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Environmental Science Department

THE EFFECT OF PESTICIDES AND ALIEN INVASIVE SPECIES ON SOIL BIOTA AND LITTER DECOMPOSITION RATES IN A MEDITERREAN-CLIMATE ECOSYSTEM OF WESTERN AUSTRALIA

En col·laboració amb/in collaboration with:

THE UNIVERSITY OF WESTERN AUSTRALIA

ERIE Ecosystem Restoration & Intervention Ecology Research Group

Memòria del Projecte Fi de Carrera

de **Ciències Ambientals** presentada

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This project has been printed on recycled, chlorine-free paper, in order to minimize its environmental impact. A summary in article format and a CD with the project and article digital files are attached.

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Contents

1. INTRODUCTION

1.1. Decomposition and nutrient cycling in ecosystems

-"Nutrient cycling involves the entry of nutrient to ecosystems, their internal transfers between plants and soils, and their loss from ecosystems" (Chapin, Matson and Mooney, 2002).

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. Chemical weathering of rocks, the biological fixation of atmospheric nitrogen, and the deposition of nutrients from the atmosphere in rain, wind-blown particles, or gases are how nutrients are introduced into ecosystems. The internal cycling processes include changes from organic to inorganic forms, from one ionic form to another, biological uptake by plants and microorganisms, and exchange of nutrients on surfaces. Ecosystem nutrient loss occurs by leaching, wind and water erosion, fire, trace-gas emission and the removal of materials in harvest. In unmanaged ecosystems it is the decomposition of soil organic matter and plant litter that supplies the nitrogen and phosphorus needed for plants to growth (Chapin, Matson and Mooney, 2002).

Ecologists have focused strongly their research on nitrogen and phosphorus for their importance in the control of plant growth, community diversity, and ecosystem-level processes such as productivity (Figures 1 and 2). Other important nutrients are potassium or sulphur in the growth of the plants, silica regulates the growth of diatoms in lakes and oceans, and calcium is an excellent predictor of the distribution of plants and plant communities (Vitousek and Matson, 2009). Some of these nutrient cycles include gaseous phases and have self-regulating feedback mechanisms (Holt, Rinehart, and Winston, 1963). Patterns of biogeochemical cycling have important differences among the different elements, and nitrogen is frequently the element that limits plant production (Chapin, Matson and Mooney, 2002).

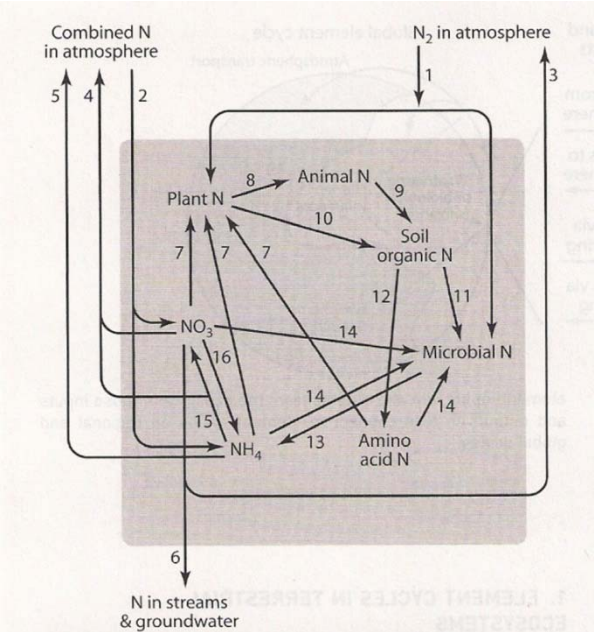


Figure 1: Pathways of nitrogen cycling in terrestrial ecosystems. The nitrogen cycle is complex, with multiple transformations and oxidation/reduction reactions; indeed, this depiction is a substantial simplification of the N cycle in that it leaves out many of the microbial populations that carry out nitrogen transformations. Major fluxes of nitrogen shown here include (1) biological nitrogen fixation; (2) atmospheric deposition of combined nitrogen; (3) denitrification to dinitrogen; (4) fluxes of oxidized trace gases of nitrogen that occur during nitrification and denitrification; (5) ammonia volatilization; (6) solution losses of nitrogen; (7) plant uptake of available nitrogen; (8) consumption of plant nitrogen by animals; (9) flux of nitrogen to soil from excretion or animal death; (10) flux of microbes that carry out decomposition; (12) mobilization of amino acids from soil organic nitrogen through the action of extracellular enzymes; (13) release of ammonium by microbes; (14) microbial uptake of available nitrogen; (15) nitrification of ammonium to nitrate; and (16) dissimilatory reduction of nitrate to ammonium. Source figure and text from: Vitousek and Matson, 2009

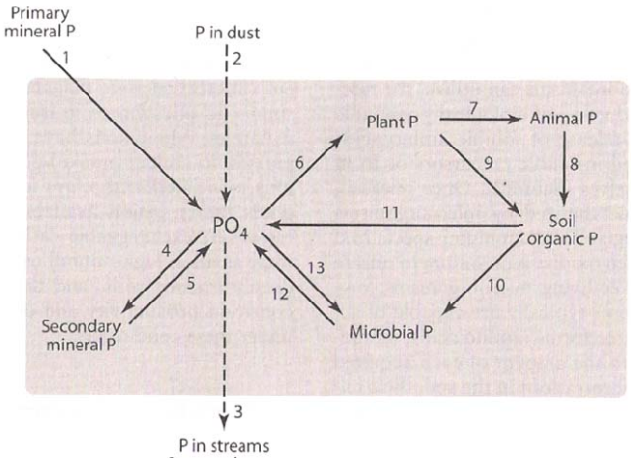


Figure 2: Pathways of phosphorous cycling terrestrial ecosystems. Although the phosphorous cycle lacks some of the biological complexity of the nitrogen cycle, it participates in a broader array of important geochemical reactions. Fluxes of phosphorous shown here include (1) weathering of primary minerals; (2) inputs of phosphorous from the atmosphere, mostly as dust; (3) leaching of phosphorous to streams and groundwater; (4) formation of secondary minerals within the soil; (5) weathering of secondary minerals; (6) plant uptake of phosphate; (7) consumption of plant phosphorus by animals; (8) flux of phosphorus to soil from excretion or animal death; (9) flux of phosphorus to soil in plant litter; (10) uptake of organic phosphorus by microbes that carry out decomposition; (11) release of phosphate from soil organic phosphorus through the action of extracellular enzymes; (12) release of phosphate by microbes; and (13) uptake of phosphate by microbes. Source figure and text from: Vitousek and Matson, 2009

Decomposition is an important process because it allows the dead organisms or plant litter[1] to be mineralized and become again available to plants (Wardle et al., 2003). 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). This recirculation of nutrients in the soil is basic to maintain the equilibrium in ecosystems, and in terrestrial ecosystems, litterfall represents the primary pathway for nutrient return to the soil (Karberg, Scott and Giardina, 2008).

The three main factors that control the decomposition rate are soil temperature, moisture content and litter quality[2]. The first two factors work in the same way: the higher is the temperature and humidity, the faster will be the breakdown of organic matter. However, there is an upper limit of temperature and moisture over which decomposition declines and, eventually, it can no longer take place. This happens because decomposition is mainly carried out by insects, worms, bacteria and fungi, either on the soil surface or in the soil, and they need intermediate conditions of temperature and moisture to function. The third factor is the quality of the litter. For the microbial decomposers different types of leaves may constitute very different substrates. Depending on its chemical composition, the leave breakdown will be faster (quality litter like nutrient–rich alder leaves) or slower (low quality litter like nutrient-

[1]Plant litter, leaf litter, tree litter, soil litter, or duff, is dead plant material, such as leaves, bark, needles, and twigs that have fallen to the ground. This detritus or dead organic material and its constituent nutrients are added to the top layer of soil, commonly known as the litter layer or O Horizon.

[2] Quality refers to characteristics of the litter like chemistry, physical attributes, etc. that influence the susceptibility of litter to decomposition.

poor conifer needles) (Karberg, Scott and Giardina, 2008). Different studies have shown that decomposition of leaf litter can be predicted by the C:N ratio (Taylor et al., 1989), by the lignin content (Meentemeyer, 1978), or by the lignin:nitrogen ratio (Melillo et al., 1982). 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).

During the initial stages of litter breakdown, 0-3 months, there is a loss of small soluble carbon molecules, like starches and amino acids, due to their easiness to break down and richer concentration of energy. After this first period, lignin, a large and complex molecule, will be decomposed. This results in a mass loss curve that resembles an exponential decay curve, first an initial short and fast breakdown, followed by a longer period of slow decomposition (Berg and Staaf, 1980; McClaugherty and Berg, 1987).

1.1.1. Soil biota

Another factor that affects decomposition is the composition of soil biota. Estimating the decomposition rates without accounting for soil faunal activities in current measurements seriously reduces the likelihood that we are properly modelling decomposition (Prescott, 2005). It is increasingly being recognized that faunal community structure, especially the influence of earthworms, are a fourth important factor determining decomposition rates and interacting with temperature and moisture conditions (Bohlen et al., 1997; Dechaine et al., 2005). Where there is enough substrate, soil microbial activity increases exponentially with soil temperature, with microbial activity often doubling with a 10°C increase in temperature ($Q_{10} \approx 2$) (Kirschbaum, 1995). But soil moisture can limit microorganisms too. When there is an increase of the temperatures, soil moisture takes an important role in maintaining high rates of microbial activity (Peterjohn et al., 1994). So, if there is a period of time where temperature and precipitation increase, rates of fresh litter decomposition would increase as a consequence of it (Meentemeyer, 1978).

Soil Animals influence decomposition by fragmenting and transforming litter, grazing populations of bacteria and fungi, and altering soil structure (Chapin, Matson and Mooney, 2002). This soil biota and litter are involved in successive phases of colonization, exploitation and exhaustion of the organic substrate during decomposition (Crossley and Witkamp, 1964; Crossley, 1970; Wallwork, 1970). Soil fauna can be divided in three groups according to body size: *microfauna* is made up by animals smaller than 0.1 mm, and they are important as predators of other soil fauna, *mesofauna* are soil organisms that fragment litter and their size is between 0.1 and 2 mm, and *macrofauna* are the large soil animals, bigger than 2 mm, that can act as ecosystem engineers.

Of the previous three groups, the mesofauna is the one which normally has more effect on decomposition, because they fragment and ingest litter coated with microbial biomass, what will produce fecal material that has greater surface area and moisture-holding capacity than the original litter (Lavelle et al., 1997). This leads to a more favourable environment for decomposition. Microorganisms condition the litter which the mesofauna organisms selectively will use as food. Collembola and mites (Acari) are generally dominant, numerically

and in terms of biomass, in the mesofauna group. Collembola are small insects that don't feed directly on litter, but rather on the soil microflora (primarily on fungi), whereas mites, a more diverse group of spiderlike animals, consume decomposing litter or feed on bacteria and/or fungi (Chapin, Matson and Mooney, 2002).

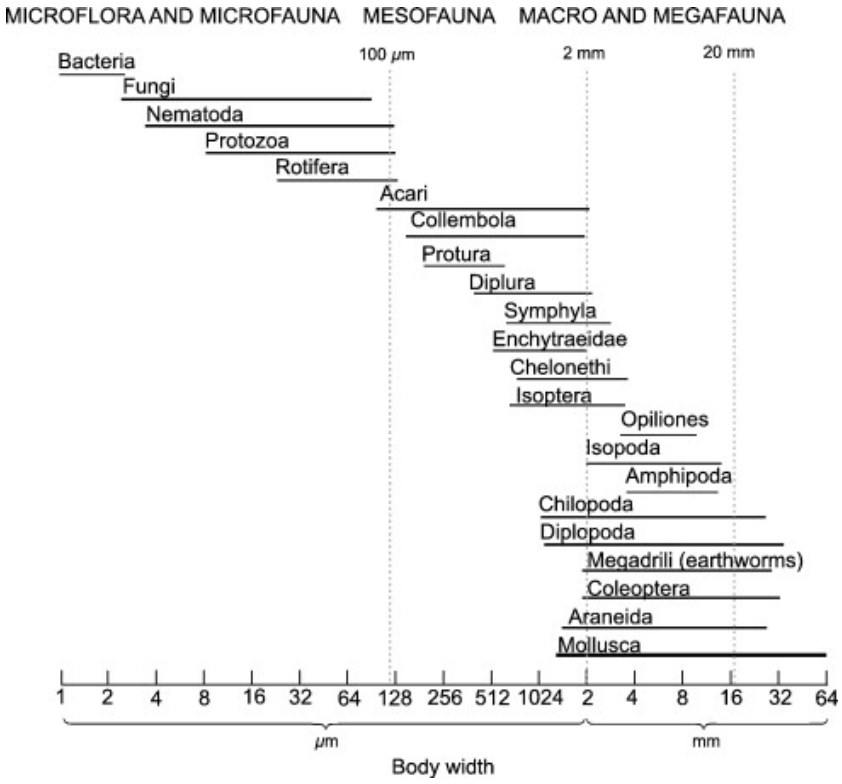


Figure 3: Representative types and sizes of soil fauna. Source: Swift et al., 1979

1.2. Global environmental change and its impacts on nutrient cycling

In addition to natural factors, humans with their activities have modified the natural development of nutrient cycles and decomposition. The growth of fossil fuels use during the last centuries has released large quantities of nitrogen and sulphur oxides to the atmosphere and has increased their inputs to ecosystems. The use of fertilizer and the cultivation of nitrogen-fixing crops to increase the crop production has increased over the last decades, and as a consequence of it the fluxes of nitrogen in agricultural ecosystems have also increased (Galloway et al., 1995, Vitousek et al., 1997a). Introduction of pesticides and other substances into the biosphere have altered the nitrogen cycle too (Cole, 1966).

Altering the natural background rate of nitrogen inputs may increase plant production enough to affect the global carbon cycle. All human disturbances such as harvest and fire can increase the amounts of nutrient pools that are available and therefore vulnerable to loss from ecosystems. Ways by which these losses can occur are by leaching of dissolved elements to groundwater, which can change soil chemistry (i.e. increasing the acidy of the soil), and increases in nutrient inputs to aquatic ecosystems. The properties of the atmosphere can be

influenced by the gaseous losses of nitrogen, and as a result of it cause air pollution and enhance the greenhouse effect (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, 1991).

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).

Currently there is strong evidence of the impact that our actions have on the natural world, for example, over the last 50 years anthropogenic activities have changed the natural ecosystems more rapidly and extensively than in any comparable period in human history. The ecosystems are the Earth's life-support systems for the human species and all other life forms. Disruption of their nutrient cycles, structure and biodiversity as a consequence of desertification, changes in chemical and physical soil structure, introduction of toxics or new species, etc. can impact on soil fertility, affecting agriculture and other lifestyles for a huge amount of people and affecting their health too. It is an important issue for the coming years to study how all these factors can affect ecosystems in order to improve their conservation and uses (Millennium Ecosystem Assessment, 2005).

1.3. Effects of pesticides

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.). Initially they were made from natural resources such as plants or insects (pyrethrin) and minerals (heavy metals), but the situation changed after the II World War, when humans achieved organic synthesis. With it appeared the possibility of synthesizing products with great pesticide power and the use of pesticides such as DDT expanded, which at first seemed to rid the world of all its plagues. Ideally, the use of pesticides has to be lethal to those target organisms that need to be controlled, and it should have no effect on the others or the environment. Unfortunately, this is not normally the case. 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).

There are many different types of pesticides, each meant to be effective against specific pests. The term "*-cide*" comes from the Latin word "to kill"; for examples, a product that kills insects is an insecti*cide*. Generally pesticides are chemicals or biological agents (biopesticides) that deter, incapacitate, kill or otherwise discourage pests, and they can be used in three different

forms: solid, liquid or aerosol. Some chemical pesticides are organophosphate pesticides, carbamate pesticides, oranochlorine insecticides and phyretroid pesticides (U.S. environmental protection agency, 2012).

Pesticides can have direct and indirect benefits. Direct benefits could be the protection of crop losses by having a control on the weeds, or reducing the pests that can affect the crops. For public health, insecticides are often used to control the insects that cause deadly diseases such as malaria, which results in an estimated 5000 deaths each day (Ross, 2005). Also pesticides have a benefit in transports, sport complexes (protecting the turf of golf fields for example) and protecting the materials of which buildings are made, like wood from the termites. An indirect benefit can arise as a consequence of the economic gains due the use of pesticides on the crops; with the use of the pesticides a farmer may have more production or less losses, and with the resulting benefits it may be possible to improve his quality of life. For example, the percentage of children with scholarship in non developed countries can grow if the benefits of the higher production are used to hire other people and children can go to the school (Aktar, Sengupta and Chowdhury 2009).

Hazards caused by pesticides, as stated before, have serious implications on the health of humans and other life forms and on the environment. Rachel Carson wrote in 1962 *Silent Spring*, where she documented detrimental effects of pesticides on the environment, particularly on birds, and played a large role in strengthening the regulations of chemical pesticides. An example of what she described was observed in US when bird populations decreased primarily due the eggshell thinning, caused by the exposure to DDT (Liroff, 2000). Nowadays, the evidence is accumulating that some of these chemical compounds pose a potential risk to life forms and may have unexpected effects on the environment (Forget, 1993). No one can be totally protected against exposure to pesticides. Within a society, there will be groups with higher risk than others depending on their economic level, profession[3], age, country, etc. (WHO, 1990). According to Environews Forum (1999) there was about 1 million world-wide deaths and chronic diseases due to pesticide poisoning in a year. OC (Organochlorine) compounds are dangerous because they can pollute almost every life form on Earth just for being in its environment. If there is a lake that has OC compounds, they will be found in the fishes that live on it, and then on the birds that feed on them (Hurley et al., 1998). Some environmental chemicals, including pesticides known as endocrine disruptors, cause adverse effects by mimicking or antagonising natural hormones in the body and it has been postulated that their long-term, low-dose exposure is increasingly linked to human

[3] The high risk groups exposed to pesticides include production workers, formulators, sprayers, mixers, loaders and agricultural farm workers. During manufacture and formulation, the possibility of hazards may be higher because the processes involved are not risk free. In industrial settings, workers are at increased risk since they handle various toxic chemicals including pesticides, raw materials, toxic solvents and inert carriers(Aktar, Sengupta and Chowdhury, 2009).

health effects such as immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer (Brouwer *et al*., 1999; Crisp *et al*., 1998; Hurley *et al*., 1998).

1.3.1. Effects of pesticides in the soil

Pesticides can pollute soil, water, and vegetation. In addition to killing weeds or insects, pesticides can be toxic to a host of other organisms including birds, beneficial insects, fish and non-target plants, and other wildlife. Insecticides are generally the more toxic class of pesticides, but herbicides pose risks to non-target organism too.

Not all the pesticides and their transformation products (TPs) have been monitored in soil, so there is still missing information about how these compounds interact in it and in other environments. Thanks to the few studies done, we know that persistency and movement of these pesticides and their TPs are determined by some parameters, such as water solubility, soil-sorption constant (K_{oc}), the octanol/water partition coefficient (K_{ow}), and half-life in soil (DT_{50}). Depending on the properties and structure of the pesticide and its TPs, they will have a determined period of life and movement around the environment and life forms. The long persistence of some of these chemicals explains why nowadays the residues of pesticides that have been banned for their contamination characteristics are still present in the environment.

The organic matter content is the most influential soil characteristic, as the larger is the organic matter content, the higher will be the adsorption of pesticides and TPs. The capacity of the soil to hold positively charged ions an exchangeable form is important in pesticides that are positively charged. For extracting these chemicals strong mineral acid will be required. The pH is also important because adsorption increases with decreasing soil pH for ionisable pesticides (Andreu and Picó, 2004). Soil fertility can be also altered by the heavy use of pesticides, causing a decline of beneficial soil microorganisms and carry over effects on plants (Pell *et* al., 1998). Also fungi can be damaged by herbicides in the soil.

Although herbicides are becoming more specific for each plant or situation, pesticide exposure can cause sublethal effects on other plants. Some herbicides can injure plants, trees or shrubs, can reduce seed quality (Locke *et al*., 1995) and increase the susceptibility of certain plants to disease (Brammall and Higgins, 1998).

1.4. Effects of alien invasive species

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.

Invasive alien species[4] (IAS) are non-native species that are deliberately or unintentionally introduced by human action outside their natural habitats where they establish, proliferate and spread in ways that cause damage to biological diversity.[5]

The National Invasive Species Management Plan (NISMP, USA), defines the term *invasive species* as "a species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic or environmental harm or harm to human health."

NOAA (National Oceanic and Atmospheric Administration) defined *alien species* as "any species, including its seeds, eggs, spores, or other biological material capable of propagating that species, that is not native" to the particular ecosystem in which it is found. The terms exotic and non-native are both synonyms for nonindigenous[6].

The characteristics that make a plant species a successful invader are the reproduction cycle, the dispersal mechanism and its physiology (Huston, 1994; Londsdale, 1999; Mack and others, 2000; Durand and Goldstein, 2001). The invasibility of a community will depend on the competition and predation relationships of the organisms, the possible disturbance processes, and its composition (Mack and D'Antonio, 1998; Levine and D'Antonio, 1999; Davis and others, 2000). The consequences of exotic invasions are changes in the community composition and in the abundance of native species, and in disturbance regimes (Cox, 1999). However, there are less obvious changes as a consequence of exotic invasions such changes below ground in the soil (Ehrenfeld and Scott, 2001), which would influence the invasibility of the ecosystem and the invasiveness of the species.

One of the ways in which alien species influence ecosystem processes is by altering the soil nutrient dynamics. This happens as a consequence of the alien being different to the native species in terms of biomass and productivity, tissue chemistry, plant morphology and phenology[7]. 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).

It has been shown that individual species can affect a variety of components of the carbon and nutrient cycles, net primary productivity, plant growth rates, chemical quality, and litterfall and

[4] means an alien species whose introduction and/or spread threatens biological diversity.
[5] European Union meeting on 22 June 2007 related to general IAS policy.
[6] So nonindigenous = alien = exotic = non-native.
[7] Is the study of periodic plant and animal life cycle events, and how these are influenced by seasonal and interannual variations in climate, as well as habitat factors (such as elevation).

C mineralization rates (Tilman and others, 1997; Hooper and Vitousek, 1998; van Breeman, 1998; Hector and others, 1999; Chapin and others, 2000). So when there is change in the species composition of a community, the nutrient cycles and other natural processes are likely to be affected. It has to be added that altering the flora will provoke changes in the physical properties of the soil, and this alters nutrient dynamics (Boettcher and Kalisz, 1989; Finzi and others, 1998: Kelly and others, 1998; Ehrenfeld 2001). Changes in the patterns of species dominance within the plant community can also have important effects, since in an ecosystem the relative abundance of a species can modulate community processes (Grime, 1998). Finally, changes in plant functional types can cause alterations in the distribution and dynamics of soil nutrients (Gill and Burke, 1999).

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).

1.5. Background

The control of pesticides in Australia is shared by the Commonwealth[8] and the States and Territories through the National Registration Scheme for Agricultural and Veterinary Chemicals. The primary role of the States and Territories is to regulate the sale, handling, use, and disposal of pesticides within the framework provided by the National Operating Principles (of Australia's Agricultural and Veterinary Chemical Management System).

The responsible for the regulation of pesticides from their manufacture or importation into Australia, from their sale until the end of they use, is a Commonwealth agency called the Australian Pesticides and Veterinary Medicines Authority (APVMA).

The legislation includes the Commonwealth Agricultural and Veterinary Chemicals Code Act 1994, which is applied in Western Australia through the Agricultural and Veterinary Chemicals (Western Australia) Act 1995.

In Western Australia, the Department of Conservation and Land Management (CALM) wrote in 1992 the environmental monitoring of pesticides[9] (policy statement nº 45), where the main objective was to monitor the use of pesticides in CALM or adjacent land, so that future consequential practice would ensure minimal contamination of the environment.

In Western Australia the use of pesticides is administered under the following Acts and Regulations:

[8] Australia is officially called the Commonwealth of Australia.
[9] Pesticides includes herbicides, insecticides and fungicides.

1. Health (Pesticides) Regulations, last one was passed on the first of February of 2011.
2. Restricted Spraying Regulations (APB[10] Act)
3. Aerial Spraying Control Act
4. Agricultural Produce (Chemical Residues) Act
5. Poisons Act

Use of insecticides and herbicides by CALM is done where there are benefits in cost and efficiency for the eradication of pests and weeds in conservation and silvicultural projects and where no other effective measures are available. Furthermore, it is a routine practice by CALM to monitor for insecticide and herbicide residues in stream or river water and soil in areas where they have been used. CALM Insect Manual is a helpful guideline for monitoring insecticide residues. In addition, a subcommittee of the Health Department's Pesticides Advisory Committee (PAC) has been established to coordinate pesticide monitoring in the environment.

Invasive species have an important impact on Australia's environment, threatening their unique biodiversity and reducing overall species abundance and diversity. For the Australian Government an invasive species include: diseases, fungi and parasites, feral animals, insects and other invertebrates, introduced marine pests and weeds. Australia has one of the most important environmental biosecurtiy[11] in the world and it occurs across the entire biosecurity continuum: pre-border preparedness, border protection and post-border management and control. The main objective of their biosecurity system is protect their unique natural landscapes and native flora and fauna. Also protect the ecosystems services they provide and the quality of life of all life forms.

The Department of Sustainability, Environment, Water, Population and Communities is who administers the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) that is the central piece of environmental legislation. It provides a legal framework to protect and manage nationally and internationally the flora, fauna, ecological communities and heritage places (defined in the EPBC Act as matters of national environmental significance). The main aims of the EPBC Act are conserve the Australian biodiversity, protect the biodiversity internationally by controlling the international movement of wildlife, provide a streamlined environmental assessment and approvals process where matters of national environmental significance are involved, protect their world and national heritage, and

[10] Agriculture Protection Board was a statutory authority to minimise the impact of Declared Plants and Animals on agriculture and related resources. This function contributed to the State Government's strategic goal of ensuring that economic activity is managed in a socially and environmentally responsible manner for the long-term benefit of the State, and to its desired outcome of protecting the productive resource base. APB Act 1950 was abolished and prescribed the Board's role and responsibilities to the Agriculture and Related Resources Protection Act (ARRPA) 1976 on 18 December of 2010.

[11] Environmental biosecurity is the protection of the environment and social amenity from the negative effects associated with invasive species; including weeds, pests and diseases.

promote ecologically sustainable development. Also the EPBC provides information about how to deal with the invasive species, including:

- List key threatening processes. These processes threaten the survival, abundance or evolutionary development of a native species or ecological community.
- Develop and implement threat abatement plants (TAPs), where research, management and other necessary actions are taken to reduce the impacts of a listed key threatening process on affected listed threatened species and ecological communities.
- Recovery plans: for threatened fauna, flora and ecological communities.

Australia, with its large size, is fortunate to have a large variety of different ecosystems and communities, which makes the protection from invasive species an important issue for the government and population of the country to maintain their biodiversity and natural landscapes. The impact of pesticides should also be kept in mind for protecting biodiversity, as they can cause changes in the composition of a community, by benefitting native or invasive species.

1.6. Objectives and hypotheses of the study

The main objective of this study is to assess the effect of herbicides and alien species on litter decomposition rates and the diversity of soil biota. This will be explored at two different sites: Ridgefied, a former cropland used for cropping and grazing and currently a research site belonging to the University of Western Australia; and Nalya, a natural reserve. Different factors and treatments will be tested, including litter type (native or invasive plants), use of herbicides, mesh size of litterbags and community type (native or invasive). Specifically, the aim is to test the following hypotheses:

(1) Decomposition rate will 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), and as a consequence are expected to decompose faster and release more nitrogen to the soil than native species. When nitrogen goes back to the soil pool in invaded areas through the invasive litter, nitrogen availability at the soil surface may increase, causing the increase of nutrient cycling rates. This implies an overall increase in the rate of decomposition (Vitousek and Walker, 1989; Witkowsky, 1991).

(2) Higher decomposition rate will be associated with higher soil biodiversity and herbicides will reduce both decomposition and soil biodiversity. It has been stated that soil-fauna diversity can significantly influence carbon and nutrient cycles, which will influence the decomposition rate. However, no general or predictable pattern has emerged (Hättenschwiler, Tiunov and Scheu, 2005).

(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. Where mesh size is big enough to allow macrofauna

communities, decomposition increases significantly. Previous studies indicate that the presence of meso- and macro-fauna increased litter decomposition rate (Bradford et al., 2002).

2. MATERIALS AND METHODS

2.1. Site description 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[12]. However, both locations have different backgrounds.

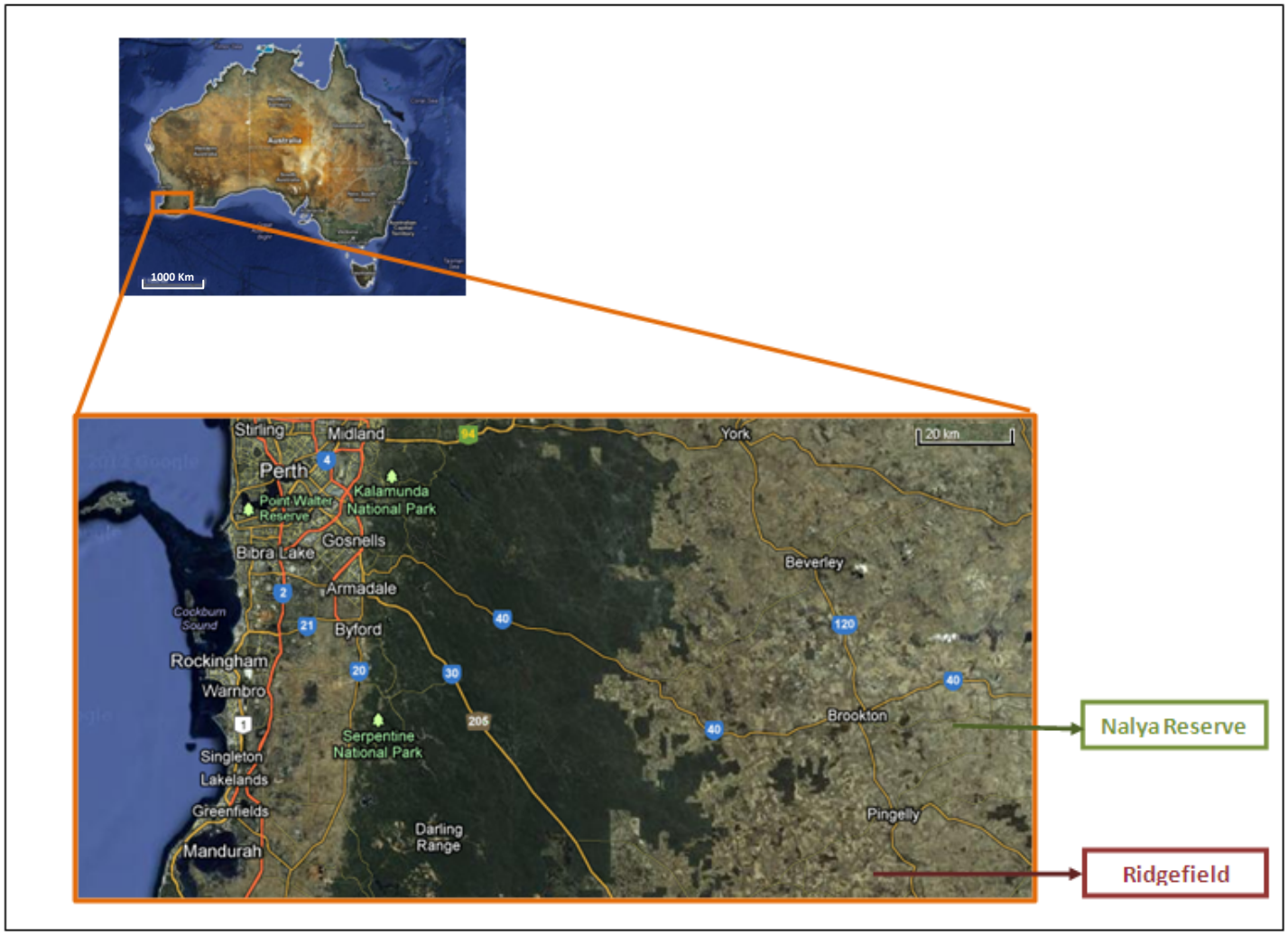


Figure 4: Study site locations. Source: Google Maps.

Nalya (32°22'S 117°12'E) is a natural reserve, where no human activities have been carried out for a long period of time. It is located in the Shire of Brookton, its average elevation is approximately 256m and the yearly average rainfall is 458.2mm. The hottest month is January (summer), with a maximum average of 33°C, and the coldest month is August (winter), with a minimum average of 4.6°C. Nalya reserve has different plant communities, some of them with

[12]From Bureau of Meteorology, 2012

invasive species like *Avena sp*. and *Erodium sp*., others just with native species like *Eucalyptus loxophleb,* shrubs of *Bankisa sessilis* and *Acacia acuminate*.

Ridgefield (32°29'S 116°58'E) was used for cropping and grazing, but nowadays is used by the University of Western Australia as a research site. It is found in the Shire of Pingelly with an average elevation of 350m and an annual rainfall of 445.1mm. In January the maximum daily average temperature is 31.8◦C and in August the minimum daily average is 5.6 ◦C. The plant community is constituted of native species: two tree species (*E. loxophleba* ssp *loxophleba* and *E. astringens*) and six shrub species (*Acacia acuminata*, *A. microbotrya*, *Banksia sessilis, Hakea lissocarpha*, *Calothamnus quadrifidus* and *Callistemon phoeniceus*).

Soil composition is slightly different at both sides. Ridgefield soil has more nitrogen and phosphorous due to prior use of fertilizers in the area for cropping, and the prior grazing activities. The slope in the two areas is nearly zero.

In both regions four blocks were delimited, each with two plots corresponding to the main treatments (see below). The dimensions of each plot within the blocks were 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[13] to prevent the growth up of non-native weed cover: herbicide (H) or control (C) plots.

Pictures nº 1 & 2: Two of the litterbags with their tag numbers, left one with large mesh, and right one with small mesh. Source: Mariona Isern Subich

2.2. Placement and harvest litterbags

Different size of mesh was used to optimize or minimize the access of different soil organisms into the bags. Two mesh sizes were used, a large one of 7 mm and a small one of 1 mm. The

[13] 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. Also Summer-active non-natives were removed with a spot application of glyphosate herbicide.

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.

Litterbags were placed in the soil ground randomly in two different moments. First bag placement was the 4th and 6th of November 2011, when 64 litterbags were placed in Nalya Reserve and Ridgefield. The second placement date was the 8th of February of 2012, 3 months after the first placement, when 32 litterbags were placed in Nalya and Ridgefield. All bags are tagged with a unique identification number.

Numbers of bags allocated to each treatment are given in tables 1 and 2 below:

Placement date in Nalya Reserve	**Treatment (Type of ecosystem)**		**Mesh size**		**Leaves type**	
			Large(L)	Small (S)	Native(N)	Invasive(I)
4th- 6th November 2011 (64 litterbags)	**Native**	32	16	16	16	16
	Invasive	32	16	16	16	16
8th February 2012 (32 litterbags)	**Native**	16	8	8	8	8
	Invasive	16	8	8	8	8

Table nº1: Number of bags (sample size) by treatments in Nalya Reserve where Native or invasive ecosystem, and bags characteristics are Large or Small mesh and Native or Invasive leaves. Source: Mariona Isern Subich

Placement date in Ridgefield	**Treatment (Use or not of Herbicide)**		**Mesh size**		**Leaves type**	
			Large (L)	Small (S)	Native(N)	Invasive(I)
4th- 6th November 2011 (64 litterbags)	**Control (C)**	32	16	16	16	16
	Herbicide(H)	32	16	16	16	16
8th February 2012 (32 litterbags)	**Control (C)**	16	8	8	8	8
	Herbicide(H)	16	8	8	8	8

Table nº2: Number of bags (sample size) by treatment in Ridgefield was the use or not of Herbicide. If plot was treated with herbicide that plot was known as H, and if it wasn't treated was known as C. Bag characteristic were the same as in Nalya Reserve, Large or Small mesh and Native or Invasive leaves. Source: Mariona Isern Subich

Bags were created in the laboratory with the help of a two decimal scales and closed with staples. They were placed in big plastic boxes (60x45x45cm aprox.) and kept until they were

taken to be placed in the field. Litterbags were subjected in the soil 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. All processes, from preparing the litterbags until they were placed in the ground, were done with care that no litter could be removed from the bags, especially with the 7-8mm mesh size bags.

Removal of the bags was done at two times. The first harvest was done the 8th of February of 2012, the same day that where placed 32 new litter bags in both study sites, and the second harvest was done the 11th of May of 2012.

Picture nº3: A litterbag before being removed from the plot. Source: Mariona Isern Subich

When a litterbag was removed from the soil floor, it was taken out with care to avoid that any kind of invertebrates or litter could fell out of the bag. Once all litterbags were collected, they were introduced in a cloth bag and transported to the facilities of the university.

After the last harvest on May 2012, numbers of bags on both study sites was:

Placement date	Harvest date	Nº of litterbags harvest	Time in field (months)
4th-6th November 2011 (64 litterbags)	8th February 2012	32	3
	11th May 2012	32	6
8th February 2012 (32 litterbags)	11th May 2012	32	3

Table nº3: Number of bags placed and harvested at Nalya Reserve. In Nalya Reserve, 64 litterbags were 3 month in the field, and 32 during 6 months. Source: Mariona Isern Subich

Placement date	Harvest date	Nº of litterbags harvest	Time in field (months)
4th-6th November 2011 (64 litterbags)	8th February 2012	32	3
	11th May 2012	32	6
8th February 2012 (32 litterbags)	11th May 2012	32	3

Table nº 4: Number of bags placed and harvested at Ridgefield. In Ridgefield, 64 litterbags stood 3 month in field and 32 stood 6 months. Source: Mariona Isern Subich

Pictures nº4 & 5: Both pictures were taken in Ridgefield. The left one is a plot with herbicide treatment (H) and the right one without herbicide treatment (C). The pictures illustrate the large difference in weed abundance between treatments. Source: Mariona Isern Subich

2.3. Measuring invertebrate biodiversity

After harvesting the litterbags, all invertebrates were taken off of the bags using the Tullgren Funnels technique[14]. The bags were left inside the funnels during three days, and they were checked three times a day to verify that all bulbs were working. After three days, the specimen jars with 70% of ethanol solution were closed and tagged.

Pictures nº 6 & 7: In the left picture the funnels where litterbags were introduced for obtaining the invertebrates can be observed. The right picture shows the inside of the funnels, where bags stayed three days. Source: Mariona Isern Subich

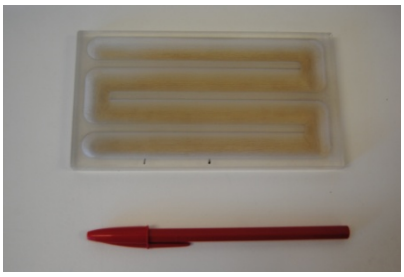
[14] This funnels are constituted of a bulb and under it there is the bag placement area. The bulb, with it warmth, make that invertebrates want to get away from it, and when they go out of the bag, by flying, walking or falling, they arrive to the end of the funnel, where there is a small plastic pot with a 70% ethanol solution, causing their dead.

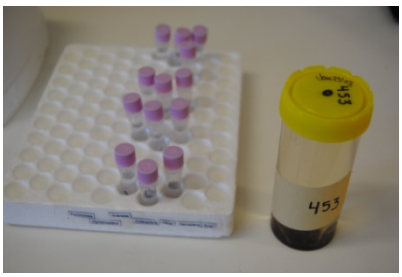
Litterbags were introduced in the funnels carefully and quickly. It had to be careful because when bags are introduced in the funnels, soil or litter can fall from them, and that would cause loses on the final litter weight and also make harder the sampling and identification of the invertebrates in the microscope due to the presence of soil in the sample. It had to be done quickly because from the moment we took out the litterbags form the cloth bag invertebrates could escape, decreasing the real numbers of found invertebrates. Also we had to check that there was no litter or invertebrates left in the cloth bag, so after placing the litterbag in the funnel, any litter left in the cloth bag was taken out and placed over the litter bag.

Once we had all invertebrates in the specimen jars, we brought them to the laboratory and before start sampling them, complete labels were introduced in the specimen jars, for checking that we had all samples and to have the information of them.

Picture nº8: These labels were introduced in every specimen jars. The first thing in the label was the study place, in this case Nalya Reserve, and then was a long line that synthesized the Litterbag characteristics: *LB* means LitterBag, *263* the LB tag number to identify it, *N03* is the plot number, *I* the treatment of the plot (in this case Invasive), the date when it was harvest, *LI* is the type of LB (in this case Large mesh and Invasive leaves) and finally the last number is the time in field (6 months). Source: Mariona Isern Subich

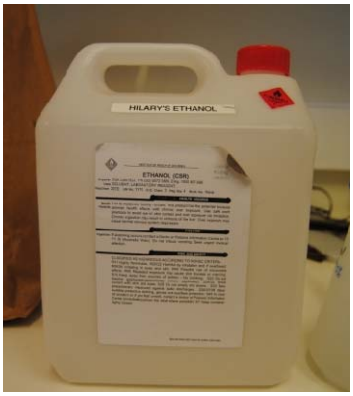
The material needed for sampling the invertebrates was a small brush (to take invertebrates out of the sample and classify them), a counter (to count *Collembola* or *Acari*, for its abundance of individuals), a plastic container (where we put the sample to be observed in the microscope), a microscope (Leica model TL4000), 70% ethanol solution (to clean the pots after using them) a funnel (to help us introduce the sample inside the small pot once invertebrates were counted and classified), small pots (where the invertebrates found in the sample were introduced) and a support placement for them.





Pictures nº 9, 10 & 11: From the left, this plastic container is where the sample was introduced to make the observation and collection of invertebrates easier, its shape helps to collect without forgetting any individual. In the middle picture there is the specimen jars where samples were stored and the small pots where invertebrates were placed after being sampled. Source: Mariona Isern Subich

All invertebrates found in the sample were collected and placed in three small pots, according to their order. The small pots were divided in *Coleoptera* (*Coleoptera larvae* or *Coleoptera adult*), *Aranae* (just spiders) and Others (all other invertebrates found in the sample). Once individuals were identified and introduced in the appropriate pot, they were recorded on a paper sheet. At the end of the sample this sheet had all invertebrates found classified by order.



Pictures nº 12 & 13: Left picture is the laboratory where samples were analysed and right picture is ethanol bottle from which ethanol 70% solution was made. Source: Mariona Isern Subich

2.4. Calculating the diversity of soil biota

A diversity index is a mathematical measure that considers the species richness (nº of species) and species abundance (nº of individuals per species). There are different diversity indices used in ecology, depending on the specific question being asked. A popular diversity index in the ecological literature is the Shannon diversity index. The Shannon index is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled. Shannon index is calculated as:

$$H = -\sum_{i=1}^{S} pi \cdot log_2\, p_i$$

p_i = is the proportion (n/N) of individuals of one particular species (n) divided by the total number of individuals found (N), S is the number of species and Σ is the sum of the values. H varies between the minimum value when H=0 (all individuals are from the same species) and the maximum value $H=\log_2(S)$ (when all individuals are distributed in the same proportion across the S species). High values of H would be representative of more diverse communities. So the H value allows us to know not only the number of species but how the abundance of the species is distributed among all the species in the community.

In this study, the Shannon index was calculated at the order level (e.g. Coleoptera, Aranae, Diptera, Hemiptera, etc.) using *Microsoft Office Excel*. The steps to calculate this index were:

1. Divide the number of individuals of orders found in the sample by the total number of individuals of all orders. This is p_i.
2. Multiply the fraction by its natural log ($p_i \cdot \log_2 p_i$).
3. This was done for all the different orders that we had.
4. Sum all the - ($p_i \cdot \log_2 p_i$) products to get the value of *H.*

2.5. Calculating the litter decomposition rate

After the invertebrates were taken off from the litterbags, they were introduced in the oven with care during two days at 40^{o}C to dry all bags. When this period of time passed, litterbags were brought to the laboratory to weight them again. Before weighting bags, a few pats were applied with care (in order not to loss litter) to remove any soil added to the bags. Once it was done, staples were taken out and bags opened. Litter was took out and put in a tray to be weighted in the scale (same precision than when bags were prepared) and after obtaining the data litter was put in paper bags to be kept in case we needed to check them again.

Once we had all data, the decomposition rate was estimated 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.

2.6. Statistical methods

Mixed linear models were used 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

3.1. Decomposition rates

	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 (month)	-0.085	0.025	-3.350	0.001

Table nº 5: Model results relating the decomposition rate with bag characteristics, treatments and time, obtained by the lineal mixed-effects model in Nalya Reserve. Source: Mariona Isern Subich

The previous table shows that in Nalya Reserve 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. Once the effect of time was accounted for, decomposition rate was influenced by treatment, with lower decomposition rates in native communities; mesh size, with lower decomposition in bags with narrower mesh; and leaf type, with lower decomposition rates for native leaves. This latter variable was the one with a stronger effect on decomposition.

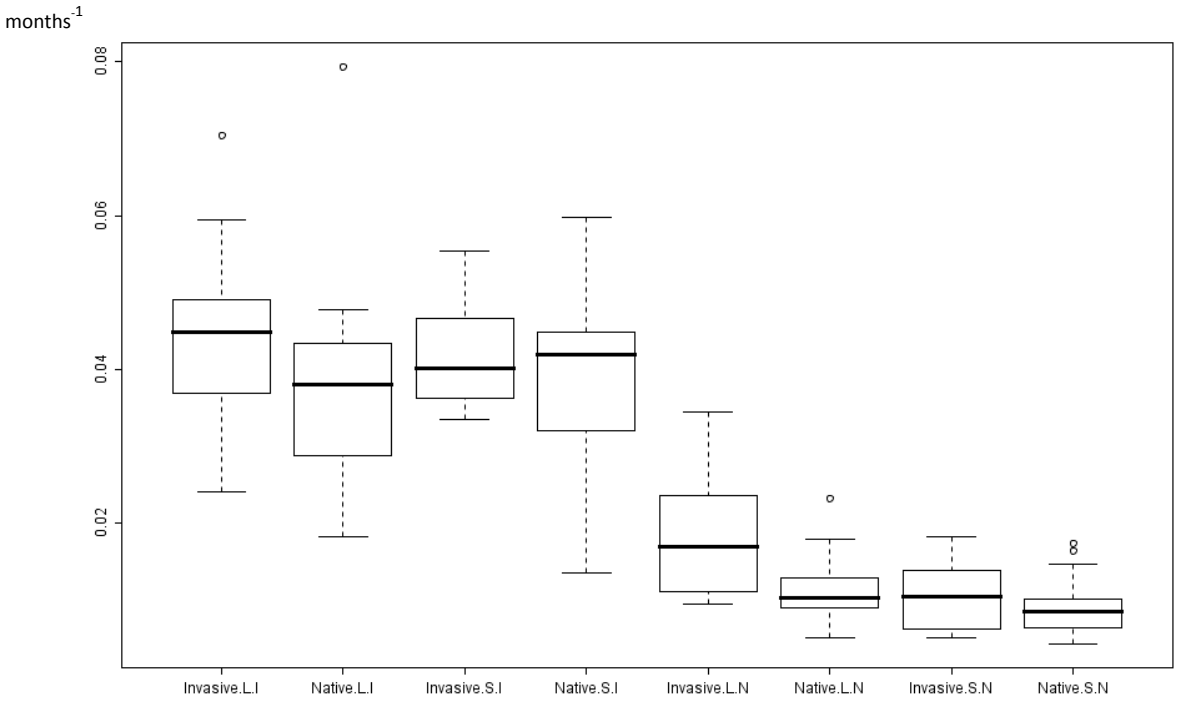


Figure nº5: Box plots showing the relationship between decomposition rate and community type (Invasive or Native), litterbag mesh size (S=Small or L=Large) and leaf type (I=Invasive or N=Native) in Nalya Reserve (see also Table 5). Source: Mariona Isern Subich

In Ridgefield, time spent in the field had again a significant negative effect on decomposition rate (Table 6). Similarly to Nalya Reserve, small mesh size and native leaf type were associated to lower decomposition rates (Table 6). Finally, the herbicide treatment resulted also in lower decomposition rates, although the effect was much lower than that of leaf type (Table 6).

	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 (month)	-0.092	0.024	-3.790	0.0003

Table nº6: Results obtained by the lineal mixed-effects model related the decomposition rate with the two treatments (herbicide and control), mesh size, leaf type and time in Ridgefield. Source: Mariona Isern Subich

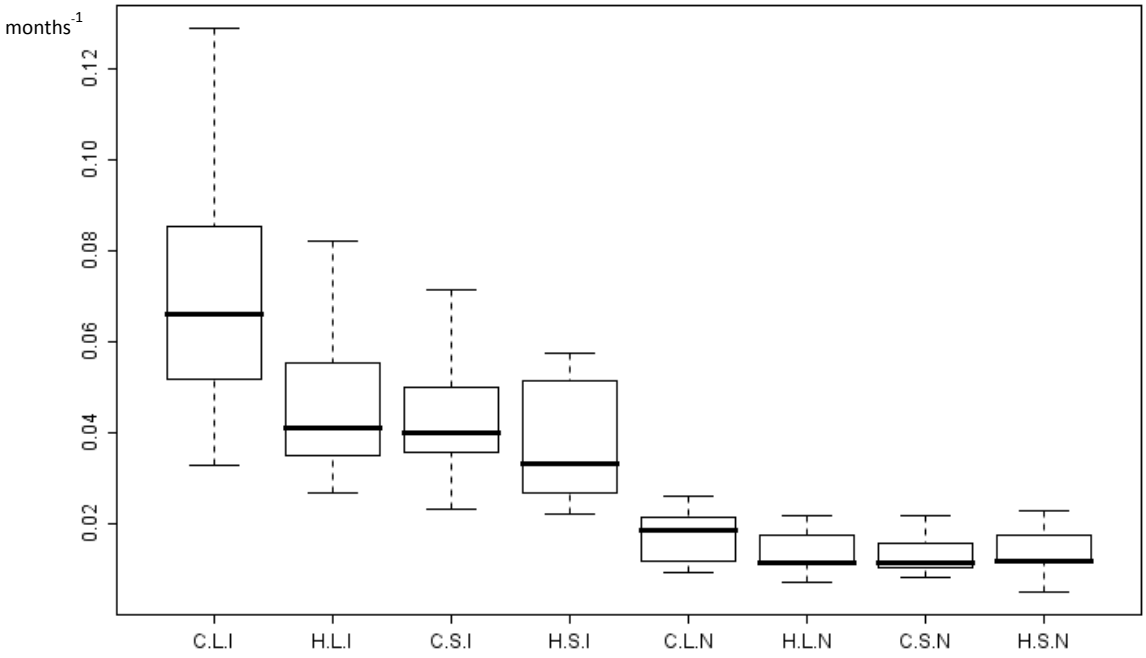


Figure nº6: Box plots showing the relationship between decomposition rate and plots where Herbicide was used (H) or not (C), litterbag mesh size (S=Small or L=Large) and leaf type (I=Invasive or N=Native) in Nalya Reserve (see also Table 6). Source: Mariona Isern Subich

3.2. Soil biodiversity

	Value	Std. Error	t-value	p-value
Intercept (Invasive treatment, Large mesh size and Invasive leaf type)	0.776	0.159	4.854	0.000
Native treatment	0.148	0. 090	1.625	0.108
Small mesh size	0.067	0.092	0.729	0.468
Native leaf type	0.0213	0.090	0.236	0.814
Time	0.0137	0.032	0.426	0.671

Table nº7: Model results relating the Shannon diversity index with treatment (native or invasive ecosystem), litterbags mesh size and leaf type and the time litterbags were left in the field (3 or 6 months), obtained for Nayla. Source: Mariona Isern Subich

The previous table shows that in Nalya Reserve Shannon Weaver index was unrelated to any of the studied variables (see also Figure 7). In Ridgefield, the results were similar (Table 8 and Figure 8), and we only observed a marginal effect of the time the samples spent in the field, with slightly higher diversity for samples that spent more time in the field.

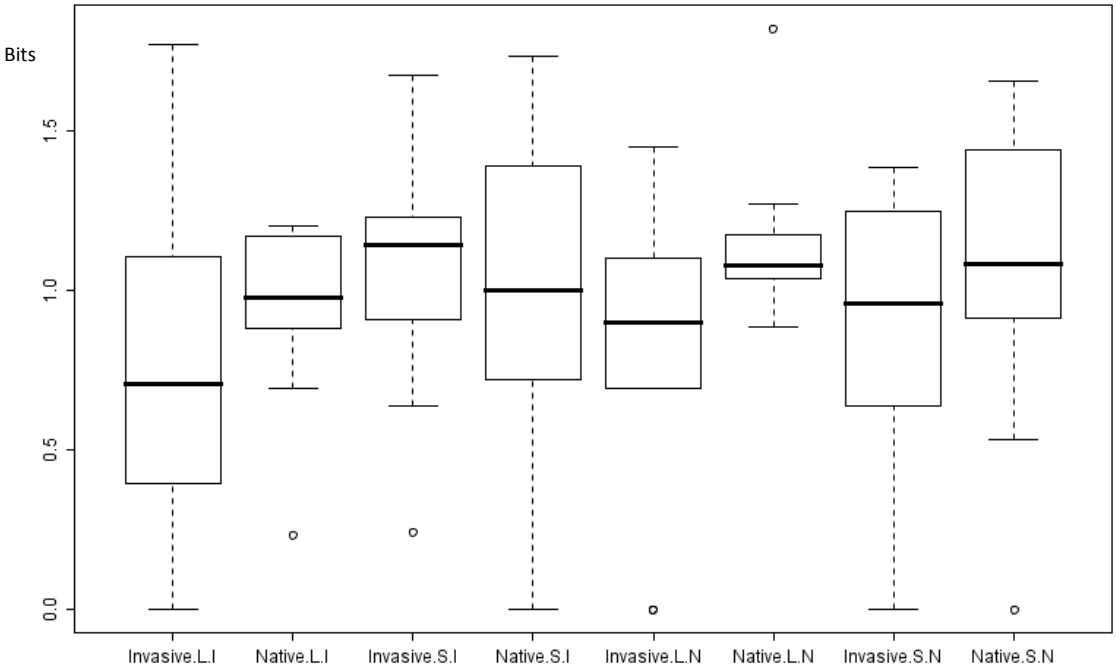


Figure nº7: Box plots showing the relationship between soil biodiversity and community type (Invasive or Native), litterbag mesh size (S=Small or L=Large) and leaf type (I=Invasive or N=Native) in Nalya Reserve (see also Table 7). Source: Mariona Isern Subich

	Value	Std. Error	t-value	p-value
Intercept (Control treatment, Large mesh size and Invasive leaf type)	0.543	0.165	3.296	0.001
Herbicide treatment	-0.141	0.095	-1.488	0.140
Small mesh size	0.034	0.095	0.356	0.723
Native leaf type	0.020	0.095	0.207	0.836
Time	0.060	0.034	1.799	0.076

Table nº8: Model results relating the Shannon diversity index with treatment (herbicide or control), litterbags mesh size and leaf type and the time litterbags were left in the field (3 or 6 months), obtained for Ridgefield. Source: Mariona Isern Subich

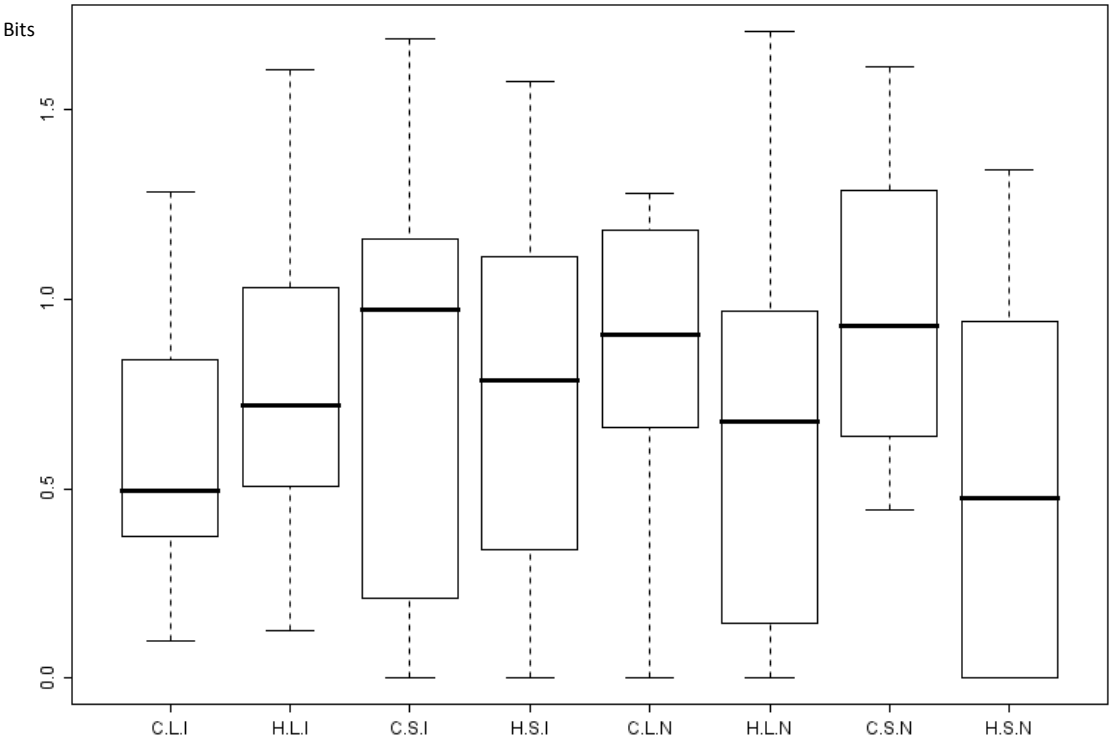


Figure nº8: Box plots showing the relationship between Shannon Weaver index and plots where Herbicide was used (H) or not (C), litterbag mesh size (S=Small or L=Large) and leaf type (I=Invasive or N=Native) in Ridgefield (see also Table 6). Source: Mariona Isern Subich

4. DISCUSSION

4.1. Temporal effects on decomposition rate and differences between sites

In both study sites decreasing decomposition rates were observed during the time of the study. These results are to be expected if we consider that two of the main factors that control the decomposition rate are soil temperature and moisture. The results show that those litterbags that spent three months in the field, from November until February, had higher decomposition rates. Spring in Western Australia takes place from September till November, and it is a wet and medium-high temperatures season, what makes a perfect environment for the decomposers. Those litterbags that spent from February to May in the field, had lower decomposition rates probably because environment conditions were not the best for carrying out decomposition. This is because from December till February is summer, with high temperatures and almost no rainfall and low moisture content, and from March till May is autumn, with more rainfall and moisture but lower temperatures. 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).

Differences were found between the decomposition rates in Nalya Reserve and in Ridgefield, where they are higher. This difference is believed to be a consequence of land-use history. Ridgefield has higher concentrations of nitrogen and phosphorus in its soil for being a crop and grazing area before being a research site for the UWA (Perring, M.P. et al., 2012), whereas Nalya have not been affected by any relevant human activity for the last 50 years. As a result, Ridgefield has more resources available in the soil, which benefits the organisms that carry out decomposition. 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). The results are in agreement with this view, implying that higher or lower concentrations of nitrogen in the soil will influence in the decomposition rates.

4.2. Effects of mesh size on decomposition.

Consistent with the initial hypothesis, decomposition rates were higher in large mesh size bags than in smaller ones. 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. Climate, leaf litter chemistry and leaf litter decomposition conforms a triangular relationship where each factor is involved in the final litter loss (Aerts R., 1997). 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 constants (Allison S. D. and Vitousek P. M, 2004). This 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's 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 the initial hypothesis.

4.5. Effects of herbicide use on decomposition

Also in agreement with the inital 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, any 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 had been used had less weed abundance, and some of them had no vegetation (pictures nº 4 and 5), which could cause a change of the microclimate of the litterbags. Decomposition rates are strongly correlated with the soil moisture and temperature (Shaw M. R. and Harte J., 2001). Therefore, the lack of weeds surrounding the litterbags could causes changes in their microclimate, and those bags that are in herbicide plots would be more expose and have different values of temperature and moisture, which interacted with the litter break down. Those bags placed in control plots had weeds and other plants surrounding them, likely keeping moisture in the litterbags and buffering temperature fluctuations at the soil, which resulted in nearly 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. CONCLUSIONS

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 in litterbags placed in plots were herbicide was used. 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.

6. LITERATURE CITED

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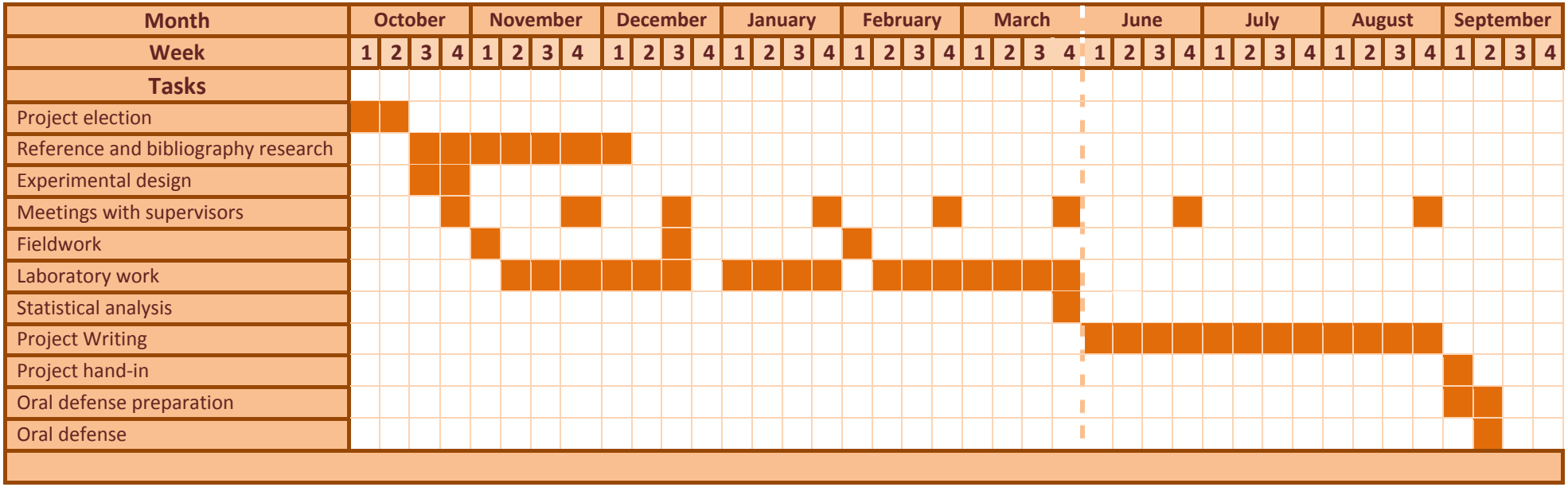
7. BUDGET

DIRECT EXPENSES			
HUMAN RESOURCES			
Research and Project elaboration			
Item			Subtotal (hours)
Experimental design and reference research			300
Meetings with Supervisors			20
Field Work			40
Laboratory Work			500
Statistical analysis			30
Writing			400
		Total (hours)	1290
			Subtotal (€)
		Cost= 15€/hour	**19.350€**
Travelling			
Item			Distance(Km)
3 Meetings with the UAB Supervisor (Bellaterra)			125
3 Field trips			1.000
1 Overseas Trip			1.300€ (not accounted in distance)
		Total distance (Km)	1.125
			Subtotal (€)
		Cost (fuel price) = 0,19€/Km	**1.514€**
		TOTAL HUMAN RESOURCES	**20.864€**
MATERIAL RESOURCES			
Item	Qty(€)	Item/price (€)	Subtotal (€)
Printing	52	0,04	2,08
Copies	3	2,08	6,24
CD copies	4	1	4
Binding	3	2,5	7,5
		TOTAL MATERIAL RESOURCES	**20€**
		TOTAL DIRECT EXPENSES	**20.884€**

INDIRECT EXPENSES	
Equipments and facilities	**Total (€)**
20% of the direct expenses	**4.177€**

Total direct expenses	**20.884€**
Total indirect expenses	**4.177€**
Basic Budget	**25.061€**
IVA (VAT) (21%)	**5.263€**
TOTAL BUDGET	**30.324€**

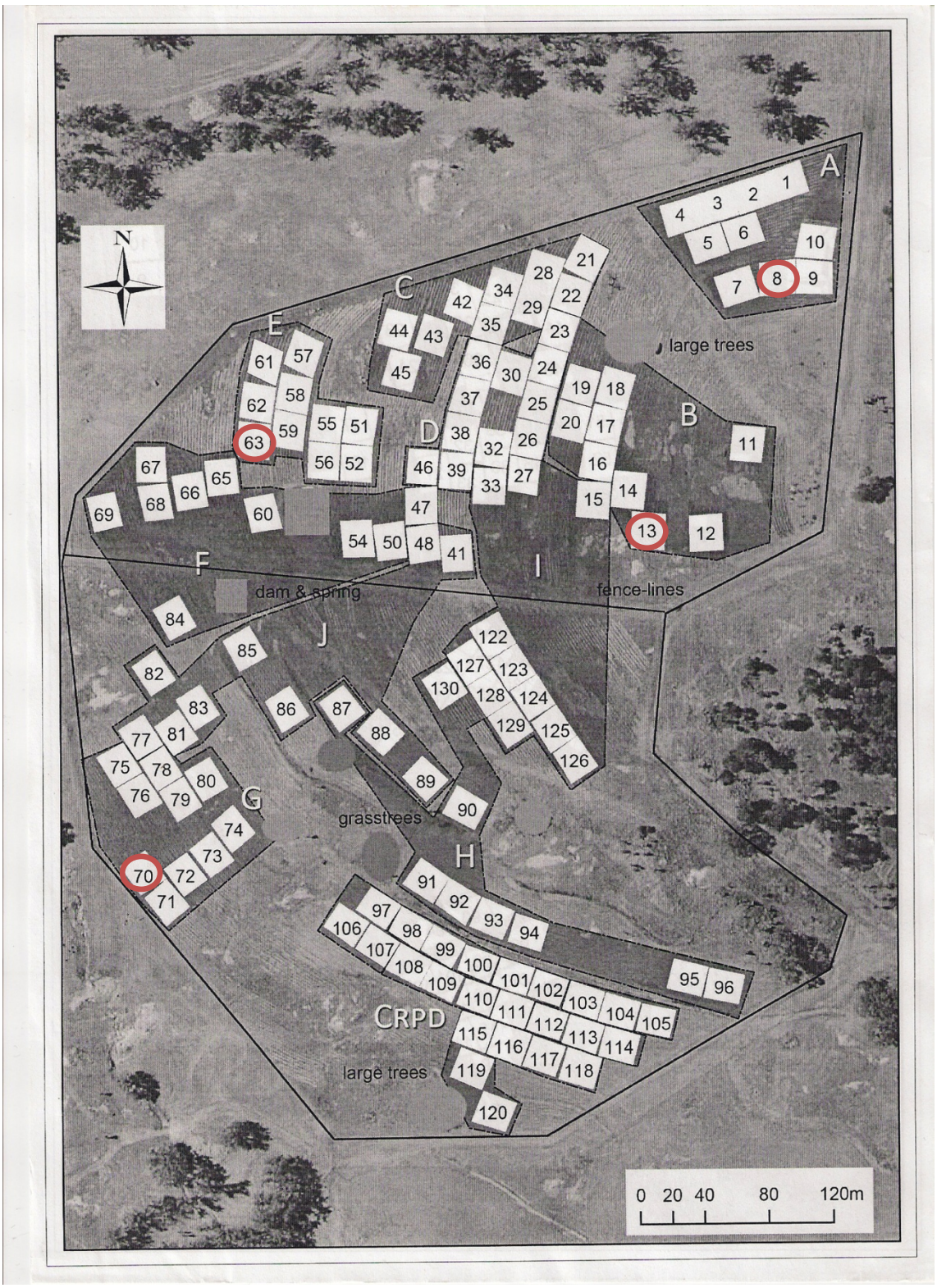
8. TIMELINE


Month
October
November
December
January
February
March
June
July
August
September
Week
1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4
Tasks
Project election
Reference and bibliography research
Experimental design
Meetings with supervisors
Fieldwork
Laboratory work
Statistical analysis
Project Writing
Project hand-in
Oral defense preparation
Oral defense

9. ANNEXES

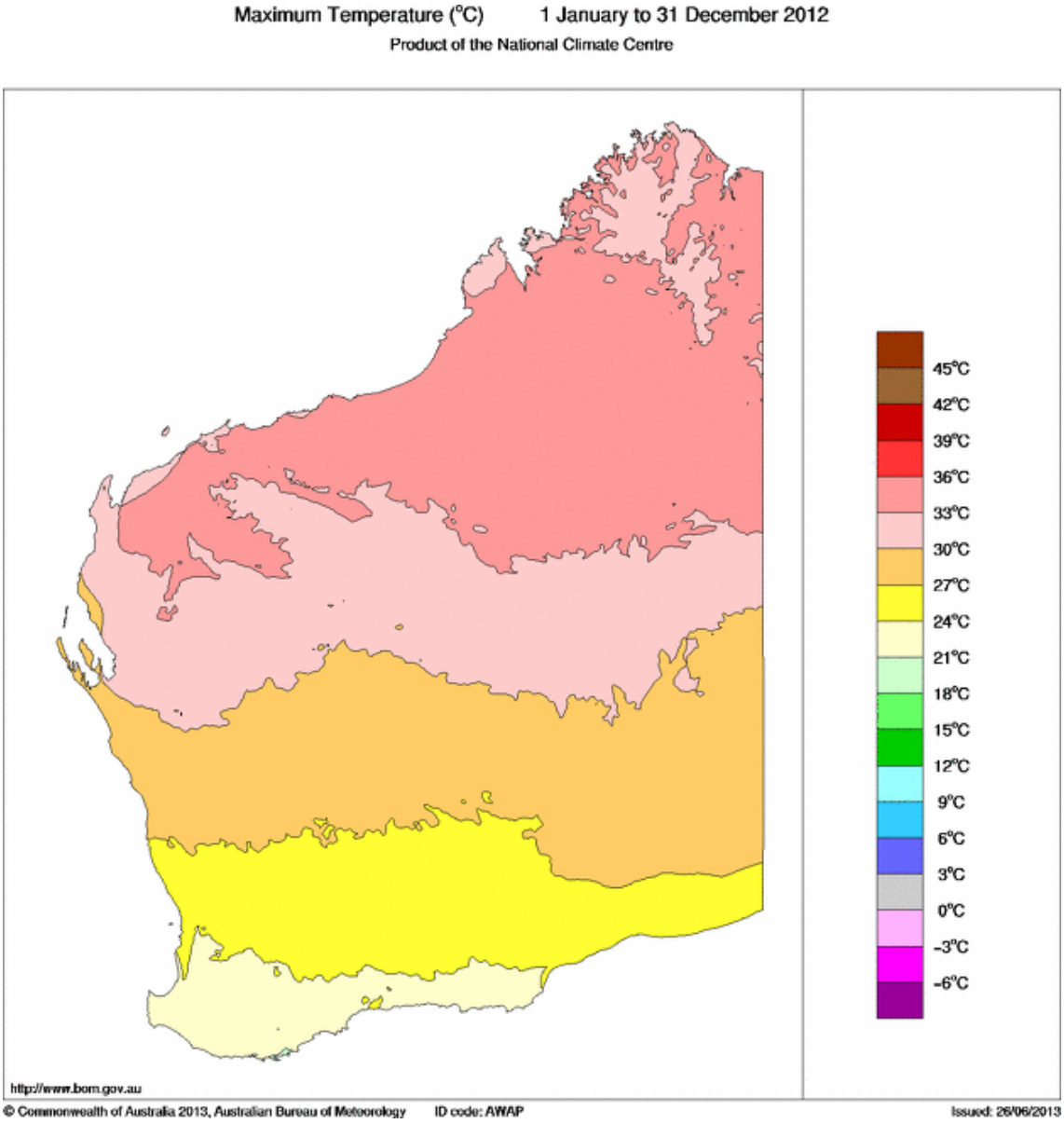
- **A- Map Ridgefield**
- **B- Climate Maps**
- **C- Litter Species used**
- **D- Invertebrate Studied Species**
- **E- Field Data (in CD)**

A – Map Ridgefield

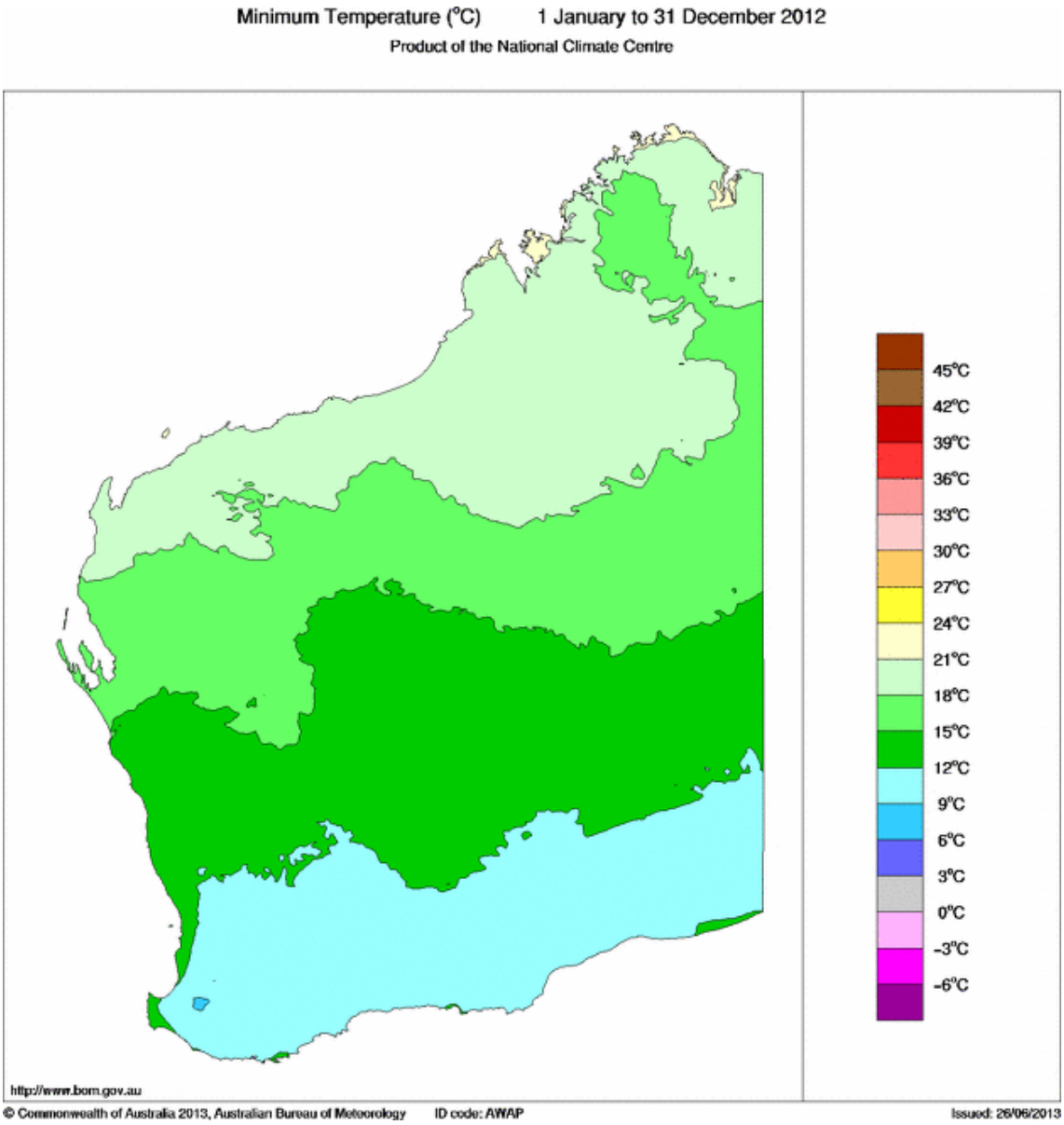


Map A1: Map of Ridgefield. Source: Perring, M.P. *et al.* (2012)

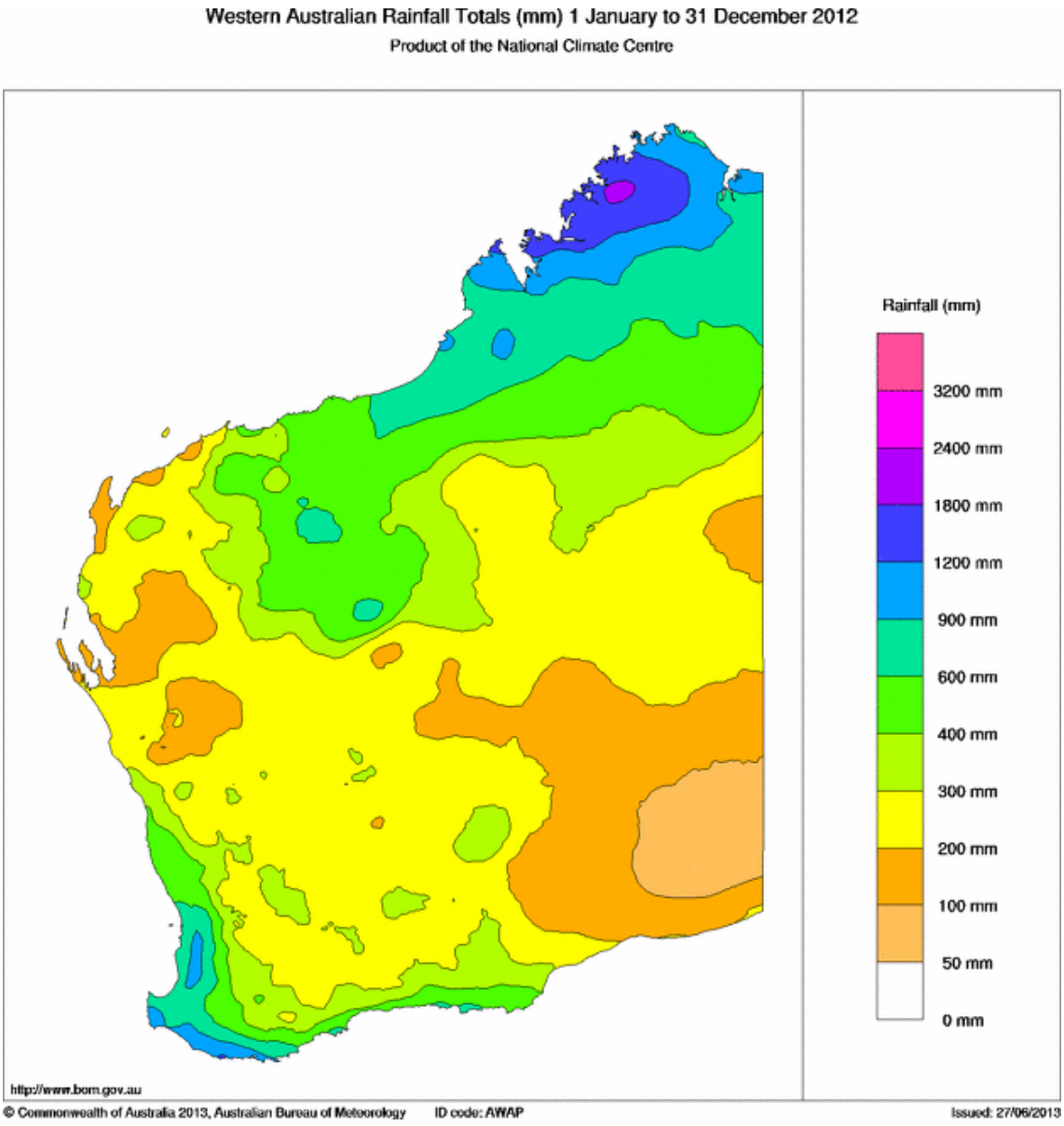
B – Climate Maps



Map B1: Maximum Temperature (°C) from 1st January to 31st December 2012. Source: Commonwealth of Australia 2013, Australian Bureau of Meteorology.



Map B2: Minimum Temperature (°C) from 1st January to 31st December 2012. Source: Commonwealth of Australia 2013, Australian Bureau of Meteorology.



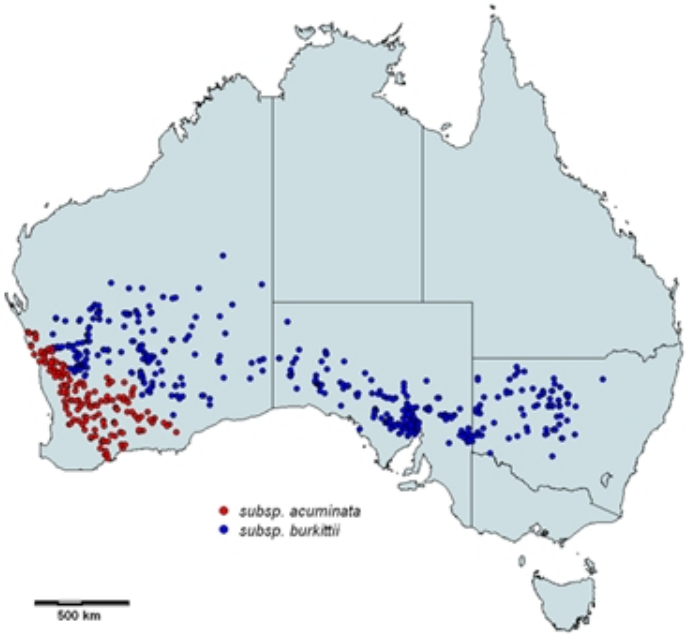
Map B3: Western Australia Rainfall Totals (mm) from 1st January to 31st December 2012. Source: Commonwealth of Australia 2013, Australian Bureau of Meteorology.

C – Litter Species used

NATIVE PLANTS

Acacia acuminate

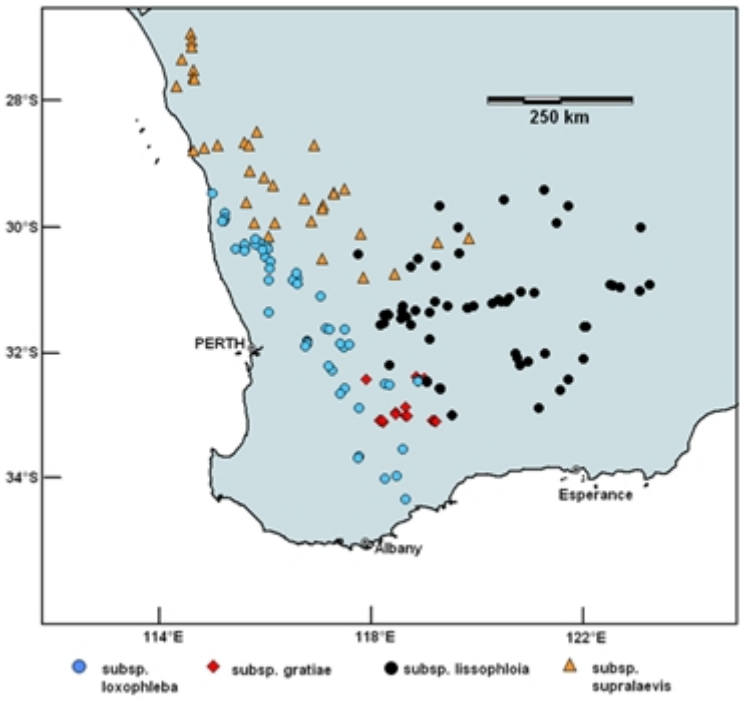
Image C1: *Acacia acuminata.* Source: Flora Base, the Western Australian Flora: J. Flint, M. Hancock, S.D. Hopper and E. Wajon.



Map C1: Distribution of *Acacia acuminata* in Western Australia. Source: Florabank.org.au

Eucalyptus loxophleba

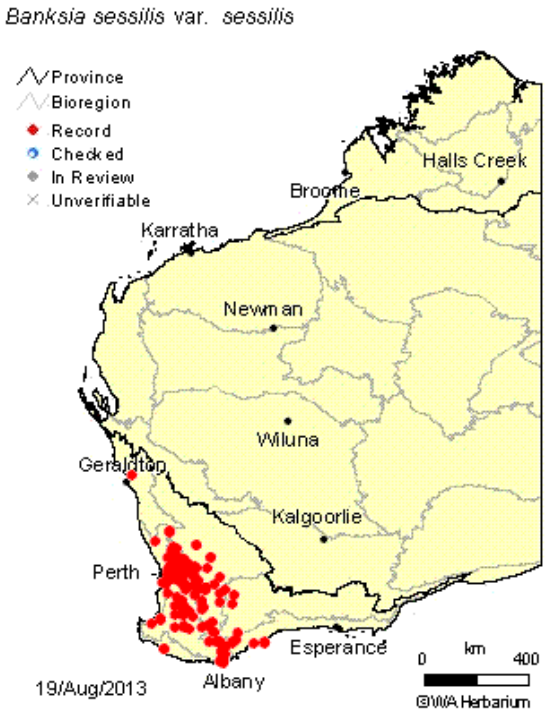
Image C2: *Eucalyptus loxophleba.* Source: Flora Base, the Western Australian Flora: B.R. Maslin and S.J. Patrick.



Map C2: Distribution of *Eucalyptus loxophleba* in the South of Western Australia. Source: Florabank.org.au

Banksia sessilis

Image C3: *Banksia sessilis var. sessilis.* Source: Flora Base, the Western Australian Flora: A. Ireland, M. Pieroni and E. Wajon.



Map C3: Distribution of *Banksia sessilis* var. *Sesillis* in Western Australia. Source: Florabank.org.au

INVASIVE PLANTS

***Avena spp*.**

Image C4: *Avena Sativa.* Source: Arizona State University

Erodium spp.

Image C5: *Erodium spp..* Source: California Academy of Sciences.

Image C6: *Erodium spp..* Source: Luigi Rignanese.

Arctotheca calendula

Image C7: *Arctotheca calendula.* Source: Flora Base, the Western Australian Flora: K.C. Richardson.

D – Invertebrate Studied Species

Image D1 & D2: Three *Coleoptera* adults in the left picture, and a larvae of *Coleoptera* in the right one. Source: Mariona Isern Subich.

Image D3 & D4: An adult of *Diptera* in the left image and a larva of *Diptera* in the right one. Source: Mariona Isern Subich.

Image D5: An ant (*Hymenoptera Formicida).* Source: Mariona Isern Subich.

Image D6: An adult of *Hymenoptera.* Source: Mariona Isern Subich.

Image D7: An adult of *Hemiptera.* Source: Mariona Isern Subich.

Image D8: An adult of *Thysanoptera.* Source: CSIRO Entomology.

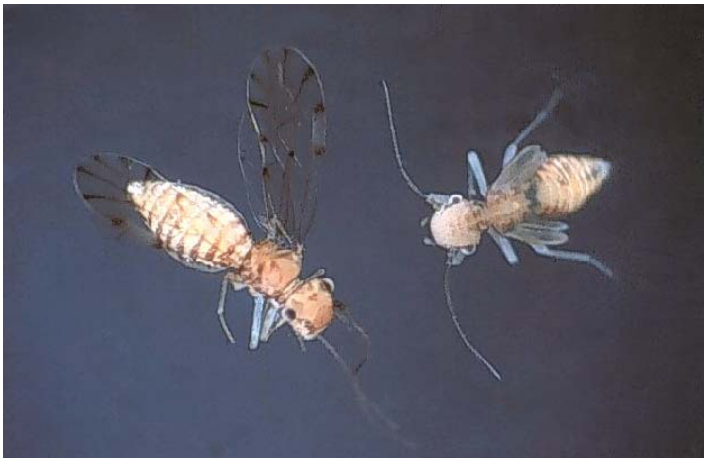


Image D9: An adult of *Psocoptera.* Source: NC State University.

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Image D10: Two adults of *Blattodea.* Source: Mariona Isern Subich

Image D11 & D12: An adult of *Thysanura.* Source: Mariona Isern Subich.

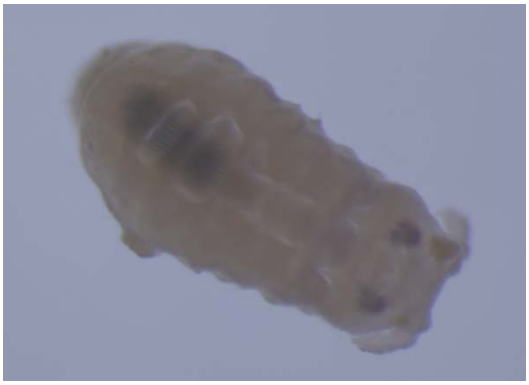

Image D13: A *Collembola.* Source: Mariona Isern Subich

Image D14: An adult of *Isopoda.* Source: Mariona Isern Subich

Image D14 & D15: In the left picture can be seen a spider (*Aranae),* and in the right image it is a *Pseudoscorpiones.* Source: Mariona Isern Subich

Image D15 & Dº16: Two different mites (*Acari*). Source: Mariona Isern Subich