error	t-value	p-value
Intercept (Invasive treatment, Large mesh size and Invasive leaf type)	-2.662	0.147	-18.078	0.000
Native treatment	-0.177	0.072	-2.475	0.015
Small mesh size	-0.223	0.073	-3.050	0.003
Native leaf type	-1.317	0.072	-18.397	0.000
Time (months)	-0.085	0.025	-3.350	0.001

Table nº 1: Model results relating the decomposition rate (month^{-1}) with bag characteristics, treatments and time, obtained by the lineal mixed-effects model in Nalya Reserve. Source: Mariona Isern Subich

	Value	Std. Error	t-value	p-value
Intercept (Control treatment, Large mesh size and Invasive leaf type)	-2.512	0.119	-20.977	0.0000
Herbicide treatment	-0.203	0.068	-2.972	0.0038
Small mesh size	-0.248	0.068	-3.637	0.0005
Native leaf type	-1.199	0.068	-17.550	0.0000
Time (months)	-0.092	0.024	-3.790	0.0003

Table nº2: Results obtained by the lineal mixed-effects model related the decomposition rate (month^{-1}) with the two treatments (herbicide and control), mesh size, leaf type and time in Ridgefield. Source: Mariona Isern Subich

The diversity of soil biota was unrelated to any of the studied variables at the two sites. Only in Ridgefield was observed a marginal effect of the time that the samples spent in the field, with slightly higher diversity for samples that spent more time in the field.

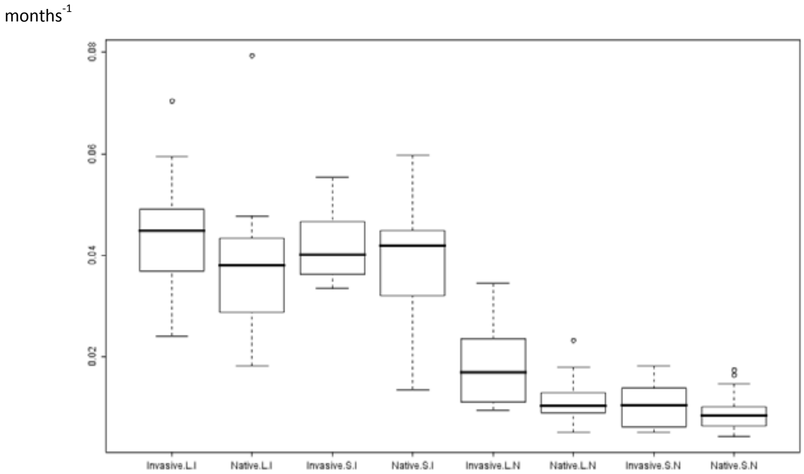
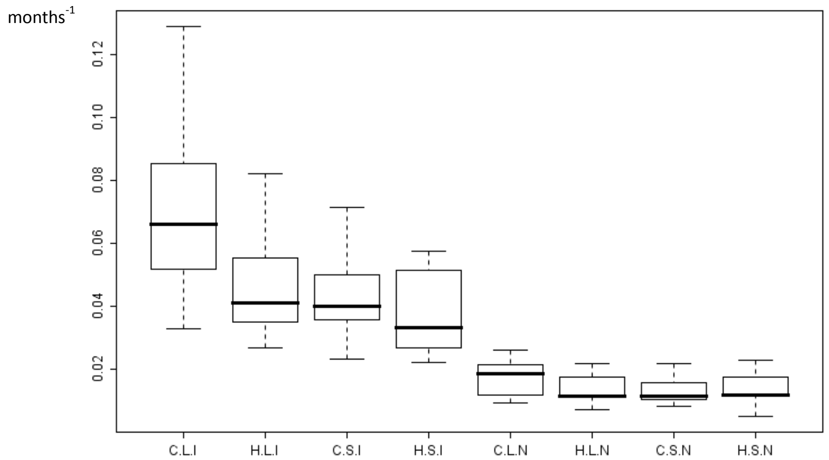


Figure nº1: Box plots showing the relationship between decomposition rate and community type (Invasive or Native), litterbag mesh size (S=Small or L=Large) and leaf type (I=Invasive or N=Native) in Nalya Reserve (see also Table 5). Source: Mariona Isern Subich



nº2: Box plots showing the relationship between decomposition rate and plots where Herbicide was used (H) or not (C), litterbag mesh size (S=Small or L=Large) and leaf type (I=Invasive or N=Native) in Nalya Reserve (see also Table 6). Source: Mariona Isern Subich

4. Discussion

4.1. Temporal effects on decomposition rate and differences between sites

Soil temperature and moisture are two of the main factors that control the decomposition rates, and these could be the reasons why in both study sites we observed decreasing decomposition rates. Spring in Western Australia takes from September till November, being a wet medium-high temperatures season, summer is from December till February, with high temperatures and almost no rainfall and low moisture content, and autumn is from March till May, with more rainfall but lower temperatures. Those litterbags that spent three months in the field, from November until February, had higher decomposition rates, probably because the climate was more favorable to decomposers. The results of the present study are then in agreement with the view that climate is the main factor that determines litter decomposition rates (Aerts R., 1997). Consequently, changes in climate may alter nutrient cycles and ecosystem functioning at local, regional and even global scales (Anderson, 1991; Shaw M.R. and Harte J, 2001).

The higher decomposition rates in Ridgefield than in Nalya Reserve were likely a consequence of land-use history.Ridgefield was used as a crop and grazing area before being a research site for the UWA (Perring, M.P. et al., 2012), so it has higher concentrations of nitrogen and phosphorus in its soil, whereas Nalya have not been affected by any relevant human activity for the last 50 years. It has been stated that the decomposition of leaf litter can be predicted by the C:N ratio, with lower C:N values implying faster decomposition (Taylor et al., 1989).

4.2. Effects of mesh size on decomposition.

Decomposition rates were higher in large mesh size bags than in smaller ones being consistent with the initial hypothesis. This is likely a result of the entrance of bigger soil fauna in the large mesh size bags, showing their important role in the breakdown of litter, as some previous studies have shown (Bohlen *et* al., 1997; Bradford Mark A., *et* al., 2002; Dechaine *et* al., 2005).

4.3. Effects of leaf type on decomposition.

The results in both study sites show that invasive species litter has higher decomposition rates than the litter of native Australian species. As hypothesized, the differences obtained in the results might be as a result of different leaf litter quality (chemistry) between native and invasive species, because the climate was the same for all litterbags in both study sites. Invasive litter usually lose nitrogen and phosphorus faster and in larger quantities than comparable native litter, resulting in higher litter decomposition rates (Allison S. D. and Vitousek P. M, 2004). These differences in the chemical composition of litter among species are likely to be the reason why invasive leaves discomposed faster and had higher decomposition rates than the native ones in Ridgefield and Nalya Reserve.

4.4. Effects of community type on decomposition

Different decomposition rates were observed between the native communities and the invasive ones in Nalya Reserve, being decomposition higher in invasive communities. As it was said in the previous section, invasive leaves usually have higher concentrations of nitrogen than those of native species (Vitousek et al., 1987; Vitousek and Walker, 1989; Witkowski, 1991; Baruch and Coldstein, 1999; Nagel and Griffin, 2001), which will be introduced into the soil after the decomposition. Therefore, a community of invasive species is likely to provide more nitrogen back to the soil than a native community, which eventually would result in more nitrogen available for other plants and for the soil biota, causing an increase in the nutrient cycles rates in general (Vitousek and Walker, 1989; Witkowsky, 1991) and in the decomposition rate in particular, in agreement with our initial hypothesis.

4.5. Effects of herbicide use on decomposition

Also in agreement with the initial hypothesis, the results showed clear differences in Ridgefield between herbicide treatments, with higher decomposition rates in control plots compared to plots where herbicide had been used. Interestingly, no difference was found in the diversity of soil biota between treatments, implying that the changes in soil biodiversity (at least for the studied groups of organisms) could not be the cause of the effects on decomposition rates (Hättenschwiler S., Tiunov A.V. and Scheu S., 2005). Plots where herbicide was used had less weed abundance, and in some of them no vegetation was present, which could cause a change of the microclimate of the litterbags. Soil moisture and temperature are strongly correlated with decomposition (Shaw M. R. and Harte J., 2001), as explained above. Those bags that were in herbicide plots were more exposed and had different values of temperature and moisture than those ones placed in control plots, which had weeds and other plants surrounding them. Having plants surrounding the litterbags keeps higher moisture content and buffering temperature fluctuations at the soil, which likely resulted in ideal conditions to carry out decomposition. Litterbags in herbicide plots had higher temperature and less moisture, causing lower decomposition rates. Other studies have supported the idea that vegetation type and cover can have a dramatic influence on nutrient cycles through changes in the micro-environmental conditions (Shaw M. R. And Harte J., 2001).

5. Conclusion

The present study has shown how different factors can affect decomposition in different environments. Differences in climate, community composition, past land use, and direct human activities such as use of herbicides all have an influence in litter decomposition rates. Each territory, ecosystem and community has its own particularities, and even small changes in them can have large impacts in the whole community and the ecosystem services we received from it. Previous studies have shown that the responses of introducing an invasive species in a given place can affect nutrient cycles, decomposition, species abundance and community structure, although the responses may be complex and it is frequently difficult to predict the exact direction of the changes (Allison S. D. and Vitousek P. M., 2004; Ehrenfeld J.G. and Scott N., 2001; Ehrenfeld J.G., 2003). Experimental studies like the ones presented here may allow predicting those directional changes under varying environmental conditions, which could inform management and allow us to prevent the alterations that are considered to be more harmful in the affected community. This study can help in situations where the affected community has similar characteristic to those found in Nalya Reserve or Rigdefield.

Herbicides have helped us along our history improving our capacity to produce food and our wellness. However, their abuse can have important harmful effects on us and all other life forms. Finding equilibrium in their use is necessary to achieve the maximum benefit of them while avoiding their negative side effects. The present study has shown that herbicide use in Ridgefield (Mediterranean-climate region) had a negative effect on decomposition rates. The results of this study, obtained from a relatively short period of time did not show any relation between decomposition rate and soil biodiversity, so it would be interesting to study in more detail how soil biodiversity is involved in the process of the decomposition, to have more information on how they influence ecosystem dynamics. Further research should be aimed at determining how general are the results obtained here and also at establishing the precise mechanisms by which herbicides affect ecosystem nutrient cycles and biodiversity in different environments. This would allow us to improve the use we make of them and to maintain the services that ecosystems provide to us, and would also give us better to restore the areas that have been already impacted by heavy herbicide use.

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