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# **Novel models and indicators for characterizing impacts on biodiversity in Life Cycle Impact Assessment**

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## Abstract

This PhD project derives from the need of expanding the focus of current biodiversity impact assessment in supply chains and improving the modeling of impacts on biodiversity in Life Cycle Impact Assessment (LCIA) to support bio-economy and the evaluation of supply chains towards sustainability.

The study falls within the research field of Sustainability Science and is focused on the development of innovative models and indicators towards the integration of ecology in Life Cycle Assessment (LCA), enabling the assessment of the sustainability of products and services by comprehensively accounting for many aspects of biodiversity.

From the explored state of the art, specific research needs at different levels of interest were identified to improve the ecological considerations in LCA. Based on these premises, this project is developed on three main interconnected levels, representing building blocks at midpoint level for the LCA framework:

- *Target species.* Insect pollinators are addressed as target group for biodiversity protection in LCA. This decision, specifically the choice of honey bees (*Apis mellifera*) as target species, derives from the functional role pollinators play both in maintaining ecosystem functioning and in relation to the socio-economic benefits they bring to humans globally (food security is a remarkable example). In this dissertation, the main anthropogenic impacts on pollinators are described, as well as the modelling needs to account for them in LCIA. Recommendations on how future research should be oriented to improve the current models and how new indicators should be developed are proposed. Based on these, a methodological study has been performed and novel characterization factors for the impacts of pesticides on honey bees have been developed as starting point for quantifying the toxicological impacts on terrestrial ecosystems.
- *Impact categories.* Enhancing the transition towards a bio-economy, while ensuring the sustainable use of resources, represents one of the main goals for sustainable growth. According to this, biotic resources, with a focus on the naturally occurring ones, are addressed in LCA by proposing a new impact category; and a novel impact pathway that shows the links between resource provision and biodiversity is defined, focusing on a midpoint indicator based on renewability rates.
- *Interaction between impacts.* The cross-cutting nature of bio-economy represents the opportunity for comprehensively addressing the inter-connected challenges, as natural resource scarcity and food security, while achieving sustainable economic growth and ensuring the integrity of ecosystems. On this background, the nexus concept (i.e. understanding and managing the interactions and connections between the sectorial demands of constrained natural resources and the role of provisioning ecosystem services), is explored and a discussion on how LCA can be applied for depicting a win-win strategy of global resources management is presented.

This PhD project highlights several limitations and research needs in the current LCA framework with respect to the assessment of impacts on ecological aspects related to biodiversity along the supply chains. Recommendations for future improvements are disclosed for each analyzed level, in order to build from theory to practice more environmentally sustainable supply chains, integrating ecological considerations and biodiversity as pivotal aspects to be preserved.

## 1. General introduction

Biodiversity in its whole represents a crucial life-sustaining element for both ecosystem functioning and socio-economic stability of human societies worldwide. The increasing pressures due to the unsustainable consumption and production patterns in both developed and developing countries are threatening biodiversity at the global scale, thus raising concerns in the political and business contexts about a potentially irreversible crisis. Traditionally, the Life Cycle Assessment (LCA) methodology is used to identify and quantify impacts and consumption of resources associated with the entire life cycle of a product, process, or service; however, so far, the existing models are not able to comprehensively capture impacts on biodiversity and its components. Therefore, there is an urgent need of methods and models for addressing the issue and for better assessing product environmental performance, integrating ecological aspects. Based on these premises, this PhD research project derives from the need of (i) expanding the focus of current biodiversity impact assessment in supply chains and (ii) improving the modeling of impacts on biodiversity in the Life Cycle Impact Assessment (LCIA) framework to support the transition to a bio-based economy, and the evaluation of supply chains and sustainable production and consumption patterns. Ultimate goal is to integrate ecological considerations in LCA, namely the inclusion of key species, of natural biotic resources and of systemic thinking.

Section 1.1 provides information about the research context of this PhD project, including a brief introduction to the problem of the biodiversity loss on the global scale due to massive human interventions (section 1.1.1) and an overview of the general LCA framework, with specific regards to the LCIA and environmental cause-effect chain modeling (section 1.1.2).

Section 1.2 reflects the state of the art, including the latest developments made in LCIA related to biodiversity, both at midpoint and endpoint level. Finally, section 1.3 focuses on the specific objectives of the PhD project, followed by section 1.4 which reports the outline of the thesis.

### 1.1. Research context

This project falls within the field of research of Sustainability Science, which focuses on the dynamic interactions between society and nature. In a world confronted with significant losses in biodiversity that threaten the stability of the living systems on which human well-being depends, it becomes urgent to understand the close relationship between the humankind (i.e. its presence and its activities) and the natural environment from the sustainability point of view. Specifically, according to the Sustainable Development Goals (UN, 2015), understanding the human pressures on biodiversity and its components represents the basis for maintaining nature's capacity to deliver those goods and services that humans need, and whose loss would come at a high price.

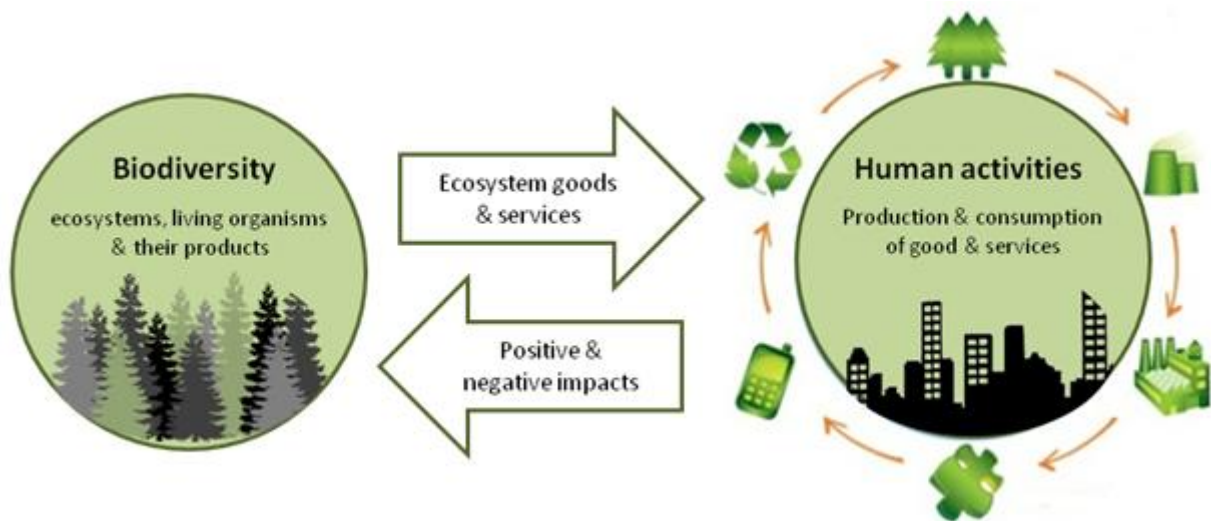
In this context, this research project aims at contributing to the ongoing discussion on the identification of innovative approaches for integrating ecological considerations in Life Cycle Assessment (LCA), enabling the assessment of the sustainability of products and services by comprehensively accounting for many

aspects of biodiversity, i.e. target species, impact categories and interactions between impacts, considered at midpoint level.

The development of comprehensive models and operational indicators is ambitious in the time horizon of a PhD program; however, this research sets the conceptual basis for a decision support system that can hold particular promise for further development and applications in LCA concerning the assessment of impacts on biodiversity and its components, highlighting main knowledge and research gaps.

### 1.1.1. Human activities and biodiversity loss

Our planet is undergoing rapid and intense environmental changes induced by human interventions that are altering the natural environment on a global scale, such that the term Anthropocene was lately coined as a geological era in which human impacts are considered to generate observable earth-scale system impacts (Lewis and Maslin 2015). Particularly, over the past few decades, the over-exploitation of natural resources e.g. for food, the extensive transformation of land for urbanization, mobility and trade, the massive use of synthetic chemicals for agricultural purposes and other processes related to the current production-and-consumption system, have been affecting the natural environment, threatening biodiversity and its ability to provide goods and services (the so-called ecosystem services, classified as provisioning, regulating, cultural and supporting services – MEA, 2005).



**Figure 1.1** Bidirectional relationship between biodiversity, which provides goods and services supporting human societies, and socio-economic activities, which may have both positive and negative impacts on biodiversity and its components.

The concept of biodiversity, or biological diversity, has a broad and complex meaning. According to the Convention on Biological Diversity (UN, 1992), it represents " *the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems*". Therefore, biodiversity cover different levels of variability, including hierarchiacal components such as

ecosystems, habitats, communities, species and genes, all of which have intrinsic and recognized social, economic, cultural and ecological values.

The bidirectional relationship between biodiversity and socio-economic activities is presented in Figure 1.1. Healthy ecosystems supply human societies with a bunch of goods (e.g. food, fibres and other natural resources) and services (e.g. protection from floods, pollination, control of climate change, etc.) that are vital for socio-economic development. However, widespread and easily observable impacts on the ecosystems, such as a decline in quality and diversity, have been documented by the Millennium Ecosystem Assessment project over the last decades (MEA, 2005). It was observed that the conversion rate of the natural areas on Earth has dramatically increased over the last 50 years, with 60% of the world's ecosystems degraded (EC, 2011). This situation is particularly critical in tropical and subtropical regions, where many biomes have undergone major changes (De Souza et al., 2013). As an example, the reduction in tropical rainforests is ongoing, with a loss of 13 million hectares each year; almost 20% of the world's tropical coral reefs have been lost, and even more are at risk (EC, 2011). A direct consequence of these actions is the drastic decline in the diversity, abundance and richness of species, leading to extinctions in extreme cases. In fact, anthropogenic impacts are contributing to an unprecedented increase in the rate of animal and plant species lost worldwide, currently estimated to be up to 1000 times higher than the average rates (Lewis and Maslin, 2015), thus potentially reducing the resilience of the ecosystems and also their ability of delivering valuable services for human societies. Moreover, The Economics of Ecosystems and Biodiversity (TEEB, 2008) measured the cost of biodiversity loss, providing realistic examples. For instance, considering that 75% of global fish stocks are overexploited or significantly depleted, the monetary annual loss associated to the current overexploitation of global fisheries has been estimated at US\$ 50 billion.

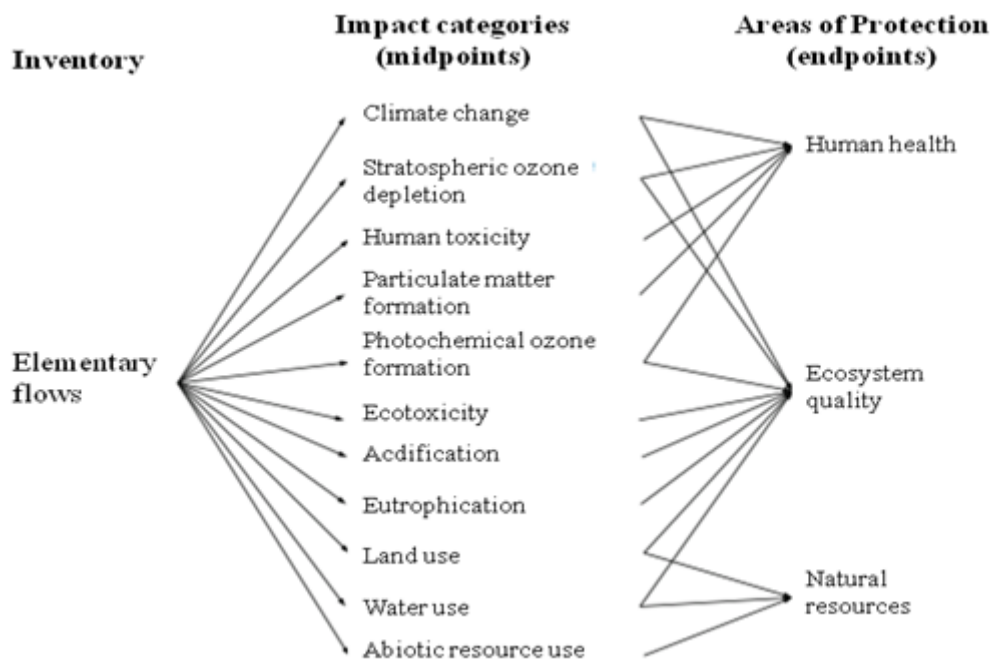
Therefore, since the loss of biodiversity could have disastrous effects on the supply of goods and services to human populations, protecting and conserving it represent nowadays an urgent problem. This is a decisive challenge for the 21<sup>st</sup> century and a fundamental goal for sustainable development in order to meet the reasonably foreseeable needs of future generations. On this background, the development of tools, from international policies to integrated methodological approaches, has been stimulated and practical actions have been put in place. Especially in Europe, the interest in biodiversity assessment has recently grown from both policy and scientific perspectives, in order to cope with this challenge and guide the transformation of the European economy. In 2011, the European Union adopted a novel strategy with the aim of halting biodiversity loss in the EU context, called "*EU Biodiversity Strategy 2020*" (EC, 2011), on the basis of the "*Strategic Plan for Biodiversity 2011-2020*" implemented by the Secretariat of the Convention on Biological Diversity (SCBD) in collaboration with the United Nations Environment Programme (UNEP) (SCBD, 2010). It was followed in 2012 by the European Commission's strategy for "*Innovating for Sustainable Growth: A Bioeconomy for Europe*" (EC, 2012), built on the Seventh Framework Programme for Research and Technological Development (FP7) (EU, 2006) and the subsequent EU Framework Programme for Research and Innovation (Horizon 2020). These policies adopt a new long-term vision, based on creating a new flourishing economy by encouraging the advancement in technologies, while promoting the



conservation and sustainable use of biodiversity. Nevertheless, biodiversity and ecological considerations are often neglected when such progresses in the industrial sectors are actually outlined. Therefore, addressing such multi-dimensional issue, as biodiversity loss, in order to identify and develop targeted solutions requires (i) biodiversity policies to be integrated to sectoral policies and be taken into account in wider policy and business concerns; (ii) a strategic, integrated and comprehensive approach, involving methodological tools in order to quantify the magnitude of impacts and the loss. Life Cycle Assessment represents such a tool.

### 1.1.2. Life Cycle Assessment

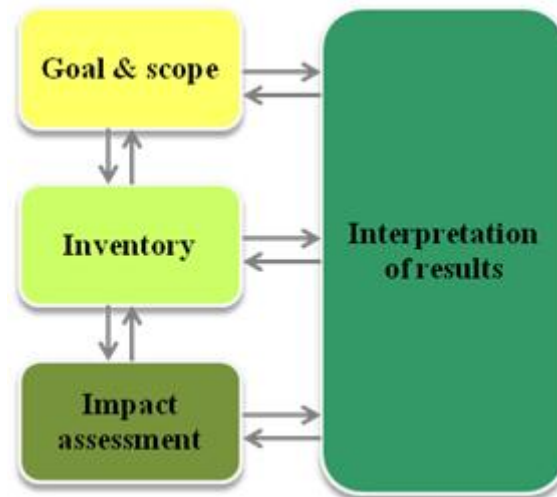
Life Cycle Assessment (LCA) is a standardized methodology widely used at international level, which supports decision makers (e.g. governmental and non-governmental organizations, corporations, etc.) for the integration of environmental consideration into the evaluation of products (i.e. goods and services). In fact, LCA is a systemic approach which considers a product as a set of input and output flows of materials and energy forms (called elementary flows), associated with all the steps of its life cycle. This methodology allows the multi-criteria assessment of the environmental impacts along the products' supply chains, by quantifying the consumption of resources and the emissions into air, water and soil using different models and indicators which cover a broad variety of pressures (classified in the so-called impact categories or midpoints) finally associated with the areas of protection (or endpoints) of human health, ecosystem quality and natural resources (Figure 1.2).



**Figure 1.2.** Simplified cause-effect chain, underpinning the Life Cycle Assessment (LCA) framework. Adapted from Hauschild & Huijbregts (2015)

LCA is one of the key tools for implementing the EU Integrated Product Policy (EC, 2013), which is a crucial component of the Sustainable Development Strategy of the European Commission. In fact, by applying LCA, possible improvements and trade-offs can be identified in order to reduce the environmental impacts of products.

According to the ISO standard (ISO, 2006), an LCA study consists of four main phases (Figure 1.3). The first step is the goal and scope definition, followed by the inventory phase, the impact assessment step (i.e. Life Cycle Impact Assessment - LCIA) and eventually the interpretation phase.



**Figure 1.3.** Life Cycle Assessment (LCA) framework, according to ISO (2006)

In the goal and scope phase, the aims of the LCA study and the main assumptions about the system (e.g. functional unit as a quantitative reference for the study, system boundaries, data quality, etc.) are defined and described, thus allowing for instance the comparison of different products or services, or the exploration of potential future improvements in the considered products.

In the subsequent Life Cycle Inventory (LCI) step, data on the amounts of extracted resources and emissions for each life cycle stage, namely from the extraction of raw material to the end of life, are gathered in a list, where each amount corresponds to the functional unit for the selected product system.

During the third phase called LCIA, the data collected in the previous step are characterized, i.e. linked to the impact categories (e.g. climate change, ecotoxicity, land use, abiotic resource use, etc.) through a cause-effect chain and translated into an environmental impact by using factors, called characterization factors, which represent the predicted contribution to a pressure per unit emission or resource consumption. These factors are calculated by using specific models, which represent the focus of the recent development in the LCIA context.

Finally, in the interpretation phase, the results of the LCIA are discussed according to the aim set in the first phase of the study.

The environmental cause-effect chain that links the elementary flow to the impact categories, or midpoints, can be extended towards the endpoints, which address the damage caused to the three areas of protection.

## 1.2. State of the art

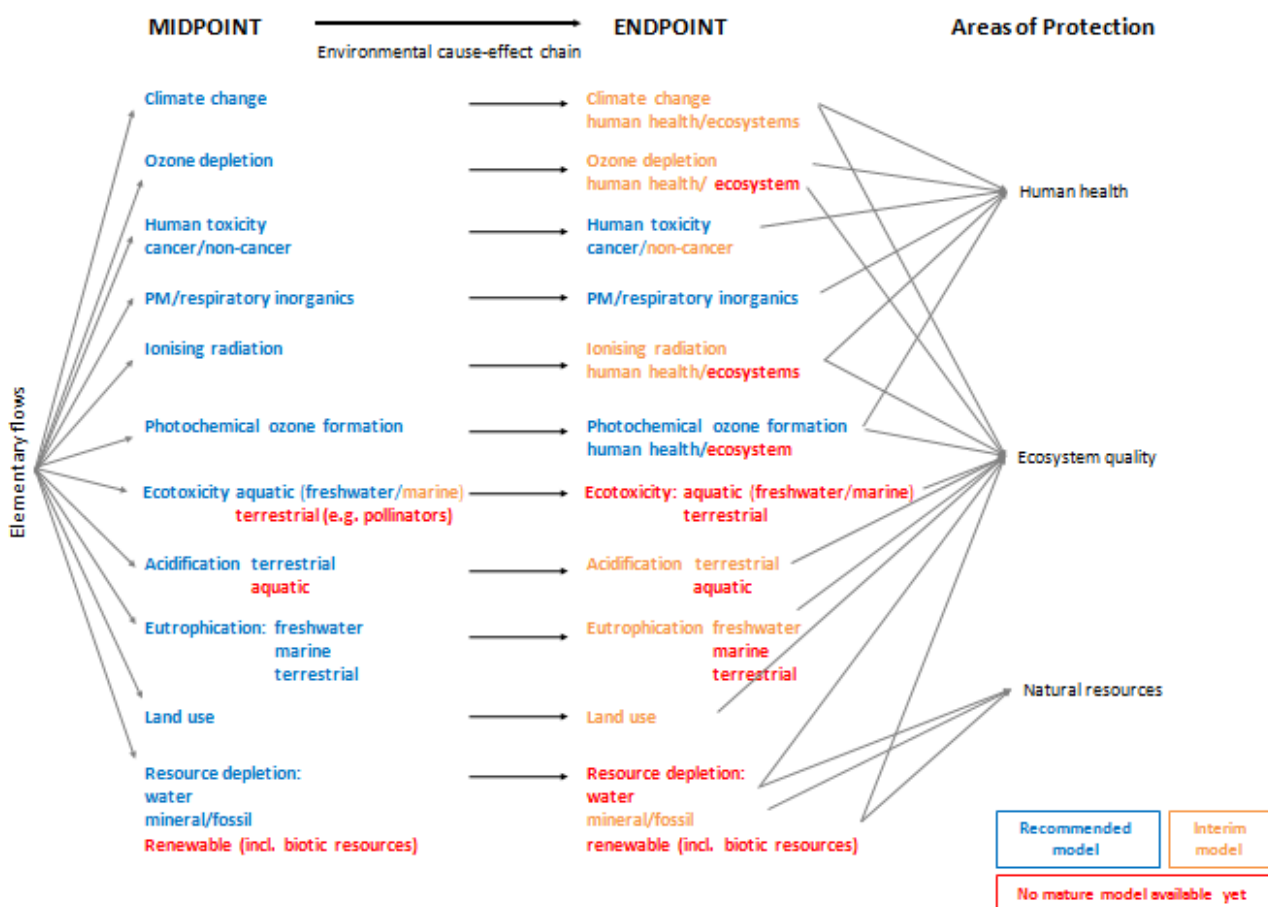
Several models accounting for a wide number of impacts/damages, as presented in Figure 1.2, have been developed over the years for being used in the common LCA practices. In 2011, the European Commission developed the International Reference Life Cycle Data System (ILCD) (EC-JRC, 2011) with the aim of supporting the use of coherent and high quality-based life cycle data and models within a common and reliable framework for decisions in both policy and business contexts. Despite its environmental assessment focus, LCA is still immature in terms of inclusion of ecological and sustainability aspects in the impact assessment modeling. In fact, LCA responds to the question of what environmental impacts are occurring at product or process level, and not yet fully to the question of what ecological impacts, and even less if these impacts are sustainable, i.e. below planetary boundaries, reversible, compatible with an intergenerational temporal horizon, etc. Thus, LCA covers only environmental aspects, without taking into account the ecological ones, and even less those related to environmental sustainability.

The necessary integration of biodiversity and its ecosystem services in life-cycle oriented methodologies has been recognized, since they represent relevant aspects to policy makers and consumers. Hence, the development of models assessing global biodiversity threats attributed to human activities represents a challenge for the LCA community. Over the last decades, many attempts have been made to quantify the impact of many drivers resulting in biodiversity loss. Researchers have been mainly inspired by the ecological literature when developing impact assessment models for biodiversity in the LCIA context, proposing different approaches to the problem both at midpoint and at endpoint level.

The most recent reviews on the inclusion of biodiversity in LCIA models are provided by Curran et al. (2011) and Teixeira (2014), who addressed both conceptual and methodological shortcomings in the current way biodiversity is considered in LCA, and the most recent review from Winter et al. (2017), built on the work of Curran et al. (2011) with a broader scope. All the authors highlighted that out of the five drivers of biodiversity loss identified by the Millennium Ecosystem Assessment (i.e. habitat change, climate change, pollution, invasive alien species and overexploitation), only the first three are represented in the existing impact categories of land use, water use, climate change, acidification, eutrophication and ecotoxicity at midpoint level (see Figure 1.4 as an overview). For what concerns the last two drivers of biodiversity loss, it is widely recognized that (i) invasive alien species represent a non-negligible threat to biodiversity, potentially causing huge losses to the economies especially at local and regional level (EC, 2017) and (ii) the overexploitation of resources, with particular regards to the unsustainable use of natural biotic ones (including overfishing and illegal hunting), may affect their natural regeneration rate, leading to their potential depletion for the future generations (Sonderregger et al., 2017). In literature, several proposals have been made to cover the missing drivers in the LCA context, e.g. Hanafiah et al. (2013) and Emanuelsson et al. (2014) assessed respectively impacts due to invasive species and overfishing (see Winter et al., 2017 for

more details). However, these models are not yet integrated in any recommended method, such as the ILCD (EC-JRC, 2011), thus not being operational yet in the common LCA practice for addressing crucial impacts to biodiversity.

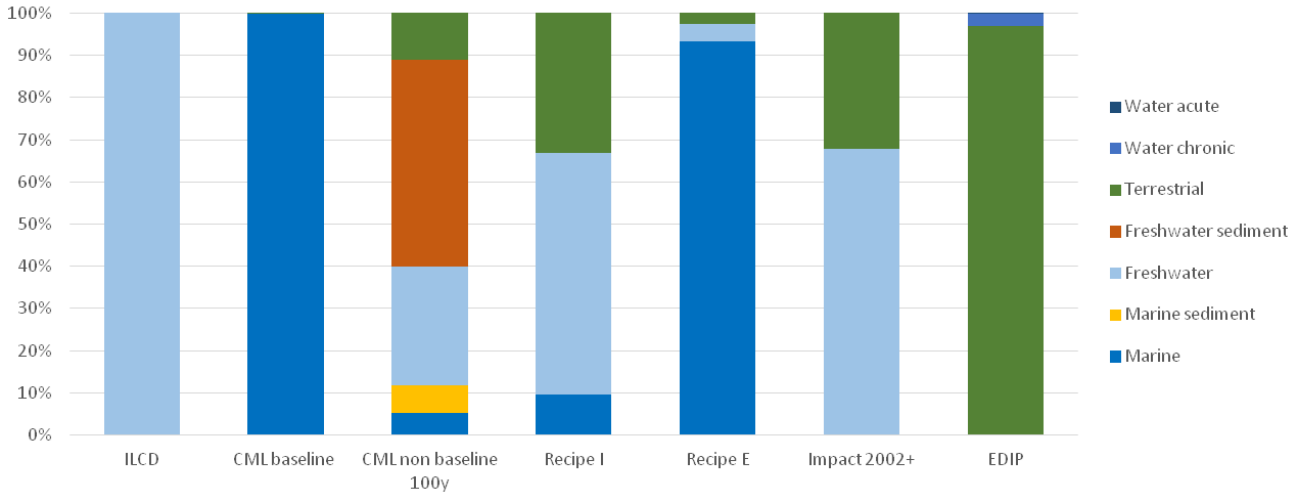
Additionally, other crucial drivers are missing in this framework, namely noise, artificial light and thermal pollution (as highlighted by Winter et al., 2017) and the international trade, which has been lately identified as a key point of biodiversity loss to be addressed in the LCA framework. In fact, in today's globalized economy, the international import-export system is accelerating habitat degradation and species loss in areas which are far from the place of consumption. It has been estimated that at least one-third of biodiversity threats across the globe are driven by the production of export goods for international trade (Lenzen, 2012; Moran et al., 2016; Moran and Kanemoto 2017).



**Figure 1.4.** Overview of the environmental cause-effect chain within the Life Cycle Impact Assessment (LCIA) framework, where the drivers of pressure (including those on biodiversity) and their integration in the LCIA framework are addressed from midpoint towards the areas of protection, according to the state of maturity of the underpinning models. Edited from Sala et al. (2012).

More recent research for specific impact categories has been performed, especially in the land use research field (e.g. Chaudhary et al., 2015 and 2016; Curran et al., 2016; Teixeira et al., 2016), which represents the focus of the majority of modeling efforts towards the inclusion of biodiversity in LCA. However, most of these models focus on compositional attributes of biodiversity only, such as species richness. Even at the endpoint level, the models currently used in LCIA and implemented in dedicated softwares for case studies

for describing the quality of ecosystems, e.g. ReCiPe (v.2008 based on Goedkoop et al., 2009; v. 2016 based on Huijbregts et al., 2017), EcoIndicator 99 (Goedkoop and Spriensma, 2000) and IMPACT 2002+ (Joliet et al., 2003), focus mainly on the flow of information at the species level. In fact, the operational indicators for biodiversity loss are *species per year* (species/yr) and the *Potentially Disappeared Fraction* of species within a spatial and temporal space (PDF.m<sup>2</sup> or, mostly used, PDF.m<sup>2</sup>.yr), which represents the extinction rate in a given area of land or water volume due to unfavorable conditions associated with land use, toxicity, increased global temperature, eutrophication, etc. The use of these metrics is driven by the fact that the loss of species is seen as indicative for a general biodiversity decline and the potential subsequent loss of resilience. In fact, the extinction of a species on the global scale is an irreversible loss of biological information, which may also have effects on other species and on the ecosystem functioning. Therefore, species extinctions among other metrics of biodiversity loss have been reflected in the LCIA modeling (Callesen, 2016). However, the taxonomic coverage of the existing LCIA models is limited and the choice of species is currently different for each stressor, namely only a relatively small number of specific taxa for individual impact pathways are used to develop impact factors. For instance, a few vertebrate and invertebrate taxa are addressed for land use related impacts on biodiversity (i.e. mammals, birds, amphibians, and reptiles in Chaudhary et al 2016, not yet implemented in the dedicated softwares for LCA case studies; additionally snails, spiders, carabids, butterflies, wild bees, and grasshoppers in the SALCA model by Jeanneret et al., 2008); while vascular plant species are adopted in other models, e.g. for acidification (e.g. ReCiPe underpinning models developed by Van Zelm et al., 2007). Even within the same impact category, the accounting of species varies according to the adopted method. Taking as an example the ecotoxicity impact category, Figure 1.5 shows the inconsistencies between 5 methods - i.e. ILCD based on USEtox (Rosenbaum et al., 2008), CML (2002) including baseline and 100-year scenario, ReCiPe (Goedkoop et al., 2009) including individualistic and egalitarian perspectives), Impact 2002+ and EDIP (Hauschild and Potting, 2005) - used for characterizing toxicity related impacts. Each among the analyzed method is based on a set of modelling assumptions and premises which are typical of the methods themselves, accounting for different organisms in terms of number of species and ecosystem type (e.g. freshwater only vs a combination of terrestrial and aquatic, etc.), thus resulting in a misleading interpretation of the overall impacts. In fact, due to the divergence of the underpinning models, some potentially relevant impacts can be lost according to the method that the users decide to adopt for their studies.



**Figure 1.5.** Comparison of the results of the impacts at midpoint on ecotoxicity, calculated with different characterisation methods, which to different extent take into consideration impacts on water (freshwater and marine), terrestrial ecotoxicological effects and effects on sediments (marine and freshwater). The results are referred to a typical European citizen’s food basket of products, according to the European Commission’s JRC.

Furthermore, considering that the substances used along the food supply chains and which are inventoried and characterized in the LCIA models are mainly from the agricultural sector (e.g. pesticides), the impacts on relevant species such as those belonging to the group of pollinators are lost due to the limitation of species included within the modeling frameworks. The loss of crucial impacts on biodiversity occurs also within the resource use related impact category, specifically referring to naturally occurring biotic resources, which are not inventoried or characterized in the LCIA models due to a lack of clarity on the underpinning impact pathway associated to their depletion.

In the adopted models for LCA, species are generally considered as equivalent, e.g. without taking into account their occurrence or distribution at local, regional and global scale, namely whether these are rare or, alternatively, widespread across many regions. Indeed, these models do not integrate in their indicators important ecological aspects such as the endemism, the recoverability and the vulnerability of species, as identified by the IUCN Red List (IUCN, 2017). These factors, which have been barely addressed in LCA so far -see the most recent work of Chaudhary et al. (2015) for more details, could be crucial for the definition of species loss on different scale, ensuring a better comparability between environmental impacts, especially when the LCIA comes to support decision making for regional purposes.

A further aspect which still needs to be clarified in this context is the definition of the ecological boundaries for biodiversity, as for example referred to in Wolff et al., (2017). Biodiversity conservation targeted solutions need to be consistently referred to the carrying capacity of the ecosystems. However, the boundary between ecosphere (i.e. the natural system of the Earth including the interactions between living and non-living organisms) and technosphere (i.e. the bunch of human activities which can exert positive or negative impacts on the ecosphere) are barely clearly set in the LCA context (Alvarenga et al., 2013), thus leading to misaccounting or misunderstanding of the actual impacts on biodiversity (see Chapter 3 for more details). Therefore, the assessment of the carrying capacity is a key element of environmental sustainability, crucially

needed for sustainability assessment and for integrated assessment methodologies as Life Cycle Assessment (LCA).

For what concerns the accounting of ecosystem services provided by biodiversity, so far there is no study that comprehensively considers the broad range of ecosystem goods and services on which the industrial system depends and their interactions. One of the latest attempts to give an ecological perspective to the LCA framework, specifically accounting for the ecosystem goods and services, is proposed by Zhang et al. (2010a, b). The *Ecologically Based LCA (Eco-LCA)* relies on the complementation of existing methods in LCA with an input-output framework and it mainly includes two out of the four types of ecosystem services recognized by the Millennium Ecosystem Assessment, namely provisioning (e.g. mineral, fuels, food) and supporting (e.g. biogeochemical cycles) ecosystem services. However, notwithstanding *Eco-LCA* aims at broadening the scope of conventional LCA with an additional ecological perspective and its methodological framework has been maintained as similar as the one of traditional LCA, many ecosystem goods and services are still ignored (e.g. wild plant and animal food) or only partially considered (e.g. pollination and wild fish) and the elements of interaction between impacts are still missing, aspect that may largely affect the results of the environmental assessments

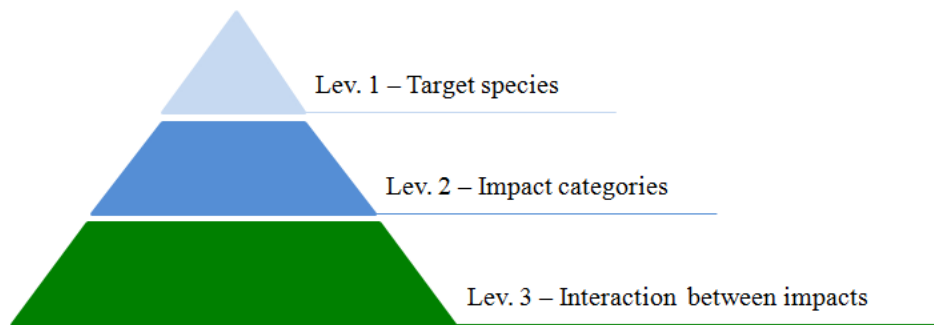
### 1.3. Research gaps and objectives

Although the need of quantifying the impacts on biodiversity and ecosystem services is widely acknowledged, this task remains difficult and the problem remains open. By definition, biodiversity is a complex and multifaceted concept that incorporates the entire range of life, including many hierarchical levels (e.g. ecosystems, species, etc.), biological attributes (e.g. functions, etc.) and a multitude of temporal and spatial interactions that make it difficult to synthesize in a single indicator. Therefore, the overall objective of this thesis is to improve the way biodiversity is accounted for in the LCA framework, furthering the development in the LCIA models via integrated models of technological and ecological systems. In particular, this research project stems from the need to bridge some of the existing conceptual and methodological gaps in LCA with regards to the assessment of impacts on specific ecological features of biodiversity.

The main background idea underpinning the development of this project is the identification of innovative approaches for integrating ecological considerations in LCA. According to this, my thesis is based on improvements and proposals on various fronts. In fact, from the explored state of the art (Section 1.2), some specific research needs at different levels of interest have been identified (Figure 1.6) in order to overcome the current limitations and improve the ecological considerations on biodiversity in the LCA framework. For instance, many target species that may have a relevant role for humans and the ecosystems are not included; biotic resources, whose depletion may undermine the development of future generation and which currently represent the key aspect in the transition from a fossil-based economy to a new form of bio-based economy, are not sufficiently considered; the interactions between impacts on biodiversity and feedbacks are not taken



into account, while their role is fundamental in determining the preference of a human intervention, while maintaining the integrity of the ecosystems.



**Figure 1.6.** Simplified overview of the three levels of interest, on which my PhD research project is based.

Based on these premises, this PhD research project is developed considering three closely connected building blocks of the LCA framework:

1. *Target species.* Insect pollinators have been considered as a target group for biodiversity protection within the LCA framework, in particular by modeling the impacts deriving from the recent intensification of agricultural practice. The decision of considering pollinating insects, specifically honey bees (*Apis mellifera*) as target species, derives from the functional role they play. In fact, they are natural providers of ecosystem services, such as pollination, which are fundamental not only from the point of view of maintaining ecosystem functioning, but also in relation to the socio-economic welfare they bring to mankind globally (e.g. food security is a remarkable example).
2. *Impact categories.* Natural resources, biotic and abiotic, are fundamental from both the ecological and socio-economic point of view, being at the basis of life-support. Reducing the demand of abiotic non-renewable resources, on which the European economy and many other developed country across the World still heavily rely, and enhancing the transition towards a bio-economy, while ensuring the sustainable use of biotic resources, represent the main goals for a Sustainable Growth (EC, 2012). Therefore, the accounting of biotic resources within the LCA framework becomes an urgent gap to be covered.
3. *Interaction between impacts.* The cross-cutting nature of bio-economy represents the opportunity for comprehensively addressing inter-connected challenges such as natural resource scarcity, food security, fossil resource dependence and climate change, while achieving sustainable economic growth and ensuring the integrity of the ecosystems. Therefore, it becomes necessary to account for natural resources and ecosystem services in an integrated and system-oriented way in order to identify a win-win strategy (namely avoiding burden shifting of impacts) for the sustainable management of global constrained resources whose uses are inter-connected.

#### 1.4. Organization of the thesis

According to the three main objectives identified in section 1.3, the thesis is organized as follows.



1. *Target species (Chapter 2)*. Section 2.1 of this thesis describes the predominant environmental and anthropogenic pressures acting on insect pollinators, potentially threatening pollination services, as results of a broad literature review. The main modelling needs in order to account for these drivers in LCIA are reported as discussed, as well. Recommendations on how future research should be oriented to improve the current models and how novel indicators should be developed are proposed in order to cover the existing conceptual and methodological gaps. Based on these recommendations, section 2.2 presents a methodological study where novel characterization factors for impacts on honey bees from agricultural pesticides are developed and proposed as starting point for quantifying the toxicity related impacts on terrestrial ecosystems.
2. *Impact categories (Chapter 3)*. In section 3, biotic resources, with a focus on naturally occurring biotic resources (NOBR), have been addressed and their inclusion in the LCA framework is discussed. A novel impact category is proposed and an impact pathway that shows the links between resource provision and biodiversity is defined, focusing on a midpoint indicator that can play a role in resource ranking. Building on the existing literature, the study in this section extensively highlights and discusses the critical aspects related to biotic resource inclusion in LCA (e.g. from the system boundaries definition up to the resource characterization).
3. *Interaction between impacts (Chapter 4)*. In section 4, the nexus concept, which is about understanding and managing the interactions and connections between the sectoral demands of constrained natural resources and the role of provisioning ecosystem services, has been explored and addressed in the LCA framework. In this section, it is discussed how LCA can be applied for depicting a win-win strategy of global resources management and supporting a holistic, system-based assessment of supply chain.

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## 2. Impacts on insect pollinators: how to address this target group in LCIA

### 2.1. Part I: Towards a framework for impact assessment

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## Abstract

Human activities are threatening biodiversity at an unprecedented scale and pace, thus potentially affecting also the provision of critical ecosystem services, including insect pollination. Insect pollinators play an essential functional role in terrestrial ecosystems, supporting ecological stability and food security worldwide. Therefore, assessing impact on pollinators is fundamental in any effort aiming at enhancing the environmental sustainability of human production and consumption, especially in the agri-food supply chains. Different drivers are leading to pollinator populations' declines. Improving a supply-chain oriented assessment of the occurrence of pressure and impacts on pollinators is needed. However, current methodologies assessing impact along supply chains, such as life cycle assessment (LCA), miss to assess impact on pollinators. In fact, none of the existing life cycle impact assessment (LCIA) models effectively accounts for pollinators. Some LCIA models have mentioned pollination, but none has presented key drivers of impact and a proposal for integrating pollinators as target group for biodiversity protection within an LCIA framework. In order to devise a pathway towards the inclusion of impacts on pollinators in LCIA, we conducted a literature review of environmental and anthropogenic pressures acting on insect pollinators, potentially threatening pollination services. Based on the evidence in literature, we identified and described eight potential impact drivers, primarily deriving from industrial development and intensive agricultural practice: (i) intensified land use as a result of uncontrolled expansion of urban areas and modern agricultural practices; (ii) use of pesticides; (iii) presence of invasive alien plants; (iv) competition with invasive alien pollinator species; (v) global and local climate change; (vi) spread of pests and pathogens; (vii) electromagnetic pollution and (viii) genetically modified crops. To account for these drivers in LCIA, there are specific modeling needs. Hence, the current study provides recommendation on how future research should be oriented to improve the current models and how novel indicators should be developed in order to cover the existing conceptual and methodological gaps

### 2.1.1. Introduction

Over the last decades, human activities related to industrial development and agricultural intensification have threatened biodiversity and the provision of ecosystem services at an unprecedented scale and pace (CBD, 1992; Curran et al. 2011), almost leading to the so-called sixth mass extinction (Ceballos et al., 2015). Ecosystem services arise when nature (in its broad definition) contributes toward meeting a human demand; they are, arguably, underpinned by biodiversity (Hooper et al., 2005; Haines-Young and Potschin, 2010). Biodiversity and ecosystem services have undergone dramatic, in some case irreversible changes: as such, also the provision of critical ecosystem services is potentially at risk (Koellner and Geyer, 2013; MEA, 2005), including those related to insect pollination. Consequently, the overall human well-being profiting from goods and services provided by nature is also potentially threatened.

To date, different classification systems for ecosystem services are in use. They invariantly discriminate among: (i) provisioning services, i.e. the goods we obtain from ecosystems, such as water, timber, fish and

agricultural products, which are all traded on markets; (ii) regulating and supporting services, i.e. the capacity of ecosystems to maintain a livable environment, which include the removal of pollutants from soil, air and water, or services which support crop production such as pollination and soil erosion control; and (iii) cultural services, i.e. the non-material benefits, essentially defined by human preferences, such as nature-based recreation and tourism.

Within the regulating and supporting ecosystem services (MEA, 2005; Soussana, 2014), pollination represents a critical life-support function which is crucial for planetary ecological stability and the provision of services and resources in the agri-food sector. Indeed, a broad variety of wild and domestic insects plays an essential functional role in both natural and managed terrestrial ecosystems (Kluser et al., 2010; Vanbergen et al., 2014). At the global level, insect pollinators are responsible for pollinating more than 80% of wild plant species and almost 75% of primary agricultural crops (Klein et al., 2007), providing mankind with global food supply and other fundamental goods and services.

Recently, the global biodiversity crisis has involved insect pollinator populations as well. Several authors have documented regional reductions in the abundance and diversity of wild bees and local decreases in other pollinator populations, such as hoverflies and butterflies (Aizen and Harder, 2009; Biesmeijer et al., 2006; Carvalheiro et al., 2013; Potts et al., 2015). Moreover, significant and constant declines in the number of managed honeybee colonies have been registered on a regional scale in both Europe and North America. This alarming situation may have serious implications. It would limit the future production of pollinator-dependent crops (vanEngelsdorp and Meixner, 2010), thus threatening the agricultural and economic systems human life relies on, and would considerably affect the maintenance of wild plant diversity and natural ecosystem stability. The services provided by insect pollinators form the basis of other important ecosystem services and their loss would limit the availability of goods for future generations (Singh and Bakshi, 2009). As a result, several international institutions, local authorities and non-governmental organizations have raised deep concerns regarding potential risks to global food security and natural ecosystem functioning (Allen-Wardell et al., 1998; Bauer and Wing, 2010; Steffan-Dewenter et al., 2005), thus appealing for the promotion of an environmentally sustainable development. An integrated approach is needed in the areas of agriculture and ecology that would reduce the trade-offs between food production, biodiversity and ecosystem services (Soussana, 2014).

Understanding and identifying the role of ecosystem services, their linkages with biodiversity and human activities and the pressures that endanger their provision have been the central point of recent research (MEA, 2005; Zhang et al., 2010a, 2010b). Previous studies have already highlighted the main threats leading to pollinator populations' declines and potentially menacing the provision of pollination services (Gonzalez-Varo et al., 2013; Potts et al., 2010; Schweiger et al., 2010; Vanbergen et al., 2013, 2014). Furthermore, numerous attempts have been made in order to quantify the magnitude of human interventions leading to biodiversity loss and ecosystem service depletion (Curran et al., 2011; Koellner and Geyer, 2013; Schmidt, 2008). Despite all those efforts and the link with supply chains related impacts, life cycle oriented methodologies still miss to account for them. A lack of accounting for regulating and supporting ecosystem

services would overthrow the goal of Life Cycle Assessment (LCA) methodology towards sustainability (Singh and Bakshi, 2009).

The development of models and indicators for biodiversity and ecosystem services in Life Cycle Impact Assessment (LCIA) has been underway for more than a decade. To our knowledge, only a few studies so far have been conducted to integrate pollinators and pollination services in the LCIA framework. Zhang et al. (2010a, 2010b) proposed a framework for an ecologically based LCA, which accounts for the contribution of a handful of ecosystem services in the life cycle of industrial activities. Nevertheless, it remains not comprehensive (Singh and Bakshi, 2009).

In an era of extreme environmental changes induced by resource exploitation, it becomes necessary assessing the sustainability of production and consumption pattern in the agri-food sector, improving the existing supporting methodologies to reach the goal of a sustainable food system (Soussana, 2014). Therefore, it is fundamental including the natural capital, particularly pollinators' biodiversity and their crucial ecosystem services, in those life cycle oriented methods, such as LCA, since none of the existing LCIA methods and models accounts for their role in a comprehensive way.

The aim of the present study is to review the anthropogenic and environmental drivers exerting pressures on pollinators. This review represents the first step towards the integration of pollinators and their services in the LCIA framework. Starting from pollination as pivotal ecosystem service and pollinators as target group for biodiversity protection, this review aims to identify the modeling needs for the impact assessment in the LCIA context. Our study represents a bridge between ecological science and global product policies. Through the implementation of LCIA models and methods capable of accounting for ecosystem services such as those delivered by pollinators, we might be able to reduce anthropogenic impacts, thus meeting the goal of a more sustainable food production and consumption system.

This review is organized as follows: section 2.1.2 is presenting the methodology adopted for the review; section 2.1.3 presents the results of the review and it is followed by section 2.1.4, where we discuss how to introduce the assessment of the drivers of impact on pollinators within LCIA. Conclusions and recommendations for future agenda are reported in section 2.1.5.

### 2.1.2. Methodology

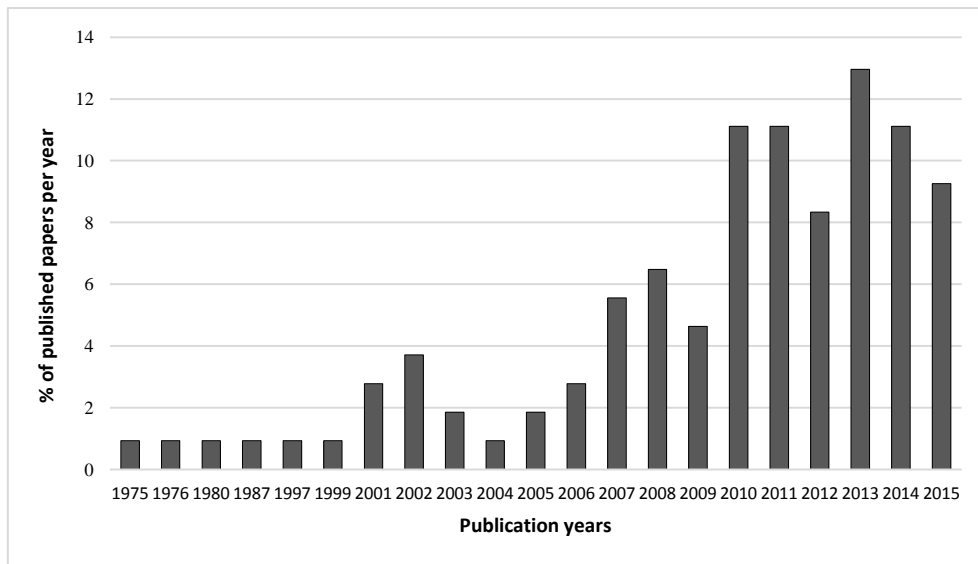
We conducted a review of scientific articles and reports focusing on evidence of impact on pollinator populations and pollination services. We carried out the literature search using the bibliographic database SCOPUS and the 'ConservationEvidence.com' website, a free authoritative information resource designed to support the protection of global biodiversity. We performed a preliminary search using headings based on combinations of broader terms related to pollination issues ((pollinator\* OR pollination) AND (decline\* OR loss\* OR threat\* OR impact\* OR risk\*)), in order to enable an early understanding of the current forces exerting pressures on pollinator populations. Then, in order to limit the results to the explicit impact drivers resulting from the preliminary search, we refined the search using more detailed criteria. We used relevant

and logical keywords referring to the specific impact driving forces as follows: ‘land use change’, (land OR habitat) AND (transformation\* OR degradation), ‘chemical emissions’, ‘pesticide\*’, ‘insecticide\*’, (invasive OR alien) AND species’, ‘invader\*’, ‘competition’, ‘climate change’, (phenological OR spatial) AND mismatch, ‘pests’, ‘pathogen\*’, ‘disease’, (electric OR magnetic) AND field\*’ and ‘electromagnetic radiation\*’, (GM OR genetically modified OR transgenic) AND crops. These keyword variations were combined with the above-mentioned broader terms on pollination issues using the Boolean command ‘AND’. The outputs included reviews, laboratory- and field-based studies, and scientific reports manifesting clear impacts on pollinator communities and pollination services and suggesting what ecological indicators are currently adopted to measure the effects of impact drivers on pollination systems. The great majority of the selected papers proceeded from peer-reviewed journals and publications of European Agencies, such as the European Food Safety Authority (EFSA) and European Academies’ Science Advisory Council. The publication years ranged from 1975 to date: we initially focused on recently published outputs (2001-2015); then, we opened a specific time window from 1975 to 2000 to include a wider variety of studies in terms of substances assessed (e.g. for ecotoxicity). We excluded studies reporting no documentation on the pressures which pollinators are subjected. We created a database (see Tables A1 and A2 in Appendix A) to enable efficient grouping and subsequent analysis of these studies. Information recorded included authors and publication date, brief paper description, impact driver categories, pollinator group affected, resulting effects on pollinators and their services, data type, modeling approach and indicators of impact and damage.

### 2.1.3. Review results: drivers and impacts responsible for insect pollinators’ decline

Applying to the abovementioned keywords and criteria, we selected 108 published studies investigating different drivers involved in the pollinator crisis. The analysis of the scientific outputs revealed that the published research in this area has recently increased (Figure 2.1). For instance, nearly 64% of the outputs were published from 2010 to the present (2015, with cut-off date on June 2015), about 30% between 2001 and 2009, leaving 6% of the outputs produced between 1975 and 2000 included.





**Figure 2.1.** Publications per year as selected in our review. X-axis reports the publication years of the literature search (from 1975 to 2015). Y-axis reports the relative number of published papers per each year, calculated as the percentage of selected paper per year divided by the total number of selected papers.

This increase can be attributable to the recent growth of awareness among the wider public towards the key role that pollinators play for the global food security and its socio-economic stability.

Of the total collected outputs, 29 were reviews, 15 scientific reports and 64 research articles, whose features are briefly described in Appendix A (Table A1). Nearly the totality of the retrieved reviews (22 out of 29) was monothematic, focusing on the identification and analysis of a single category of impact, whereas the remaining seven reviews had a more holistic approach. We referred to these latter outputs as “multi-impact” reviews, since they gave a comprehensive understanding of the main possible pressures contributing to the decline of insect pollinator populations. In some “multi-impact” reviews authors reported descriptive or experimental analyses of interactive effects between biotic and/or abiotic stressors on pollinators (see Table A3 in Appendix A). Amongst the selected reports, nine of them proceeded from European institutions such as the European Food Safety Authority (EFSA 2013a, b, c, d; 2014; 2015a, b, c) and the European Academies' Science Advisory Council (EASAC, 2015).

Authors investigated the relationships between human and environmental pressures and pollinator population declines through laboratory- and field-based experiments with the aim of identifying a cause-effect chain.

The majority of the selected papers tended to focus on the European honey bee (*Apis mellifera*), and to a lesser extent on bumblebees (*Bombus* spp.). Among non-Hymenoptera pollinators, dipterans, especially hoverflies (*Syrphidae* family), and lepidopterans resulted to be the most investigated (Table 2.1).

**Table 2.1.** Overview of the total number of outputs published for each type of investigated pollinator taxon.

\* a) Multi-impact, b) land occupation and transformation, c) ecotoxicity, d) presence of invasive alien plant species, e) competition with invasive alien pollinator species, f) climate change, g) pests and pathogens, h) electro-magnetic pollution and i) genetically modified crops.

\*\* One paper can cover one or several types of pollinator taxa. Therefore, in the last column, the sum of the number of papers for each pollinator type is not necessarily equal to the total number of papers.

	Impact driver categories *									Total **
	a	b	c	d	e	f	g	h	i	
Total n°. of outputs	16	23	23	7	5	11	9	10	4	108
N.° of papers on:										
Honeybees ( <i>Apis mellifera</i> )	10	19	20	5	1	4	8	10	4	81
Bumblebees ( <i>Bombus</i> spp.)	11	20	12	7	4	5	1	0	1	61
other Hymenoptera (solitary bees, wasps)	9	19	7	4	1	5	1	0	1	47
Coleoptera	3	5	1	2	0	2	0	0	0	19
Diptera	5	9	1	4	0	3	0	0	0	22
Lepidoptera	5	5	1	4	0	3	0	0	2	14
other or not specified pollinators	4	5	0	1	1	2	0	0	1	14

The review led to the identification of eight impact drivers menacing insect pollinator populations, namely: 1) intensified land use as a result of uncontrolled expansion of urban areas and modern agricultural practices; 2) use of pesticides; 3) presence of invasive alien plants; 4) competition with invasive alien pollinator species; 5) global and local climate change; 6) spread of pests and pathogens; 7) electro-magnetic pollution (including electro-magnetic radiations, electric charges and magnetic field fluctuations) and 8) genetically modified (referred to as GM) crops (Tables 2.2 and 2.3). For instance, nearly 21% of the outputs dealt with land use related issues, representing the most investigated impact driver, whereas GM crops and their potential impacts represent the least covered area, with only 4% of retrieved outputs. A more detailed analysis for each driver is reported in Table 2.4 and in the sections below.

**Table 2.2.** Total number and percentage of outputs, divided per impact category, reporting impacts on pollinator populations. Output types are reported for each impact category. Invasive alien plant and pollinator species have been included in a macro-category named “invasive alien species”; the category named “electro-magnetic pollution” includes electro-magnetic radiations, electric charges and magnetic field fluctuations.

Output type						
Impact driver category	Reviews	Reports	Research articles	Tot. n.° outputs	% outputs	
0 Multi-impact	7	4	5	16	14.8	
1 Land occupation and transf.	6	-	17	23	21.3	
2 Ecotoxicity	5	8	10	23	21.3	
3 Invasive alien plant species	-	-	7	7	6.5	Invasive alien species: 12 tot. outputs; 11.1%
4 Invasive alien pollinator species	2	-	3	5	4.6	
5 Climate change	3	-	8	11	10.2	
6 Pests and pathogens	5	2	2	9	8.3	
7 Electro-magnetic pollution	-	-	10	10	9.3	
8 Genetically Modified crops	1	1	2	4	3.7	
<b>Total</b>	<b>29</b>	<b>15</b>	<b>64</b>	<b>108</b>	<b>100</b>	

**Table 2.3.** Number of multi-impact outputs that report the effects of a specific impact driver category. Invasive alien plant and pollinator species have been included in a macro-category named “invasive alien species”; the category named “electro-magnetic pollution” includes electro-magnetic radiations, electric charges and magnetic field fluctuations.

\* Each multi-impact output deals with more than one driver; therefore, in the second and third columns, the sum of the number of paper for each driver does not necessary corresponds to the sum of multi-impact outputs.

Impact driver category	N°. of multi-impact outputs *	% of multi-impact outputs *	
<b>0 Multi-impact</b>	<b>16</b>	<b>100</b>	
1 Land occupation and transf.	9	56.3	
2 Ecotoxicity	11	68.8	
3 Invasive alien plant species	8	50.0	Invasive alien species
4 Invasive alien pollinator species	11	68.8	
5 Climate change	11	68.8	
6 Pests and pathogens	11	68.8	
7 Electro-magnetic pollution	2	12.5	
8 Genetically Modified crops	3	18.8	

**Table 2.4.** Summary of the potential direct and indirect effects of each impact driver category on insect pollinators and pollination services.

Impact driver category		Potential effects on insect pollinators and pollination services	
		Direct effects	Indirect effects
1	Land occupation and transformation	Loss of natural and semi-natural habitats meaning loss of favourable nesting sites and food supply; pollinators tend to remain in isolated fragments, which act as barrier to gene flow; subsequent loss of pollinator species richness and abundance	Local species extinction; consequent lower visitation rate to flowering plants in fragmented areas; resulting pollination deficit. Biotic homogenization, with loss of specialist pollinators; resulting pollination deficit Wild plant biodiversity loss due to the tendency of pollinators to forage in huge monoculture fields where the density of floral resources is higher than in natural margins; resulting pollination deficit
2	Ecotoxicity	Potential toxic lethal (i.e. premature individuals' death and colony collapse) and sub-lethal effects, due to poisoning for direct exposure to pesticide spray and dust or for ingesting contaminated pollen and nectar (e.g. disrupted foraging activity, impaired homing ability, reduced learning performances)	Pollination deficit as a result of pollinators' loss
3	Invasive alien plant species	Double effect: 1. Invasion by non-native plants, which compete with native plants for pollination. 2. Facilitation of both the survival of native pollinators when food resources are scarce, and native plant reproduction.	Reduced pollination success of native species Positive effect on pollination of native plants. Potential for hybrid formation, which may have poor germination rate and limited growth; resulting pollination deficit.
4	Invasive alien pollinator species	Competition for food resources and nest sites; displacement of native organisms toward less profitable forage leading to limited quantity of pollen carried to the hive.	Spread of exotic pests and pathogens causing infections leading in some cases to death; resulting pollination deficit.
5	Climate change	Loss of synchrony (phenological mismatch) between insect pollinator activity and flowering/fruitlet time; geographic shifts (e.g. migration) with species either losing or expanding their range.	Local species extinction with negative consequences on the structure and the functioning of plant-pollinator systems; resulting deficit in the provisioning of pollination ecosystem services; reduction in reproductive success and species richness of both pollinators and plants (e.g. reduced seed production in crop plants); competitive relationships among species or hybridization
6	Pests and pathogens	Infections predominantly reducing colony growth, disrupting foraging activity, orientation skills and behavioral performances.	Spread of other parasites, particularly virus, causing secondary infections leading in some cases to death; resulting pollination deficit.
7	Electro-magnetic pollution	Behavioral and physiological changes (e.g. increased aggressiveness, irritability and hyperactivity, increase in piping signal, disrupted homing ability, decline in colony growth, decrease in the activities of seminal enzymes in drones)	Death due to hyperactivity, disorientation and premature swarm; resulting pollination deficit.

Impact driver category	Potential effects on insect pollinators and pollination services	
8 Genetically Modified crops	Potential toxic sub-lethal effects similar to those caused by pesticide exposure, for ingestion of nectar/pollen containing toxins (e.g. antifeedant effect, reduced learning ability, altered flight activity).	Tendency of GM plants to hybridize with sexually compatible native plants, increasing the risk of plant diversity extinction; resulting loss of pollination services.

### 2.1.3.1. Land occupation and transformation

Recently, research has been focused predominantly on land use and the impacts on pollinator populations derived from its changes. The intensification of agricultural practices as well as the uncontrolled expansion of urban and sub-urban areas have severely modified the natural environment. Natural and semi-natural habitats have been deteriorated, with negative consequences for pollinators and their services (Burkle et al., 2013; Gonzalez-Varo et al., 2013; Kluser et al., 2010; Lautenbach et al., 2011; Ollerton et al., 2014; vanEngelsdorp and Meixner, 2010; Winfree et al., 2009). Almost all the authors agreed that monoculture expansion and the subsequent natural habitat fragmentation are the primary causes of pollinators' abundance and diversity loss (Holzschuh et al., 2011; Kells et al., 2001; Kluser and Peduzzi, 2007; Ricketts et al., 2008; Le Feon et al., 2010; Morandin and Winston, 2005; Rands and Whitney, 2010; Vanbergen et al., 2013, 2014; Winfree et al., 2011). The massive introduction of monoculture crops such as maize, oilseed rape and sunflowers has played a crucial part in reducing ecosystem biodiversity, leading to a significant decline of wild floral plant abundance and diversity which insect pollinators depend on for nesting and foraging (Holzschuh et al., 2011; Kennedy et al., 2013; Klein et al., 2007; Weiner et al., 2011). Extreme changes in landscape structure include the fragmentation of natural and semi-natural habitats associated with the expansion of agricultural crop fields. These changes result in the rise of barriers to gene flow between populations (Garibaldi et al., 2011; Goverde et al., 2002; Nielsen et al., 2012; Steffan-Dewenter and Tschardtke, 1999), potentially causing their isolation from one other, thus increasing the risk of pollinator species extinction in the long term (Kremen et al., 2007) and facilitating the disruption of plant-pollinator mutualisms with resultant severe pollination deficit. In fact, as a consequence of this progressive amalgamation at the landscape level in favor of monoculture croplands, insect pollinators have gone through a sort of "biotic homogenization", thereby altering the structure and the stability of plant-pollinator communities at local and regional scales (Carvalho et al., 2013; Rands and Whitney, 2010; Winfree et al., 2011). The pollination service is driven by both generalist and specialist pollinators. Both the two groups contribute to maintain biodiversity, which underpins pollination services. Their vulnerability to environmental and anthropogenic pressures is different due to their ecological traits: specialists are more susceptible to changes than generalists are, since they rely on limited varieties of plants for feeding and nesting. For instance, in the long term, specialist pollinator species that depend on floral and habitat resources threatened by land transformations, are expected to be lost in favor of generalist species, which in turn will dominate anthropogenic habitats (Donaldson et al., 2002; Steffan-Dewenter et al., 2002; Vanbergen et al., 2013; Winfree et al., 2011). Indeed, loss of specialist pollinators' species means loss of species

richness and abundance; it consequently means loss of a certain amount of pollination service, since generalists would not be able to completely supply pollination services provided by specialists.

The expansion of urban and sub-urban areas has similar negative effects on the environment and its inhabitants, as agricultural intensification. Ahrne et al. (2009) and Bates et al. (2011) observed that urban sprawl towards the countryside has a significant impact on flower-visitor communities. Indeed, the abundance of insect pollinator populations significantly changes through the urban-rural gradient, with mainly generalist species populating urban degraded sites.

#### 2.1.3.2. Ecotoxicity

According to the results of our review, pesticides represent another important threat to biodiversity of pollinators that visit cultivated fields and natural edges nearby. It has long been known that pesticides are a cause of concern for pollinators, especially for bees. The increasingly massive use of plant protection products in modern agriculture and their potential impacts on pollinators have received considerable attention especially over the last decades. Within the various classes of insecticides, recent research has been focused on neonicotinoids.

Almost all of the analyzed studies (such as Kessler et al., 2015) proposed an experimental approach, predominantly based on controlled experimental settings in laboratory, focusing almost exclusively on chronic oral exposure of adult bees. Honey bees and, to a lesser extent, bumblebees were fed with a sucrose solution containing field realistic, sub-lethal concentrations of pesticide, within the range found in crop nectar and pollen in the field, in order to evaluate the effects of the exposure as close as possible to real conditions. In some cases, such as in Gill et al. (2012) and Henry et al. (2012), experiments were performed under semi-field conditions: pollinators received contaminated nectar or pollen in laboratory and then were let free to move and forage into the field. Only some authors have investigated the effects of neonicotinoids on solitary wild bees, both under laboratory and field conditions, assessing both contact and oral exposure (Blacquiere et al., 2012; Brittain and Potts, 2011; Rundlof et al., 2015).

Pollinators are not target organisms of neonicotinoids, but they may recurrently be directly or indirectly exposed to such chemicals because of their foraging activities. As systemic pesticides, neonicotinoids are taken up by the plant and transported to all the tissues (leaves, flowers, roots and stems, as well as pollen and nectar). Both lethal and sub-lethal effects have been identified through the literature search. All the retrieved articles mentioned at least a sub-lethal effect, whereas registered evidence of seasonal individuals' mortality in pollinators was limited (e.g. Kessler et al., 2015). Laboratory- and field-based studies allowed authors to record disruption of some pollinators' abilities. In particular, chronic exposure to field realistic, sub-lethal concentrations of neonicotinoids can affect pollinator reproductive performance and social behavior, leading in some cases to loss of species richness and decline in population size (Sandrock et al., 2014). The majority of authors recorded altered foraging activities (EFSA 2013a, b, c, d; EFSA 2015a, b, c; EASAC, 2015; Gill et al., 2012; Kessler et al., 2015) or behavioral changes like impaired olfactory memory, learning dysfunction

and alteration of navigation skills leading to failure in the ability of relocating the hive (Blacquiere et al., 2012; Brittain and Potts, 2011; EASAC, 2015; Godfray et al., 2014; Goulson, 2013; Henry et al., 2012; vanEngelsdorp and Meixner, 2010). Especially in bumblebee colonies, authors observed reduced colony growth due to a decline in brood and queen production, potentially resulting in premature colony collapse (Gill et al., 2012; Godfray et al., 2014; Goulson, 2013; Rundlof et al., 2015; Whitehorn et al., 2012). The exposure to sub-lethal doses of neonicotinoids may also significantly elevate vulnerability to certain pathogens, as described in Doublet et al. (2015) and Pettis et al. (2012), increasing the mortality rate of bees.

Overall, neonicotinoids used on mass flowering crops may affect pollinator health and performance, but still represent a controversial topic in scientific and policy context. Based on scientific findings indicating that some insecticides belonging to neonicotinoid group showed high risks for bees (EFSA 2013a, c, d), in 2013 the European Commission restricted the use of three pesticides. However, even though there is strong evidence for important sub-lethal effects, to date there is little evidence outside controlled experimental settings (Godfray et al. 2014), being Rundlof et al. (2015) one of the few studies reporting field condition of the experiments.

Through a specific search for other plant protection products, we selected five additional papers showing effects on pollinators exposed to non-neonicotinoid pesticides. Similar to neonicotinoids, other pesticides such as pyrethroids, organophosphates and carbamates may alter learning, foraging and homing ability of pollinators and impair their biological development (Taylor et al., 1987; Thomson, 2003). Evidence of reduced survival in adult bees exposed to other pesticides different from neonicotinoids was registered as well (Balanca et al., 1997; Barker et al., 1980; Kevan, 1975).

### 2.1.3.3. Invasive alien species

Along with chemical emissions, invasive alien species (hereafter referred to as IAS) are considered another leading cause of biodiversity loss worldwide, after habitat alteration (EC-JRC, 2015). They are novel species in their non-native range that act through the modification of plant-pollinator communities, thus having mainly adverse effects such as resource depletion and competition (EC, 2014).

IAS, introduced accidentally or intentionally for economic purposes especially in the agricultural sector, may alter natural plant-pollinator communities and the structure of their networks. The majority of the studies showed that non-native plants generally invade and monopolize ecological interactions, competing with native plants for pollination, thus potentially provoking disruption of native species connections and reducing the pollination success of native species (Aizen et al., 2008; Brown and Mitchell, 2001; Brown et al., 2002; Chittka and Schurkens, 2001; Kluser and Peduzzi, 2007; Larson et al., 2006; Lopezaraiza-Mikel et al., 2007; Vanbergen et al., 2014). Only a few authors (Bartomeus et al., 2008; Muñoz and Cavieres, 2008) described how invasive alien plants might, in some cases, facilitate both the survival of native pollinators when food resources are scarce, and native plant reproduction. Indeed, non-native plants can attract native

pollinators to areas populated by both native and non-native species that otherwise they would not visit, positively affecting the pollination of native plants.

Even the introduction of alien insects can have strong impacts on native ecological communities. Non-native insects may prey on native pollinators (Monceau et al., 2014) or compete with them for floral resources or nesting sites (Goulson, 2003; Nagamitsu et al., 2010; Stout and Morales 2009; Thomson 2004, 2006; Traveset and Richardson, 2006; Vanbergen et al., 2013, 2014). Alternatively, they may modify native plant-pollinator communities, with a possible displacement of one or more native pollinator species towards other areas and leading to local losses of pollinator specialists. These events, associated with the increasing role of non-native generalist species and their potential to hybridize (Schweiger et al., 2010), may have alarming consequences such as biodiversity and pollination loss in natural ecosystems (Aizen et al., 2008; Stout and Morales, 2009). Non-native pollinators can also act as dispersal vectors of exotic parasites and related diseases, potentially leading to the collapse of native pollinators' colonies (Goulson, 2003; Traveset and Richardson, 2006).

#### 2.1.3.4. Climate change

Several authors have investigated how changes in climatic conditions are likely to affect plant-pollinator networks. Changes in climatic conditions may act on the occurrence of insect and plant species (Ewald et al. 2015), thus causing temporal or spatial mismatches between pollinator populations and the floral resources they rely on. These mismatches can potentially lead to local species extinction with expected consequences on the structure and the functioning of plant-pollinator systems and, as a result, on the provisioning of ecosystem services such as yield derived from pollinator-dependent crops (Bellard et al., 2012; Polce et al., 2014). Fluctuations in the flowering and fruiting periods and a general contraction of the growing season may partially or completely disrupt the natural time-sensitive relationships between plant blooming time and pollinator flight period, resulting in potential negative consequences which alter the rates of reproduction and survival of both plants and pollinators (Kluser et al., 2010; Robbirt et al., 2014). Indeed, under climate warming, plant and insect phenology may not respond equally to changes in climatic conditions, and the natural synchrony may be lost (Gordo and Sanz, 2005; Schweiger et al., 2010). Moreover, climate change may trigger modifications in the geographic distribution of floral resources, influencing the composition of pollinator populations and the spatial dislocation of processes like pollination (Polce et al., 2014; Vanbergen et al., 2014). As a result of the above-mentioned aspects, a decline in nectar production and pollen availability may occur, bringing about concerning consequences such as reductions in pollinator fitness and species richness (Le Conte and Navajas, 2008; Memmott et al., 2007; Petanidou et al., 2014) and declines in plant reproductive success (Hegland et al., 2009; Kudo and Ida, 2013). Climate-induced temporal and spatial shifts may therefore be particularly detrimental for specialized plant-pollinator mutualisms (Kuhlmann et al., 2012; Le Conte and Navajas, 2008; Polce et al., 2014).

As reported by Schweiger et al. (2010) and Pradervand et al. (2014), climate change may also affect morphological matching of plant and pollinator species, homogenizing morphological diversity and



modifying population patterns with the prevalence of more generalized species, which are more adaptable to climate variations.

#### 2.1.3.5. Pests and pathogens

During the last decades, the enormous increase in trading and the degradation of ecosystems caused by human activities such as the sprawl of urban or peri-urban areas and the expansion of intensive farming have facilitated the spread of parasites and other pathogens that may affect both managed and wild pollinators (Gonzalez-Varo et al., 2013; Kluser et al., 2010).

Most of the evidence on threats to pollinators from pathogens and diseases around the world comes from managed honeybees, which represent the model species in the nearly totality of retrieved papers. The analysis of the outputs highlighted that an assembling of pathogens has been clearly implicated in the so-called “Colony Collapse Disorder” (CCD), a recent documented phenomenon of sudden bee colony death, with a loss of healthy adult bees in the hives, that has occurred especially in Europe and North America (Kluser et al., 2010). Infections with the acarine mite *Varroa destructor* (Le Conte et al., 2010; Rosenkranz et al., 2010) and the small hive beetle *Aethina tumida* (Charrière, 2011; Cuthbertson et al., 2008; Ellis and Delaplane, 2008; FERA, 2013) are unanimously considered the most detrimental pathologies to honeybees worldwide, with high impact on terrestrial ecosystems (EC-JRC, 2015). Both parasites affect bee colonies predominantly reducing the number of adult foragers and increasing the mortality of brood (Ellis and Delaplane, 2008). Moreover, *V. destructor* contributes to transmit a broad array of other pathogens, particularly viruses such as Deformed Wing Virus (DWV), Acute Bee Paralysis Virus (ABPV), Israeli Acute Paralysis Virus (IAPV) and Kashmir Bee Virus (KBV) (Charrière, 2011; FERA, 2013; Kluser and Peduzzi, 2007; McMenamin and Genersch, 2015; Meeus et al., 2011; Vanbergen et al., 2014; vanEngelsdorp and Meixner, 2010), which are implicated in secondary infections leading to colony immune weakness and death. Microsporidia of the genus *Nosema*, such as *Nosema ceranae* (Dussaubat et al., 2013), and bacterial diseases such as European (Forsgren, 2010) and American foulbroods (EFB and AFB respectively), are other causes of increased mortality of infected bees and reduced performance and productivity of colonies (Charrière, 2011; Dussaubat et al., 2013; FERA, 2013; Vanbergen et al., 2013). All these diseases and disease-causing agents may potentially cause the failure of pollinator communities; with likely negative effects also on the services they provide, e.g. pollination of crop and natural vegetation.

#### 2.1.3.6. Electro-magnetic pollution

Most recent research has identified electro-magnetic pollution as a potential additional threat to insect pollinators. We decided to include electric charges, magnetic fields and electro-magnetic radiations in the same impact category because of their similar effects on insect pollinators, and a similar underpinning cause-effect chain.

The majority of studies dealt with electro-magnetic radiations and was carried out by the same authors who improved their own investigations with supplementary experiments in subsequent years. Honey bees were

unanimously chosen as a model organism since they are good biological indicators for electro-magnetic pollution (Ferrari, 2014). Radiations transmitted by cell towers and cell phones have been recognized to be the major sources of electro-magnetic pollution, significantly affecting the biological and physiological processes in bees (Kumar, 2012). There is clear evidence that honey bees exposed to high or low energy fields or electro-magnetic radiations tend to suffer dramatic behavioral and physiological changes in both laboratory- and field-based experiments. Exposed honey bees showed increased aggressiveness, irritability and hyperactivity (Dalio, 2015; El Halabi et al., 2013; Kumar et al., 2011; Warnke, 1976), resulting in a premature swarming process (Favre, 2011). Cell phone radiations can alter even navigational skills of bees: numerous authors measured statistically significant decreases in the number of adult bees returning to their colonies under field conditions (Dalio, 2015; El Halabi et al., 2013, 2014; Ferrari, 2014; Sahib, 2011; Sharma and Kumar, 2010). Several authors observed also that colonies exposed to electro-magnetic pollution were subjected to a strong decline in their brood productivity with a reduction in egg laying rate of queen (Dalio, 2015; El Halabi, et al., 2013, 2014; Sahib, 2011). In addition, Kumar et al. (2011) and Kumar (2012) recorded a considerable increase in the concentration of biomolecules such as carbohydrates, proteins and lipids in the semen and a significant decrease in the activities of seminal enzymes in drones exposed to electro-magnetic radiations from cell phones. These deviations from the normality represent clear signs of disturbance in the normal physiology of drone semen. Hence, there is concern that changes in reproductive behavior and physiology of insect pollinators may potentially lead to inadequate mating and reproduction, both of which can further contribute to the global pollinator crisis.

#### 2.1.3.7. Genetically Modified crops

Potential impacts on pollinators and their services associated with the expansion of the currently commercialized genetically modified (GM) crops correspond to the least covered area, with an exiguous number of retrieved papers. This limited quantity of outputs is probably due to the barely recent interest within scientific circles about the safety of GM crops. The effects of GM crops are studied mainly on honey bees, chosen unanimously as model organisms under controlled conditions.

GM crops were developed as a substitute for pesticides in order to ensure crop yield and plant health: enabling plant species to produce naturally occurring pesticides, for instance, allows them to become resistant to the actions of certain pest insects, without the need to use insecticides (Sanvido et al., 2007). However, this ultimate purpose of crop protection has raised concerns that commercial transgenic crops with insecticidal properties would result in potential adverse effects on the environment, especially on flower-visitor insects. Currently, there is little evidence of sub-lethal effects linked to toxicity of Bt-proteins (*Bacillus thuringiensis* toxins). Bt-proteins are toxins with insecticide properties commonly used for the production of GM crops; they can be traced in nectar and pollen, with potential negative effects on non-target insects feeding on them. Only a few experiments showed negative consequences for pollinators' behavior, such as reduced foraging efficiency and disrupted learning performances (Han et al., 2010; Malone and Burgess, 2009; Ramirez-Romero et al., 2008; Sanvido et al., 2007). Beside their potentially toxic effects,

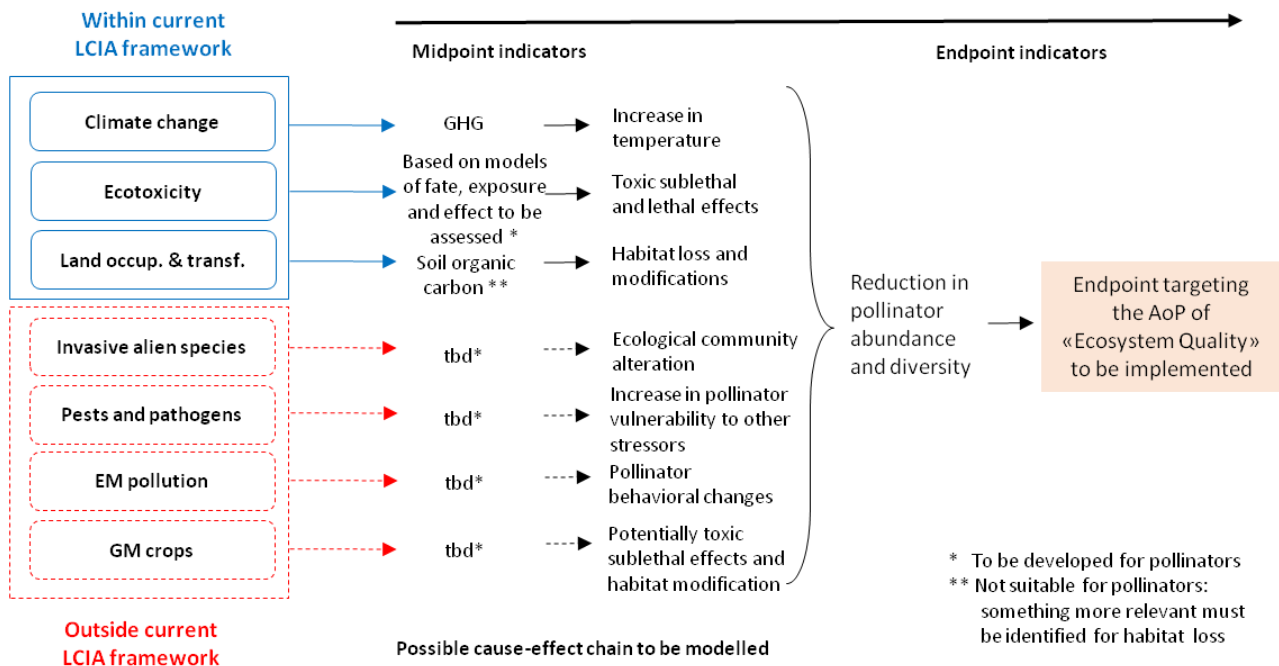
which in the long-term would tend to reduce pollinator populations, GM crops may also act as a pressure in indirect ways: their tendency to hybridize with sexually compatible native plants may increase the risk of plant diversity extinction (Sanvido et al., 2007), consequently leading to contingent pollinator and pollination losses.

Actually, studies related to transgenic crops gave controversial results, since the toxicity depends on the real exposure level of organisms. There is ambiguous evidence that GM plants, which constitutively express insecticide properties, have such negative impacts on pollinators (Kluser and Peduzzi, 2007; vanEngelsdorp and Meixner, 2010). Some authors, such as Morandin and Winston (2005), recognize the urgent need to study more deeply this topic, to manage agroecosystems and to promote the sustainability of food production.

#### 2.1.4. Pollinators in LCA: where we are and where to go

Despite the recognized importance of pollinators and the services they deliver for human well-being and for the maintenance of terrestrial biodiversity, current LCIA frameworks appear missing these components. In fact, considering the LCIA frameworks of several LCA methods currently used (e.g. CML (2002); ReCiPe 2008 (Goedkoop et al., 2009), LIME (Itsubo and Inaba, 2003), Impact 2002+ (2002), TRACI (Bare, 2002), ILCD (EC-JRC, 2011), pollinators are not considered by any approach as target organisms of any impact. Even the most advanced proposals, for example for land use (Chaudarhy et al., 2014; Verones et al., 2015) do not include pollinators, predominantly because of the lack of data on species richness and geographic range. However, in the last years, LCA specialists from the UNEP/SETAC life cycle initiative have advanced several LCIA models to characterize land use-driven impacts on ecosystem services (Koellner et al., 2013). Despite there is no mentioning to pollination services, those models are certainly more conceptually advanced than current LCIA operational methods like ReCiPe or ILCD. To date, the most advanced attempts to include ecosystem services are those of Koellner and Geyer (2013) and Saad et al. (2011, 2013). Recently, the models proposed by Saad et al. (2013) have been implemented in Impact world+ (2015), which is the only methodology presenting an area of protection devoted to resources and ecosystem services. The only approach that specifically mentions pollinators is EcoLCA (Zhang et al., 2010a, 2010b), using an input-output framework (Baral et al., 2012). In fact, the authors introduced, for the first time, a life cycle framework to assess the dependency of target industrial sectors on pollination services and the model is under further development (Chopra et al., 2015). EcoLCA is probably the most advanced life cycle-oriented approach to link pollination services to economic/technological systems. However, the model is not fully operation for what concerns the quantification of impacts on pollinators.

Overall, the different available LCIA frameworks incorporate some of the above-mentioned threats to pollinators as impact categories (i.e. land use, ecotoxicity and climate change, see Figure 2.2) while lacking an impact pathway leading to assess damage on pollinators. Besides, some threats are completely missing, namely an impact category is not existing, although there is evidence of potential environmental concern related to the topic.



**Figure 2.2.** Identified drivers of impacts on pollinators. In some cases, an impact category already exists within the traditional LCIA framework (blue boxes), whereas in other cases new impact categories should be included (red boxes). Reduction in the provision of ecosystem services, such as pollination, may lead to subsequent loss in the global economic system, nutrition supply and genetic resources. EM=Electro-magnetic; GM=Genetically modified; AoP=Area of Protection

In detail, assessing e.g. the ILCD LCIA framework, the threats that are already included are:

- Climate change. Assuming that a midpoint indicator as GWP (Global Warming Potential) is useful, from midpoint to endpoint a link with pollinators is missing. In models such as LIME (Itsubo and Inaba, 2003), where biotic production is taken into account, the role of pollinators might be considered as intermediated step in the cause-effect chain leading to a reduction in productivity.
- Ecotoxicity. Over the last years, freshwater species and relative responses to chemical emissions have received the most attention in LCIA (Curran et al. 2011). Although models assessing terrestrial biodiversity responses to chemical pollution exist, they do appear to be unsuitable for pollinators as well as for the area of protection related to ecosystem quality. The current consensus model for ecotoxicity in LCA (USEtox, 2015) is a multimedia box model, which calculates three components: fate, exposure and effects on freshwater organisms for a given chemical emitted into the environment. USEtox is applied for calculating characterization factors as a result of the multiplication of a fate factor, an exposure factor and an effect factor. Each of these three elements needs an adaptation for pollinators. In fact, there is need of an improved estimation of plant uptake for some substances (e.g. neonicotinoids), the definition of equations reflecting the peculiar elements of the exposure pathways of pollinators (e.g. contact exposure (Barmaz et al., 2010)), and the calculation of effect factors for pollinators that are not currently included. Thereby, integrating fate, exposure and effects of chemicals affecting pollinators in the ecotoxicity models is of high priority. This will allow us to better assess impacts in terrestrial ecosystems, especially the agricultural ones.

- Land occupation and transformation (commonly referred to as land use changes in the most of models). Notwithstanding the role of habitat loss and fragmentation is increasingly discussed and considerable efforts have been recently made with the proposal of novel methodologies aiming at assessing land use related biodiversity impacts (Maia de Souza et al., 2015; Teixeira et al., 2015), current LCIA models are still unable to capture impacts at landscape level, e.g. accounting for relevant elements of habitat composition and configuration (Maia de Souza et al., 2013; Teixeira et al., 2015) such as the presence or absence of field margins in agroecosystems. A new approach is necessary to integrate in the inventory those features that highlight the loss of relevant pollinator habitats in the current land use models (such as field margins). Representing the most important resources of food and nesting sites for all pollinators (Kells et al., 2001; Rands and Whitney, 2010), field margins and their role should be taken into consideration. Additional future challenges are related to inventory issues: it is necessary to improve life-cycle inventories, including land management details as mentioned, in primis, presence and typology of field margins. These improvements would move the approach from a “field focus” to a “landscape focus”, enabling us to better represent the characteristics of the landscape such as the variety of its habitats, in other words representing a landscape as a mosaic rather than through each single piece.

Other four drivers of impacts are currently missing: invasive alien species, pests and pathogens, electromagnetic pollution and GM crops. From an LCA point of view, there is a potential of linking processes and products with: invasive alien species (e.g. traded goods and risk of invasive species introduction, as in Hulme, 2009), GM crops and electro-magnetic fields (e.g. associated with the presence of specific infrastructure in a system). Despite being a relevant source of impacts, pests and pathogens are more difficult to be linked to a specific process or product, therefore their inclusion in the impact framework is unlikely.

Finally, current LCIA framework does not effectively account for the functional role of pollinators in providing pollination services at endpoint level. The existing indicators for biodiversity are based on data of species richness (PDF/PAF = Potentially Disappeared/Affected Fraction of species), but they do not take into account the functional aspect of biodiversity in the landscapes. Ecosystem services need to be introduced in the current LCIA framework. Thereby, a further goal is to overcome the classical biodiversity measurements in the LCIA framework to embrace novel concepts, such as those related to functional diversity (Maia de Souza et al., 2013) for land use related impacts: species are not equal as they offer a wide range of functions supporting ecosystem processes, which in some cases are not replaceable. Functional diversity has a strong ecological importance, since it influences ecosystems’ dynamics and consequently socio-economic productivity, being a more understood option for an ecosystem service indicator. Current biodiversity land-use modeling tends to oversimplify the real dynamics and complexity of the interactions of species among each other and with their habitats (Maia de Souza et al. 2015). Of course, the inclusion of pollinators may need to expand the elements currently covered by the area of protection “ecosystem quality”, checking whether current metrics are suitable for expressing and then aggregating ecosystem-related results.

There are serious conceptual shortcomings in the way the current models are built. It is necessary to overcome the existing weaknesses, setting new models based on meaningful and robust indicators of impact

and damage for biodiversity. These should cover not only the part related to ecosystem diversity, but also the key role that some species such as pollinators play and that could not be replaced if lost.

#### 2.1.5. Conclusion and outlook

This review contributes to our current understanding of the factors leading to pollinator populations' declines and represents the first step to overcome problems related to the lack of appropriate LCIA models for assessing impacts on biodiversity. Our study aims at bridging ecological and environmental sciences and global product strategies. We discussed existing conceptual and methodological gaps between LCIA and the assessment of key ecosystem services, such as pollination.

Several authors have long recognized the main drivers of impact acting on pollinators, potentially threatening also pollination services. Intensive agricultural practices are responsible for the majority of the identified threats, which are 1) intensified land use as a result of uncontrolled expansion of urban areas and modern agricultural practices, 2) use of pesticides, 3) presence of invasive alien plants; 4) competition with invasive alien pollinator species; 5) global and local climate change; 6) spread of pests and pathogens; 7) electromagnetic pollution (including electro-magnetic radiations, electric charges and magnetic field fluctuations) and 8) genetically modified crops.

Notwithstanding the importance of pollination for environmental and socio-economic reasons, existing LCIA methods and models appear to be incomplete with respect to pollinators. This is principally due to a general lack of knowledge on how different anthropogenic pressures affect changes in pollinator biodiversity and pollination services, and on how species diversity is connected to ecosystem functioning and human well-being. Therefore, there are specific research needs towards the integration of pollinators as a target group for biodiversity protection in the LCIA framework. Firstly, future investigations are to be oriented to improve the models and the indicators currently used in the LCIA framework. Thus, it is of high priority integrating within inventories those features that highlight the loss of relevant pollinator habitats in the current land use models as well as the fate, exposure and effects of the chemicals affecting pollinators in current models of ecotoxicity. Then, for other categories of impacts, novel models and indicators both at midpoint and endpoint levels should be developed to cover the existing conceptual and methodological gaps. Particularly, new impact categories and related models should be developed and the feasibility of including them in the LCIA methodology should be assessed.

We also investigated models and indicators proposed in the studies we selected for the review; however, easily implementable models are not yet available. The only exception would be for ecotoxicity, where the procedure proposed by Barmaz et al. (2010) could be used for estimating the exposure of pollinators to plant protection products. The authors developed a procedure for predicting pesticide exposure for pollinators based on the foraging behavior of honeybees (*Apis mellifera*). This approach is overcoming the current official procedures to assess pesticide risk -based on a Hazard Quotient- and may be a starting point for

integrating the assessment of pollinators in multimedia box models used in LCA (such as USEtox), particularly for calculating the exposure factor.

Moreover, given that at the endpoint level, different target organisms are considered for different impact categories (e.g. plants, freshwater organisms, mammals etc), the use of indicators of impact for pollinators may be a promising unifying endpoint for different impact categories.

Considering the role of crucial ecosystem services for sustaining life, including impact on pollinators is an impelling step for increasing the comprehensiveness of LCA. The services provided by pollinators represent an important function supporting the global food security and its socio-economic stability. Thereby, accounting for them is fundamental in any effort aiming at achieving sustainable growth and sustainable use of natural resources.

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## 2.2. Part II: Development of characterization factors for insect pollinators

Chapter developed during the Erasmus Traineeship at the Technical University of Denmark (DTU) in the research group of Assoc. Prof. Peter Fantke.

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## Abstract

Over the last decades, insect pollinators' populations have suffered significant decline in abundance and diversity. Especially, honey bee colonies have experienced large-scale and rapid losses of adult workers, a phenomenon called Colony Collapse Disorder (CCD), with adverse feedback on the development of the bee hives. This has raised concerns about a potential global crisis in the agro-food sector. In this context, the use of agricultural pesticides along the supply chains has been identified as one of the main contributing causes for CCD. In fact, due to the dependency of insect pollinators on a large number of crops for collecting nectar and pollen, where pesticide residues can accumulate, there is high potential for pollinating insects to be exposed to these pesticides.

Life Cycle Assessment (LCA) aims at comprehensively covering all relevant environmental impacts and insect pollinators start to become a relevant issue in this context. The impact pathway associated with the exposure of insect pollinators to agricultural pesticides has not been developed yet and no underlying models to characterize this type of impacts in Life Cycle Impact Assessment (LCIA) are available. Conceptual models for agrochemical risk assessment are generally used outside the LCA context for addressing issues related to pollinators' safety; however, they are not enough. In a world characterized by a rapid increase in the demand for goods and services, products-related impacts on pollinators linked to the massive use of pesticide (for the purpose of incrementing agricultural yields and food production) should be taken into consideration in order to quantify the actual impacts that insect pollinators are subject to along the supply chains. Therefore, given the relevant ecological and socio-economic role of insect pollinators on the global scale and the importance of better characterizing the toxicity-related pressures along the agro-food supply chains, impacts on insect pollinators need to be accounted for in the existing models for ecotoxicity in LCIA. To address this need, we developed an impact characterization framework to quantify the in-field exposure of forager honey bees (i.e. *Apis mellifera*, chosen as target species) to agricultural pesticides and related potential ecotoxicity impacts. We applied the modeling framework in a first illustrative case study to characterize the impacts of two selected pesticides on honey bees: an insecticide (lambda cyhalothrin) and a fungicide (boscalid) applied to oilseed rape. We observed a relatively higher impact of the insecticide on forager honey bees with respect to the fungicide application; in fact, the impact score calculated for lambda cyhalothrin is three orders of magnitude higher than the one of boscalid. According to our results, a specific type of forager bees, namely nectar foragers, is the most affected for both pesticides. The case study supports the identification of areas of improvement for the model in order to improve applicability and wider implementation in LCIA and potentially other frameworks where pollinator-related exposure is relevant.

### 2.2.1. Introduction

Over the last decades, wild and managed insect pollinators have been declining in abundance and diversity (Potts et al., 2010). Especially honey bee colonies have experienced large-scale and rapid losses of adult workers, a phenomenon called Colony Collapse Disorder (CCD) (Watson e Stallins, 2016), with adverse feedback on the development of the hives, thus raising concerns about a potential global crisis for the

agricultural industry and consumers. In this context, the use of agricultural pesticides, here referred to as the biologically active ingredient in commercial plant protection products, has been identified as one of the main contributing causes for CCD (Goulson et al., 2015; Woodcock et al., 2017).

In Europe, the authorization of pesticides and their use are strictly regulated (EU, 2009). Moreover, the promotion of good agricultural practices (GAP) attempts ensuring a high level of protection of the environment. However, due to the dependency of insect pollinators on a large number of crops for collecting nectar and pollen – where pesticides can accumulate after root and leaf uptake and subsequent translocation into other crop components –, there is high potential for pollinating insects to be exposed to pesticides.

Given the important economic and agronomic role of insect pollinators on the global scale and the importance of better characterizing the toxicity-related pressures along the agro-food supply chains, as highlighted in Crenna et al. (2017) [see the previous section 2.1], impacts on pollinating insects need to be further investigated and accounted for in the existing impact assessment models.

Traditionally, the impact assessment is conducted in the context of risk assessment. Models for estimating the potential exposure of pollinating insects to pesticides have been developed over the years considering different exposure pathways (e.g. dermal contact and dietary) (Barmaz et al., 2012; Poquet et al., 2014; Baveco et al., 2016; Sanchez-Bayo e Goka, 2016), with the aim of predicting the pressures of pesticides on pollinators. However, none of these models has been developed to deal with pesticide exposure in a way coherent with conditions of the life cycle impact assessment (LCIA) framework. In fact, the existing models are rather conceptual models for agrochemical risk or product safety assessment, generally based on the worst-case analysis procedure that diverges from the typical exposure assessment underpinning environmental impact assessment methodologies embracing all stages of a supply chain, such as life cycle assessment (LCA). On the other hand, LCIA-based models are adopted in order to comprehensively depict impacts associated to certain production and consumption patterns while informing on the most environmental sustainable performance profile. So far, ecotoxicity in the LCIA context has covered mainly freshwater and hypogean terrestrial organisms as starting point to quantify the impacts in terrestrial ecosystems deriving from chemical emissions. In fact, LCIA has traditionally focused on freshwater species and relative responses to chemical emissions, disregarding the potential impacts related to terrestrial ecosystems, particularly the air compartment (Rosenbaum et al., 2008). However, in a world characterized by a rapid increase in the demand for agricultural-based goods and services (e.g. food and its derivatives that we daily use), impacts on pollinators linked to the massive use of pesticides for the purpose of increasing agricultural yields and food production should be taken into consideration. In this context, risk assessment-oriented models are not enough, since they are not comparative and do not help minimizing impacts that insect pollinators are subject to along all the stages of a supply chain. LCIA-aligned models do allow for a comparison among products, hot-spotting the main drivers of impacts along the supply chains of those products that we daily consume or use. Therefore, in order to better support decision making along the agro-food supply chains and address the ecotoxicity of chemicals in LCA in a consistent and more comprehensive

way also covering the terrestrial ecosystems, it becomes of high priority to understand and quantify the exposure of insect pollinators to pesticides and the related adverse effects.

To address this need, the present study aims at characterizing impacts of agricultural pesticides on honey bees (*Apis mellifera*), chosen as target species, consistently with the LCIA framework. We defined three specific objectives, namely (i) to develop an impact characterization framework to quantify the exposure of honey bees to agricultural pesticides and related negative ecotoxicological effects, (ii) to calculate a set of ingestion and dermal exposure factors for different types of honey bees within the worker caste, referred to as honey bee forager types, and (iii) to apply the framework in an illustrative case study to characterize impacts for two selected pesticides applied to oilseed rape (*Brassica napus*).

### 2.2.2. Methodology

LCIA is a methodological framework that connects emissions into the environment with impacts on three areas of protections, including natural ecosystems. One of the first steps is the identification of the so-called “environmental cause effect chain”. For the toxicity-related impact categories, LCIA requires to enable the calculation of fate, exposure and effects (Rosenbaum et al., 2008).

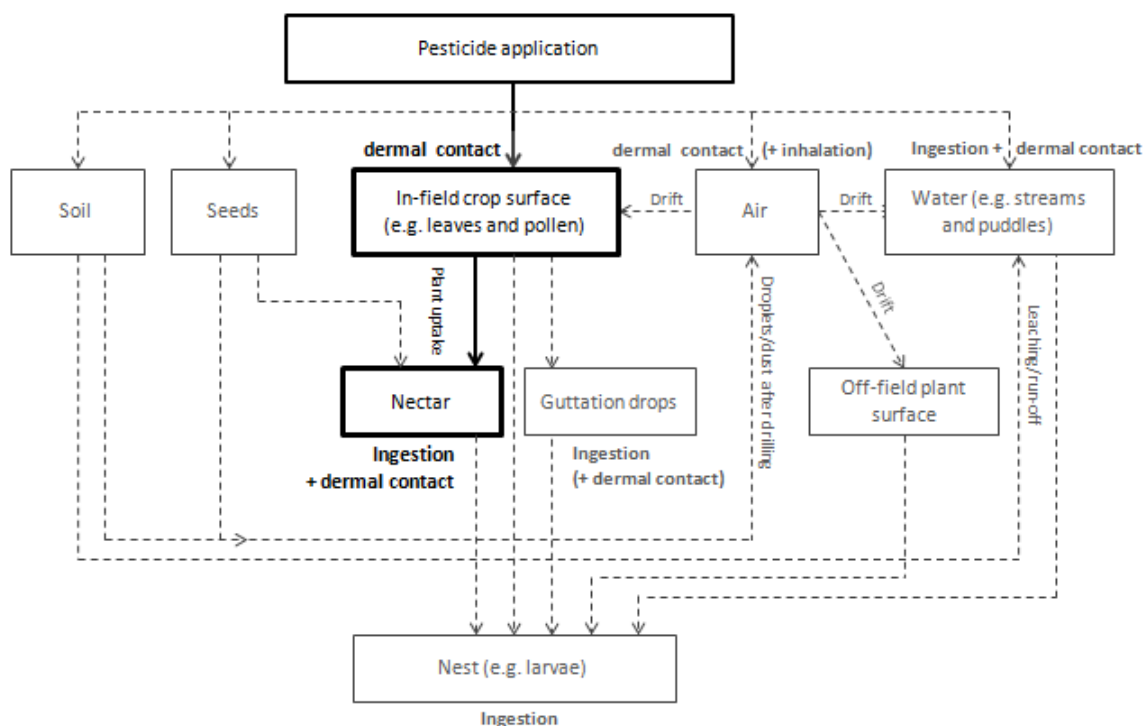
The pollinator ecotoxicity characterization framework presented in this study was developed according to the following steps:

- review of possible exposure pathways of insect pollinators to agricultural pesticides;
- development of an exposure model suitable for being integrated into the LCIA framework;
- specification of honey bees exposure pathways towards the calculation of dermal and ingestion exposure, as well as the pesticide dissipation rates from nectar and pollen; and
- calculation of effect factors for honey bees.

A case study has been conducted to test the developed model and to highlight future improvements needed toward full integration into the existing LCIA characterization framework.

#### 2.2.2.1. Overview of fate and exposure pathways for insect pollinators

The exposure of insect pollinators to agricultural pesticides has been extensively studied and various exposure pathways are reported in the literature (Rortais et al., 2005; Johnson et al., 2010; Thompson, 2012; Sanchez-Bayo and Goka, 2016). A summary of the fate and exposure pathways is presented in Figure 2.3.



**Figure 2.3.** Conceptual overview of the possible exposure pathways of insect pollinators to agricultural pesticides. The main exposure pathway, i.e. the focus of this study, is presented in bold. Other potential exposure pathways, including transport processes, are presented with dashed lines.

Depending on the pesticide application method (e.g. foliar spray, seed treatment, etc.) and on their intrinsic physico-chemical properties, pesticides can distribute into different environmental compartments and persist there, making residues available to honey bees and other insect pollinators (EFSA, 2012). There are different ways through which pollinating insects can be exposed to agricultural pesticides, whose relevance generally depends on the life stage of the organisms (Thompson, 2012). Exposure can be (i) via direct dermal contact (e.g. when insects fly in the field while spraying, although the use of pesticide during the flowering period of crops – i.e. the activity period for pollinators – is normally forbidden by the regulations (EU, 2009; Renzi, 2013); (ii) via indirect dermal contact, i.e. through contact with treated surfaces (e.g. contaminated pollen, nectar, water and guttation fluids, which are collected both in- and off-field by insect pollinators (Krupke et al., 2012; Kasiotis et al., 2014)) or through dust dispersed after spray or seed treatments; and (iii) via ingestion of contaminated pollen, nectar and water.

Direct dermal contact with contaminated pollen and ingestion of residues found in nectar represent the most relevant exposure pathways for the foragers of honey bees and other pollinating insects, which are in charge of the most energy-demanding tasks (Sanchez-Bayo and Goka, 2016; Sponsler and Johnson, 2017); while the exposure of larvae is predominantly via ingestion of residues in processed pollen and nectar (Rortais et al., 2005; Thompson, 2012).

#### 2.2.2.2. Characterization framework for honey bees' exposure to pesticides

The framework for characterizing honey bees' exposure to pesticides includes a mathematical model that allows quantifying the exposure of honey bees to agricultural pesticides via different pathways and the associated adverse effects. In fact, the characterization factors (CFs, in equation 1) resulting from the model, determine the number of affected honey bees per mass of pesticide applied in the agricultural environment, thus allowing the comparison of the results of impact assessments across a broad variety of agricultural pesticides.

The CFs [(bees<sub>affected</sub> ha<sup>-1</sup>) / (kg<sub>applied</sub>·ha<sup>-1</sup>)], whose underpinning approach follows the environmental cause-effect chain on which is built the LCIA modeling (as presented at the beginning of the methodology section), are calculated from ecotoxicity effect factors (EF) for honey bees and intake fractions, adapting to bees the human intake fraction initially defined by Bennett et al. (2002). Specifically, the CFs introduce novel metrics for honey bees, named *dermal uptake fraction* and *ingestion intake fraction*, shortened as  $uF(\Delta t)$  and  $iF(\Delta t)$  respectively, which include environmental fate and exposure processes together.

$$CF = uF(\Delta t) \times EF_{\text{dermal}} + iF(\Delta t) \times EF_{\text{oral}} \quad (\text{Eq. 1})$$

where  $uF(\Delta t)$  [(kg<sub>uptake</sub> ha<sup>-1</sup>) / (kg<sub>applied</sub> ha<sup>-1</sup>)] and  $iF(\Delta t)$  [(kg<sub>intake</sub> ha<sup>-1</sup>) / (kg<sub>applied</sub> ha<sup>-1</sup>)] give information on the mass of pesticide taken up via respectively dermal or ingestion exposure by the organisms (i.e. the honey bees, differentiated per type) per unit of applied pesticide over a given exposure duration  $\Delta t$  (days);  $EF_{\text{dermal}}$  [bees<sub>affected</sub>/kg<sub>uptake</sub>] is the effect factor characterizing the contact exposure (i.e. dermal) of the honey bee foragers and  $EF_{\text{oral}}$  [bees<sub>affected</sub>/kg<sub>intake</sub>] represents the effect factor for the oral exposure (i.e. ingestion) of honey bees. The elements of the CFs equation (1) and the assumptions behind are extensively described in the following sections 2.2.2.3 and 2.2.2.4.

Our characterization model focuses on the in-field exposure of honey bees to pesticides, which depends on several factors, including the specific exposure pathway (in bold in Figure 2.3), the characteristics of the organisms (e.g. their tasks in the colony, their behavior in field), the physico-chemical properties of the pesticide, the environmental conditions and the crop species. The behavior of honey bees foraging in field is well documented in literature (Mohr e Jay, 1988; Corbet et al., 1991; Abou-Shaara, 2014). Moreover, the crop field, especially when mass-flowering crops such as sunflower and oilseed rape are set as monoculture, represents the area where honey bees are active the most due to the abundance of food and energy resources. According to the existing LCIA framework (EC-JRC, 2011), we assume for most impact categories steady state conditions, namely we aim at parameterizing the influential factors contributing to bee exposure variability, such as seasonal fluctuations and the change in the foraging resources, that may push the colony to adjust the ratios of individual bees engaged in the different tasks (Robinson, 1992). We thus consider honey bees acting as individuals (individual-based modeling), in a crop field rich in food resources, i.e. during the flowering period of crop species, which does not have a specific spatial extent or shape, according to the recommendations of the Glasgow consensus (Rosenbaum et al., 2015).

Our characterization framework uses simple differential equations which are based on the assumption that a fixed number of honey bees forage only in the crop field, showing flower constancy as individuals (Bohart and Nye, 1956), and following a simple set of rules, namely: (i) they fly out of the nest, also called beehive, to a patch of flowers, (ii) collect pollen or nectar at the flowers in the crop field, (iii) fly back to the nest, (iv) unload the food at the beehive and then (v) set out again on their next trip. The nest is imaginarily located at the edges of the field, considering that, when food is enough abundant in the vicinity of the nest, honey bees forage within a radius of 1 km (Seeley, 1995; Villa et al., 2000). Therefore, the CFs account for the exposure of honey bees to the pesticide residues in pollen and nectar while foraging, flying loaded back to the nest, and finally during the unloading inside the beehive.

In this context, we collected information on the most relevant aspects of honey bees' in-field behavior (e.g. foraging activity, flying period and time, etc.), and on pesticides application (e.g. application time and period, etc.) mainly from the ecological literature, pesticide labels and risk assessment reports.

In order to better compare the contribution of pesticides to ecotoxicity to honey bees, we also quantified the fraction of bees affected per application, identifying an impact score (IS) as follows:

$$IS [\text{bees}_{\text{affected}}/\text{ha}] = CF [\text{bees}_{\text{affected}}/\text{kg}_{\text{applied}}] \times m_{\text{applied}} [\text{kg}_{\text{applied}}/\text{ha}] \quad (\text{Eq. 2})$$

From this, we then derived the potentially affected fraction (PAF) of honey bees as:

$$\text{PAF} [\% \text{ affected bees}] = IS [\text{bees}_{\text{affected}}/\text{ha}] / N [\text{bees}/\text{ha}] \quad (\text{Eq. 3})$$

#### 2.2.2.3. Honey bees' exposure pathways

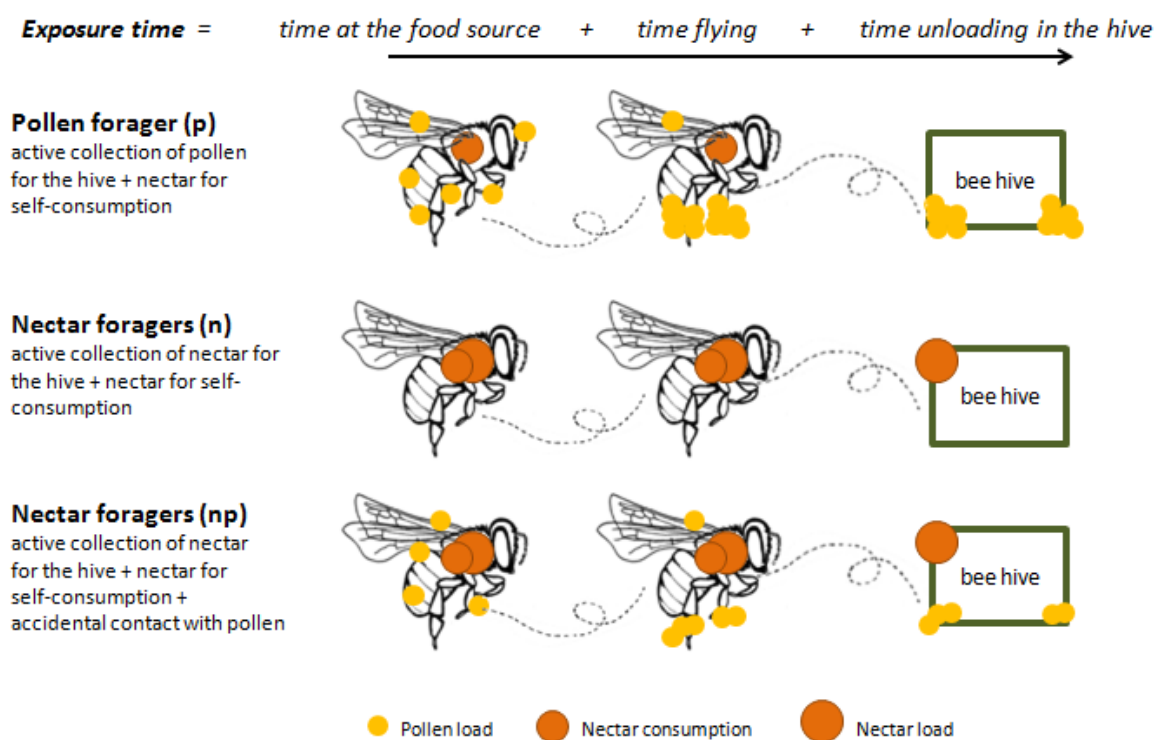
Division of labor in honey bee colonies is characterized by tasks performed by specialized individuals. Our characterization framework is applied to two main types of honey bees within the worker caste, namely (i) pollen foragers and (ii) nectar foragers, as foragers generally aim either for pollen or for nectar. However, nectar foragers may get unintentionally in touch with pollen (Bohart and Nye, 1956) (Figure 2.4). We quantify the exposure of pollen and nectar foragers separately, considering pollen and nectar as different pesticide residue compartments, due to their different behavior in the field and inside the hive, as explained in the following sections.

Pollen foragers use their mandibles or legs to move the anthers of the flowers, allowing the pollen to stick to the hair that covers their body. Then, the bees clean themselves, packing the pollen grains in form of balls, together with some nectar used as glue. The pollen balls, called pollen load, are stored in the baskets on the hind legs. When the full load is reached, the bees return to the hive, where the pollen is unloaded in cells (Seeley, 1995). While, nectar foragers use their tongues to suck the nectar out of the flowers, found in the nectarines which usually are at the base of the flower, and store it in a secondary stomach, called "honey stomach", which is separated from the digestive stomach (Bohart and Nye, 1956). As soon as the honey stomach is full, nectar foragers return to the hive and transfer the nectar to the other non-foraging workers. Depending on the shape of the flower and on the foragers' attitude, nectar foragers may also get in contact with the anthers and pollen grains can stick to their body hair. For example, honey bees that forage on oilseed rape plants can either push their tongues between the petals "thieving" nectar from the side without

contacting the anthers or enter directly the front of the flowers getting in contact with pollen (Westcott and Nelson, 2001). Then, as pollen foragers do, nectar foragers pack pollen in balls on the hind legs, to be then brought to the hive (Kleinjans et al., 2012). Generally, nectar foragers return to the hive due to a full nectar load before the pollen baskets are full (Bohart and Nye, 1956).

Notwithstanding honey bees' efficiency in cleaning their body hair, some pollen grains can remain on the bees' bodies during foraging, enhancing crop pollination (Kleinjans et al., 2012). For the sake of model simplicity, the exposure of honey bees via these residues is considered negligible, as well as the exposure to the nectar used for sticking pollen grains into the baskets.

Additionally, all honey bees' foragers collect nectar for self-consumption, since foraging and flying activities are energy demanding tasks.



**Figure 2.4.** Exposure of honey bees' forager types to pesticide residues in pollen and nectar along their foraging trips.



For the sake of clarity, in Table 2.5 we summarize the keywords used along the manuscript in the modeling framework.

**Table 2.5.** Relevant keywords used in the presented modeling framework

<b>Keywords</b>	<b>Definition</b>
Pollen	Protein rich food, collected from flowers
Nectar	Sugar rich food, collected from flowers
Pollen forager	Honey bee forager type that collects actively pollen and delivers it to the hive
Nectar forager	Honey bee forager type that collects actively nectar, either with or without getting in contact with pollen, and delivers it to the hive. In case of pollen contact, the fraction of nectar foragers collecting pollen delivers also pollen to the hive
Pollen load	Amount of fresh pollen, in shape of balls formed by honey bees, stored on both the hind legs in the pollen baskets and carried to the hive as a source of food for the colony, especially for the larvae
Nectar load	Amount of fresh nectar stored by a honey bee in the so-called honey stomach (or secondary stomach) and carried to the hive as a source of food for the colony, especially for the larvae
Nectar consumption	Amount of fresh nectar taken in by all the honey bees forager types as a source of immediate energy for supporting foraging and flying activities.
Exposure time fraction	Fraction of time over 24 hours, during which individual honey bee forager types are exposed to agricultural pesticides, via dermal contact of either pollen or nectar.
$uF(\Delta t)$	Dermal uptake fraction, i.e. the amount of a certain pesticide transferred via contact exposure from pollen or nectar load to the surface (i.e. either skin or honey stomach) of the specific honey bees forager type, per unit of the same pesticide applied in the crop field, over a certain exposure time. It is measured as $(\text{kg}_{\text{uptake}} \text{ha}^{-1}) / (\text{kg}_{\text{applied}} \text{ha}^{-1})$
$iF(\Delta t)$	Ingestion intake fraction, i.e. the amount of pesticide taken in via consumption of nectar by honey bees per unit of the same pesticide applied to the crop field. It is measured as $(\text{kg}_{\text{intake}} \text{ha}^{-1}) / (\text{kg}_{\text{applied}} \text{ha}^{-1})$

The calculations of dermal uptake fraction  $uF(\Delta t)$  and ingestion intake fraction  $iF(\Delta t)$  are presented in sections 2.2.2.3.1 and 2.2.2.3.2 respectively, followed by section 2.2.2.3.3 concerning the estimate of dissipation rates of pesticide residues in pollen and nectar.

#### 2.2.2.3.1 Calculating dermal exposure

Dermal exposure occurs when an exposed population of honey bees gets in contact with contaminated pollen or nectar via body contact after a given exposure duration. Contact may occur externally (i.e. at skin level, via pollen contact) or internally (i.e. at secondary stomach or “honey sack” level, via nectar contact). Therefore, the dermal uptake fraction  $uF(\Delta t) [(\text{kg}_{\text{uptake}} \cdot \text{ha}^{-1}) / (\text{kg}_{\text{applied}} \cdot \text{ha}^{-1})]$  describes the exchange of residual mass of a pesticide applied to a crop from the food/energy source (i.e. pollen/nectar) to the honey bees’ external or internal surface during a given exposure time per unit of applied pesticide. Dermal exposure fraction is calculated in Eq. 4 for both types of honey bees within the worker caste, namely: (i) pollen



foragers (p) and (ii) nectar foragers (n), as the fraction of chemical transferred to the honey bees' body divided by the original applied mass:

$$uF(\Delta t) = \frac{N_i \cdot M_{i,j} \cdot fr_i \cdot \int_{t_0}^{t_1} C_{x,y}^j dt}{m_{x,y}^{appl}} \quad (\text{Eq. 4})$$

where  $N_i$  [bees/ha] is the density of the specific type of honey bee foragers on field, for  $i \in \{p, n\}$ ;  $M_{i,j}$  [kg/(bee. d)] is the daily load carried by each specific type of honey bee forager, for  $i \in \{p, n\}$  and  $j \in \{\text{pollen, nectar}\}$ ;  $fr_i$  [d/d] represents the daily exposure time fraction for  $i \in \{p, n\}$ , namely the fraction of time over a day during which a forager honey bee is exposed to pesticide residues;  $\int_{t_0}^{t_1} C_{x,y}^j dt$  [(kg/kg).d] for  $j \in \{\text{pollen, nectar}\}$  is the integral mean value of the residual concentration of pesticide  $x$  in nectar/pollen of crop species  $y$  within the flowering period, multiplied by the exposure period (i.e.  $\Delta t = t_1 - t_0$  [days]); and  $m_{x,y}^{appl}$  [kg<sub>applied</sub>/ha] is the applied mass of pesticide  $x$  to crop  $y$ .

Additionally,  $uF(\Delta t)$  is calculated for quantifying the exposure of the fraction of nectar foragers which accidentally may get in contact with pollen ( $i=np$ ).

$N_i$  depends on the general characteristics of a colony, i.e. size and structure, which in turn rely on several external and internal factors, such as the availability of food and, in case of managed colonies, on beekeeping practice. Honey bee colonies are dynamic; it means that the colony's worker population can vary in size and structure over time depending mainly on the season and on the needs of the hive. However, in the LCIA context, we consider an average fixed fraction for each type of honey bee forager, according to the available ecology-based literature (Table 2.6). The average density of bees per each forager type is finally derived as the product of the fraction of the specific honey bee forager type  $i$  [%], the fraction of foragers out of a colony [25.5%], the average hive population [18500 bees/hive] and the hive density in the field [3 hives/ha]. This latter parameter is mainly linked to beekeeping practice. We adopted an average value of hives per hectare for the representative crop species of our case study, i.e. oilseed rape.

**Table 2.6.** Numerical values used for building the model with regard to the main scenario-independent, constant parameter  $N_i$ . When available in literature, the range of variation is reported.

Parameters	Unit	Value	Range of variation
Fraction of honey bee pollen foragers ( $i=p$ ), out of the total forager population in an average hive [1]	%	25	-
Fraction of honey bee nectar foragers ( $i=n$ ), out of the total forager population in an average hive [1]	%	75	-
Fraction of honey bee nectar foragers potentially in contact with pollen ( $i=np$ ), out of the total forager population in an average hive [1]	%	17	-
Fraction of forager honey bees, out of the total population of honey bees in an average hive [2]	%	25.5	-
Number of bees per hive during the spring/summer period [3]	bees/hive	18500	7000 to 30000
Number of hives per hectare recommended for oilseed rape crop fields [4; 5]	hives/ha	3	2 to 4

[1] (Adeva, 2012); [2] (Torres et al., 2015); [3] (Van Der Steen, 2015); [4] (Mcgregor, 1976); [5] (Westcott and Nelson, 2001)

$M_{i,j}$  varies according to honey bee forager type and their specific foraging behavior (Table 2.7).  $M_{i,j}$  for  $j$ =pollen corresponds to an average full pollen load for pollen foragers ( $i=p$ ); while nectar foragers which get in contact with pollen ( $i=np$ ) generally return to the hive before the pollen baskets are full (Bohart and Nye, 1956). Therefore, for the former we set this parameter at the average amount of pollen daily carried by individual honey bees, while for the latter we set the value at the minimum amount of pollen load found in the literature, since no more details are available at this stage. The value of  $M_{i,j}$  for  $j$ =nectar is fixed at the average daily nectar load for all nectar foragers.  $M_{i,j}$  is finally obtained as product of the average pollen or nectar load per trip [ $\text{kg}_{\text{uptake}}/(\text{bee. trip})$ ] and the average number of trips per day made by the forager honey bees [trips/d].

**Table 2.7.** Numerical values used for building the model with regard to the main scenario-independent, constant parameter  $M_{i,j}$ . When available in literature, the range of variation is reported.

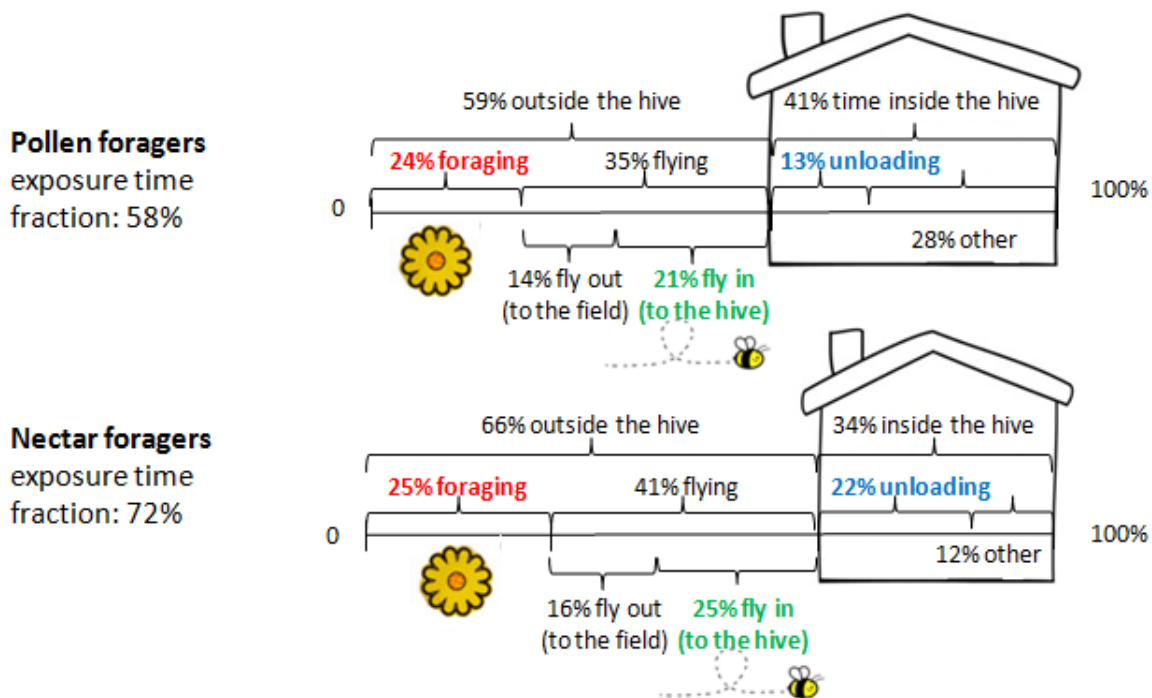
Parameters	Unit	Value	Range of variation
Average pollen load per trip carried by an individual pollen forager ( $i=p$ ) [1; 2]	$\text{kg}_{\text{uptake}}/(\text{bee. trip})$	$2.00 \times 10^{-5}$	$1 \times 10^{-5}$ to $3 \times 10^{-5}$
Average pollen load per trip carried by an individual nectar forager in contact with pollen ( $i=np$ ) [1; 2]	$\text{kg}_{\text{uptake}}/(\text{bee. trip})$	$1.00 \times 10^{-5}$	-
Average nectar load per trip carried by an individual nectar forager ( $i=n$ ) [1; 2]	$\text{kg}_{\text{uptake}}/(\text{bee. trip})$	$3.25 \times 10^{-5}$	$2.5 \times 10^{-5}$ to $4 \times 10^{-5}$
Average number of trips per day for both honey bee pollen and nectar foragers [3]	trips/d	10	-

[1] (Godfray et al., 2014) ; [2] (Van Der Steen, 2015); [3] (Rortais et al., 2005)

The exposure time fraction  $fr_i$  [d/d] is derived in Eq. 5 as the fraction of time over a day that an individual honey bee spends collecting, actively or not, pollen and nectar in the crop field, flying back into the nest and unloading:

$$fr_i = fr_{\text{foraging}} + fr_{\text{flying\_in}} + fr_{\text{unloading}} \quad (\text{Eq. 5})$$

The foraging behavior is derived from the field of ecology and the exposure time fraction depends on the honey bees' forager type, i.e. pollen or nectar forager (Figure 2.5). For the fraction of honey bees' nectar foragers that may get in contact with pollen ( $i=np$ ), we assume the same foraging behavior inside and outside the hive as all the other nectar foragers ( $i=n$ ).



**Figure 2.5.** Exposure time fractions for the specific honey bees' forager types during a whole foraging trip, according to their type-specific behavior in field.

Generally, pollen foragers spend more time in the hive after unloading compared with nectar foragers, which in turn spend more time in the hive from the arrival until the end of the unloading process. This is mainly due to the fact that pollen foragers need less time to end the unloading because they do not need to wait for a store bee, namely they go directly to the combs and unload the pollen in the empty cells. However, it takes more time to them for preparing leaving the hive since they communicate with other bees in order to receive information on the needs of the hive for pollen (trophallactic interactions) (Seeley, 1995). Additionally, both nectar and pollen foragers make the same number of trips to the flowers over a day (10 trips per day on average, see Table 2.7), but pollen foragers spend less time in the field for getting a full pollen load. In fact, pollen foragers make shorter trips (10 minutes per trip on average, see Table 2.8) since they travel shorter distances compared to nectar foragers (55 minutes per trip on average, see Table 2.9) (Rortais et al., 2005).

Nectar foragers are in charge of more energy- and time-demanding tasks, spending more time in the field for foraging.

The estimate of the fractions of time spent inside versus outside the hive is based on the study of Seeley (1994). We assume that nectar foragers behave as the studied foragers which fly long distances from the hive, i.e. to a far feeder; while pollen foragers behave as the foragers flying shorter distances, i.e. to a near feeder. The fraction of time spent flying back to the nest is principally based on the speed that a honey bee can reach while it is loaded with food, according to Kacelnik et al. (1986); whereas, the fraction of unloading time is calculated according to the in-field data from Seeley (1995) and Weidenmüller and Tautz (2002) for pollen foragers and from Seeley et al. (1991) for nectar foragers. Detailed information, namely average values and their ranges of variation as reported in literature, and the specific calculations are reported in Table 2.8 and 2.9.

**Table 2.8.** Numerical values used for building the model with regard to the main scenario-independent, constant parameter  $fr_i$  for pollen foragers. When available in literature, the range of variation is reported.

Parameters	Unit	Value	Range of variation*
Average time per round trip (Park, 1929)	min.d <sup>-1</sup>	20	10 to 30
Fraction of time outside the hive, out of the total round trip time (Seeley, 1994)	%	59 <sup>(a)</sup>	58 to 60%
Fraction of time spent flying, out of the time spent outside the hive (Seeley, 1994)	%	35 <sup>(b)</sup>	34 to 36%
Fraction of time flying out (i.e. from the hive to the food source), out of the total time spent flying while outside the hive (Kacelnik et al., 1986)	%	13 <sup>(c)</sup>	-
Fraction of time flying in (i.e. from the food source back to the hive), out of the total time spent flying while outside the hive (Kacelnik et al., 1986)	%	21 <sup>(d)</sup>	-
Fraction of time spent foraging at the food source, out of the time spent outside the hive (Seeley, 1994)	%	24 <sup>(e)</sup>	-
Fraction of time spent inside the hive, out of the total round trip time (Seeley, 1994)	%	41 <sup>(f)</sup>	40 to 42%
Fraction of time exposed in the hive (i.e. from the arrival to the end of the unloading process), out of the total time spent in the hive (Weidenmüller e Tautz, 2002)	%	13 <sup>(g)</sup>	11 to 15%
Fraction of time spent in other activities after the end on the unloading process, out of the total time spent in the hive (Weidenmüller e Tautz, 2002)	%	28 <sup>(h)</sup>	26 to 30%
$fr_{i=p}$	d/d	0.08 <sup>(i)</sup>	-

- (a) Calculated as average of the ratios between the time spent outside the hive and the time of a round trip for near-feeder cases, as  $(143s/246s + 133s/223s)/2$
- (b) Calculated as average of the ratios between the total time spent flying and the time spent outside the hive for near-feeder cases, multiplied by the percentage obtained from (a), as  $(82s/143s + 80s/133s) \times 59\%$
- (c) According to (Kacelnik et al., 1986), the speed of a unloaded honey bee is 8 m/s, while the flying speed linearly decreases at 5 m/s when the honey bee is loaded with 36 mg of sugar solution. We assume that a honey bee carrying a full load of pollen has the same speed, as confirmed by a beekeepers' association (PRBKA, 2017). Assuming a unit distance food-hive (1 m/trip), we calculate the time per trip that takes a honey bee when unloaded (0.13 s/trip, i.e. 38% out of the total time spent flying) and loaded (0.20 s/trip, i.e. 62% out of the total time spent flying). Therefore, the fraction of time flying out with respect to the time spent outside the hive is derived as 38% multiplied by 35% (i.e. the fraction obtained from (b))
- (d) According to (c), the fraction of time flying in is derived as 62% multiplied by 35% (i.e. the fraction obtained from (b))
- (e) Calculated as (a)-(b), i.e. 59%-35%
- (f) Calculated as difference between 100% (i.e. fraction of a total round trip) and 59% (i.e. fraction obtained from (a), referred to the fraction of time spent outside the hive out of a total round trip)
- (g) Calculated as average of the ratios between the time spent from the arrival at the hive up to the end of the unloading process and the total time spent in the hive for each case reported, as  $(80s/290.9s + 109.7s/316.2s + 89.9s/247.3s + 139.6s/375.5s + 100.6s/362.6s + 86.3s/278.3s)/6=32\%$ . This fraction is confirmed also in (Seeley, 1995). Then, the fraction of exposure time in the hive out of the total time spent in the hive is derived as 32% multiplied by 41% (i.e. the fraction obtained from (f), which is the fraction of time spent inside the hive, out of the total round trip time).
- (h) According to (g), the fraction of time spent in other activities after the end on the unloading process is derived as  $(1-32\%)$  multiplied by 41% (i.e. the fraction obtained from (f), which is the fraction of time spent inside the hive, out of the total round trip time)
- (i) Calculated as  $[20 \text{ min/trip} \times 10 \text{ trip/d} \times (21\% + 24\% + 13\%)]/1440 \text{ min/d}$
- \* calculated by considering the minimum and maximum value or fraction for each parameter.

**Table 2.9.** Numerical values used for building the model with regard to the main scenario-independent, constant parameter  $fr_i$  for nectar foragers. When available in literature, the range of variation is reported.

Parameters	Unit	Value	Range of variation*
Average time per round trip (Rortais et al., 2005)	min.d <sup>-1</sup>	55	30 to 80
Fraction of time outside the hive, out of the total round trip time (Seeley, 1994)	%	66 <sup>(a)</sup>	65 to 67%
Fraction of time spent flying, out of the time spent outside the hive (Seeley, 1994)	%	41 <sup>(b)</sup>	39 to 42%
Fraction of time flying out (i.e. from the hive to the food source), out of the total time spent flying while outside the hive (Kacelnik et al., 1986)	%	16 <sup>(c)</sup>	-
Fraction of time flying in (i.e. from the food source back to the hive), out of the total time spent flying while outside the hive (Kacelnik et al., 1986)	%	25 <sup>(d)</sup>	-
Fraction of time spent foraging at the food source, out of the time spent outside the hive (Seeley, 1994)	%	25 <sup>(e)</sup>	25 to 26%
Fraction of time spent inside the hive, out of the total round trip time (Seeley, 1994)	%	34 <sup>(f)</sup>	33 to 35%

Parameters	Unit	Value	Range of variation*
Fraction of time exposed in the hive (i.e. from the arrival to the end of the unloading process), out of the total time spent in the hive (Seeley et al., 1991)	%	22 <sup>(g)</sup>	19 to 25%
Fraction of time spent in other activities after the end on the unload process, out of the total time spent in the hive (Seeley et al., 1991)	%	12 <sup>(h)</sup>	9 to 15%
$fr_{i=n}$	d/d	0.28 <sup>(i)</sup>	-

(a) Calculated as average of the ratios between the time spent outside the hive and the time of a round trip for far-feeder cases, as  $(294s/440s + 233s/359s)/2$

(b) Calculated as average of the ratios between the total time spent flying and the time spent outside the hive for far-feeder cases, multiplied by the percentage obtained from (a), as  $(184s/298s + 140s/233s) \times 66\%$

(c) According to (Kacelnik et al., 1986), the speed of a unloaded honey bee is 8 m/s, while the flying speed linearly decreases at 5 m/s when the honey bee is loaded with 36 mg of sugar solution. We assume that a honey bee carrying a full load of nectar has the same speed, as confirmed by a beekeepers' association (PRBKA, 2017). Assuming a unit distance food-hive (1 m/trip), we calculate the time per trip that takes a honey bee when unloaded (0.13 s/trip; 38% out of the total time spent flying) and loaded (0.20 s/trip, i.e. 62% out of the total time spent flying). Therefore, the fraction of time flying out with respect to the time spent outside the hive is derived as 38% multiplied by 41% (i.e. the fraction obtained from (b))

(d) According to (c), the fraction of time flying in is derived as 62% multiplied by 41% (i.e. the fraction obtained from (b))

(e) Calculated as (a)-(b), i.e. 66%-41%

(f) Calculated as difference between 100% (i.e. fraction of a total round trip) and 66% (i.e. fraction obtained from (a), referred to the fraction of time spent outside the hive out of a total round trip)

(g) Calculated as average of the ratios between the time spent from the arrival at the hive up to the end of the unloading process and the total time spent in the hive for each case reported, as  $(68s/91s + 50s/70s + 46s/68s + 64s/115s)/4 = 66\%$ . Then, the fraction of exposure time in the hive out of the total time spent in the hive is derived as 66% multiplied by 34% (i.e. the fraction obtained from (f), which is the fraction of time spent inside the hive, out of the total round trip time)

(h) According to (g), the fraction of time spent in other activities after the end on the unloading process is derived as  $(1 - 66\%)$  multiplied by 34% (i.e. the fraction obtained from (f), which is the fraction of time spent inside the hive, out of the total round trip time)

(i) Calculated as  $[55 \text{ min/trip} \times 10 \text{ trip/d} \times (25\% + 25\% + 22\%)]/1440 \text{ min/d}$

\* calculated by considering the minimum and maximum value or fraction for each parameter.

The residual concentration of pesticide  $x$  in pollen and nectar of crop  $y$  over the considered exposure period,

$\int_{t_0}^{t_1} C_{x,y}^j dt [(\text{kg/kg}).\text{d}]$ , depends on the dissipation rate of the pesticide in pollen and nectar along the

flowering period of crop species  $y$ , which in turn depends on the physico-chemical properties of the pesticide. The details on the estimate of the dissipation rate are presented in section 2.2.2.3.3.

### 2.2.2.3.2 Calculating ingestion exposure

Ingestion exposure occurs when a honey bee gets in contact with contaminated nectar via ingestion.

The ingestion intake fraction  $iF(\Delta t)$  [(kg<sub>intake</sub> · ha<sup>-1</sup>)/(kg<sub>applied</sub> · ha<sup>-1</sup>)] for honey bees is calculated in Eq. 6

for both pollen foragers and nectar foragers, as follows:

$$iF(\Delta t) = \frac{N_i \cdot Q_i^{nectar} \cdot \int_{t_0}^{t_1} C_{x,y}^{nectar} dt}{m_{x,y}^{appl}} \quad (\text{Eq. 6})$$

where  $N_i$  [bees/ha] is the density of the specific type of honey bee foragers on field, for  $i \in \{p, n\}$ ;  $Q_i^{nectar}$

[kg/(bee · d)] for  $i \in \{p, n\}$  is the daily nectar consumption rate;  $\int_{t_0}^{t_1} C_{x,y}^{nectar} dt$  [(kg/kg) · d] is the integral mean

value of the residual concentration of pesticide  $x$  in nectar of crop species  $y$  within the flowering period, multiplied by the exposure period (i.e.  $\Delta t = t_1 - t_0$  [days]); and  $m_x^{appl}$  [kg<sub>applied</sub>/ha] is the application rate of pesticide  $x$  to crop  $y$ .

As for the dermal uptake fraction,  $N_i$  depends on the specific type of honey bee foragers and on the general characteristics of a colony. Therefore, it is calculated following the same procedure explained in previous section 2.2.2.3.1 (Table 2.6).

$Q_i^{nectar}$  depends on the activities of the honey bees, thus being honey bees forager type-specific. Its value is given by the average consumption rate according to the USEPA Guidance for Assessing Pesticide Risks to Bees (USEPA, 2014), which provides specific information on the amount of nectar consumed by each type of honey bee forager (Table 2.10). We do not refer to EFSA guidelines (EFSA, 2013), since the data reported by EFSA are not forger type-specific, i.e. the consumption rate of nectar is not differentiated according to the pollen or nectar foraging activity.

**Table 2.10.** Numerical values for scenario-independent, constant parameter  $Q_i^{nectar}$  used in the model, referred to nectar consumption. Source: USEPA, 2014.

Parameters	Unit	Value	Range of variation
$Q_i^{nectar}$ for $i=p$	kg <sub>intake</sub> /(bee · d)	$4.35 \times 10^{-5}$	$3.5 \times 10^{-5}$ to $5.2 \times 10^{-5}$
$Q_i^{nectar}$ for $i=n$	kg <sub>intake</sub> /(bee · d)	$2.92 \times 10^{-4}$	-

As for the dermal exposure, the residual mass of pesticide  $x$  in pollen and nectar of crop species  $y$  over the

exposure period,  $\int_{t_0}^{t_1} C_{x,y}^j dt$  [(kg/kg) · d], depends on the dissipation rate of the pesticide in pollen and nectar

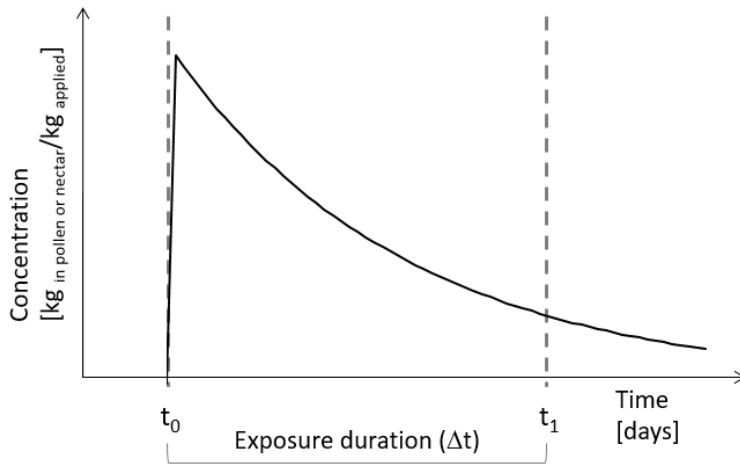
during the flowering period, which in turn depends on the physico-chemical properties of the pesticide. The details on the estimate of the dissipation coefficient are presented in section 2.2.2.3.3.

### 2.2.2.3.3 Calculating dissipation rate from nectar and pollen

To solve the equations (4) and (6), we need the respective dissipation rates  $k_{x,y}^j$  [ $d^{-1}$ ]. Under the assumption of first-order kinetics, the dissipation of the pesticide is expressed according to the following equation:

$$C_{x,y}^j(t) = C_{x,y}^j(t_0) \cdot e^{(-k_{x,y}^j \cdot t)} \quad (\text{Eq. 7})$$

where  $C_{x,y}^j(t_0)$  [kg/kg] is the initial concentration of pesticide  $x$  in pollen or nectar of crop  $y$ , and  $k_{x,y}^j$  [ $d^{-1}$ ] represents the first-order rate constant for the exponential dissipation of the pesticide in pollen and nectar over time (Figure 2.6).



**Figure 2.6.** Example of pesticide residual concentration over the exposure duration, according to first-order kinetics.

To calculate  $k_{x,y}^j$ , we started from studies reporting measurements of the residual concentration (kg/kg) in pollen and nectar at different times after pesticide application, which is the mass of a residual pesticide per mass of a collected pollen or nectar sample measured. We estimated  $k_{x,y}^j$  in pollen and nectar separately, by the linear least-square regression in Eq. (8):

$$\ln[C_{x,y}^j(t)] = \ln[C_{x,y}^j(t_0)] - k_{x,y}^j \cdot t \quad (\text{Eq. 8})$$

thus  $k_{x,y}^j$  [ $d^{-1}$ ] graphically represents the slope of the line fitting the experimental residual pesticide data in pollen/nectar.

Finally, solving the integral referred to the mean integral concentration of pesticide over the exposure period in Eq. (4) and (6), we obtain:

$$\int_{t_0}^{t_1} C_{x,y}^j dt = \frac{C_{x,y}^j(t_0)}{k_{x,y}^j} \left[ e^{-k_{x,y}^j t_0} - e^{-k_{x,y}^j t_1} \right] \quad (\text{Eq. 9})$$



#### 2.2.2.4. Ecotoxicity effect factors for honey bees

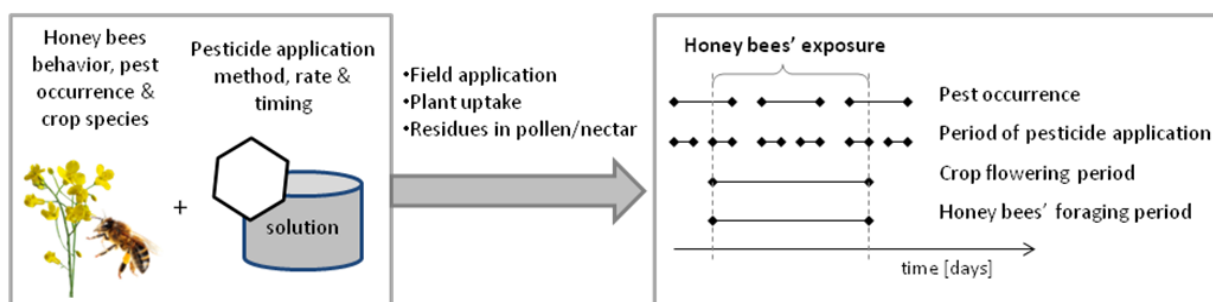
The effect factor (EF) [bees/kg] converts the uptake/intake fractions into the number of affected honey bees per kilogram of pesticide applied. This factor depends on the toxicity that the pesticide exerts on bees and is derived for both dermal and ingestion exposure, as follows:

$$EF_s = \frac{0.5}{(LD_{50}^s)} \quad (\text{Eq. 10})$$

where  $LD_{50}^s$  [kg/bee] for  $s \in \{\text{dermal, ingestion}\}$  is the amount of pesticide taken up or in by an exposed honey bee population, that affects 50% of the exposed bee population over background terms of lethal effects.  $LD_{50}^s$  are generally available from acute oral and contact toxicity tests, conducted on adult worker honey bees under the environmental risk assessments for the European registration process. However, in LCA a long-term perspective is considered and hence lifetime exposure and related chronic effects in bees would be needed. To the authors' knowledge, data from chronic oral and contact toxicity tests on adult forager honey bees are not available in the current literature; therefore in this study we assume that acute  $LD_{50}$  represent chronic  $LD_{50}$ .

#### 2.2.2.5. Case study

We applied the characterization framework to a case study, for which we chose oilseed rape (*Brassica napus*) as reference crop species. Honey bees are the main pollinators of oilseed rape and can account for up to 95% of all insect pollinators of this crop (Viik, 2012). In fact, oilseed rape has a high level of attractiveness for honey bees due to its abundant production of pollen and nectar (Free and Nuttall, 1968; Westcott and Nelson, 2001; Nedić et al., 2013) and it represents one of the most cultivated crops in Europe. We collected data as presented in Figure 2.7.



**Figure 2.7.** Overview of the information collected for the case study on the exposure of honey bees' foragers to selected pesticides applied on oilseed rape (*Brassica napus*) in the European context.

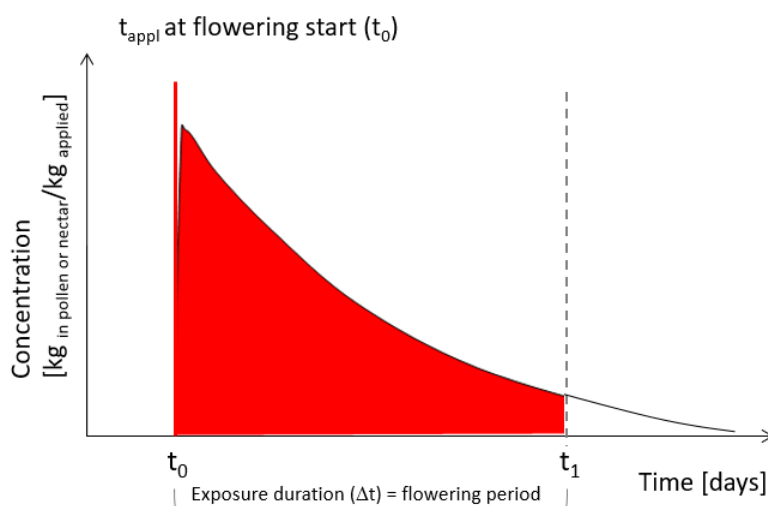
First of all, we retrieved information on the possible pests occurring on oilseed rape in Europe during its flowering period (Williams, 2010), which corresponds to the honey bees' active foraging season. According to the exposure pathways identified in section 2.2.2.1, we considered only pesticides applied as foliar spray treatment. We identified a set of two pesticides, namely lambda-cyhalothrin (insecticide pyrethroid, CAS 91465-08-6) and boscalid (fungicide carboxamide, CAS 188425-85-6), which are authorized in the European

Union and registered by the Member States to be applied on the blooming oilseed rape plants and, in particular lambda cyhalothrin, are extensively studied for effects on bees. We collected data on their application rate. Then, due to the fact that flowering period of oilseed rape may differ between countries according to the weather conditions, we estimated an average flowering scenario for oilseed rape in Europe (Table 2.11), by consulting the AppDate software (Klein, 2012). AppDate was developed for calculating reasonable application dates for different crops at selected locations in Europe based on crop life-cycle stages.

**Table 2.11.** Average duration of the flowering period for oilseed rape (*Brassica napus*) in Europe, based on Klein et al. (2012).

Crop	Location	Average start flowering period (Julian day)	Average end flowering period (Julian day)	Average duration flowering period	Duration uncertainty range
oilseed rape	Europe	134	159	24 days	8 to 32 days

The length of the exposure period, measured in terms of number of days, is necessary for calculating the cumulative residual concentration in pollen and nectar over the entire exposure period. By setting the application time of pesticide in an example scenario at the beginning of the flowering period ( $t_0$ ), we define our scenario of exposure. In this scenario, we assume a single application at the beginning of flowering period, as a conservative scenario (see example in Figure 2.8). According to the GAP, a second application is not always necessary and it generally falls outside the flowering period.



**Figure 2.8.** Residual concentration of pesticide  $x$  in pollen or nectar of crop  $y$  over the flowering period, i.e. when honey bees' foragers are exposed.

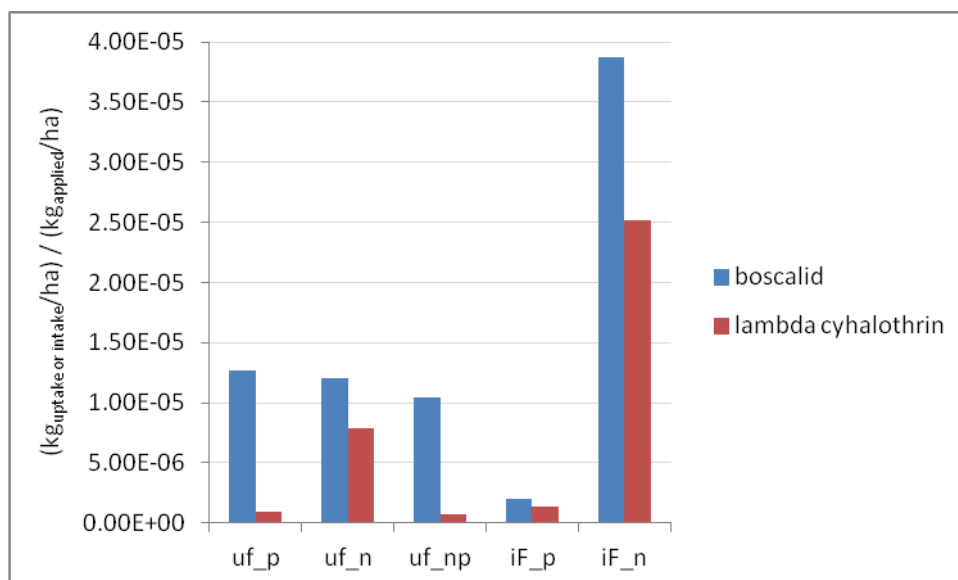
Finally, we collected data on the acute toxicity (either oral or contact) of both pesticides to honey bees from the Pesticide Properties Database (PPDB, 2017), and we used these values, assuming that acute  $LD_{50}$  represent chronic  $LD_{50}$ , for calculating the effect factors (EFs). In case of “higher-than” value, as for acute contact toxicity of boscalid, we selected the reported absolute value based on a conservative assumption.

### 2.2.3. Results

The dermal uptake fractions ( $uF$ ) and ingestion intake fractions ( $iF$ ), calculated according to the methodology section, are reported in Table 2.12 for both pesticides in analysis and for each honey bee forager type. The highest uptake fraction is found in pollen foragers for boscalid, which is at least one order of magnitude higher than the correspondent uptake fraction for lambda cyhalothrin; while the highest intake fraction are found in nectar foragers for both pesticides (see Figure 2.9).

**Table 2.12.** Dermal uptake fractions and ingestion intake fractions of two selected pesticides applied to oilseed rape during its flowering period, according to the case study.

Parameters	Boscalid	Lambda cyhalothrin
$uF (\Delta t)$ for all pollen foragers (i=p) [(kg <sub>uptake</sub> /ha)/(kg <sub>applied</sub> /ha)]	$1.27 \times 10^{-5}$	$9.49 \times 10^{-7}$
$uF (\Delta t)$ for all nectar foragers (i=n) [(kg <sub>uptake</sub> /ha)/(kg <sub>applied</sub> /ha)]	$1.20 \times 10^{-5}$	$7.83 \times 10^{-6}$
$uF (\Delta t)$ for the fraction of nectar foragers potentially in contact with pollen (i=np) [(kg <sub>uptake</sub> /ha)/(kg <sub>applied</sub> /ha)]	$1.04 \times 10^{-5}$	$7.77 \times 10^{-7}$
$iF (\Delta t)$ for all pollen foragers (i=p) [(kg <sub>intake</sub> /ha)/(kg <sub>applied</sub> /ha)]	$2.06 \times 10^{-6}$	$1.34 \times 10^{-6}$
$iF (\Delta t)$ for all nectar foragers (i=n) [(kg <sub>intake</sub> /ha)/(kg <sub>applied</sub> /ha)]	$3.87 \times 10^{-5}$	$2.52 \times 10^{-5}$



**Figure 2.9.** Comparison between uptake ( $uF$ ) and intake ( $iF$ ) fractions of each honey bee forager type, namely pollen foragers (p), nectar foragers (n) and nectar foragers potentially in contact with pollen (np), for the fungicide boscalid and the insecticide lambda cyhalothrin.

Generally, boscalid shows higher uptake and intake fractions, compared to lambda cyhalothrin. This is likely associated with the combination of the physico-chemical properties of pesticides, such as their persistence in

the environmental matrices and capability of accumulate in terrestrial plant biomass, and the initial concentration found in the same matrices (i.e. pollen and nectar). The dissipation rate in both pollen and nectar of the selected pesticides and other pesticide-specific data are presented in Table 2.13.

**Table 2.13.** Numerical values used for quantifying the concentration of pesticides in either pollen or nectar over the flowering period, based on empirical residual data. Data come from the references in brackets below each pesticide, unless otherwise specified.

Parameters	Boscalid (Wallner, 2009)	Lambda cyhalothrin (Choudhary and Sharma, 2008)
CAS number	188425-85-6	91465-08-6
Pesticide class	Fungicide/Carboxamide	Insecticide/Pyrethroid
Mass applied [kg a.i./ha]	0.250	0.075
$k_{j=p}$ , i.e. dissipation rate in pollen [ $d^{-1}$ ]	0.25 <sup>(a)</sup>	1.33 <sup>(a)</sup>
Initial concentration in pollen [kg <sub>in pollen</sub> /kg <sub>applied</sub> ]	$1.39 \times 10^{-5}$	$1.64 \times 10^{-6}$ <sup>(b)</sup>
$k_{j=n}$ , i.e. dissipation rate in nectar [ $d^{-1}$ ]	0.43 <sup>(a)</sup>	1.33 <sup>(a)</sup>
Initial concentration in nectar [kg <sub>in nectar</sub> /kg <sub>applied</sub> ]	$1.43 \times 10^{-6}$	$8.73 \times 10^{-7}$ <sup>(c)</sup>
log $K_{oa}$	12.72 <sup>(d)</sup>	11.21 <sup>(e)</sup>

(a) Dissipation rate of the selected pesticide in pollen/nectar, calculated according to equation (2)

(b) Initial concentration in pollen of mustard (*Brassica juncea*), used as proxy for oilseed rape (*Brassica napus*), measured at the application day. This value is calculated as average between the residual concentrations in pollen measured at the application day by using chromatographic methods during the seasons 2003-2004 ( $1.67 \times 10^{-6}$  kg/kg<sub>applied</sub>) and 2004-2005 ( $1.61 \times 10^{-6}$  kg/kg<sub>applied</sub>). Source: Choudhary and Sharma (2008).

(c) Initial concentration in nectar of mustard (*Brassica juncea*), used as proxy for oilseed rape (*Brassica napus*), measured at the application day. This value is calculated as average between the residual concentrations in nectar measured at the application day by using chromatographic methods during the seasons 2003-2004 ( $9.09 \times 10^{-7}$  kg/kg<sub>applied</sub>) and 2004-2005 ( $8.36 \times 10^{-7}$  kg/kg<sub>applied</sub>). Source: Choudhary and Sharma (2008).

(d), (e) Logarithm of the octanol-air partition coefficient ( $K_{oa}$ ). Data from USEPA (2017a, b)

The octanol-air partition coefficient ( $K_{oa}$ ) is an indicator of the capability of a chemical to be incorporated in terrestrial plant biomass; therefore, it can be taken as an indicator of pollen and nectar uptake capability (Villa et al., 2000). Substances with high log  $K_{oa}$  values ( $> 11$ ) tend to concentrate more readily in organic matter. Hence, chemicals with high log  $K_{oa}$  values may be of concern because they have the potential to be incorporated in living organisms. According to this, a high capability of concentration in the biotic compartment and a low dissipation rate (namely, longer persistence) may lead to potentially high uptake/intake by organisms such as honey bees which are exposed to contaminants in pollen and nectar. This is likely the case for boscalid, for which a combination of higher log  $K_{oa}$  and lower dissipation rate than lambda cyhalothrin results in higher uptake and intake fractions.

By using the derived values presented above, namely  $uF$  and  $iF$ , and the effects factors calculated according to section 2.2.2.4, we obtained the characterization factors for boscalid and lambda cyhalothrin as presented in Table 2.14. CF of boscalid is at least two orders of magnitude lower than CF of lambda cyhalothrin; this is

likely due to the differences in the effect factors, based on the toxicity of the specific pesticide. As we expected, the insecticide lambda cyhalothrin shows greater impact on honey bees' foragers than the fungicide boscalid, based on its high potential bee ecotoxicity effects. Often but not always, it is possible to observe a correlation between high  $K_{ow}$  (octanol-water partition coefficient) and high toxicity potential. According to the data available in literature, lambda cyhalothrin has higher  $\log K_{ow}$  than boscalid (6.80 versus 2.96, from Kim et al. (2016)), meaning that lambda cyhalothrin has greater potential of bio-concentrate in living organisms, thus having the potential of exerting higher toxicity.

**Table 2.14.** Effect factors (for both dermal contact and oral exposure) and characterization factors of two selected pesticides applied to oilseed rape during its flowering period, according to the case study.

Parameters	Boscalid	Lambda cyhalothrin
EF dermal [bee/kg <sub>uptake</sub> ]	$2.50 \times 10^6$ <sup>(a)</sup>	$1.32 \times 10^{10}$ <sup>(b)</sup>
EF oral [bee/kg <sub>intake</sub> ]	$5.00 \times 10^6$ <sup>(c)</sup>	$5.49 \times 10^8$ <sup>(d)</sup>
CFs [(bees ha <sup>-1</sup> )/(kg <sub>applied</sub> ha <sup>-1</sup> )]	$2.92 \times 10^2$	$1.40 \times 10^5$

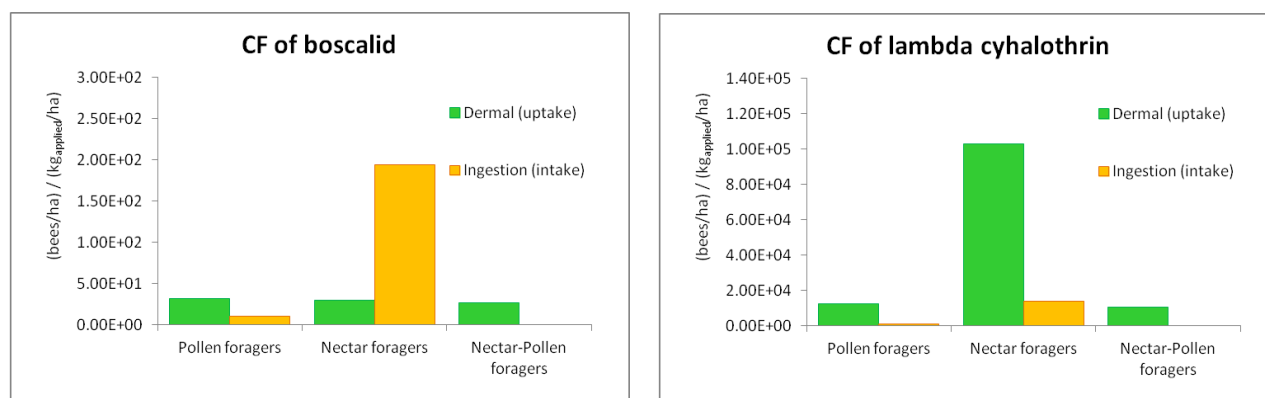
(a) Based on acute LD<sub>50</sub> contact 200 µg/bee, obtained over 48h of experimental test (PPDB, 2017)

(b) Based on acute LD<sub>50</sub> contact 0.038 µg/bee, obtained over 48h of experimental test (PPDB, 2017)

(c) Based on acute LD<sub>50</sub> oral 100 µg/bee, obtained over 48h of experimental test (PPDB, 2017)

(d) Based on acute LD<sub>50</sub> oral 0.91 µg/bee, obtained over 48h of experimental test (PPDB, 2017)

Furthermore, characterization factors aggregated over exposure pathway for each honey bees' forager type are shown in Figure 2.10 and 2.11.



**Figure 2.10 and 2.11.** Contribution of each impact pathway, aggregated over honey bees' forager type, to the characterization factor of boscalid and lambda cyhalothrin respectively

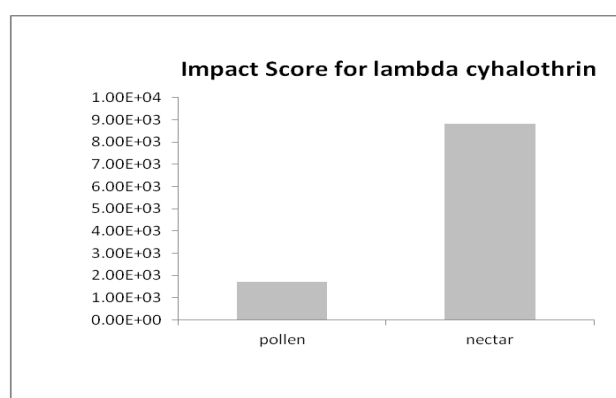
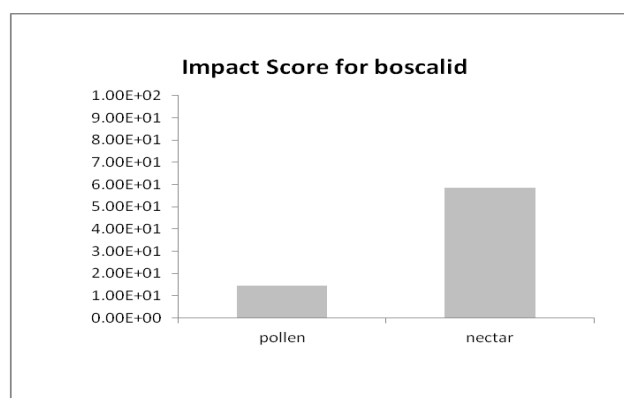
Differences in impacts across pesticides are highlighted, especially for nectar foragers. In fact, for this forager type, the analyzed pesticides show opposite patterns, namely for boscalid the ingestion pathway has a greater impact than the uptake pathway of at least one order of magnitude; whereas for lambda cyhalothrin the uptake pathway is responsible of a higher number of affected bees than intake by ingestion of nectar. This may depend on whether pesticides are more active when ingested versus taken up dermally.

Then, by multiplying the mass of the pesticide applied [kg<sub>applied</sub>/ha] according to the GAP with the corresponding characterization factor [(bees/ha)/(kg<sub>applied</sub>/ha)], we obtained the toxicity impact score (IS) associated to the selected pesticides (see Table 2.15). The IS of lambda cyhalothrin is three orders of

magnitude higher than the IS of boscalid, as already observed in the comparison of CFs. Toxicity effect factors are highest for insecticides such as lambda cyhalothrin, but, for example, solubility and the  $K_{ow}$  of other pesticides might yield higher exposure than for insecticides, which is why it is important to model all of them through instead of focusing only on the pesticides with high EF. Nectar foragers represent the group of honey bees mostly affected by both pesticides. The major contributor to the IS of lambda cyhalothrin is represented by the exposure pathway via dermal contact with nectar which affects 7720 nectar foragers out of 9907 total nectar foragers in an average colony located in an oilseed rape field; while the ingestion of nectar by nectar foragers is the main contributor to the IS of boscalid, with about 48 nectar foragers affected.

**Table 2.15.** Impact scores (IS) associated to two selected pesticides and the contribution of each impact pathway named as the related exposure fraction.

IS [bees affected]	Boscalid	Lambda cyhalothrin
Contribution from:		
$uF(\Delta t)$ for $i=p \times EF_{dermal} \times m_{applied}$	7.95E+00	9.37E+02
$uF(\Delta t)$ for $i=n \times EF_{dermal} \times m_{applied}$	7.50E+00	7.72E+03
$uF(\Delta t)$ for $i=np \times EF_{dermal} \times m_{applied}$	6.51E+00	7.67E+02
$iF(\Delta t)$ for $i=p \times EF_{oral} \times m_{applied}$	2.57E+00	5.54E+01
$iF(\Delta t)$ for $i=n \times EF_{oral} \times m_{applied}$	4.84E+01	1.04E+03
IS tot	7.29E+01	1.05E+04



**Figure 2.12 and 2.13.** Impact scores (IS) of boscalid and lambda cyhalothrin, aggregated over exposure to pollen (only contact) and nectar (both contact and ingestion).

In general, the exposure of honey bees' foragers to pesticide residues in nectar, both via oral and dermal contact, represents the most noticeable issue for both pesticides, although the insecticide lambda cyhalothrin shows highest impacts on honey bees' forager population compared to the fungicide boscalid (Figure 2.12 and 2.13). This is highlighted by the potentially affected fraction of honey bees (PAF), measured as percentage of bees affected and calculated as ratio between the pesticide-related IS and the total honey bees' forager population (Table 2.16) for each group of foragers (Table 2.17). In fact, for example out of the entire forager population, nearly 65% of nectar foragers are affected by lambda cyhalothrin, while boscalid affects less than 1% of nectar foragers.

It is worth noticing that the PAFs obtained for honey bees are referred to a single species; whereas in the current LCIA framework for ecotoxicity indicators, PAF is generally referring to an exposed set of distinct species living in the same (e.g. freshwater) ecosystem.

**Table 2.16.** Potentially affected fractions of honey bees (PAF) due to the application of two selected pesticides and the contribution of each impact pathway named as the related exposure fraction.

PAF [% bees affected, out of total exposed foragers]	Boscalid	Lambda cyhalothrin
Contribution from:		
$(uF(\Delta t) \text{ for } i=p \times EF_{\text{dermal}} \times m_{\text{applied}}) / N_{i=p+n}$	0.06%	6.97%
$(uF(\Delta t) \text{ for } i=n \times EF_{\text{dermal}} \times m_{\text{applied}}) / N_{i=p+n}$	0.06%	57.45%
$(uF(\Delta t) \text{ for } i=np \times EF_{\text{dermal}} \times m_{\text{applied}}) / N_{i=p+n}$	0.05%	5.70%
$(iF(\Delta t) \text{ for } i=p \times EF_{\text{oral}} \times m_{\text{applied}}) / N_{i=p+n}$	0.02%	0.41%
$(iF(\Delta t) \text{ for } i=n \times EF_{\text{oral}} \times m_{\text{applied}}) / N_{i=p+n}$	0.36%	7.74%
PAF tot	0.54%	78.27%

**Table 2.17.** Potentially affected fractions of honey bees (PAF) out of each type of foragers, due to the application of two selected pesticides and the contribution of each impact pathway named as the related exposure fraction.

PAF [% bees affected, out of each group of foragers ( $i \in \{p, n, np\}$ )]	Boscalid	Lambda cyhalothrin	
Contribution from:			
$(uF(\Delta t) \text{ for } i=p \times EF_{\text{dermal}} \times m_{\text{applied}}) / N_{i=p}$	0.22%	26%	% affected out of all pollen foragers
$(iF(\Delta t) \text{ for } i=p \times EF_{\text{oral}} \times m_{\text{applied}}) / N_{i=p}$	0.07%	2%	
$(uF(\Delta t) \text{ for } i=n \times EF_{\text{dermal}} \times m_{\text{applied}}) / N_{i=n}$	0.08%	78%	% affected out of all nectar foragers
$(iF(\Delta t) \text{ for } i=p \times EF_{\text{oral}} \times m_{\text{applied}}) / N_{i=n}$	0.49%	11%	
$(uF(\Delta t) \text{ for } i=np \times EF_{\text{dermal}} \times m_{\text{applied}}) / N_{i=np}$	0.38%	45%	% affected out of the fraction of nectar foragers in contact with pollen

#### 2.2.4. Discussion

For the first time, the impact pathway associated to the exposure of honey bees to residual concentration of pesticide in pollen and nectar has been developed and characterized. The CFs proposed in this study are thus to be considered as a starting point for characterizing the impacts of agricultural pesticides on honey bees and other insect pollinators, developed by combining exposure and ecotoxicological effects.

Applying and testing the model to an illustrative case study enabled a comparison between the characterization factors and impact scores of different pesticides, leading to understand which chemical is potentially more dangerous for bees that collect either pollen or nectar, in line with the LCA framework's purposes. The strength of this model is, indeed, based on the fact that its structure and its underpinning features may fit into the existing LCA framework. Particularly, for what concerns the inventory, it would be important to implement the existing list of pesticides with additional information about their application rate during the flowering period; while information on honey bees are fixed average values already included in the model. The inventory flows are to be entered in terms of amount (kg) applied per ha, in line with Rosenbaum et al. (2015).

However, the model proposed in this study is a novel model which also brings with it several limitations of applicability. The model is built on a single set of measured pesticide residual concentrations in pollen or nectar found in the literature, due to the poor availability of similar information. Generally, data of residues in mono-specific pollen and nectar are limited to a very few studies due to the fact that (i) for economic purposes and to protect the health of consumers, honey (or food, in general) is the most studied compound, not pollen or nectar themselves; and (ii) residues are generally measured as multi-residues, namely without differentiating among the crop species of origin. Additionally, measured residue content in matrices like pollen and nectar may present high variability, depending on the application rate and technique, the selected crop species, season, location, etc., that may influence their persistence (Bonmatin et al., 2015). Therefore, to improve and operationalize the model in a reliable way for use in LCIA, some important parameters should be extrapolated or predicted from other dissipation data (Fantke and Juraske (2013) and related Fantke et al. (2014)) when not available, such as pollen and nectar concentrations linked to the mass of pesticide applied to specific crops under certain conditions and half life in pollen and nectar, which can help estimate the dissipation rate. The modeling framework still needs to be applied to a wide range of pesticides for showing that it can be operationalized in LCIA and then the uncertainty associated to its parameters evaluated and tested. Another critical aspect is related to the actual pesticide application. In fact, to avoid the toxic effects of pesticides on honey bees, the application of insecticides especially is often not allowed during the flowering period of given crops. However, residues can still contaminate nectar and pollen in sub-lethal doses via both active and passive transport (Viik, 2012). Therefore, it becomes important to extend the model by taking into account the application of pesticide outside the time constraint of the flowering period, namely by exploring application scenarios with application time starting some days before or after the beginning of the flowering period. It would be crucial as well to consider the specific features of landscape and their relevance in the impact assessment on honey bees. In fact, there is evidence that both the presence of small plots in a heterogeneous agricultural mosaic and other aspects of the landscape context, such as field margins, may help incrementing the density of honey bees and other pollinating species (Le Féon et al., 2013; Nicholls and Altieri, 2013), thus influencing the results of the impact assessment above performed. Linked to this, another aspect on which it would be important to further reasoning is given by the assumption that, in our modeling framework, honey bees forage only in the crop field. In fact, assuming only in-field exposure, disregards that pesticide, due to drift, may have reached the field margins, thus leading us to a potential underestimation of the overall “off-field” exposure.

Concerning the effect factors, we calculated them by using acute toxicity data based on 48 hours of experimental test (according to OECD guidelines for the testing of chemical on honey bees (OECD, 1998a; b), namely by using acute toxicity as an approximation of a chronic or sub-chronic exposure. In fact, acute toxicity assesses the immediate effects of a chemical sample and is based on the administration of a single dose, while for sub-chronic and chronic toxicity assessment multiple doses are administered over a period of time which is usually equal to 10% of the life of the studied animal.



The typical experimental duration for acute toxicity corresponds to 4% of a honey bee forager's life cycle, since, according to Tremolada et al. (2011) the biological cycle of worker honey bees, including forager bees, is about 40–45 days in the active period, i.e. in summer. In LCA a long-term perspective is considered and hence lifetime exposure and related chronic effects in bees would be needed. Therefore, in order to be compliant with the existing LCIA framework for ecotoxicity, it would be crucial to derive the chronic-equivalent ecotoxicity endpoint per species by applying an acute-to-chronic extrapolation factor, as it is already done for freshwater ecotoxicity (Henderson et al., 2011).

What is finally missing is how to effectively operationalize this framework in the LCA context, particularly its match with the ecotoxicity category since this type of model is closer to the characteristics underpinning the assessment of human exposure, namely (i) it is based on the concept of intake fractions instead of taking into account the available fraction and (ii) it is referred to a single species and not to the ecosystem community.

### 2.2.5. Conclusions and outlook

We developed a characterization framework able to quantify the in-field exposure of forager honey bees to agricultural pesticides via different pathways and the associated adverse effects. Our model, built on simple mathematical equations which follow the environmental cause-effect chain of the current LCA framework, introduces novel metrics for honey bees, based on the concept on intake fraction and named *dermal uptake fraction* and *ingestion intake fraction*. Our model is built on ecological data accounting for the behavior of foraging honey bees and on data regarding the environmental fate of pesticides.

For future improvement and operationalization of the model, some aspects are to be considered, as follows:

- The limited availability of crop-specific measured residual concentrations in pollen and nectar is a crucial aspect to be taken into account for future agenda. In fact, important parameters such as pollen and nectar concentrations linked to the mass of pesticide applied to specific crops under certain conditions and half life in pollen and nectar, which can help estimate the dissipation rate, should be evaluated carefully when not available by alternatively proposing scientifically strong estimation procedures.
- The identification of pollen and nectar as new environmental compartment, both in-field and off-field, represents a fundamental area of research need on both inventory and model sides. In fact, the information on fate of pesticides to pollen and nectar need to be specifically addressed and quantified, in contrast to what currently occurs in inventories such as Ecoinvent, where 100% or fixed fractions of applied pesticide mass are assumed to be emitted exclusively to soil and air.
- In order to be consistent with the existing and recommended LCIA models for ecotoxicity, it would be crucial to derive the chronic-equivalent ecotoxicity endpoints for honey bee species to be used for calculating the effect factors by applying an acute-to-chronic extrapolation factor.
- Since the loss of forager honey bees may lead to adverse feedback on the development of the hives, as the growth of larvae, thus raising concerns about a potential global crisis for the agricultural industry and consumers, it would be crucial to include the exposure assessment of honey bees larvae who are fed with

contaminated pollen and nectar brought by the foragers. According to this, data on larvae's behavior (i.e. ingestion and contact exposure) and ecotoxicity related data will be needed in order to extend the modeling framework to a comprehensive assessment of the cause-effect chain.

- As already mentioned, the exposure of honey bees to pesticides is not limited to in-field scenario. In fact, forager bees can search for food and other important elements for the hive (e.g. water, or other sources of pollen and nectar) also, for example, in the flowering patches of the field margins. According to Figure 2.3 at the beginning of the methodology section, other pathways are also potentially relevant for honey bees' impact assessment in the LCA context. Therefore, the modeling framework can be extended in the future, by exploring and covering other pathways such as the ingestion of or contact with contaminated water, off-field exposure, etc.
- Additionally, it would be interesting to cover more crop-related scenarios, by collecting data on pesticides application on several crop species in different locations across Europe. The development of archetypes built on the combination of information on representative crop species, landscape features, geographical location and weather condition will be critical for better exploring the agro-food supply chain related impacts on pollinators.

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### 3. Impacts on biotic resources: towards including a new impact category in LCIA

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## Abstract

Natural resources, biotic and abiotic, are fundamental from both the ecological and socio-economic point of view, being at the basis of life-support. However, since the demand for finite resources continues to increase, the sustainability of current production and consumption patterns is questioned in both developed and developing countries. A transition towards an economy based on biotic renewable resources (bio-economy) is considered necessary in order to support a steady provision of resources, representing an alternative to an economy based on fossil and abiotic resources. However, to ensure a sustainable use of biotic resources, there is the need of properly accounting for their use along supply chains as well as defining a robust and comprehensive impact assessment model. Since so far naturally occurring biotic resources have gained little attention in impact assessment methods, such as life cycle assessment, the aim of this study is to enable the inclusion of biotic resources in the assessment of products and supply chains. This paper puts forward a framework for biotic resources assessment, including: i) the definition of system boundaries between ecosphere and technosphere, namely between naturally occurring and man-made biotic resources; ii) a list of naturally occurring biotic resources (NOBR, e.g. wild animals, plants etc) which have a commercial value, as basis for building life cycle inventories; iii) an impact pathway to identify potential impacts on both resource provision and ecosystem quality; iv) a renewability-based indicator (NOBRri) for the impact assessment of naturally occurring biotic resources, including a list of associated characterization factors. The study, building on a solid review of literature and of available statistical data, highlights and discusses the critical aspects and paradoxes related to biotic resource inclusion in LCA: from the system boundaries definition up to the resource characterization.

### 3.1. Introduction

The secure access to natural resources, both abiotic and biotic, provided by the Earth, i.e. metals, minerals, wood, water, air and soil, is the basis for human life and socio-economic well-being. In fact, natural resources have direct or indirect functions for humans representing both a building block in the supply chains as material inputs, thus enhancing the economic growth, and being fundamental for the provision of services and functions by ecosystems, for instance climate regulation.

Historically, economies in developed countries have been characterized by a high level of both abiotic and biotic natural resources consumption, including fossil resources used for energy, transport, materials and chemicals production (Mancini et al., 2015). However, in a globalized world where population is expected to reach 9 billion people by 2050 and demand and competition for finite resources continue to increase, the sustainability of the existing production and consumption patterns is raising concerns for environmental implications and in terms of security of resource supply (UNDESA, 2015). On such a background, economies worldwide need to radically revise the current approach to production and consumption, improving the efficiency in resource use for both abiotic and biotic resources, in order to meet challenging objectives such those included in the Sustainable Development Goals (UN, 2016). As a response to the conventional fossil-based economic model, the transition towards a bio-based economy (bio-economy) has

been put forward. Bio-economy refers to the economic activities relating to the production, use and development of biological products and processes (OECD, 2009). It is specified as the sustainable production and efficient use of renewable biological resources proceeding from agriculture, forestry, fishery etc., and their subsequent conversion into value added products, such as food, feed, industrial materials and energy (EC, 2012). The concept has been already translated in action plans in different contexts. For example, some EU policies such as the Biomass Action Plan (EC, 2005) and the Renewable Energy and Fuel Quality Directives (EU, 2009a, b) already promote the bio-economy (EC, 2010; 2012).

The intrinsic renewability of biotic resources make them, theoretically, more available compared to finite resources. However, their supply could be considered critical as well, if the carrying capacity of the ecosystems responsible for their provision is overcome, namely when resources are extracted at a rate higher than their regeneration capability. In fact, renewable resources do not continue to grow indefinitely and they can be depleted beyond the point of renewability, for instance when commercially valuable species are harvested to extinction. The assessment of the carrying capacity is a key element of environmental sustainability, crucially needed for sustainability assessment and for integrated assessment methodologies as Life Cycle Assessment (LCA) (Sala et al., 2013a, b).

Notwithstanding renewable biotic resources or bio-based products (i.e. products wholly or partly derived from biomass) are often claimed to represent the appropriate solution for a sustainable post-fossil carbon society, it is clear that “renewable” and “bio-based” are not necessarily synonymous with “sustainable”. This may seem a paradox, but biotic resources could be as critical as fossils or abiotic ones, if the processes underpinning their renewability are affected beyond the ecosystem's carrying capacity or beyond the sustainability of the underpinning bio-geological cycles. For example, agriculture products could not be indefinitely produced without certain quality of soil, pollination service, an adequate amount of available water, etc. Moreover, bio-based products and energy may anyway imply a significant amount of embodied fossil-based energy (Arvidsson et al., 2012) for their production, as well as may be associated with direct and indirect land-use impacts. The nexus between the use of biotic and abiotic resources for competing uses (e.g. food, energy, materials) (Karabulut et al., 2017; see Chapter 4 for more details) and the interplay between ecosystems and resources is still not fully explored. For instance, several studies have already highlighted that large-scale cultivation of biomass for biofuel may affect global food security and natural ecosystem functioning (e.g. Smith et al., 2010). From the material point of view, the criticality of biotic resources is increasingly recognized, e.g. from 2014, three biotic raw materials –rubber, pulpwood and sawn softwood– have been included in the list of European critical raw materials (EC, 2014).

In this context, holistic methodologies such as LCA (ISO, 2006) are necessary to ensure that different drivers of environmental impacts are simultaneously considered and burden shifting avoided. However, biotic resources have been barely considered within LCA and specific improvements are needed to better capture impacts related to biotic resource extraction and use. Now, in an LCA study, if a product is based on natural occurring biotic resources, the resources themselves are not accounted for the majority of the cases since elementary flows are mainly missing and no characterization of the resources is performed.



Firstly, the transition towards bio-economy requires more specificity in resource accounting. Statistics usually report data on biomass from harmonized statistical sources such as agricultural crop, forestry and fishery databases; however, at a high level of aggregation (e.g. wood) (Eurostat, 2016). Besides, a comprehensive and robust model of impact assessment needs to be developed for natural biotic resources, to ensure the sustainable use thereof. In the last decades, several methodologies and indicators, including life cycle oriented approaches for biotic resource depletion, have been developed with different purposes. However, typical concerns for biotic resources, such as the large global capture of fish from wild fisheries and the overharvesting of natural wood resources, are not generally accounted in such methodologies. Moreover, within supply chain management, a robust characterization of biotic resources and a reliable set of indicators are actually missing in a framework of impact assessment. Therefore, additional investigations are necessary in order to cover the research gaps, by integrating biotic resources as an impact category within the Life Cycle Impact Assessment (LCIA) framework.

This study aims at unveiling the challenges in the accounting of biotic resources and the limitations associated with the characterization step towards their inclusion within LCIA. A model for the assessment of biotic resources is proposed, encompassing: i) a definition of system boundaries between ecosphere and technosphere, namely between naturally occurring and man-made resources; ii) a list of biotic resources focusing on commercially valuable resources coming from the wild (i.e. naturally occurring biotic resources, referred to as NOBR, e.g. wild animals, plants, etc.), as basis for building life cycle inventories; iii) an impact pathway entailing an impact assessment model and associated characterization factors (CFs) for ranking resources based on one key element of their sustainability: the renewability potential. The study, building on a solid review of literature and of available statistical data, highlights and discusses the critical aspects and paradoxes related to biotic resource inclusion in LCA: from the system boundaries definition up to the resource characterization

The chapter is organized as follows: section 3.2 focuses on the state of the art of the accounting and characterization of natural biotic resources in LCA; section 3.3 presents an overview of the methodological steps adopted in this study towards the inclusion of biotic resources in LCA, encompassing the system boundaries, the impact pathway, the biotic resources to be considered in the inventory and their characterization; in section 3.4 we present the results of the extensive literature review, discussing the role of natural biotic resources into the current LCA framework and the proposed indicators to characterize natural biotic resources; sections 3.5 presents the results and the discussion on the key aspects to be taken into considerations for improving the accounting and the impact assessment of natural biotic resources, proposing a new indicators for the characterization; finally, section 3.6 encloses the conclusions and suggestions for further research.

### 3.2. State of the art

In the last decades, several methodologies and indicators have been developed in order to take into account the environmental, social and economic relevance of natural biotic resources in a context of resource depletion:

- Resource accounting-based methods, e.g. Material Flow Analysis (MFA) (Hinterberger et al., 2003), are mass-based accounting approaches seeking to quantify environmental pressures from resource consumption. They include exchanges of biotic material, such as biomass from agriculture, forestry and fishery, quantifying their use in terms of mass, without characterizing the intrinsic properties of each material.
- Resource characterization-based methods, instead, relate resources to physical, chemical or biological factors that describe their relevant properties. Characterization is an essential component of many impact assessment frameworks. Among these approaches:
  - Footprinting, e.g. ecological footprint (Global Footprint Network, 2016), developed to measure human pressures on bio-capacity of the Earth. Specifically, ecological footprint accounts for the flows of energy and matter, including biotic resources, to and from any economic system and converts them into the corresponding proportion of the Earth's bio-productive land or water areas required to supply resources to a particular human activity.
  - Impact assessment-based methods, founded on the LCA, where biotic resources and their renewal rates have so far received relatively little regard (Klinglmair et al., 2014).

Despite many efforts, current LCA frameworks miss a specific focus on natural biotic resources. In fact, LCA inventories lack of a complete list of elementary flows for natural biotic resources (Table 3.1) as well as models for a comprehensive characterization. Hence, there is the need to cover this conceptual gap, overcoming the current limited coverage of biotic resources, especially with regards to some categories like top-soil, forest biomass and fish stocks which have relevance for the global economies (EC-JRC, 2016).

**Table 3.1.** List of natural biotic resources and their units currently included in one LCA inventory, i.e. Ecoinvent v.3.3 (2016)

Resource name in Ecoinvent v. 3.3	unit
Animal matter	kg
Biomass	kg
Biomass, feedstock	MJ
Carbon, organic, in soil or biomass stock	kg
Fish, unspecified, in sea	kg
Peat	kg
Wood (16.9 MJ/kg)	kg
Wood and wood waste, 20.9 MJ per kg, oven dry basis	kg
Wood and wood waste, 9.5 MJ per kg	kg
Wood, dry matter	kg

<b>Resource name in Ecoinvent v. 3.3</b>	<b>unit</b>
Wood, feedstock	kg
Wood, hard, NE-NC, standing	m <sup>3</sup>
Wood, hard, standing	m <sup>3</sup>
Wood, primary forest, standing	m <sup>3</sup>
Wood, soft, INW, standing	m <sup>3</sup>
Wood, soft, NE-NC, standing	m <sup>3</sup>
Wood, soft, standing	m <sup>3</sup>
Wood, soft, US PNW, standing/m3	m <sup>3</sup>
Wood, soft, US SE, standing/m3	m <sup>3</sup>
Wood, unspecified, standing/kg	kg
Wood, unspecified, standing/m3	m <sup>3</sup>

NE-NC: Northeast North Central; INW: Inland West; US PNW: United States Pacific Northwest; US SE: United States Southeast

The identification of the elementary flows needs a considerable effort in order to establish a harmonized and unified reference terminology within the inventories. Then, an inventory analysis based on mass accounting of the elementary flows referred to a product or service would not be enough to perform an impact assessment. Some specific aspects, like the ecological properties together with the geographical localization of the extraction of the resources, are fundamental in view of a complete analysis of natural resources. To date, there is no consensus on how to properly address the area of protection “Natural Resources” in LCA (Dewulf et al., 2015). In the case of biotic resources, beyond no consensus on how to derive impact factors for assessing their depletion (Klinglmair et al., 2014), there are very few impact assessment models addressing the issue. This is principally due to the complexity of assessing the impacts, the need of further clarifying the boundaries between ecosphere and technosphere as well as the fact that characterizing a mass-based accounting is challenging in terms of suitable metrics for assessing impacts. Currently, a model for the characterization of biotic resources is not available within the ILCD recommendation for LCIA by the EC-JRC (2011). Although impacts on habitats of biotic resources are assessed in the area of protection “Ecosystem Quality”, damages to biotic resources related to depletion (such as overharvesting, overfishing and overhunting) remain not accounted within the ILCD recommended framework.

To date, the attempts made over time to include biotic resources in a life cycle framework use different models and indicators (Table 3.2).

**Table 3.2.** Natural biotic resource coverage according to the models existing within the current literature

Model	Indicator	Unit	Natural Biotic Resources	References
Exergy	Cumulative Energy Extracted from the Natural Environment	MJex/unit	Wood	Dewulf et al. (2007); Alvarenga et al. (2013); Taelman et al. (2014)
Emergy	Solar Energy Factor (SEF)	MJse/unit	Wood	Rugani et al. (2011)
EPS 2000	Environmental Load Units (ELU)	ELU/kg	Wood, fish & meat	Steen (1999)
BRD-fish (Biotic Resource Depletion)	1/maximum sustainable yield (MSY) or 1/current fish catches (Ct) [to be applied in case of overexploitation]	yr/t	Fish	Langlois et al. (2014)
LPY-fish (Lost Potential Yield)	Lost Potential Yields (LPY)	dimensionless	Fish	Emanuelsson et al. (2014)
BIRD	Biotic Resource Availability (BRA)	dimensionless	Terrestrial biotic materials	Bach et al. (2017)

The EPS 2000 (Steen, 1999) is a damage oriented model, with the highest coverage of biotic resources, including fish, meat and wood. In the EPS system, Willingness to Pay (WTP) to avoid damages to natural resources' availability is chosen as indicator for characterizing biotic resources in monetary terms.

Other models are based on thermodynamic features of resources. For example, exergy-based LCA models, such as Dewulf et al. (2007), Alvarenga et al. (2013), Taelman et al. (2014), aim at assessing the quality of resources depending on the amount of useful energy needed for producing them and that could be obtained from them. Besides, emergy-based LCA model (Rugani et al., 2011) aims at measuring the Solar Energy Demand (SED) associated with the extraction of resources, including both naturally occurring and man-made biotic ones.

Recent attempts seeking to develop a characterization model to assess human-related impacts on natural biotic resources within the LCIA framework come from the studies of Langlois et al. (2014), Emanuelsson et al. (2014) and Bach et al. (2017).

Langlois et al. (2014) proposed quantitative approaches to address overfishing at the midpoint level. In fact, the authors reviewed the use of the sea in LCA and developed a methodological framework, then implemented by Helias et al. (2014), to assess impacts of fish depletion at both species and ecosystem levels. The model of Langlois et al. (2014) is based on the concept of biotic resource depletion for fish, which aims to characterize the current biomass uptake related to either the Maximum Sustainable Yield (MSY, based on fisheries science) or the current fish catches in case of overexploitation. This model, which provides characterization factors for 127 fish species, allows evaluating the environmental pressures on fisheries in a context where one third of global fish stocks is already overexploited (FAO, 2016c). The study is the first attempt to assess impacts on the use of biotic resources taking into account ecological aspects such as the resource recovery capacity.

In parallel with the study of Langlois et al. (2014), Emanuelsson et al. (2014) focused on the concept of Lost Potential Yield (LPY) for fish, proposing new characterization factors for 31 European fish species. The model aims at measuring and characterizing the current overexploitation of natural fish stocks, suggesting a midpoint indicator that allows identifying the impacts on the reduction of future fish supply.

Bach et al. (2017) propose the BIRD approach, inspired by the abiotic depletion potential (Van Oers et al., 2002). The BIRD model focuses on terrestrial biotic resources, as the majority of the LCA models above-mentioned, and it measures the availability of biotic resources by using the BRA indicator (defined as availability to use ratio). In their proposal, the authors include considerations on the replenishment rate and the identification of a reference species. Differently from other models, in this approach several aspects beyond the ecological constraints are taken into account (e.g. socio-economic aspects).

All in all, the applicability of these approaches is limited in the context of current LCA since elementary flows and life cycle inventory for terrestrial resources and fish are still missing.

For what concern wood, several of the above-mentioned models consider this material, which is included in the LCIA framework as an energy resource. Wood is one of the most versatile raw materials, employed in a variety of industrial processes and domestic uses (Schweinle, 2007). It is generally taken into account as simple “wood”, or just making a distinction between “hardwood” (i.e. wood stemming from broadleaves) and “softwood” (i.e. wood coming from coniferous), without addressing the species, the distribution around the globe, the original habitat (natural forests versus human-made plantations) or species vulnerability. When coming to the impact assessment, this lack of specificity in the accounting could generate an over- or under-estimation of the impacts which biotic resources are subject to, due to ecological features that may results in population dynamics of plants being more or less sensitive to human interventions. For instance, the impacts on biotic resources due to the extraction of wood from widespread oaks is likely to be different from the impact which may affect the extraction of wood from a threatened and endemic plant species growing in specific locations. The focus on the species, their distribution and their ecological status is fundamental in order to effectively assess the effects of extracting biotic resources and characterize them.

Regarding soil, the threats to soil as a biotic resource may be the result of a physical removal (e.g. erosion or physical loss due to building construction or other human interventions) or a detriment in its quality, meaning that the resource is not available anymore for a specific life-support function (e.g. due to salinization, compactation, etc). However, the variety of soils and their properties, their widespread distribution around the globe and the heterogeneity of pressure they undergo suggest the need of a comprehensive impact assessment scheme, which has not been standardized so far. In a recent review (Vidal Legaz et al., 2017), soil quality models for impact assessment were analyzed, highlighting that current models for soil related impacts are not yet able to comprehensively cover both the areas of protection “Ecosystem Quality” and “Natural Resources”. An additional research by Curran et al. (2010), in which the authors reviewed the use of indicators and approaches to model biodiversity loss due to land/soil use within

the LCA framework, outlined serious conceptual and methodological gaps to be covered in the way the soil related impacts are modeled for addressing biodiversity concerns.

Overall, human driven impacts on marine and most terrestrial biotic resources still remain unaccounted within current LCA framework since these models are not yet operational and no official guidelines exist for biotic impact assessment (Emanuelsson et al., 2014; Woods et al., 2016).

### 3.3. Methodology

Based on the state of the art, it is clear that several aspects are still missing for a complete inclusion of naturally occurring biotic resource in LCA. Hence, the focus of the study is the improvement of the LCA framework towards a better inclusion and assessment of biotic resources and their availability. For each methodological step, our study is grounded on a thorough review of existing literature and available statistical data. Information and data were collected and integrated into a larger database in order to classify and characterize naturally occurring biotic resources (NOBR).

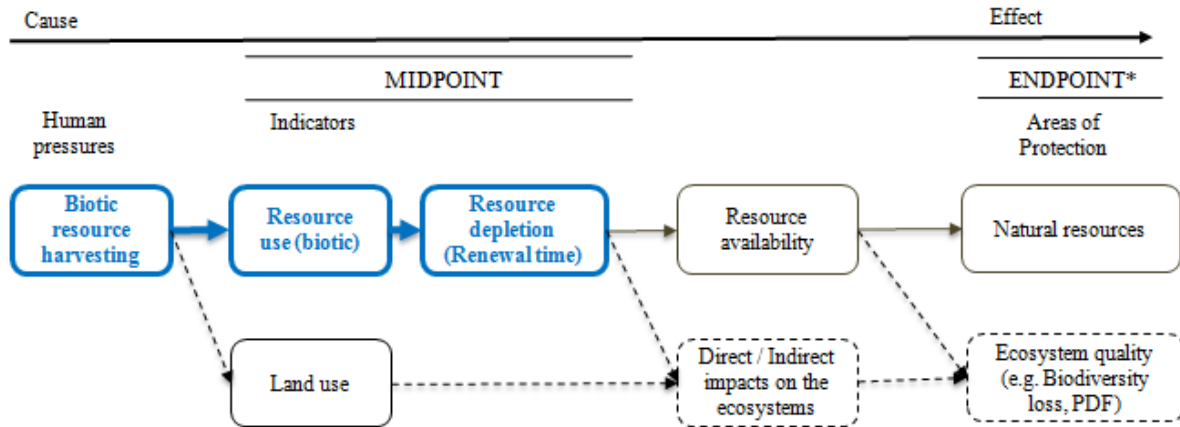
The methodology of this study involved several steps:

1. Illustration of the impact pathway (cause-effect chain) that links biotic resources with impact on the areas of protection “Natural Resources” and “Ecosystem Quality”;
2. Definition of the system boundaries, namely the criteria to identify which are the biotic resources that should be addressed by the resource accounting and impact modelling;
3. Collection of available data on naturally occurring biotic resources for providing a list of biotic resources. This list could be used for building elementary flows to be used in future life cycle inventories.
4. Proposal of an indicator based on renewability to characterize and rank biotic resources, based on an extensive review of literature in the ecology domain.

#### 3.3.1. The impact pathway associated to biotic resources

Life cycle impact assessment models are built to model impacts on the environment due to emissions or resource uses. Hence, the first step is the illustration of the impact pathway, in terms of the potential cause-effect chain along which human interventions may generate impacts. In principle, the overexploitation of biotic resources may be associated with two different areas of protection (Figure 3.1). On one hand, using resources beyond their carrying capacity may be detrimental for the future supply of the resource for human needs, leading to an impact on the area of protection “Natural Resources”. On the other hand, the overexploitation may imply consequences on the area of protection “Ecosystem Quality”, namely when the use is associated with a direct biodiversity loss (the extinction of those species which are used as a biotic resource, e.g. a fish species used as food) or an indirect biodiversity loss (the reduction of biotic resources which leads to an impact on e.g. a trophic chain). Several threats to biodiversity have been associated to the use of naturally occurring resources (e.g. in Lenzen et al 2012, focusing on impacts due to trade). In this study, biotic resources are addressed limiting the focus on their role in supporting human activities, i.e. as

input material in the socio-economic system, and not for their contribution to ecosystem quality and functioning (which would fall into the area of protection “Ecosystem Quality”).



**Figure 3.1.** Cause-effect chain outlining the scope of the paper about the accounting of biotic resources and the characterization of their availability. PDF = Potentially Disappeared Fraction of species.

Light blue box = here proposed to be included in the LCA framework

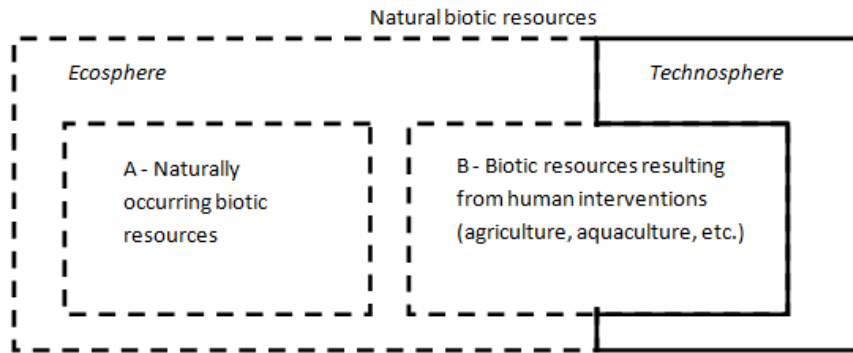
Dashed-line box = potentially alternative pathway for biotic resources, out of the scope of this study.

\*Areas of Protection at endpoint are beyond the scope of the study

### 3.3.2. The definition of the biotic resources to be assessed

In LCA, the impacts associated to natural resources produced by human interventions are usually captured assessing the environmental profile of the processes underpinning their production (e.g. cultivation), whereas the impacts linked with naturally occurring biotic ones are usually barely covered in life cycle inventories. Although the processes for their extraction from biosphere are accounted for (e.g. emission due to harvesting of woods), the impacts of their extraction are neither characterized in the area of protection “Natural Resources” nor in the “Ecosystem Quality”.

According to the approach proposed by Alvarenga et al. (2013), we then provided the clarification of the system boundary. We identified and set the boundary between ecosphere and technosphere when coming to define what natural biotic resources are, i.e. when dealing with biotic resources extracted from natural environment and used by humans (“A - Naturally occurring biotic resources” in Figure 3.2) versus biotic resources produced by human interventions such as crops from agriculture (B). The B box in the figure refers to the fact that crops, which are the result of human interventions in the technosphere, require natural inputs from ecosphere and the boundary is often difficult to be defined in agricultural systems compared to industrial systems.



**Figure 3.2.** System boundary for natural biotic resources which are distinct in those naturally occurring (A) and those resulting from human interventions (B). Adapted from the approach developed by Alvarenga et al. (2013).

In the present study, we focused on naturally occurring biotic resources, i.e. (A), those commercially valuable resources proceeding from biological sources (i.e. plants, animals and other organisms) that are caught or harvested from ecosphere as input material for human purposes like wild feed and food, wood and other products from natural forests, etc. Natural organic topsoil has been included into our analysis, since it is one of the critical underpinning renewable resources (in the ecosphere) that sustain the production of crops (in the technosphere).

### 3.3.3. Towards building an inventory of natural occurring biotic resources

The first step for including naturally occurring biotic resources in the LCA framework is the identification of which biotic resources should be accounted for. In order to build a list of the resources commercially valuable proceeding from biological sources (i.e. plants, animals and other organisms) that are caught or harvested from ecosphere as input material for human purposes, we consulted specific reports and databases, such as, just to name a few, FAO databases for forestry and fishery statistics (FAO 2016a, b), Artemis-face database from the European Federation of Hunters for game hunting information (FACE, 2016) and the International Union for Conservation of Nature (IUCN) red list of threatened species (IUCN, 2016). Thus, by collecting and combining data from different sources, we identified and integrated a list of NOBR in a database, excluding those proceeding from agriculture, aquaculture and livestock, since they depend on human interventions. This list could be the basis for a list of elementary flows to be used in life cycle inventories. Moreover, we sought for data on biotic resource availability, use, and consumption to understand how these resources are distributed and shared within the markets at different scales, both local and global. The data on availability and consumption have been collected to demonstrate that the resource is use somewhere in the economy, so is a resource used by humans. This step is fundamental to identify the biotic resources currently used and, hence, for which of them data may be available ,in future, for populating life cycle inventories.



### 3.3.4. A life cycle impact assessment indicator based on renewability

As mentioned earlier, in this study we aim at identifying an indicator to be used for the impact assessment of naturally occurring biotic resources. The indicator should increase comprehensiveness and ecological relevance in assessing biotic resources approach, beyond the state of the art (section 3.2). Since the scope is to rank the resources based on the likelihood of a reduction in their availability, we propose an indicator for biotic resource based on the renewability rate. Renewability rate of natural biotic resources is a key ecological concept that could be adopted as a basis to identify the ecological and environmental features of the analyzed biotic resources, thus to characterize them. This indicator could be considered a midpoint indicator that could be further complemented in future with indicators of e.g. carrying capacity, availability-to-use ratios or species vulnerability, towards endpoint modeling either in the related to area of protection “Natural Resources” or “Ecosystem Quality”.

The renewability is one of the bases for assessing the potential reduction of the future availability of biotic resources, assuming that resources with longer renewability rate may be more exposed to a wide range of pressures ultimately undermining their provision. Therefore, we referred to the renewability and regeneration time, which represent key elements assessed in ecology, specifically, in population dynamics, as a potential proxy indicator for the capability of a species to grow and regenerate over time. The literature on population dynamics is vast; however, systematized information on renewability and regeneration time for species with a commercial value was not available (to the knowledge of the authors). On this basis, we conducted a systematic review of the literature, initially searching the ISI Web of Science database, which provides access to peer-reviewed studies, in order to have a preliminary understand about the spread and the actuality of this issue. The detailed criteria for this search are reported in Table 3.3; all the literature consulted, underpinning the list of natural occurring biotic resources, is reported in Appendix B.

**Table 3.3.** Terminology and other details used for the literature search.

<b>Literature search details</b>	
<i>Source:</i>	ISI Web of Science database
<i>Research domains:</i>	SCIENCE TECHNOLOGY
<i>Research areas:</i>	ENVIRONMENTAL SCIENCES ECOLOGY OR BIODIVERSITY CONSERVATION OR ZOOLOGY OR PLANT SCIENCES
<i>Key words for Topic:</i>	TOPIC: (natural biotic resources*) AND TOPIC:(ecosystem* or environment*) NOT TOPIC: (farm* or crop* or agro* or pasture* or grass* or agriculture* or agricultural* or livestock*) AND TOPIC: (restor* or re-creat* or rehabilitat* or enhance* or recovery* or resilience*) NOT TITLE: (industrial* or production* or aquaculture*) NOT TOPIC: (genetic* or microbiology* or genus* or gene*).
<i>Key words for renewability and regeneration:</i>	General terms → renewal time regeneration period growth rates recovery time restoration time

### Literature search details

For wildlife →	Population doubling time (or simply doubling time) Doubled population Population life cycle
For plants →	Rotation period Reproduction time Regeneration time

Conforming to the increasingly widespread cross-interest in the concept of renewability and resilience (Curtin et al., 2014), we set up a “renewability-based” database. Through a detailed search of all types of published literature and using a snowball search, we identified relevant references to point out potential reliable indicators for biotic resources. To carry out our refined search, we started identifying key terminology related to renewability and regeneration, such as: renewal time, regeneration period, growth rates, recovery time, restoration time and similar others. Those terms were selected according to the terminology predominantly used in the ecological domain. As an example, we combined in a concatenated string of words either the common or the scientific name of biotic resources (e.g. “sturgeon\*” or “*Acipenser oxyrinchus*”), or even categories of them, like “freshwater fish” or “marine fish” and key words such as those presented above, using the Boolean command AND. We queried online bibliographic databases such as Google Scholar, SCOPUS, Web of Science and the libraries of specific journals like Global Ecology and Conservation. To improve the results, we refined the investigation focusing on a reduced sample of species, namely the most valuable species from the commercial point of view (i.e. the most commercialized globally or locally) and the most representative from the conservation point of view, which means the most threatened by the risk of extinction. Therefore, we searched for studies focusing on single species. In the case of both terrestrial and marine fauna, we were interested in the features of the population dynamics. Thus, we adopted terms of search like “population doubling time” (or simply “doubling time”), “doubled population” or “population life cycle”; while for plants, we used silviculture terms such as “rotation period”, “reproduction time” or “regeneration period”, in combination with the common or specific name.

The outputs included different types of scientific literature, predominantly reports on conservation and management of species and ecological studies. However, the overwhelming majority of the selected papers proceeded from grey literature and reports from international organizations. The publication years of the whole research ranged from the 1970’s to date.

Finally, we created a database (splitted in two tables according to the specific features reported, see Table B1 and Table B2) to collect the list of naturally occurring biotic resources and their related ecological characteristics in order to analyze and discuss the pattern of distribution and renewability of biotic resources and eventually estimate the characterization factor to be used within the LCIA phase. In particular, the recorded information included living organism category, commercial group, common species name, scientific species name, family name, distribution (local or global) and habitat, the vulnerability score according to IUCN criteria where available, renewability indicator type and its quantification.

### 3.4. Results and discussion

According to the steps illustrated in section 3.3, our proposal for the inclusion of naturally occurring biotic resources in the LCA framework and their characterization build on the following results:

- the identification of natural occurring biotic resources and the population of a database of commercial valuable naturally occurring biotic resources, reporting their renewability rates;
- the proposal of a renewability based indicator for biotic resource characterization, built from a review of available information on resources renewability and regeneration time.

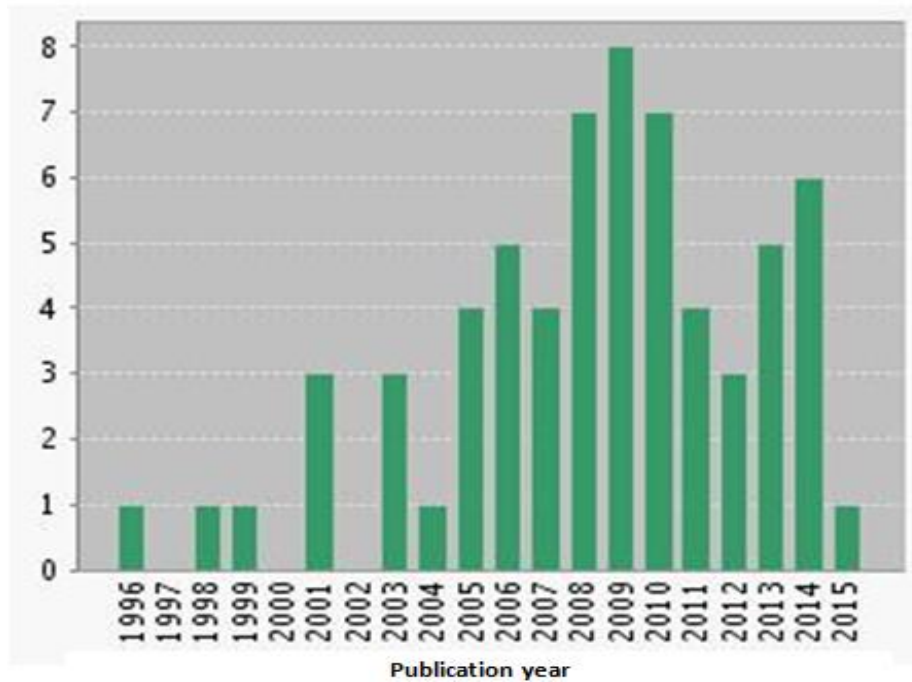
#### 3.4.1. Natural occurring biotic resources with a commercial value: classification and data availability

Based on the review of the literature and statistical available data, naturally occurring biotic resources have been classified in the following major categories, according to their taxonomic level: aquatic and terrestrial vertebrates, aquatic and terrestrial invertebrates, terrestrial plants, aquatic plants and algae, fungi, aquatic and terrestrial animal products, terrestrial plant products (for major details see Table B1, including figures on availability, use, consumptions and references; Table B2, including species names, ecological features and related references). As mentioned above, we included soil as well.

According to our analysis, naturally occurring biotic resources are most commonly used as material input in a broad array of industrial sectors, ranging from food to chemical and pharmaceutical sectors, up to production of e.g. furniture. Together with their derived products, they are generally used as commercial goods marketed at global level, in terms of food and feeding, as source of energy, in the cosmetics, as medicines and for the production of other accessories in different branches of the industrial sector (e.g. natural pearls, natural latex). Several natural biotic resources, such as wild plants, are used in local communities, especially in the developing countries, as dyes, poisons, shelter, fibers and in religious and cultural ceremonies (Heywood, 1999).

It is worth noting that, even though naturally occurring biotic resources are spread around the world and the overwhelming majority of them are commercially used on a global scale, so far a complete list is missing within the available literature. An important attempt was made by Schulp et al. (2014), who synthesized and mapped the ecosystem service called “wild food”, quantifying the supply of terrestrial edible species (i.e. game, mushrooms and vascular plants) across Europe. Gathering a broad list of around 150 species based primarily on their commercial use allows us to start connecting natural biotic resources to elementary flows within the LCA framework.

The literature search showed that, recently, the issue related to the stock of natural biotic capital and its renewability has become central in the ongoing discourse about resources depletion within the scientific circle, with an increasing publication about the topic in the recent years (Figure 3.3).



**Figure 3.3.** Publications per year concerning renewability of natural biotic resources, as selected in our search. X axis reports the publication years of the literature search from 1996 (significant starting point according to the number of retrieved data) to date. Y axis reports the absolute number of published papers per each year.

In spite of the recognized role of naturally occurring biotic resources in human daily life, accurate data on their availability and renewability rate were difficult to gather among scientific literature. On one hand, this may be because most countries, especially the developing ones, have less or no official supervision on the volume of biotic material harvested from the wild and quantities collected are scarcely inventoried. On the other hand, it is often difficult to distinguish between wild and cultivated resources, especially in the case of wild plants, since such primarily wild-collected products are often sold as cultivated (Kuipers, 1997). Some information exists on a reduced number of natural biotic products; however, the available data are extremely variable in coverage and reliability. In fact, the majority of retrieved data were scattered among reports and databases proceeding from different sources, disciplines and institutions, reporting information limited to some specific locations.

The availability of data on biotic resources is generally linked to: i) their use as material input in the industrial sectors of developed countries; ii) their consumption as food; or iii) to the vulnerable state of their populations (IUCN, 2016). In most cases, statistics are provided by national authorities. However, figures are often incomplete due to the considerable variation in the consumption patterns among continents, countries and communities. On the other hand, this type of information is predominantly related to biotic resources linked to human interventions such as those products of agriculture, aquaculture, livestock or to the re-introduction following conservation action plans.

We found predominantly data on resource availability related to few group of organisms, especially the most commercialized animals such as targeted marine fish species (see FAO, 2016a) and game mammals and birds, since they are likely the most easily captured and consumed natural products all over the globe.

Moreover, the overexploitation and the potential subsequent collapse of marine and freshwater fish populations is a well-known issue that may affected populations up to not being available in the future (Hutchings, 2000).

Wild plants, particularly those used in rural communities, and wild mushrooms tend to receive less recognition. Data on their availability are general scarce and rarely published in literature since their collection and consumption are at subsistence level and no legislative and policy support for wild harvesting procedures is arranged. Bais et al. (2015) started covering the gap in the substantial lack of knowledge related to the amount and use of hardwood in a reduced number of world regions. However, overall there are no precise global figures available on the total volume of wild-collected natural resources and on their spatio-temporal patterns in the world market. Furthermore, understanding the magnitude of illegal logging and hunting, which represent a serious problem for biotic resources all over the world, is not immediate. Although many NGOs and other institutions such as UNEP have so far collected information about these activities (UNEP, 2017), comprehensive country-specific statistics on illegal forest logging and wildlife hunting are difficult to quantify and use in an LCA perspective due to their fragmentary nature. Generally, indirect methods are adopted for these estimations, mainly focused on illegal international trade for commercial use (Kleinschmit et al., 2016). Nevertheless, the loss of biotic resources is measured in terms of percentage of logging activity (as reported by Kleinschmit et al., 2016), or in million cubic metres roundwood equivalent (RWE) volume (according to WWF, 2007) or, referring to illegal trade in wildlife, in terms of dollars annually (according to Nellemann et al., 2012). These values are not equivalent, thus making difficult to combine and compare the available data. Moreover, in the case of monetary measurement, they are not properly representative of biotic resource depletion since they do not account for either the availability or the renewability rate of resources.

#### 3.4.2. Indicator for characterizing biotic resources in LCIA

The current available approaches to biotic resource characterization are relatively limited for what concern the ecological relevance of the approach. Hence, in order to identify suitable characterization factors for natural biotic resources to be employed within LCIA frameworks, a better understanding of the effects of human interventions on availability of biotic resources is needed. The indicator that we are proposing is an attempt to fill the ecological gap focusing on renewability of natural resources. Two steps were followed to define the indicator:

- a literature review on renewability rate and regeneration time;
- the calculation of characterization factors based on renewability.

#### 3.4.2.1. Renewability rate and regeneration time

Information related to renewability or recovery rate (reported in Table B2) was found to be species specific, and in many cases population specific, depending on the magnitude of the environmental or human stressors they are subject to. This aspect may represent a limitation when coming to gather data, since a huge amount of data (e.g. population growth, spatial distribution of the species, etc.) should be taken for describing the depletion of a biotic resource's stock, thus restraining the possibility of elaborating a model for characterizing natural biotic resources.

The ecological data on fish species were represented by the renewability rate of species, called “resilience” within the Fishbase dataset (Fishbase, 2016), a global information system about fishes which gathers information provided by different professionals such as research scientists, fisheries managers, and zoologists. The indicator used is the “population doubling time” (i.e. the amount of time that takes for a population to double in size or value at a constant growth rate), an indicative measure of renewability that can make possible the comparison with other animal species in order to elaborate physical indicators and models for the impact assessment within the LCA framework. Data on the renewability rate within the population dynamics domain are available for several species, but just in particular contexts and just in few cases it is possible to generalize the data to a global or national level as it is necessary in LCA, being the studies very context-dependent. Furthermore, although changes in population size following medium-long term pressures have been measured for several species in terms of percentage of increase or decrease over time (e.g. elephants, see Ogutu et al., 2014 and Okello et al., 2008), population- or species-specific indicators based on renewal time and their estimations were not available as a systematic list to be used for our purposes.

Given the differences in population dynamics and modeling thereof, there is not an equivalent renewability measures or indicators on species others than fish, thus making the comparison between species problematic. Among the indicators about species renewability, the most suitable in the view of falling into the LCIA scheme were the following: a) “biomass at maximum sustainable yield (MSY)” (OECD, 2017), mainly applied to fish stocks, which is considered as the largest yield (in tons) that can be caught from a specific fish stock over an indefinite period of time under constant environmental conditions; and b) recovery of population size, to be considered as the time needed to a population to return to its pristine conditions following a decline.

MSY has been heavily criticized for both practical and theoretical reasons, namely: (i) a general lack of reliability of data to make a clear determination of the population's size and growth rate; (ii) it misses the fact that populations undergo natural fluctuations in abundance and would become severely depleted under a constant-catch strategy; (iii) the tendency to ignore the broad variety of aspects of population structure, such as age classes and their differential rates of growth, survival and reproduction (Townsend et al., 2008).

Data on renewability time of natural forests and other commercial plants from natural habitats were difficult to gather as well. In fact, it was complicated distinguishing between data proceeding from man-made

plantations or from natural forest management, especially in the case of tropical forests harvested for hardwood, fruit and latex. On this basis, we identified those species or categories of products that may come not only from natural forests, but also from cultivation (i.e. cork from the bark of cork oaks, etc.).

Generally, the rotation period (defined as the period between regeneration establishment and final cutting - SAP, 1994) and natural regeneration or reproduction period (i.e. the time between the initial regeneration cutting and the successful reestablishment of a new age class by natural means, planting, or direct seeding - SAP, 1994) are reported. However, these parameters vary depending on altitude and soil fertility, and in forest management, regeneration time can be set depending on current market demand, generating a sort of human-dependency, which overcomes the natural life cycle and recovery of resources.

Ideally, the characterization should encompass all biotic resources. Comparisons between impacts across geographic areas, ecosystems and temporal scales (see Figure B1 in Appendix B) would be possible with a standardization of indicators. Considering the renewability of resources would allow adding a temporal element to resource depletion, as Cummings and Seager (2008) already underlined, and would help generate reliable characterization factors accounting for the sustainable use of natural biotic resources. However, there is still need of systematizing the species renewability concept (Woods et al., 2016).

#### 3.4.2.2. Renewability-based model and associated characterization factors

According to our literature review, we identified a number of renewability indicators (Figure B1 in Appendix B) to be potentially adopted in the calculation of characterization factors. It was difficult to find homogeneous renewability indicators for all biotic resources, except for fish and a few other animals and plants. Therefore, by capitalizing on the available indicators and data, we selected two indicators, namely population doubling time for wildlife and rotation period for plants to be used as a practical example, since so far they represent the best quantitative proxy of key feature affecting resource availability potentials.

Characterization factors as calculated by us are reported in Table 3.4. When a single data was not available, we calculated CFs as arithmetic mean between the maximum and the minimum values of the renewal time range proposed in the retrieved literature. In several cases (e.g. brown trout; Atlantic sturgeon), due to the lack of a properly defined range of time, we used the maximum or minimum presented value as absolute average value for the calculation of CFs. In order to multiply each CF by the related elementary flow, the CF unit of measurement is in terms of years/kg and is to be multiplied by the elementary flow expressed in terms of mass.

The resulting indicator is called NOBRri, namely Naturally Occurring Biotic Resource renewability indicator.



**Table 3.4.** Examples of Characterization Factors (CFs) for NOBRri based on the mean of renewal time ranges, expressed in terms of “population doubling time” (D) and “rotation period” (R) for the most commercially valuable species. The list is presented according to the alphabetical order of commercial groups within each system (aquatic animals; terrestrial animals; terrestrial plants). Chromatic scale for CFs ranges from red (lowest renewability rate) to light green (highest renewability rate).

Commercial group	Species	Common name	Renewal time - Range from literature (years)		Average renewal time (years/kg)	Ref.
Amphibians	<i>Lithobates catesbeianus</i>	Bullfrog	> 3	D	3	1
Anchovies	<i>Engraulis encrasicolus</i>	European anchovy	1.4 - 4.4	D	2.9	2
Aquatic mammals	<i>Balaena mysticetus</i>	Bowhead whale	52	D	52	3
	<i>Balaenoptera musculus</i>	Balaenoptera	31	D	31	3
	<i>Orcinus orca</i>	Killer whale	23	D	23	4
Barbels	<i>Barbus barbus</i>	Barbel fish	4.5 - 14	D	9.25	2
Carp	<i>Cyprinus carpio</i>	Common carp	1.4 - 4.4	D	2.9	2
Crocodiles & alligators	<i>Crocodylus acutus</i>	American crocodile	< 20	D	20	5
	<i>Crocodylus niloticus</i>	Nile crocodile	< 23	D	23	6
	<i>Alligator mississippiensis</i>	Alligator	< 50	D	50	7
Flounders	<i>Platichthys flesus</i>	European flounder	1.4 - 4.4	D	2.9	2
Halibuts	<i>Hippoglossus hippoglossus</i>	Atlantic halibut	> 14	D	14	2
Herrings	<i>Clupea harengus</i>	Atlantic herring	1.4 - 4.4	D	2.9	2
Paddlefishes	<i>Polyodon spathula</i>	Mississippi paddlefish	4.5 - 14	D	9.25	2
River eels	<i>Anguilla anguilla</i>	European anguilla	4.5 - 14	D	9.25	2
Salmons	<i>Salmo salar</i>	Atlantic salmon	1.4 - 4.4	D	2.9	2
	<i>Oncorhynchus gorbuscha</i>	Pink salmon	1.4 - 4.4	D	2.9	2
Sardines	<i>Sardina pilchardus</i>	European pilchard	1.4 - 4.4	D	2.9	2
Shads	<i>Hemitaenichthys polylepis</i>	Alosina	< 1.3	D	1.3	2
Sharks	<i>Alopias vulpinus</i>	Common thresher	6.7 - 11.6	D	9.15	8
	<i>Carcharhinus leucas</i>	Bull shark	17.1 - 30.8	D	23.95	8
	<i>Carcharodon carcharias</i>	White shark	12.2 - 20.1	D	16.15	8
	<i>Isurus oxyrinchus</i>	Mako shark	9.4 - 15.9	D	12.65	8
	<i>Lamna nasus</i>	Porbeagle	> 14	D	14	2
	<i>Mustelus californicus</i>	Gray smooth hound	3.3 - 5.8	D	4.55	8
	<i>Prionace glauca</i>	Blue shark	7.7 - 13.2	D	10.45	8
	<i>Pristis pectinata</i>	Smalltooth sawfish	4.5 - 14	D	9.25	2
	<i>Pristis perotteti</i>	Large-tooth sawfish	4.5 - 14	D	9.25	2
	<i>Rhizoprionodon terranovae</i>	Atlantic sharpnose	5.0 - 9.2	D	7.1	8
	<i>Sphyrna tiburo</i>	Bonnethead	4.2 - 7.5	D	5.85	8
	<i>Squalus acanthias</i>	Spiny dogfish	28.9 - 46.2	D	37.55	8
	<i>Triakis semifasciata</i>	Leopard shark	14.9 - 25.7	D	20.3	8
Smelts	<i>Osmerus eperlanus</i>	European smelt	1.4 - 4.4	D	2.9	2
Sturgeons	<i>Acipenser oxyrinchus</i>	Atlantic sturgeon	> 14	D	14	2
Tilapias & other cichlids	<i>Gadus morhua</i>	(Eastern) Baltic Cod	1.4 - 4.4	D	2.9	2
	<i>Oreochromis mossambicus</i>	Mozambique tilapia	1.4 - 4.4	D	2.9	2
	<i>Oreochromis niloticus</i>	Nilotic tilapia	1.4 - 4.4	D	2.9	2



Commercial group	Species	Common name	Renewal time - Range from literature (years)		Average renewal time (years/kg)	Ref.	
Trouts	<i>Salmo trutta</i>	Brown trout	< 1.3	D	1.3	2	
	<i>Istiophorus platypterus</i>	Sailfish	2.9 - 4.4	D	3.45	8	
Tunas. bonitos. billfishes	<i>Kajikia audax</i>	Striped marlin	3.7 - 5.6	D	4.65	8	
	<i>Katsuwonus pelamis</i>	Skipjack tuna	1.3 - 2.9	D	2.1	8	
	<i>Makaira nigricans</i>	Blue marlin	3.7 - 5.5	D	4.6	8	
	<i>Thunnus alalunga</i>	Albacore tuna	4.2 - 6.2	D	5.2	8	
	<i>Thunnus albacares</i>	Yellowfin tuna	1.4 - 4.4	D	2.9	2	
	<i>Thunnus maccoyii</i>	Southern bluefin tuna	5.2 - 7.5	D	6.35	8	
	<i>Thunnus obesus</i>	Bigeye tuna	2.5 - 4.9	D	3.7	8	
	<i>Thunnus orientalis</i>	Northern bluefin tuna	4.6 - 6.5	D	5.55	8	
	<i>Thunnus thynnus</i>	Atlantic bluefin tuna	4.5 - 14	D	9.25	2	
	<i>Xiphias gladius</i>	Swordfish	4.8 - 6.9	D	5.85	8	
	Terrestrial crustaceans	<i>Scylla serrata</i>	Crabs	1.4 - 4.4	D	2.9	9
Fur or skin terrestrial vertebrates	<i>Canis lupus</i>	Wolf	4.7	D	4.7	3	
	<i>Kinixys belliana</i>	Bell's hinge-back tortoise	15	D	15	10	
	<i>Martes martes</i>	Pine marten	7	D	7	11	
	<i>Mustela erminea</i>	Stoat	10	D	10	12	
	<i>Mustela vison</i>	European mink	10	D	10	13	
	<i>Ondatra zibethicus</i>	Muskrat	10	D	10	14	
	<i>Vulpes vulpes</i>	Red fox	10	D	10	15	
Game birds	<i>Branta canadensis</i>	Canada goose	3	D	3	16	
Game mammals	<i>Bison bonasus</i>	European bison	5.0 - 6.0	D	5.5	17	
	<i>Cervus elaphus</i>	Red deer	10.0 - 14.0	D	12	18	
	<i>Odocoileus virginianus</i>	deers				19	
	<i>Odocoileus bezoharticus</i>	deers	2.0 - 3.0	D	2.5		
Hardwood	<i>Acer platanoides</i>	Maple	100 - 120	R	110	20	
	<i>Acer pseudoplatanus</i>	Sycamore maple	100	R	100	20	
	<i>Acer rubrum</i>	Red maple	50 - 110	R	80	21	
	<i>Platanus spp.</i>	Sycamore spp.	60 - 80	R	70	20	
	<i>Populus spp.</i> ( <i>alba.nigra. tremula</i> )	Poplar	80 - 120	R	100	22	
	<i>Prunus avium</i>	Wild cherry	60 - 80	R	70	20	
	<i>Quercus spp.</i>	Oak spp.	60 - 120	R	90	23; 24	
	<i>Quercus suber</i>	Cork oak	10 - 12	R	11	25	
	<i>Robinia pseudoacacia</i>	Black locust	5	R	5	26	
	<i>Sorbus torminalis</i>	Wild service tree	120 - 150	R	135	27	
	<i>Tectona grandis</i>	Teak	20 - 40	R	30	28	
	Softwood	<i>Betula spp.</i>	Birch	70 - 140	R	105	29
		<i>Fraxinus spp.</i>	Ash	60 - 80	R	70	20
<i>Picea spp.</i>		Spruce	100	R	100	30	
<i>Pinus strobus</i>		White pine	90 -150	R	120	31	
<i>Pinus sylvestris</i>		Red pine	150-200	R	175	30	

**References:** (1) Amphibian Survival Alliance, 2016; (2) Fishbase, 2016; (3) IUCN, 2016; (4) Olesiuk et al., 2005; (5) US Fish and Wildlife Service, 2016; (6) GBIF, 2016; (7) Naturalis Biodiversity centre, 2016; (8) Camhi et al., 2009; (9) Shelley and Lovatelli, 2012; (10) WCT, 2016; (11) Storch et al., 1990; (12) ADW, 2016; (13) DAISIE, 2016; (14) COSEWIC, 2016; (15) Grzimek, 1975; (16) US Fish and Wildlife Service, 2005; (17) Deinet et al., 2013; (18) Langvatn and Loison, 1999; (19) The Northeast Deer Technical Committee, 2016; (20) Spiecker and Hein, 2009; (21) WDNR, 2015; (22) Klimo and Hager, 2001; (23) DeStefano et al., 2001; (24) Dey et al., 1996; (25) PFAF, 2016; (26) Bassam, 2013; (30) Frelich and Reich, 1995; (27) Nicolescu et al., 2009; (28) Ladrach, 2009; (29) United States Forest Service, 1975; (30) Frelich and Reich, 1995; (31) Martin and Lorimer, 1997.

The evaluation of the renewal time is only one feature related to the concept of ecosystems' carrying capacity. However, it allows characterizing natural resources with an indication of the potential time-related constraints associated with their provision, ranking e.g. different species inside a kingdom. However, it is worth noting that the resulting CFs could be used at this stage for hotspots analysis only, when comparing biotic resources coming from different kingdoms (e.g. plant and animals). The hotspots would help identify resources which are longer available to be used by humans. However, among different kingdoms, CFs are not fully comparable, considering that they quantify different ecological aspects for the mentioned naturally occurring biotic resources. Indeed, this limitation is dictated by several aspects: (i) the fact that there is no functionally comparability between the aquatic and terrestrial systems since resources are principally affected by different factors (e.g. removal from original stock vs land use changes, respectively); (ii) the fact that natural biotic resources we considered, such as animals and plants, are taxonomically distant. The concept of renewability for a plant species may not match with the renewability for an animal species. This happens because the calculation of their renewability is based on different ecosystemic aspects. However, they all may share the same unit of measurement, i.e. the renewal time in years/kg. A similar case within the LCA framework exists for the category "Human Toxicity – non cancer", where disparate diseases having independent intrinsic characteristics are listed and characterized with the same unit of measurement. On this basis and considering the missing of accounting of spatial aspects in CFs when coming to measure the renewability of biotic resources' stocks, it results necessary to deepen the research for a common indicator, which could measure a consistent and coherent aspect of renewability.

### 3.5. A research agenda for improving accounting and characterization of naturally occurring biotic resources

Several steps are to be considered in order to cover the conceptual and methodological gaps in the accounting and the impact assessment of natural biotic resources within the LCA framework. Characterization- aiming at covering the "Natural Resource" area of protection- should be focused on measuring potential constraints to the availability of resources, ensuring a sustainable harvesting. We analyzed the issue of natural biotic resources focusing on elements that may interfere with the natural process of providing biomass. Consideration on how those interventions may lead to depletion is based on the potential of being renewed in relatively short time frame. On this basis, renewal or regeneration time may be adopted as a proxy, taking into consideration the current level of resource stocks. In fact, the scarcity aspect, related to current level of consumption versus availability, requires further methodological steps to be developed. Our choice of adopting the renewal time (or similar indicator) as a measure for characterizing

biotic resource is a preliminary attempt to characterize possible drivers of depletion towards the more complex concept of scarcity.

A number of research gaps need to be covered by future research, as reported hereinafter.

- **Completeness of the inventory:** several issues are related to this aspect. First of all, elementary flows and inventories for biotic resources are very limited in number. In order to develop future inventories for LCA studies, elementary flows related to commercially valuable and natural occurring biotic resources (i.e. resources that represent an input material in supply chains) should be developed. Moreover, information and metadata on elementary flows should be included and better defined (e.g. how to describe the resources, whether in terms of gross or net material, wet or dry weight). For example, details about the usable volumes such as the carcass weight are needed (e.g. eating 1 kg of fish requires a certain fraction of the entire fish body). Another important issue to overcome is that information is sometimes provided for commercial groups (e.g. tunas) and not for specific species. This lack of specificity may hamper the impact assessment of the biotic resource as different species within the same group may have ecologically different traits. Generally, the terminology still needs to be defined, harmonized and standardized, in order to differentiate between naturally occurring biotic resources and farmed/cultivated ones. Of course, the risk of assessing the same input material in two ways (as coming from ecosphere directly or as results of a production system) is something to be discussed and solved.
- **Comparability with biotic resources from the technosphere (e.g. from agriculture and livestock).** Currently, resources that derive from processes along a supply chain (resource as a product) are not characterized and, when they enter a new supply chain, their use does not generate any impact on the resource depletion category. On the other hand, a similar type of resource taken from nature (resource as an input from ecosphere) could be hypothetically characterized as done for abiotic resources from the ecosphere, and their impacts on resource depletion category will have a certain value different from zero. Research should go towards the direction of defining how to treat these apparently different resources. A key element is related to how soil is modeled for the cultivation of biotic resources. In fact, the biotic resource to be protected in agricultural production is the soil, which underpins the production, and not the produced biomass (e.g. the crop).
- **Boundary between ecosphere and technosphere.** Based on the current way adopted to assess and quantify the impact, i.e. as a transition of materials and energy from the ecosphere to the technosphere, we may face with some issues for biotic resources that need particular attention. For instance, this may be the case of the breeding, restocking and reintroduction of wild animals. In fact, it remains unclear if, in this case, resources taken from a reintroduced population are to be considered as proceeding from the ecosphere or as a product deriving from human intervention, therefore belonging to the technosphere. This issue can occur also in the case of forestry management, especially for short rotation. Hence, it becomes fundamental to identify and set the boundary between ecosphere and technosphere when coming to define natural biotic resources.

- **Overlaps between assessments of impacts associated with resource depletion and land use.** According to our goal, i.e. the focus on resource availability and not on ecosystem quality per se, double counting of impacts is, in principle, avoided as the land use related impacts are focusing on different impact mechanisms. However, both land use change when exploiting a resource and the overexploitation itself may lead to biodiversity impacts. Moreover, there are several naturally occurring biotic resources which are not related to a land use occupation or transformation (e.g. fishing) or others which are used in concomitance with other land uses (e.g. harvesting herbs in forest for pharmaceutical uses may be barely related with “forest occupation” as we usually address it in LCA – using the forest for exploiting wood).
- **Paradoxes.** While certain biotic resources are affected by the removal from their available stock (e.g. fish stocks), other resources (such as some terrestrial animals) may suffer the most for the loss of habitat, which is actually associated with impacts from land use category rather than properly resource removal and consequent stock depletion. The CFs we propose are based on the same criteria of depletion both for aquatic and terrestrial systems. However, substantial differences between the two environments exist, that are not negligible.
- **Alien and invasive species.** Within the database (Table B2), we included a specific focus on environmental aspects about alien and invasive species. We felt that considering the alienness and invasiveness of species might be a key element to approach life cycle of biotic resources and their impact assessment. In fact, alien and invasive species may represent a threat to the availability and renewability of the most marketable existing species worldwide. For instance, *Oreochromis* spp. (i.e. *O. mossabicus* and *O. niloticus*), which are addressed as invasive species (GISD, 2016), are exerting significant pressures on water ecosystems because of their aggressiveness, their high fecundity rate and resistance to poor water quality and infectious diseases. Therefore, the likelihood of their occurrence and the potential effect on native species should be considered carefully in a framework of impact assessment.
- **Spatial and temporal dimensions.** Compiling the database allowed us to outline the temporal and spatial issues associated with commercial wild annual species, particularly when coming to plants. In this case, the differences in the temporal scales between technosphere and ecosphere are evident. Technosphere processes, which are analyzed by an LCA study, act on a static anthropocentric equilibrium, without taking into account interactions with the ecosystem; whereas ecosphere processes follow a dynamic equilibrium (i.e. in constant change) (Commoner, 1972). *Matricaria chamomilla* (common name: chamomile) can be presented as a tangible example of the temporal and spatial paradoxes which involve natural biotic resources in LCA. This wild species is a naturally occurring annual herb, generally harvested for its medicinal properties. Its growth and availability depends on its interactions with the ecosystem, in particular with soil and nutrients, and with its vulnerability to natural disasters like fires and pests. Therefore, any change in ecosystem quality, soil components and nutrient cycle may generate variations on annual availability and physico-chemical properties of chamomile (Harborne, 1982). This evolving status of nature, which is not currently taken into account in the LCA-based “static” system, should be considered in future modeling.

- **Spatial definition of CFs.** The pressures exerted by human interventions on biotic resources may depend on their localization in space. Particularly, the availability of biotic resources may depend on a combination of factors, among others the magnitude of human pressures (e.g. the rate of harvesting) and the geographic area where the resources are localized. For instance, the same species may react in different ways to impact of the same magnitude but localized in different geographical areas, due to the size of the biotic resource stock and their stability. Therefore, when dealing with the proposal of CFs for biotic resources, it becomes important to account for the location specificity of impacts.
- **Non-linearity of impacts.** Populations undergo natural fluctuations in abundance and would become severely depleted (ultimately leading to local extinctions) under a constant-catch strategy. In fact, it becomes an issue including such a non-linear growth rate in the LCA system, which presumes the linearity of production scale and of environmental impacts. Therefore, it should be important to take into consideration the current level of consumption versus availability.
- **Ecological features.** According to the pressures put by human interventions, an additional step that should be taken into account is related to the importance of considering the ecological conservation status of species. On this basis, the CFs values proposed could be then weighted by considering the vulnerability of the species, for example according to the IUCN red list values (IUCN, 2016). In fact, renewability is just one of the elements affecting availability of resources; other ecological features such as resistance, resilience, vulnerability, etc. may play a role and should be taken into consideration in order to avoid compromising the natural system.
- **Globalization and international trade.** In a globalized economy, where imports and exports represent the ordinary way of sharing products and services, another important step to be overcome to devise a valid pathway for characterizing impacts on natural biotic resources is to understand to what extent the trade of products on a global scale generates impacts on natural biotic resources. Global trade has been addressed as a potential source of biodiversity threats by several studies (Lenzen et al., 2012) and impacts on the environment associated to globalized supply chains are generally known; however the impact on species extracted as resources (e.g. to what extent a forest is affected by the removal of a plant for extracting wood) and the impact on species that are secondarily put at risk as a consequence of another resource extraction (e.g. to what extent a populations of forest birds are affected by the extraction of a tree for wood) are not clear nor quantified. Moreover, it is important to understand this aspect in order to incorporate these details in the LCA of products and services in view of a sustainable and equitable economy at global level.

### 3.6. Conclusions

Human population derives many essential goods from natural ecosystems, including seafood, game animals, wood, herbs for domestic and industrial processes, cosmetics and pharmaceutical products. These goods represent a fundamental part of the global economy and an important life-support for human societies. In a world where growing population is consuming natural resources at an unprecedented scale and pace, it is

clear that there is the need to go towards the direction of a sustainable development according to the Sustainable Development Goals (UN, 2016). European policies promote the efficient use of resources and set the need to manage natural biotic resources in order to ensure sustainable production, distribution and consumption of biomass. In light of this, a more specific accounting of natural biotic resources, in particular those naturally occurring in the ecosphere, is necessary to be improved. In fact, the impacts associated with these resources are barely covered in life cycle inventories, also due to the urgent need to clarify the boundaries between ecosphere and technosphere in LCA. Besides, a consistent and robust impact assessment scheme, which evaluates how much our market demands influence and endanger the availability of natural resources, needs to be developed.

In this study, we reached the target of improving the inventory of naturally occurring biotic resources, identifying and listing the most commercially valuable species, which could be entered as elementary flows in LCA inventories. Terminology still needs to be defined, harmonized and standardized and the list needs to be evidently implemented with additional information, such as those about usable volume. We acknowledge that it is unrealistic to develop a fully comprehensive list of resources; however, consultations with experts are crucial in order to build a reliable database.

Additional research is needed in order to overcome the issue related to the heterogeneity of indicators and to address their feasibility in describe the impacts on resource availability within the LCA context. For example, future effort should be made in order to understand how to deal with resources of different nature (such as plants versus animals).

In this study, through the proposal of the new indicator based on renewability, NOBRri, we identified the notable potential of using renewability time as a basis for calculating the characterization factors for biotic resources and their depletion. Undoubtedly, more research is needed in this field, and the collaboration between different disciplines (e.g. ecology, engineering, etc.) is required in order to make progress towards an impact assessment model and framework fully implementable in LCA.

In our opinion, this work could be the basis for a coherent framework for improving the modelling of biotic resource in life cycle assessment, a step needed to ensure that the methodology comprehensively account for crucial elements of environmental sustainability.

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## 4. Nexus: a new era for addressing interaction between impacts in LCIA

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## Abstract

Ensuring secure access to food and energy worldwide relies on win-win share of sectoral use of constrained natural resources such as land and water, taking also into account the crucial role of ecosystems and their services. The increase in global population and the related growing demand for food and other services are exerting unsustainable pressures on natural resources, compromising their use within the ecosystems' carrying capacity. Progressively, studies and initiatives have been developed with the aim of identifying win-win share strategies, which may compensate the sectoral demands of natural resources, addressing the need of a holistic and interdisciplinary nexus approach. In this study, while emphasizing the importance of a holistic approach and highlighting the fundamental role of ecosystems, we propose a synthesis matrix system that describes the complex and closely bound relationship between natural resources use for food (specifically water and land), energy (defined as ecosystem service flows) and ecosystems, along the lines of the concept of ecosystem-water-food-land-energy nexus. Our synthesis matrix system, which could address different scales, i.e. both from the global to the local, has been designed to include impacts and their interactions, as well as with climate change. The matrix aims at integrating quantitative and qualitative aspects, which are often neglected in traditional impact assessment approaches. The complexity of the interactions between the different components of the nexus (i.e. resources, energy, ecosystem) requires relying not only on quantitative evidences, but also on expert judgment. A sensitivity analysis has been therefore conducted to illustrate and verify the convergence of judgments from different experts. Moreover, being the matrix meant for supporting holistic assessment of supply chain, in the present study the integration of the matrix within life cycle assessment (LCA) is proposed. However, in order to support the analysis of interconnections among impacts, further methodological development of the LCA methods is still needed. An illustrative example related to the competition for water, land and food for bioenergy production is depicted. The matrix system shows that there are predominantly negative impacts given by sectoral uses of resources on the provision of ecosystem services, an issue that requires most focus on resource efficiency and on the environmental and economic impacts of natural resources use while reducing the trade-offs between the sectoral demands.

### 4.1. Introduction

Food, water, land and energy are fundamental and closely linked life-sustaining needs for human well-being as well for sustainable development (FAO, 2014; UNWWAP, 2014; Harvey and Pilgrim, 2011). Changes in the availability of water, land and energy supplies would strongly affect production of food, including the secure access thereof, with severe implications for human health (MEA, 2005; Gerbens-Leenes et al., 2009), particularly in developing countries that already experience serious resource scarcity conditions (Tilman et al., 2009).

The rapid increase in global population occurred over the last decades has exerted unmanageable pressures on natural resources, compromising their sustainable use (Hoff, 2011; Tilman et al., 2009). In particular, population growth, acting as an independent driver of impacts, has played a significant part in not only

increasing food (De Fraiture et al., 2007), water, land and energy demand worldwide (Gerbens-Leenes et al., 2009; Harvey and Pilgrim, 2011), but also correspondingly amplifying the adverse environmental and economic impacts of other enchain drivers such as urbanisation, land degradation, economic inequity and climate change (Tilman et al., 2009). As a result of this continuum chain, the competition for natural resources between sectors such as agriculture and industry has increased, with unpredictable consequences for human well-being, economic growth and the environment (FAO, 2014; Ringler et al., 2013). It is all to the good that the increasing awareness of consumers and societies towards the selection of goods and environmentally friendly produced services, at least in the developing countries, is pushing the agricultural producers and industry to adopt more functional and sustainable production systems with the aim of improving their sectoral productivity, while protecting ecosystems and their functions. In fact, natural resources are shared by different sectors with different social and economic expectations. Political economy-based factors, particularly short term governmental economic plans aiming for the maximum economic benefit and economic growth, which may usually ignore minimizing environmental impacts, constrain the sustainable use of natural resources. Therefore, synergies with shared benefits among sectoral users of natural resources are still missing, also because the management of water, land and energy generally takes place in isolation, without concrete consideration of the positive or negative impacts they have on other sectors.

Recently, to answer to the need of assessing the interplay between the different sectoral demands together to identify a win-win sectoral strategy of global resource management, a nexus concept among food, water, energy sectors (Hoff, 2011; Bazillian et al., 2011; Rasul, 2014; FAO, 2014) and ecosystems (ICIMOD, 2012; Bizikova et al., 2013), including land use (Ringler et al., 2013) has been proposed. Nexus concept describes the complex and closely bound relationship of human needs, natural resources and ecosystems on which human well-being rely to achieve socially, economically and environmentally sustainable goals (FAO, 2014).

Building on over 30 years of initiative for sustainable development, the recently released Sustainable Development Goals (SDG, 2015) aim at safeguarding the long-term well-being of humankind by ensuring that agreed development is socially, economically and environmentally sustainable (Weitz et al, 2015). In short, long-term sustainability requires integrated sectoral strategies by acknowledging that many natural resources such as water, land, fossil fuels and minerals are on the decrease because of their current unsustainable over-exploitation.

To better ensure the sustainable use of natural resources, resulting from the cooperation among sectors, in synergy with ecosystems and biodiversity, and to improve decisions in a Green Economy framework, the nexus-oriented approach requires the integration of life cycle thinking for each type of sectoral use, in order to avoid burden shifting and assess trade-offs among different pressures and impacts. Life cycle thinking could be applied using a broad variety of methodologies, namely life cycle assessment (LCA, ISO 14040, 2006; and ISO 14044, 2006), life cycle costing (LCC), social life cycle assessment (sLCA) and a further integrated methodology which is the life cycle sustainability assessment (LCSA) (Sala et al., 2013a; 2013b).

So far, very few studies have attempted an integration of life cycle thinking within the nexus framework. For example, Dale and Bilec (2014) developed a model for calculating life-cycle environmental impacts of regional energy and water supply scenarios (the so-called REWSS model) using the water-energy nexus approach. The model was used to discuss future energy pathways in Pennsylvania, future electricity impacts in Brazil, and future water pathways in Arizona. Scott et al. (2011) proposed a study on LCC aiming at building policy awareness on the role of life cycle thinking to achieve the objectives of sustainable development, as well as sustainable service flows. However, there are not sufficient studies which enlighten the uncertainties and gaps on a holistic nexus perspective to refer the whole conceptual frame. Recently, Hang et al. (2016) develop a “two-stage process systems” engineering tool, an integrated design of the food-energy-water nexus that allows potential interactions between productions, combined with the concept of resource accounting by using exergy, for the design of local production systems in a designated eco-town in the UK. The authors demonstrated the advantages of an integrated system making design that centralizes the supplies in local productions to meet local demands. In another work, Sanders and Masri (2016) proposed a novel approach adopting the remote sensing into integrated resource management communities, leading to the development of technologies for assisting in achieving sustainable development. The authors highlighted the opportunities and challenges that can guide technology development and created new interdisciplinary research partnerships using the concept of food-energy-water nexus.

The Nexus concept has an intricate nature due to the complexity of its components (namely ecosystem, water, land, food and energy), that should be addressed together in order to provide solutions aiming at improving resource use efficiency (Bazillian et al., 2011; Olsson, 2013). In this context, ecosystems represent the most important pillar of the nexus, since they incorporate all the features that support food, water, land and energy availability and production. However, this aspect increases the complexity of the nexus itself. Therefore, in this study, we develop a nexus-based synthesis matrix system for food security, which places ecosystems and their services at the centre of the nexus and refers to the Common International Classification of Ecosystem Services tables, hereinafter referred to as CICES (Haines-Young and Potschin, 2013). The objective of this system thinking is to identify the interrelations among the service flows related to the different sectors mentioned in the ecosystem-water-food-land-energy nexus. For instance, the effects of different sectoral energy, water and land demands onto the ecosystem services can be evaluated together by considering the life cycle impact assessment of each service flow, addressing the role of climate change and relevant EU policies as well.

The standard LCA misses elements of interaction between impacts, which may largely affect the results of the assessment. The synthesis matrix coupled with standard LCA may improve substantially the analysis of agricultural systems. Therefore, the aim of this paper is to discuss the role of life cycle assessment of each service flow associated to different sectoral use of resources such as water and energy as pivotal complementary part of the proposed Ecosystem Water Food Land Energy (EWFLE) nexus. The issue of improving the impact assessment stage of LCA integrating qualitative aspects into quantitative ones has already emerged as a need, especially when bio-based/ agricultural systems are involved (Sala et al., 2017,

Notarnicola et al., 2017). Illustratively, the study shows how nexus and life cycle assessment can be integrated using a synthesis matrix system for food security to define better potential drivers of impacts and hotspots.

This chapter is organized as a discussion paper, as follows: section 4.2 presents the methodology adopted for investigating current nexus concepts and building the nexus-oriented matrix, linking the previously mentioned EWFLE nexus and the life cycle assessment framework; section 4.3 presents the resulting matrices and their integration in a life cycle assessment system thinking; in section 4.4, the utility of the synthesis matrix system in the challenges towards achieving sustainability goals, particularly within the agri-food sector is discussed; finally, section 4.5 encloses the conclusions and suggestions for further research.

## 4.2. Methodology

A synthesis matrix which illustrates relationship between interventions and impacts has been developed, focusing on Ecosystem Water Food Land Energy (EWFLE) nexus. The steps to build the matrix and to link the matrix to the life cycle assessment framework are:

- an evidence-based step, built upon a literature review to identify links between the elements of the nexus (section 4.2.1) towards the development of the synthesis matrix itself;
- an expert-based step, which aims at identifying the type of link between impacts (positive/ negative/ neutral), assigning scores to the impacts (section 4.2.2 and section 4.2.3);
- the matrix integration within a life cycle assessment framework (section 4.2.4).

### 4.2.1. Literature screening of possible nexus framework

There is a wide number of international initiatives to frame the water-food-land-energy nexus and define the close relationships between its components. The literature suggests that a holistic nexus-oriented approach is necessary to achieve sustainable growth and to reduce unintended consequences and trade-offs of adopting narrowly sectoral approach (Hoff, 2011; WEF, 2011; ICIMOD, 2012; Bizikova et al., 2013).

Among the most important initiatives about the nexus concept, we reported the following:

- The nexus framework, developed as a part of the *Bonn2011 Nexus Conference on Water, Energy and Food Security Nexus*, focuses on integrated solutions for a more efficient resource management across sectors in space and time to move towards a green economy. The proposed framework approach, which is mainly centred on water supply, represents an effort to clarify the interdependencies between food and energy sectors, which are connected to available water resources. The goal of promoting water, energy and food security for all sectors with equitable sustainable growth could be achieved through action fields and specific measures by integrating the society, optimized economy and sustained ecosystem services (Hoff, 2011; Bizikova et al., 2013).



- The framework approach proposed by the World Economic Forum (WEF, 2011) represents an attempt to generate more consciousness among decision makers about the major global environmental risks, e.g. air pollution, biodiversity loss, earthquakes, storms, ocean governance and volcanic eruptions, and to prepare them to better react in case of crises. This nexus framework, which symbolizes the major global risk area together with economic imbalances, underlines the interconnections between food and water security, economic inequality, energy security and economic risks resulting in energy shortages with impact on growth and social persistence. Economic growth and environmental pressures affecting resource supply are also included into this framework.
- The framework developed by the International Centre for Integrated Mountain Development (ICIMOD, 2012) focuses on ecosystems and their functions, as the synthesis matrix system we propose in our study, and poses predominantly emphasis on the mountain perspective of the nexus in the Himalayas and South Asia area. In this framework, the provisioning, regulating, supporting and cultural ecosystem services are considered to crucially contribute to the food-water-energy nexus, thus becoming inevitable to protect the ecosystems that characterize the Himalayan area in order to achieve sustainable contributions. However, although a holistic nexus-oriented approach for Himalayan area has been structured; it has not been implemented yet.
- Similar to the ICIMOD framework, the International Institute for Sustainable Development (IISD) offers a framework centred on ecosystem management (Bizikova et al., 2013) for optimizing water, energy and food security. A practical implementation guidance is provided to strengthen the integration of research, policy, investment and other related actions within the nexus framework. According to the authors, focusing on ecosystems and their services while emphasizing the importance of the biotic components of the landscape as a common connection between water, food, energy would fill the gap associated to the lack of accounting of ecosystem services in previous frameworks.
- Another example is proposed by Daher and Mohtar (2015), who explored the link between water, resources and food security performing a WEF nexus-based project. They designed and tested an innovative integrated resource management tool for decision makers, based on the water-energy-food nexus framework for Tunisia, Jordan and Qatar. With this nexus-oriented modelling tool, they defined the linkages between the interconnected resources as water, energy and food, and enabled explicit corresponding quantifications. The integrated nexus-based model provides a common platform for scientists and policy-makers for evaluating scenarios and identifying strategies of sustainable national resource allocation which focus on Qatar.

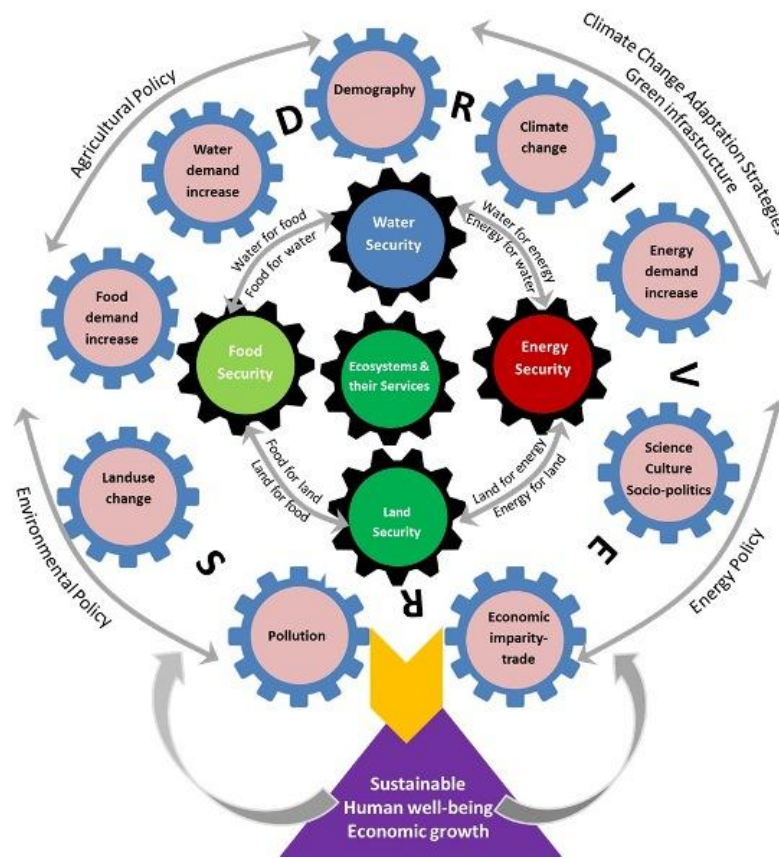
The shared focus of all the frameworks presented above is to promote joint actions with multi-purposed policy strategies aiming to reduce trade-offs, enhance synergies across sectors and provide sustainable growth.

Apart from the frameworks, there are studies that: (i) highlight the importance of and deeply argue the nexus-oriented approach (Beck and Walker, 2013; FAO, 2014; Kibaroglu and Gürsoy, 2015; Wong and Pecora, 2015; Wong, 2015); (ii) recommend solutions based on holistic approaches which simplify the complex nexus cyclic system by using different combination of nexus components (Allouche et al., 2015; Bhaduri et al., 2015, FAO, 2014); (iii) and analyze regionally or globally the environmental status in order to facilitate the implementation of a nexus-oriented approach (Lawford, 2013; Holtermann and Nandalal, 2015; Daher and Mohtar, 2015; Mohtar and Daher, 2016; Mohtar, 2016). For presenting the practical consequences and the interactions of joint actions and policy strategies, Gain and Giupponi (2015) analyzed nexus-related institutional issues in Bangladesh, then suggesting integration for policy implementation. Halbe et al. (2015) presented a methodological framework to analyze sustainability innovations in the WEF nexus and strategies for governing transition processes in Cyprus. Hensengerth (2015) analyzed the limited influence that a water-centred organization may have on hydropower development addressing the issue of authority and hegemony in the field of international development and hydropower policy, by using as an example the case study of the Xayaburi Dam in the Lower Mekong Basin.

By proposing our nexus-oriented framework in this study, we believe that the involvement in life cycle assessment of each sectoral action included into the holistic nexus approach and defined with a synthesis matrix system would provide different beneficial dimensions, included those related to governance. For instance, this could facilitate the involvement of the stakeholders in the solutions and decision making process towards multi-participation.

#### 4.2.2. Matrix development: water, energy and ecosystem for food

As proposed by ICIMOD (2012) and Bizikova et al. (2013), we emphasize the importance of ecosystems and their services in providing water, energy and food supply, thus posing them in the centre of our nexus-oriented framework (Figure 4.1).



**Figure 4.1.** Framework for the ecosystem-water-land-food-energy (EWLFE) security nexus. Political, socio-economic and environmental drivers are posing at risk the provision of ecosystem services, which are central life-sustaining elements for human well-being and resource supply as well as green growth. The close relationship between ecosystem services, secure access and supply of natural resources and external drivers are explained through the holistic and integrated approach proposed in the EWLFE nexus-oriented framework.

Ecosystems and their services are central in our scheme as they are crucial and vital elements for human well-being. This is also recognized through their incorporation into environmental policies and initiatives at international level (MEA, 2005; TEEB, 2010; Scarlett and Boyd, 2011; EC, 2011).

In this matrix system, we took into consideration only provisioning ecosystem services; the remaining services (i.e. supporting, regulating and cultural) were not included in the paper, since this research work represents the first attempt to explain how this newly developed matrix basically works. In addition to this, in our matrix, land has been included under the concept of ecosystem with the thought that the term of land embraces soil ecosystems as well as different land uses and land covers e.g., grasslands, heathlands, forests, agricultural, urban ecosystems.

According to the framework we proposed in Figure 4.1, we prepared a synthesis matrix system consisting of three matrices for the EWLFE nexus in form of a double entry table in order to identify the relationships between sectoral uses of resources and the role of provisioning ecosystem services, accounted as reported in the CICES classification (Haines-Young and Potschin, 2013).

Given the complexity of the interactions between the different components of the nexus, it requires relying not only on quantitative evidences but also on expert judgment. Examples exists of integration of historical evidence, expert judgment and statistical approaches to improve impact assessment (e.g. Muxika et al., 2007)

According to our experiences based on integrated watershed modeling to support mapping ecosystem services (Karabulut et al., 2015), we listed the possible nexus service flows, meaning flows of water, energy and food used in a specific sector, like water for drinking, water for agriculture etc. We classified these service flows according to the possible types and sub-types of sectoral uses, referring to either final or intermediate services which directly or indirectly affect human well-being. For instance, within the types of “water for food” service flows, we accounted for “water for drinking”, “water for any food (crops and meat) productions”, “water for all household uses for food” etc. as final services, while we considered available soil water as intermediate service supporting the food production as green water. Then we built our double entry table, in which we localized sectoral uses of resources (which are sectoral service flows) in the rows and provisioning ecosystem services of CICES in the columns. The cell resulting from the meeting between a row and a column defines the relationship between a specific sectoral use of resources and the selected ecosystem service. In this way, we obtained three matrices setting up our synthesis matrix system for food security, one for each resource category considered, i.e. “water for food”, “energy for food” and “ecosystem for food”. These matrices are detailed in section 4.3 (Table 4.1, 4.2, and 4.3).

The main purpose of the proposed synthesis matrix system is to build a frame that gathers around the ecosystems and their services to support the nexus approach. Therefore, we used the CICES table to demonstrate also the synergies and the conflicts between the nexus service flows, explained in the paragraph below.

#### 4.2.3. Scoring systems for assessing the directionality of the relationships within the nexus

In this study, we assumed that an illustrative study area is located in a region undergoing possible water and energy stress. Based on qualitative expert judgment, we determined the possible impacts of several service flows underpinning food security (e.g. the use of water for drinking or irrigation, energy for agriculture, etc.) on the ecosystem provisioning services. Within the matrix system, we defined the interrelations between the service flows (also called sectoral actions, reported in therows of the matrices) and the ecosystem provisioning services (in the columns of the matrices), by considering possible impacts. Differently from the CICES classification system, we included abiotic outputs from natural systems -abiotic provisioning such as minerals, fuels, clays, marbles etc.- together with biotic ecosystem services, since they might also act as drivers affecting each other positively or negatively. For instance, agricultural machineries, such as fertilizer spreaders, sowing machines, harvesting machines etc., which are required to produce crops, generally consume fossil fuel; it represents an abiotic output of ecosystems, namely a non-renewable abiotic energy source. While fossil fuel is supporting the services for food, food production is creating an antagonistic effect on fossil fuel resource by consuming it due to the use of agricultural machineries and afterwards the use for

transporting food. All these connected conflicts and supporting actions could be determined better by using LCA.

Integrated holistic impact assessments require an integrated modeling approach (Boumans et al., 2002; Tallis and Polasky 2009; Villa 2009; Müller et al., 2010), enabling to involve in and assess all related sectoral dynamics in the model. Physical, geographical and geomorphological characteristics of the interested area, current and suggested policies related to relevant sectors to be implemented, socio-cultural and economic structures are major input data for such models. Due to the difficulties of obtaining such data and the complexity of the interconnected impacts, we classified the possible impacts of each service flow based on expert judgment. Other examples in literature are using expert judgment to integrate quantitative and qualitative aspects, especially when the latest are very complex and interconnected (see e.g. Burkhard et al. (2012) for assessing ecosystem services) and the actions and ecosystem services of the synthesis matrix system could be changed depending on the interested area. The approach we used can be transformed into such an integrated model to assess interrelated impacts at local, national and global scale elaborately and somehow quantitatively. As stated by Burkhard et al. (2012), by linking real information from, e.g. remote sensing, land survey and GIS with data from monitoring, statistics, modeling or interviews, ecosystem service supply and demand can be assessed and transferred to different spatial and temporal scales. In our study, we classified the directionality of the impacts of each service flow of the nexus on provisioning ecosystem services within a specific case-study as follows:

- direct positive and supporting (secondary positive) effects, i.e. synergic, colored in green color;
- direct negative and conflicting (secondary negative) effects, i.e. antagonistic, colored in red;
- not clear, colored in grey, used in situations in which we were not sure of the direction of the impact or if there is no provision of those services in the relevant study area. The neutral environmental and economic impacts are considered in this class as well. Within the matrix, indirect effects were addressed within the supporting and conflicting classes.

While some service flows have explicit negative or positive impacts, some others can have either positive or negative impacts on ecosystems or on their capacities of providing services, according to the thresholds they may have. In some cases, over time there can be a transition from positive to negative effects when the above-mentioned threshold (in a real case study) is overcome. For the sake of example, very severe water scarcity in the Atacama region in Chile, which results from being located in a hyper-arid climate, creates a serious conflict between copper mining industries which necessitates considerable amount of water in their production process and ingenuous public water demand (drinking water, and household water use) and agricultural water demand (Budds, 2010). Actually, the conflict does not entail only water quantity, but also pollution contributions to water quality in that region. In such a region where priority should be assigned to water for drinking and household's use, while water is used primarily for the advantage of mining activities, this obviously creates negative impacts on drinking water and irrigation water provisioning services, thus resulting in a decrease in biomass production. There are indicators or indices to determine thresholds

quantitatively. For example, water scarcity indices, e.g. Falkenmark Water Scarcity index (FLK) (Falkenmark, 1989) or Water Exploitation Index (WEI+) (EEA, 2010), are generally used to define the thresholds for water scarcity. In another example, the allowable nitrate concentrations in water resources are determined for Europe with Nitrates Directive (91/676/EEC). However, interrelations between the sectoral actions and ecosystem services, and their thresholds representing the degree of the impacts (direct positive/supporting or direct negative/conflicting), can be defined by means of integrated models and scenarios based on real data. In another example, according to our matrix, energy for wastewater treatment may either affect crop production positively, since water returning to the environment is cleaner and has less negative effect in terms of water quality; or negatively; since a huge amount of energy is used for water treatment in case of water scarcity and competitions among water user sectors arise. Similarly, drained water returning to the riverine, lentic ecosystems or ground waters from agro-ecosystems could be a gain in terms of quantity, while this agricultural returning water might have antagonistic affect in terms of quality if there is overuse of fertilizer or pesticide in those agricultural areas. However, we cannot claim that this returning water could be either a positive or a negative driver for the abiotic material (except for table salt resources) or non-renewable energy resources of provisioning services. Therefore, being unable to define the direction of the impact, we identify this relationship as “not clear” in our subjective assessment.

Another open issue is related to the distinction between services and benefits, which is still under discussion due to different terminology used in different scientific disciplines. As an example, while some consider the recreation as a cultural ecosystem service, some others claim that this is the benefit given by ecosystem's physical characteristics (Haines-Young and Potschin, 2013). Therefore, without distinguishing between services and benefits, we adopted either final ecosystem services or benefits to represent the service flows of nexus.

To disclose the role of climate change into the nexus concept, we signed tables with stars, which represent the potential of ecosystem services to be directly or indirectly affected by climate change. It is to be noted that this is an impact analysis exercise, expert judgment-based, which could be subject to consultation with other experts when it has to be used to run specific case studies at local scale. The matrix we present has an illustrative purpose on a fictive example. Optionally -and beyond experts- local stakeholders may be involved in the evaluation of the elements of the matrix, improving transdisciplinarity of the impact assessment and relevance for local conditions. Moreover, this also ensures that the synthesis matrix could be adjusted related to local conditions, namely involving specific experts in the definition of the matrix and even local stakeholders.

#### 4.2.4. A conceptual framework for linking the developed matrices to LCA

Life cycle thinking and assessment have been initially conceived as concept and method, respectively, for integrated impact assessment. Few examples exist on studies mentioning the use of LCA for exploring nexus, but they have a relatively limited scope, e.g. Feng et al. (2014) focusing on the water energy nexus in the Chinese context; Qui et al. (2014) assessing effect on agriculture. Methodologically, the assessments are

conducted using input-output approaches, hence with a sector-based scale (e.g. Mekonnen et al., 2016; Wang and Chen, 2016). Hence, exploring the potential of integrating the nexus concept and matrix within the LCA method is herein discussed. In fact, each service flow in the matrix may be related to certain stages of a life cycle of a product and/or element of the environmental cause-effect chain adopted in life cycle impact assessment (LCIA). Moreover, the provision of a product may generate impacts that may, following a causal chain, negatively affect other service flows. In the example of crop production for food versus bioenergy, the water withdrawn from either groundwater or surface water is delivered to the agricultural areas to irrigate the crops. The type of irrigation system and the type of crop also make difference on the amount of water used, on the economic conditions of the producer, and on the impacts to the environment. However, water deprivation derived from the production may affect ecosystem services that are needed for a future production. For this reason, each service flow in the synthesis matrix system should be evaluated in terms of its link with a life cycle stage, its effects on the environment, economy and society. Therefore, in this chapter we explore the potential integration of ecosystem-water-food-land-energy nexus with life cycle assessment at two levels: (i) LCA results informing the assessment of the impacts in the synthesis matrix; (ii) the results of the synthesis matrix, to be used as a qualitative complement of LCA applied to e.g. agricultural systems, supporting the identification of potential hotspots of impacts neglected by traditional impact assessment models.

### 4.3. Results

The matrices of “water for food”, “energy for food” and “ecosystem for food” that we obtained and some practical related examples are presented in the following sections 4.3.1, 4.3.2 and 4.3.3. Each cell of the matrices reports the qualitative assessment of the possible impact, according to the majority of the experts. Since assessing such matrices takes long time, we used a limited number of expert judgments to make inferences about population of interest. Estimation on sensitivity involves the calculation of confidence intervals (95% confidence intervals- 95% CI) for a proportion of experts’ judging each cell of the matrices. Although the results may vary from sample to sample, we are “confident” because the margin of error would be satisfied for 95% of all samples. The expected lower rate of experts (the lower level of confidence interval) judging the alternatives with 95% confidence interval (Gardner and Altman, 1989) in the matrices is illustrated in the tables in Appendix C (Table C1, C2 and C3). Logically, the value 100 indicates that all experts have voted the same alternative. The degree of the agreement among the experts on the possible impacts in the matrices is illustrated with Cohen’s kappa indicator (Cohen, 1960) in Appendix C as well. We used Cohen’s kappa indicator because it is a statistical measure on inter-rater-agreement commonly used for categorical items (Warrens, 2010), as we have in our matrices case and, in general, it is a more robust measure than the percentage of agreement because the kappa takes account of the agreement occurring by chance.

The general level of concordance and agreement of the experts is very high for the majority of the statements of the survey (see 100% values in Appendix C - Table C1, C2 and C3). However, the experts do not agree

with the possible impacts of water for food actions on the non renewable fossil energy (referred in Table C1 as NRBioE), as well as the energy for food actions on biomass (BioM in table C2).

#### 4.3.1. Water for food

At the global level, the greatest amount of freshwater withdrawals, namely about 80% of blue water plus a large fraction of green water, is used for agricultural purposes in order to support food production (Hoff, 2011; MEA, 2005). This feature is projected to increase by 10 % by 2050 (FAO, 2014). Moreover, “virtual agricultural water”, i.e. water consumed during food production processes and embedded in national or international food trade or import, represents an increasing argument in the challenges about global water scarcity (Allan, 2003). On the other side, rain-fed croplands require fewer water supplies due to the use of only available soil water content (green water) than the irrigated crops. In addition, about 10% of irrigation water in developing countries comes from reused wastewater (MEA, 2005). The sharing of water among different agricultural activities in water-poor regions creates an antagonism because these activities compete against each other to receive sufficient amount of water supply.

We may interpret the following matrix (Table 4.1) observing how, in water scarcity condition, a sectoral use of water (e.g. water used for drinking purpose) affects an ecosystem service (e.g. biomass production, which is an example for crop production). The relationship may be addressed as direct and negative because the amount of potable water, which is taken from the environment and processed for drinking, becomes unavailable for producing crops. As already mentioned, it is crucial to put the accent on the water scarcity condition, because if water was abundant in a region and there was no water scarcity issue, it may not be possible to recognize the positive or negative effects in short term.



**Table 4.1.** Synthesis matrix for water-food-energy nexus versus ecosystem provisioning services: “water for food” case

<b>SYNTHESIS MATRIX for ECOSYSTEM-WATER-FOOD- ENERGY NEXUS</b>  (Modified after CICES_V3 and V4.3)  <b>WATER FOOD ENERGY SERVICE FLOWS</b>  <b>WATER (GW&amp;SW) FOR FOOD (withdrawn, consumed, returned)</b>	ECOSYSTEMS' PROVISIONING SERVICES						
	Nutrition		Materials			Energy	
	BioM	PoW	BiotM	AbioM	NPoW	RBioE	NRBioE
Water for drinking	★	★	★	★	★	★	★
Water for irrigation	★	★	★	★	★	★	★
Available soil water (green water) for crop production	★	★	★	★	★	★	★
Water for livestock fattening							
Water for slaughter house							
Water for managing farms							
Water for agri-industrial productions (agricultural machineries, fertilizer, pesticides, herbicides, etc.)							
Water for apiculture							
Water for fisheries							
Water for aquaculture							
Water for forest farming/agroforestry							
Water for horticulture							
Water for transferring foods (inland navigations)							
Water for manufacturing, refining, packing the foods							
Water for washing vegetables, fruits etc., by end-users (eg., at home, restaurants and hotels)							
Water for prepare food at home, restaurants, hotels, refectories etc.							
Water for cleaning food remains by end-users							
Water for waste treatment							
BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RABioE: Renewable abiotic energy sources (hydropower, wind, etc...)							
Direct positive (Dp) /Supporting (S)	Direct negative (Dn) /Conflicting (C)	Dp or S/ Dn or C	Dn or C/ Dp or S	Neutral /Not clear			

Given that in the water-scarce hypothetical study area the sectoral water demands and sectoral service flows are as in Table 4.1, conflicts in water scarce area may explicitly become clearer than those of water abundant areas, due to the fact that food security might have priority among other, e.g. energy security (Carpenter et al., 2005; UN-UNITAR, 2016). Therefore, water use for interventions such as crop cultivation, horticulture,

livestock flattening and apiculture to ensure food security may have positively adjuvant impacts on biomass provisioning services. However, if there are also mining activities supporting abiotic resource provisioning or fossil fuel productions in this water-scarce area, given the water use priority assigned to the food security, water allocated to food security may affect negatively other sectoral provision services e.g. potable water (PoW), biotic materials (BiotM), renewable biomass based energy sources (plant, animal) (RBioE), renewable abiotic energy sources (hydropower, wind, etc.) (RABioE). As previously mentioned, provision services of abiotic materials (ABioM) and non-renewable fossil energy (NRBioE) (grey colored in Table 4.1) might be negatively affected (thus turning in red color) by securing food production if there are mining activities or fossil fuel production in competition for water supply. Moreover, if there are crops dedicated to biofuel energy production and crops for food provision, the use of water might be in competition in case of water scarcity conditions. On the other hand, available soil water content, depending on the precipitation amount and timing, increases not only the rain-fed (green water) crop production, but also biomass of natural vegetation covering other natural food production such as food derived from forest farming. It should not be forgotten that those conflicts may not be explicit in water abundant areas.

Concerning the impacts of forest farming, we may consider that forest farming is an agro-forestry system applied as an alternative to conventional agricultural farming, which provides marginal land becoming productive for food security as well as wildlife preserved, pollution decreased, the beauty of landscapes enhanced (Douglas and Hart, 1976) and flood risks controlled (Calder 2002). According to the matrix, we may say that water for forest farming and related agro-industrial production support biomass growth; on the contrary, the use of water for those same farming and manufacturing processes can create a conflict with the water required for drinking, irrigation and other energy use.

Furthermore, water required for aquaculture or fisheries in the dam lakes may support sufficient amount of water for renewable abiotic water-based energy, while increasing water demand for aquaculture can turn into a negative conflicting effect. In fact, in water scarcity conditions, the increasing amount of water reserved for aquaculture or fisheries related needs can create a conflict with, for example, the potable or irrigation water needs.

Because water resources recharge depends on meteorological factors, such as precipitation, evapotranspiration, etc., any change in the climate conditions may directly affect freshwater resource supply and its provisioning services (Hamlet and Lettenmaier, 1999; Vörösmarty et al., 2000; Christensen et al., 2004; Jentsch and Beierkuhnlein, 2008; Piao et al., 2010). The IPCC technical paper (Bates et al., 2008) on climate change and water states that, globally, the negative impacts of future climate change on freshwater systems are expected to be drastic. By the 2050s, the area of land subject to increasing water stress due to climate change is projected to be more than double that what would happen with decreasing water stress. An increase in global population means a consequent increasing demand for agriculture, greater use of water for irrigation and more water pollution. Therefore, changes in water quantity and quality due to climate change are expected to affect food availability, stability, access and utilisation. This may lead to decreased food security and increased vulnerability of poor rural farmers, especially in the arid and semi-arid regions (Bates

et al., 2008). Under these assumptions, we signed with stars the nexus actions that are likely to be directly and mostly affected by climate change. However, this does not mean that the non-signed actions are not affected by climate change. Using this approach, we assumed that most of non-signed actions are affected by climate change indirectly. The table shows clearly that food security is highly affected by climate change as indicated by Bates et al. (2008).

#### 4.3.2. Energy for food

Food production and its supply chain consume significant quantity of energy, particularly in developed countries (Olsson, 2013) where mechanization and other modernization measures are adopted. In fact, at the global level the food sector accounts for nearly 30% of the total energy consumption and more than 70% of that energy is used beyond the farm gate (FAO, 2014). Within the agriculture and food production context, energy is used in different ways. For instance, it is employed directly to heat and cool buildings, operate agricultural equipment such as tractors, pump water for irrigation, transport products to the market, etc. Moreover, energy use is interconnected between various sectors, from agriculture to industry. As an example, modern farms that produce meat and dairy products by raising numerous animals in industrial livestock need access to large quantities of feed such as grain fodder. Fodder production, in turn, requires huge amounts of pesticides and synthetic fertilizers, which are produced by pharmaceutical industry using notable energy input deriving from the use of fossil fuels as raw material. Besides, fodder production requires fossil or bio fuels for crop cultivation. Consequently, any type of energy source is crucial for global food security. The status of energy, which is inadequately assessed and primarily used to ensure food security (in terms of all the actions needed to produce food as included in the synthesis matrix), may have negative impact on non-renewable fossil energy and abiotic material provisioning (NRBioE and AbioM in Table 4.2).

Benchmarking the components of “energy for food” versus provisioning services provided by ecosystems may vary depending on the energy sources used. For instance, the production of fossil fuels consumes a considerable amount of water during its life cycle chain. The production of shale gas is more water-intensive than conventional natural gas due to water required for hydraulic fracturing (Clark et al., 2013). The amount of water used in fossil fuel production can result in conflicts between the non-renewable fossil energy and non-potable water and/or potable water in areas with high water stress/low water availability or in times of drought (Clark et al., 2013). Therefore, if energy is procured from fossil fuel for agricultural management practices (i.e. striping, bulking, seeding, planting, fertilizing, sowing, applying and controlling weeds, pests or disease, harvesting etc.), it will have supporting effects on biomass production, while having conflicting effects on potable and non-potable water provisioning because of water consumption in the fossil fuel production processes. In addition, energy required for irrigation in food production may compete against the energy required for bio-fuel production or for other sectors.

**Table 4.2 – part a.** Synthesis matrix for water-food-energy nexus versus ecosystem provisioning services: “energy for food” case.

<b>SYNTHESIS MATRIX for ECOSYSTEM-WATER-FOOD- ENERGY NEXUS</b>  (Modified after CICES_V3 and V4.3)  <b>WATER FOOD ENERGY SERVICE FLOWS</b>  <b>ENERGY FOR FOOD</b>	ECOSYSTEMS' PROVISIONING SERVICES						
	Nutrition		Materials			Energy	
	BioM	PoW	BiotM	AbioM	NPoW	RBioE	NRBioE
Energy for agricultural management practices (Striping, bulking, seeding, planting, fertilizing, sowing, applying&controlling weeds, pests or disease, harvesting etc.)	Green	Red	Green	Red	Red	Green	Red
Energy for management of farms or state hatchery	Green	Red	Green	Red	Green	Green	Red
Energy for delivering water to agricultural areas	Green	Red	Green	Red	Red	Green	Red
Energy for operating irrigation system	Green	Red	Green	Red	Green	Green	Red
Energy for livestock fattening	Green	Red	Green	Red	Red	Green	Red
Energy for poultry husbandry	Green	Red	Green	Red	Red	Green	Red
Energy for apiculture	Green	Red	Green	Red	Red	Green	Red
Energy for fisheries	Green	Red	Green	Red	Red	Green	Red
Energy for aquaculture	Green	Red	Green	Red	Red	Green	Red
Energy for horticulture	Green	Red	Green	Red	Red	Green	Red
Energy for disposing of waste from an agricultural activity	Green	Green	Green	Red	Green	Green	Red
BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RAbioE: Renewable abiotic energy sources (hydropower, wind, etc...)							
Direct positive (Dp) /Supporting (S)	Direct negative (Dn) /Conflicting (C)	Dp or S/ Dn or C	Dn or C/ Dp or S	Neutral /Not clear			

**Table 4.2 – part b.** Synthesis matrix for water-food-energy nexus versus ecosystem provisioning services: “energy for food” case

SYNTHESIS MATRIX for ECOSYSTEM-WATER-FOOD- ENERGY NEXUS  (Modified after CICES_V3 and V4.3)	ECOSYSTEMS' PROVISIONING SERVICES						
	Nutrition		Materials			Energy	
	BioM	PoW	BiotM	AbioM	NPoW	RBioE	NRBioE
WATER FOOD ENERGY SERVICE FLOWS							
ENERGY FOR FOOD							
Energy for transporting food (crop, livestock, etc.) to process (hauling) (transporting in-country, intercountry)	Green	Red	Green	Red	Red	Green	Red
Energy for processing food (manufacturing, refining, grading, packaging)	Green	Red	Green	Red	Red	Green	Red
Energy for transporting food to the markets (transporting in-country, intercountry)	Green	Red	Green	Red	Red	Green	Red
Energy for per capita to have food at home	Red	Red	Green	Red	Red	Green	Red
Energy for preparing food at home or restaurant, hotels, refectories etc.	Red	Red	Green	Red	Red	Green	Red
Energy for collecting organic wastes	Green	Red	Green	Red	Red	Green	Red
Energy for other food procedures (energy for pruning etc.)	Green	Red	Green	Red	Red	Green	Red
Energy for agri-industrial productions (fertilizer, pesticides, herbicides industrial productions etc.)	Green	Red	Green	Red	Red	Green	Red
Solar energy for net primary productivity (NPP) in terrestrial and marine ecosystems (The rate at which new biomass accrues in an ecosystem)(Higher in humid and warmer climate, less in dry and hot climate)	★	★	★	★	★	★	★
BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RAbioE: Renewable abiotic energy sources (hydropower, wind, etc...)							
Direct positive (Dp) /Supporting (S)	Direct negative (Dn) /Conflicting (C)	Dp or S/ Dn or C	Dn or C/ Dp or S	Neutral /Not clear			

According to the matrix in Table 4.2, collecting, transporting and disposing organic waste (included in the table as “energy for disposing of waste from agricultural activity”) are energy-consuming activities that directly affect fossil fuels availability, if the fossil fuel is used. However, there are systems that can produce their bio-energy using the waste. In most of cases, the organic waste is accumulated as biomass source to produce biogas, which is also used in public transportations. For instance, since 1994 buses in Lille in the

North of France and in Sweden, especially in Linköping and Uppsala, have been using biogas fuel (EBA, 2015). In this way, biogas plays an important role in reducing the dependence on fossil fuels as renewable environmentally friendly energy production.

As another example from the matrix, fertilizers are used to provide nutrients and support soil productivity for increasing crop production, while pesticides and herbicides are used to protect the crops and keep yield in the maximum level. However, at the same time, these activities have the potential of damaging the environmental sustainability as contaminants if they are overused. Herbicides and pesticides may also damage some living organisms in the soil. In addition, ammonia and urea can be produced from different hydrocarbon feedstocks such as natural gas, coal and oil and one of the end uses for ammonia and urea is as fertilizers. Therefore, the use of natural gas as an input in the production of fertilizer (Ammonia Outlook, 2002; Urea Outlook, 2002; Ramirez and Worrel, 2006), and petroleum and natural gas as input for pesticides production (Ware, 1983; Lee, 1991) can create conflicts with the supply of abiotic material and fossil fuels, since such agrochemicals consume great amount of abiotic material during their processing phase. On one hand, their use supports the food biomass production (Kuniuki, 2001; Cooper and Dobson, 2007), while overuse may increase the environmental pollution (Kovach et al., 1992; Miller, 2004).

The solar energy required by the net primary productivity in terrestrial and marine ecosystems should also be considered in the “energy for food” loop in order to better define the conditions and environmental life cycle impact of these drivers. For instance, greenhouse gases with their contribution to climate change are the main drivers affecting the functions and intensity of solar energy. Proper amount of solar energy can support biomass provisioning to ecosystems in a way that higher in humid and warmer climate, less in dry and hot climate (Ruimy et al., 1999). However, anomalies related to the increase in solar energy that reaches the Earth’s surface could create conflicts by sometimes causing drought; on the contrary, less solar energy creates colder climate, thus proportionally reducing the biomass provisioning. As an example, an increasing trend in solar energy and temperature against the decreasing or stable rainfall trend may negatively affect water and biomass provisioning because of draughtiness (Schmidhuber and Tubiello, 2007; Chaouche et al., 2010).

In Table 4.2, focusing on flow actions related cells with stars, it is possible to observe that climate change is directly responsible for changes in the provision of energy for net primary productivity in both land and marine ecosystems, e.g. agricultural crop yields (Rosenzweig and Parry, 2004), forest and marine biomass productions (Beier et al., 2004). Therefore, under our assumptions, the other cells within the matrix are considered as indirectly affected by climate change. For the sake of example, climate change may alter the season cycle affecting directly the provision of solar energy for biomass production; this can be considered as a direct action of climate change. On the other hand, an example of indirect impact would be the same in a case where biomass is used as a source of energy. That is, climate change can directly affect the production of biomass of biofuel plants; while indirectly affect the transportation of food or other actions that use biofuel as a source of energy for transporting the goods.

### 4.3.3. Ecosystem for food

The availability of adequate resources (e.g. water, fuels) and energy for food production depends on ecosystems' quality and their ability to provide services. Particularly, agro-ecosystems, marine ecosystems and forests represent the main source of nutrient provisioning services in many regions. Forest ecosystems provide a wide range of food such as different varieties of berries, mushrooms, nuts, roots, tubers and wild animals, which are very important particularly in the rural economy. In most cases, forests also support water-provisioning and regulation services. Correspondingly, agro-ecosystems provide humankind with crops and livestock. However, beside their importance in nutrient provisioning services, agro-ecosystems compete with other water demanding sectors to receive sufficient amount of water for irrigation. Furthermore, due to over-fertilization, agro-ecosystems still may threat waters and ecosystems like lotic, lentic and marine aquatic ecosystems, which have great importance on food security as well.

**Table 4.3.** Synthesis matrix for water-food-energy nexus versus ecosystem provisioning services: “ecosystems for food” case.

SYNTHESIS MATRIX for ECOSYSTEM-WATER-FOOD- ENERGY NEXUS  (Modified after CICES_V3 and V4.3)	ECOSYSTEMS' PROVISIONING SERVICES						
	Nutrition		Materials			Energy	
	BioM	PoW	BiotM	AbioM	NPoW	RBioE	NRBioE
WATER FOOD ENERGY SERVICE FLOWS							
ECOSYSTEM FOR FOOD							
Marine ecosystems (fish, crustacean, molluscs, echinoderms etc.)	★	★	★	★	★	★	★
Lentic (lakes, reservoirs, ponds) ecosystems (fish, some vertebrates, duck,)	★	★	★	★	★	★	★
Lotic ecosystems (fish, some vertebrates,)	★	★	★	★	★	★	★
Wetlands (Fish, duck, etc.)	★	★	★	★	★	★	★
Forest ecosystems (all berries, honey, mushroom, seed, nut, root, tuber, gums, sap, wild animals (pork) etc.)	★	★	★	★	★	★	★
Agro-ecosystems (all cultivated crops)	★	★	★	★	★	★	★
Grasslands and heathlands (rabbits, wild pork, etc.)	★	★	★	★	★	★	★
BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RAbioE: Renewable abiotic energy sources (hydropower, wind, etc...)							
Direct positive (Dp) /Supporting (S)	Direct negative (Dn) /Conflicting (C)	Dp or S/ Dn or C	Dn or C/ Dp or S	Neutral /Not clear			



According to the matrix in Table 4.3, ecosystems play a pivotal role in supporting the provision of services within the context of a sustainable system. They predominantly have direct and positive effects on the availability of energy and matter for food production. An important example is represented by pollination. Pollination is an essential ecosystem service for primary producers in both wild and managed terrestrial ecosystems (Klein et al., 2007; Kremen et al., 2007; Zulian et al., 2013). In fact, it represents a crucial ecosystem service primarily supporting the provisioning of food such as honey, which is a direct food production by bees, and crops, fruits etc. in the agri-food sector. Intensified agricultural practices, particularly the massive use of pesticides, are likely to affect pollination mediated by insects, posing a risk to global food security. Agro-chemicals can quickly degrade pollination services acting through the loss of species (Potts et al., 2010; Sandrock et al., 2014), while the remaining species being unable to compensate for the difference (Kremen, 2005). Since pollinators are particularly vulnerable to environmental stress (Kevan, 1999), pollination requires healthy environmental conditions in order to provide mankind with functioning ecosystems and services. Hence, pollination is a vital service not only for biomass provisioning (e.g. crops), but also for regulating the provision of final goods and services, such as water provisioning for its healthy ecological requirements.

Grounded the fact that the fossil fuels is formed from decayed plants and animals over hundred millions of years (i.e. ecosystem biomass services existed hundred millions of years ago) (Hubbere, 1949), it should be acknowledged that the ecosystems will continue their role of fossil fuel generation for the future time. Therefore, in the synthesis matrix we considered that ecosystems will continue its positive impacts on NRBioE generation.

Ecosystems and their biodiversity are highly dependent on climate factors (Beier et al., 2004; Jeppesen et al., 2009). Therefore, all the ecosystem service flows within the matrix are considered as directly affected by climate change (Table 4.3). In a similar vein, according to USEPA (2010), climate change warms up the oceans and affects the temperature both at the surface and at depths, thus potentially resulting in a change in the habitats and food supplies for many kinds of marine life—from plankton to polar bears just to name a few.

#### 4.3.4. Integration between the nexus-based synthesis matrix and LCA

Life cycle assessment method is devoted to quantify and assess the environmental impacts of supply chains in an integrated way, namely accounting for more than one indicator (ISO 14040 and ISO14044, 2006). Since the nexus concept may be linked to several stages of a product life cycle (e.g. agricultural phase, production and transformation, distribution, consumption and end of life stages), the integration of the nexus concept within the LCA framework should follow two steps, namely: a first one related to the identification of the nexus-based matrix elements within the system boundary of an illustrative LCA case study; a second one based on the identification of the impacts related to the pre-selected elements that may lead to modifications in the input to the system (e.g. affecting water availability, soil quality etc). This means that the life cycle inventory and the life cycle impact assessment are to be improved in order to account for the



elements of the nexus-oriented matrix, namely the sectoral use of resources. In literature, the issue of improving the impact assessment step of LCA by integrating qualitative aspects into a quantitative system has already emerged as a need, especially when bio-based/agricultural systems are involved (Sala et al., 2017, Notarnicola et al., 2017). Standard LCA applied to the agricultural system misses several potential drivers of impacts and hotspots whose identification could be helped by the application of the nexus-oriented synthesis matrix.

To illustrate the process of integration of the nexus-oriented matrix within the LCA framework, we present an illustrative example addressing the possible life cycle assessment of biodiesel crop production within a system in competition with crop production for food (Figure C1 in Appendix C).

In details, the system boundaries of the study may be defined to support the identification of the potential occurrence of interrelations between input and output of the system. Specifically, this requires to explicitly mentioning which elements are crossing the boundary between ecosphere and technosphere. As an example, this would mean that cubic meters of water or square meters of land are identified as input of the cultivation stage; however, they are in between ecosphere and technosphere, due to their close relationship and interdependency with ecosystems (indeed, soil of a certain quality is the result of bio-geological cycles, while water availability is related to climatic conditions and to the texture of soil, etc.). Beyond the inputs explicitly mentioned in inventories (such as water, land, nutrients), other elements such as ecosystem services of different nature (e.g. pollination) are essential for an agricultural supply chain; however, they are not even mentioned. Although their quantification is difficult, their existence and role should be acknowledged and addressed.

Based on the inputs reported above, a production chain of crops may be depicted, leading to the production of food or bioenergy. The system boundary, as presented in Figure C1 (Appendix C), is usually adopted as reference for building the life cycle inventory (i.e. list of emissions and resource used in each stage) and for, in a second step, calculating the impacts through a set of impact assessment models. However, in order to apply the matrices based on the nexus concept to the LCA framework, the usual procedure is not enough. In fact, moving from linear (namely the environmental cause-effect chain of LCA) to circular systems (such as the one in our example), feedback back to the ecosphere are to be taken into account, especially for those elements explicitly accounted for (land/water) or not accounted for (ecosystem services) in the LCA-based inventories which are both crucial for the delivery of the final output in the technosphere.

Biofuel related impacts have mainly been evaluated in relation to their positive contribution to climate change, i.e. through reducing greenhouse gas emissions, and to meet green energy demand; while the associated potential increase in water use, which may generate competition with other sectors, has received minor attention (Brentrup et al., 2005; Kim and Dale, 2005; Emmenegger et al., 2011). In fact, neglecting to consider the overall environmental impacts related to a process or product may cause misleading incentives. However, very limited work has been performed to assess the impacts deriving from freshwater use in LCA studies on biofuel crop production (Núñez et al., 2012 (Spain); Emmenegger et al., 2011 (Argentina); Pfister

et al., 2009; Chiu et al., 2009 (USA); Service, 2009; Dominguez-Faus et al., 2009). This is due to the lack of regional inventory data and more focus on climate change adaptation, which is perceived by the audience as a more urgent issue to be dealt with.

Basically, in future, three typologies of nexus should be addressed in an LCA framework:

- Primary nexus. The one occurring between elements within the system boundaries and that may lead to the identification of competitions between uses;
- Negative feedback-based nexus. The one related to the impacts occurring due to the production of a certain good, that may imply the reduction of ecosystem quality and services, as well as of critical inputs (e.g. water, land), leading to negative effects on future production;
- Positive feedback-based nexus. The one related to the positive feedbacks that could be related to the identification of possible sources of resources to be used in a synergetic manner (e.g. recovery of nutrients that may support the substitution of a chemical fertilizer)

Some of these concepts, e.g. elements that may lead to competition of uses, are already embedded in the so-called consequential LCA, while others not (such as the interplay between impacts), thus requiring in future a dedicated analysis in order to be further explored and systematized. Indeed, the consequential approach intends to describe how physical flows can change as a consequence of an increase or decrease in demand for the product system under study, including unit processes inside and outside of the product's immediate system boundaries (Earsel et al., 2011). The traditional consequential assessment covers the life cycle inventory, whereas the proposed nexus-oriented approach add on top impact assessment aspects and could be considered a sort of consequential impact assessment. The two level of consequential analysis (inventory and impact assessment) may then complement each others.

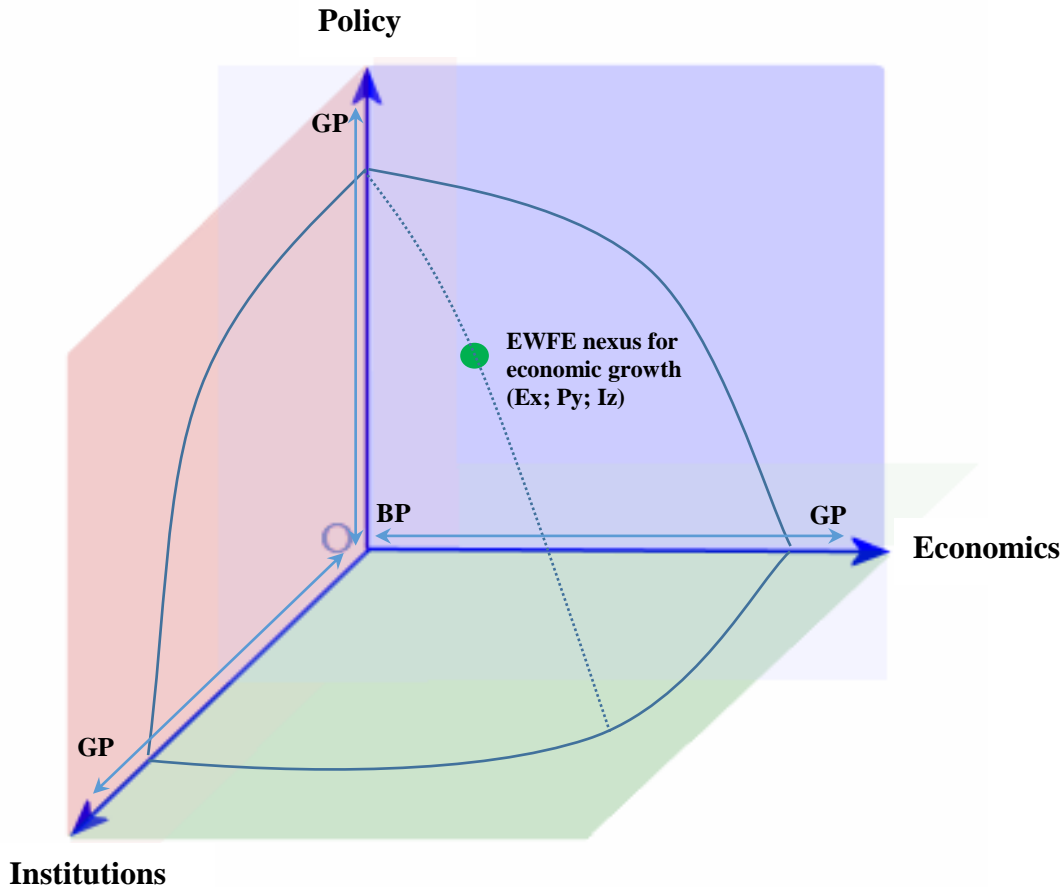
The life cycle-based assessment done for the nexus service flows could be further developed and integrated with the nexus-oriented synthesis matrix system. It is crucial to assess all these impacts, either spatially explicit or at least regionally; for this aim, spatially resolved LCIA models could be used. However, since each region has its own different sectoral uses or ecosystem service flows, it is more convenient to set up site-specific local nexus-oriented synthesis matrices with their own service flows and their life cycle assessments for each region.

Moreover, the synthesis matrix itself could be used for a qualitative hotspot analysis to complement the quantitative results of LCA, highlighting potential impacts not covered by current impact assessment methods.

#### 4.3.5. Supporting economic growth by adopting the nexus-based synthesis matrix

The EWFLE nexus-based matrix, particularly if integrated with supply chain related considerations made through LCA, may be used to analyze the current environmental and economic impacts of all service flows in a target area. This may be done by adopting a site-specific synthesis matrix system, which could result from the consultation of several experts as well as local stakeholders. In this regard, it is inevitable to include

explicitly economic, institutional and policy aspects into the EWFLE nexus (Markantonis et al., 2015) in order to promote economic growth in the target region. Thereby, we propose a three-dimensional (3D) structure for the EWFLE nexus to support economic growth (Figure 4.3).



**Figure 4.3.** Three dimensional EWFLE nexus definition model for promoting sustainable economic growth. Each axis (x, y and z) represent a feature underpinning the achievement of economic growth.  
*GP: Good practices-positive LCI; BP: Bad Practices-negative LCI; Ex: Optimum benefit of Economics; Py: Optimum benefit of Policy; Iz: Optimum benefit of Institutions.*

The socio-economic growth based on the holistic nexus-oriented approach can be achieved by optimization, namely using the feedback of three dimensions (economy, policy and institutions, as reported in Figure 4.3). For examples, policies devoted to energy or water security may create negative effects on food security or vice versa. Therefore, for enhancing synergies between sectoral uses and provisioning ecosystem services, policy-institution-economy dimensions should be all taken into account, as well as for achieving optimal cross-cutting policies and ensuring economic benefits from nexus components. Figure 4.3 illustrates the point (Ex; Py; Iz) where an optimisation of cross-cutting objectives is reached, covering both economic, institutional and political optimum. The green point is a function of the policy-economic-institution interconnection that represents the optimal combination of environmental impacts and economic benefits shared by all dimensions.

Sustainability related impacts on environmental and socio-economic dimensions should be evaluated in a life cycle thinking framework, namely towards the maximization of the societal benefit while minimizing negative environmental and socio-economic impacts. Some preliminary attempts of coupling optimization models and LCA have been performed (e.g. You et al. 2012). However, the challenge of including the feedback into the input system accounting for nexus is not yet developed.

#### 4.4. Discussion

The synthesis matrix system we developed for food security is an example of (i) how the nexus-oriented system thinking enables to highlight the relevance of the ecosystem functioning and (ii) how it is important to integrate it with LCA, under a holistic perspective.

The nexus concept can be linked to several stages of a product life cycle; therefore, the life cycle inventory and the life cycle impact assessment can be further improved in order to account for the matrix elements. In this context, the synthesis matrix should be considered as a methodological guidance to assess interactions between environmental pressures in specific contexts, which are missing in the traditional LCA. The matrices depicted by our work refer to a general tendency in nexus between environmental aspects. This means that if the matrix is applied in a specific context where, e.g. water stress conditions are extreme, the interplay between the elements of the matrix may change significantly.

The synthesis matrix system explains the interrelationships between the service flows (actions in the nexus) and the ecosystem provisioning services, by using an imaginary case-assessment. The interrelationships presented in the results' section are to be considered as speculative, i.e. the relationships are evaluated in qualitative terms, without having been ranked on a quantitative assessment basis and are examples from the author's knowledge and experience. By presenting these cases, we believe that assessing the impacts with real quantitative data using a life cycle-based approach for each action would give more concrete and reliable results.

Comparing tables 4.1, 4.2 and 4.3, it is obviously seen that ecosystems have almost completely direct or supporting positive impacts on food security, thus enhancing food production. While the increasing human demand for natural resources poses a risk to resource supply itself and thus on the provision of ecosystem services, functioning ecosystems are able to enhance food production and ensure access to food globally. This positive impact of ecosystems explicitly shows that, to be sustainable, it is inevitable to include also the ecosystems and their services security into each policy and implementation related to the food, water and energy, as also mentioned in Bizikova et al. (2013).

Even though obtaining the holistic view of every impact involved in the activities of those sectors that use resources is very complex and difficult, mostly due to the lack of reliable empirical data, it is necessary to evaluate actual environmental and economic impacts of any sectoral service flow in the nexus, in order to avoid any conflict between sectoral uses in the future projections. Thus, assessments built on setting simplified local synthesis matrix system may solve the complexity, while enhancing a holistic view of the system under study. To simplify and better determine quantitatively the interrelationships between the

service flows within the nexus and the provisioning ecosystem services, multi-disciplinary biophysical and economical models can be integrated or interactively used, such as LEAP (SEI, 2015a), WEAP (SEI, 2015b), InVEST (Tallis et al., 2011), SWAT (Arnold et al., 1998), EPIC&APEX (Texas A&M AgriLife Research, 2015) IMAGE (Stehfest et al., 2014), just to name a few. More in detail, SWAT and WEAP models are commonly used for watershed managements, EPIC&APEXS is used for land management impacts and crop production, LEAP is used for sustainable energy analysis, IMAGE is used to assess global environment, and InVEST is used to model ecosystems and their services.

Furthermore, policy, economics and institutions, i.e. the most important primary management mechanisms of the nexus, could be linked to the synthesis matrix system to identify the cross-cutting policies or good/bad implementations by each institution with different economic perspectives regarding each component of the nexus (namely ecosystem, water, food, energy).

Increasing demand and unbalanced sectoral share not only induce drastic depletion of natural resources, but also make explicit the need of sectoral interdisciplinary management strategies. Therefore, the method proposed in this study could be interpreted as a holistic, interdisciplinary, system-based way to acquire well-matched cross-cutting policies, inter-institutional relations between the nexus components. As mentioned before, by taking advantage of enhancing synergies between sectoral actions and provisioning ecosystem services (described by the nexus-oriented synthesis matrix system) using LCA assessment and integrated models, an interdisciplinary and holistic policy making system can be constituted as well, considering the policy-institution-economy triangle dimensions related to the EWFLE nexus to ensure sustainable green economic growth.

#### 4.5. Conclusions

Water, energy and food are essential for human well-being and economic growth. Agriculture has the dominant use of natural resources, primarily for food production, involving approximately 50 % of the land area used in the EU-27 and 2.4 % of the European agricultural area devoted to organic production (Eurostat, 2015). Within this context, ecosystems, water, food and energy are inextricable interrelated, in that concept called Ecosystem-Water-Food-Land-Energy (EWFLE) nexus. The nexus concept represents a challenge towards achieving sustainability goals, especially within the agri-food sector. Although their use is shared by different sectors such as agriculture and industry, water and energy have so far been considered in isolation. The lack of cooperation between sectors has led to significant competition between the different uses of energy and water for food or for other purposes. This is reflected in contradictory strategies and policies, which do not effectively permit to reach either economic or environmental sustainability, posing a risk to global food security. Water, energy and food need to be addressed simultaneously, in order to develop strategies to enhance positive synergies between sectoral uses, share benefits and meet the increasing demand, still protecting the ecosystems and their functioning. Therefore, given the importance of the environmentally friendly agriculture sector for ensuring secure access to food, we felt the need to exercise the interrelationships among EWFLE nexus sectors and integrated assessment methods, such as life cycle

assessment. With the proposal of a synthesis matrix system, we aim to march forward, towards the identification and simplification of the trade-offs between sectoral service flows as well as their life cycle impacts for food security. However, the current LCA framework requires adaptations to improve the comprehensiveness of the impact evaluation, in light of capitalizing the theoretical underpinning of the nexus and translating it into a quantitative assessment of the impacts. The novelty of the matrix we proposed, in fact, goes for this purpose since it stands in the proposal of a qualitative assessment to evaluate the effective relationships between natural resource uses and ecosystems, which underpin resource' supply. The nexus-oriented matrix can be adapted from elaborative local scale to global scale for investigating the trade-offs, either synergies or conflicts, with research-based quantitative thresholds. We hope that it will inspire further analysis and lead to in-depth studies on how to make food security as functional and sustainable as possible, improving synergies among sectoral demands and uses.

For future work, we highly recommend multi-disciplinary studies to improve this system thinking approach to clarify the gaps or uncertainties while working globally or locally on the matrix. Therefore, any recommendation on terminology or on categorization of the service flows and on identifying the synergy is welcome to improve the matrix for different purposes and locations.

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## 5. General conclusions and outlook

The research outlined in this PhD project aims at contributing to the ongoing discussion on the identification of innovative approaches for integrating ecological considerations in Life Cycle Assessment (LCA), enabling the assessment of the sustainability of products and services, by comprehensively accounting for many aspects of biodiversity, i.e. target species, impact categories and interactions between impacts, considered at midpoint level. This research project, in fact, stems from the need to bridge some of the existing conceptual and methodological gaps in LCA with regards to the assessment of impacts on biodiversity and its components.

In this PhD thesis, the relevance of biodiversity for humankind and the crucial need of accounting for it in the assessment of impacts along supply chains have been disclosed. Biodiversity plays a pivotal role for human societies and ecosystem functioning. However, the magnitude of the pressure that humans are currently placing on it is enormous. Therefore improving the modeling of impacts on biodiversity in Life Cycle Impact Assessment (LCIA), by integrating ecologically relevant features about three main building blocks (i.e. target species, impact categories, interactions of impacts), to support bio-economy and the evaluation of supply chains towards sustainability represents an urgent issue to deal with.

LCA is a powerful methodology that can be used to estimate the effects of products on several aspects of biodiversity. In fact, as it aims at disclosing the most environmental friendly performance of products, it is important for LCA to address all the impacts affecting human well-being and environmental functioning, such as those impacts resulting in biodiversity loss. LCA might help prioritize what should be targeted along the supply chain, by using an integrated, holistic, system-based approach, while increasing transparency in the communication to different stakeholders (i.e. producers and consumers).

In this PhD thesis, specific theoretical and methodological gaps regarding the integration of ecologically relevant features of biodiversity into LCA have been identified. In particular, through an extensive literature review, relevant pressures on target species, such as honey bees (*Apis mellifera*) and other insect pollinators, have been identified, thus contributing to the current understanding of the factors leading to biodiversity decline and moving the first step towards overcoming problems related to the lack of appropriate LCIA models for assessing impacts on biodiversity. Then, with a specific focus on agricultural pesticides as one of the main contributing causes of biodiversity loss, preliminary characterization factors for ecotoxicity have been calculated for quantifying impacts on insect pollinators, particularly honey bees, and applied and tested in a first illustrative case study. The underpinning model has been built on ecological data accounting for the behavior of honey bees and on data regarding the environmental fate of pesticides. This represents the first step to extend the existing ecotoxicity characterization models with respect to terrestrial organisms, of which honey bee represents a specific target species which has not been included in the life cycle assessment yet. After additional improvements, the modeling framework will be potentially proposed for inclusion in the scientific consensus model for ecotoxicity related impact assessment, thus furthering the integration of ecological modelling in LCA.

Moreover, a novel impact category related to biotic resources has been proposed. In this context, the target of improving the inventory of naturally occurring biotic resources has been reached, by identifying and listing the most commercially valuable species, as a starting point for generating elementary flows in the life cycle inventories, through a solid review of literature and available statistical data. Terminology still needs to be defined, harmonized and standardized and the list of biotic resources needs to be evidently implemented with additional information, such as the usable volume. In order to enable the inclusion of biotic resources in the assessment of products, a model for biotic resources assessment has been developed, encompassing: (i) a definition of system boundaries between ecosphere and technosphere, namely between naturally-occurring and man-made biotic resources; (ii) a novel impact pathway, a renewability-based indicator for the impact assessment of biotic resources and the associated characterisation factors. The proposal of a model approach based on renewability rate has notable potential for calculating characterization factors for biotic resources and their potential scarcity. Renewability is just one of the elements affecting availability of resources; other ecological features such as resistance, resilience, vulnerability, etc. may play a role and should be taken into consideration in order to avoid compromising the natural system. More research is undoubtedly needed in this field, and the collaboration between different disciplines (e.g. ecology, engineering, etc.) is required in order to make progress towards an impact assessment scheme implementable in LCA.

For what concerns the interplay of impacts, which are not accounted for in the current LCA framework, and the underpinning nexus approach, a novel synthesis matrix has been proposed as well as a qualitative assessment to evaluate the effective relationships between natural resource uses and ecosystems, which underpin the supply of resources. The nexus-oriented approach, which aims at optimizing benefits, while minimizing trade-offs, based on an integrated approach that finely matches with LCA methodology, is crucial to guarantee the access to natural resources and their management towards sustainable development. The theoretical matrix showed that there are predominantly negative impacts given by sectoral uses of resources on the provision of ecosystem services, an issue that requires most focus on resource efficiency and on the environmental and economic impacts of natural resources use while reducing the trade-offs between the sectoral demands. Thanks to its link to policy targets and management goals, in future the synthesis matrix could be adapted from the local to the global scale and coupled with research-based quantitative thresholds, to create synergies among sectorial demands and uses.

It is evident that more effort is necessary and many critical aspects are still to be taken into account. Several challenges are still open to discussion, such as those related to the poor availability of data and modeling issues, as the clarification of the boundary between ecosphere and technosphere. Nevertheless, this PhD thesis wants to be a solid starting point, in order to help future research in covering the presented gaps towards the integration of ecological considerations in LCA and to continue stimulating the interest of stakeholders in a common denominator for human well-being called biodiversity.



## Appendix to Chapter 2 (Appendix A)

**Table A1.** For each paper included in the review of Section 2.1, the addressed impact categories, the investigated pollinator taxa, a brief description of the contents and the effects on pollinator populations and/or pollination service are reported.

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Traveset and Richardson, 2006	review	Multi-impact, including: presence of invasive alien plants; competition with invasive alien pollinator species	Native wild bee species (honey bees are excluded), butterflies and other insect pollinators	Review on the effects that the introduction of invasive alien species (pollinators, predators or plants) have on plant-pollinator mutualistic interactions.	Changes in foraging behaviour of native pollinators; decrease in native flower visitation rate by native pollinators; loss of native pollinators which are replaced by invasive alien species (competition); disease due to the indirect introduction of parasite
Kluser and Peduzzi, 2007	scientific report	Multi-impact, including: land use alteration (habitat fragmentation); use of agrochemicals; competition with alien pollinator species; climate change; pests and pathogens; electro-magnetic pollution; genetically modified crops	Honey bees; wild bee species	Scientific report on factors potentially leading to honeybees' Colony Collapse Disorder (CCD) in the U.S.A.	Loss or limitation of food resources and nesting habitats; overwintering mortality; loss of adult bees by poisoning; loss of orientation and homing ability; competition with invasive species; disease and stress leading to immune weakness and CCD
Aizen et al., 2008	research article	Multi-impact, including: presence of invasive alien plants; competition with invasive alien pollinator species	Wild bumblebees and solitary bees, dipterans, butterflies, beetles	Research on the effects of the introduction of alien species on ten native plant-pollinator mutualistic webs.	Loss of specialist native pollinators, increasing role of super-generalist alien species; alien species tend to monopolize ecological interactions provoking displacement of native species and disruption of their connections with plants in the native ecosystem; potential decline of biodiversity and pollination quantity and/or quality



Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Stout and Morales, 2009	review	Multi-impact, including: presence of invasive alien plants; competition with invasive alien pollinator species	Wild bumblebees and solitary bees	Review of experimental case studies about the direct and indirect effects of the accidental or intentional introduction of alien species (plants and insects) on native bee populations across the globe. Suggestions for future studies are provided.	Reduced fitness (fecundity, survival and population size) of pollinators, particularly specialist species; limitation of accessible pollen/nectar to native bee species; loss of native nesting sites for pollinators; competition with invasive pollinators for resources and nesting sites; disease due to the indirect introduction of parasite; changes in foraging behaviour of native pollinators, with workers spending less time foraging on flowers; reproductive disruption via interspecific mating between native and invasive congeners (hybridization)
Kluser et al., 2010	scientific report	Multi-impact, including: land use alteration (habitat fragmentation); use of pesticides; competition with alien pollinator species; climate change; pests and pathogens; electromagnetic radiations	Honey bees, bumblebees, butterflies	Scientific report on the driving forces which have recently resulted in pollinators' declines around the world, focusing on wild and managed bee populations. Mitigating measures and recommendations to decision-makers are provided.	Loss or limitation of food resources and nestingsites; poisoning which impairs bee reproduction; phenological and geographic mismatch between plants and their pollinators; loss of orientation and homing ability; competition with alien invasive pollinator species; disease and stress leading to immune weakness and CCD; change in bee behaviour
Potts et al., 2010	review	Multi-impact, including: land use alteration (habitat fragmentation), use of agrochemicals (insecticides/herbicides), introduction of alien plant species, competition with alien pollinator species, climate change, pests and pathogens, <i>INTERACTIONS between unspecified stressors</i>	insect pollinators in general; honey bees, bumblebees and other hymenoptera, lepidoptera, hoverflies (diptera) are mentioned	Review of knowledge on the status and trends of wild and managed pollinators and on the main drivers acting on pollinator populations.	Loss of pollinators abundance and species diversity; loss of specialist pollinators; loss of floral and nesting resources; lethal and sub-lethal effects of insecticides; competition for resources as a consequence of the introduction of alien pollinators (especially managed bees); infections of colonies by pathogens; species spatial shifts; changing in the temporal activity of bees

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Schweiger et al., 2010	review	Multi-impact, including: competition with invasive alien pollinator species ( <i>Apis mellifera</i> , <i>Bombus terrestris</i> and some species of solitary bees are mentioned as invasive); introduction of invasive plant species; climate change; pests and pathogens; <i>INTERACTIONS</i> (see Table A3)	insect pollinators	The authors investigate both individual and combined effects of two key drivers of anthropogenic environmental change on plant-pollinator interactions, identifying potential positive and negative consequences for pollinator species and living communities.	Phenological mismatch between plants and pollinators leading to decreased pollinator availability for a particular plant or pollen limitation to a particular specialist pollinator; changes in pollen sucrose content leading to poor quality of pollen; change in pollinators' body size.
VanEngelsdor p and Meixner, 2010	review	Multi-impact, including: land use alteration; use of pesticides; climate change; pathogens; genetically modified crops; <i>INTERACTIONS</i> (see Table A3)	Honey bees	Overview of the status of managed honeybee populations in Europe and in the United States of America. The main factors leading to colony collapse and their effects are described.	loss of food resources, impaired learning ability, behavioural changes, disrupted flying ability and disorientation, reduced individual survival; paralysis, poor queen quality
Pettis et al., 2012	research article	Multi-impact, including: pesticides, pathogens and their <i>INTERACTIONS</i> (see Table A3)	Honey bees	Experimental research to test the hypothesis that bees chronically exposed to sub-lethal levels of pesticides (i.e. imidacloprid) are more vulnerable to infections by pathoges (e.g. <i>Nosema</i> spp.)	Bees from colonies chronically exposed to pesticide had higher levels of <i>Nosema</i> spores, it means that <i>exposure to pesticide makes bees more susceptible to infections by pathogens</i> such as <i>Nosema</i> spp.; reduced weight in queen bees exposed to pesticide sub-lethal levels.
Burkle et al., 2013	research article	Multi-impact, including: land use alteration (habitat fragmentation), climate change	bee species (solitary and social bees included <i>Apis mellifera</i> and <i>Bombus</i> spp.)	Using historic data sets, the authors quantified how global change over the last 120 years affected network structure, local bee diversity, and phenologies of forbs and bees in a temperate forest community in Illinois, USA. Above all, they found that quantity and quality of pollination services have declined through time.	Loss of floral resources and nest sites, extirpations of bee species participating in the interactions (especially those rare and specialized species, species occupying higher trophic levels, and cavity-nesting species), lack of spatial co-occurrence of species in modern fragmented landscapes (phenological mismatch), at communiti level: reduced redundancy in network structure, weakened interaction strengths, declined quantity and quality of pollinator service through time.

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
González-Varo et al., 2013	review	Multi-impact, including: land use alteration (habitat fragmentation); use of agrochemicals (pesticides/insecticides); invasive plant and pollinator species (commercial honeybees <i>Apis mellifera</i> and bumblebees <i>Bombus spp</i> are mentioned); climate change; pathogens; <i>INTERACTIONS</i> (see Table A3)	insect pollinators (wild bees are mentioned)	Overview of the effects of the main global change pressures acting on insect pollinators, reporting empirical evidence of their combined effects and suggestions for future research.	Geographic and/or phenological shifts; pollen limitation; change in community composition; reduction of food and nesting resources; reduction in species richness and abundance; competition for resources; loss of specialist pollinators; inbreeding depression; spread of disease
Vanbergen et al., 2013	review	Multi-impact, including: land use alteration; use of pesticides; invasive alien plant and pollinator species; climate change; pests and pathogens; <i>INTERACTIONS</i> (see Table A3)	Wild and managed bees, butterflies, flies	Overview of the multiple anthropogenic pressures and the interactions between them leading to insect-pollinator decline or loss worldwide. Recommendation on risk assessment and land management for future decision-making are provided.	Loss of nesting and foraging area; reduced species richness; changes in pollinator behaviour, impaired reproductive performance; shifts in home ranges and phenology, spatial dislocation of pollinatio; competition with invasive species; “Colony Collapse Disorder”; co-infection with viruses and other pathogens
Vanbergen et al., 2014	scientific report	Multi-impact, including: land use alteration (habitat fragmentation); use of pesticides; competition with invasive alien pollinators; introduction of invasive alien plants; climate change; pests and pathogens; <i>INTERACTIONS</i> (see Table A3)	Honey bees, wild and managed bumblebees, solitary bees, hoverflies, butterflies and moths	Report on status of pollinators and pollination service in the UK, focusing on how human interventions affect pollinator population and communities in England.	Loss of specialist pollinators; loss of nesting areas and foraging sources; loss of genetic diversity due to geographic isolation; impaired bee performances leading to species richness loss; reduced foraging activity, growth rate and queen production; phenological and geographic mismatch; competition with invasive pollinator species; colony immune weakness and death.
European Food Security Authority (EFSA), 2014	scientific report	Multi-impact, including: land use alteration (habitat fragmentation), use of agrochemicals, invasive alien species (pollinators and predators), pests and	Honey bees and bumblebees	Report on recent work carried out by EFSA (16 papers), Member States (181) and the European Commission (20) on bee health topic, identifying knowledge gaps to work	Overwintering colony mortality, seasonal mortality, disruption of normal foraging and mating behaviour, malnutrition

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
		pathogens, climate change, genetically modified crops		towards an integrated environmental risk assessment of multiple stressors on bees.	
Doublet et al., 2015	research article	Multi-impact, including: pesticides; pathogens; <i>INTERACTIONS</i> (see Table A3)	Honey bees	Controlled laboratory experiments to assess the effects on larvae/adult bees provoked by the combination of three stressors: the exposure to sublethal doses of a commonly used pesticide and escalating doses of two pathogens.	Synergistic interaction between two pathogens induced higher mortality rate in individual honeybees; the exposure to the pesticide increased viral loads, leading to higher rates of mortality
Ewald et al., 2015	research article	Multi-impact, including: use of pesticides, climate change, <i>INTERACTIONS</i> (see Table A3)	Honey bees	Experimental research on the correlation of changes in climatic conditions and the increasing use of agrochemicals with changes in abundance of 26 invertebrate taxa	Changes in invertebrate abundance in response to extreme weather events; general decline in invertebrate abundance with increasing pesticide use
Steffan-Dewenter & Tscharntke, 1999	research article	Land occupation & transformation	Honey bees, bumblebees, solitary bees	Experimental research on the influence of habitat fragmentation on bee populations and seed set.	Decreased abundance and species richness of flower-visiting bees with increasing distance between fragments, increasing pollinators' body size, reduced seed set, decline in flower visitation rate, lack of favorable nesting sites and food supply
Kells et al., 2001	research article	Land occupation & transformation	bumblebees ( <i>B. terrestris</i> , <i>B. lapidarius</i> ) and honeybees	Experimental research assessing the role of natural unmanaged field edges in comparison with cropped field margins as important source of food and shelter for bumblebees, honeybees and other pollinator species.	Decrease in forage resources and nest sites, in addition to loss of floral diversity (it means loss of food variety) in managed cropped margins
Donaldson et al., 2002	research article	Land occupation & transformation	36 insect species, included solitary bees, honey bees, beetles, butterflies, moths, flies and wasps	Experimental research on the effects of habitat fragmentation on insect-pollinator diversity and plant reproductive success in South Africa.	Decrease in pollinators' abundance with increasing distance from large fragments (isolation); pollination failure appears to be more likely on small and isolated fragments than on large or well connected ones; potential high failure rate among pollination systems involving specialized

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
					pollinators and synchronously flowering plants
Goverde et al., 2002	research article	Land occupation & transformation	bumblebees	Operating under natural conditions, the authors experimentally assessed the involvement of habitat fragmentation occurred in the northwestern Swiss on bumblebee-plants interaction.	Pollinators tend to remain in isolated fragment (barrier to gene flow) with consequent lower visitation rate to flowering plants; change in pollinator behaviour potentially reducing pollen quantity and dispersal among flowers
Steffan-Dewenter et al., 2002	research article	Land occupation & transformation	Honey bees, bumblebees, solitary bees	Research on the effects of landscape context on bee abundance and diversity at different spatial scales. In particular, the relationship between bee distribution and the percentage of semi-natural habitats were analyzed.	Decreased abundance and species richness of flower-visiting bees with decreasing proportion of semi-natural habitats, loss of specialist wild bee species in favour of managed social bees which may lead to changes in plant populations, increased bees' body size, decline in flower visitation rate
Morandin and Winston, 2005	research article	Land occupation & transformation	Honey bees (only 2% of total captures), wild bees	Using an experimental field approach, the authors investigated how different land use categories (organic, conventional and GM crop) affect wild bee abundance in Canada.	Reduced wild bee abundance; reduced production of seed (pollination deficit) in GM crop fields.
Klein et al., 2007	review	Land occupation & transformation	Honey bees, solitary and stingless bees, bumblebees, wasps, hoverflies and other dipters, beetles	The authors evaluated the importance of insect pollination to human society and economic development and assessed the influence of land-use changes (agricultural intensification) at different scales on wild pollinator communities.	Loss of pollinator abundance and diversity
Kremen et al., 2007	review	Land occupation & transformation	wild bee species and honey bees	Development of a conceptual model in order to evaluate, even from the economic and social points of view, how changes in land use may affect pollinator communities and pollination	Changes in foraging behaviour: limited visitations to flowering plants; loss of food sources and nesting sites; pollinator diversity loss; alteration of plant-pollinator interactions

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
				services.	
Ricketts et al., 2008	review	Land occupation & transformation	Honey bees, bumblebees, wild bees, coleoptera, diptera, lepidoptera	Synthesis of 23 published studies in order to estimate the relationship between pollination services by insect pollinators and the distance from natural or semi-natural habitats.	Reduced visitation rate; decline in pollinator species richness with increasing distance from natural habitat; reduced fruit/seed set (weak evidence)
Ahrné et al., 2009	research article	Land occupation & transformation	bumblebees	Research on the effects of urbanization on pollinator populations. In particular, the abundance and diversity of bumblebee species along an urban-periurban gradient (including urban gardens) were studied.	Decreased diversity (species richness) of bumblebees with increasing urbanization level, decline in abundance of bumblebees in response to habitat quality change (loss of floral resources), potential loss of nesting sites
Winfrey et al., 2009	review	Land occupation & transformation	wild bees (bumblebees and other wild bees), feral and managed honey bees	Meta-analysis of 54 published studies recording bee abundance and/or species richness in order to evaluate the effects of anthropic disturbance (deriving from land use occupation/transformation) on bees.	Bee abundance and species richness were significantly, negatively affected by disturbance produced by human land occupation/transformation, although the magnitude of the effects was not large.
Le Féon et al., 2010	research article	Land occupation & transformation	Bumblebees, honey bees, solitary bees	Experimental research on the effects of both intensified agricultural practice and landscape composition on bee communities in four Western European countries.	Decrease in bee species richness, abundance and diversity and loss of food resources due to agricultural intensification; increase in bee species richness in semi-natural habitats, which provide bees with food resource and nesting areas
Rands and Whitney 2010	research article	Land occupation & transformation	1 bumblebee species ( <i>Bombus impatiens</i> )	Using a simplified model, the authors examined the effects of habitat fragmentation (monoculture crop fields separated by margins containing wild flowers) and flower density on pollinator behaviour.	Pollinators tended to forage in huge monoculture fields where the density of floral resources is higher than in natural margins, leading to wild plant biodiversity and related ecosystem functioning loss.

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Bates et al., 2011	research article	Land occupation & transformation	Honey bees, bumblebees, solitary bees and dipterans (included hoverflies)	Research on the potential impacts of urbanization on the structure of pollinator communities in the urban, suburban and rural areas across a UK city.	Lower abundance and diversity (species richness) of pollinators in urban and suburban areas than rural sites, changes in community composition along a urban-rural gradient with mainly generalist species in low-quality urban areas
Lautenbach et al., 2011	research article	Land occupation & transformation	solitary wild bees	Research on the effects that land-use changes over time may have on ecosystem services, such as pollination, at regional scale in Germany. The loss of ecosystem services in degraded ecosystems was analyzed.	Loss of potential nesting habitats; decrease in the number of pollinator visitations to flowers
Garibaldi et al., 2011	review	Land occupation & transformation	bumblebees, honey bees, solitary bees, beetles, ants, hoverflies and other dipterans	Review on the knowledge acquired through 29 pollination studies: the authors analyzed the impacts of isolation from natural and semi-natural areas on temporal and spatial stability of pollination service in crop fields.	Decline in pollinator species richness in fragmented habitats, lower visitation rate to flowering crops when distance from natural habitats is high
Holzschuh et al., 2011	research article	Land occupation & transformation	solitary bees, bumblebees, honey bees	Through a large-scale field study, the authors assessed the interactions between the abundance of pollinators and the expansion of lands for agricultural use at the expense of natural and semi-natural areas.	Strong landscape-scale dilution of bees in crops: lower visitation rate to crop flowering plant when natural habitats are removed, loss of nesting sites, loss of bee diversity and abundance.
Weiner et al., 2011	research article	Land occupation & transformation	Honey bees, bumblebees, solitary bees, butterflies, beetles, heteropterans and dipterans (included hoverflies)	Experimental research on how different degrees of land-use intensity may affect insect pollinator populations.	Reduced specialist pollinator diversity and abundance in high intensity land-use context

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Winfree et al., 2011	review	Land occupation & transformation	Apiformes, included bumblebees, solitary bees and hoineybees; in addition butterflies, hoverflies, birds and bats are mentioned	Synthesis of the current literature on the effects of land-use changes (from natural and seminatural to anthropogenic habitats) on the main groups of pollinator, included insects.	Loss of specialist pollinators (biotic homogeneization), reduced flower visitation rate in fragmented areas, loss of nesting sites, decrease in native species
Nielsen et al., 2012	research article	Land occupation & transformation	Honey bees, bumblebees, solitary bees and hoverflies	Experimental research on how human induced habitat fragmentation influences the composition of pollinator communities in five European countries	There appears to exist a relationship between the size and density of local plant aggregations and visitation frequencies of important pollinators: the spatial distribution of plants (fragment area and poulation density) affect the composition of pollinator communities depending on their behavioural attitude (social vs solitary)
Kennedy et al., 2013	research article	Land occupation & transformation	solitary bees, honey bees, bumblebees	The authors synthetized recent information on the impacts produced by land use on wild pollinators, modelling data of landscape composition, landscape structure and local management in order to predict how bee diversity and abundance change.	Loss of bee diversity and abundance, in addition to loss of nesting sites and food resources in low-quality habitats
Carvalho et al., 2013	research article	Land occupation & transformation	native wild bees (included bumblebees), hoverflies and butterflies	Comparing periods (1930-1990) characterized by intensive land use with significative natural habitat loss and periods (post-1990) with increased conservation investment, the authors investigated how biodiversity, especially pollinator richness, has changed in three European countries.	Loss of species richness, biotic homogeneization particularly with loss of specialist pollinators and spread of generalist pollinators in the period of intensified land use



Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Ollerton et al., 2014	research article	Land occupation & transformation	aculeate pollinators (Hymenoptera)	Using historical data, the authors assessed the extinction rate of bee and flower-visiting wasp species in Britain over the last 160 years, finding evidence that supports the relationship between the phases of Aculeata extinction and changes in agricultural practices.	Decrease in flower diversity which means decrease in food resources with consequent loss of species in those periods of dramatic changes in agricultural practices
Kevan, 1975	research article	Ecotoxicity	Wild bees including <i>Bombus</i> spp.	The author investigated the relationship between the application of fenitrothion pesticide for forest protection and the decline in abundance and species richness of wild bees in the neighboring crop fields in a Canadian region.	Reduced abundance and diversity of wild pollinators in crop fields close to sprayed forests
Barker et al., 1980	research article	Ecotoxicity	Honey bees	Experimental research on the toxicity and repellency of field-level dimethoate insecticide to honeybees.	Reduced survival of exposed adults
Taylor et al., 1987	research article	Ecotoxicity	Honey bees	Experimental research in controlled laboratory conditions in order to evaluate the effects of the exposure to pyrethroid insecticides on the odor-mediated learned responses of honeybees.	Reduced learning response rate (measured in terms of proboscis extensions) compared with untreated bees, meaning inhibition of foraging activity
Balanca and De Visscher, 1997	research article	Ecotoxicity	hymenoptera (Apoides were included), coleoptera, orthoptera	Field experiment to test the impacts of fipronil insecticide on non-target insects, included bee species.	Mortality (lethal effect) of non-target insects

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Thompson, 2003	review	Ecotoxicity	bee species (primarily <i>Apis mellifera</i> and <i>Bombus</i> spp.; wild bees are mentioned)	Review of the potential behavioral (sub-lethal) effects on bees exposed to different classes of chemicals (e.g. pyrethroids, organophosphates, carbamates, juvenile hormone analogues).	Inhibited hypopharyngeal gland development; disrupted foraging activity; reduced flower visitation; decreased learning ability, included olfactory learning performance; decreased flight activity; failure to return to colony; decreased brood production; reduction in surviving larvae/brood; increased queen supersedure; adults emerging with malformations;
Brittain and Potts 2011	review	Ecotoxicity	Honey bees, bumblebees and solitary bees	Overview of the potential impacts of pesticides exposure on bee species, seeking to identify how life-history traits of bees (sociality, floral specialization, nesting location and construction, ecc...) could be linked to their sensitivity to pesticides and how this feature might reflect changes in community structure of bees and flowers pollinated by them.	Reduction in bee life expectancy, decline in population size and species diversity, reduced visitation frequency to flowering plant due to abnormal foraging behaviour, reduction in orientation ability, impaired olfactory learning and memory.
Gill et al., 2012	research article	Ecotoxicity	bumblebees	Experimental research on the effects of two combined pesticides at field-level concentration on bumblebees at individual and colony level.	Reduction in colony queen and worker production; reduction in foraging activity; increase in worker mortality as a potential result of chronic exposure
Henry et al., 2012	research article	Ecotoxicity	Honey bees	Experimental research performed in order to verify the hypothesis that a sublethal exposure of honeybees to a neonicotinoid pesticide (thiamethoxan) may reduce adult foraging success potentially leading to the increase of colony death rate.	Reduced olfactory memory; learning dysfunctions; alteration of navigation skills; decrease in foraging success leading to reduction of bee survival rate and colony collapse

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Blacquière et al., 2012	review	Ecotoxicity	Honey bees, bumblebees, solitary bees	Review on the results of 15-year research about the risks that neonicotinoids pose on pollinators. The effects (especially sublethal effects derived from chronic oral/contact exposure) of the different pesticides toward bees are summarized.	Decreased foraging activity with reduction in sucrose uptake; affected mobility; changes in olfaction learning; altered learning ability; reduced breeding performance (delay in the time needed for honeybee larvae to hatch or develop as an adult when fed with contaminated food), leading to minor colony vitality; potential worker mortality (especially in winter bees)
Whitehorn et al., 2012	research article	Ecotoxicity	bumblebees	Experimental research on the effects of a neonicotinoid pesticide (imidacloprid) on bumblebees' colony success.	Reduction in colony queen production and colony growth rate
EFSA 2013a,b,c,d; EFSA 2015 a,b,c: conclusions on pesticide peer review (clothianidin, fipronil, imidacloprid, thiamethoxam)	4 scientific reports	Ecotoxicity	predominantly honey bees	The European Food Safety Authority drew the conclusions on four pesticide peer reviews elaborated by the European Commission, reporting evidence of lethal and sublethal effects of neonicotinoids on bee species, especially honey bees, and providing toxicological endpoints for adult honeybees.	Poisoning for direct exposure to spray and dust or for ingesting contaminated pollen and nectar leading to reduction of foraging activity, negative change in behavior with impaired learning performance, impaired homing ability, mortality of individuals which potentially leads to colony collapse
Goulson, 2013	review	Ecotoxicity	Honey bees, bumblebees	Review on the effects of neonicotinoids on wildlife, included insect pollinators (especially honeybees and bumblebees).	Potential direct mortality, reduced learning and foraging ability, impaired homing ability
Godfray et al., 2014	review	Ecotoxicity	Honey bees and bumblebees	The authors present a project in which the pressures exerted by neonicotinoids on insect pollinators are exposed, with evidence statements reported.	Prolonged exposure reduces longevity of pollinators leading to individuals' death and colony collapse; sub-lethal exposure causes metabolic, neurological and behavioural changes in adults, with olfactory, learning, memory and feeding behaviour affected and worker and queen production reduced; delayed larval and

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
					pupal developments
Sandrock et al., 2014	research article	Ecotoxicity	Honey bees	Research on the effect of sublethal dietary neonicotinoid exposure on honeybee colony performance.	Decrease in bee performance in the short- and long-term: loss of adults and broods, reduction in honey production and pollen collections; queen failure (increased supersedure rate); reduced tendency to swarm (it means reduced colony strenght)
European Academies Science Advisory Council (EASAC), 2015	scientific report	Ecotoxicity	bumblebees, solitary bees, moths, butterflies and dipterans are mentioned, even if the interest on neonicotinoid exposure is focused most on honey bees	EASAC report reviews the relationships between agricultural systems and natural ecosystem services, i.e. pollination, focusing on the impact that neonicotinoids have on organisms providing ecosystem services to agriculture.	Mortality (lethal effect) or fitness reduction (sublethal effect) through reduced cognitive abilities and location finding, impaired foraging behaviours, decreased memory, especially after prolonged exposure.
Kessler et al., 2015	research article	Ecotoxicity	Honey bees, bumblebees	Through a manipulative experiment, the authors tested whether bumblebees and honeybess avoid nectar containing different neonicotinoid pesticides.	Attractive effect of neonicotinoids; reduced total consumption of food, leading to reduced survival and premature mortality; neonicotinoids affect the neural mechanism involved in learning about the location of rewarding food
Rundlöf et al., 2015	research article	Ecotoxicity	bumblebees, solitary bees, honey bees	Research study with replicated and matched landscapes in order to assess the effects of neonicotinoid insecticides on bees under field conditions.	Reduced density of wild bees (solitary bees and bumblebees); reduced nesting activity of solitary bees, reduced colony growth and reproduction of bumblebees. No lethal effects were observed.
Chittka and Schurkens, 2001	research article	Invasive alien plant species	bumblebee species	Experimental research on the effects of alien plants ( <i>Impatiens glandulifera</i> ) on native plants' fitness. The results highlighted the high invasiveness of <i>I. glandulifera</i> , which deprives native plants of pollination service.	Reduced pollinators' visitation rate and seed set in native plants when in competitive patches

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Brown and Mitchell, 2001	research article	Invasive alien plant species	Honey bees, bumblebees	Through an experimental approach, the authors assessed the quantity of pollen produced by native plants when in competition for pollination with co-flowering invasive congener plants.	Lower seed production in native plants due to changes in pollinator behaviour with reduced visitation and subsequent reduced conspecific pollen quantity, potential for hybrid formation (reduced pollen quality within the community) which may have poor germination rate and limited growth
Brown et al., 2002	research article	Invasive alien plant species	bumblebees and honey bees	Experimental research on the effects of invasive species ( <i>Lythrum salicaria</i> ) on pollinator visitation rate and seed set in native congeners ( <i>L. alatum</i> ). Additional study on potential heterospecific pollen transfer via pollinators that represents a threat to biodiversity conservation.	Declined reproductive success of native plants given by reduced floral visitation rate and subsequent lower seed production, reduced loads of conspecific pollen transferred between native plant; potential for hybrid formation (reduced pollen quality within the community)
Larson et al., 2006	research article	Invasive alien plant species	Honey bees, bumblebees, solitary bees, wasps, dipterans, Lepidoptera	Experimental research on the effects of alien plants ( <i>Euphorbia esula</i> ) on pollinator communities and on the quantity of pollen deposited on native plant species during a 2-year period.	Reduced floral visitation rate (over 1-year period), decline in conspecific pollen delivered to native plants in infested plots (over 2-year period). Potential for significant pollen shortfalls in rare native plant species.
Lopezaraiza-Mikel et al., 2007	research article	Invasive alien plant species	Hymenoptera (31 species, among these <i>Apis mellifera</i> and <i>Bombus spp.</i> Solitary bees are included), Diptera, Hemiptera, Coleoptera, Lepidoptera, Dermaptera, Mecoptera	Experimental research on the effects of alien plants ( <i>Impatiens glandulifera</i> ) on a community of co-flowering native plants and their interactions with native pollinators. Alien plants have been shown to dominate the pollen transport networks.	<u>Double effect</u> : in the plots where alien species was removed, species richness of flower visitors on native plants decreased, whereas in the mixed plots where alien and native plants were together, an increasingly high number of insects visited both native and alien flowers. Generally decrease in quantity of conspecific pollen transferred between native plants, leading to potential hybrid formation (reduced pollen quality within the community) and no increase in native seed set production; overall dominance of generalist pollinators

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Bartomeus et al., 2008	research article	Invasive alien plant species	Honey bees, bumblebees, solitary bees, coleoptera, dipterans, lepidoptera	Assessment of the potential effects of two alien plant species ( <i>Caprobotus</i> spp. and <i>Opuntia</i> spp.) on a native plant-pollinator community. Provided evidence for both positive and negative effects on pollination services.	<u>Different effects in different species:</u> <i>Caprobotus</i> did not compete with native plants, enhancing pollinator visitations to native plants in plot where alien species was present, whereas <i>Opuntia</i> competed with native plants monopolizing the available pollinators and depriving native plant species of their service (highly decreased visitation rates to native plants in plots where alien species was present). Invasive species did not affect species richness
Munoz and Cavieres 2008	research article	Invasive alien plant species	Social and solitary bees (excluded honey bees), butterflies and dipterans	Experimental research on the effects of an invasive plant species ( <i>Taraxacum officinale</i> ) on pollination service delivered to two co-occurring native plant species ( <i>Perezia carthamoides</i> and <i>Hypochaeris thrincioides</i> ) in Chile. Results showed different responses depending on invasive floral density.	<u>Double effect:</u> reduced seed set production in native plants when high density of alien plants is present; neutral or facilitative effects on pollination of native plants <i>Perezia carthamoides</i> and <i>Hypochaeris thrincioides</i> respectively when alien plant species was absent or present at low density (density-response)
Goulson, 2003	review	Invasive alien pollinator species	Wild bee species	Overview of the potential consequences that exotic bees bring about when they are introduced in native ecosystems. Analysis of manipulative case studies; provided evidence of competition between exotic and native bees.	Ecological niche overlap leading to competition for floral resources (displacement of native organisms toward less profitable forage leading to limited quantity of pollen carried to the hive) and nest sites; spread of exotic pests and pathogens
Thomson, 2004	research article	Invasive alien pollinator species	bumblebees	The author assessed how competition with invasive European honeybees affects colony foraging behaviour, growth and reproductive success of native pollinators <i>Bombus occidentalis</i> in California.	2004: reduced rate of forager return; lower ratio of foraging trips for pollen; reduced both male and female reproductive success.
Thomson, 2006					2006: decline in bumblebees' abundance

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Nagamitsu et al., 2010	research article	Invasive alien pollinator species	bumblebees	Through a field manipulative experiment over a 3-year period, the authors evaluated the competitive impact of invasive <i>Bombus terrestris</i> on the abundance and the body size of three abundant native bumblebee species in Japan.	Competition for food resources and nest sites; negative effects on native queen abundance
Monceau et al., 2014	review	Invasive alien pollinator species	Honey bees	Review on the current information on <i>Vespa velutina</i> biology and the effects of this invasive predator on honeybees and other pollinators.	Loss of worker honeybees, leading to potential colony collapse
Gordo and Sanz, 2005	research article	Climate change	Honey bees, butterflies, beetles, flies	Analysis of temporal data series in order to clarify the role of climate change in some plant-insect pollinator interactions in a Mediterranean locality over the last century.	Temporal shift toward earlier flowering dates in plant species which may lead to a temporal mismatch between mutualistic partners
Memmott et al., 2007	research article	Climate change	1419 insect pollinators and one species of hummingbird	The authors investigated the potential disruption of mutualistic interaction in plant-pollinator systems, modelling empirical data and making prediction about future global situation under climate change.	Disrupted overlap in seasonal timing of flower production and pollinator flight activity that means pollinators suffering disruption in food supply (curtailments or loss of food resources); particularly specialist pollinators are at risk of extinction
Le Conte and Navajas, 2008	review	Climate change	Honey bees	Review on the potential effects driven by climate alterations on <i>Apis mellifera</i> behaviour, physiology and geographic distribution.	Distribution range shifts (migration) leading to competitive relationships among species and hybridization (loss of genetic variability); loss of food resources (nectar/pollen) due to climate induced-changes in flower species distribution leading to bee death for starvation

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Hegland et al., 2009	review	Climate change	Honey bees, wild bumblebees, solitary bees, butterflies	Overview of the possible effects of climate change on plant-pollinator interactions; review on evidence of temporal and spatial mismatches in plants and pollinators as a response to climate warming; potential consequences for pollination interactions are presented.	Limitation of nesting sites and food resources (nectar/pollen) leading to reduction in reproductive success/survival especially in specialized pollinator species
Bellard et al., 2012	review	Climate change	biodiversity, included insect pollinators	The authors examined the involvement of climate change in species' temporal and spatial shifts and biodiversity loss. In addition, they present principles and weakness of the main ecological approaches used at global and regional scales to projecting species loss, highlighting the needs for future implementation.	Phenological shift in flowering plants and insect pollinators leading to species extinction; geographic mismatch; physiological alterations; behavioural modifications of diet, activity and energy budget
Kuhlmann et al., 2012	research article	Climate change	solitary bees	Research on the effects of climate change on bees' geographic range in South Africa; modelling bee species distribution in the present and prediction of a potential future scenario distribution.	Spatial shifts of solitary bee species with potential expansions or contractions in habitat range.
Kudo and Ida, 2013	research article	Climate change	bumblebees	Research on the ecological impacts of climate change on plant ( <i>Corydalis ambigua</i> ) – pollinator (bumblebees) interactions in Japan over a 14-year period.	Phenological mismatch between pollinators and their host plants due to earlier flowering time, which lead to lower levels of pollination services and subsequent reduced seed production



Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Petanidou et al., 2014	research article	Climate change	Honey bees, bumblebees, solitary bees, flies, beetles, wasps, butterflies and true bugs in a Greek pollinator community	Experimental research on the interactions between plant and pollinator species within a Greek ecological community under climate change conditions	Temporal shift toward earlier seasonal timing of activity (phenological shift) in flowering plant species which may lead to a temporal mismatch between mutualistic partners causing a reduction of pollinator species richness and loss of pollination services
Polce et al., 2014	research article	Climate change	Wild bumblebees and solitary bees, hoverflies	Research on the present and predicted (for 2050) distribution of orchards species and their pollinators in Great Britain in order to understand how climate change may affect plant-pollinator interactions.	Climate-induced geographic shifts of bee species; altered plant-pollinator interactions; loss of pollination service
Pradervand et al., 2014	research article	Climate change	bumblebees	Research on climate change influences on plant-pollinator mutualistic interactions, particularly studying bumblebee species distribution and community composition in Swiss mountain regions. Application of climate change projection scenarios	Geographic shifts in bumblebee species distribution toward higher elevations, with species either losing or expanding their range under climate change; change in community functional composition with homogenization of proboscis diversity along elevation gradient; loss of bee diversity (climate change appears more probable to affect specialist pollinators)
Robbirt et al., 2014	research article	Climate change	One solitary bee species	Using specimen- and recent field-based records, the authors assessed the influence of climate change on phenological synchrony of a solitary bee species ( <i>Andrena nigroaenea</i> ) and the orchid it pollinates ( <i>Ophrys sphegodes</i> ).	Loss of synchrony (phenological mismatch) between insect pollinator activity and flowering time leading to lower pollination success rate in plants.

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Ellis and Delaplane, 2008	research article	Pests and pathogens	Honey bees	Experimental approach in order to to evaluate how two nest invaders species ( <i>Varroa destructor</i> and <i>Aethina tumida</i> ) affect pollination efficacy both at colony and community level in <i>Apis mellifera</i> . Negative consequences to pollination especially in the long-term.	Decrease in bees' honey production, body weight, life span, sperm load in drones, size of mandibular glands, flight activity and insecticide tolerance
Le Conte et al., 2010	review	Pests and pathogens	Honey bees	Overview of the current knowledge on the biology of <i>Varroa</i> mites; explanation about how these pathogens are involved in bees' Colony Collapse Disorder.	Colony infection, spread of parasites particularly virus, premature death of infected bees.
Forsgren, 2010	review	Pests and pathogens	Honey bees	Review on historical results and recent molecular data about European foulbrood disease that affects honeybees worldwide.	Deformed larvae, high mortality in larvae with high load of <i>Melissococcus</i> bacteria, co-infection with other bacteria
Rosenkranz et al., 2010	review	Pests and pathogens	Honey bees	Overview of <i>Varroa spp.</i> mites, focusing on <i>Varroa destructor</i> biology and damage to the host honeybees.	The loss of hemolymph during the ontogenetic development within the brood cell significantly decreases the weight of the hatching bee; parasitized drones lose their body weight that led to decreased flight performance. Worker bees start earlier with foraging and have a significantly reduced life span. The parasitized foragers display a decreased capability of non-associated learning, prolonged absences from the colony and a lower rate of return to the colony (reduced ability to navigate). Co-infection with various viruses, which leads to scattered brood, crawling or even crippled bees, supersedure of queens and reduction of the bee population. Less swarm. Overall, colony fitness declines.

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Meeus et al., 2011	Scientific report	Pests and pathogens	Honey bees	Report on the negative effects of various parasites, viruses and other pathogens on honeybee colonies.	Weakened colonies, reduced growth of colonies, increased brood/adult mortality, co-infections, reduced flight ability, deformed wings and body
Charrière, 2011	review	Pests and pathogens	bumblebees	Review of the potential impacts of some parasites which are transmitted from commercial to wild populations of bumblebees, reporting evidence from manipulative experiments for such negative effects	Spread of diseases resulting in rapid colony decline; reduced queen fitness and subsequent brood production, increased mortality rates, reduced foraging ability, deformed body
Dussaubat et al., 2013	research article	Pests and pathogens	Honey bees	Experimental research on the potential negative effects <i>Nosema ceranae</i> parasites may bring about in honeybee colonies	Changes in Ethil Oleate (EO) pheromone levels which reduce homing and orientation skills, precocious and higher flying activity, subsequent altered foraging behaviour which lead to reduced life span
Food and Environment Research Agency (FERA), 2013	Scientific report	Pests and pathogens	Honey bees ( <i>Apis mellifera</i> ) and <i>Apis ceranae</i>	Scientific report on the impacts and distribution of those pathogens and pests that primarily affect honeybee species health in Europe.	Colony infection; disorientation and inability to fly in adult bees leading to disruption of foraging behaviour; pollinators' death (different pathogens act at different pollinators life stages leading to death of larvae, pupae or adults)
McMenamin and Genersch, 2015	review	Pests and pathogens	Honey bees	Review of the recent evidence provided by previous monitoring studies of the impacts which <i>Varroa destructor</i> mites and associated spread viruses have on honeybee colonies.	Paralysis of adult bees, impair cognition and homing ability, learning deficits, deformed adults, colony infection and subsequent collapse with adult and brood mortality
Sharma and Kumar, 2010	research article	Electro-magnetic radiations	Honey bees	Experimental research on the pressure that phone radiations exert on exposed honeybee colonies in terms of foraging performance and colony productivity.	Decline in colony strength, decreased egg laying rate of the queen and brood development, disruption of foraging activity with decreased collected pollen, decline in amount of produced honey and number of bees returning to the colony

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Kumar et al., 2011	research article	Electro-magnetic radiations	Honey bees	Research on the effects of cell phone radiations on bee metabolism and study of the subsequent changes in biomolecules concentration of worker honeybees.	Increased concentration of biomolecules (lipids, proteins, carbohydrates, glycogen, glucose, cholesterol) correlated to change in behaviour (bees become more aggressive and agitated)
Favre, 2011	research article	Electro-magnetic radiations	Honey bees	Experimental research (acoustic experiments) on the effects of electromagnetic waves from mobile phones on honeybee behaviour.	Increase in piping signal (alarm sound linked to swarming process) leading to adult losses
Sahib, 2011	research article	Electro-magnetic radiations	Honey bees	Research on the effects of towers and mobile phone radiations on honeybees in order to clarify their decline in India. Experimental research with exposure of colonies to electrowaves radiation.	Loss of orientation, disruption in navigational skills; reduced egg laying rate (reduced queen prolificacy)
Kumar, 2012	research article	Electro-magnetic radiations	Honey bees	Experimental research on consequences of exposing honeybee drone semen to cell phone radiations. Concentrations of various biomolecules were studied.	Increased concentration of biomolecules (lipids, proteins, carbohydrates, glycogen, glucose, cholesterol) and decreased activity of seminal enzymes as indication of metabolic disturbance in spermatozoa and semen quality; loss of orientation
El Halabi et al., 2013	research article	Electro-magnetic radiations	Honey bees	Experimental research on the influence of mobile phone radiations on honeybee navigational system.	Increasing activity, loss of orientation, loss of broods
El Halabi et al., 2014	research article	Electro-magnetic radiations	Honey bees	The research is a complementary process to the previous paper written by the same authors in 2013. It aims to investigate the effects of mobile phone antennas' radiations on honeybee behaviour.	Loss of orientation (affected magnetic reference of bees and memory performance); reduced production of broods

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Dalio, 2015	research article	Electro-magnetic radiations	Honey bees	Experimental research on the effects of electromagnetic radiations at field level emitted by cell phones on honeybee colonies.	Changes in behavioural pattern with increasing degree of aggressiveness of adult bees; decreased strength of colony, loss of orientation leading to loss of worker bees, decline in pollen and honey stores, reduced queen prolificacy, potentially leading to CCD.
Warnke, 1976	research article	Electric charges	Honey bees	Research on the effects of external electric fields, such as those generated by high-tension wires or by natural storms, on honeybees at both individual and colony level.	Behavioural changes: hyperactivity, irritability and aggressivity leading bees to leave the hives or kill their broods; disability in imbibing food; disorientation
Ferrari, 2014	research article	Magnetic field fluctuations	Honey bees	Experimental research on the effects of magnetic field fluctuations and solar-induced geomagnetic storms interference on forager homing abilities.	Loss of orientation due to a "magnetoreception disorder" (MRD) in foragers leading to loss of adult returning home
Sanvido et al., 2007	scientific report	GM crops	non-target arthropods. Honeybees, moths and butterflies are mentioned.	Overview of the ecological impacts brought about by genetically modified crops on agryecosystems and on their biodiversity, included pollinators.	Changes in consumptions resources due to hybridization with wild plants; sub-lethal toxic effects on bees, caused by feeding on toxic pollen, which lead to behavioural changes; some Bt-toxins are selectively toxic to Lepidoptera
Ramirez-Romero et al., 2008	research article	GM crops	Honey bees	Experimental research on the potential impacts of Cry1Ab protein, expressed in GM crops, on life traits of young adult honey bees (survival rate, foraging behaviour and learning performance),	Higher concentrations of Cry1Ab protein in GM crops do not induce lethal effects on honeybees, but are responsible for disruption in honeybee food consumption (reduced foraging efficiency) and memorization processes

Reference	Output type	Impact driver category	Investigated pollinators	Brief paper description	Showed effects on insect pollinators and pollination
Malone and Burgess, 2009	Review	GM crops	bumblebees, honey bees, solitary bees, lepidoptera	Overview of the effects of GM crops, both herbicide-tolerant and expressing Bt toxins, on bee pollinators.	The majority of the analyzed studies showed no effects on bees, but there appears to be some experiments that found at least sub-lethal effects provoked by GM crops on bees' species. For example: Bt oilseed rape (Cry1Ac) has been seen to produce less nectar with low sugar content; CpTi seems to reduce adult bee longevity; other insecticidal proteins may have sub-lethal effects such as altered flight activity, reduced learning ability, decline in food consumption, inhibition of some protease, reduced size of hypopharyngeal glands and their protein content; loss of weed as food source or nesting sites
Han et al., 2010	research article	GM crops	Honey bees	Case study in China. Assessment of the lethal and sub-lethal toxicity of GM cotton pollen to honeybee adults and larvae.	No lethal effect on bees; disturbed feeding behaviour, reduced quantity of pollen consumed by bees (antifeedant effect) potentially leading to insufficient food resources and malnutrition of broods in the colony.

**Table A2.** For the papers included in the review presented in Section 2.1, the available modelling approach and indicators of impact and damage for pollinators are reported.

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Traveset and Richardson, 2006	Multi-impact	/	/	The authors indirectly suggest native bee population growth and seed set production as predictors for damage to ecosystem services
Aizen et al., 2008	Multi-impact	Comparison between couples of plant-pollinator webs with different degrees of invasion	Proportion of flower visitation frequencies of alien plants and for flower-visitors as proxies for functional importance of invading plants and animals respectively. The average between the previous proportions is the index of the degree of invasion (0 = no interacting alien species; 1 = interaction exclusively between alien species)	Mutualism strenght; distribution of asymmetries in interaction webs.
Schweiger et al., 2010	Multi-impact	/	Pollinator body size; nectar quantity and quality (sucrose content);	Pollinators' appareance; species richness
VanEngelsdorp and Meixner, 2010	Multi-impact	/	LD <sub>50</sub> or LC <sub>50</sub> can be considered as impact factor associated to pesticides	/
Pettis et al., 2012	Multi-impact	Experimental approach; Dose-response tests; comparison between treatment and control groups	Queen bee weight; number of <i>Nosema</i> spores after pesticide exposure	(Mortality)
Burkle et al., 2013	Multi-impact	Quantitative analysis of bee and forbs co-occurrence in monitored land parcels over time	/	Species richness and abundance; quantity and quality of pollen delivered as a proxy for pollination service
European Food Security Authority (EFSA), 2014	Multi-impact	/	For chemicals: LD <sub>50</sub> (ug/bee), LC <sub>50</sub> (ug/bee per day or ug/larvae per development period), NOEC, Effective Concentration (Ec <sub>10</sub> or EC <sub>20</sub> ) →Toxic Unit approach	/
Doublet et al., 2015	Multi-impact	Experimental approach; treatment vs control groups; dose-response tests	Increase in viral/fungal loads after pesticide exposure	Larval/adult mortality to treatments

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Ewald et al., 2015	Multi-impact	Statistical analysis	Monthly mean temperature, total monthly precipitation, patch density (number of patches of habitat measured in ha), field boundary density (m/ha), crop types, pesticide application (% spray area)	Change in the abundance of invertebrate (annual change index)
Steffan-Dewenter & Tschardt, 1999	Land occupation & transformation	Experimental approach based on the negative correlation between habitat characteristics (isolation distance) and pollinators' distribution	Linear distance from the nearest natural habitat fragments; distance from the nearest apiary	Bee abundance and species richness; bee body size; flower visitation rate; reproductive success of plant based on seed set as a proxy for pollination efficiency
Kells et al., 2001	Land occupation & transformation	Experimental approach: treated (spontaneous and unmanaged margin) vs control (cropped and managed margin); relationship between bee and land-use variables	Management level (qualitative data)	Index of flower preferences of individual bees (PI), based on number of foraging visits
Donaldson et al., 2002	Land occupation & transformation	Distribution and abundance of insect pollinators	Fragment size, vegetation cover, distance between fragments	Pollinator diversity in each analyzed fragment measured using species richness index, Brillouin index and Shannon's diversity index; fruit set as a proxy for pollination service
Goverde et al., 2002	Land occupation & transformation	Experimental approach: control (connected) vs treated (fragmented) plots	/	Percent of visited inflorescences, visiting time per patch related to inflorescence number, ratio of mean visiting distances, ratio of total visiting distances
Steffan-Dewenter et al., 2002	Land occupation & transformation	Experimental approach; relationship between landscape context (proportion of seminatural habitat and diversity of habitat types) and bee abundance and diversity	Proportion of semi-natural habitat and distances from them (correlated with habitat diversity measured using Shannon-Wiener index)	Abundance and diversity of bees (species richness) in response to land-use changes
Morandin and Winston, 2005	Land occupation & transformation	Experimental approach based on the comparison between land use management practices (crop types as land-use categories)	/	Wild bee abundance; seed and fruit set (difference in mean seed/fruit number between supplementally and open-pollinated flowers) as a proxy for pollination deficit
Klein et al., 2007	Land occupation & transformation	/	Variables proposed by mentioned papers: linear isolation distance from near-natural habitat, proportion of natural areas	Variables proposed by mentioned papers: fruit set, seed set, flower visitation rate, pollinator diversity and abundance...



Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Kremen et al., 2007	Land occupation & transformation	/	The authors do not directly propose indicators of impact on pollinators/pollination service, but the text suggests that there are some aspects of land use to be taken in account since they are linked to pollinator decline/loss, such as distance to natural habitats and proportion of natural areas, GM crops effects and organic field management.	The text suggests some feature that could be used to evaluate the damage to pollinators communities or directly to pollination service: changes in pollinator richness due to isolation or the economic calculation of pollination service loss based on how much people would have to pay for pollinated food products or on the proportion of the total value of pollinated crop loss
Ricketts et al., 2008	Land occupation & transformation	Statistical analysis: model of exponential decline and comparative analysis	Isolation from natural habitats (linear distance or proportional area of natural habitat)	Pollinator species richness, pollinator visitation rate, fruit or seed set
Ahrné et al., 2009	Land occupation & transformation	Urban-periurban gradient approach; relationship between pollinators data (abundance and species richness) and land use data (abundance and diversity of flowering plants, proportion of different land-cover types, size of urban gardens)	Environmental variables with significant effect: proportion of impervious surface, flower abundance and plant species richness	Diversity and abundance of bumblebees
Winfree et al., 2009	Land occupation & transformation	Meta-analysis	/	Bee abundance and species richness
Le Féon et al., 2010	Land occupation & transformation	Experimental approach: relationship between bee species indices with both chemical and land use variables and semi-natural habitat proportions	Pesticide input, livestock density, number of crops, semi-natural habitat proportions	Bee abundance, bee species richness, bee diversity calculated with the Shannon index at each analyzed site
Rands and Whitney 2010	Land occupation & transformation	Simplified density-preference model	Flower density within the pollinators' foraging range is the variable with statistically significant effect	/
Bates et al., 2011	Land occupation & transformation	Urban-rural gradient approach; relationship between pollinators data (abundance and species richness) and land use data (abundance and diversity of flowers, percentage of built space)	Habitat variables: diversity and abundance of flowers, percentage of built landscape	Diversity and abundance of pollinators

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Lautenbach et al., 2011	Land occupation & transformation	Temporal distribution analysis	/	Area of potential nesting sites (m <sup>2</sup> ) which is the areas providing the ecosystem function, as a proxy for the supply with pollination; visitation probability as indicator for pollination service, calculated in two ways: euclidian distance between potential nesting habitats and the nearest arable land cell (m), and number of visitation of native pollinator on arable fields, based on a k-nearest neighbour approach
Garibaldi et al., 2011	Land occupation & transformation	Association between each response variable (temporal and spatial coefficient of variation and mean of visitation rate to crop flowers, pollinator species richness, seed/fruit set) and isolation	Distance to natural areas	Seed or fruit set as a proxy for pollination service level; pollinator species richness + visitation rate as proxies for pollinator community depauperation and pollination stability
Holzschuh et al., 2011	Land occupation & transformation	Relationship between bee diversity/species abundance and land-use variables (crop filed size and % of presence, natural area size and % of presence, flower diversity primarily)	Crop filed size and % of presence, natural area size and % of presence, flower diversity	Bee diversity and species abundance
Weiner et al., 2011	Land occupation & transformation	Correlation between floral availability and pollinator visitation rate; comparison of relative species composition on "treated" (high intensity) and "control" (low intensity) fields.	Flower abundance; flower diversity and evenness based on Simpson's diversity indices	Pollinator diversity and evenness based on Simpson's diversity indices; pollinator visitation rate
Winfree et al., 2011	Land occupation & transformation	/	Proportion of natural area surrounding disturbed study site, linear distances from the disturbed site to the nearest natural habitat, size of the natural habitat fragment where data are collected; floral resources (floral density) can be a limiting factor for bee populations	Flower visitation rate is a strong predictor of pollination; species abundance and richness; reproductive success, nest density (according to the authors, last 2 variable have seldom been reported probably because they are complicated to measure); bee extinction debt
Nielsen et al., 2012	Land occupation & transformation	Experimental approach; relationship between pollinator visitation frequencies and spatial variables	Plant community variables averaged values: population area (m <sup>2</sup> ), population density (flowers per m <sup>2</sup> ), fragment area (m <sup>2</sup> ), fragment density (flowers per m <sup>2</sup> )	Mean pollinator visitation rate

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Kennedy et al., 2013	Land occupation & transformation	Relationship between land use variables and bee data	Landscape composition (measured through LLI -Lonsdorf Landscape Index-which is a quality index based on nesting suitability and floral resources), landscape configuration (measured as a combination of perimeter-area ratio distribution, euclidean nearest neighbour distance distribution and interspersion and juxtaposition index), farm management (measured with qualitative indicators)	Bee abundance and richness
Carvalho et al., 2013	Land occupation & transformation	Comparison between pollinator and plant species distribution over time and space	Land use transformation is mentioned as the predictor for impacts on biodiversity, but here it was evaluated only in qualitative terms	Pollinator species richness; Simpson dissimilarity or spatial turnover
Ollerton et al., 2014	Land occupation & transformation	Relationship between pollinator distribution and changes in land-use practice over time	Change in land-use practice (Qualitative data)	Annual and decadal extinction rates, qualitatively correlated with changes in land use
Kevan, 1975	Ecotoxicity	Study performed under field conditions. Statistical analysis on the correlation between pesticide application and pollinator abundance/diversity.	/	Index of diversity-and-abundance (Margalef's index of diversity multiplied by the abundance of pollinators)
Barker et al., 1980	Ecotoxicity	Experimental approach under laboratory conditions: treated vs control individuals.	/	Mortality measured in LT <sub>90</sub> (toxicity test)
Taylor et al., 1987	Ecotoxicity	Experimental approach under laboratory conditions: treated vs control individuals. Conditioned response to a treatment.	Response rate (proboscis extension rate) after pyrethroid insecticide exposure	(Mortality)
Balanca and De Visscher, 1997	Ecotoxicity	Experimental approach under field conditions: treated vs control plots.	/	Abundance of non-target insects
Thompson, 2003	Ecotoxicity	Review of studies predominantly under laboratory conditions.	/	/
Gill et al., 2012	Ecotoxicity	Experimental approach: dose-response relationship	(Field-realistic) chemicals' concentration	Individual level: number of foragers, foraging bout frequency, amount of pollen collected, duration of pollen foraging bouts. Colony level: worker production, brood number, nest structure mass, worker mortality, worker loss colony failure (n.failed/n.survived)

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Henry et al., 2012	Ecotoxicity	Experimental approach under semi-field conditions: dose-response relationship; honeybee population dynamics model	Neonicotinoid exposure	Foragers mortality induced by homing failure, calculated as the proportion of nonreturning treated foragers relative to the expectations given by the proportion of returning control foragers
Whitehorn et al., 2012	Ecotoxicity	Experimental approach under laboratory conditions; chronic oral exposure; dose-response relationship	(Field-realistic) neonicotinoid concentration	Queen production, colony growth rate (measured as weight of all biological material such as wax, food stores, brood and adult bees accumulated within the colony)
EFSA 2013a,b,c,d; EFSA 2015 a,b,c: conclusions on pesticide peer review (clothianidin, fipronil, imidacloprid, thiamethoxam)	Ecotoxicity	/	Acute and chronic toxicity endpoints (LD <sub>50</sub> , NOEC)	/
Goulson, 2013	Ecotoxicity	Review of studies predominantly under laboratory and semi-field conditions.		
Godfray et al., 2014	Ecotoxicity		Estimations of LD <sub>50</sub> (available for different neonicotinoid-pollinator combinations, although the majority of the studies have considered only honeybees)	
Sandrock et al., 2014	Ecotoxicity	Experimental approach: control (unexposed) vs treated (chronically exposed to contaminated pollen) individuals and colonies; interaction between bee colony variables and exposure to field-realistic concentration of neonicotinoids	Neonicotinoid concentrations	Pollen collection and consumption, honey production; colony propensity to swarm as an indicator for colony fitness; queen supersedure rate; number of adults, pupae, eggs and larvae as a proxy for colony growth rate

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
European Academies Science Advisory Council (EASAC), 2015	Ecotoxicity	/	EASAC report EFSA's ETR <sub>acute</sub> : exposure to toxicity ratio between the amount of residues that may be ingested by and adult bee in 1 day and the LC50 value as an indicator of risk	/
Kessler et al., 2015	Ecotoxicity	Manipulative experimental approach under laboratory conditions; two choice test with exposure to increasing concentrations of neonicotinoid pesticides	Pesticide concentration	Premature mortality, reduced survival period due to reduced amount of food consumed
Rundlöf et al., 2015	Ecotoxicity	Matched landscapes: treatment (application of insecticides) vs control. Experiment performed under field conditions.	Pesticide concentration	Number of flower visiting wild bees as a proxy for wild bee density; number of solitary bees' cocoons and open cocoons as a proxy for nesting activity; number of queen and worker/male cocoons, weight of cocoons, larvae and nest structure and the number of cells used for nectar and pollen storage as proxies for bumblebee colony growth and reproduction; number of adult bees per colony as a proxy for honeybee colony strength
Chittka and Schurkens, 2001	Invasive alien plant species	Experimental approach; manipulative field experiment with comparison between invaded and uninvaded patches	Invasive floral attractiveness through higher sugar production	Floral visitation frequencies; seed set production
Brown and Mitchell, 2001	Invasive alien plant species	Experimental approach under field conditions: comparison between three treatments (3 set of native plants pollinated with native pollen, alien pollen, mixture of native and alien pollen respectively)	Alien pollen load	Number of foraging visits; seed set production
Brown et al., 2002	Invasive alien plant species	Experimental approach; comparison between treatments (monospecific between competitive plots)	/	Number of foraging visits; seed set production
Larson et al., 2006	Invasive alien plant species	Experimental approach under field conditions: comparison between invaded and uninvaded areas' variables	Invasive floral density	Floral visitation frequencies; native and alien pollen loads

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Lopezaraiza-Mikel et al., 2007	Invasive alien plant species	Experimental approach; manipulative field experiment (removal of invader species individuals), making then a comparison between treatments (monospecific vs competitive plots)	/	Visitation data (abundance of insects visiting flowers and frequency of visits); pollen transport data (pollen loads)
Bartomeus et al., 2008	Invasive alien plant species	Experimental approach with paired sites (field replicated comparative study): comparison between visitation frequencies received in uninvaded and invaded plots	/	Number of floral visitation in terms of number of visiting pollinators
Munoz and Cavieres 2008	Invasive alien plant species	Experimental approach: comparison between treatments (uninvaded, slightly invaded and highly invaded areas)	Relative Neighbour Effect index (RNE) evaluates the effects of different invasive floral density on pollinator visitation rates and seed production in native species	Pollinator visitation rate, seed set production
Thomson, 2004 Thomson, 2006	Invasive alien pollinator species	Experimental approach: effects of distance from introduced <i>Apis</i> colonies on bumblebees' measured variables	Distance from introduced <i>Apis</i> colonies	Foraging behaviour: mean forager return rate and pollen foraging effort (ratio of returns with pollen to total return) per colony; Growth: total number of cocoons per colony as a proxy for colony size; Reproductive success: number of gyne cocoons, ratio of gyne cocoons to non-gyne cocoons, mean gyne size as a proxy for quality of produced gynes, number of sighted flying males
Nagamitsu et al., 2010 Monceau et al., 2014	Invasive alien pollinator species Invasive alien pollinator species	Experimental approach based on the effects of invasive species removal: treatment (removed invaders) vs control sites	Number of exotic species' individuals, particularly queens and workers as indicator of population growth	Native queen abundance, native workers' body size as head width (mm)
Gordo and Sanz, 2005	Climate change	Dynamic factor analysis (DFA) in order to analyze temporal changes and effects of climatic variables in plant life cycle events; regression analysis used to test temporal changes and climatic variables for the timing of insect appearance	Change in monthly means of temperature; rainfall; flowering dates (plants' trend)	Adult pollinators' appearance (pollinators' trend)

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Memmott et al., 2007	Climate change	Relationship between plant flowering and pollinator activity dates. Models of temporal shift prediction under climate change conditions are used.	Mean annual temperature; flowering dates	Pollinator flight timing
Hegland et al., 2009	Climate change	/	Nectar/pollen amounts as proxy for food availability	Reproductive success, survival rate
Bellard et al., 2012	Climate change	Review of ecological modelling approaches which quantitatively assess the prospects for biological diversity in the face of global climate change: Bioclimatic Envelope Models (BEMs), Dynamic Vegetation Models (DVMs), Species-area relationships (SAR), IUCN status metrics, Dose-response relationships (DRR), other models that "attempt to quantify the impact of human activities on species abundance" (i.g. GLOBIO model)	Species and community level. Climate niche (BEMs), Plant Functional Type distribution (DVMs), Extinction risk (SAR and IUCN status metrics), change in species loss in relation to the importance of a driver (DRR), Biodiversity Intactness Index, Mean Species Abundance (MSA).	Extinction risk (SAR and IUCN status metrics)
Kuhlmann et al., 2012	Climate change	Species distribution models (SDMs), in which climate and environmental variables are used as predictors. The model express the % contribution of the different variables to the bee species distribution. Climate projection have been made.	Size of range shift (average predicted loss of habitat)	/
Kudo and Ida, 2013	Climate change	Correlation between flowering onset and phenological mismatch; correlation between seed set and both flowering onset and phenological mismatch	First flowering day as an index of phenological shift in plants; delay in initial bee activity from flowering onset as indicator of phenological mismatch	Seed production as a proxy for pollination success
Petanidou et al., 2014	Climate change	Correlation between the number of visiting pollinators and flowering plants' variables	Mean of first flowering dates as an index of phenological shift	The number of pollinator species visiting a plant species (i.e. pollinator species richness) is used as a proxy for the success of that species in obtaining pollination (thus, as a proxy for pollination services)

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Polce et al., 2014	Climate change	Species distribution models (SDMs) in which climate data are used as environmental descriptors for present and future distributions. Climate projections have been made.	Climate variables	Species level. Index of Pollinator availability (PA, resulted from the contribution of each species probability of occurrence predicted by SDMs) as a proxy for pollination service
Pradervand et al., 2014	Climate change	Stacked species distribution models (S-SDMs) in which climate data are used as environmental descriptors for present and future distribution; three IPCC-based climate change projection scenarios are used to forecast future species distribution.	Climate variables	Change in suitable surface for bumblebee species, proboscis length along elevation gradient
Robbirt et al., 2014	Climate change	Correlations between bee mean flight dates and orchid peak flowering time with mean spring temperature and subsequent comparison between bee and orchid data	Mean spring temperatures; flowering date;	Bee flight dates
Ellis and Delaplane, 2008	Pests and pathogens	Experimental approach based on the effects of different pathogen loads (treatments vs control) to pollination variables	Parasite loads	Seed/fruit set and their number/weight and rate of honeybee flower visits as proxies for pollination efficacy
Dussaubat et al., 2013	Pests and pathogens	Experimental approach: treated (experimental infected) vs control (healthy) individuals	Infection level (spore loads)	Ethil Oleate (EO) levels; Flight activity based on the daily number of exits; mortality
Sharma and Kumar, 2010	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	EMF (Electromotive field) power density; electrowaves frequencies; exposure duration	Foraging activity in terms of flight activity (n.° leaving bees), pollen foraging efficiency (n.° returning bees with pollen) and returning ability (n.° returning bees); brood area; queen prolificacy measured in terms of egg laying rate; colony growth in terms of bee strenght, honey stores and pollen stores
Kumar et al., 2011	Electro-magnetic radiations	Experimental approach: treated (exposed) vs control (unexposed) colonies; response to a disturb	EMR exposure (frequencies + duration)	Biomolecules concentration



Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Favre, 2011	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	Cell phone SAR (specific energy absorption rate) values; EMR intensity and frequencies + exposure duration → increase piping sound in intensity and frequency	/
Sahib, 2011	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	EMF (Electromotive field) power density; electrowaves frequencies; exposure duration	Number of worker bees returning to the colony as a proxy for flight activity and bee performance; egg laying rate as a proxy for queen prolificacy and colony productivity
Kumar, 2012	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	Duration of electromagnetic radiation exposure and EMR frequencies	Biomolecules concentration, enzymes activity
El Halabi et al., 2013	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	Frequency of electromagnetic waves emitted by mobile phones and exposure duration which produce > frequency and > intensity of the sounds produced by honeybees	Number of bees in the colony, developed eggs
El Halabi et al., 2014	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	Combination of antenna power density, frequencies of emission and distance from the source	Number of bees in the colony, area of wax, larvae and eggs state as proxies for colony health and performance
Dalio, 2015	Electro-magnetic radiations	Experimental approach: treated vs control colonies; response to a disturb	EMR exposure (frequencies + duration)	Degree of aggressive behaviour, n°. of outgoing bees/min/colony, % of returning bees, strength of colonies --> egg laying rate, area under brood, area under honey/nectar/pollen stores
Ferrari, 2014	Magnetic field fluctuations	Experimental approach: control vs treated individuals; sensitivity to environmental stress	Magnetic field intensity	Species level. Forager return rates (% returned bees over minutes); correlation between honeybee losses with total hours of n. K-indices.
Sanvido et al., 2007	GM crops	/	The authors suggest that the potential hazard of Bt-crops depends on the amount of toxin expressed in the pollen	/
Ramirez-Romero et al., 2008	GM crops	Dose-response relationship (toxin concentration-syrup consumption-mortality)	Concentration of Cry1Ab toxin → proboscis extension reflex (PER) as indicator of olfactory response (memorization process); feeding duration on contaminated syrup as indicator of foraging efficiency	Individual and colony level: % bee mortality

Reference	Impact driver category	Modeling approach	Indicators of impact	Indicators of damage
Malone and Burgess, 2009	GM crops	/	Concentration of toxins in transgenic pollen which may cause sub-lethal effects on bees	/
Han et al., 2010	GM crops	Dose-response relationship (toxin concentration-pollen consumption-mortality)	Concentration of toxins → pollen consumption	% bee mortality

**Table A3.** Papers included in the review of Section 2.1, dealing with the interactions between impact drivers acting on pollinator populations.

	Land occup. and transf.	Ecotoxicity	Invasive alien plant species	Invasive alien pollinator species	Climate change	Pests and pathogens	Electro-magnetic radiations	Electric charges	Magnetic field fluctuations	GM crops
Land occup. and transf.	/	Gonzalez-Varo et al., 2013	Gonzalez-Varo et al., 2013	Gonzalez-Varo et al., 2013	Gonzalez-Varo et al., 2013; Vanbergen et al., 2013; 2014	Gonzalez-Varo et al., 2013; Vanbergen et al.; 2013, 2014	/	/	/	/
Ecotoxicity	Gonzalez-Varo et al., 2013	Gill et al., 2012	/	/	Ewald et al., 2015	Doublet et al., 2015; Gonzalez-Varo et al., 2013; Pettis et al., 2012; Vanbergen et al., 2013; 2014	/	/	/	/
Invasive alien plant species	/	Gonzalez-Varo et al., 2013	/	/	Schweiger et al., 2010; Vanbergen et al., 2014	/	/	/	/	/
Invasive alien pollinator species	/	Gonzalez-Varo et al., 2013	/	/	Gonzalez-Varo et al., 2013; Schweiger et al., 2010; Vanbergen et al., 2014	Gonzalez-Varo et al., 2013; Schweiger et al., 2010; Vanbergen et al., 2014	/	/	/	/
Climate change	Gonzalez-Varo et al., 2013; Vanbergen et al., 2013; 2014	Ewald et al., 2015	Schweiger et al., 2010; Vanbergen et al., 2014	Schweiger et al., 2010; Gonzalez-Varo et al., 2013; 2014	/	Gonzalez-Varo et al., 2013 (positive); Schweiger et al., 2010; vanEngelsdorp and Meixner, 2010	/	/	/	/

	Land occup. and transf.	Ecotoxicity	Invasive alien plant species	Invasive alien pollinator species	Climate change	Pests and pathogens	Electro-magnetic radiations	Electric charges	Magnetic field fluctuations	GM crops
Pests and pathogens	Gonzalez-Varo et al., 2013; Vanbergen et al., 2013; 2014	Doublet et al., 2015; Gonzalez-Varo et al., 2013; Pettis et al., 2012; Vanbergen et al., 2013; 2014	/	Schweiger et al., 2010; Gonzalez-Varo et al., 2013; 2014	Gonzalez-Varo et al., 2013 (positive); Schweiger et al., 2010; vanEngelsdorp and Meixner, 2010	Doublet et al., 2015	/	/	/	/
Electro-magnetic radiations	/	/	/	/	/	/	/	/	/	/
Electric charges	/	/	/	/	/	/	/	/	/	/
Magnetic field fluctuations	/	/	/	/	/	/	/	/	/	/
GM crops	/	/	/	/	/	/	/	/	/	/

## Appendix to Chapter 3 (Appendix B)

**Table B1.** List of naturally occurring biotic resources, reported as main commercial groups, including information on their use, availability in the wild, harvesting and consumption at global, European and country levels where available. Complete references (accessed between March and June 2016) are reported below the table in alphabetical order.

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Aquatic Vertebrates	Marine and freshwater fishes	food; fish oil; fishmeal; fish glue; isinglass for beer clarification; skin for accessories (e.g. eel skin belt)	Captures in 2012: 77.6 million tonnes	Captures in 2012 (including crustaceans, molluscs and other aquatic animals): about 13.1 million tonnes	Data of capture production and consumption are reported for 57 countries all over the world until 2012	FAO, 2014
	Other aquatic vertebrates (e.g. whales, seals, crocodiles, etc.)	food (meat); oil (from blubber); fur for coats; other accessories (e.g. skin for bags, shoes, belts, etc.)	Captures in 2012: about 580 Ktonnes			
Terrestrial Vertebrates	animals for fur or skin (e.g. snake, fox, mink, etc.)	fur, skin, accessories, trophy	-	-	Data available at country level: specific national datasets are reported in the Artemis-FACE database (e.g. <a href="https://riistaweb.riista.fi/">https://riistaweb.riista.fi/</a> for Finland game bag)	FACE, 2016
	animals for fur or skin (e.g. snake, fox, mink, etc.)	fur, skin, accessories, trophy	Every year, foreign hunters export the carcasses (usually just heads and hides) of 665 wild lions from Africa: on average nearly 2 lions each day	-	e.g. in Zimbabwe, hunters exported 49 lion trophies in 2013 alone.	Cronin, 2015
	game mammals (e.g. deers, etc.)	food, trophy	-	-	Data available at country level: specific national datasets are reported in the Artemis-FACE database (e.g. <a href="https://riistaweb.riista.fi/">https://riistaweb.riista.fi/</a> for Finland game bag)	FACE, 2016

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Terrestrial Vertebrates	game birds (e.g. Partridge, etc.)	food, trophy	-	-	Data available at country level: specific national datasets are reported in the Artemis-FACE database (e.g. <a href="https://riistaweb.riista.fi/">https://riistaweb.riista.fi/</a> for Finland game bag)	FACE, 2016
	game birds (e.g. Partridge, etc.)	food, trophy	-	Average annual shooting bag for birds (2005): about 102 million tonnes	Data of annual shooting bags are reported for 27 European countries	Hirschfeld et al., 2005
	Edible game species (both mammals and birds)	food	-	-	Study at EU-scale (17 countries): 97 game species hunted in Europe.	Schulp et al., 2014
Invertebrates	marine and freshwater crustaceans	food	Captures in 2012: about 6.3 million tonnes	-	-	FAO, 2014
	marine and freshwater molluscs	food, accessories	Captures in 2012: about 7 million tonnes	-	-	FAO, 2014
	other aquatic invertebrates (e.g. sea-urchins, etc.)	food, accessories	Captures in 2012: about 110 Ktonnes	-	-	FAO, 2014
	terrestrial insects	food; feed; dyes; ornaments; accessories; used for their products (honey, wax, etc.); source of medical substances	FAO registered ca. 1900 edible insect species and estimates about 2 billion consumers worldwide in 2005, mainly in the developing (tropical) countries. Providing definitive figures is difficult due to the use of common names and more than one vernacular name in many cultures.	41 species consumed in 11 European countries	-	Van Huis et al., 2013

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Invertebrates	terrestrial insects	food; feed; dyes; ornaments; accessories; used for their products (honey, wax, etc.); source of medical substances	-	-	About 96 insect species are eaten in Central Africa. About 9.5 billion caterpillars are harvested annually in southern Africa. In Asia, between 150 and 200 species of edible insects are consumed in Southeast Asia, 81 in Thailand, 57 in South Asia, 39 in Papua New Guinea and the Pacific Islands. In Brazil a total of 135 edible insect species are consumed. Up to 27 caterpillar species in Mexico.	Durst et al., 2010
	terrestrial insects	food; feed; dyes; ornaments; accessories; used for their products (honey, wax, etc.); source of medical substances	2037 species (2015)	-	-	Jongema, 2015
	terrestrial insects	food; feed; dyes; ornaments; accessories; used for their products (honey, wax, etc.); source of medical substances	-	-	over 470 species of insects are eaten in Africa	Kelemu et al., 2015
Terrestrial plants	wood (roundwood, pulpwood, sawnwood, etc.)	A broad variety of industrial and domestic uses: as a source of energy (fuel), production of paper and paperboard, panels, furnitures, sports equipments, building and construction, etc.	<i>Data on wood production and trade are available; However, it is not specified if wood products come from natural forests</i>	-	-	FAO, 2016a; b

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Terrestrial plants	wood (roundwood, pulpwood, sawnwood, etc.)	A broad variety of industrial and domestic uses: as a source of energy (fuel), production of paper and paperboard, panels, furnitures, sports equipments, building and construction, etc.	About 93% of global forest area is natural forest (i.e. primary forest and other naturally regenerated forests), corresponding to 3.7 billion ha in 2015	-	Data on area and volume of natural forests by country across the world are reported	FAO, 2015
	wood (roundwood, pulpwood, sawnwood, etc.)	A broad variety of industrial and domestic uses: as a source of energy (fuel), production of paper and paperboard, panels, furnitures, sports equipments, building and construction, etc.	-	Primary forests cover about 277 million ha (2015). The area of forests available for wood supply in 2015 amounted to 166 million ha. <i>However, it is not specified if this area completely corresponds with natural forests</i>	-	Forest Europe, 2015
	(hardwood)	mainly used as a energy source	-	-	Total biomass appropriation, consumption and net trade in 11 world regions in 2010 are reported	Bais et al., 2015
	Herbaceous plants	Food (subsistence level in rural communities); medical use; veterinary and traditional uses such as ritual and ornaments	<i>wild plants tend to receive little recognition from the development community for: (i) lack of information about the extent of their use and importance in rural economies; (ii) lack of information, especially statistics, concerning the</i>	-	-	-



taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Terrestrial plants			<i>economic value of wild plants;(iii) lack of information and reliable methods for measuring their contribution to farm households and the rural economy;(iv) lack of world markets, except for a small number of products;(v) irregular supply of wild plant products;(vi) lack of quality standards;(vii) lack of storage and processing technology for many of the products;(viii) availability of substitutes;(ix) bias in favour of large-scale agriculture</i>			
	Herbaceous plants	Used as aromatics and for medical purposes	<i>Most countries have little or no regulations controlling the collection of material from the wild. Thus, it is difficult to provide accurate global data on the volume of wild harvested medicinal plants, also because it is very difficult to distinguish between wild and cultivated material. Although data can be indicated for some specific cases, there is very little actual global data on the volume of wild harvested medicinal plants</i>	-	Although the major part of wild harvested material is sourced from developing countries, a surprisingly high amount is also gathered in developed countries. For example, in France, more than 500 species were wild harvested during 1988-89, including those used homeopathically, i.e. in minute quantities.	Kuipers, 1997
	Herbaceous plants	Medical use	More than 253 different plant species.	-	-	Censkowsky et al., 2007

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Terrestrial plants	Herbaceous plants	Used as aromatics and for medical purposes	-	-	<i>Unfortunately, there are no exact figures available of the total production of herbs within a country including plant material collected for own purposes, purchased for the domestic market, or destined for export either as raw material or processed commodity.</i>	Lange, 2002
	Herbaceous plants	Medical use	-	-	e.g. About 500-600 tonnes of Egyptian herbane are collected from wild sources annually in Egypt and are exported to Germany. 800 tonnes of Serpent Wood are collected from wild sources in the western coast of Africa, mainly in Zaire, Mozambique and Rwanda, from where it is exported to Italy and West Germany.	Iqbal, 1993
	Herbaceous plants	Medical use	<i>Information on trade in wild-harvested medicinal plants is scarcely collected or published at a national level, since production and consumption are generally at subsistence level. As a consequence, the economic importance of these activities is largely under-estimated.</i>		-	-
Seaweed and aquatic plants	Seaweed and aquatic plants	food; cosmetics and personal care; fertilisers; industrial gums and chemicals	Captures in 2012: about 1.1 million tonnes	-	-	FAO, 2014

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Wild mushrooms	Fungi	food	-	Study at EU-scale. Quantities of wild food collected are very rarely inventoried	-	Schulp et al., 2014
	Fungi	food; medial use	<i>For many countries there is little or no published information on wild edible fungi; information on trade and export is often incomplete, data are missing for important exporting countries</i>		-	Boa, 2004
	yeast	food and food supplement	-	-	-	<i>to be improved</i>
Aquatic animal products	Pearls, mother-of-pearl, shells	accessories (e.g. Jewelry, etc.)	-	-	-	<i>to be improved</i>
	corals	accessories (e.g. Jewelry, etc.)	70 nations imported a total of 19,262t (or 34,600,000 pieces) from 120 exporting nations over the period 1982-1997	European imports of coral in 1997: 14.9 tonnes	-	Green & Shirley, 1999
	sponges	cosmetics, bath, or industrial purposes; extraction of bioactive substances for medical purposes	-	-	-	<i>to be improved</i>
Terrestrial animal products	honey	food, medicine	-	-	In Bangladesh 220 tonnes of forest honey are produced and annually; about 350 tonnes of honey are collected from forests in India; in Vietnam, about 200 to 400 tonnes of forest honey are marketed annually;	Iqbal, 1993

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
Terrestrial animal products	beeswax	food supplement; personal care; medical purposes; accessories (e.g. Candles, etc.)	-	-	In Bangladesh 55 tonnes of wax are produced annually; about 28 tonnes of wax are collected from forests in India	Iqbal, 1993
	lac (i.e. resinous secretion from insect <i>Laccifera lacca</i> )	edible dye; variety of uses in plastics, electrical goods, adhesives, leather, wood finishing, lacquer work, printing, polish and varnish, ink, etc.	-	-	India and Thailand are the major lac producers, producing on the average, 17 Ktonnes annually	Iqbal, 1993
	cochineal (from insect <i>Dactylopius coccus</i> )	colours / dye carmine	Total world production of cochineal, though fluctuating, is estimated at 150 to 180 tonnes per year	-	-	Iqbal, 1993
	insect galls	dyes; medical purpose	<i>not found</i>	-	-	Iqbal, 1993
	leather	furniture; sports equipment (e.g. Gloves, etc.); clothing and other accessories (e.g. belts, bags, etc.)	-	-	-	<i>to be improved</i>
	ivory ( <b>ILLEGAL TRADE</b> )	trophy, accessories	Ivory estimated to weigh more than 23 metric tons (i.e. a figure that represents 2500 elephants) was seized in the 13 largest seizures of illegal ivory in 2011	-	-	WWF, 2016a
	Horns ( <b>ILLEGAL TRADE</b> )	trophy, accessories	rhino poaching to fuel the demand for the illegal rhino horn trade reached an all-time high in 2011, with 448 rhinos poached in South Africa alone.	-	-	WWF, 2016b

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
	musk	cosmetics (component of fragrances and as a fixative in perfumes); medical purposes	-	-	-	<i>to be improved</i>
Terrestrial plant products	fruit, berries, spices, and other terrestrial plant products as wild food (defined as provisioning ecosystem service)	food	-	Study at EU-scale (17 countries): 592 edible species were identified	e.g. Production of berries reported for Swedish forest in 1979: 250 Ktonnes blubberies, 155 Ktonnes lingonberries, 79-89 Ktonnes cloudberries	Schulp et al., 2014
	(certified) organic wild collected products, including roots, leaves, nuts and fruits	food, cosmetics and personal care, natural remedies / medicine, natural textiles and other industrial uses	In 2005, approximately 440 different organic wild collected products (totalling almost 250 Ktonnes) have been identified.	About 33 Ktonnes of organic wild products (including berries and fruits) are collected in Europe (28 countries)	In this report, wild collected products and their harvested quantities are reported per country in each continent	Censkowsky et al., 2007
	rubber	waterproof and elastic material, especially in automotive industry; playground equipment, shoes, mats, flooring, healthcare supplies, household supplies, balls, toys, etc.	-	-	-	<i>to be improved</i>
	resins	production of varnishes, adhesives and food glazing agents; basis for the synthesis of other organic compounds; component of incense and perfume	-	-	-	<i>to be improved</i>

taxonomic level category	Commercial group	Use	Estimated quantities (available and harvested/consumed)			Ref. on availability in the wild, use, consumption
			World	Europe	specific region/country	
	cork	Building insulation; floor and wall tiles; sports equipment; wine bottle taps; other industrial and domestic applications	-	-	-	<i>to be improved</i>
	wax	food coatings; car and furniture polish; floss coating, surfboard wax and other domestic and industrial uses	-	-	-	<i>to be improved</i>
	fibers	food and supplements	-	-	-	<i>to be improved</i>
	tree oil	personal care; medical purposes; component in perfumes	-	-	-	<i>to be improved</i>
	wild fodder, forage		-	-	-	<i>to be improved</i>
<b>Soil</b>	soil organic matter		-	-	-	<i>to be improved</i>
<b>(fossil origin)</b>	peat		-	-	-	<i>to be improved</i>

## References for Table B1

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**Table B2.** Natural Occurring Biotic Resources (NOBR) database. Complete references are reported below the table.

Legend:

**Bold** = species with high commercial value

\* = species both harvested in the wild and cultivated in plantations

(§) = estimate based on expert judgment, uncertainty high

n.a. = not available so far

BMSY = Biomass Maximum Sustainable Yield

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	River eels	European anguilla	<b><i>Anguilla anguilla</i></b> *	Ocean & rivers: Atlantic coast from Scandinavia to Morocco; Baltic, Black and Mediterranean Seas	CR	minimum population doubling time	4.5 - 14 years	Fishbase, 2016; DAISIE, 2016	European part of Russia and Israel
	Salmons	Atlantic salmon	<b><i>Salmo salar</i></b> *	North Atlantic Ocean: temperate and arctic zones in northern hemisphere	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
		Pink salmon	<b><i>Oncorhynchus gorbuscha</i></b> *	Arctic and Pacific drainages from Canada to California, USA; Asia from North Korea to Arctic Russia	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
	Trouts	Brown trout or Sea trout	<b><i>Salmo trutta</i></b> *	Europe and Asia: Atlantic, North, White and Baltic Sea basins	LC	minimum population doubling time	Less than 15 months	Fishbase, 2016	Italy - Mediterranean Sea
	Smelts	European smelt	<b><i>Osmerus eperlanus</i></b> *	North Atlantic, the Gironde estuary is the southern limit of his distribution. Landlocked populations in lakes of coastal areas of North, Baltic, White and Barents Sea.	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
	Sturgeons	Atlantic sturgeon (caviar)	<b><i>Acipenser oxyrinchus</i></b> *	Distributed along the Atlantic coast of the USA, in Baltic Sea + aquaculture reintroduction.	NT	minimum population doubling time	more than 14 years	Fishbase, 2016	-



Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Paddle fishes	Mississippi paddlefish	<i>Polyodon spathula</i>	North America: Mississippi River system, including the Missouri River into Montana, the Ohio River, and their major tributaries	VU	minimum population doubling time	4.5 - 14 years	Fishbase, 2016	Europe
	Carps	Common carp	<i>Cyprinus carpio*</i>	Europe to Asia: Black, Caspian and Aral Sea basins.	VU	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	Europe
	Barbels	Barbel fish	<i>Barbus barbus*</i>	Europe	LC	minimum population doubling time	4.5 - 14 years	Fishbase, 2016	Italy
	Tilapias and other cichlids	Mozambique tilapia	<i>Oreochromis mossambicus</i>	Central african basins	NT	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	European part of Russia and East-Europe
		Nilotique tilapia	<i>Oreochromis niloticus*</i>	Africa + widely introduced on global scale for aquaculture	Not Evaluated	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	Ukraine
	Cods, hakes, haddocks	Arctic cod (or polar cod)	<i>Arctogadus glacialis</i>	Arctic and Northeast Atlantic	Not Evaluated	recovery time of BMSY	5-7 years to recover from overfished to fished above MSY	BalticSea 2020, 2016	-
		(Eastern) Baltic Cod	<i>Gadus morhua*</i>	North Atlantic and Arctic	VU	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
					stock recovery time	generally no, or slow, recovery 5-15 years after [stock] depletion	Myers and Worm, 2005	-	

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Flounders	European flounder	<i>Platichthys flesus</i>	Eastern Atlantic. Introduced into the USA and Canada accidentally through transport in ballast water. Iran.	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	European part of Russia
	Halibuts	Atlantic halibut	<i>Hippoglossus hippoglossus*</i>	Eastern Atlantic: Bay of Biscay to Spitsbergen, Barents Sea, Iceland and eastern Greenland. Western Atlantic: southwestern Greenland and Labrador in Canada to Virginia in USA	EN	minimum population doubling time	more than 14 years	Fishbase, 2016	-
						stock recovery time	often rapid recovery, i.e. 5-10 years after [stock] depletion	Myers and Worm, 2005	-
	Herrings	Atlantic herring	<i>Clupea harengus</i>	North Atlantic	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
	Sardines	European pilchard or Sardine	<i>Sardina pilchardus</i>	Northeast Atlantic; Mediterranean (common in the western part and in Adriatic Sea, rare in the eastern part), Sea of Marmara and Black Sea	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
	Anchovies	European anchovy	<i>Engraulis encrasicolus</i>	Eastern Atlantic; Mediterranean, Black and Azov seas	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
	Shads	Alosina	<i>Hemitaurichthys polylepis</i>	Northeast Atlantic: Iceland (rare) and North Sea, southward to Bay de Gorée, Senegal. Mediterranean (common in the western part and in Adriatic Sea, rare in the eastern part), Sea of Marmara and Black Sea.	LC	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
Sharks	Smalltooth sawfish	<i>Pristis pectinata</i>	Circumglobal: Western Atlantic; Caribbean Sea; eastern Atlantic; possibly in the Mediterranean Sea; Indo-West Pacific	CR	minimum population doubling time	4.5 - 14 years	Fishbase, 2016	-	

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Sharks	Smalltooth sawfish	<i>Pristis pectinata</i>	Circumglobal: Western Atlantic; Caribbean Sea; eastern Atlantic; possibly in the Mediterranean Sea; Indo-West Pacific	CR	population doubling time	5.4 - 8.5 years	Simpfendorfer, 2000	-
	Sharks	Large-tooth sawfish	<i>Pristis pristis</i>	North Atlantic; Eastern and Western Central Pacific; Indo-China; South America	CR	minimum population doubling time	4.5 - 14 years	Fishbase, 2016	-
						population doubling time	10.3 - 13.5 years	Simpfendorfer, 2000	-
	Sharks	Porbeagle	<i>Lamna nasus</i>	Circumglobal, amphitemperature with centers of distribution in the North Atlantic and temperate water of the southern hemisphere; not in equatorial seas	VU	minimum population doubling time	more than 14 years	Fishbase, 2016	-
						Population recovery	30 - 60 years	Camhi, et al. 2008	-
						recovery time of BMSY	70–100 years	Lotze et al., 2011	-
	Sharks	Leopard shark	<i>Triakis semifasciata</i>	Eastern Pacific: Oregon to the Gulf of California.	LC	population doubling time	14.9-25.7 years	Camhi et al., 2008	-
	Sharks	Shortfin Mako shark	<i>Isurus oxyrinchus</i>	Offshore temperate and tropical seas worldwide.	VU	population doubling time	9.4-15.9 years	Camhi et al., 2008	-
Sharks	Great White shark	<i>Carcharodon carcharias</i>	Northeast USA and California, South Africa, Japan, Oceania, Chile, and the Mediterranean.	VU	population doubling time	12.2-20.1 years	Camhi et al., 2008	-	
Sharks	Gray smooth hound	<i>Mustelus californicus</i>	continental shelves of the subtropical eastern Pacific, from northern California to the Gulf of California	LC	population doubling time	3.3-5.8 years	Camhi et al., 2008	-	
Sharks	Bonnethead	<i>Sphyrna tiburo</i>	Western Atlantic: North Carolina to southern Brazil, including Cuba and the Bahamas. Caribbean and Gulf of Mexico; rare in Bermuda. Eastern Pacific: southern California to Ecuador.	LC	population doubling time	4.2-7.5 years	Camhi et al., 2008	-	

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Sharks	Atlantic sharpnose	<i>Rhizoprionodon terraenovae</i>	Western Atlantic: New Brunswick, Canada to the Gulf of Mexico. Southwest Atlantic: coasts of Brazil.	LC	population doubling time	5.0-9.2 years	Camhi et al., 2008	-
	Sharks	Blue shark	<i>Prionace glauca</i>	Circumglobal in temperate and tropical waters. Western, Central and Eastern Atlantic: Canada to Argentina, Norway to South Africa, Mediterranean. Indo-West Pacific: East Africa to Indonesia, Japan, Australia, New Caledonia and New Zealand. Eastern Pacific: Gulf of Alaska to Chile. Highly migratory species	NT	population doubling time	7.7-13.2 years	Camhi et al., 2008	-
	Sharks	<b>Bull shark</b>	<i>Carcharhinus leucas</i>	Cosmopolitan in tropical and subtropical waters: widespread in warm oceans, rivers and lakes. Western to Eastern Atlantic. Indo-Pacific: Kenya and South Africa to India, Vietnam to Australia; southern Baja California, Mexico to Ecuador and possibly occurring in Peru. In freshwater it can be found in rivers of West Africa from Gambia River to Ogowe River.	NT	population doubling time	17.1-30.8 years	Camhi et al., 2008	-
	Sharks	<b>Common thresher</b>	<i>Alopias vulpinus</i>	Cosmopolitan in temperate and tropical seas. Population reduced in the US Atlantic waters; lower risk/conservation dependent in US Pacific waters; data deficient in the rest of Atlantic and rest of Pacific	VU	population doubling time	6.7-11.6 years	Camhi et al., 2008	-

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Sharks	Spiny dogfish	<i>Squalus acanthias</i>	Western Atlantic: Greenland to Argentina; Eastern Atlantic: Iceland and the Barents Sea to Western Sahara and the Canary Islands; Angola to South Africa. Indo-Pacific; the Mediterranean and Black Seas.	VU	population doubling time	28.9-46.2 years	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Yellowfin tuna	<i>Thunnus albacares</i>	Worldwide in tropical and subtropical seas, but absent from the Mediterranean Sea	NT	minimum population doubling time	1.4 - 4.4 years	Fishbase, 2016	-
						population doubling time	2.5-4.2 years	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Atlantic bluefin tuna	<i>Thunnus thynnus</i> *	Western and Eastern Atlantic, including the Mediterranean and the southern Black Sea	EN	minimum population doubling time	4.5 - 14 years	Fishbase, 2016	-
	Tunas, bonitos, billfishes	Northern bluefin tuna	<i>Thunnus orientalis</i> *	North Pacific: Gulf of Alaska to southern California and Baja California and from Sakhalin Island in the southern Sea of Okhotsk south to northern Philippines. The species occurs mainly in the northern Pacific but ventures into New Zealand waters for at least three months during spring and early summer.	VU	population doubling time	4.6-6.5 years	Camhi et al., 2008	-
Tunas, bonitos, billfishes	Southern bluefin tuna	<i>Thunnus maccoyii</i> *	Atlantic, Indian and Pacific: temperate and cold seas, mainly between 30°S and 50°S, to nearly 60°S. During spawning, large fish migrate to tropical seas, off the west coast of Australia, up to 10°S. Highly migratory species	CR	population doubling time	5.2-7.5 years	Camhi et al., 2008	-	

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Tunas, bonitos, billfishes	Skipjack tuna	<i>Katsuwonus pelamis</i>	Cosmopolitan in tropical and warm-temperate waters. Not found in the Black Sea. Highly migratory species	LC	population doubling time	1.3-2.9 years	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Bigeye tuna	<i>Thunnus obesus</i>	Worldwide in tropical and subtropical seas, but absent from the Mediterranean Sea. Highly migratory species	NT	population doubling time	2.5-4.9 years	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Albacore tuna	<i>Thunnus alalunga</i>	Cosmopolitan in tropical and temperate waters of all oceans including the Mediterranean Sea but not at the surface between 10°N and 10°S.	NT	population doubling time	4.2-6.2	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Sailfish	<i>Istiophorus platypterus</i>	Indo-Pacific: tropical and temperate waters approximately 45°- 50°N and 40°-35°S in the western Pacific, 35°N and 35°S in the eastern Pacific; 45°S in western Indian Ocean and 35°S in eastern Indian Ocean. Entered Mediterranean Sea	LC	population doubling time	2.9-4.4 years	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Striped marlin	<i>Kajikia audax</i>	Indo-Pacific: tropical, subtropical and temperate waters. Highly migratory species. Horseshoe-shaped pattern from the northwest Pacific through the eastern Pacific to the southwest Pacific. Indian Ocean, fish are more densely distributed in equatorial regions with higher concentrations off eastern Africa, in the western Arabian Sea, the Bay of Bengal and off northwestern Australia.	NT	population doubling time	3.7-5.6 years	Camhi et al., 2008	-

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Tunas, bonitos, billfishes	Blue marlin	<i>Makaira nigricans</i>	Atlantic Ocean: in tropical and temperate waters.	VU	population doubling time	3.7-5.5 years	Camhi et al., 2008	-
	Tunas, bonitos, billfishes	Swordfish	<i>Xiphias gladius</i>	Atlantic, Indian and Pacific: tropical and temperate and sometimes cold waters, including the Mediterranean Sea, the Sea of Marmara, the Black Sea, and the Sea of Azov.	LC	population doubling time	4.8-6.9 years	Camhi et al., 2008	-
	Crocodiles and alligators	American crocodile	<i>Crocodylus acutus</i>	USA: Atlantic, from Caribbean to Mexico	VU	population doubling time	Less than 20 years	U.S. Fish and Wildlife Service – SE region 2016	-
	Crocodiles and alligators	Nile crocodile	<i>Crocodylus niloticus</i> *	Africa + widely introduced for aquaculture on global scale	LC	population doubling time	Less than 23 years	GBIF, 2016	Spain and Israel
	Crocodiles and alligators	Alligator	<i>Alligator mississippiensis</i> *	Native USA; Southeastern part, from Texas to Carolina and Florida	LC	population doubling time	Less than 50 years	Catalogue of life, 2016	Germany and Canary
	Amphibian	Bullfrog	<i>Lithobates catesbeianus</i> *	Global	LC	population doubling time	more than 3 years (§)	Amphibian Survival Alliance, 2016	Europe
	Sea turtles	-	-	-	-	n.a.	n.a.-	-	-
	Whales, seals	Grey whales and Blue whales	(e.g. <i>Eschrichtius robustus</i> and <i>Balaenoptera musculus</i> )-	-	LC/EN-	population size recovery time	Gray whales: 18,3 years; Blue whales: 16,2 years	Brown, J.H., 2000	-
	Whales, seals	Balaenoptera (Blue whale)	<i>Balaenoptera musculus</i>	North Atlantic, North Pacific and Southern Hemisphere and vicinity to Northern Indian Ocean.	EN	population doubling time	31 years	IUCN, 2016	-

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Aquatic vertebrates	Whales, seals	Bowhead whale	<i>Balena mysticetus</i>	Arctic and subarctic regions	LC	population doubling time	52 years	IUCN, 2016	-
	Whales, seals	Killer whales	<i>Orcinus orca</i> *	USA North Pacific	DD	population doubling time	population almost doubled in size from about 125 in 1973 to 213 animals by 1996 (23 years)	Olesiuk, et al., 2005	-
	Eared seals, hair seals, walruses	-	-	-	-	n.a.	n.a.	-	-
	Sea otters	Common sea otter	<i>Enhydra lutris</i>	Russia, Alaska, British Columbia, Washington, California, Oregon	EN	population size recovery time	About 50 years to recover the population in Alaska from human exploitation	Lotze et al., 2011	-
	Marine species	General data on finfish & invertebrates	-	-	-	recovery time of BMSY	Documented <b>finfish and invertebrate</b> stock recovery times ranged from 3 to 30 years, with demersal recoveries generally being longer than pelagic ones.	Lotze et al., 2011	-



Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Terrestrial Vertebrates	Fur/skin terrestrial animals	Python	<i>Python reticolatus</i> *	Asia	Not Evaluated	n.a.	n.a.	The Animal Diversity Web, 2016	-
	Fur/skin terrestrial animals	Bell's hinge-back tortoise	<i>Kinixys belliana</i> *	sub-Saharan Africa such as in Sudan, Tanzania, DR Congo and down to southern Africa	LC	population doubling time	15 years	World Chelonian Trust, 2016	-
	Fur/skin terrestrial animals	Eurasian red squirrel	<i>Sciurus vulgaris</i> *	Globally	LC	population size recovery time	1 year	IUCN, 2016	-
	Fur/skin terrestrial animals	European Beaver	<i>Castor fiber</i> *	Globally	LC	population size recovery time	20 years (\$)	Wilson and Reeder, 2005	-
	Fur/skin terrestrial animals	Muskrat	<i>Ondatra zibethicus</i> *	over most of Canada and the United States and a small part of northern Mexico. Introduced in Europe at the beginning of the 20th century	LC	population size doubling time	10 years (\$)	COSEWIC., 2016	[invasive species in north-western Europe]
	Fur/skin terrestrial animals	Red fox	<i>Vulpes vulpes</i> *	distributed across the entire northern hemisphere from the Arctic Circle to southern North America, Europe, North Africa, the Asiatic steppes, India, and Japan. The European subspecies was introduced into the eastern United States	LC	population size doubling time	10years (\$)	Grzimek, 1975; Wildlife online, 2016	Corse, Greece, Sardinia and Cyprus
	Fur/skin terrestrial animals	Raccoon	<i>Procyon lotor</i> *	Distributed in North America, and introductions in the mid-20th century, the raccoon is now distributed in several European and Asian countries.	LC	n.a.	n.a.	[Other ref. for distribution: The Animal Diversity Web, 2016]	Europe
	Fur/skin terrestrial animals	Stoat	<i>Mustela erminea</i> *	Arctic, China, Europe, Indian subcontinent, North America, Russia	LC	population size doubling time	10years (\$)	The Animal Diversity Web, 2016	-

Taxa	Commercial group	Common name	Species	Distribution on global scale (mainly based on information from IUCN, 2016)	Vulnerability based on IUCN criteria	Renewability indicator type	Renewability & regeneration time (after human disturbance)	Other ref. for distribution and for the indicator	Where the species is alien (DAISIE, 2016)
Terrestrial vertebrates	Fur/skin terrestrial animals	European mink	<i>Mustela vison</i> *	Native Range: North America, excluding the north of the Arctic Circle, Mexico. Known Introduced Range: Europe, the former Soviet Union, Argentina and Chile. Possibly Japan and other Asian countries	LC	population size doubling time	10years (§)	DAISIE, 2016	-
	Fur/skin terrestrial animals	Pine marten	<i>Martes martes</i>	West and central Palaearctic, across most of Europe, Asia Minor, northern Iran, the Caucasus, and in westernmost parts of Asian Russia (western Siberia).	LC	population doubling time	about 7 years (extrapolated datum)	Storch et al., 1990	Sardinia and Balears
	Fur/skin terrestrial animals	Wolf	<i>Canis lupus</i> *	Canada, Alaska, northern USA, Europe, and Asia.	LC	population doubling time	4.7 years	Mech et al., 2016	-
	Fur/skin terrestrial animals	Snowshoe hare	<i>Lepus americanus</i> *	Boreal and mixed deciduous forests of North America.	LC	population cycle	8-11 years	Env. and Natural Resources, 2016	UK and Italy
	Fur/skin terrestrial animals	Eastern Cottontail	<i>Sylvilagus floridanus</i> *	widely distributed throughout the USA, Central America, southern Canada, northern part of South America	LC	population cycle	8-11 years	DAISIE, 2016	Europe
	Game mammals	deers	<i>Odocoileus virginianus</i> *, <i>bezoharticus</i> *	North america	LC	population doubling time	When predation and other losses are low and food resources are plentiful, deer populations can double every 2 to 3 years.	Riehlman et al., 2016	North and East Europe

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Terrestrial vertebrates	Game mammals	Red deer	<i>Cervus elaphus</i> *	Europe and North Africa introduced in Australia, New Zealand, Argentina and Chile.	LC	population doubling time	14 years	Langvatn and Loison, 1999	European part of Russia, UK, Italy, Ireland, Poland, Sweden, Austria
	Game mammals	European bison	<i>Bison bonasus</i> *	Europe	VU	population doubling time	After releasing from hunting pressure, over 10 years population density doubled	Coulson et al., 2004	-
	Game birds	Ruffed grouse	<i>Bonasa umbellus</i> *	Canada; United States	LC	population cycle	Population numbers can fluctuate widely. Over most of their range, the population highs and lows are cyclical, every 9 to 11 years.	DeStefano et al., 2001	-

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Terr. Vert.	Game birds	Canada goose	<i>Branta canadensis</i>	America, Eurasia and New Zeland	LC	population doubling time	When goslings reached breeding age, the population increase doubling every 3 years	U.S. Fish and Wildlife Service, 2005	Europe
Freshwater and terrestrial invertebrates	Crustacean	Giant mud crab	<i>Scylla serrata</i> *	Indo-Pacific. It is found from South Africa, around the coast of the Indian Ocean to the Malay Archipelago, as well as from southern Japan to south-eastern Australia, and as far east as Fiji and Samoa. Introduced to Hawaii and Florida.	LC	population doubling time	1.4 - 4.4 years	U.S. Fish and Wildlife Service, 2005	-
	Crustacean	Redclaw Crayfish	<i>Cherax quadricarinatus</i> *	Native to freshwater creeks and water bodies in tropical Queensland, the Northern Territory and south-eastern Papua New Guinea. Widely translocated around the world	LC	population recovery time	4 years	IUCN, 2016	Spain [invasive species]
	Amphipoda, Decapoda and Isopoda	(general data)	-	-	-	population size recovery time	reported recovery times for macro-crustaceans were scarce and ranged between 4 month and 5 years, with an overall mean of 1.7 years	Gergs et al., 2016	-

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Freshwater and terrestrial invertebrates	Insects - Hymenoptera	Honey bee	<i>Apis mellifera</i> *	Africa; Australia; Caribbean; Europe & Northern Asia (excluding China); Middle America; North America; Oceania; South America; Southern Asia	DD	n.a.	n.a.	-	-
	Insects – Coleoptera	(general data)	-	-	there are VU species	population size recovery time	2.2 years (average for aquatic spp)	Gergs et al., 2016	-
	Freshwater Molluscs	(general data)	-	-	-	population size recovery time	between 1 week and 8 years, with an overall mean recovery time of 2.5 years. Longer recovery times of 12 and 26 months were reported for coal-ash exposure and crude-oil spill, respectively	Gergs et al., 2016	-
	Freshwater Oligochaeta	(general data)	-	-	-	population size recovery time	mean of 2.2 years. Recovery within 6 months was found in case studies of small-scale physical disturbance.	Gergs et al., 2016	-
Terr. Plant	Hardwood	(general data)	-	-	-	rotation period	Based on a weighed assessment of economic and	Centeno, 2016	-

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Terrestrial plants							silvicultural considerations, a rotation of 25 years to 40 years may at present be considered as the optimum cycle to achieve a viable balance between financial returns and the production of market quality timber		
	Hardwood	Date palm	<i>Phoenix dactylifera</i> *	Native country unknown, possibly the oases of the Sahara, widely cultivated in the Middle East and dry regions of Africa, introduced into California, Queensland, Australia and elsewhere.	Not Evaluated	rotation period	(short)	Zaid, A., 2002; EOL, 2016	Spain and South Europe
	Hardwood	Teak	<i>Tectona grandis</i> *	Native to India, Indonesia, Myanmar, northern Thailand, and northwestern Laos.	Not Evaluated	rotation period	Although teak was once managed on rotations of 80 to 100 years, current rotation lengths have been shortened to 20 or 25 years for commercial wood production	Ladrach, 2009	-

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Terrestrial plants	Hardwood	Oaks	-	-	-	rotation period	60-80 years	DeStefano et al., 2001	-
						rotation period	80-120 years	Dey et al., 1996	-
						regeneration period	approximately the first 20 years after final harvest. This period coincides with Oliver and Larson's (1990) stand initiation and stem exclusion stages of stand development.	Dey et al., 1996	-
	Hardwood	White oak	<i>Quercus alba</i> *	Native range spans the entire eastern half of the USA from the Atlantic seaboard in the east, to the Missouri River in the west, from the Gulf Coast to the Great Lakes, extending north into southern Ontario and Quebec	LC	regeneration period	from 10 to 20 years or longer.	U.S. Forest Service Northeastern Area, 2016	-
	Hardwood	Cork oak	<i>Quercus suber</i> *	Native to southwest Europe and northwest Africa.	Not Evaluated	rotation period	10 - 12 year	Plants for a Future, 2016	-
	Hardwood	Sycamore spp.	(e.g. <i>Acer pseudoplatanus</i> )	-	-	rotation period	60-80 years	Spiecker and Hein, 2009	-
	Hardwood	Sycamore maple	<i>Acer pseudoplatanus</i>	Native to central and eastern Europe and western Asia. Introduced to suitable locations outside Europe.	Not Evaluated	rotation period	it is quite normal to have sycamore rotations equal to 100 years.	Spiecker and Hein, 2009	North Europe
Hardwood	Poplar	<i>Populus</i>	Europe, Mediterranean incl. N.	<i>P nigra</i> :	rotation	80 to 120-year	Klimo and	-	

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Terrestrial plants			<i>spp.(alba,nigra,tremula)*</i>	Africa and Middle East, temperate Asia, India, Pakistan, Sri Lanka, and North America.	LC	period		Herbert, 2001	
	Hardwood	Maple	<i>Acer platanoides*</i>	Europe. In North America is invasive species.	Not Evaluated	rotation period	100-120 years	Spiecker and Hein, 2009	North Europe and Spain
	Hardwood	wild service tree	<i>Sorbus torminalis</i>	Europe	Not Evaluated	rotation period	120-150 years	Spiecker and Hein, 2009	-
	Hardwood	Silver maple	<i>Acer saccharinum*</i>	North America	Not Evaluated	regeneration time	The minimum seed bearing age for silver maple is 11 years. Large seed crops are produced annually	U.S. Forest Service, 2016a	Europe
	Hardwood	Red maple	<i>Acer rubrum</i>	eastern North America	Not Evaluated	rotation period	from 50 to 110 years	WDNR, 2016	-
	Hardwood	Wild cherry	<i>Prunus avium*</i>	Native to Europe, Anatolia, Maghreb, and western Asia, from the British Isles south to Morocco and Tunisia, north to the Trondheimsfjord region in Norway and east to the Caucasus and northern Iran, with a small isolated population in the western Himalaya.	Not Evaluated	rotation period	60-80 years	Spiecker and Hein, 2009	Bulgaria, Estonia, Balears.
	Softwood	Birch	<i>Betula spp. (e.g. Betula pendula)</i>	<i>Betula pendula</i> : native distribution is more or less continuous from Europe to the Far East and into northwest Africa (Morocco). Extensively cultivated throughout its temperate range;	LC/CR	rotation period	70 to 140 years	U.S. Forest Service, 1975	-
					regeneration period	up to 30 years	U.S. Forest Service, 1975	-	



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Terrestrial plants	Softwood	Pines	<i>Pinus spp.</i>	One species ( <i>Sumatran pine</i> ) crosses the equator in Sumatra. Various species are distributed across the North Hemisphere	LC/EN	rotation period (after natural fires)	The natural disturbance regime originally consisted of catastrophic crown or intense ground fires with rotation periods dependent on the dominant species and site: -50 years in jack pine, -100 years in spruce-aspen-birch, and 150-200 years in red and white pine	Frelich and Reich, 1995	-
	Softwood	White pine	<i>Pinus strobus</i> *	western USA and Canada	Not Evaluated	rotation period	The rotation length depends on the productivity of the site and forest management goals. It may be as short as 90 years or as long as 150 years.	Martin and Lorimer, 1997	-
	Softwood	black locust	<i>Robinia pseudoacacia</i>	Native to the southeastern United States, but it has been widely planted and naturalized elsewhere in temperate North America, Europe, Southern Africa and Asia	LC	rotation period	5 years	El Bassam, 1998	Europe [invasive species]

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Terrestrial plants	Softwood	Lebanon cedar	<i>Cedrus libani</i>	Turkey, Syria and Lebanon, Eastern Mediterranean region.	VU	n.a.	n.a.	-	UK, Ireland, France, Corse, Portugal, Croatia.
	Softwood	Spruce	<i>Picea spp.</i>	-	-	n.a.	n.a.	-	-
	Softwood	Fir	<i>Abies spp.</i>	-	-	n.a.	n.a.	-	-
	Softwood	Ash	<i>Fraxinus spp.</i>	-	-	rotation period	60-80 years	Spiecker and Hein, 2009	-
	Softwood	Beech	<i>Fagus spp.</i>	-	-	n.a.	n.a.	-	-
	Softwood	rubber tree	<i>Hevea brasiliensis*</i>	Native to the Amazon region; Brazil, Venezuela, Ecuador, Colombia, Peru, and Bolivia. Introduced to many other tropical regions of the world, as Indonesia, Malaysia, Liberia, India, Sri Lanka, Sarawak, and Thailand (Reed, 1976).	Not Evaluated	rotation period	continuous rotational cropping	Duke, 1983	-
		Common hazel	<i>Corylus avellana*</i>	Native to Europe and western Asia, from the British Isles south to Iberia, Greece, Turkey and Cyprus, north to central Scandinavia, and east to the central Ural Mountains, the Caucasus, and northwestern Iran.	LC	rotation period	(short)	Buckley, 1992	Azores, Cyprus, Malta
	Oil	Olive	<i>Olea europaea*</i>	Worldwide	NT	rotation period	(Unrotated)	García-Mozo et al., 2008	Spain
aromatic herbs	Chamomile	<i>Matricaria chamomilla</i> and <i>M. recruta*</i>	All over Europe and temperate Asia, and it has been widely introduced in temperate North America and Australia.	Not Evaluated	rotation period	(Continuos)	Barbieri, 2013	North Europe, Baleares, Azores.	

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	aromatic herbs	Artemisia	<i>Artemisia absinthium</i> *	Naturalized across the northern USA and in Canada. It occurs from Nova Scotia west to British Columbia; south to Oregon and Utah; and east through Colorado and Nebraska to Virginia.	Not Evaluated	rotation period	(unrotated)	U.S. Forest Service, 2016b; EC, 2003; Lym et al., 2010	“North Europe.
Aquatic plants	Aquatic macrophyte		(Submerged): <i>Haloragaceae</i> spp. <i>Ceratophyllaceae</i> spp. <i>Characeae</i> spp. <i>Potamogetonaceae</i> spp. (Emergent): <i>Cyperaceae</i> spp.	-	-	recovery was investigated with regard to area coverage or plant biomass	from 1 to 15 years (mean 2.75 years). Rapid recovery, usually below 1 year frequently found for submerged macrophytes. Long recovery times usually associated with emergent plant after dredging events	Gergs et al., 2016	-
Algae	Algae	Green algae and Cyanobacteria	<i>Chlorella vulgaris</i> ; <i>Haematococcus pluvialis</i> , <i>Dunaliella salina</i> ; <i>Cyanobacteria</i> <i>Spirulina maxima</i>	-	Not Evaluated	population size recovery time, i.e. Time to return to pre-disturbance population size (total biomass recovered)	3 days to 20 weeks	Gergs et al., 2016	-

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Algae	Algae	Green algae	<i>Chlorella vulgaris</i> *	worldwide, since very adaptable	Not Evaluated	cell doubling time	2.26-4.50 days	CODIF Recerche & Nature, 2016; Sacasa Castellanos, 2013	-
Fungi	Wild mushrooms	Penny-bun Bolete	<i>Boletus edulis</i> *	Fairly frequent throughout Britain and Ireland as well as on mainland Europe and in Asia, Also in the USA, where it is known as the King Bolete, although it is a matter of ongoing debate whether the American mushroom is in fact the same species as that found in Europe. Introduced to southern Africa, Australia and New Zealand.	Not Evaluated	rotation period	it depends on symbiotic ectomycorrhizal associations	Peintner et al., 2007	-
Aquatic animal products	Natural pearls, shells	(general data)	-	-	-	regeneration period	2-6 years	Sustainable Pearls, 2016; Southgate and Lucas, 2011	-
	Natural pearls, mother-of-pearl, shells	Pearl oysters	<i>Pinctada maxima</i> *	Australia, Fiji, Tahiti, Indonesia and the Philippines.	Not Evaluated	n.a.	n.a.	[Other ref. on distribution: Hawes et al., 2011]	-

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Aquatic animal products	corals	(general data)	-	-	-	population size recovery time, i.e. time to return to predisturbance population size (total biomass recovered)	After benthic trawling, slower-growing sponges and corals took up to 8 years to recover compared with <1 years for polychaetes.	Lotze et al., 2011	-
	corals	Precious coral	<i>Corallium rubrum</i>	Western and Eastern Mediterranean: from Greece and Tunisia to the the Straits of Gibraltar including Corsica, Sardinia and Sicily. Eastern Atlantic: South Portugal, Morocco, Canary and Cape Verd Islands.	Not Evaluated	time recovery population	15 to 25 years	FAO Fisheries & Aquaculture, 2016; Montero-Serra et al., 2015	-
	sponges	(general data)	-	-	-	individual regeneration population size recovery time, i.e. Time to return to pre-disturbance population size (total biomass recovered)	200-300 days After benthic trawling, slower-growing sponges and corals took up to 8 years to recover compared with <1 years for polychaetes.	Bell, 2002 Lotze et al., 2011	-

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-	soil organic matter	(general data)	-	-	-	recovery	50 yr is defined an adequate time for recovery (from cultivation) of active soil organic matter and nutrient availability, but recovery of total soil organic matter pools is a much slower process	Burke et al., 1995	

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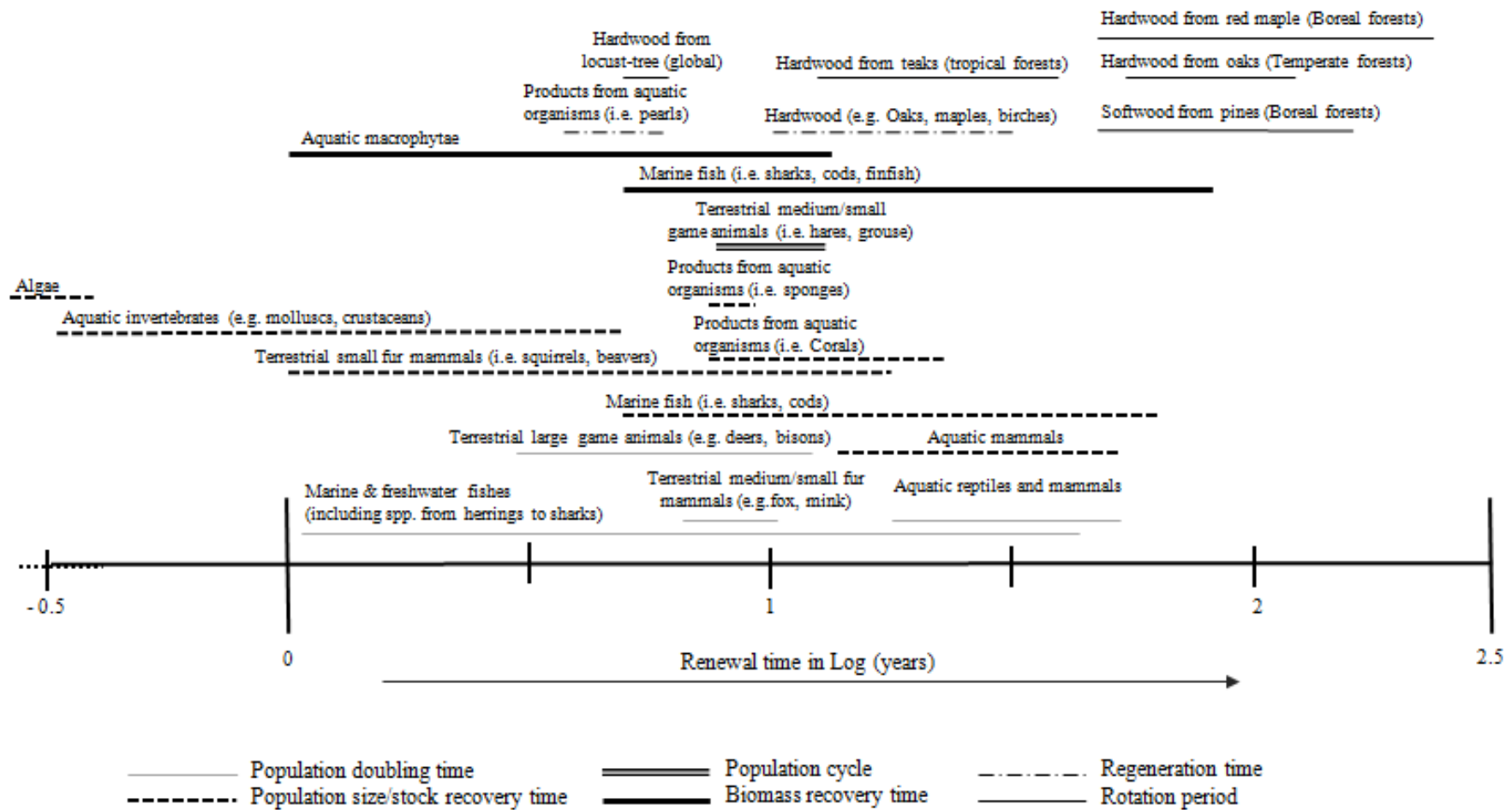
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**Figure B1.** Renewability rate of several naturally occurring biotic resources, expressed in Log (years)



## Appendix to Chapter 4 (Appendix C)

**Table C1.** Results of the sensitivity analysis of the “water for food” matrix. The lower level of agreement between the expert judgments (white cells, where 100 means complete agreement between experts) and the Cohen’s kappa indicator, namely the degree of agreement (grey cells), are reported for each service flow action.

<b>WATER (GW&amp;SW) FOR FOOD</b>	<b>BioM</b>	<b>PoW</b>	<b>BiotM</b>	<b>AbioM</b>	<b>NPoW</b>	<b>RBioE</b>	<b>NRBioE</b>	<b>Cohen's Kappa: AGREEMENT on the possible impacts of the sectoral action on all ecosystem provisioning services</b>
Water for drinking	100	64.6	100	100	100	100	45	<b>71.4</b>
Water for irrigation	100	100	100	100	100	100	45	<b>85.7</b>
Available soil water (green water) for crop production	100	100	100	100	29	100	100	<b>85.7</b>
Water for livestock fattening	100	100	100	100	100	100	45	<b>85.7</b>
Water for slaughter house	100	100	100	100	100	100	45	<b>85.7</b>
Water for managing farms	100	100	100	100	100	100	100	<b>100.0</b>
Water for agri-industrial productions (agricultural machineries, fertilizer, pesticides, herbicides, etc.)	100	100	100	100	100	100	64.6	<b>85.7</b>
Water for apiculture	100	100	100	100	100	100	29	<b>85.7</b>
Water for fisheries	100	100	100	100	100	100	29	<b>85.7</b>
Water for aquaculture	100	100	100	100	100	100	29	<b>85.7</b>
Water for forest farming/agroforestry	100	64.6	100	100	100	100	29	<b>71.4</b>
Water for horticulture	100	100	100	100	100	100	29	<b>85.7</b>
Water for transferring foods (inland navigations)	100	100	64.6	100	100	100	64.6	<b>71.4</b>
Water for manufacturing, refining, packing the foods	100	100	64.6	100	100	100	45	<b>71.4</b>
Water for washing vegetables, fruits etc., by end-users (eg., at home, restaurants and hotels)	100	100	100	100	100	100	100	<b>100.0</b>
Water for prepare food at home, restaurants, hotels, refectories etc.	100	100	100	100	100	100	100	<b>100.0</b>
Water for cleaning food remains by end-users	100	100	100	100	100	100	100	<b>100.0</b>
Water for waste treatment	100	100	100	100	100	100	100	<b>100.0</b>
<b>AGREEMENT on the possible impacts of water for food on each ESS</b>	<b>94.74</b>	<b>84.21</b>	<b>84.21</b>	<b>94.74</b>	<b>89.47</b>	<b>94.74</b>	<b>31.58</b>	

BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RAbioE: Renewable abiotic energy sources (hydropower, wind, etc...)

**Table C2.** Results of the sensitivity analysis of the “energy for food” matrix. The lower level of agreement between the expert judgments (white cells, where 100 means complete agreement between experts) and the Cohen’s kappa indicator, namely the degree of agreement (grey cells), are reported for each service flow action.

<b>ENERGY FOR FOOD</b>	<b>BioM</b>	<b>PoW</b>	<b>BiotM</b>	<b>AbioM</b>	<b>NPoW</b>	<b>RBioE</b>	<b>NRBioE</b>	<b>Cohen's Kappa: AGREEMENT on the possible impacts of the sectoral action on all ecosystem provisioning services</b>
Energy for agricultural management practices (Striping, bulking, seeding, planting, fertilizing, sowing, applying&controlling weeds, pests or disease, harvesting etc.)	100	100	100	100	100	100	100	100.0
Energy for management of farms or state hatchery	100	100	100	100	100	100	100	100.0
Energy for delivering water to agricultural areas	100	100	100	100	100	100	100	100.0
Energy for operating irrigation system	100	100	100	100	100	100	100	100.0
Energy for livestock fattening	64.6	100	100	100	100	100	100	85.7
Energy for poultry husbandry	64.6	100	100	100	100	100	100	85.7
Energy for apiculture	64.6	100	64.6	100	100	100	100	71.4
Energy for fisheries	64.6	100	100	100	100	100	100	85.7
Energy for aquaculture	100	64.6	100	100	100	100	100	85.7
Energy for horticulture	64.6	64.6	100	100	100	100	100	71.4
Energy for disposing of waste from an agricultural activity	64.6	64.6	100	100	100	100	100	71.4
Energy for transporting food (crop, livestock, etc.) to process (hauling) (transporting in-country, intercountry)	100	100	100	100	100	100	100	100.0
Energy for processing food (manufacturing, refining, grading, packaging)	64.6	100	100	100	100	100	100	85.7
Energy for transporting food to the markets (transporting in-country, intercountry)	100	100	100	100	100	100	100	100.0
Energy for per capita to have food at home	100	100	100	100	100	100	100	100.0
Energy for preparing food at home or restaurant, hotels, refectories etc.	45	100	100	100	100	100	100	85.7
Energy for collecting organic wastes	100	100	45	100	100	100	64.6	71.4
Energy for other food procedures (energy for pruning etc.)	100	100	100	100	100	100	64.6	85.7
Energy for agri-industrial productions (fertilizer, pesticides, herbicides industrial productions etc.)	64.6	64.6	64.6	64.6	64.6	64.6	64.6	33.4
Solar energy for net primary productivity (NPP) in terrestrial and marine ecosystems (The rate at which new biomass accrues in an ecosystem)(Higher in humid and warmer climate, less in dry and hot climate)	100	100	100	100	100	100	100	100.0
<b>AGREEMENT on the possible impacts of energy for food on each ESS</b>	<b>55.00</b>	<b>80.00</b>	<b>85.00</b>	<b>95.00</b>	<b>95.00</b>	<b>95.00</b>	<b>85.00</b>	

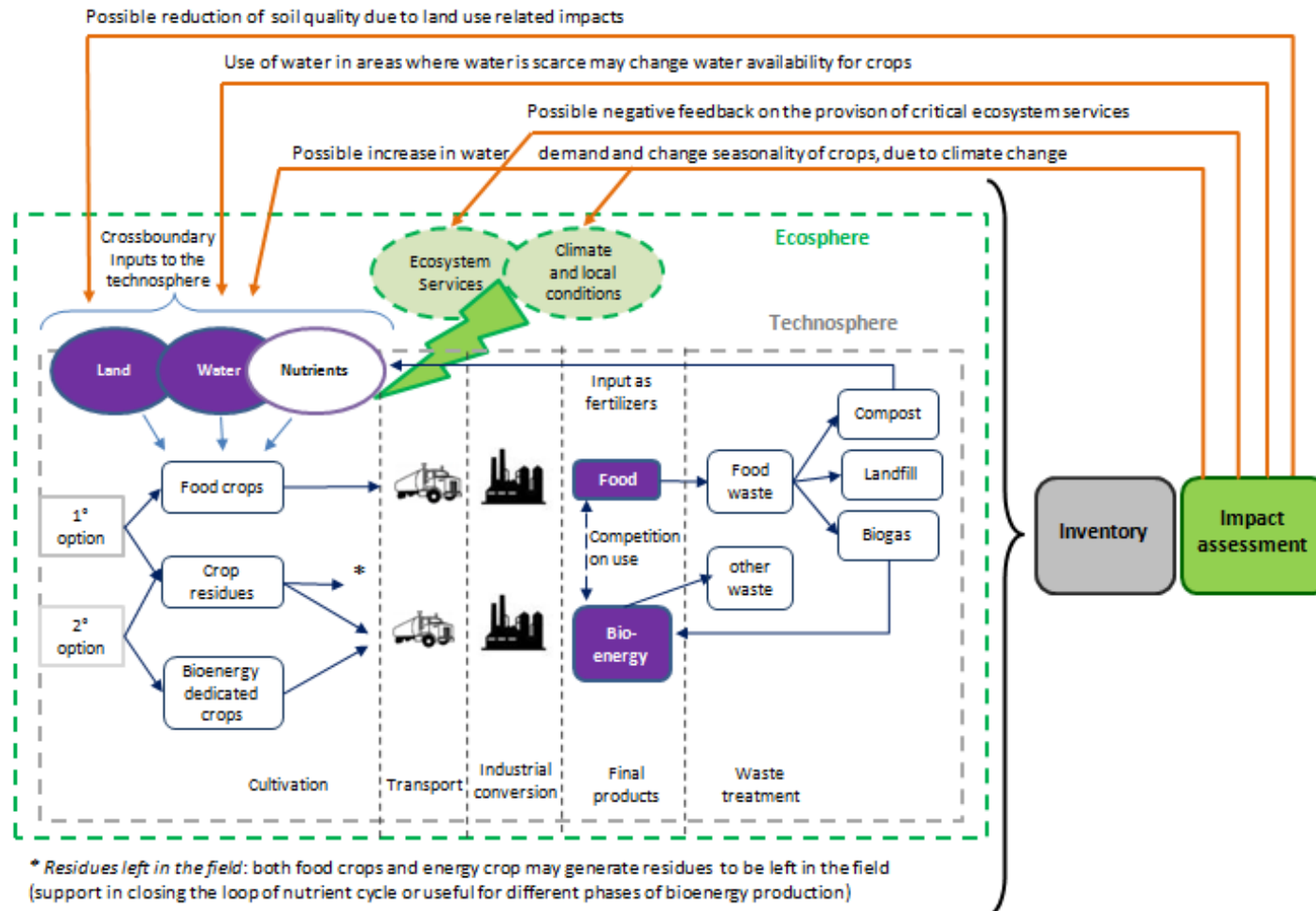
BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RAbioE: Renewable abiotic energy sources (hydropower, wind, etc...).

**Table C3.** Results of the sensitivity analysis of the “ecosystem for food” matrix. The lower level of agreement between the expert judgments (white cells, where 100 means complete agreement between experts) and the Cohen’s kappa indicator, namely the degree of agreement (grey cells), are reported for each service flow action.

<b>ECOSYSTEM FOR FOOD</b>	<b>BioM</b>	<b>PoW</b>	<b>BiotM</b>	<b>AbioM</b>	<b>NPoW</b>	<b>RBioE</b>	<b>NRBioE</b>	<i>Cohen's Kappa: AGREEMENT on the possible impacts of the sectoral action on all ecosystem provisioning services</i>
Marine ecosystems (fish, crustacean, molluscs, echinoderms etc. )	100	100	100	100	100	100	100	<b>100.0</b>
Lentic (lakes, reservoirs, ponds) ecosystems (fish, some vertebrates, duck,)	100	100	100	100	100	100	100	<b>100.0</b>
Lotic ecosystems (fish, some vertebrates,)	100	100	100	100	100	100	100	<b>100.0</b>
Wetlands (Fish, duck, etc.)	100	100	100	100	100	100	100	<b>100.0</b>
Forest ecosystems (all berries, honey, mushroom, seed, nut, root, tuber, gums, sap, wild animals (pork) etc.)	100	100	100	100	100	100	100	<b>100.0</b>
Agro-ecosystems (all cultivated crops)	100	100	100	45	100	100	100	<b>85.7</b>
Grasslands and heathlands (rabbits, wild pork, etc.)	100	100	100	100	100	100	100	<b>100.0</b>
<b>AGREEMENT on the possible impacts of ecosystem for food on each ESS</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	<b>85.71</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>	

BioM: Biomass, PoW: Potable water, BiotM: Biotic materials, AbioM: Abiotic materials, NPoW: Non-potable water, RBioE: Renewable biomass based energy sources (plant, animal), NRBioE: Non-renewable fossil energy, RAbioE: Renewable abiotic energy sources (hydropower, wind, etc...)

**Figure C1.** Conceptual framework for the integration of the nexus-oriented matrix into life cycle assessment, through the illustration of an example of competition between crop production for food and bioenergy. The impacts related to different uses may trigger negative consequences (or feedback), such as damage to human well-being, resource supply and ecosystem functioning, and in turn these may generate feedback back to critical inputs to the production system of either food or bioenergy.





## Publications

- Crenna, E., Jolliet, O., Sala, S., Collina, E., Fantke, P. (2017, in preparation). Characterizing exposure and ecotoxicity of pollinators to pesticides in life cycle impact assessment.
- Rosa, F., Crenna, E., Collina, E., Fantke, P. (2017, in preparation). Modelling the exposure of pollinators to pesticides via water contact and related effects in life cycle impact assessment.
- Crenna, E., Sala, S., Polce, C., & Collina, E. (2017). Pollinators in life cycle assessment: towards a framework for impact assessment. *Journal of Cleaner Production*, 140: 525-536.
- Crenna, E., Secchi, M., Benini, L., Sala, S. (2017). Global environmental impacts in 2010: data sources and methodological choices for calculating normalisation factors. To be submitted to *The International Journal of Life Cycle Assessment*
- Crenna, E., Sozzo, S., Sala, S. (2017, in press). Natural biotic resources in LCA: towards an impact assessment model for sustainable supply chain management. *Journal of Cleaner Production*, <http://dx.doi.org/10.1016/j.jclepro.2017.07.208>
- Karabulut, A., Crenna, E., Sala, S. (2017, in press). A proposal for the integration of the Ecosystem-Water-Food-Land-Energy (EWFLE) nexus concept into the life cycle assessment: A Synthesis Matrix for food security. *Journal of Cleaner Production*, <https://doi.org/10.1016/j.jclepro.2017.05.092>
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## Conference communications

- Crenna, E., Sala, S. (2016). Pollinators in LCA: relevance and challenges. Poster for SETAC Europe 26<sup>th</sup> Annual Conference, Nantes, 2016
- Crenna, E., Sala, S. (2017). Making biotic resources count in Life Cycle Impact Assessment. Poster for SETAC Europe 27<sup>th</sup> Annual Conference, Brussels, 2017
- Crenna, E., Sala, S., Polce, C., Collina, E. (2015). Pollinators in LCA: towards a framework for impact assessment. Proceedings of the International Conference LCA for "feeding the Planet and energy for life". ENEA. ISBN 978-88-8286-321-0.
- Crenna, E., Saouter, E., Sala, S. (2016). Pollinators in Life Cycle Impact Assessment. Proceeding of the 10<sup>th</sup> Conference of Associazione Rete Italiana LCA. ENEA. ISBN 978-88-8286-333-3.
- Crenna, E., Saouter, E., Sala, S. (2017). A trophic chain-based approach for ranking chemicals in LCA. SETAC Europe 27<sup>th</sup> Annual Conference, Brussels, 2017
- Sala, S., Benini, L., Crenna, E., Secchi, M. (2017). Searching for the right number: which are the most suitable reference values to be compared to the planetary boundaries? Poster for SETAC Europe 27<sup>th</sup> Annual Conference, Brussels, 2017
- Sala, S., Benini, L., Crenna, E., Secchi, M. (2017). European and global consumption: to which extent are they surpassing planetary boundaries? Poster for Life Cycle Management (LCM) Conference, Luxembourg, 2017