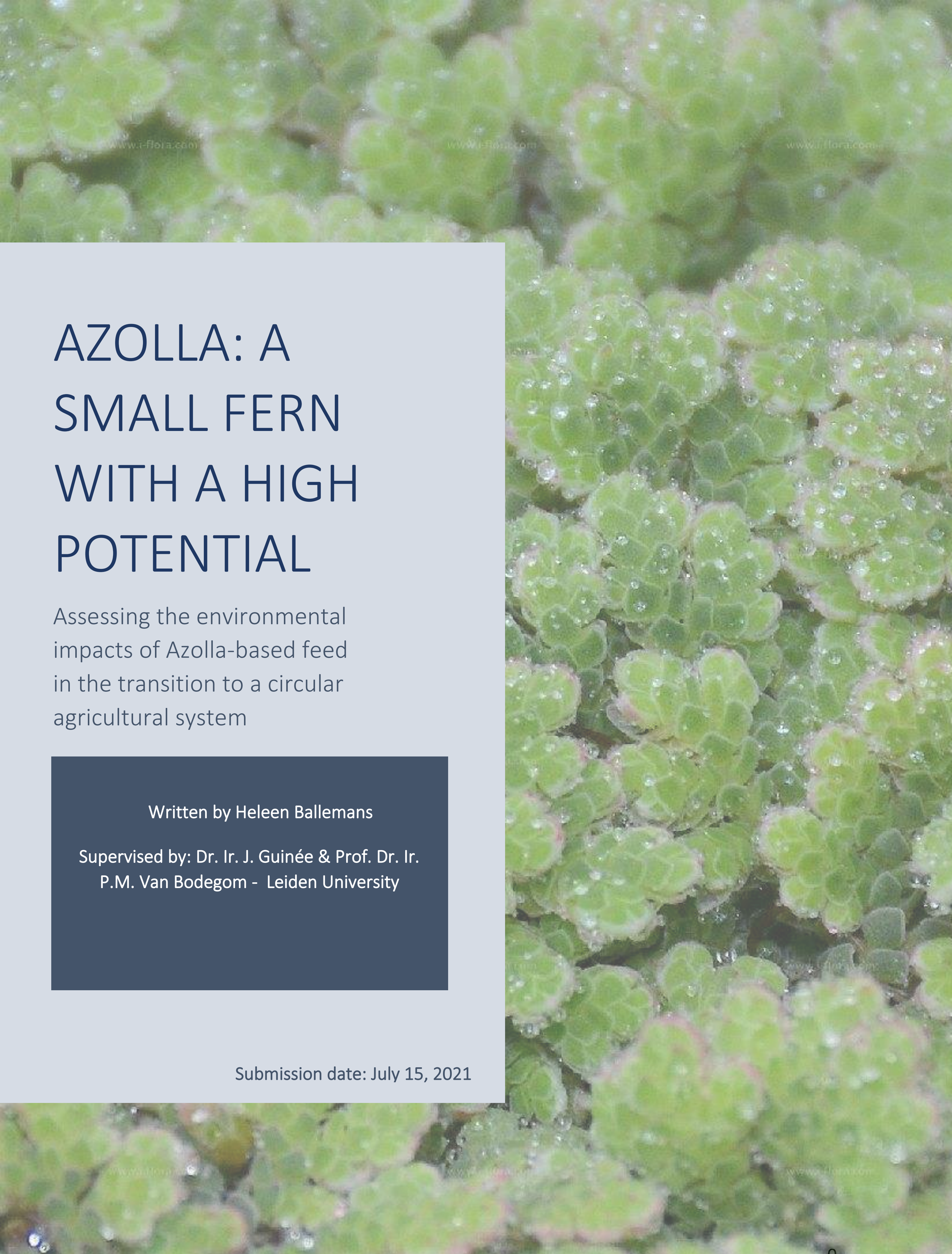

AZOLLA: A SMALL FERN WITH A HIGH POTENTIAL

Assessing the environmental impacts of Azolla-based feed in the transition to a circular agricultural system

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Universiteit Leiden

TU Delft

INDUSTRIAL ECOLOGY INNOVATIVE BY NATURE

AEQUATOR groen & ruimte

Azolla: a small fern with a high potential
Assessing the environmental aspects of *Azolla*-based feed in the transition to a circular agricultural system

By

Heleen Ballemans

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“The circular economy gives us the opportunity to build a system that can run in the long term, and the time is right for it to reach scale.”

Ken Webster, Head of Innovation at the Ellen MacArthur Foundation

“Since nature has the most sustainable ecosystem and since ultimately agriculture comes out of nature, our standard for a sustainable world should be nature’s own ecosystem.”

Wes Jackson, member of the World Future Council

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Executive summary

Since the mid-20th century, the unprecedented population growth and increase in demand for animal-based commodities have among other things driven the intensification and specialisation of the Dutch livestock sector. The lack of access to domestic, protein-rich feeds that has arisen as a result, is dealt with by the import of large quantities of soymeal. This system causes a variety of detrimental environmental impacts. Hence, the present study proposes to substitute soy-based feed by an alternative feed, based on the aquatic plant species *Azolla Filiculoidis*. In this study, it is examined *to what extent the large-scale implementation of* Azolla-*based feed production will affect the future environmental performance of the Dutch livestock feed sector, in the context of the transition to a circular agricultural system (i.e., CAS).*

First, it was investigated *by which indicators the environmental performance of the life cycle of an alternative,* Azolla-*based feed production system (i.e., FPS) and a conventional, soy-based feed production system (i.e., C-FPS) may be evaluated*. To this end, a systematic review was conducted of the literature on environmental impact assessments featuring FPSs. In total, 22 indicators were identified, and subdivided over six groups of impact categories. Based on a set of predefined criteria, 12 indicators were selected that encompass the broadest range of input- and output-related environmental burdens measurable in LCA. These include the depletion of abiotic resources, water and land as well as emissions of nutrients, toxics, greenhouse gases, and pollutants. No consistent methods exist yet to quantify all relevant environmental burdens (e.g., linked to the biodiversity and the ES) in an LCA context.

Afterwards, it was examined *how* Azolla-*based feed production could be operationalised such that it has the highest potential of fitting in a CAS*. Desk research was conducted to unveil which unit process options exist for the different life cycle stages of *Azolla*-based feed production. For each stage, the unit process with the supposedly lowest virgin resource inputs and waste and emission outputs, as well as the highest economic throughputs and opportunities for resource reuse (i.e., IROT), were selected and combined into two alternative *Azolla*-based FPS alternatives. These novel systems are referred to as the local, *Azolla*-based FPS (i.e., LA-FPS) and the regional, *Azolla*-based FPS (i.e., RA-FPS), and form each other's opposite ends in terms of technological advancement. In both FPSs, *Azolla* biomass is cultivated in a paludiculture setting, due to its potential for buffering water and carbon, as well as cycling nutrients.

Next, *the environmental sustainability performance of different feed production scenarios, involving the* Azolla-*based and conventional FPSs,* was assessed. Three narratives for distinct feed production trajectories were developed. Each scenario is characterized by the share which the FPSs take in the total feed market. The BAU (i.e., business-as-usual) scenario represents a future in which the C-FPS, implemented in a linear agricultural system (i.e., LAS) remains dominant, while the local farming projects (i.e., LFP) and regional supply chains (i.e., RSC) scenarios represent a future in which the LA-FPS and RA-FPS, respectively, take over. In an ex-ante LCA, executed in the Activity Browser (i.e., AB) software tool, the scenarios were quantified against the background of the second shared socioeconomic pathway (i.e., SSP2), representing the middle-of-the-road in the IMAGE energy model. Parametrization was used to add a temporal dimension, up to 2050, to important technology variables.

Given their optimistic assumptions on core parameters, the normative LFP and RSC scenarios performed substantially better on all selected input- and output-related environmental indicators. In terms of the land use-, nutrients-, and pollutants-related categories, the reductions in adverse impacts were especially pronounced. These effects can be explained by the high protein yield/hectare of *Azolla* cultivation, the high nutrient-efficiency of *Azolla* biomass, the low biogenic C losses, and the short transport distances along the *Azolla* life cycle. In the abiotic resource-, water use- and toxicity-related impact categories, improvements were smaller, yet substantial. The low demand for artificial fertilisers and pesticides, the absence of irrigation, and the short transport distances in the *Azolla*-based FPSs, mainly attribute to these outcomes.

Thus, despite its limitations regarding data collection and the ex-ante LCA method, this study shows that, based on the examined scenarios, *Azolla*-based FPSs could indeed enhance the future environmental performance of the Dutch livestock sector in the transition to a CAS. The implementation of large-scale *Azolla* production practices is expected to significantly reduce an array of environmental impacts, compared to the incumbent C-FPS. This study opens the way for more profound future-oriented modelling research, particularly focussing on the role of *Azolla* in the agricultural system, and recommends follow-up studies on the biogeochemical C cycle of agro-industrial processes, the development of fore- and background databases for (circular) feed production, and further advancement of the AB software tool.

Table of contents

List of figures

List of tables

List of common abbreviations

AB	Activity Browser
AD	abiotic resource depletion
AHSS	animal husbandry sub-system
ALO	agricultural land occupation
BAU	business-as-usual
CAS	circular agricultural system
C	conventional
CC-B/WB	climate change (with biogenic/without biogenic)
ES	ecosystem service
FE	freshwater eutrophication
FET	freshwater ecotoxicity
FPS	feed production system
FPSS	feed production sub-system
FTA	freshwater and terrestrial acidification
GHG	greenhouse gas
HL	harvesting loss
HT	human toxicity
IROT	input, reuse, output, throughput
LCA	life cycle assessment
LA	local *Azolla*-based
LAS	linear agricultural system
LFP	local farming projects
NLT	natural land transformation
OD	ozone depletion
POF	photochemical oxidant formation
RA	regional *Azolla*-based
RQ	research question
RSC	regional supply chains
SQ	sub-question
TE	terrestrial eutrophication
TER	terrestrial ecotoxicity
WD	water depletion

Chapter 1. Introduction

1.1. Background

1.1.1. The specialisation and intensification of agriculture

Since the 1950s, the global population has tripled, from 2.5 to 7.8 billion people, while the average demand for animal-based products has doubled, from 20.5 to 43.0 kg/capita (Food and Agricultural Organisation, FAO, 2016; World Bank, 2020). In response, the agricultural sector has evolved rapidly, supported by scientific and technological advancements, like the invention of synthetic fertiliser and the establishment of large infrastructural networks (Tzachor, 2019). These developments have marked a turning point for agriculture, resulting in the separation of cropping and livestock practices, that no longer depend on the exchange of resources (Rauw et al., 2020). The emergence of specialised cropping and livestock sectors is characteristic for many present-day developed economies, including the Netherlands.

In the Netherlands, the agricultural sector boomed after the Second World War, driven by the continuously expanding population with changing dietary preferences. As the demand for animal-based products surged, intensive livestock farmers were subsidised for producing large quantities of meat, dairy and eggs at marginal cost (Clark and Tilman, 2017). As such, livestock production delivered essential services, by creating employment opportunities and generating income, while contributing to food security and human wellbeing (Van Merriënboer, 2006). Once the government withdrew, giving way to free market competition, the dynamics of supply and demand kept favouring a model of large-scale, specialised, intensive production over small-scale, mixed, extensive production (Van der Hoeven, 2019).

At the onset of the 21st century, the process of intensification had transformed the Dutch livestock sector completely. By means of illustration, the number of farms currently amounts to 50,000, compared to 410,000 in 1950 (Central Bureau for Statistics, CBS, 2020). Simultaneously, the size of farms has increased. The average number of cows per farm, for instance, has risen from 13 in 1950 to 160 as of today (totalling 3.9 million animals) (CBS, 2017b). Furthermore, improvements in health conditions, animal genetics and feed conversion ratios (FCR, i.e. the kg feed input/kg of product output) have reinforced the productivity of the livestock sector (Mottet et al., 2017). A Dutch dairy cow, for example, produced 4,000 kg milk/y in 1950, which over the next seven decades doubled to 8,200 kg/y (CBS, 2017a). Considering that the number of people to be fed by 2050 will grow to almost 10 billion, with an average meat consumption of 52 kg/capita, the intensification of agriculture seems favourable in the sense that it could aid in fulfilling the increasing demand for livestock commodities (FAO, 2016).

1.1.2. The Dutch dependency on feed imports

The combination of a small surface area, high livestock breeding intensity and high productivity has its downsides. One major consequence is that the Netherlands faces insufficient access to domestically produced feed resources. A distinction is made here between crude feed, staple crops and protein-rich feed. On the one hand, the Dutch production capacity for crude, carbohydrate-rich feed (i.e. grass and maize silage, complemented by hay, straw and fodder beet) and starchy staple crops (i.e. maize, wheat and barley), nearly meets domestic demands (Vijn et al., 2019). These crops cover about 610,000 hectares (ha): about a third of the total area reserved for agricultural production in the Netherlands (Compendium for the Living Environment, CLO, 2020).

Of protein-rich feedstock, on the other hand, the gap between domestic supply and demand has grown notably (Vijn et al., 2019). Also, persistent droughts and stricter manure regulations have recently reduced the protein content of staple crops (Bista et al., 2018; Erisman and Verhoeven, 2019). For these reasons, a substantial amount of protein-rich feed has to be imported (Taelman et al., 2015). In fact, of the protein-rich feed demanded by the Dutch livestock sector in 2018, 87% was produced abroad, mainly beyond the European Union (EU) (Vijn et al., 2019). This is a sizable increase compared to the 1970s, when 60% of it was imported, mainly from within the EU (Cormont and Van Krimpen, 2019). It highlights the growing reliance of the Netherlands on the international feed market (de Visser et al., 2014).

1.1.3. Protein-rich co-products as animal feed

When it comes to protein-rich feed, the Dutch livestock sector relies for the bigger part on co-products, with an annual total of approximately 5,010 kilotonnes (kt). Co-products are secondary commodities with an economic value, generated during the manufacturing process of primary products in the human food industry. Yet, in many cases, co-products are also edible by people (Boland et al., 2013). The main types of protein-rich co-products in the Netherlands are scraps and pulps from soy, grains, rapeseed, palm kernel and sunflower seed (Vijn et al., 2019). These protein-rich co-products are either fed separately to livestock, or in combination with energy-rich, fibre-rich and oily co-products in the form of a feed concentrate.

Table 1.1. Common types of protein-rich co-products

Co-product	Origin	Netherlands	Europe	World	Total
Soybeans and scraps	S/N-AM	0	100	1660	1760
Crude protein grains	EU/NL	1000	500	0	1500
Rapeseed scrap	NL/EU	100	600	0	700
Sunflower scrap	NL/EU/S-AM	130	120	100	350
Palm kernel scrap	AS/EU	0	150	550	700
Total		**1230**	**1470**	**2310**	**5010**

The most common types of protein-rich co-products consumed by animals in the Netherlands, as well as the main region(s) of origin (in kt), based on Vijn et al. (2019).

Table 1.1 reveals that soy is the dominant protein-rich co-product in the Dutch livestock sector. In 2018, the industry required 1,760 kt of soybeans and soy scrap, of which 94% was produced outside of the EU. This corresponds to 2,100 kt of soybean equivalent (i.e., the production of a certain weight of soybean needed to meet the demand for scrap and/or oil, including losses, excluding the hull). Assuming an average yield of 3.5 t/ha/y, the total demanded surface area for the cultivation of soy for the Dutch livestock sector amounted to just below 600,000 ha, which exceeds the surface area dedicated to cropland for livestock feed production within the Netherlands (de Visser et al., 2014; Picoli, 2018; Tzachor, 2019).

Although since 2015 the overall consumption of co-products has declined, soy imports have increased steadily (Hoste, 2014; Vijn et al., 2019). At the moment, an average of 232, 648 and 967 g of soy is needed for the production of a kg of beef, pork and poultry, respectively (de Visser et al., 2014). At the current growth rate, global demand for soy is expected to increase by another 80% up until 2050 (Tzachor, 2019). This trend is underpinned by its year-round availability and favourable nutrition profile (Willis, 2003; de Visser et al., 2014). Moreover, in the General Trade and Tariff Agreement, dating back to 1962, the tax-free entrance of oilseeds (including soy) to the European market was negotiated, taking away the incentive of the Dutch farmers to invest in domestically produced, protein-rich crops (Rauw et al., 2020).

1.1.4. The environmental impacts of imported feed

Despite being a convenient source of high quality, plant-based protein, soy has its downsides too. The cultivation of soybean goes accompanied by various environmental issues (de Visser et al., 2014). Because of the rise in demand, monocultural, intensive farming practices have established across North- and South-America. In these regions, the large-scale application of fertilisers, pesticides and irrigated water to croplands, has put an immense pressure on vulnerable ecosystems (Clark and Tilman, 2017; Lathuillière et al., 2017). Where insufficient surface area for cultivation is available, aggravated by the feed-food-fuel competition, (oftentimes illegal) deforestation practices are employed to clear the land, most notoriously in the Brazilian Amazon and Cerrado ecoregions (Lathuillière et al., 2017).

Moreover, livestock feed exerts adverse impacts down the value chain, for instance during the transport of commodities over long distances in freight ships and trucks, and by contributing to excess manure at farm-level (Piecyk and McKinnon, 2010; Willis, 2003; Withers et al., 2018). All in all, the life cycle of livestock feed is considered as the main contributor to environmental impacts related to livestock production (Eriksson et al., 2005; Van Zanten et al., 2016).

By and large, the Dutch agricultural sector neither gains all feed inputs needed for livestock breeding from its own land, nor does it return its by-products (e.g., manure) back onto the land without creating wastes and emissions into the ecosystem. Instead, it relies on the import and export of commodities and wastes across the border. As a result of its intensification and specialisation, the current linear agricultural system (i.e., LAS) is unable to optimally recover value from local resources and minimise residual streams for treatment, and thereby strongly contributes to a range of environmental problems (Taelman et al., 2015).

1.1.5. The transition to a circular agricultural system

The present study suggests that transitioning to a circular agricultural system (i.e., CAS) could improve the environmental performance of the agricultural sector. This resonates with the ambition of the Dutch Ministry of Agriculture, Nature and Food Security, to achieve full circularity by 2050 (Erisman and Verhoeven, 2019; Rijksoverheid, 2019).

The term CAS is based on the concept of the circular economy (CE). As Stahel (2016, p. 1) puts it, the CE is an output-driven economic system that aims to "turn goods that are at the end of their service life into resources for others, closing loops in industrial ecosystems and minimizing waste." Ward et al. (2016) point out that this definition of a CE is not compatible with environmental sustainability per se, even if the terms are often used interchangeably. Non-renewable fossil inputs, for instance, may be propagated even in a fully circular system.

For this reason, the Ellen MacArthur Foundation (2015, p. 7) adds that industrial ecosystems must be "restorative or regenerative by intention and design", tapping into renewables with a lower environmental impact whenever possible. As such, the CE stands in sharp contrast with the linear, input-driven economy, founded on the "take-make-dispose" principle, in which natural resources are converted into commodities and subsequently into wastes, discarded against the lowest possible cost and with little attention for ecological ramifications (Ghisellini et al., 2016; Withers et al., 2018).

In the domain of agriculture, the concept of circularity is still very much in its infancy (Withers et al., 2018). Yet, traditional models have long resorted to the CE principles of resource preservation and recovery (Devendra and Thomas, 2002). Recent years have witnessed a revived interest in the application of the CE to the agricultural sector. According to Toop et al. (2017), a wealth of agricultural wastes, co-products and by-products (AWCB), could be cycled in the economic domain, substituting raw materials and minimising waste flows. Possible applications of AWCB include the production of fertilisers and soil amendments, the recovery of energy, the production of chemicals and of livestock feed (Diacono et al., 2019).

Therefore, a CAS is defined as *a system that ensures the more sustainable production of food and feed commodities with a minimum use of material and energy, aimed at closing resource loops while reducing adverse environmental impacts* (adjusted from Babu et al., 2020, p. 2). This definition puts forward the prominent role of feed commodities, on top of food commodities, in the agro-industrial complex, and the importance of closing the loops for all involved resources, as opposed to a linear agricultural system (LAS). In other words, in a CAS, the input (I) of external resources is to be minimised, the reuse (R) of resources within the system is to be maximised, and the output (O) of waste and emissions is to be minimised. On top of the aforementioned, the throughput (T) of products with an economic value is maximised, such that the environmental impacts per production unit are lower than in its LAS counterpart. Together, these elements, hereafter referred to as the IROT concept, are at the core of the present study.

1.2. Problem definition and research questions

1.2.1. Problem definition

It has become apparent that the Dutch livestock sector grapples with a lack of locally, sustainably produced, protein-rich feed resource alternatives to imported feed (Taelman et al., 2015). Therefore, to shift fully to a CAS by 2050, it is crucial that novel feed production systems (FPSs) are developed (de Visser et al., 2014; Van der Weide et al., 2016; Termeer, 2019; Tzachor, 2019).

Such FPSs have to satisfy certain conditions before deemed suitable for implementation in a CAS. Firstly, the feed must be nutritious, yet may only contain crops or waste streams unfit for human consumption (Gontard et al., 2018; Hussein et al., 2017; Mottet et al., 2017; Ran et al., 2017; Van der Weide et al., 2016). Secondly, feed may only be cultivated at locations unsuitable for human food, and contain as little external inputs as possible (Mottet et al., 2017; Ran et al., 2017). Thirdly, the environmental impacts of the FPS should be less than those of the conventional system (Taelman et al., 2015; Veldkamp and Bosch, 2015). Finally, the system must be able to cover a sizable fraction of the total livestock feed demand (Slätmo et al., 2017).

Based on the above conditions, the *Azolla Filiculoidis* (hereafter: *Azolla*), a sub-species of the *Azolla* genome, has emerged as a promising candidate. *Azolla* (see *Figure 1.1.*) is the world's smallest, free-floating macrophyte (i.e., aquatic plant that can be spotted with the naked eye) (Kollah et al., 2016; Lumpkin and Plucknett, 1980). Research on its biochemical profile has revealed that the fern contains high concentrations of crude proteins, amino acids and other nutritious elements, comparable to an average soymeal (Leterme et al., 2010; Brouwer et al., 2018). Besides, the plant fixates atmospheric N as well as efficiently recovers P from water, reaching high relative growth rates of 0.5 days^{-1} (De Vries and de Vries, 2018; Muradov et al., 2014). Since its biomass floats on water, *Azolla* may be cultivated on ditches and inundated zones unfit for human food production (Smolders and Van Kempen, 2015).

Figure 1.1: A close-up of *Azolla Filiculiodis*, which under favourable conditions spreads rapidly on freshwater surfaces (Taylor, 2011).

1.2.2. Research gaps

Nevertheless, the environmental performance of *Azolla*-based livestock feed, based on a standardised set of indicators, so far is unexamined (Brouwer, 2017; Paramesh et al., 2019). Furthermore, an exploration of the fern's potential role in achieving full circularity in de Dutch agricultural sector, by aid of different feed production scenario's, is absent (Kumar and Chander, 2017). Knowledge on these aspects is crucial for determining the benefits of replacing conventional types of protein-rich livestock feed by *Azolla* (Sasu-Boakye et al., 2014).

1.2.3. Research questions

The current study seeks to fill the abovementioned knowledge gaps, by answering the following research question:

To what extent does the large-scale implementation of Azolla-*based feed production affect the future environmental performance of the Dutch livestock feed sector, in the context of the transition to a circular agricultural system?*

To answer the main research question (RQ), several sub-questions (SQ) have been formulated:

SQ 1 By which indicators can the environmental performance of the life cycle of an *Azolla*-based, aquatic and a conventional, terrestrial FPS be evaluated?

SQ 2 How could *Azolla*-based feed production be operationalised such that it has the highest potential of fitting in a CAS?

SQ 3 What is the environmental sustainability performance of different feed production scenarios, involving *Azolla*-based and conventional FPSs?

1.3. Thesis structure

This thesis report consists of seven chapters, each presenting a component towards answering the main RQ (see *Figure 1.2*). In *Chapter 2*, the choice of research approach is motivated. Afterwards, *Chapter 3* seeks to answer the first SQ, by identifying a set of relevant indicators based on which the environmental performance of distinct FPSs can be evaluated. Then, *Chapter 4* addresses the second SQ by designing *Azolla*-based FPS alternatives that could replace a conventional FPS in the transition to a CAS. In *Chapter 5*, several feed production scenarios are developed and compared in an ex-ante environmental impact assessment, with the selected indicators. Finally, *Chapter 6* discusses the key findings and limitations of the study, while *Chapter 7* draws a conclusion and presents recommendations for future research.

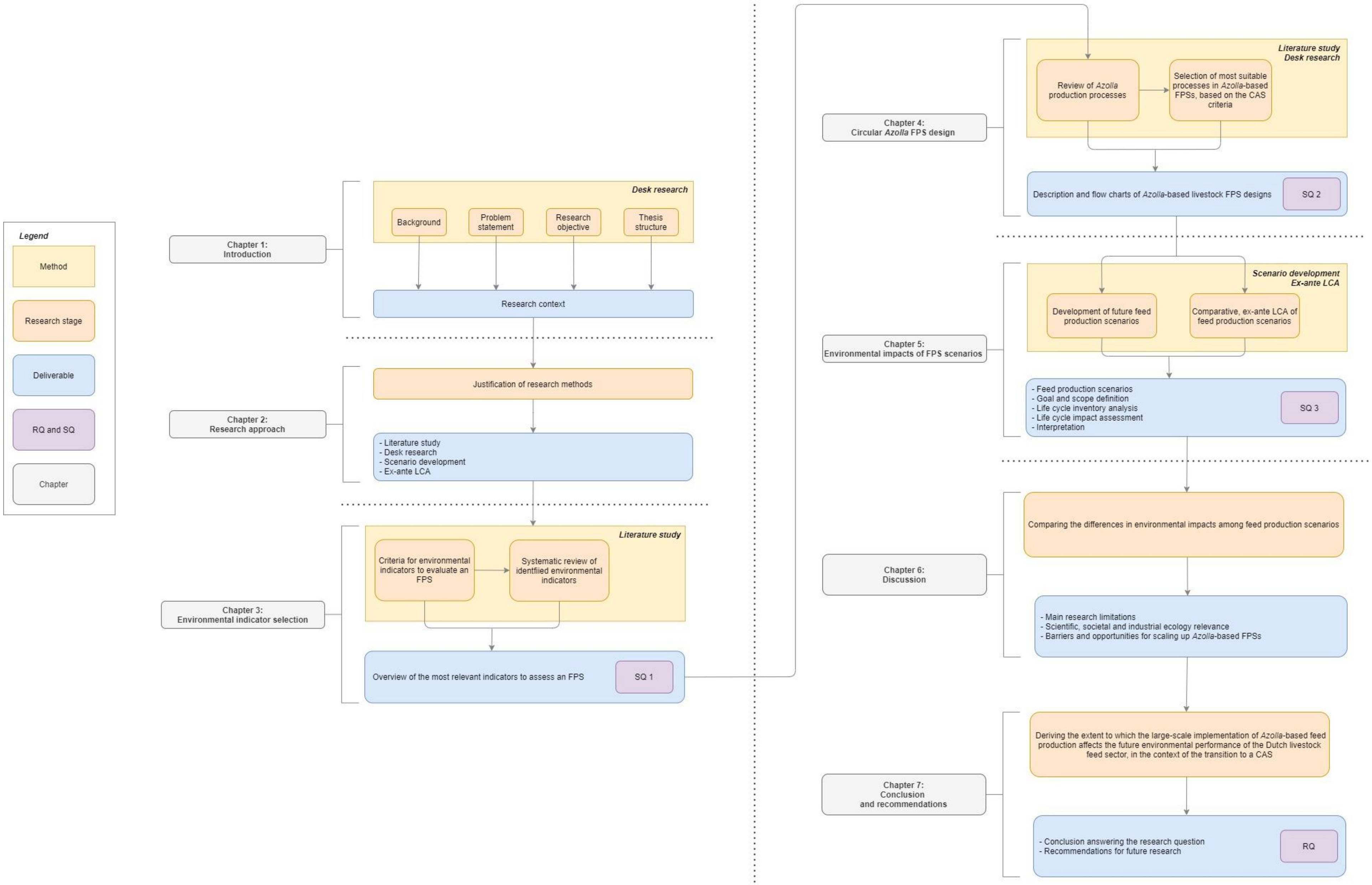


Figure 1.2. A flow diagram of the present study, organised by chapter. Zoom in for more detail.

Chapter 2. Research approach

This chapter provides a motivation for the chosen research approach, and describes how this approach will be used throughout the current study.

2.1. SQ 1: Environmental indicator selection

It is hypothesized that shifting from conventional feed production in an LAS to *Azolla*-based feed production in a CAS, will result in the reduction of adverse environmental impacts caused by the livestock sector. To test this hypothesis, a comparative assessment of different FPSs needs to be performed by means of relevant indicators. Various studies propose the use of indicators to evaluate the environmental performance of production systems (e.g., Heink and Kowarik, 2010; Niemeijer and de Groot, 2008). Among them is Jackson et al. (2000, p. vii), according to whom indicators provide a "sign or signal that relays a complex message, potentially from numerous sources, in a simplified and useful manner".

However, many different indicators exist, and which of the numerous ones "characterize the entire system yet are simple enough to be effectively and efficiently monitored and modelled" (Dale and Beyeler, 2001, p. 4) can be debated. Although indicators are applied ubiquitously across domains, problems and locations, their selection is often subject to arbitrary decisions. Studies of similar issues or geographical areas may adopt widely varying sets of indicators, which may lead to different conclusions. Also, including too many indicators complicates the validation and interpretation of the results, and affects its scientific credibility and usability by practitioners in policy-making procedures (Guinée et al., 2002).

In order to deliver rigorous results, *Chapter 3* aims to report transparently and logically on its indicator selection procedure. It starts off determining a set of research-specific indicator identification criteria (*section 3.2*) and, based on these, setting clear system boundaries that encompass the system's relevant in- and outputs (*section 3.3*). Next, indicators fitting in these system boundaries will be identified by means of a systematic literature review (*section 3.4*). An overview of the utilised key words can be found in *Table A1* of *Appendix A*. Finally, by aid of several pragmatic criteria, the most useful indicators will be selected that enable a comparison of environmental impacts between different conventional and *Azolla*-based FPSs (*section 3.5*).

2.2. SQ 2: Circular *Azolla* FPS scenario design

The environmental indicators identified in *Chapter 3* will be used to compare the environmental impacts exerted by a conventional FPS (in an LAS) and *Azolla*-based, alternative FPSs (in a CAS). Therein, the conventional FPS (i.e. C-FPS) represents the status quo, covering well-examined practices, common in protein-rich feed production, and readily implemented at a large scale. Alternative, *Azolla*-based FPSs, however, do not exist in practice yet. The knowledge available on *Azolla* production technologies stems from experimental, small-scale lab and pilot studies, focussing on a single unit process (i.e., cultivation, harvesting, or processing and storage) rather than integrated into a life cycle.

In *Chapter 4*, information on technologies applicable to *Azolla*-based feed production will be gathered from relevant studies, and complemented with expert consultations, scientific reports, and manufacturing websites, to the end of designing circular *Azolla*-based FPS alternatives. Distinct production technologies demand for different types and quantities of inputs, while generating different types and quantities of through- and outputs. Hence, each identified technology will be tested against the IROT concept (*section 4.1* up to *4.3*), which are inherent to the definition of a CAS as stated in *section 1.1.5*. Revisiting these elements, when designing a circular, *Azolla*-based FPS:

- The inputs (I) of (non-)renewable natural resources are to be *minimised*;
- The reuse (R) of wastes and emissions as a secondary resource is to be *maximised*;
- The outputs (O) of wastes and emissions are to be *minimised*;
- The throughputs (T) of goods are to be *maximised.*

Based on a predominantly qualitative analysis, the production technologies discerned as most suitable will be incorporated in two *Azolla*-based FPS designs that form each other's opposite ends in terms of technological advancement *(section 4.4*). Where possible, key variables are quantitatively estimated to support the analysis. Note that each process is considered individually, yet embedded in the production system as a whole to enable a thorough assessment of the environmental impacts. Opting for a particular harvesting method, for instance, may result in different processing requirements, thereby changing the system's overall environmental burden.

Because the design of an *Azolla*-based FPS is in its early, exploratory stage, experimental studies with the plant genus *Lemna* (i.e. duckweed) are considered, but only if the relevant physical characteristics are demonstrably similar.

2.3. SQ 3: Ex-ante environmental impact assessment

In *Chapter 5*, the environmental impacts of a C-FPS and the *Azolla*-based FPSs designed in *Chapter 4*, will be examined. To that end, an ex-ante life cycle assessment (LCA) will be performed on a number of feed production scenarios. In the following sections, the methods used for production scenario analysis (*section 2.3.1*), as well as the LCA framework (*section 2.3.2* up to *2.3.7*), its limitations (*section 2.3.8*) and details on software use and data collection (*section 2.3.9.*) are elaborated on.

2.3.1. Production scenario analysis

The following sections briefly review the relevant theory on scenario development in the light of ex-ante LCA. These include the goal of scenario development (*section 2.3.1.1*), approaches to scenario development (*section 2.3.1.2*), the differentiation of scenarios (*section 2.3.1.3*), and the diffusion trajectory of novel production systems (*section 2.3.1.4*).

2.3.1.1. Goal of scenario development

Scenario development is a tool for assessing the future environmental performance of a system. It seeks to enable a better preparation for emerging circumstances, or to actively guide the construction of one's own future (Pesonen et al., 2000). As a tool, scenario development is often applied in prospective modelling studies, including ex-ante LCA. Ex-ante LCA studies differ from ex-post LCA studies in that they compare an incumbent production system with an alternative, novel production system (between t_0 and future time t_f), rather than comparing readily existing production systems, where empirical information is available (at time t_0) (Van der Giesen et al., 2020). Hence, ex-ante LCA may be used in an attempt to deal with the so-called design paradox, or Collingridge dilemma (Arvidsson et al., 2018). This dilemma states that, at an early stage of artefact design, the possibility to alter and control is high, yet the knowledge about the artefact is scarce. At a later development stage, more knowledge has arisen, but the possibility of altering the artefact has decreased (see *Figure 2.3*; Hirooka, 2006).

In the current, early stage of the design procedure, it is of the utmost importance to pursue an *Azolla*-based FPS with minimal environmental impacts. However, since *Azolla*-based feed production is not yet implemented in the Netherlands, process and data uncertainties are inevitably high. Therefore, distinct feed production scenarios will be sketched that allow for an environmental performance assessment of the C-FPS and *Azolla*-based FPS alternatives.

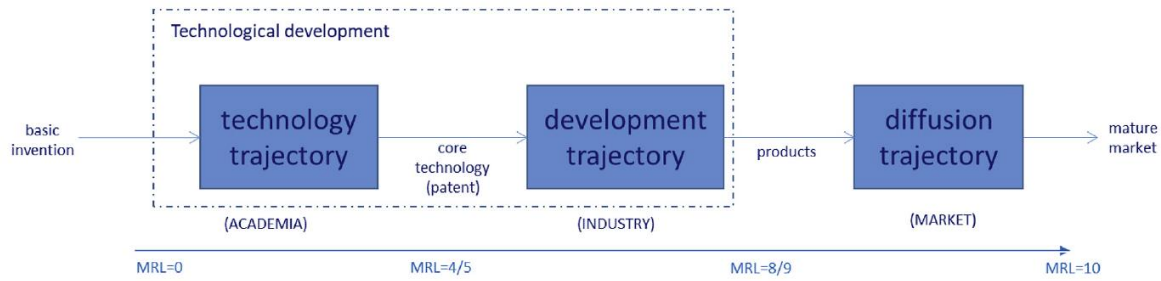


Figure 2.1: The innovation and development trajectory of production systems over time, as well as the manufacturing readiness levels (Hirooka, 2006).

2.3.1.2. Approaches to scenario development

Different approaches to scenario development exist. Which approach is most appropriate, depends on type of question it addresses. Höjer et al. (2008) distinguish between three categories of scenarios: predictive, explorative and normative scenarios. Each scenario category demands for a different scenario development approach. First, predictive scenarios seek to answer questions such as “What will happen?”, to be approached by forecasting the most *probable* futures. Second, explorative scenarios focus on “What could happen?”, to be examined by constructing different *possible* futures. Third, normative scenarios respond to the question “How can a certain target be reached?”, to be considered by developing different pathways to achieving a *desirable* future.

As stated before, the aim of the present study is to research the role of substituting a C-FPS by *Azolla*-based FPSs in the transition to a CAS. Given the clear objective of reaching full circularity by 2050, a normative approach (i.e. “what-if exploration”) is perceived most useful. This approach will aid in providing strategic information for long-term policy planning by representing distinct trajectories toward sustainable feed production (Höjer et al., 2008). When developing normative scenarios, optimistic assumptions are made with regard to the future performance of the *Azolla*-based FPSs, under favourable market conditions and policy interventions (Cucurachi et al., 2018).

2.3.1.3. Differentiation between scenarios

With regard to deciding on how many scenarios to include, ex-ante LCA comprises of at least one baseline, or business-as-usual scenario, and one (or several) alternative scenario(s). Each of these scenarios should be based on a clear narrative, as well as a set of explicit assumptions. Pesonen et al. (2000) state that analysing more than four scenarios in an ex-ante LCA study will the decision-making unmanageable. Instead, they suggest adopting a total of three scenarios: one business-as-usual scenario, and two others, that represent desirable future outcomes, while focussing on critical uncertainties. These scenarios should be internally consistent, and sufficiently different.

2.3.1.4. Innovation diffusion trajectories

To ensure that the modelled scenarios are sufficiently different, Arvidsson et al. (2018) emphasize the importance of varying the scale at which each production system is implemented. Therefore, a useful production scenario entails a product diffusion trajectory. It was decided to utilise the theory underlying to the life cycle of environmental innovations (*Figure 2.1;* Huber, 2003) and the accompanying customer adoption curve (*Figure 2.2*; Treloar, 1999) to simulate and describe the diffusion trajectories of *Azolla*-based FPSs.

In the first stage of the life cycle of environmental innovations, the novel production system is introduced to a select group of technology enthusiasts, who are keen to be part of the experimental development process. The share of the novel system in the total market is insignificant. In the second stage, the system gains popularity, sparking the interest of a larger group of early adopters, or visionaries, causing the market share to gradually increase. In the third stage, market diffusion speeds up as an early majority of pragmatists transitions from the incumbent to the novel production system. In the fourth stage, the novel system reaches maturity, attracting a late majority of conservatives, and the diffusion curve deflects from progressive to regressive. In the fifth and final stage, market saturation is achieved as the remaining sceptics, or laggards, are converted. By now, the novel production system is commercially deployed, (almost fully) replacing the incumbent, conventional production system. In the meantime, a life cycle may have started for yet another novel innovation, so that the initially novel production system falls into decline, to be phased out eventually.

As *Figure 2.2* shows, the operational scale of a novel production system affects its technological performance. As time proceeds and the system gains popularity, its production capacity increases, and thereby its market position improves. This evolvement also results in the change of technology performance parameters linked to, for instance, material and energy efficiency. Reductions in energy use or changes in the energy mix affect the system's environmental performance, and is considered by means of parametrization (see *section 5.4.3.4*).

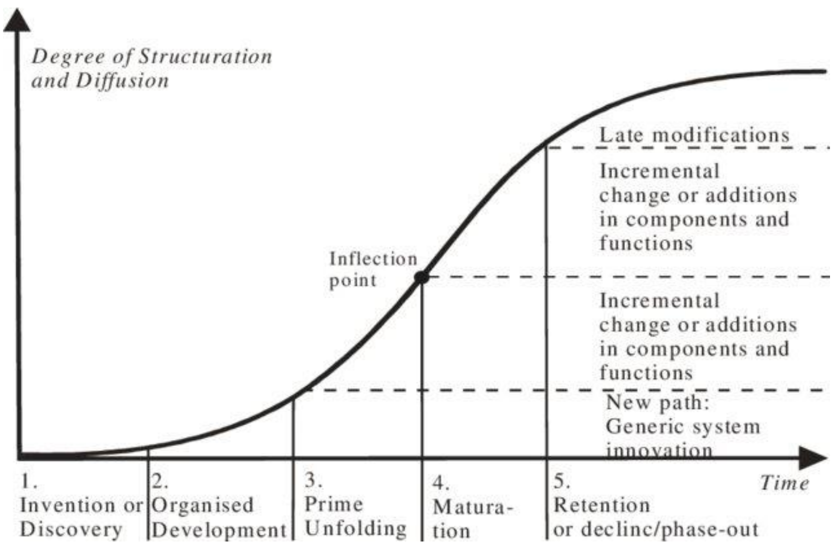


Figure 2.2. The life cycle of environmental innovations, visualising the phases that each production system passes, from the moment of invention to maturation and retention (Huber, 2003).

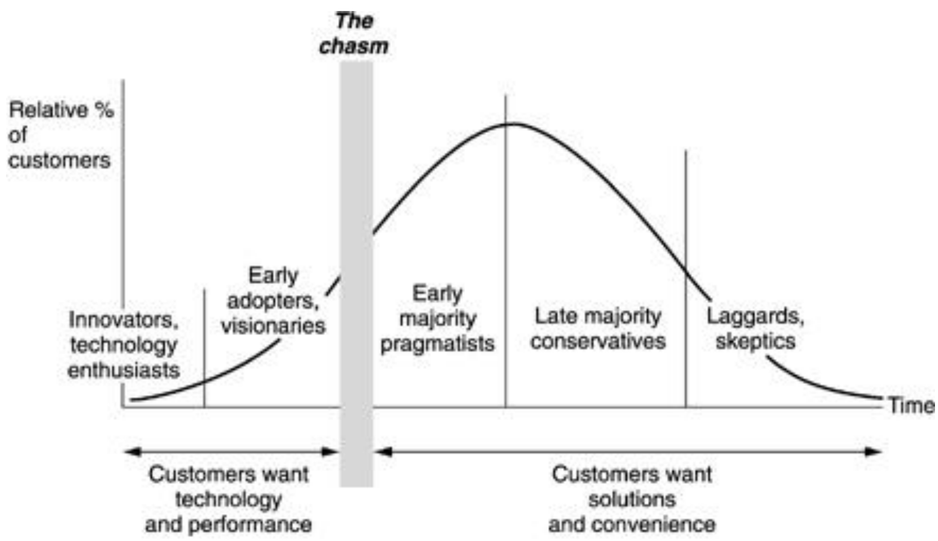


Figure 2.3. The change in customer adoption of an innovative technology or system over time (Treloar, 1999).

2.3.2. Ex-ante LCA definition

Ex-ante LCA is widely endorsed as a comprehensive approach to quantifying environmental burdens occurring along the life cycle of product systems, from raw material extraction and processing through to consumption and the end-of-life stage (Guinée et al., 2002). In this way, it allows for the detection of environmental hotspots, i.e. the life cycle stages with the largest contribution to resource depletion or ecological pollution. Subsequently, the method can be used for developing interventions aimed at maximising the value of resources and preventing losses, without shifting environmental burdens in time and space, in the design of novel production systems (Kjaer et al., 2019).

2.3.3. Life cycle assessment framework

In the present study, the guidelines of the International Organisation for Standardisation (ISO) 14040 series are followed. As depicted in *Figure 2.4*, conducting an LCA in accordance with the ISO guidelines demands for an iterative approach, comprising of four main phases, including a goal and scope definition (*section. 2.3.4*), an inventory analysis (*section 2.3.5*), an impact assessment (*section 2.3.6.*), and a final interpretation (*section 2.3.7*) (Finkbeiner et al., 2006).

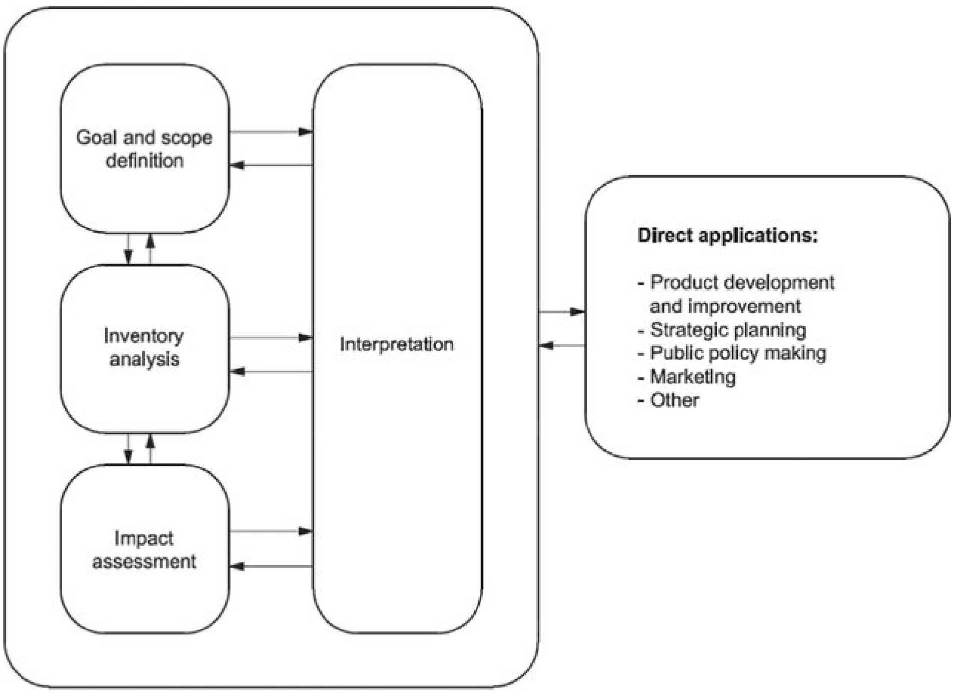


Figure 2.4. The life cycle assessment framework, its phases and applications (Finkbeiner et al., 2006).

2.3.4. Goal and scope definition

In the first phase (*section 5.3*), the study objectives and targeted audience are described. Also, the technological, spatial and temporal coverage of the study are defined, which determine the inclusion of relevant processes and flows, and therewith the required level of detail. Special attention is paid to the technology readiness level (TRL) of the incorporated technologies, as suggested by Moni et al. (2020). TRL is a systematic, qualitative scaling method that describes the maturity of a technology, starting with TRL1 (i.e., scientific breakthrough) to TRL9 (i.e., technology commercialization), and in the case of emergent technologies, assists in setting out a transition pathway. Afterwards, the system's function, functional unit (FU), alternatives and reference flows, which are directly linked to the examined scenarios, are discussed (Pesonen et al., 2000).

2.3.5. Life cycle inventory analysis

In the second phase (*section 5.4*), a flow chart is developed for the production system of each alternative, and its corresponding FU. The flow charts visualise the main inputs (i.e. of energy and materials) and outputs (i.e. of wastes and emissions) of each unit process of the production system, within the demarcated Technosphere-ecosphere system boundaries. Based on these predefined in- and outflows, back- and foreground data is gathered and compiled in a life cycle inventory (LCI), quantifying the systems' impacts on the environment in terms of elementary flows (i.e. flows to soil, water and air), with consideration for multifunctional processes.

2.3.6. Life cycle impact assessment

In the life cycle impact assessment (LCIA) phase (*section 5.5*), the magnitude of environmental impacts linked to each production scenario is evaluated. To this end, a set of impact categories, either mid-point or end-point oriented, is decided upon. In the present study, these categories are based on the selected indicators presented in *Chapter 3*, and grouped into an impact family. Next, the elementary flows within the LCI table are assigned to impact categories, and multiplied by the appropriate characterisation factor (CF). Such CF relies on mathematical models, and seeks to express the assembled flows in a single, standardised unit. For instance, all GHG emissions are expressed in terms of CO_2-equivalents. Consequently, the initially extensive list of flows is reduced to a comprehensive overview of environmental impacts, to facilitate a comparison between the scenarios.

2.3.7. Interpretation

In the final phase (*section 5.6*), the implications of each alternative scenario for the LCIA results, are discussed (Pesonen et al., 2000). Besides, a consistency and completeness check are performed to assess the extent to which the absence of homogeneous data of good quality and realistic assumptions affect the model's validity and reliability. Also, a contribution (i.e. hotspot) analysis is performed to derive the extent to which process in the investigated life cycle scenarios links to certain environmental impacts, indicating which processes should be targeted to minimise the burden of the overall production system. Finally, sensitivity analyses are conducted to reveal the model's response to a change in an individual assumption or parameter, to test the robustness of its outcomes.

2.3.8. Software and data collection

2.3.8.1. Software use

The conventional and *Azolla*-based FPSs are modelled in the Activity Browser (AB) software, a graphical user interface for the Brightway2 advanced LCA framework, making use of Qt for Python under the LGPLv3 license. This software is convenient due to its fast, flexible calculations, and its functions for scenario incorporation and data parametrization (Mutel, 2017).

2.3.8.2. Data collection

Data for foreground processes are obtained from literature, scientific reports, factsheets, laboratory experiments and expert consultations. Background processes are sourced from a modified ecoinvent database, version 3.7, under allocation, cut-off by classification (Wernet et al., 2016). This means that the activities already present in ecoinvent are changed, and new activities are added, in accordance with scenario data from, in this case, the Image Energy Regional (TIMER) model. Based on different scenarios, versions of ecoinvent are created that represent future states of production systems. This method enhances the temporal consistency of the environmental assessment. Moreover, it allows for an integrated approach, as process modifications become effective in the database as a whole. Consequently, it offers insights in the interactions between the future agro-industrial supply chains and energy supply. In the section below, the TIMER model is elaborated on.

2.3.8.3. Modelling energy scenarios

The Image Energy Regional Model (TIMER) was used to model future fluctuations in regional energy mixes and efficiencies. This electricity module of the integrated assessment model (IAM) IMAGE, seeks to simulate the long-term environmental consequences of human activities worldwide, building on the Shared Socioeconomic Pathways (SSPs). Each pathway consists of a baseline scenario, i.e., how the future develops without additional policies, and various mitigation scenarios (Riahi et al., 2017). From those pathways, the SSP2 (i.e. middle-of-the-road) pathway was selected. This pathway represents a future in which current trends continue without considerable change. From SSP2, two extremes, being SSP2 (i.e. its baseline scenario) and SSP2-2.6 (i.e. its strongest mitigation scenario) were selected. SSP2-2.6 embodies the strongest mitigation efforts to reach the two-degree target of 450 ppm CO_2-eq. (Fricko et al., 2017).

Chapter 3. Environmental indicator selection

The present chapter will lay the basis for conducting an ex-ante comparative assessment of the environmental performance of different FPSs. In doing so, it seeks to answer the following sub-question: *by which indicators can the environmental performance of the life cycle of an aquatic, Azolla-based and a conventional, terrestrial FPS be evaluated?*

3.1. Linear versus circular feed production

In order to find out whether the *Azolla* production may result in reduced environmental impacts, its future implementation should be compared with a C-FPS. This C-FPS exists in an LAS. *Figure 3.1a* and *3.1b* below schematically depict the differences between an LAS and a CAS. The box in the centre represents the Technosphere, or manmade environment, where industrial activities take place (Ayres, 1989).

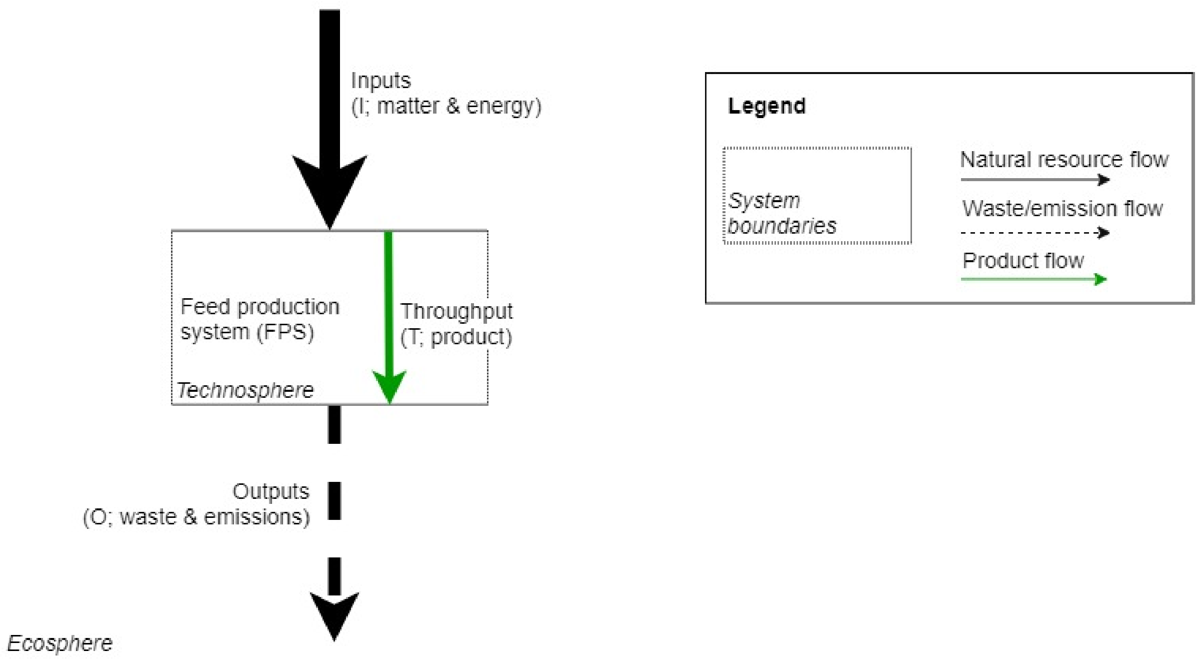


Figure 3.1a: feed production in a *linear* agricultural system, with large in- and outputs.

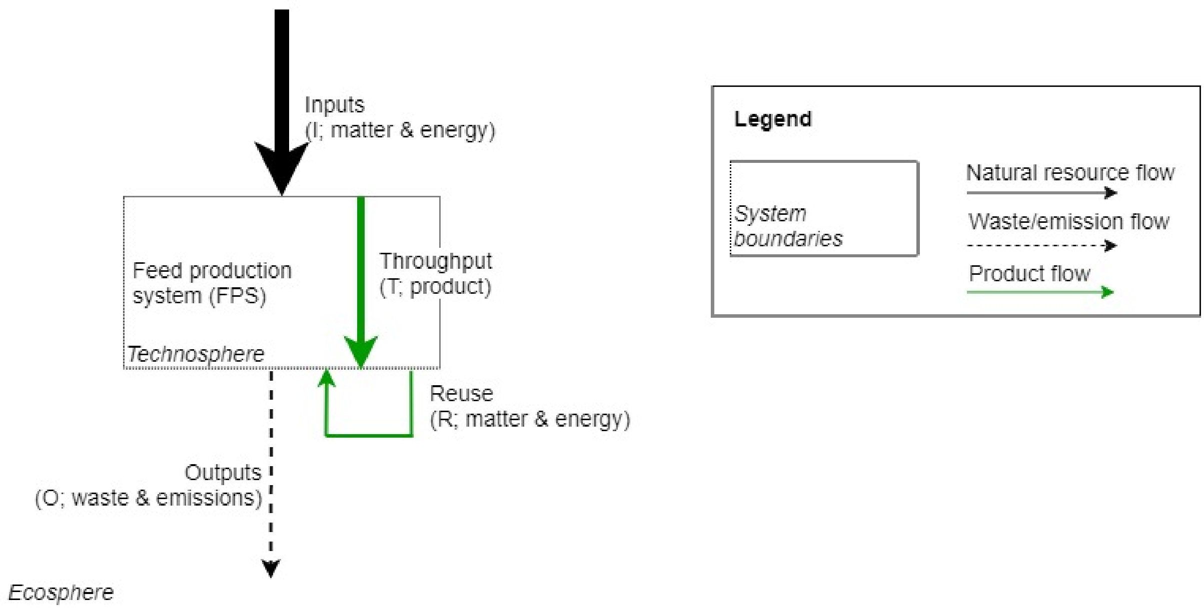


Figure 3.1b: feed production in a *circular* agricultural system, with reduced in- and outputs.

In this study, the Technosphere refers to the agro-industrial practices involved in feed production. The dotted line represents its boundary with the ecosphere, or natural environment, including soil, water and air. The arrows represent flows, linking the ecosphere to the Technosphere through ecosphere inputs (I; of matter and energy) outputs to the ecosphere (O; of waste and emissions), as well as throughputs (T; economic flows within the Technosphere) (Frosch and Gallopoulos, 1989). The thickness of the arrows roughly corresponds with the flow magnitude.

In *Figure 3.1a*, the external inputs into the FPS are large. This means that substantial amounts of virgin resources are withdrawn from the ecosystem to produce a unit of feed. At the same time, the large output flow indicates the discharge of potentially harmful substances from the FPS into the ecosystem. As such, feed production in an LAS adversely affects the ecosphere upstream, by contributing to resource depletion, and downstream, by causing environmental pollution (Korhonen et al., 2018). In contrast, the CAS in *Figure 3.1b* involves substantially less external resource inputs and emission outputs. In line with the definition presented in section *1.1.5*, a CAS strives to minimise the demand for external material and energy, by making outputs re-enter the production system, therewith (partially) closing the resource loop and avoiding environmental impacts (Ghisellini et al., 2016). Hence, its in- and outputs and reuse of resources determine how circular an FPS is, and therewith strongly affect its environmental performance.

3.2. Indicator identification criteria

Several indicator selection criteria, specific to the present study, are formulated. These criteria affect which domains, processes and factors are either covered or omitted by the indicators.

Firstly, since this study evaluates the environmental performance of feed, the selected indicators must link to an environmental flow (i.e. extension) relevant to the examined FPSs. Indicators measuring impacts that do not directly relate to these flows (e.g., biodiversity, soil erosion, and pest control) are disregarded.

Secondly, a life-cycle perspective is taken, meaning that the chosen indicators must allow for an assessment of an FPS in its entirety. Rather than only evaluating the primary production of feed, the chosen indicators of environmental flows should link to the processes in an FPS, enabling a systematic, comparative assessment of the relative contribution of each process to the system's overall performance. In this way, the trade-offs, synergies and burden shifts among indicators and processes can be detected (McClelland et al., 2018).

Thirdly, the indicators must be relevant for both a terrestrial cropping system and a freshwater aquatic cropping system, to facilitate a comparison of the conventional, linear FPS and the *Azolla*-based, circular FPS (Paramesh et al., 2019).

Finally, only indicators assessing natural resource inputs to and waste and emissions outputs from the system will be taken into consideration, as these form a direct measure of the system's environmental impact. The product throughput and internal resource reuse, which offer a relevant yet less direct measure of the system's environmental impact (i.e. a heightened productivity or internal resource cycling do not per se result in lower environmental burdens), will be addressed in *Chapter 4* (Ward et al., 2016).

3.3. System boundaries of an FPS

To compare the magnitude of environmental impacts associated with conventional and *Azolla*-based feed, standardised boundaries need to be set that apply to both FPSs. As stated in section *3.3*, the environmental in- and outflows of the system can be used to evaluate its performance. Hence, the system boundaries used for indicator selection will encompass these relevant flows, as well as the processes along the life cycle that require or emit them.

3.3.1. Processes in system boundaries

The highly simplified representations of FPSs, shown in section *3.1,* can be broken down into two sub-systems: the feed production sub-system (FPSS) and the animal husbandry subsystem (AHSS). Despite variations in terms of crop type, production methods and targeted farm animal, the processes related to the life cycle of crop-based feeds appear similar, and hold for both a terrestrial and an aquatic cropping FPS (e.g., Eriksson et al., 2005; Taelman et al, 2015; Tallentire et al., 2018). *Figure 3.2* shows these standardised processes as boxes. In the FPSS (either inside the Netherlands or abroad), feed crops are cultivated, harvested, processed (e.g., dried, milled and extracted), and transported to the farm. In the AHSS, the feed is consumed by the animal. Processes beyond the farm gate, such as slaughtering, retail and human consumption are excluded, as these are not directly affected by the type of feed examined (Post et al., 2020).

3.3.2. Flows in system boundaries

The processes in FPSs rely on the input of primary resources and the output of waste and emissions, represented in *Figure 3.2* by solid and dashed arrows, respectively. In the FPSS, the cultivation process mainly requires fossil resources (e.g., fuel, synthetic fertilisers and pesticides), nutrients (e.g., mineral and synthetic fertilisers), water and land as inputs. The subsequent processes along the life cycle mainly draw on fossil resources (e.g., electricity and heat) and land (e.g., road infrastructure and buildings). Upon entering the AHSS, the feed is converted into several outputs, including animal-based commodities and manure, that is processed, stored and distributed in the manure management process. All processes exert impacts on the ecosphere (i.e., soil, water and air) by releasing greenhouse gas (i.e., GHG) emissions, nutrients and other pollutants. In a CAS, (some of) the Technosphere flows in which these outputs are embedded (e.g., manure) are fed back into the FPS as a secondary resource, therewith supposedly reducing the system's inputs and outputs. The flows of matter that

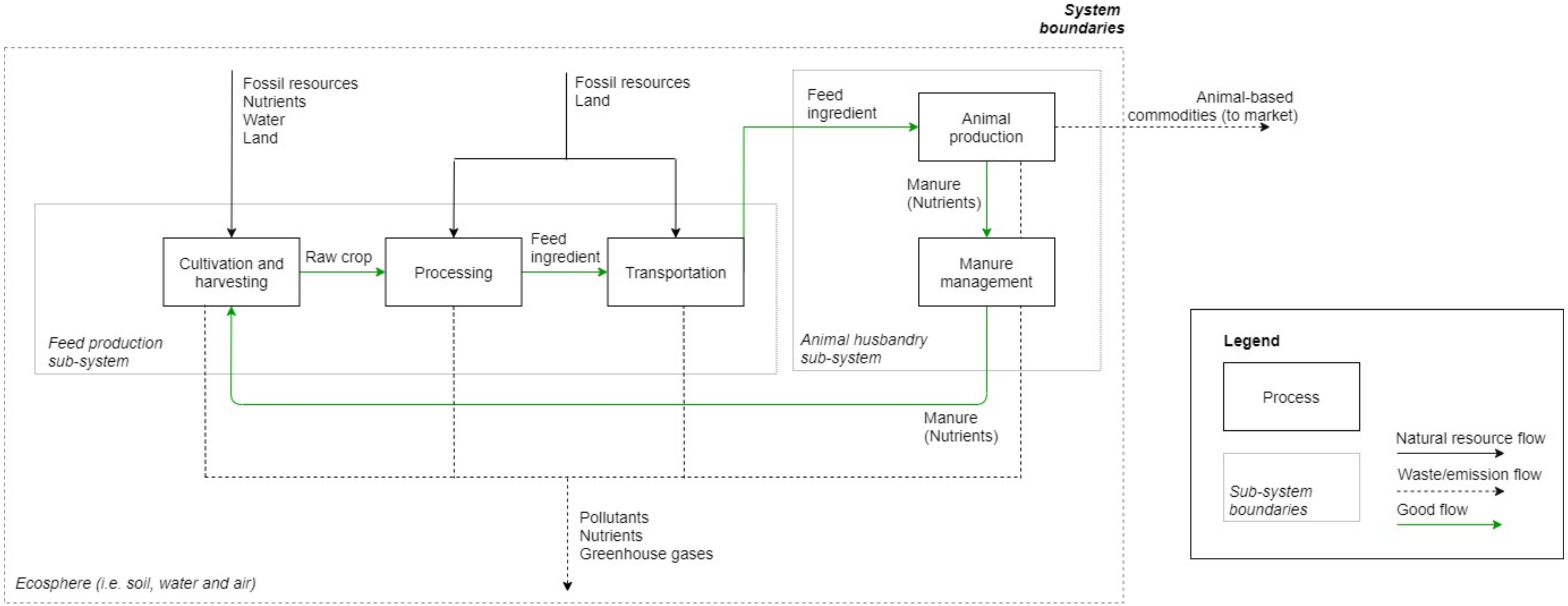


Figure 3.2. A schematic representation of the system boundaries central to the present study, including its subsystems. Zoom in for more detail.

depart from the Technosphere, entering the ecosphere, to eventually return to the FPS, are also considered as a feedback loop in the figure.

It must be noted that even in a conventional, predominantly linear FPS, there will always be a certain degree of circularity, due to the myriad of complex interactions between the soil, water, air and the cropland. Likewise, in a CAS, some wastes and emissions into the environment will persist, yet these flows are intended to be substantially smaller than in an LAS (Babu et al., 2020; Ghisellini et al., 2016).

The inputs of natural resources and outputs of emissions and wastes are linked to environmental impact types (see *Table 3.1*). According to Baumgartner et al. (2008) and Post et al. (2020), each of these categories has been recognised as a source of particular concern, has been studied intensively and monitored regularly and therefore can be considered useful for identifying relevant indicators to assess the environmental performance of FPSs.

Table 3.1. Environmental impacts of an FPS

Input-related impacts	Output-related impacts
Abiotic resources-related impacts	Nutrients-driven impacts
Water use-related impacts	Toxicity-driven impacts
Land competition-related impacts	Pollutants-driven impacts

The environmental impact types linked to an FPS' in-and outputs, used for indicator selection.

3.4. Indicator identification

A review of scientific literature review was conducted to identify indicators related to the environmental impact categories listed in *Table 3.1.* Four commands were executed in the digital database of Scopus, combining the terms "circular" "sustainability", "environmental impacts", "livestock", "animal", "feed", "indicator", and "production system". The full search commands and a disaggregation of the hits can be found in *Table A1* (see *Appendix A*). In total, 25 papers were selected, either directly or indirectly (through snowballing). These papers comply with the searching criteria outlined below.

Firstly, studies conducted by for-profit companies were excluded, as their independence could not be guaranteed. Secondly, studies that did not represent FPSs for the three large animal husbandry segments (i.e., pig, poultry and cattle) were excluded. Also, studies focussing on agricultural production in developing countries, lacking (highly) industrialised FPSs were left out. Thirdly, studies focusing on an entire livestock or food production system or only on the primary production stage, without explicitly addressing the environmental impacts of FPSs as a whole, were disregarded. Fourthly, the focus was limited to alternative crops (either terrestrial or aquatic) or side-streams of crops, excluding for example insects, yeasts and bacteria, as their FPSs, and therewith the indicators for assessing them, diverge.

Table A2 (see *Appendix A*) shows the indicators found per paper, and *Table 3.2* shows the collected papers, assigned to the environmental impact category for which they propose (an) indicator(s). These studies encompass a range of quantitative methods, including material flow analyses (MFA, e.g., Jouan et al., 2020), ex-post life cycle assessments (LCA, e.g., Lathuillière et al., 2017), agent-based modelling studies (e.g., Fernandez-Mena, 2020), or a mix of these (e.g., Eriksson et al., 2005). Others take on a more qualitative approach, presenting a literature review (e.g., Clark and Tilman, 2017), or a systemic description (e.g., Withers et al., 2018). Note that several papers assess the environmental performance of FPSs in different impact categories.

3.5. Indicator selection

The identified indicators meet a set of research-specific criteria, ensuring their relevance for assessing the environmental performance of different FPSs. Yet, not all of these indicators can be utilised in this study, because of academic and pragmatic constraints. Importantly, the selected indicators must be specific, measurable, comparable, sensitive to temporal change and representative type of impact they seek to evaluate (Niemeijer and De Groot, 2008; Post et al., 2020). Furthermore, it must be possible to quantify the impacts expressed by the indicator by means of a life cycle assessment (LCA). Finally, sufficient data should be available to conduct an analysis based on the chosen indicators, given the limited timeframe of the present study. In the section below, the identified indicators will be described briefly, and one or more will be selected per impact type. With this set of indicators, the overall environmental performance of the conventional FPS and the *Azolla*-based FPS will be assessed.

3.5.1. Input-related indicators

In terms of input-related indicators, eight different indicators were found, of which four were selected.

3.5.1.1. Abiotic resource-related impacts

Regarding abiotic resource-related impacts, two indicators were found: energy use (EU) and abiotic depletion (AD) (e.g., Baumgartner et al., 2008; Philis et al., 2018). EU, or cumulative energy demand, on the one hand, accounts for the (non-)renewable energy consumed by a product-system. AD, on the other, typically concerns the extraction of natural, non-living resources, including minerals and fossil fuels (e.g., iron ore, mineral phosphorus (P), crude oil and wind energy) from reserves (Guinée et al., 2002). It was decided to only include AD, as it offers a more optimal coverage of resource demand, beyond the use of energy alone. Although it among the most widely used impact categories, a standardized problem definition does not exist, meaning that results may vary across quantification methods (Guinée et al., 2002).

3.5.1.2. Water use-related impacts

As for water use, three indicators were found: the water use ratio (WUR), consumptive water use (CWU) and blue water use (BWU). Firstly, the WUR seeks to compare the efficiency of human food and livestock feed production in terms of water demand, which is not the aim of the present study. Secondly, the CWU allows for a complete picture of the water demand by considering different sources (i.e. (grey) waste water, (green) rain water and (tap) blue water), which is especially useful in agricultural processes, yet cannot be quantified effectively in an LCA context (Ran et al., 2017). Thirdly, the BWU reflects the unit of water consumed per unit of water extracted from surface water bodies or groundwater aquifers (e.g.., Huijbregts et al., 2017; Post et al., 2020; Rauw et al., 2020). It assumes that the depletion of blue water affects the green water cycle, and as such it does regard these indirect impacts (Huijbregts et al., 2017). Therefore, BWU is deemed the best option in the present LCA effort.

Table 3.2. Identified indicators per impact category

Impact type	Impact category	Explanation (indicator)	Equation of indicator	Unit	Applicable in LCA?	Citati-ons
Abiotic resources-related	Energy use	Amount of energy, fossil or renewable, used (energy consumed).	$EU = \sum i\ E_i$	MJ-eq.	Yes	11
	Abiotic depletion	Amount of minerals, fossils and metal ores withdrawn from deposits (depletion of the ultimate reserve in relation to annual use).	$AD = \sum i\ ADP_i\ \times m_i$	kg Sb-eq.	Yes	5
Water use-related	*Blue water use*	Amount of blue water required (blue water consumed from blue water extracted).	$BWU = \sum i\, W_i$	m³	Yes	6
	Consumptive water use	Amount of blue, green and grey water required (water consumed from water extracted).	$CWU = \sum i\ W_{i,green} + W_{i,grey} + W_{i,blue}$	m³	No	1
	Water use ratio	Amount of water needed to produce one unit of human digestible protein from animal-based versus human food products.	$WUR = \frac{CWU \times HDP_{food}}{HDP_{feed}}$	dn	No	1
Land use-related	*Land competition*	Amount of land transformed an maintained in the transformed state (land occupation).	$LC = \sum s\, U_s$	km/y	Yes	15
	Land use risk	Amount of land transformed and occupied in response to direct land use (direct and indirect land occupation).	$LURI = LC_{direct} + LC_{indirect}$	km	No	2
	Land use ratio	Relative amount of land needed to produce one unit of human digestible protein from animal-based versus human food products.	$LUR = \frac{LO \times HDP_{food}}{HDP_{feed}}$	dn	Yes	2

Nutrients-driven	Nutrient balance	Amount of nutrients present in soil (nutrient inputs minus nutrient outputs).	$NS = IN_N + IL_N - OUT_N - \Delta S_N$	kg/t	No	7
	Nutrient efficiency	Amount of nutrients input that is needed for a desired output (nutrient inputs over nutrient outputs).	$NE = \frac{OUT_N}{(IN_N + IL_N - \Delta S_N)}$	%	No	3
	Nitrate pollution of groundwater	Average nitrate concentration in the shallow groundwater layer under agricultural land.	$NPG = \frac{S_N}{LO}$	%	No	2
	Terrestrial eutrophication	Impact of nutrients on the functioning of the soil surface (deposition N/P eq. in biomass).	$TE = \sum i \ TEP_i \times m_i$	kg PO_4^{3-}-eq.	Yes	11
	Aquatic eutrophication	Impact of nutrients on the quality of freshwater/marine bodies (deposition N/P eq. in biomass).	$AE = \sum i \ AEP_i \times m_i$	kg PO_4^{3-}-eq.	Yes	11
	Aquatic and terrestrial acidification	Impact of nutrients on the functioning/quality of the soil and surface water bodies (deposition/ acidification critical load).	$ATA = \sum i \ AP_i \times m_i$	kg SO_2-eq.	Yes	8
	Relative crop and grass production area	Impact of nutrients on the functioning/quality of soil and surface water bodies (land used for crop production over total area of livestock feed production).	$RCGPA = \frac{LUCP}{LU}$	%	No	1
	Chemical water quality standard exceedance	Impacts of nutrients on the functioning/quality of surface water bodies (water bodies with exceeding nutrient levels over all water bodies).	$CWQSE = \frac{WBENL}{TWB}$	%	No	1
Toxicity-driven	*Terrestrial ecotoxicity*	Impact of heavy metals and toxic substances to soil quality (predicted environmental concentration).	$TET = \sum i \sum ecom \, TETP_{ecom,i,t} \times m_{ecom,i}$	kg 1,4-DCB-eq.	Yes	3

	Freshwater aquatic ecotoxicity	Impact of heavy metals and toxic substances to freshwater quality (predicted environmental concentration).	$FAET = \sum i \sum ecom\, FAETP_{ecom,i,t} \times m_{ecom,i}$	kg 1,4-DCB-eq.	Yes	4
	Human toxicity	Impact of heavy metals and toxic substances on human health (acceptable daily intake).	$HET = \sum i \sum ecom\, HTP_{ecom,i,t} \times m_{ecom,i}$	kg 1,4-DCB-eq.	Yes	4
Pollutants-driven	*Climate change*	Impact of greenhouse gases emissions on climate change (infrared radiative forcing).	$CC = \sum i\ GWP_{a,i} \times m_i$	t CO_2-eq.	Yes	15
	Stratospheric ozone depletion	Impact of substances on the depletion of the atmospheric ozone layer (stratospheric ozone break down).	$SOD = \sum i\ ODP_{\infty,i} \times m_i$	kg CFC-11-eq.	Yes	3
	Photo-oxidant formation	Impact of nitrogen oxides (i.e. NO_x) and volatile organic compound (i.e. VOC) emissions on human health (tropospheric ozone formation).	$POC = \sum i\ POCP_i \times m_i$	kg ET-eq.	Yes	4

An overview of the indicators identified in the literature review, with a brief description, the corresponding equation, unit and number of citations. The indicators selected for further analysis are marked in italic. The meaning of abbreviations shown in the table can be found below.

Abbreviations:

a: year

t: time

U: land

m: mass

i: substance

s: state

$ecom$: emission compartment

dn: dimensionless

km: kilometre

kg: kilogram

t: tonne

mm: millimetre

S: stock

N: nutrient

E: energy consumption

W: water consumption

IN: input

OUT: output

IL: indirect losses

L: litre

MJ: megajoule

ABP: animal-based product

TWB: total water bodies

HDP: human digestible protein

$WBENL$: water bodies with exceeding nutrient level

GWP: global warming potential

ODP: ozone depletion potential

$POCP$: photochemical ozone creation potential

TEP: terrestrial eutrophication potential

AEP: aquatic eutrophication potential

AP: acidification potential

$TETP$: terrestrial ecotoxicity potential

$FAETP$: freshwater aquatic ecotoxicity potential

$MAETP$: marine aquatic ecotoxicity potential

HTP: human toxicity potential

3.5.1.3. Land use-related impacts

For land use, three indicators were identified: the land use ratio (LUR), land competition (LC) and land use risk (LURI). The LUR is excluded for the same reason as is the WUR (Van Zanten et al., 2014). The LC considers the temporary unavailability of land due to human occupation (e.g., Dekker et al., 2013 and Tzachor, 2019). Since LC is a direct driver of, for example, species diversity and soil disturbance, it can offer a proxy for these impacts, and hence will be applied in the present study (Borelli et al., 2020; De Baan et al., 2013). It is assumed that upon land conversion, the land passively recovers to a (semi-)natural habitat. Besides, it must be noted that only land occupied directly by the examined FPS will be accounted for. In contrast, the LURI does include land occupied indirectly, in response to a change in demand for another product, which is highly relevant in the light of this study, but a lack of data and methods for inclusion into an LCA obstructs its application (e.g., Leinonen et al., 2013; Meul et al., 2012).

3.5.2. Output-related indicators

With regard to output-related indicators, fourteen indicators were identified, of which eight were selected.

3.5.2.1. Nutrient-driven impacts

With respect to nutrient-driven impacts, eight different indicators were found. Of these, only three can, and will, be applied in LCA, being: terrestrial and aquatic eutrophication (TE and AE) as well as aquatic and terrestrial acidification (ATA). The TE and AE represent the excessive transfer of nutrients, most importantly of nitrogen (N) and P to the soil and water, causing undesirable shifts in species composition and biomass production (e.g., Eriksson et al., 2005). The ATA quantifies the impact of emissions of acidifying substances to the air, and subsequent deposition to the soil and water (e.g., De Alvarenga et al., 2012). All three represent a variety of impacts on ecosystems, human health and materials. The remaining indicators, which among other things represent the state of a particular ecosystem (e.g., the chemical water quality standard exceedance, or CWQSA) or the efficiency of a product-system (e.g., nutrient efficiency, or NE), cannot be measured by means of an LCA and are therefore excluded (e.g., Jouan et al., 2020; Post et al., 2020).

3.5.2.2. Toxicity-driven impacts

For toxicity-driven impacts, three indicators, all applicable in an LCA, were identified: terrestrial and freshwater aquatic ecotoxicity (TET and FAET, respectively), as well as human ecotoxicity (HET). The first two indicators measure the impact of a predicted concentration of heavy metals and toxic substances on the quality of a particular environmental compartment. Since the present study focuses on terrestrial and freshwater cropping systems, it is expected that the TET and FAET will provide an adequate representation of damage inflicted, hence both are included. On top, the HET will be used to assess the impacts of heavy metals and toxics on human health by, aid of the acceptable daily intake of harmful components. Because the discussion on modelling techniques these impact categories is far from settled, results must be considered with caution (Baumgartner et al., 2008; Guinée et al., 2002).

3.5.2.3. Pollutants-driven impacts

Turning to the remaining pollutants-driven impacts, indicators include climate change (CC), stratospheric ozone depletion (SOD), and photo-oxidant formation (POF). Again, all are quantifiable in the context of an LCA, and sufficient data is available to carry out an assessment of the FPSs of interest. To begin with, the CC quantifies the impact of (anthropogenic) greenhouse gas (GHG) emissions on atmospheric radiative forcing (e.g., Clark and Tilman, 2017). Besides, the SOD refers to the thinning of the stratospheric ozone layer, caused by emissions such as chlorofluorocarbons (CFCs), and leading to a heightened fraction of ultraviolet (UV) radiation reaching the earth's surface (e.g., Eriksson et al., 2018). Finally, the POF, also known as summer smog, concerns the formation of reactive chemical compounds, like ozone (O_3), as a result of the interaction between UV light and primary air pollution (e.g. of NO_x and VOC) (e.g., De Alvarenga et al., 2012). Since these indicators complement one another by assessing different effects on organisms, ecosystems, and natural resources, each will be included for a full representation of the pollutants-driven impacts (Guinée et al., 2002).

3.5.3. Excluded environmental impacts

Because of exploratory character of this study, a set of indicators is selected that offers a starting point for a more all-encompassing environmental assessment. As a result, a variety of indicators are left out of consideration, which are elaborated on further in the *Chapter 6*.

Chapter 4. Circular *Azolla* FPS design

In *section 1.2.1*, four criteria were mentioned that an FPS should meet to suitable for incorporation in a CAS. Based on these criteria, *Azolla* was selected as a potential candidate for replacing conventional, protein-rich livestock feed. Nevertheless, since a standardised, *Azolla*-based FPS does not exist in practice yet, knowledge is lacking on what unit processes such a system should consist of. Therefore, this chapter seeks to identify the different options for producing *Azolla*-based livestock feed*,* and integrate these into alternative production systems. In combination, this will answer the following question: *How could Azolla-based feed production be operationalised such that it has the highest potential of fitting in a CAS?*

The following sections will consider the main unit processes that together make up an *Azolla*-based FPS. These include cultivation (*section 4.1*), harvesting (*section 4.*2), and processing and storage (*section 4.3*). Finally, the options that score best in terms of the IROT concept, integral to the CAS definition, will be used to compile two alternative, *Azolla*-based FPSs (*section 4.4*).

4.1. Cultivation

Azolla requires few inputs in order to thrive. In terms of fossil resources, fuel is needed to power the machines for dispersing *Azolla* sporophyte culture. From cultivation experiments, it has appeared that the application of insecticides is not necessary, because of a fern-specific gene that confers a strong insect resistance (Li et al., 2018). In terms of insects, the most substantial threat to *Azolla* is the weevil, an invasive species that feeds on the fern, and can be fought with the insectivore endoparasite *Boveria* (B. van de Riet, personal communication, September 14, 2020). In addition, a low dosage of fungicide might be needed to ensure the spread of moulds (J. Adema, personal communication, November 2, 2020).

In terms of nutrients, the fern’s main growth-limiting factor is P, which it absorbs efficiently when diffused in water. Its symbiotic relationship with the blue-green algae *Anabenae azollae* provides ample ammonium (NH_4^+) through the fixation of atmospheric N (Brouwer, 2017). Ideally, *Azolla* cultivation is combined with phytoremediation (i.e. the ability of aquatic plants to recycle nutrient in polluted water) on a residual flow of nutrients, such as slurry (i.e. manure effluent of livestock production), or taps into P present in the soil, unavailable to arable crops (Afkairin et al., 2021; Muradov et al., 2014; Tallentire et al., 2018). As for water, the fern requires a layer of ±10-15 cm of (rain)water to grow on in order to develop its roots (Sawant, 2018). Evidence exists that surface water covered with *Azolla* hardly evaporates, which limits the water demand (Kollah et al., 2016).

4.1.1. Locations for cultivation

The question remains what sort of location is most optimal for the cultivation of *Azolla*. As summarised in *Table 4.1a*, a suitable location preferably requires minimal external fossil resources, nutrients, and water, tapping into locally available, reusable resources, while only occupying marginal land and providing a high yield.

Table 4.1a. Criteria for *Azolla* cultivation in a CAS

Flow	Applied to cultivation
Input (I)	Minimal use of fossil resources, nutrients, water and (marginal) land.
Reuse (R)	Maximal reuse of water and nutrients.
Output (O)	Minimal emissions of GHGs and other pollutants.
Throughput (T)	Maximal yield of usable *Azolla*, preferably year-round.

An overview of the IROT concept, applied to *Azolla* cultivation.

One option is to cultivate *Azolla* in an open pond, outdoors (see *Figure 4.1a*). In such a setting, a foil is spread out to cover the soil, and properly secured between clay or sand embankments. The pond is filled with water, mixed with for example, pig or poultry slurry (Utomo et al., 2019). Alternatively, the manure-enriched cultivation pond is installed indoors, for instance on top of the livestock shed, with the heat radiated by the animals accelerating the growth rate of *Azolla* (see *Figure 4.1b*) (Kroes et al., 2016). Furthermore, *Azolla* can be cultivated in ditches and canals surrounding agricultural land (see *Figure 4.1c*). Finally, *Azolla* can be applied in paludiculture, referring to the practice of crop production on inundated wetland (see *Figure 4.1d*). Especially in regions where water levels are lowered to facilitate cattle grazing, like in the northern and western provinces of the Netherlands, paludiculture is posed as a solution for counteracting soil subsidence (Joosten et al., 2016; Hardeveld et al., 2020).

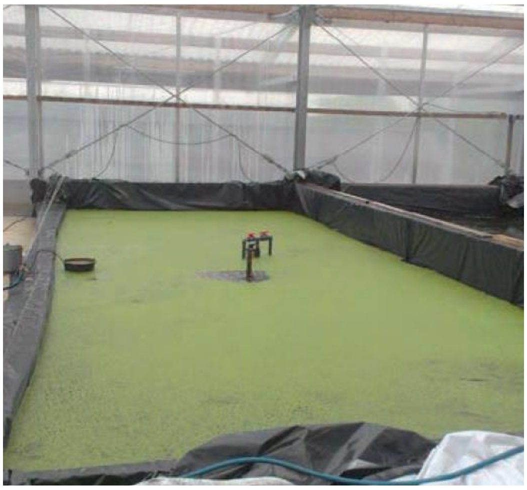
 

Figure 4.1a. Outdoor pond cultivation (Van de Riet, n.d.) and *b*. Indoor pond cultivation (Kroes et al., 2016).

Figure 4.1c. Ditch with *Azolla*-cultivation (Van de Riet, n.d.).

Figure 4.1d. An experimental setting of wetland inundation with *Azolla* cultivation (Van de Riet, n.d.).

Table 4.1b shows these options, as well as their main (dis)advantages in terms of resource inputs (both external and through reuse), emission outputs, and an estimation of the annual yield. The main trade-off is between the possibility of cultivating *Azolla* year-round, at high quantities (i.e., maximal throughput), and the input of resources (i.e., minimal input). For instance, the outdoor systems appear to have a relatively low yield, yet with a minimal input of external resources (J. Adema, personal communication, November 2, 2020). For the indoor, closed-loop system, the opposite holds, as the controlled conditions could potentially facilitate a high yield, yet require controlled settings, accompanied by a high energy intensity (Brouwer et al., 2017). However, experiments conducted with indoor duckweed cultivation have so far not proven successful, due to the high investments and dissatisfying yields (Kroes et al., 2016).

Furthermore, the addition of slurry as a mineral fertiliser gives rise to two problems. Firstly, the *Azolla* biomass will have to be washed at a later point, to remove the smell and taste of dung, increasing the water demand downstream (Sawant, 2018). Secondly, the heavy metals embedded within manure are released into the water, implying that to prevent contamination, further treatment will be needed before the water can be discharged safely into the environment (Timmerman and Hoving, 2016).

The outdoor systems (except for the outdoor pond) offer the benefit of nutrient reuse, and simultaneously act as a water and carbon buffer, reducing the need for external inputs and thereby enhancing to the system's circularity. Especially in wetland agriculture, these benefits have been demonstrated repeatedly with a variety of crops, including reed and cattail (Jurasinski et al., 2020). In recent studies, it has been found that *Azolla* cultivation in Dutch wetland microcosms can actively contribute to mining immobile P soil legacies (Wang et al., 2019b), storing soil organic carbon (Smolders et al., 2013) and retaining surface water (Smolders et al., 2019), while enabling a high biomass yield (Jurasinski et al., 2020). Therefore, from a circularity perspective, the *Azolla* cultivation in the Dutch flooded wetland setting is considered as the most promising option for the first stage in the *Azolla*-based FPS design.

Table 4.1b. A comparison of different *Azolla* cultivation systems on the criteria of *Table 4.1a*

Option	*Advantages*	*Disadvantages*	*Yield*
Open pond installation (e.g., Dairy farm in Lemmer, Friesland and Landsmeer, North-Holland)	+ Applicable on marginal land (I). + Suitable for removing nutrients from slurry, reducing nutrient emissions (R, O, I).	- Not feasible all year around (T). - Requires water and energy for washing to remove the slurry (I). - Heavy metal emissions from slurry may occur (O).	5-20 t dm/ha/y (T)
Indoor pond installation (e.g., Ecoferm experimental farm)	+ Applicable on top of the farm, not requiring additional land (I). + Feasible all year round (by capturing heat of the animals) (I, T). + Suitable for removing nutrients from slurry, reducing nutrient emissions (R, O, I).	- Requires extra infrastructure and resources, including (LED) lights and heating during winter time (I). - Requires water for washing to remove the slurry (I). - Heavy metal emissions from slurry may occur (O).	10-35 t dm/ha/y (T)
Inundated wetland (e.g., Polder Zuiderveen, Groningen)	+ Applicable on marginal land (I). + Suitable for reusing nutrients present in the soil (e.g., P mining) and buffering water on the land, reducing nutrient and GHG emissions (R, O, I).	- Not feasible all year around (T).	15-20 t dm/ha/y (T)
Ditches (e.g., Dairy farm in Stolwijk, South-Holland)	+ Suitable for reusing nutrients present in the soil (e.g., P mining), storing carbon and buffering water on the land, reducing nutrient and GHG emissions (R, O, I).	- Not applicable on marginal land, because waterways are needed for drainage, and cultivating *Azolla* may causes clogging (I).	5-20 t dm/ha/y (T)

An evaluation of the different options for *Azolla* cultivation, based on the CAS definition.

4.2. Harvesting

Once matured (having reached a size of ±2-2.5 cm), *Azolla* is to be removed from the surface water. Typically, *Azolla* doubles in biomass every two to three days, under favourable weather conditions (Brouwer, 2017). In the outdoors, *Azolla* can be harvested between April and October. For duckweed, which has a similar growth rate to *Azolla*, removing 20% of the total biomass at three-day intervals is regarded optimal. This frequency has appeared most effective to enhance nutrient recovery and prevent algae growth as well as double bedding (i.e. when plants start overlapping), resulting in a stagnation of growth and subsequent rotting (Hoving et al., 2012; Tallentire et al., 2018). A similar harvesting regime is assumed suitable for *Azolla*.

4.2.1. Methods for harvesting

Ideally, the applied harvesting method consumes minimal energy, where possible renewable, to avoid emissions. Also, it preferably enables the harvesting of high-quality *Azolla*, implying that only little water, dirt and remnants of deeper (water) plants are scooped up. *Table 4.2a* summarises the criteria that a harvesting method should live up to qualify for implementation in a CAS, and *Table 4.2b* compares the methods based on these criteria.

Table 4.2a. Criteria for *Azolla* harvesting in a CAS

Flow	Applied to harvesting
Input (I)	Minimal use of fuel, electricity and water.
Reuse (R)	Not applicable.
Output (O)	Minimal emissions of GHGs and other pollutants.
Throughput (T)	Maximal yield of high-quality *Azolla*.

An overview of the IROT concept, applied to *Azolla* harvesting.

Different methods exist to remove plant biomass from the top layer of the surface water, most of which have been utilised to remove excess duckweed to unclog ponds and ditches. Yet, these methods are also applicable for harvesting *Azolla*. The four most promising are highlighted in the present section (Timmerman and Hoving, 2016).

First of all, a wheeled excavator may be used to remove plants from the water surface. An excavator can be equipped with either a flat sieve pan (see *Figure 4.2a*), or a dredge covered with a fine gauze (see *Figure 4.2b*), dipping in the surface water to remove the weed, and relocating it to a tip-truck (Timmerman and Hoving, 2016). Secondly, *Azolla* can be harvested with a paddle wheel that floats on the water, automatically transferring the water top layer to the waterside over a solar-powered conveyor belt (see *Figure 4.2c*). Thirdly, the aquatic weed band constitutes of a reel with 100 meters of band, attached to a floating unit (see *Figure 4.2d*). A person unrolls the band to enclose the area of the water that is to be harvested, after which the reel coils the band and draws in the weed. This method can be combined with a wheeled excavator or a solar-powered conveyor belt to speed up the harvesting rate. Therefore, it is most useful in case a high harvesting rate is desirable, or on large patches of land (G.J. Bom, personal communication, November 20, 2020). Finally, the aquatic weed skimmer consists of a small floating unit, which creates a vortex of water, drawing in the water plants and pumping it through hoses to an onshore filtration unit, from where the water returns to the water body (see *Figure 4.2e* and *4.2f*) (Meers and Coudron, 2018).

Figure 4.2a. and *4.2b.* Wheeled excavator with dredge and wheeled excavator with flat sieve (Timmerman and Hoving, 2016).

Figure 4.2c. and *4.2d.* Solar-powered conveyor belt and aquatic weed brace (BomTechniek BV, n.d.).


Figure 4.2e and f. Aquatic weed skimmer, featuring the onshore filtration unit and hoses (Meers and Coudron, 2018).

Each harvesting method has its advantages and limitations. The main trade-off identified, concerns the speed of *Azolla* harvesting, and quality of the harvested biomass. For instance, the wheeled excavator with flat sieve pan rapidly removes *Azolla* from the surface water, but the purity of the material is low, as it includes a lot of dirt and water plants. As a result, the volume of the biomass is much higher, thereby increasing the transport load. Moreover, biomass has to be subjected to a more extensive treatment of separation and rinsing, adding to the energy- and water demand later on in the value chain (Timmerman and Hoving, 2016). Another trade-off occurs between the harvesting speed and the energy- and emission-intensity. While the diesel- or petrol-fuelled machines harvest more efficiently, the slower techniques can be powered by (renewable) electricity, resulting in lower emissions (G.J. Bom, personal communication, November 20, 2020).

Table 4.2b. A comparison of different *Azolla* harvesting methods on the criteria of *Table 4.2a*

Method	*Advantages*	*Disadvantages*	*Rate*
Wheeled excavators	Flat sieve pan: + High harvesting quality (T).	Flat sieve pan: - Low harvesting efficiency, hence time-consuming (T). - High energy demand, and diesel-fuelled, so high in emissions (I, O).	±4-8 t wm/d (T)

	Dredge with fine gauze: + High harvesting efficiency (T).	Dredge: - Low harvesting quality (T). - High energy demand, and diesel-fuelled, so high in emissions (I, O).	±10-18 t wm/d (T)
Solar-powered conveyor belt	+ High harvesting quality (T). + Low energy demand, (solar) powered entirely, so low in emissions (I, O).	- Low to medium harvesting efficiency, hence time-consuming (T).	± 6-10 t wm/d (T)
Aquatic weed skimmer	+ Low energy demand, (solar) electricity-powered entirely, so low in emissions (I, O).	- Low harvesting quality (T). - Low to medium harvesting efficiency, hence time-consuming (T).	± 6-10 t wm/d (T)
Aquatic weed band with electric motor	+ High harvesting quality (T). + High harvesting efficiency (T). + Low energy demand, so low in emissions (O) (if combined with a solar-powered conveyor belt).	- Cannot be used by itself, requires to be combined with another harvesting method.	±10-18 t wm/d (T)

An evaluation of the different options for *Azolla* harvesting, based on the CAS definition.

4.3. Processing and storage

Fresh *Azolla*, with a moisture content of ±93.3 wt%, is highly perishable and needs to be processed in order for it to be stored longer (Brouwer, 2017). Also, to safeguard feed security (e.g., killing pathogens, like E. coli bacteria), to maintain its nutritional value, ensure feed homogeneity and remove the sandy taste, subjecting the harvested biomass to a treatment is essential (Hoving et al., 2012; J. Adema, personal communication, November 2, 2020).

4.3.1. Methods for processing and storage

To achieve the highest attainable degree of circularity, the applied processing method should consume minimal fuel, electricity and water, while releasing as little GHGs and pollutants as possible. Its aim should be to generate a high-quality feed that can be stored without losing its nutritious value. *Table 4.3a* summarises the criteria that processing options should meet, and *Table 4.3b* compares the identified options based on these criteria.

Table 4.3a. Criteria for *Azolla* processing in a CAS

Type	Flow
Input (I)	Minimal use of fuel, electricity and water.
Reuse (R)	Not applicable.
Output (O)	Minimal emissions of GHGs and other pollutants.
Throughput (T)	Maximal high-quality, nutritious feed, suitable for storage year-round.

An overview of the IROT concept, applied to *Azolla* processing and storage.

First of all, after the *Azolla* has been harvested, it may be pressed directly to a moisture content of about 90 wt% and ensiled with a sugar-rich additive (e.g., molasses, pulp or maize) (see *Figure 4.3a* and *b*) (Hoving et al., 2011). Alternatively to ensilage, *Azolla* may be dried. Since sun-drying of *Azolla* is not considered feasible in the temperate climate of the Netherlands, an energy source is needed instead. An experiment has shown that in a gas-heated drying room of 40°C, it takes about 30 hours for a batch of about 4 t wm of duckweed to be dried to a product with a moisture content of 10 wt% (Holshof, 2009).

After the ensilage or drying phase, further treatment could be opted for. A milling machine (typically a hammer mill) may be utilised to grind the dried *Azolla* into a powder (see *Figure 4.3c* and *d*). Subsequently, this powder can be combined with a binding agent (e.g., starch or oilseed by-products) and pressed into feed pellets. Holshof et al. (2009) produced such pellets of 96% duckweed and 4% molasses, suggesting that *Azolla* can be processed in the same way (see *Figure 4.3e* and *f*) (Hoving et al., 2011).

Table 4.3b. A comparison of different *Azolla* processing methods on the criteria of *Table 4.3a*

Method	*Advantages*	*Disadvantages*	*Capacity*
Pressing of fresh biomass	+ Low energy requirement (I). + Lowers the volume, reducing transport movement, avoiding emissions (O). + Reduces nutrient emissions occurring during silage (O).	Not applicable.	3-15 m^3 wm/h (depending on the machinery sub-type and processing efficiency)

Ensiling of pressed biomass	+ Low energy requirement (I). + Can be done on-site, preventing transport movement, avoiding emissions (O).	- Nutrient emissions may occur as the biomass content is pressed to reduce water content (O).	1-33 t wm/h, ensiling one batch takes ±6 weeks (T)
Drying of fresh biomass	+ Lowers the volume, reducing transport movement, avoiding emissions (O). + Could make use of (industrial) waste heat (R).	- High energy requirement (I). - Cannot be done on-site, requiring transport movement (I, O).	4-10 t wm/round, drying one batch takes ±30 hours (T)
Milling into powder	+ Lowers the volume, requiring less transportation, avoiding emissions (O).	- High energy, requirement (I). - Cannot be done on-site, requiring transport movement (I).	1-26 t dm/h (depending on the machinery sub-type and processing efficiency) (T)
Pelletising into (compound) feeding granules	+ Lowers the volume, requiring less transportation, avoiding emissions (O).	- High energy requirement (I). - Cannot be done on-site, requiring transport movement (I).	1-15 t dm/h (depending on the machinery sub-type) (T)

An evaluation of the different options for *Azolla* processing and storage, based on the CAS definition.

Figure 4.3a. Screw press *Azolla* treatment and *b.* ensilage treatment (Hoving et al., 2011).

Figure 4.3c. Feed hammer mill machine (ABCmachinery, n.d.-a) and *d*. *Azolla* after milling treatment (Holshof, 2009).

Figure 4.3e. Feed pelleting machine (ABCmachinery, n.d.-b) and *f. Azolla* after pelleting treatment (Holshof, 2009).

Some form of treatment is needed to maintain the quality of *Azolla* biomass until consumption. Yet, the more processing steps are added, the more energy-intensive the treatment becomes. If the fresh *Azolla* is merely pressed and ensiled on-farm, energy use could be kept at a minimum and transport movements are averted. Yet, the end product can only be preserved for weeks up to months, meaning that feed shortages may emerge during the winter months, when low temperatures impede *Azolla* cultivation. Also, the pressing and ensiling of *Azolla* may result in nutrient losses, at the expense of its nutritive value (Hoving et al., 2012).

Alternatively, the wet *Azolla* biomass may be dried, milled and granulated into pellets. These steps would delay its expiry date, enabling farmers to store the feed up to a year. However, in a feasibility study, Derksen and Zwart (2010) found that the milling and pelletising of unconventional, protein-rich crops demands for a processing capacity of at least 10,000 t dry mass (dm)/y (the equivalent to 500 ha, assuming 20 t dm/ha/y). Including these processing options in an *Azolla*-based FPS would only be viable with access to a regional processing facility. The fuel needed for transport to such an off-site facility, plus the electricity and gas needed for treatment, render these options energy-intensive. Also, according to Hoving et al. (2012), drying duckweed biomass only is attractive if a high protein content is guaranteed, or a cheap flow of residual heat (e.g., from an agro-industrial complex) is available.

4.4. *Azolla*-based FPS alternatives design

As laid out in *section 2.3.1.3*, scale is a key aspect in the development of future production scenarios. The production capacity of an FPS strongly affects crucial technology performance parameters, such as energy efficiency. At the moment, the scale at which *Azolla*-based feed will be produced as a substitute for conventional protein-rich feed, is highly uncertain. Its implementation in the agricultural system is complicated by a variety of demographic, economic, social, technological, ecological and political factors, such as resistance to change among Dutch farmers, fluctuations in the market price of soy, and changes in legislation (Faber, J., personal communication, December 2, 2020; Hoving et al., 2012).

Because of the clear target of reaching full circularity in the agricultural sector by 2050, the future environmental impacts of *Azolla*-based FPSs are determined by aid of normative scenarios (Arvidsson et al., 2018). To this end, it is decided to design two *Azolla*-based FPSs, which both represent another production scale extreme. At first, a local, *Azolla*-based FPS is designed, enabling feed production entirely at farm-level. Afterwards, a regional, *Azolla*-based FPS is compiled, for production at a regional level. It is assumed that differences in scale will affect resource use and efficiency, determining the systems' environmental performance.

4.4.1. Local *Azolla*-based FPS

The main feature of the local FPS is that it is implemented entirely on a single farm. This implies that the fewer processing steps needed to transform the fresh *Azolla* biomass into the final feed product, the better. Also, the production system ideally requires no advanced technological equipment and does not rely on regionalised supply chains, as to safeguard on-farm production autonomy insofar possible.

From the above analysis, the inundated wetlands appear most suitable as a location for *Azolla* cultivation in the context of a CAS. It does not necessitate any large upfront investments, contrary to the indoor pond installation, or additional processing step to remove slurry, as for the outdoor open pond (Kroes et al., 2016; J. Adema, personal communication, November 2, 2020). Therefore, it is most fit for local use. As for harvesting, the solar-powered conveyor belt scores best in terms of circularity. Since its mechanism is low-maintenance and easy to use, it is considered appropriate for implementation at a local level. Moreover, its relatively low harvesting capacity is assumed to be sufficient for farming businesses with a small to medium acreage (G.J. Bom, personal communication, November 20, 2020). With this harvesting method, the removed biomass is typically clean and of high quality, hence an additional rinsing step is avoided. Since the *Azolla* feed will only be used directly on-farm, no extensive conservation period is needed. To that end, pressing and ensilage, both solely requiring simple machinery, generally available on pasture-based farms, as is the case in Dutch wetland farming environments, are regarded as realistic and satisfactory processing methods (Hoving et al., 2011). Until consumption, the ensiled *Azolla* feed is stored in reusable big bags. *Figure 4.4a* presents a diagram of the local, *Azolla-based* FPS.

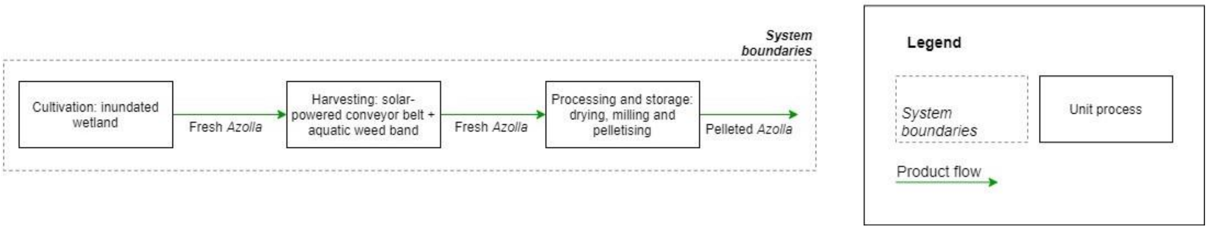


Figure 4.4a. A schematic representation of the local *Azolla*-based FPS. Zoom in for more details.

4.4.2. Regional *Azolla*-based FPS

In contrast to the local *Azolla*-based FPS, the regional alternative does not solely rely on the assets of an individual farm. Instead, the FPS is integrated into a regional supply chain, in which each link adds value to the feed product. On a regional level, options for more advanced processing techniques are unlocked.

In a regional level FPS, the scalability of *Azolla* cultivation is a major concern. After all, the feed is supposed to play a role on the regional livestock feed market. While indoor cultivation ponds as well as ditches and canals are limited in size, drained wetlands are omnipresent in the Dutch agricultural landscape, covering over 220,000 ha in the north and west alone (Smolders et al., 2019). Due to this high scaling potential, *Azolla*-based inundated wetland agriculture will be integrated as the first stage in the regional *Azolla*-based FPS design (Brouwer et al., 2017). Next up, a method is required that enables harvesting at a high rate, in order to upkeep biomass throughput for meeting the regional demand. To that end, it is decided to pair up the solar-powered conveyor belt and the aquatic weed brace (G.J. Bom, personal communication, November 20, 2020; Tallentire et al., 2018). Afterwards, the fresh *Azolla* biomass is to be processed such that it complies with the regional standards regarding feed security, health and homogeneity (Hoving et al., 2012). In a regional facility, advanced processing options become viable (Derksen and Zwart, 2010). Here, fresh *Azolla* biomass flows from farms situated across the region are gathered, combined and subsequently dried, milled and pelletised. The final feed product is stored in big bags, and sold on the regional feed market. *Figure 4.4b* shows a diagram of the regional, *Azolla-based* FPS.

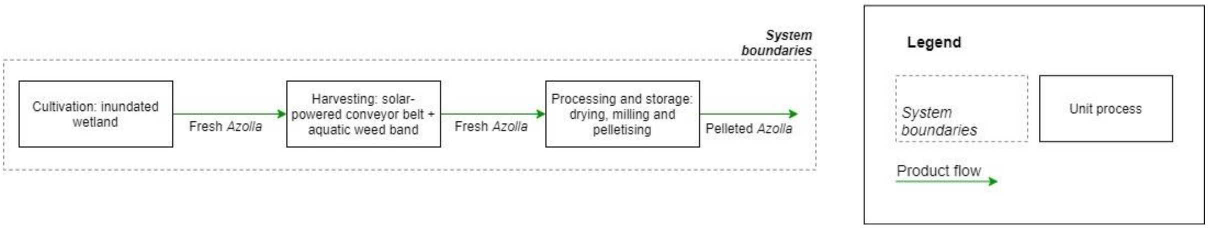


Figure 4.4b. A schematic representation of the regional *Azolla*-based FPS. Zoom in for more details.

Chapter 5. Environmental impacts of FPS scenarios

In the previous chapter, two alternative, *Azolla*-based FPSs were designed in alignment with the CAS definition. A distinction is made between a local *Azolla*-based FPS (i.e., LA-FPS) and a regional *Azolla*-based FPS (i.e., RA-FPS), both consisting of different unit processes. The present chapter seeks to combine these alternatives and a C- FPS into future feed production scenarios and quantify the associated environmental burdens. Therewith, the third SQ is addressed: *What is the environmental sustainability performance of different feed production scenarios, involving* Azolla-*based and conventional FPSs?* Since within this chapter, the LCA methods and results cannot be treated as strictly separate, *section 5.3* up to *5.6* will start with a brief methods paragraph, followed by the results.

5.1. Feed production scenario development

As laid out in *sub-section 2.3.1*, a way of dealing with the absence of certainty on how the future will unfold, yet with an end goal in mind, is to use normative scenarios. These scenarios are described in terms of the innovation life cycle model (Huber, 2003) and the customer adoption curve (Treloar, 1999).

The results are described in *sub-sections 5.1.1* up to *5.1.3*. Note that in the normative scenarios, the year 2035 marks an inflection point. By then, 50% of the conventional feed is replaced by *Azolla*-based feed. From then onwards, the progressive growth-curves display a saturated behaviour, meaning that the expansion of *Azolla*-based FPSs decreases as there are fewer and fewer new customers.

5.1.1. Scenario 1: Business-as-usual

The business-as-usual (BAU) scenario construes how the FPSs currently in place roughly remain unchanged in the decades to come. Low-cost conventional livestock feeds continue to be available in vast quantities, so that the agricultural sector is not encouraged to invest in novel feeds. Likewise, political decision-makers barely give financial incentives to support the adoption of local feed resources, nor do they alter legislation as to allow the entrance of novel feeds to the market. In the meantime, some bottom-up, collaborative pilot projects are initiated that aim to experiment with the production of *Azolla*, resulting in the emergence of an LA-FPS at a small scale. Yet, such initiatives merely operate in a niche, characterised by early technology enthusiasts with a high willingness to improve their farm's environmentally-friendliness. Therefore, *Azolla*-based FPSs do not get the chance to diffuse, so that by the middle of the 21st century, the C-FPS still very much prevails in the agro-industrial complex.

5.1.2. Scenario 2: Local farming projects

The local farming projects (LFP) scenario describes how the innovative, *Azolla*-based FPSs gradually, yet pervasively, replace the C-FPS. This scenario can be divided into two phases. In the first phase (2020-2035), a raised interest in sustainable feed alternatives causes the LA-FPS to gain ground. A number of early adopters prove the novel feed to be a worthy substitute, and soon a majority of pragmatists follows in their footsteps. By the start of the second phase (2036-2050), even conservative farming business and initial sceptics start converting to an LA-FPS, so that the system becomes widely diffused. Simultaneously, a small group of technology enthusiasts seeks to invest in an RA-FPS. Although the market share of the RA-FPS slowly increases, the vast majority of farmers is convinced of the benefits of local production, and holds on to it. Hence, following this pathway, by 2050, the LA-FPS is dominant, succeeded by the RA-FPS, while the C-FPS has disappeared completely.

5.1.3. Scenario 3: Regional supply chains

Similar to the LFP scenario, the regional supply chains (RSC) scenario represents a trajectory in which *Azolla*-based FPSs rapidly outpace the C-FPS. In the first phase, a rising awareness rises on the negative side-effects of the agricultural sector's dependency on imported feed, and drives a group of early adopting farmers to switch to an LA-FPS. The political domain observes this development and implement several advantageous legislative and financial instruments, enabling *Azolla*-based feed to secure a competitive position on the feed market. In the second phase, increased demands for *Azolla*-based feed push actors to set up a RA-FPS supply chain, enabling large-scale production for the majority of pragmatists, conservatives and, eventually sceptics, causing it to rapidly diffuse and even outgrow the share of the LA-FPS. Consequently, by 2050, *Azolla* produced in an RA-FPS is widely accepted as an affordable commodity, fully replacing conventional feed.

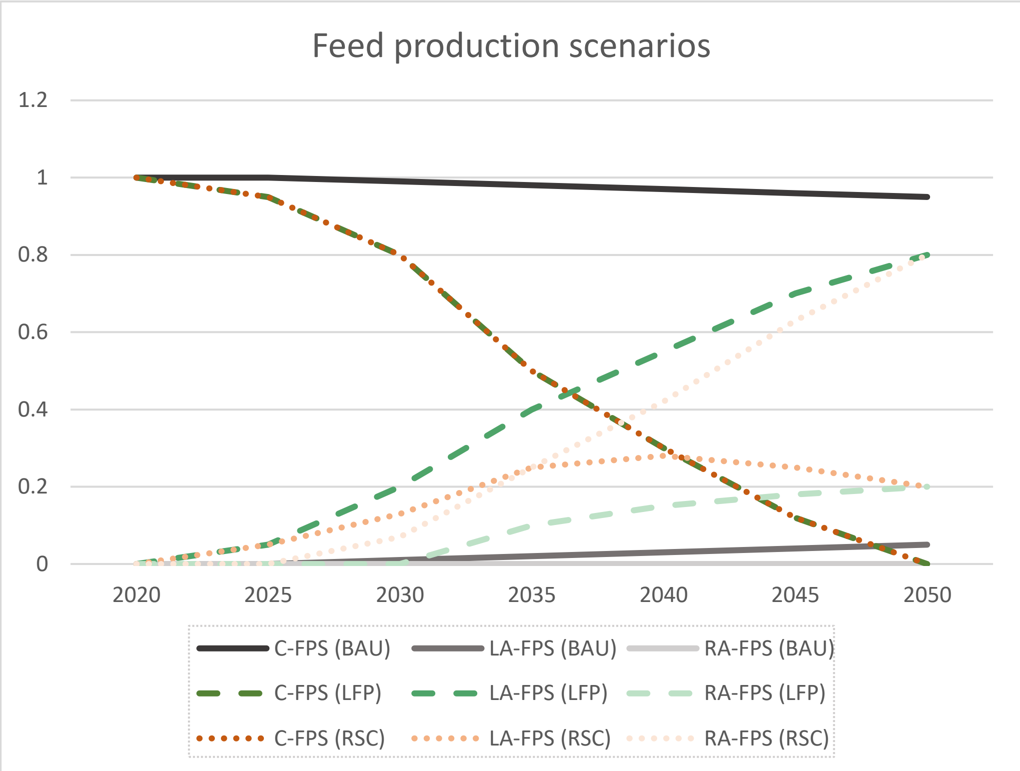


Figure 5.1. A visual representation of the feed production scenarios, including a baseline (BAU, black shade, continuous line) scenario, and two normative scenarios, of which one dominated by the LA-FPS (LFP, green shade, dashed line) and the other by the RA-FPS (RSC, orange shade, dotted line).

Figure 5.1 summarises the feed production trajectories elaborated on in the sections above, by graphically representing their respective share in the total feed market between 2020 and 2050. *Table B1* in *Appendix B* shows the exact percentages of these shares.

5.2. Choice of conventional FPS

For the C-FPS, it is decided to consider soy as the primary feed. As shown in *sub-section 1.1.3*, soy is the most consumed protein-rich feed in the Dutch livestock sector (Hoste, 2014; Vijn et al., 2019). Moreover, examinations of their nutritional profiles indicate that the amino acid (AA) concentrations of *Azolla* and soy are similar (see *Table B5B* of *Appendix B*) (Leterme et al., 2010; Brouwer et al., 2018). With regard to some AAs (e.g., lysine, methionine and threonine), a growth-limiting factor in dairy cows, *Azolla Filiculoidis* is even slightly superior (Brouwer et al., 2018). Given its favourable protein content (see *Table B5C* of *Appendix B*), it is assumed that soy and *Azolla* can serve interchangeably as a protein-rich feed resource.

However, *Azolla* also contains certain anti-nutritional factors that may reduce its digestibility in monogastric livestock (e.g., pigs and poultry). Hence, the current environmental assessment will focus on its application to polygastric, ruminating animals (Brouwer et al., 2018). More specifically, the switch from soy to *Azolla*-based feed in dairy cows is considered, given that the Dutch wetlands to be utilised for *Azolla* cultivation are at the moment primarily occupied by dairy cattle farms (Van der Peet, 2018).

5.3. Goal and scope definition

In the first phase of this study, its objectives, and target audience are described (*sub-section 5.3.1*). Afterwards, the technological, spatial and temporal coverage of the model are defined to establish its appropriate level of detail (*sub-section 5.3.2)*. Then, the system's function, FU, alternatives and reference flows, are discussed (*sub-section 5.3.3*, Pesonen et al., 2000).

5.3.1. Goal

The goal of this ex-ante, attributional LCA (ALCA) is to compare the environmental performance of different dairy cow feed production scenarios that include soy produced in a linear C-FPS, and *Azolla* produced in a circular LA-FPS and RA-FPS. Each FPS encompasses a variety of processes, responsible for exerting various impacts. "Life cycle thinking" offers a useful, holistic approach to capture the full picture of these impacts. Moreover, an LCA study allows the researcher to identify environmental trade-offs among LC stages, production systems and scenarios (Guinée et al., 2002).

Ultimately, this exploratory study seeks to take a first step towards further scenario-based research on sustainable protein-rich livestock feed alternatives. Its results may contribute to informing policy makers and guide research and development decisions on the implications of replacing soy by *Azolla*-based feed in the nationwide transition to a CAS by 2050 (Rijksoverheid, 2019). In this way, avoidable environmental burdens and regrettable investments in technologies with a low TRL may be prevented, and changes in legislation may be anticipated on, which is key early on in the design process of a novel production system before pursuing market diffusion (Cucurachi et al., 2018).

5.3.2. Scope

5.3.2.1. Geographical scope

In the C-FPS, upstream processes take place in Brazil, where the Netherlands imports over 90% of its soybean meal from. It is assumed that the soy is cultivated in Mato Grosso, in the Central-West of Brazil, known as the country's largest soybean producing region (Bicudo da Silva, 2020). Downstream processes occur in the Netherlands, at a conventional, non-organic dairy farm in Lemmer, situated in the province of Flevoland, adjacent to the Noordoostpolder.

The entire life cycle of the *Azolla*-based FPSs, on the other hand, is situated in the Netherlands. In the LA-FPS, all life cycle processes occur on the farm's property in Lemmer. Only when necessary, inputs are sourced from external entities. In the RA-FPS, *Azolla* cultivation and milk production are located on the farmer's land, while the feed processing is done in Proeffrabriek Leusden, a subsection of ABZDiervoeding, known for producing unconventional livestock feed (ABZDiervoeding, n.d.).

5.3.2.2. Temporal scope

To ensure that the LCA model reflects the state-of-the-art of feed production, the most recent data available is used. This data has been recorded between 2001 and 2021 (and reported on in the research papers by among others Brouwer et al., 2017; Brouwer et al., 2018; Holshof et al., 2009; Hoving et al., 2011; Hoving et al., 2012; Joosten et al., 2015, and Smolders et al., 2013, and datasets by Di Santi Barrantes, 2018; Maxime, 2018; Picoli, 2018 and Sugawara, 2018). Whereas the C-FPS seeks to reflect the status quo of protein-rich feed production, the *Azolla*-based alternatives are not yet implemented, and therefore represent possible future systems that could aid in achieving a CAS. The model covers the time period of 2020 to 2050 in five-year intervals.

5.3.2.3. Technological scope

Soy has been a dominant protein-rich feed in Dutch dairy cattle farming for over five decades (FAO, 2021). As a result, the C-FPS has become the incumbent FPS. Since the technologies used for soy production have been optimized for many decades, these are assumed to not change notably within the timeframe of this study (Arvidsson et al., 2018). In fact, the C-FPS is at TRL9, meaning that the actual system functions in a fully operational environment. Status quo data on mass production is believed to sufficiently represent the future development of soy-based feed and will be utilised in the model (Moni et al., 2020).

Contrary to the C-FPS, the technologies incorporated in the LA-FPS and RA-FPS, are at a very early stage of development. *Azolla* cultivation, to begin with, has been tested lab scale, and currently a pilot scale experiment is conducted by the Wetland Innovation Centre (*in Dutch*: Veenweide Innovatiecentrum, or VIC) (Brouwer et al., 2017; B. van de Riet, personal communication, September 14, 2020). Despite these ongoing research efforts, which lift *Azolla* cultivation to TRL6 (i.e. validation of a system in its relevant environment), the pilot scale data is not yet available. Therefore, *Azolla* cultivation will be regarded as if in TRL4, meaning that the system or its components are validated in a laboratory environment, so that he LCA model relies on lab-scale data (Moni et al., 2020).

The other technologies incorporated in the LA-FPS and RA-FPS (i.e., for harvesting, drying, ensiling, and pelleting) are readily available and in use, yet in different contexts. Several published research reports have demonstrated how these technologies have been applied successfully to duckweed, which has similar biochemical characteristics as *Azolla* (e.g., Holshof et al., 2009; Hoving et al., 2011). Hence, it is assumed that these technologies are applicable to *Azolla*-based feed production, positioning it at TRL2, referring to the formulation of a technology concept and/or application, implying that the LCA model will build on a mix of inventory data, manufacturing factsheets, and literature.

It was decided on only model the cradle-to-farm-gate processes in any of the FPSs, as to focus on the impacts of feed production, rather than of livestock commodity production as a whole. For each technology within the LA-FPS and RA-FPS, core parameters, such as energy use and processing capacity, as well as their variability throughout time, were estimated. To this end, the data will be parametrized, as explained in *sub-section 5.4.3.4*.

5.3.3. Function, functional unit, alternatives and reference flows

As the modelled system only involves cradle-to-farm-gate processes, the function is to provide the dairy market with cow milk. In terms of the functional unit (FU), the environmental impacts related to the in- and outputs needed for producing 1 kg of cow milk will be assessed. Since the trajectory of each scenario is dominated by a particular FPS, the alternatives and reference flows will be defined accordingly. Hence, the three alternatives are BAU scenario cow milk, LFP scenario cow milk and RSC scenario cow milk. Consequently, the reference flows are the provision of 1 kg of BAU scenario cow milk, the provision of 1 kg of LFP scenario cow milk, and 1 kg of RSC scenario cow milk, to the market for cow milk.

An average Dutch dairy cattle meal composition is taken as the starting point for the life cycle calculations of the different alternatives. *Table B5A* in *Appendix B* shows that, in general, about 12% of the feed requirement of a Dutch dairy cow is met with soy (i.e., beans and meal). In total, approximately 1.46 kg of protein-rich feed (here: soy) is needed to produce 1 kg of milk. Hence, the calculations only reflect the processes and flows related to 0.1692 kg of soybean meal, disregarding the impacts of the other feed ingredients (Remmelink et al., 2019). In the *Azolla*-based alternatives, this soybean meal fraction is substituted by ensiled or pelleted *Azolla* respectively, such that the animal's protein demand is met (see *Table B5D* of *Appendix B*).

5.4. Life cycle inventory analysis

In the life cycle inventory (LCI) phase, the model's system boundaries are set (*sub-section 5.4.1*) and flow charts are developed for the production system of each alternative, to visualise the main in- and outputs of the unit processes within each production system (*sub-section 5.4.2*). Next, back- and foreground data is collected on these in- and outputs, including flows from and to the bio- and Technosphere, and the parametrization procedure for scenario data is elaborated on (*sub-section 5.4.3*). Finally, the problem of multi-functionality in the model is considered (*sub-section 5.4.4*), and the LCI results are presented *(sub-section 5.4.5*).

5.4.1. System boundaries

As indicated in *section 3.4*, standardised system boundaries are set that apply to all examined FPSs, to enable a valid comparison. Especially in LCAs involving agricultural processes, such as performed in the current study, it is important to clearly determine the system's economy-environment boundaries. Here, the soil, water and air compartments are, in spite of their economic value, considered as belonging to the environment (i.e., ecosphere). The ecosphere interacts with the Technosphere by delivering natural resource inputs and receiving emission or waste outputs. Hence, despite the boundary separating them, they are closely connected.

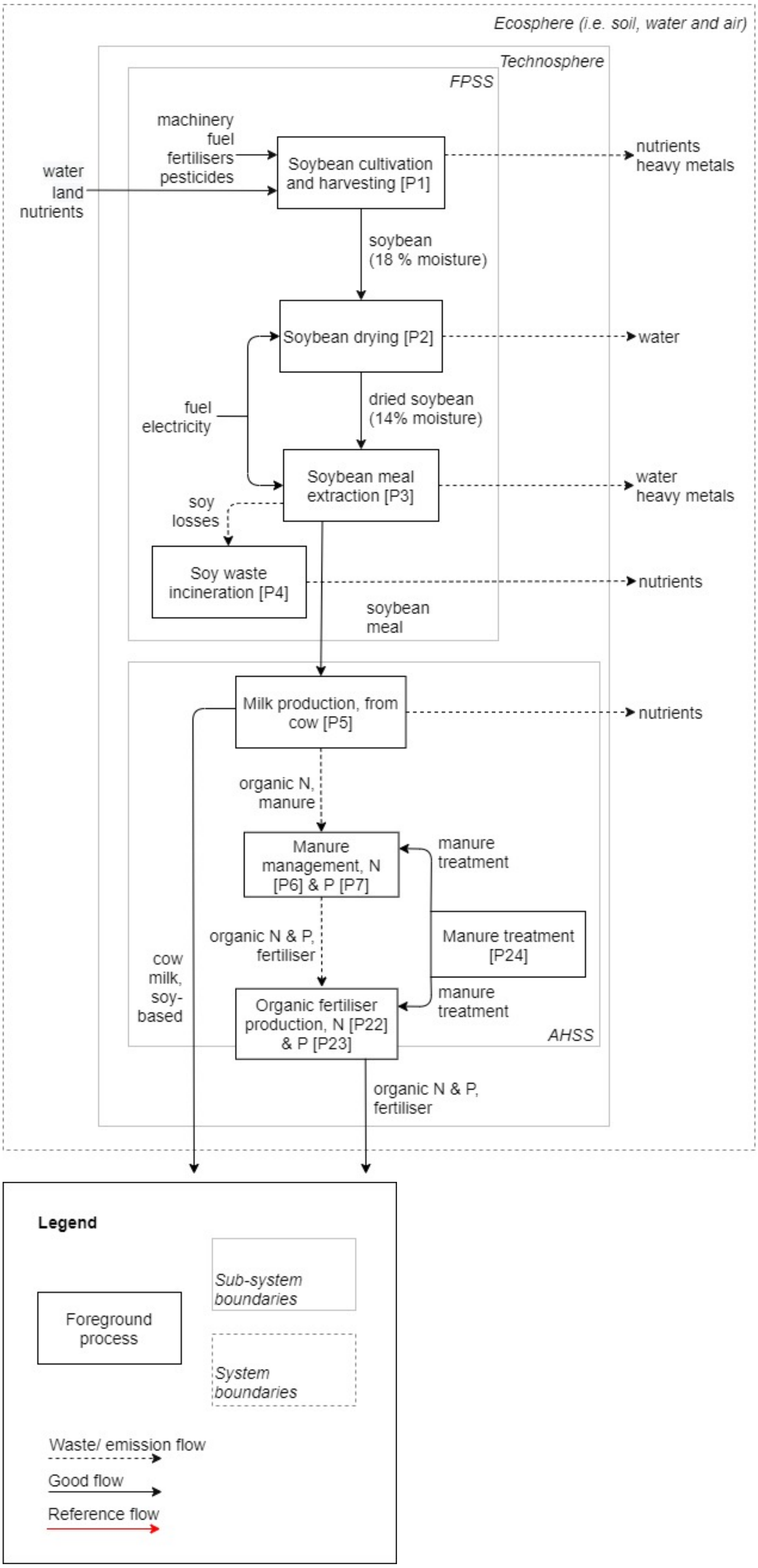


Figure 5.2a. Flow chart of the processes and flows in the C-FPS, for soy-based cow milk production.

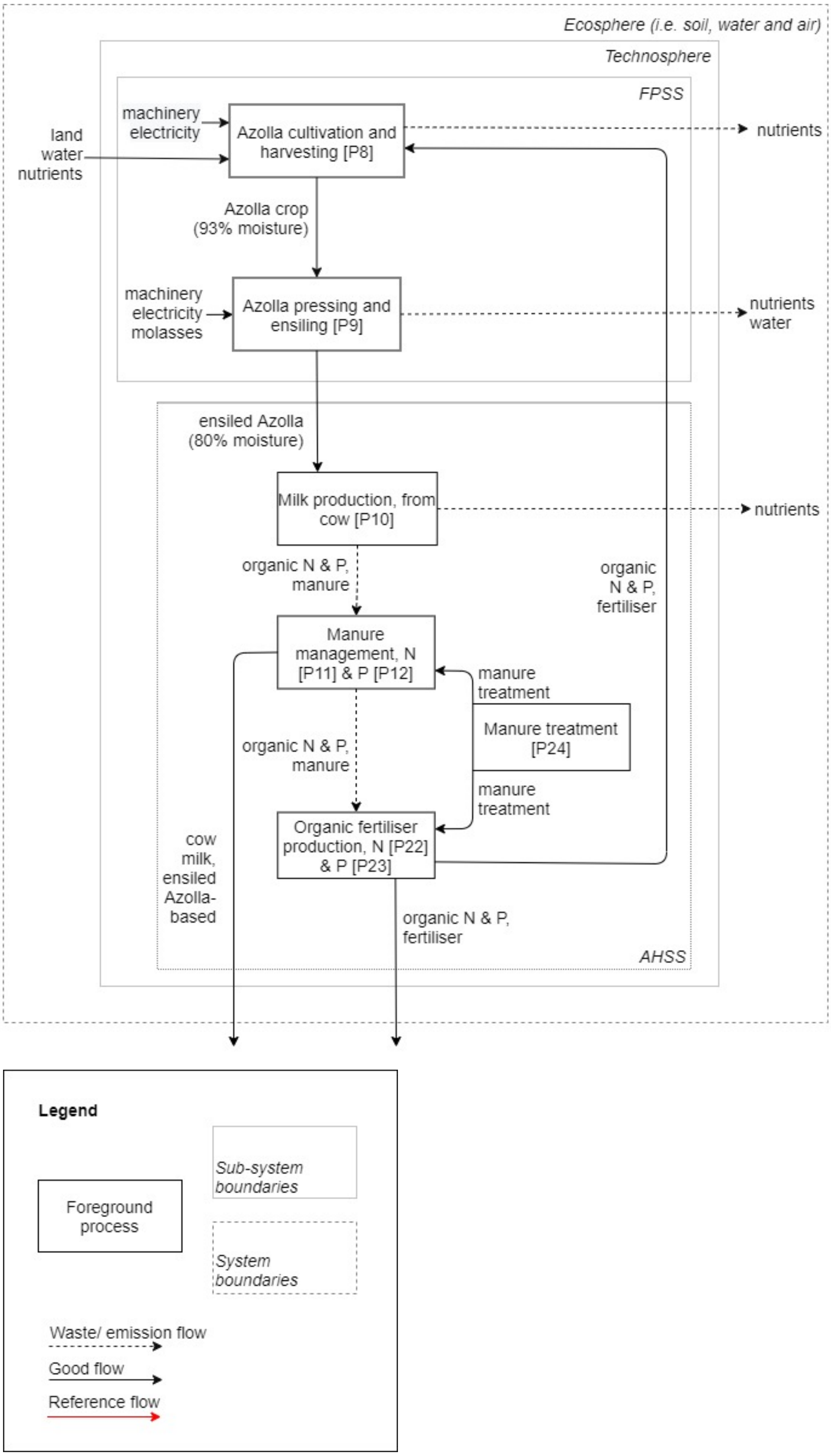


Figure 5.2b. Flow chart of the processes and flows in the LA-FPS, for ensiled *Azolla*-based cow milk production.

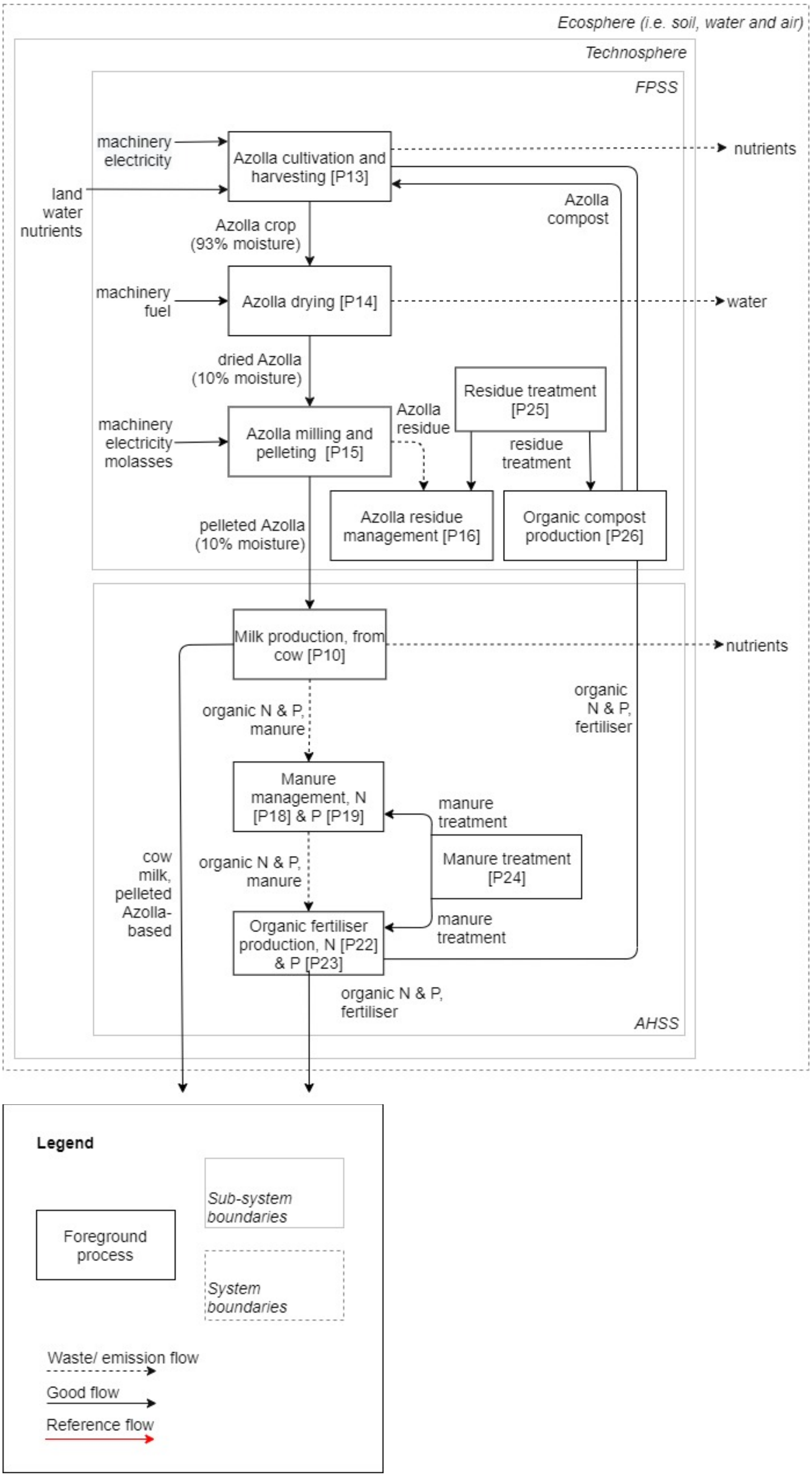


Figure 5.2c. Flow chart of the processes and flows in the RA-FPS, for dried *Azolla*-based cow milk production.

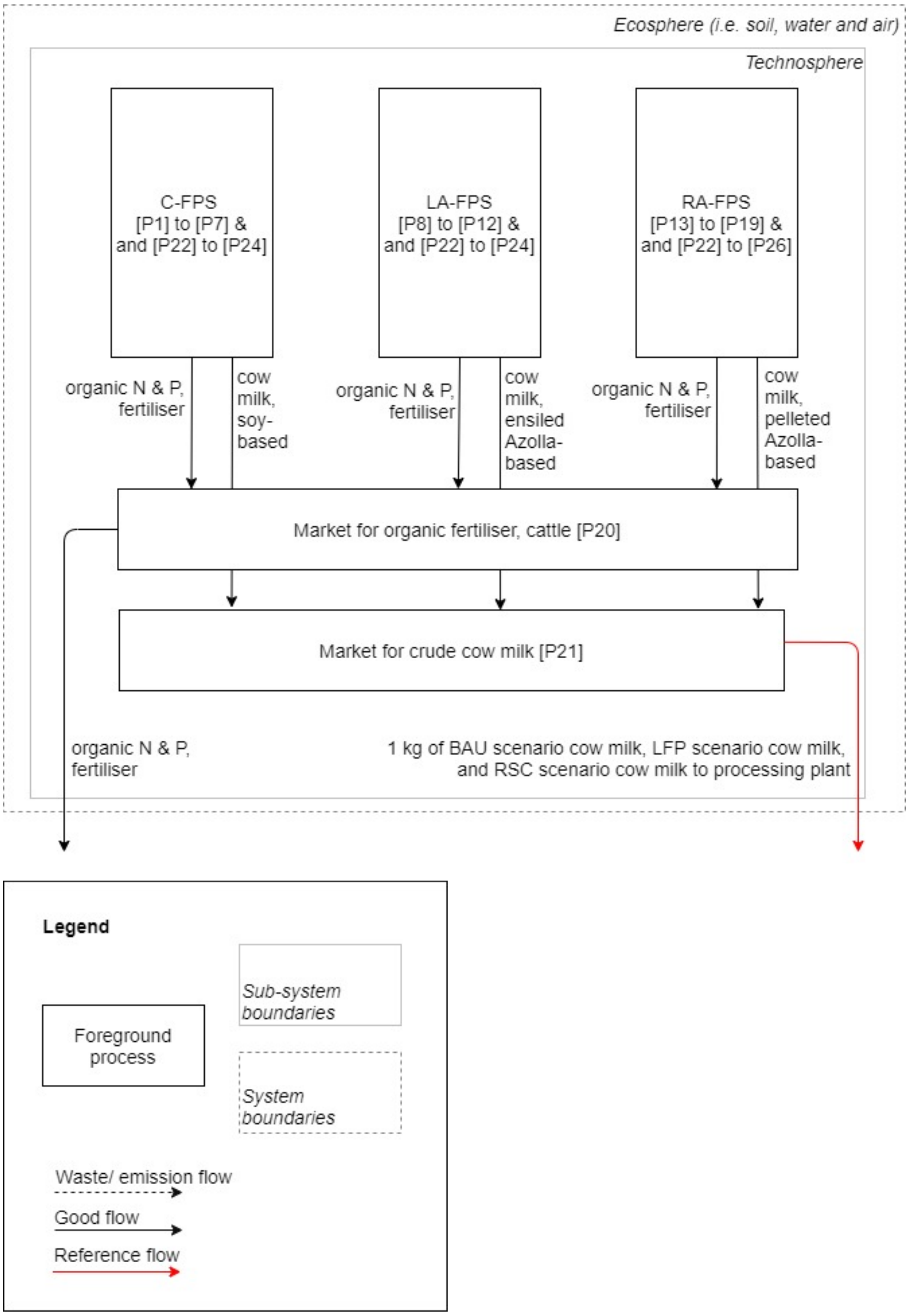


Figure 5.2d. Flow chart of C-FPS, LA-FPS and RA-FPS, and the departing cow milk and N & P fertiliser flows entering their respective markets.

In the next sub-sections, these system boundaries are used to draw flow charts of the relevant processes and flows, and operationalise them for the C-FPS, LA-FPS and RA-FPS.

5.4.2. Flow charts

In brief, within the Technosphere boundaries, two sub-systems exist: the feed production sub-system (FPSS) and the animal husbandry sub-system (AHSS). The FPSS includes feed cultivation and processing, while the AHSS includes milk production and manure management. Including the AHSS in the model is needed to examine the extent to which nutrients leaving the livestock system re-enters the cultivation process in the FPSS, with the goal of closing the feed-manure cycle, which is at the centre of the CAS. *Figures 5.2a-d* graphically represent the system structure in which the unit processes are organized, as well as in-and outflows of each FPS, and visualise how the crude milk outflow enters the market.

5.4.3. Data collection and unit processes

5.4.3.1. Data collection

Data was collected from a variety of sources. For the C-FPS, most data for processes in the FPSS was obtained from the ecoinvent v3.7 database (e.g., Di Santi Barrantes, 2018; Maxime, 2018; Picoli, 2018; Sugawara, 2018), in combination with literature (e.g., Hoste, 2014; Salvagiotti et al., 2008; Wolf et al., 2015), while for the AHSS, literature and scientific reports were consulted in addition (e.g., Alvarez-Fuentes et al., 2016; Remmelink et al., 2019).

Regarding the *Azolla*-based FPSs, data on both the FPSS and AHSS was gathered from scientific reports (e.g., Brouwer et al., 2017; Brouwer et al., 2018; Holshof et al., 2009; Hoving et al., 2011; Hoving et al., 2012; Joosten et al., 2015, Smolders et al., 2013; Sawant, 2018), national statistics (e.g., Netherlands Enterprise Agency, RVO, 2018), factsheets (e.g., VIC, n.d.), laboratory data (e.g., Brouwer, 2017), machinery manufacturers (e.g., ABZmachinery, n.d-a,b; Smicon, n.d.), and expert consultations. The sub-sections below describe the main assumptions underlying the unit processes, as well as the parametrization procedure used to model changes in core parameters. A complete overview of all processes, assumptions, calculations and sources, can be found in the tables of *sheets 2-9* of *Appendix B.* An elaborate documentation of developing an LCA model and (re)producing results in the AB, can be found in *Appendix C*.

5.4.3.2. C-FPS alternative

The FPSS of the C-FPS involves Soy cultivation and harvesting [P1], Soybean drying [P2] and Soybean meal extraction [P3]. First, soy is cultivated at an annual yield of 3,000 kg dry dm/ha in an intensive, non-irrigated, rotational cropping system. It includes the activities of sowing seeds, applying artificial fertiliser (note that 48% of the N demand is met in this way, and the remaining is fixed as atmospheric N (see *Table B3cA*) (Salvagiotti, 2008), pesticides for weed, pest and pathogen control, and combine-harvest, transport from field to farm (with a distance of ±15 km) and on-farm storage (Picoli, 2018).

Although the original ecoinvent cultivation dataset includes LUC emissions, it assumes that soy cultivation in Mato Grosso does not directly contribute to deforestation (Picoli, 2018). However, according to the literature, this assumption results in a severe underestimation of LUC emissions. Therefore, it was decided to reflect a more realistic situation in which half of the soy cultivation causes direct forestation in the tropics, while the other half is linked to indirect deforestation (e.g., through conversion of forests to grazing pastures to soy fields), assuming a tillage system (Castanheira and Freire, 2013; Gollnow et al., 2018) (see *Table B7a*). As for biogenic C, the original dataset only included C uptake by plant matter during photosynthesis. It assumes that the biogenic C embedded in the harvested produce is not released elsewhere. Here, C sequestration in soil organic matter (SOC), and emissions from non-harvested soybean plant residue as a result of decomposition, were estimated by means of a mass balance (see *Table B3bA* and *B3bD;* Reijnders and Huijbregts, 2008; Wolf et al., 2015).

Although the soy cultivation dataset originally includes soybean drying (Picoli, 2018), it was decided to separate this process, because its high energy demand is suspected to strongly affect the system's total impacts (Tallentire et al., 2018). The energy consumption is based on the amount of water evaporated to bring the moisture content down from the original 18 wt% to 14 wt%, as computed in a mass balance (see *Table B3aA*). The drying is done in an Easel Dryer, fuelled by firewood, under humid, tropical conditions (Di Santi Barrantes, 2018).

Afterwards, the dried soybeans are transported to an oil mill for treatment. The beans are washed, cracked, dehulled, the oil and meal are extracted (with hexane as a solvent), and the soybean meal is processed. The demand for electricity and natural gas for heating, phosphoric acid as a bleaching agent, and tap water are included (Sugawara, 2018). A soybean meal loss of 2 wt%, is assumed (see *Table B2B,* Hoste, 2014). These lost soybeans are collected and incinerated, and nutrients and biogenic CO_2-emissions were estimated in Soybean waste incineration [P4] (see *Table B3cE* and *B3bA*). Transportation is modelled as an input to the milk production process, rather than as an individual unit process, for the sake of consistency.

Based on a weighted mix of soy farms in the Central-West of Brazil, it is assumed that the soy is transported by lorry and diesel-powered freight train to the Port of Santos (Gindroz, 2018; da Silva, 2010). The soy crosses the Atlantic Ocean by steam-turbine container ship, entering the Port of Rotterdam. A lorry delivers it to the dairy cattle farm in Lemmer (Google Maps, n.d.; Valsasina, 2018a,b) (see *Table B7B*). The data includes the lifetime of each transport mode, the combusted fuel and (non-)exhaust emissions (e.g., from tyre, break and road wear). Soy losses during transport are on average <1 wt%, hence neglected (Barbosa et al., 2020).

The soy-based AHSS consists of Milk production, from cow [P5], Manure management, N [P6] and P [P7], Manure treatment [P24], and Organic fertiliser production, N [P22] & P [P23]. On-farm, the soy is mixed with other types of feed to gain the desired dietary composition, and fed to cattle (see *Table B5A,* Remmelink et al., 2019). CH_4 emissions from enteric fermentation as well as ammonium (NH_3) and nitrous oxide (N_2O) emissions from manure were retrieved from ecoinvent (Maxime, 2018). Besides, the N and P content of milk and manure were estimated, based on literature and Dutch statistics on conventionally-fed dairy cattle (Alvarez-Fuentes et al., 2016; RVO, 2018). It is assumed that the nutrient content of mature cow tissue is in equilibrium, hence the input of N and P in feed equals the output in manure or milk (*Table B3cA*).

Since the C-FPS is implemented in an LAS, the manure generated in this system is not returned to the local farmland. Instead, the treated manure is passed on to the Market for organic fertiliser, cattle [P20] (Smolders et al., 2019). The milk flows to the Market for crude cow milk [P21].

5.4.3.3. LA-FPS and RA-FPS alternative

The FPSS of both *Azolla*-based feed alternatives starts with *Azolla* cultivation and harvesting [P8], [P13] (Smolders et al., 2019). In the model, the cultivation process includes biogenic C sequestration in *Azolla* biomass, P uptake and atmospheric fixation of N (Brouwer et al., 2018;

Smolders et al., 2013; Vermaat and Hellmann, 2010) (see *Table B4aA*). It is assumed that no *Azolla* biomass HL occurs, meaning that all photosynthesised C is removed at harvesting (see *Table B3bB-C*). *The* use of groundwater to inundate the drained wetland, land transformation to wetland and occupation as well as land use-related CH_4 emissions were estimated (Cheng et al., 2010) (see *Table B4aB*). The latter estimation was based on results from the greenhouse gas emissions site types (GEST) method presented by Joosten et al. (2015), assuming that 5.1 t CO_2-eq./ha/y can be saved by rewetting a short grassland under temperate climate conditions. Regarding harvesting, in the LA-FPS, the material and energy demand for a solar-powered conveyor belt are included in the model. In the RA-FPS, this conveyor belt is combined with an electric motor to speed up the harvesting rate (see *Table B4aC*).

After harvesting, *Azolla* is processed distinctly in the LA-FPS and RA-FPS. In the LA-FPS, the fresh *Azolla* remains on-farm for processing. In *Azolla* pressing and ensiling [P9], the excess water is removed with a screw press and the *Azolla* biomass is ensiled in bales (see *Table B3aB*). This process includes the machinery, electricity and fuel, sugar beet molasses and plastic film for pressing and baling (see *Table B4aD*) (Blaser, 2007a; Blaser, 2007b). Data regarding the energy and material use and lifetime of machinery were retrieved from the website of a manufacturer (Smicon, n.d.). Nutrient losses during treatment were derived from an experimental analysis of duckweed ensilage (Hoving et al., 2011) (see *Table B3cB-C*). As these spillages are very small, they are assumed as lost to the environment.

In the RA-FPS, the fresh *Azolla* is transported by truck to Leusden for treatment (Google Maps, n.d.) (see *Table B7B*). In *Azolla* drying [P14], it is first dried in a gas-heated drying room to reduce the moisture content from 93 wt% to 10 wt% (Holshof et al., 2009) (see *Table B3aC* and *B4aD*). In Azolla milling and pelleting [P15], the dried *Azolla* is processed in a hammer mill and ring die machine. The material and energy demand, lifetime, processing capacity were retrieved from the website of a manufacturer (ABC Machinery, n.d.-a & n.d.-b). Besides, 4 wt% of sugar beet molasses were included to this process, acting as the primary binding agent. Like in the C-FPS, a biomass loss of 2 wt% is assumed. Yet, in this FPS the biomass is gathered and handled in *Azolla* residue management [P16], Residue treatment [P25] and Organic compost production [P26]. The compost output is fed back on the land to improve the soil organic carbon (SOC), of which the biogenic C emissions are estimated (see *Table B3bC*).

As the LA-FPS entirely takes place on-farm, the ensiled product can be fed directly to the dairy cattle, which takes place in the AHSS. In the RA-FPS, the feed is transported from the processing facility to the farm. Since pelleted *Azolla* has a lower moisture content than ensiled *Azolla*, its nutritive density is higher and therefore less volume is needed to fully replace soy (see *Table B5D*). Because more accurate data is lacking, emissions of CH_4, NH_3 and N_2O in Milk production, from cow [P10], [P17] are kept the same as in the C-FPS (A. Bannink, personal communication, January 5, 2021). A prior experiment demonstrated that adding up to 15% of *Lemna* to a cattle diet, did not significantly impact the CH_4 emissions from enteric fermentation, which backs this assumption (Tirado-Estrada et al., 2018). The urea content of *Azolla*-based milk is assumed to be equal to the N content of soy-based milk, and the distribution of P output over milk and manure is computed in the same way as for the C-FPS (see *Table B3cC-D*).

Both in the LA-FPS and the RA-FPS, a fraction of manure generated by dairy cattle in Manure management, N [P11], [P18] and P [P12], [P19], is subjected to Manure treatment [P24], and Organic fertiliser production, N [P22], P [P23]. The organic fertiliser is fed back onto the land with a vacuum tanker. Since *Azolla* satisfies its own N demand, inevitably an excess of organic N arises as manure is applied to the wetland. Since most Dutch wetlands are not N-saturated, the N is assumed to accumulate in the agricultural soil. The surplus of manure is handled on the Market for organic fertiliser, cattle [P20] (see *Table B6*).

5.4.3.4. Parametrization procedure

Parametrization can be utilized to model future changes of core parameters. To model these changes, Arvidsson et al. (2018) suggest using scenario ranges. This implies that a realistic lower and an upper limit are determined for relevant parameters, and a curve is developed to connect these on a temporal dimension. Here, it is assumed the *Azolla* yields, technology production capacities and source of P withdrawal change over time, as briefly discussed below.

Firstly, it is assumed that the *Azolla* yields increase, from 15 t dm/ha/y in 2020, to 17 t dm/ha/y (in the LA-FPS) or 20 t dm/ha/y (in the RA-FPS) by 2050 (VIC, n.d.). This difference in upper limit is explained by the fact that in the RA-FPS, the application of a more advanced harvesting technology results in a higher biomass removal rate, hence a faster *Azolla* growth rate (Hoving et al., 2012). The values of several parameters depend on the amount of *Azolla* harvested. For example, if the *Azolla* yield increases, while the total water consumption and land use emissions remain the same, the water consumption and land use emissions per unit of *Azolla* biomass, decrease (see *Table B4bA*).

Secondly, scenario ranges are used to indicate improvements in the energy and material efficiencies of processing technologies in the LA-FPS and RA-FPS. For each technology, the production capacity is fixed at a lower end value, and as the share of *Azolla*-based cow milk on the total market rises, resource efficiencies improve in accordance with the technology performance curve of Huber (2003) presented in *Figure 2.2*, which is generally applicable to novel technologies, until reaching the upper limit by 2050 (see *Table B4bB-bC*). Note that this curve merely illustrates the diffusion trend of a novel innovation. It does not quantify the expected percentage change, as this depends strongly on time- and place-specific factors. The lower and upper limit were determined based on factsheets from manufacturer websites and the growth curve was composed to visually resemble Huber's (2003) work.

Thirdly, *Azolla* efficiently absorbs P accumulated in intensive agricultural landscapes, depleting the P legacy over time (Van Diggelen et al., 2013). Therefore, the model reflects how in the current situation, 60% of the P demand arrives from available deposits, while in 2050, this P demand is partially fulfilled by manure generated on-farm, in an attempt to close the feed-manure cycle. Since modelling the depletion of agricultural P reserves requires complex techniques, it was decided to assume a simplified, linear decrease in agricultural P reserves and corresponding increase in P sourced from manure (see *Table B6*). The consequences of this development for N emissions and manure export are considered as well.

5.4.4. Multi-functionality and allocation

A multi-functionality problem occurs when one process is linked with the management of two or more functional flows (Guinée, 2002). *Table B10a* of *Appendix B* presents four steps to identify multi-functional foreground processes in the modelled FPSs, as well as to determine an appropriate way of dealing with these. In this study, allocation was used to solve the multi-functionality problem, meaning that the in- and outputs of several unit processes were partitioned to account for those relevant to the system of interest.

To begin with, in the C-FPS, economic allocation is performed to the Soybean meal extraction [P3] process, which originally co-produces soybean meal and soybean oil (Sugawara, 2018).

Also, economic allocation was applied to the Milk production [P5], [P10] and [P17] process, which originally co-produces cow milk and cattle for slaughtering (Maxime, 2018). Additionally, in all three FPSs, Manure treatment [P24] represents a recycling process, converting manure into fertiliser. Physical allocation is applied to account for waste input (i.e., P and N from manure), and good output (i.e., P and N in organic fertiliser). Similarly, physical allocation is used to partition the waste input (i.e., *Azolla* residue) and good output (i.e., *Azolla* compost) of the Residue treatment [P25] process as well. An explanation of how allocation factors are implemented in the AB is found in *Step 5* of *Appendix C*.

5.4.5. Results of inventory analysis

In the inventory analysis phase, a complete life cycle inventory (LCI) was calculated, containing all biosphere and Technosphere flows per FU (see *Table B10bA-B* of *Appendix B*).

5.5. Life cycle impact assessment

In the life cycle impact assessment (LCIA) phase, the magnitude and significance of potential environmental impacts linked to each scenario are evaluated. To that end, a set of impact categories is selected (*sub-section 5.5.*1). Then, each elementary flow in the LCI is multiplied by the appropriate CF (e.g., global warming potential, or GWP, for climate change), within that impact category, and expressed with a standardized unit (e.g., in kg CO_2-eq./kg emission, for climate change) to obtain the characterization results (*sub-section 5.5.2*) (Guinée, 2002).

5.5.1. Selection of impact categories

In *Chapter 3,* twelve indicators were selected for assessing the environmental performance of FPSs. In order to quantitatively compare the FPSs of interest in an LCA study, impact categories have been selected from the AB that correspond closely with the relevant indicators.

For a start, it was decided to include only midpoint-oriented impact categories. This means that the results will show the system's impacts early on in the environmental cause-effect chain (e.g., increased P run-off to surface water bodies), which are abstract yet relatively certain. Midpoint-oriented categories offer a more detailed insight in the effect of each FPS on a broader range of impacts (De Haes and Heijungs, 2009).

Looking at the families available in the AB, the ReCiPe midpoint V1.13 framework includes the largest set of midpoint-oriented impact categories (i.e., 17 in total). This framework draws on the state-of-the-art scientific knowledge with respect to, for instance, time horizons and damage pathways, where some of its predecessors have become outdated (Huijbregts et al., 2017). Hence, the impact categories in the ReCiPe family that cover a selected environmental indicator, are included, and the remaining categories are picked from other families. An overview of the impact categories utilised in this study is found in *Table B11* of *Appendix B.* The sub-sections below present a brief rationale for choosing each impact category.

5.5.1.1. Selection of input-related impact categories

In *sub-section 3.5.1*, three input-related environmental indicators were selected. First, the only impact family available in the AB that covers abiotic depletion (AD) is the obsolete CML2001, with a total of 158 CFs for a wide range of natural resources. Neither the updated AD category from CML, nor a version from another impact family can be used yet, and therefore the obsolete version was included (Sonderegger et al., 2020; Van Oers and Guinée, 2016). As for consumptive water use (CWU), the ReCiPe V1.13 indicator water depletion (WD) is opted for, which includes 5 CFs for water extraction from different types of water bodies. Regarding land competition (LC), 81 impact categories exist in the AB, of which the ReCiPe V1.13 natural land transformation (NLT) and agricultural land occupation (ALO) are chosen. The former comprises of 120 CFs, and the latter of 48 CFs, and both include the biomes relevant in the present studies (i.e., pastures, tropical rainforests, and wetlands). Together, these categories are thought to sufficiently represent the effect of feed production on input-related impacts.

5.5.1.2. Selection of output-related impact categories

Sub-section 3.5.2 presents a total of nine relevant output-related environmental indicators. ReCiPe V1.13 does not offer a terrestrial eutrophication (TE) impact category, while the ILCD 2.0 family is the most up-to-date one that does. Therefore, the AE (here: freshwater eutrophication, or FE) category from ReCiPe, with 13 CFs, and the terrestrial eutrophication (TE) category, with 16 CFs, from ILCD 2.0 2018, were included. The same holds for freshwater and terrestrial acidification (FTA). With its 21 CFs, the ILCD 2.0 category accounts for impacts caused by sulphur and N-related emissions where a ReCiPe alternative is absent. The terrestrial, freshwater and human (eco)toxicity (TET, FET and HT, respectively) from ReCiPe

V1.13 were found most extensive in terms of flows accounted for, with 1578, 1158 and 1124 CFs for toxic compounds and heavy metals respectively, and hence included.

Turning to climate change (CC), two categories were included: one with biogenic C (CC-B) and one without (CC-WB). Unlike fossil carbon, biogenic carbon is sequestered from the atmosphere during biomass growth, and partially released from plant material after harvesting due to combustion or decomposition; a mechanism that takes a central position in the examined agro-industrial production systems (Levasseur et al., 2013). In the AB, the ILCD 2.0 and IPCC 2013 family recognize this distinction, but the latter was considered more suitable as it includes biogenic CO_2 flows, while the former only comprises of CFs for biogenic CH_4, which is less relevant in the context of the present study. The CC-WB category comprises of 211 CFs, CC-B includes 6 CFs in addition (e.g., the emission “Carbon dioxide, non-fossil” and natural resource “Carbon dioxide, in air”), totalling 217 CFs. Fluxes of CO_2 to or from soil or biomass stocks, which represent LUC effects on CC, are not regarded as biogenic.

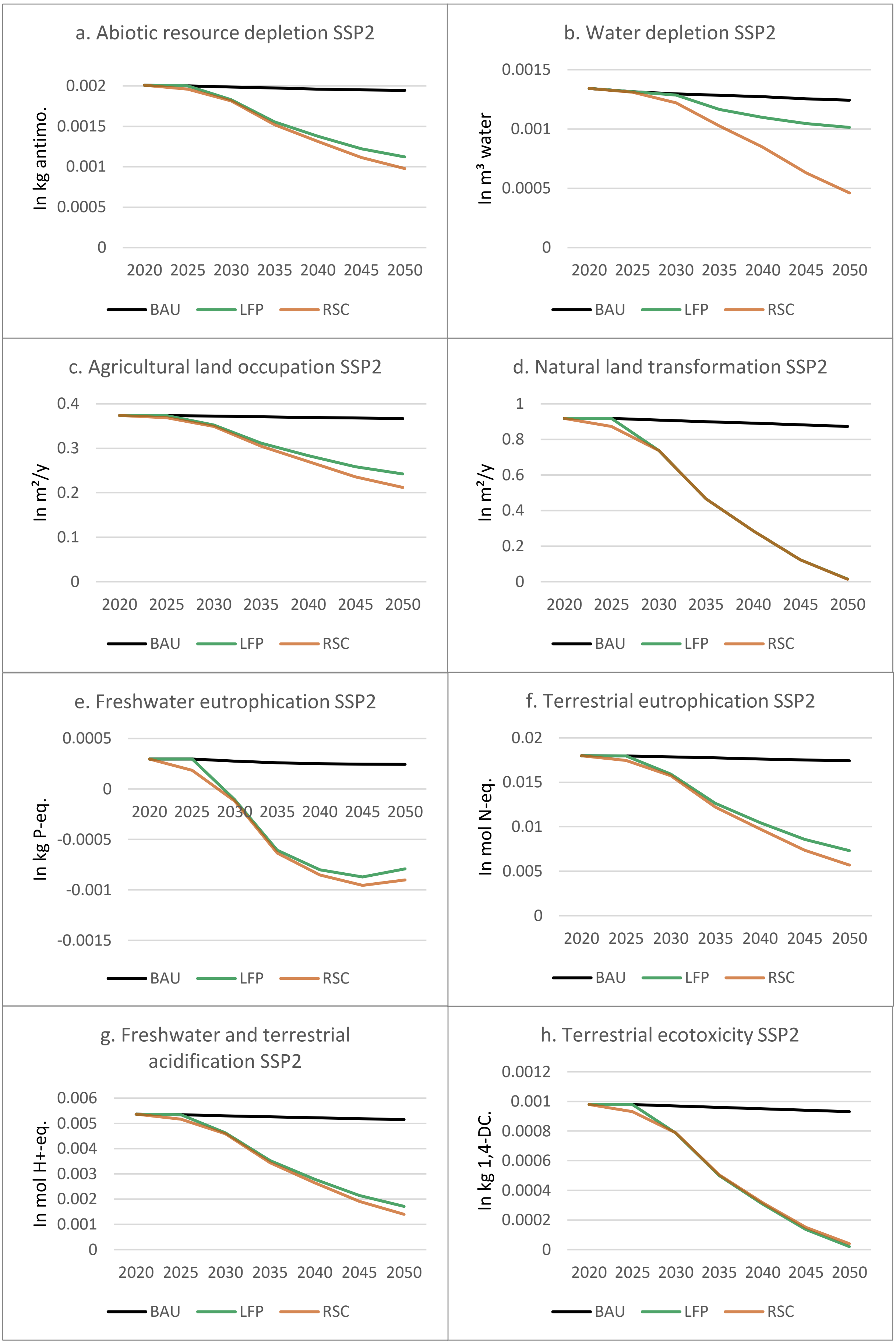
Finally, ozone depletion (OD) and photochemical oxidant formation (POF) were both drawn from the ReCiPe V1.13 family. With 57 CFs for halogenated hydrocarbons and 210 CFs for secondary air pollutants respectively, these are thought to appropriately indicate the system’s environmental burdens.

5.5.2. Characterization results

The characterization results were calculated, reducing the extensive LCI list of emissions and wastes to a more comprehensive set of environmental impacts (see *Table B11* of *Appendix B*) in the given time period for each scenario. The results per kg of milk produced are visualized in *Figure 5.3a-n* for SSP2, representing the baseline pathway. The outcomes for pathway SSP2.6, representing the strongest mitigation pathway, do not appear notably different from SSP2, hence are included in *Table B12A-N* and corresponding figures in *Appendix B*.

5.5.2.1. Characterization results input-related impacts

The effects of feed production on the four input-related impact categories are shown in *Figure 5.3a-d*. From 2020 to 2050, the LFP and RSC scenarios exhibit a strong decrease in terms of AD (by 44% and 51%, respectively) and WD (by 24% and 66%, respectively). In the same categories, the BAU impacts decrease by 3% and 7%, respectively. The LFP and RSC scenarios also result


a. Abiotic resource depletion SSP2
In kg antimo.
0.002
0.0015
0.001
0.0005
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
b. Water depletion SSP2
In m³ water
0.0015
0.001
0.0005
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
c. Agricultural land occupation SSP2
In m²/y
0.4
0.3
0.2
0.1
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
d. Natural land transformation SSP2
In m²/y
1
0.8
0.6
0.4
0.2
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
e. Freshwater eutrophication SSP2
In kg P-eq.
0.0005
0
-0.0005
-0.001
-0.0015
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
f. Terrestrial eutrophication SSP2
In mol N-eq.
0.02
0.015
0.01
0.005
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
g. Freshwater and terrestrial acidification SSP2
In mol H+-eq.
0.006
0.005
0.004
0.003
0.002
0.001
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC
h. Terrestrial ecotoxicity SSP2
In kg 1,4-DC.
0.0012
0.001
0.0008
0.0006
0.0004
0.0002
0
2020 2025 2030 2035 2040 2045 2050
BAU LFP RSC

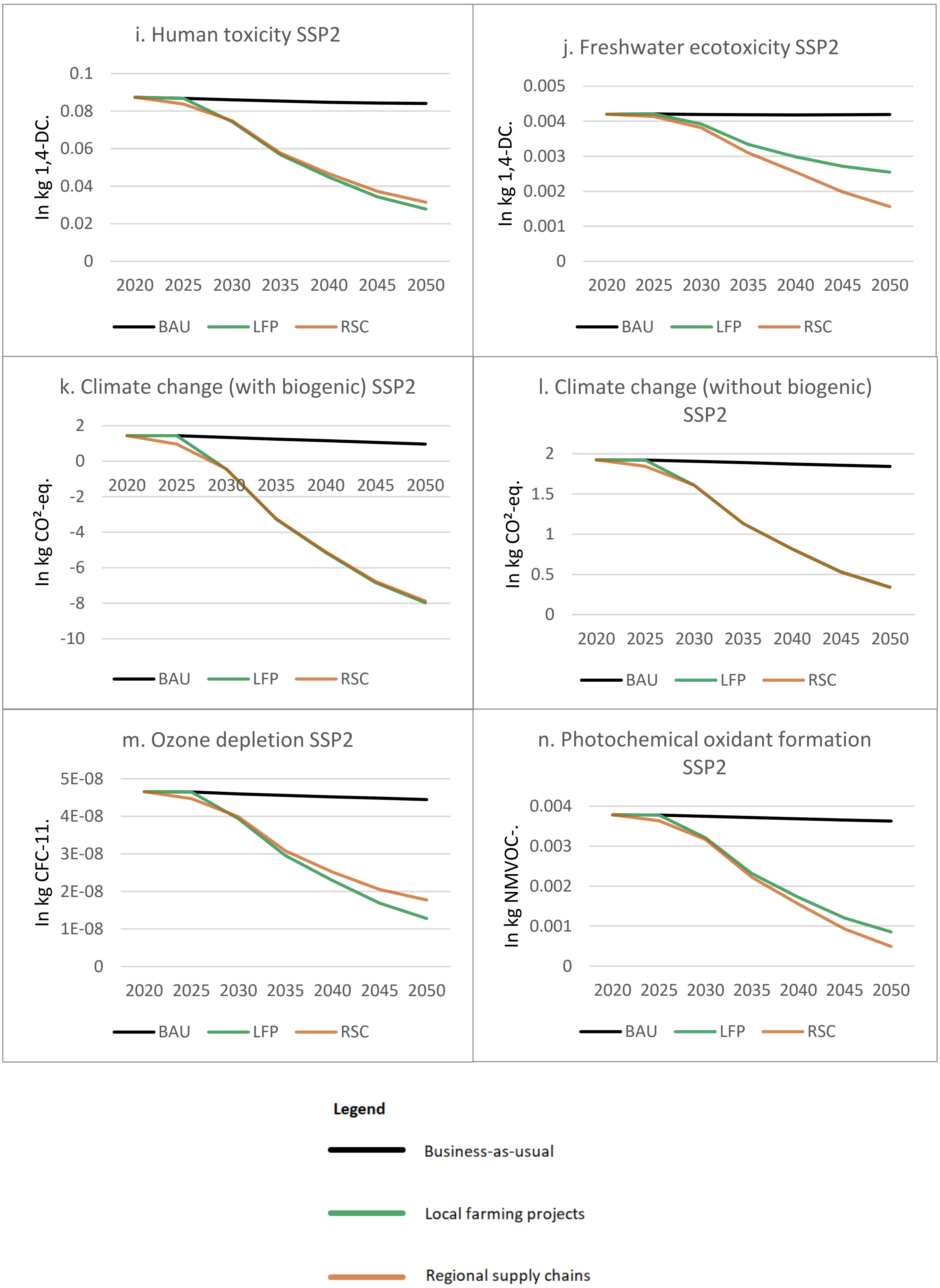


Figure 5.3a-n. Visualization of the characterization results for the three examined feed production scenarios, per kg of cow milk, to processing plant.

in a reduction of ALO (by 35% and 43%, respectively) and NLT (by about 98%), where the BAU impacts reduce slowly (by 2% and 5%, respectively).

5.5.2.2. Characterization results output-related impacts

The results of the ten output-related impact categories are shown in *Figure 5.3e-n*. Nutrients-driven impacts, which include FE, TE and FTA, are expected to decrease drastically, by 59-367% (LFP) and 68-404% (RSC), relative to the starting point in 2020. Feed production in the BAU scenario leads to a smaller reduction of impacts, amounting to 3-18%. A declining trend is also visible in all scenarios in terms of toxicity-driven impacts, comprising of the TET, HET and FET categories. In the LFP scenario, impacts decrease up to 98% and in the RSC scenario up to 97%, while in the BAU scenario the reduction is merely 0-5%.

Turning to the remaining pollutants-driven indicators, it appears that the choice of including or omitting biogenic carbon from climate change impacts strongly affects the results. In the CC-B category, the impacts of the LFP and RSC scenarios both decrease by about 650%, while in the BAU scenario this reduction is only 33%. In the CC-WB, impacts decrease by about 80% in the LFP and RSC scenarios, but it only 4% in the BAU scenario. Furthermore, the decrease in OD is highest for the LFP scenario (by 73%), followed by the RSC scenario (by 62%) and the BAU scenario (by 4%). Finally, POF shows decreases by 4%, 77% and 87% for BAU, LFP and RSC, respectively.

5.6. Interpretation

In the final phase, several checks were performed in order to evaluate the consistency (*sub-section 5.6.1*) and completeness (*sub-section 5.6.*2) of the present LCA study. These are followed by a contribution analysis, to identify which processes are responsible for inducing certain environmental impacts (*sub-section 5.6.3*) and a sensitivity analysis, to evaluate the model's sensitivity to a change in core parameters (*sub-section 5.6.4*) (Guinée, 2002).

5.6.1. Consistency check

To begin with, in the C-FPS, the production of seeds for soy cultivation is included, while in the LA-FPS and RA-FPS, *Azolla* sporophyte culture production is not. There is reason to believe, however, that *Azolla* spore production contributes only marginally to the system's overall environmental performance, due to its high reproduction rate and suspected high survival rates in the mild winters typical for the Netherlands (Brouwer et al., 2014).

Also, conventional cultivation of soy requires the input of multiple herbicides (e.g., paraquat), insecticides (e.g., glyphosate) and fungicides (e.g., azoxystrobin), whereas *Azolla* cultivation is assumed to use organic methods for combating pests (Picoli, 2018). This may have resulted in an unfair comparison between the FPSs, especially since the production of *Boveria* (an insectivore endoparasite that fights the spread of weevils), is not quantified in the *Azolla*-based FPSs model due to a lack of data (B. van de Riet, personal communication, September 14 2020).

On the other hand, soy cultivation enjoys the advantages of a long history of genetic modification (GM), for example to improve its drought tolerance and protein quality. *Azolla* could benefit from GM as well, for instance by reducing its anti-nutritional factors to enhance digestion in ruminants, which diminishes CH_4-emissions from belching. These inconsistencies should be kept in mind when interpreting the modelling results, as they may undermine the validity of the model.

5.6.2. Completeness check

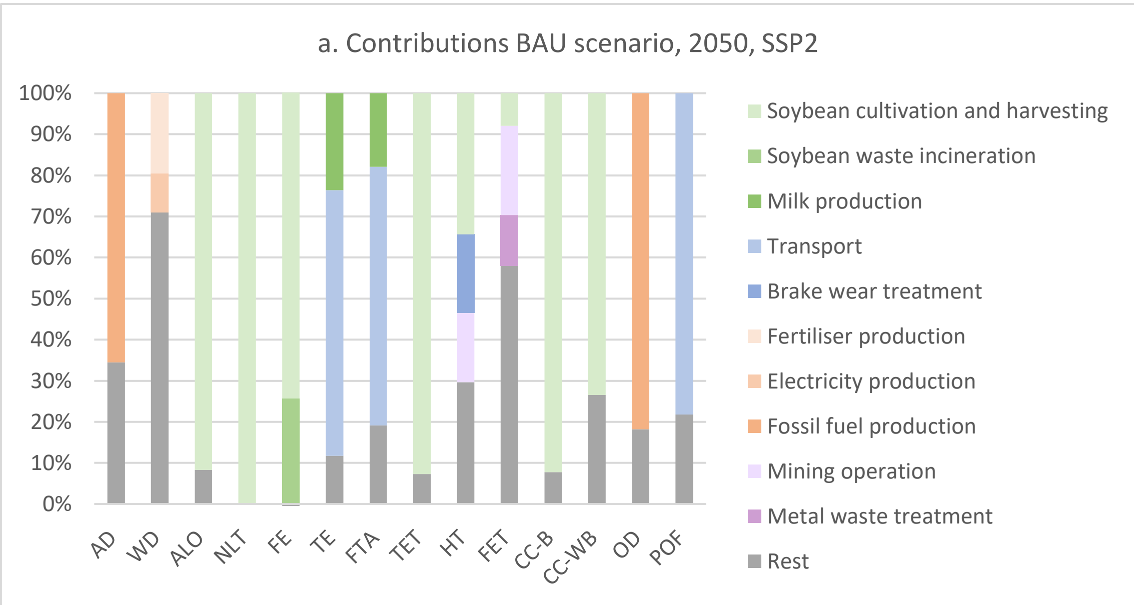
First of all, the uptake of heavy metals (e.g., cobalt, chromium and lead) in biomass during crop cultivation was omitted in all three FPSs. The removal of polluting compounds from agricultural soil, or in the case of *Azolla* from the water body it floats on, would make it seems as if causing a reduction in toxicity impacts (Muradov et al., 2014). Yet, a complete mass balance is necessary to trace toxic compounds down the value chain, where excretion occurs as the metals are released from livestock manure. The same issue holds for biogenic C, for which some of flows have been omitted, which may have caused an underestimation of CC-B impacts. *Sub-section 6.1.2* elaborates on how this issue arises and is dealt with in the current study.

Also, the preparation of a wetland for *Azolla* cultivation (e.g., the installation of sand embankments, installation of mash screen for wind protection) or of land for soy cultivation was not regarded (Picoli, 2018; S. van der Salm, personal communication, August 31, 2020). Moreover, occupation and transformation of land was not taken into account for any process except cultivation, while in the C-FPS and RA-FPS, the long-term storage of feed could in fact put a considerable pressure on land use. Indirect emissions resulting from soy or *Azolla* occupying land at the expense of the crop originally cultivated there, which migrates and potentially contributes to the conversion of natural area, was not included in the model either.

On top, emissions of heat and dust during the drying process of both soybean and *Azolla*, and emissions from manure treatment, were not regarded. Finally, no packaging materials were included in the FPSs, except for the plastic foil needed to bale the ensiled *Azolla*. The choice of packaging materials (e.g., plastic or cardboard) and packaging end-of-life treatment (e.g., incineration, recycling or reuse) are expected to affect the system's environmental impacts (Molina-Besch et al., 2019). Disregarding these may have led to an underestimation of the total FPSs impacts (Di Santi Barrantes, 2018). Adding process data with regard to these aspects would render the present analysis more complete.

5.6.3. Contribution analysis

The contribution results show the extent to which each unit process exerts different impacts, and thus aids in identifying the system's major environmental hotspots. In the present analysis, the unit processes accounting for 95% of the total impacts were included, and the remaining 5% were grouped under "Rest". *Figure 5.4a-c* displays the contribution results for the end point of each scenario. Note that similar processes were assigned to process groups (e.g., Milk production, Transport, and Mining operation), marked by different colours. In *Table B13aA-bD* of *Appendix B*, the unaggregated data and process groups can be found.



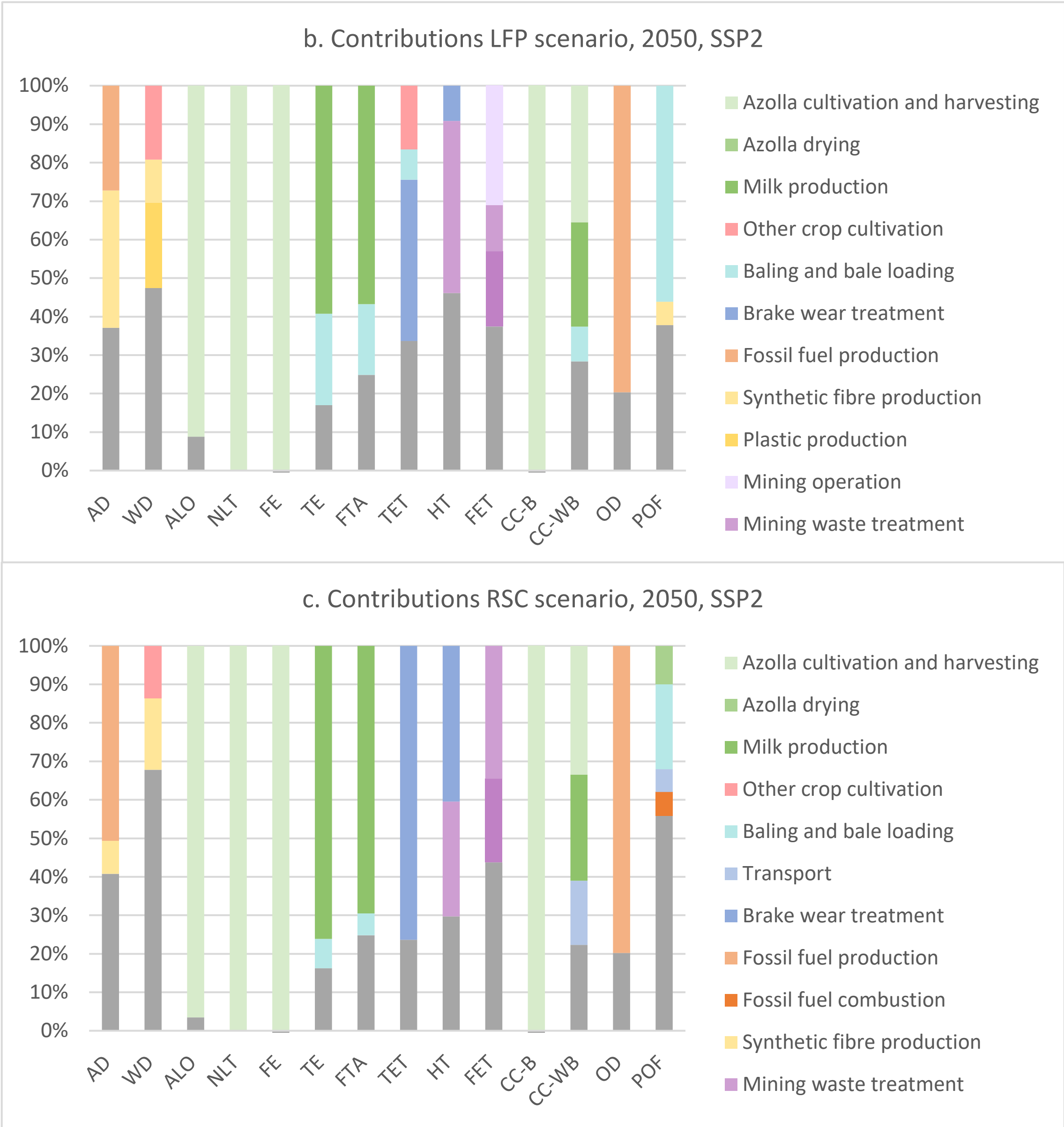


Figure 5.4a-c. Visualization of the contribution results for the three examined feed production scenarios, divided in the categories of related processes. Note that the bars also represent processes contributing to negative impacts.

5.6.3.1. Contribution results input-related impacts

Starting with AD, most impacts in the BAU scenario (i.e., 66%) are caused by the production of fossil fuels, particularly on-shore petroleum and gas, needed for transporting soybeans overseas and on-land. The results of the RSC scenario are similar to those of BAU, although the fossil fuels are primarily used to transport *Azolla* from the farm to the central processing facility and vice versa. In the LFP, however, the largest share of AD impacts (i.e., 36%) are effectuated by synthetic fibre – specifically ethylene – production, utilised for *Azolla* baling.

Most of the WD effects in 2020 in the BAU scenario are due do the production of anhydrous NH_3 and urea, which form the foundation for synthetic N-based fertilisers applied to soybean crops. In the other scenarios, the demand for these fertilisers virtually disappears. In LFP, 33% of de WD results from plastic film extrusion and ethylene production, for *Azolla* baling. Another 19% can be followed back to the cultivation of sugar beet, which molasses are used in *Azolla*-based feed production. In RSC, the same processes underlie WD as in LFP.

Regarding land use-related impacts, 91-97% of ALO can be attributed to soybean cultivation (in the BAU scenario) and *Azolla* cultivation (in the LFP and RSC scenarios). The remaining impacts arrive from sugar beet cultivation. As for NLT, all impacts originate in the cultivation processes, as the model only includes data on the transformation and occupation of land for crop cultivation purposes.

5.6.3.2. Contribution results output-related impacts

In terms of FE, nutrient losses during the cultivation and incineration of soybean (26% and 75%, respectively, in BAU) and cultivation of *Azolla* (100% in both the LFP and RSC) are the largest contributor. Considering TE, 66% of the impacts in the BAU scenario, are directly caused by the transport of soybeans, mainly in lorries and freight trains on the Brazilian mainland. Another 25% of the TE and FTA impacts emanate from milk production. Since transport movements are drastically lower in the FSC and RSC, milk production takes a sizable share in TE (of 59% and 76%, respectively), as well as FTA (of 56% and 70%, respectively), followed by *Azolla* baling (with shares ranging between 6% and 24%).

Furthermore, the application of crop protection substances is responsible for most of the TET and HT impacts (with 93% and 34%) in the BAU scenario. In LFP and RSC, these substances are phased out. Instead, heavy metal emissions from brake wear treatment take a prominent position in TET (by 42% and 76%, respectively) and HT (by 9% and 41%, respectively). In the LFP scenario, another 17% of the impacts are caused by the protection of sugar beet crops. In all three scenarios, FET impacts are mainly the result of pollution from mining operations (e.g., treatment of sulfidic tailings and spoil from lignite mining), as well as the waste processing (e.g., incineration of scrap copper), which link to the raw resources needed in transport networks.

With respect to CC, the impacts in the CC-B category, on the one hand, overwhelmingly take place during soybean and *Azolla* cultivation for all three scenarios. The impacts in the CC-WB category, on the other hand, are more diverse. Emissions from machinery used for cultivation and harvesting (e.g., tractors and manure vacuum pumps) remains dominant in the BAU (with 73%), and less so in LFP and RSC (with 35% and 33%, respectively). Besides, in the LFP and RSC, CH_4-emissions from milk production contributed strongly (with 27% and 28%, respectively), followed by baling (with 9% and 17% respectively) to the overall CC-WB impacts.

For OD, the contribution results are uniform across scenarios, as 80-82% of the impacts arise from oil and gas production, used for transportation. Finally, most POF impacts in the BAU scenario (with 79%), emerge from transportation, whereas in LFP and RSC, baling and bale loading is a major contributor (with 57% and 22%, respectively).

5.6.4. Sensitivity analysis

5.6.4.1. Harvesting loss during Azolla *cultivation*

In the original LCA model, it is assumed that no harvesting loss (HL) from *Azolla* biomass occurs to the soil. However, in an operational setting, some loss is inevitable. This loss sinks from the surface water to the bottom, where most of the C embedded within it is sequestered (i.e., ±80%) and the remaining is emitted as CH_4 (i.e., ±20%). Hence, in the first sensitivity analysis, the HL from *Azolla* biomass is increased from 0% to 12%, which is equal to the HL during soy cultivation (Wolf et al., 2015). *Figure 5.5a* (based on *Table B14A*) shows the subsequent effect in terms of CC-B.

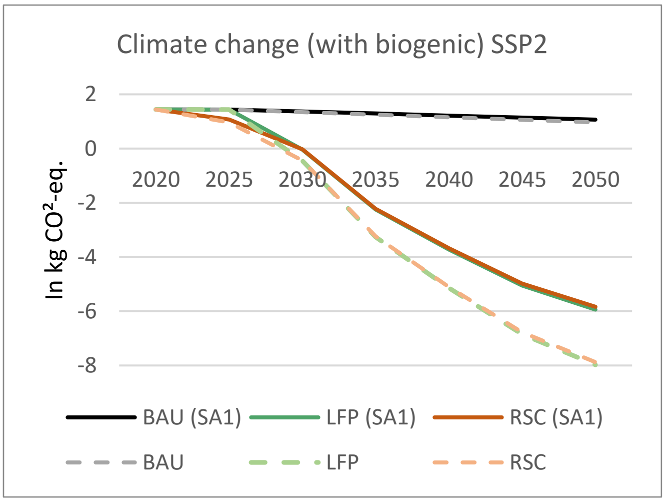


Figure 5.5a. The effect of including a HL of 12% in the *Azolla* cultivation process, on the system's overall CC-B. The dashed lines in light shades represent results of the original model. SA = sensitivity analysis.

The rise in HL translates to a factor 1.3 increase in impacts from CC-B in both the LFP and RSC scenario, relative to the original model. Impacts in the BAU scenario increase by a factor 1.1. This signifies that the model is sensitive to changes in biogenic C emissions from residual HL in *Azolla*-based FPSs, which can be explained by the high GWP of CH_4.

5.6.4.2. *Methane emissions* Azolla-*based feed*

Another assumption of the original LCA is that CH_4-emissions from enteric fermentation in *Azolla*- and soy-fed dairy cattle are equal. In reality, these emissions may be higher in the former, as *Azolla* contains anti-nutritional factors (e.g., condensed tannins) that potentially obstruct its digestion in ruminating livestock and contribute to higher enteric CH_4-emissions (Brouwer, 2017). Hence, in the second sensitivity analysis, emissions from *Azolla*-fed dairy cattle were increased by 20%, while kept constant for soy-fed dairy cattle. *Figure 5.5b* (based on *Table B14B*) shows the subsequent effect on CC-WB.

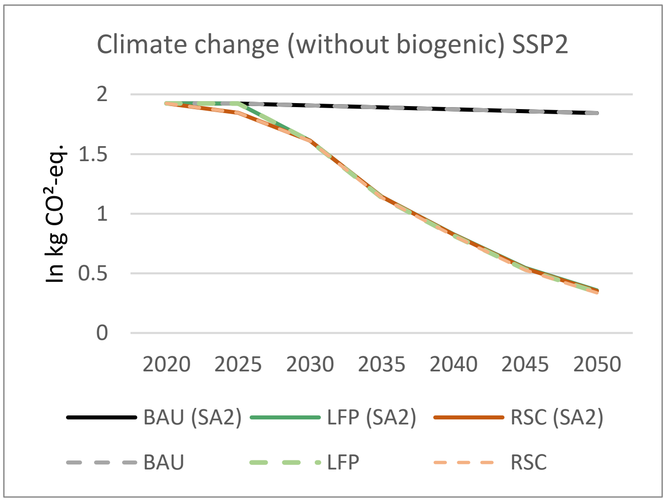


Figure 5.5b. The effect of increasing the CH_4-emissions in *Azolla*-fed dairy cattle on the system's overall CC-WB. The dashed lines in light shades represent results of the original model. SA = sensitivity analysis.

It is observed that augmenting the CH_4-emissions in *Azolla*-fed dairy cattle barely affects the total CC-WB effects of the different scenarios. For BAU, the results remain the same, while increasing sightly (i.e., by a factor 1.04) in the LFP and RSC, relative to the original model. This suggests that the model is relatively insensitive to an alteration of the magnitude of CH_4, in favour of the model's robustness.

5.6.4.3. Insecticide application during Azolla *cultivation*

The original LCA model builds upon the assumption that unlike soy, *Azolla* thrives organically, without the use of crop protection substances. Nonetheless, to ensure stable yield outputs, *Azolla* cultivation may benefit from the application of a fungicide (J. Adema, personal communication, November 2, 2020). The ensuing effect is examined in the third sensitivity analysis. A small dosage of 2.86e-05 kg/kg dm *Azolla* (≈0.5 kg/ha) of azoxystrobin (i.e., a common multi-use fungicide) is added to the cultivation process (Lu et al., 2019). Just as in the C-FPS, the mass balance is maintained, assuming that all of the active ingredient entering the crop, is lost to the soil and is modelled as emission to agricultural soil. *Figures 5.5c-d* (based on *Table B14C-D*) show the subsequent effect in the TET and FET categories.

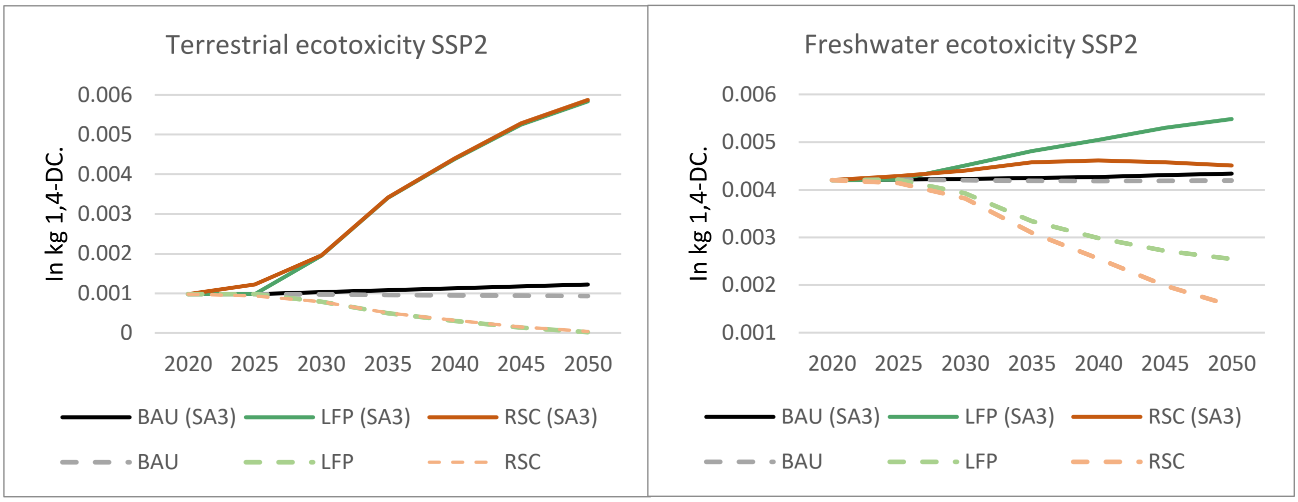


Figure 5.5. Visualization of the effect of applying a fungicide to *Azolla* cultivation on the system's *c.* TET and *d*. FET. The dashed lines in light shades represent results of the original model. SA = sensitivity analysis.

It appears that adding a fungicide to the *Azolla* cropland leads to a sizable increase in toxicity-driven impacts. In the TET category, impacts increase by 1.3 for BAU, by a factor 284 for LFP and by a factor 146 for RSC, relative to the original model. In the FET category, impacts increase remain the same in BAU, and increase by a factor 2.2 in LFP and 2.9 in RSC, relative to the original model. These outcomes indicate that changing an assumption on crop protection, significantly affects the model's performance in terms of TET, and to a lesser extent, of FET.

Chapter 6. Discussion

This chapter reflects on the key findings and lessons-learnt of the present study. It starts off with a comparison of the examined feed production scenarios and the limitations pertaining to these (*section 6.1*), followed by some remarks about its scientific, societal and IE relevance (*section 6.2*), and eventually, an outlook on future barriers for scaling up *Azolla*-based feed production (*section 6.3*).

6.1 Comparing feed production scenarios

This study has examined to what extent replacing the C-FPS by the LA-FPS and RA-FPS could improve the future environmental performance of the Dutch livestock feed sector up to 2050. In doing so, three feed production scenarios were introduced, with scale as the core differentiating variable. The sections below elaborate on the most remarkable differences in environmental impacts, among scenarios, as well as on the limitations that may affect the validity of these results.

6.1.1 Comparing input-related impacts

The BAU scenario performs worse than the LFP and RSC scenarios on all input-related impact categories. By 2050, the largest difference in impacts among scenarios is found for NLT (where LFP and RSC score lower than BAU by a factor 59 and 64, respectively), which shows the land use benefits of transforming wetland-based pastures to extensive paludiculture croplands paired with a decreased transformation of the Amazon rainforest basin.

Smaller differences in impacts were found for LFP and RSC than BAU in terms of AD (by a factor 1.7 and 2.0, respectively), WD (by a factor 1.2 and 2.7), as well as ALO (by a factor 1.5 and 1.7, respectively). These results reveal that *Azolla* cultivation and processing, despite aiming to consume as little inputs as possible, continue to rely on increasingly scarce abiotic, land, and freshwater resources. This ongoing dependency is attributed largely to background processes that represent data for upstream supply chains, including the production of fossil fuels (e.g., needed for transportation) and synthetic fibres (e.g., needed for baling and bale loading), which deliver resource inputs to the FPSs foreground processes.

However, the reliability of these results may be questioned, most importantly because in the LA-FPS and RA-FPS, the foreground processes represent novel technologies with a low TRL (i.e., TRL <5). This means that little knowledge exists on the current, let alone future, types and quantities of input requirements, (Van der Giesen et al., 2020). The lack of access to sufficient primary data to model the *Azolla*-based FPSs foreground in an operational environment is a major limitation (Moni et al., 2020). To obtain the necessary insights on parameters at the core of *Azolla* production, lab and proxy data sources were consulted, and revised by experts insofar possible (Van der Giesen et al., 2020). Most data on aquatic biomass processing was, for example, obtained from trials with duckweed as a feed. Yet, due to the novelty of this research field, as well as a lack of transparency on data quality and replicability, doubt may be cast on the precision and credibility of the retrieved data.

Besides that, a lack of consistent background data poses a problem in this study. Although the foreground processes within the *Azolla*-based FPSs have been designed for increased circularity, the background system (comprising up to 99% of all unit processes) relies on LCI data reflecting production practices that are all but circular. This incongruity is referred to as the mismatch between fore- and background data (Van der Giesen et al., 2020). For example, the LA-FPS and RA-FPS foreground systems source inputs like single use plastics for baling, natural gas for drying, and petrol for transport from background processes. These processes build on virtual data from the SSP2, middle-of-the-road, pathway (i.e., in which current trends continue without considerable change). Scenario data reflecting a more circular mitigation and adaptation trajectory, including more sustainable processes (e.g., including the reuse or recycling of plastic, the substitution of natural gas generation by waste heat, and of petrol production by hydrogen or renewable electricity) could yield more favourable outcomes for the LFP and RSC scenarios, and prevent the clustering of AD and WD impacts in the background system. To this end, it would be interesting to harmonize background data with the SSP1, taking-the-green-road, pathway (i.e., a world of sustainability-focused growth and equality). Improvements in such a pathway may, however, may also apply to the incumbent C-FPS, which would again result in a convergence of impacts among BAU and the normative scenarios.

Moreover, due to their "what-if" nature and reliance on virtual data, ex-ante LCAs draw on a multitude of assumptions about pathways from the present to possible futures (Pesonen et al., 2000). Several assumptions that take a central position in the current study could be disputed.

For instance, in terms of WD, it may be doubted whether a wetland for *Azolla* cultivation only needs to be rewetted once a year, or that harvested *Azolla* does not require a rinsing step. As for NLT and ALO, the main uncertainty is believed to lie in the presumption that the annual *Azolla* yield rises steadily over time, without being threatened by harvest failures (e.g., pests, cold winters, or high wind speeds). Furthermore, the belief that the technologies incorporated in the LA-FPS and RA-FPS will improve when scaled up and reach their optimal production efficiencies by 2050, resulting in a low AD, may be challenged.

Changing these or other optimistic assumptions on crucial variables, may significantly alter the scenarios' overall performance (Moni et al., 2020). Therefore, these must be treated as "What-if" scenarios, aiming to "investigate the consequences of specific, discrete assumptions and uncertainties" (Pesonen et al., p. 26, 2020). "If", for example, outdoor *Azolla* cultivation appears extremely sensitive to exposure to pests, then the harvest outputs may be destabilized such that water and land demands sky-rocket. Consequently, there is reason to assume that in reality, the input-related impacts linked to *Azolla*-based feed production are less favourable than the present study indicates. In the worst-case scenario, assuming a repeated harvest failure, AD, WD and ALO inputs per unit of *Azolla* biomass may even surpass those per unit of soy, rendering *Azolla*-based FPSs unattractive alternatives to the C-FPS. However, given the fast growth of *Azolla* biomass, under a broad range of environmental conditions, this worst-case scenario seems unlikely to occur.

6.1.2 Comparing output-related impacts

In the original LCA model, the BAU scenario scores worse than the LFP and RSC scenarios on all output-related impact categories. To begin with, by mid-century, the major difference among scenarios is found for TET (where LFP and RSC score lower than BAU by a factor 45 and 23, respectively). This result suggests the ostensibly beneficial effects of omitting crop enhancement substances, hence assuming *Azolla* cultivation to be organic.

However, adding a low dosage of fungicide to the *Azolla* cropland, increases the system's TET impacts by a factor 125 (and by a factor 2.2 and 2.9 for FET) in the LFP and RSC scenarios, respectively, relative to the original model. This drastic rise shows the responsiveness of the model to an adjustment in toxicity-related variables, and is perceived as a major trade-off for safeguarding high *Azolla* harvest outputs. Again, since data on the effects of applying pesticides to *Azolla* crops in a fully operational environment is not available, their potential effects on TET and FET may be under- or overestimated.

Also, pronounced differences were found among scenarios for CC-B (where LFP and RSC score lower than BAU by a factor 9) and for CC-WB (where LFP and RSC score lower than BAU by a factor 5). For CC-B, it was initially assumed that *Azolla* cultivation does not involve a harvesting loss (i.e., HL). Equating the HL from *Azolla* cultivation to the soy cultivation HL, led to a converging performance of the scenarios (with LFP and RSC scoring lower than BAU by a factor 7). These results support the expectation that implementing *Azolla*-based FPSs dramatically lowers GHG outputs, due to the low biogenic C emissions from *Azolla* cultivation, combined with an increased use of renewable energy sources and improved energy-efficiencies.

Yet, these outcomes are limited in two main respects. Firstly, the LCA method at present is unequipped for facilitating an in-depth, robust analysis of the impacts associated with the complex biogenic C cycle, as noted in other agro-industrial LCA studies (e.g., Head et al., 2019). The absence of coherent definitions for (non-)biogenic C flows obstructs an accurate modelling representation of the element's behaviour. For example, in this study, the biosphere flow "Carbon dioxide, in air" was used to configure C uptake in biomass (IPCC, 2013). Yet, flows representing the decomposition, humification, and sequestration of biogenic C, do not exist in ecoinvent. While the flow "Carbon dioxide, to soil or biomass stock" at first sight seems to serve this purpose, its CF reveals it to be accounted for as non-biogenic. Therefore, and to prevent double counting, this study offers a strongly simplified LCI representation of the intricate biogeochemical C cycle, possibly resulting in a severe underestimation of CC-B effects.

Secondly, choices on the scope of the LCA model have in all likelihood affected the magnitude of negative CC-B impacts. The system boundaries of each FPS are restricted to the cradle-to-farm-gate. Technosphere flows that depart from the dairy cattle farm (i.e., milk, cattle, and manure), are cut-off. Although biogenic C is tracked through the processes within the model's scope (from cultivation to feed production), C embedded in flows which are further processed or channelled to the market, are beyond the system boundaries. To gain a more complete view on the total CC-B effects, a mass balance would have to be composed that includes downstream processes like cattle slaughtering, leather, meat and milk consumption too. Because of a lack of data, this study assumes that the CC-B impacts linked to these downstream processes are similar, while in reality, this may not hold. The approach of assessing a mere fragment of the supply chain is common in ex-ante LCA for novel food systems, while it may distort conclusions on FPSs comparisons (Hospido, 2010).

The same limitation applies to (negative) nutrients- and toxicity-driven impacts. The present study shows that the adverse effects in the FE, TE and FTA categories are lower by a factor 4.2, 2.4 and 3.0, respectively (in the LFP scenario), and by a factor 4.7, 3.1 and 3.7, respectively (in the RSC scenario), compared to BAU. These strong reductions suggest the high nutrient-efficiency of *Azolla*-based FPSs, proceeding from the reuse of N and P in a (semi-)closed feed manure loop. In reality, these nutrient- and toxicity reductions are expected to be much less high, since the negative emissions from crop uptake are taken into account, but the emissions back into the ecosystem, departing from processes beyond the system boundaries, are not.

Turning to the remaining impact categories, differences among scenarios are substantially lower in LFP and RSC than BAU for HT (by a factor 3.0 and 2.7, respectively), OD (by a factor 3.5 and 2.1, respectively) and POF (by a factor 4.2 and 7.4, respectively). These outcomes indicate that by implementing *Azolla*-based FPSs, the feed sector's reliance on polluting mining and metal (waste) treatment operations as well as petroleum and gas extraction (linked to the production of artificial fertilisers, pesticides and fuel for transport) could reduce substantially.

It must be noted that there is no emission data available on the foreground processes of the LA-FPS and RA-FPS. For instance, it is unknown what quantities of heavy metals leak into the soil or water during *Azolla* cultivation, and how much is absorbed and released only later on in the life cycle. More real-life data and complete mass balances are needed to fully determine the environmental implications of each scenario. In addition to that, like for AD and WD, most adverse impacts in the pollutants- and toxicity-driven impact categories stem from background processes. As explained before, the mismatch between fore- and background process data is in part responsible for this outcome (Van der Giesen et al., 2020).

6.1.3 General limitations on key findings

In addition to the limitations above, two more shortcomings are discussed that do not relate to a particular (group of) impact categories, but rather to the study at large.

6.1.3.1. *Excluded environmental indicators*

A multitude of indicators which could be of considerable importance for understanding the full range of impacts caused by FPSs, for instance on biodiversity and ecosystem services (ES), were not regarded (Joosten et al., 2016). Prior research suggests the positive effect of paludiculture on regulatory and maintenance services, such as water purification and pest and weed control (Smolders and Van Kempen, 2015; Smolders et al., 2019) as well as support services, like soil formation, water buffering and nutrient recycling (Lamers et al., 2018; Smolders and Van Kempen, 2015). Disregarding these impacts may have led to an underestimation of the environmental benefits of the *Azolla*-dominated feed production scenarios, as it was found that *Azolla*-based paludiculture could contribute to such ES, but not soy production (Jurasinski et al., 2020; Smolders et al., 2013; Smolders et al., 2019; Wang et al., 2019b). Besides that, reflecting back on the IROT concept, the indicators used in this study, all involved I (i.e., input) and O (i.e.., output) related impacts, not R (i.e., reuse) or T (i.e., throughput) related impacts. At the moment, no consistent methods or datasets exist yet that allow for a quantification of such impacts in an LCA context (Alejandre et al., 2019; Cucurachi et al., 2019).

6.1.3.2. *Shortcomings of the AB software tool*

Since the AB software is in an early development stage, some noteworthy limitations were encountered during this study. At present, the AB is missing some functionalities that are crucial for LCAs. First, it does not enable its users to retrieve classification results or flows lacking a CF, nor does it allow for normalising the results over a reference point or for conducting a combined process-intervention contribution analysis. Performing these steps manually is possible, though time-consuming. Second, the built-in Sankey diagram, useful for tracing back environmental impacts to their original source, solely displays the results for the starting point of the temporal horizon (here: 2020) in scenario-based LCA. Besides, it is currently not possible to export the Sankey-diagram from the AB, which could aid in visualising the results in a report. Third, not all up-to-date impact families (e.g., CML-IA or FEP) are available, impeding LCA practitioners to use standardized methods, or to conduct sensitivity analyses based on different characterization models. Fourth, the AB does not distinguish between goods and wastes, which renders the modelling of multi-functional processes and the application of an appropriate allocation method more cumbersome than in other software tools, like CMLCA (CML, 2014).

At the same time, the AB could profit from the wealth of options that its programming language (i.e., Python) offers. Linking the tool to geospatial analysis software, or facilitating mass balance calculations within the modelling environment, would increase its user-friendliness and suitability for answering a wealth of questions in a more detailed manner. In the light of *Azolla*-based paludiculture, for example, it would be interesting to incorporate statistical differences in nutrient deposits or groundwater levels across Dutch wetlands, to enrich policy-makers with more geographically specific insights.

6.2 Relevance of this study

In several respects, this study contributes to the pursuit of academic knowledge acquisition, to various solutions for issues facing the Dutch society, and to the IE research discipline.

6.2.1 Scientific relevance

To begin with, this study shows that including a broad range of impact categories, justified on the basis of a thorough literature review, enables the researcher to better identify environmental trade-offs among systems and scenarios. Although the number of multi-category LCA studies (i.e., covering 4≥ impact categories) on livestock commodities has increased over time, simplified LCAs (i.e., covering 1-3 impact categories) remain dominant in the literature. This study supports the view that comprehensive studies of FPSs are sorely needed (McClelland et al., 2018). In addition, this study transparently and extensively addresses the issues arising when setting system boundaries, collecting data, making scale-up assumptions, and interpreting results in an ex-ante LCA context. Consequently, it may aid in establishing a consistent, integrated framework for ex-ante LCA, therewith complementing the already expanding literature body (e.g., Arvidsson et al., 2018; Cucurachi et al., 2019; Moni et al., 2020; Van der Giesen et al., 2020). Simultaneously, this study has identified several shortcomings on the analysis of complex biogeochemical processes (e.g., nutrient cycling and carbon buffering) which draw on the ecoinvent database and need further exploration.

6.2.2 Societal relevance

First of all, this study suggests that *Azolla*-based paludiculture may enhance carbon storage in rewetted wetland soils. Thereby, it may aid to counteract soil subsidence and comply with the

Dutch Climate Agreement goals, which demand an annual 1 Mt reduction of CO_2-eq. emissions from peat oxidation by 2030 (Joosten et al., 2016; Smolders et al., 2019). Fully replacing the C-FPS by the LA-FPS and RA-FPS could reduce the total GHG emissions from drained wetlands from 6.5 Mt CO_2-eq. to below 4.5 Mt CO_2-eq. by 2030 (assuming that the demand for livestock feed remains the same) (see *Table B15A-C* of Appendix B). Secondly, *Azolla* cultivation may enhance water regulation and retention, by allowing for heightened groundwater levels in dairy farming areas, mitigating the detrimental effects of increasingly extreme droughts (Smolders et al., 2019). Thirdly, *Azolla* appears employable as a phytoremediation agent, suited for recovering nutrients or heavy metals from contaminated soils or water bodies (Wang et al., 2019b). Especially in the light of the "nitrogen crisis", *Azolla* could be an attractive crop due its fast growth rate, even on marginal lands (Brouwer et al., 2017).

Regarding these themes, knowledge on *Azolla*-based feed production may offer useful input for a broader dialogue between actors, including (dairy cattle) farmers, agricultural advisory bodies, policy makers and academics, in their common interest of enhancing the sustainability performance of the agro-industrial complex.

6.2.3 Industrial ecology relevance

This study was conducted as a Master's thesis for the Industrial Ecology (IE) programme. It exemplifies the approach of life cycle thinking to designing novel, innovative FPSs (Frosch, 1992). Also, it identifies beneficial biophysical characteristics of *Azolla*, and uses these as a source of inspiration for agro-industrial activities (Erkman, 1997). Consequently, it promotes the conservation of virgin materials and optimal utilisation of materials at end-of-life, and reduces the need for waste treatment by closing resource cycles (e.g., of N, P and C) in the agricultural domain (Frosch, 1992; Ghisellini et al., 2016).

On top, it takes a systems perspective on the complex interactions between the Techno-and biospheres in the agro-industrial domain, by modelling their exchanges of material and energy (i.e., the system's industrial metabolism) (Ayres, 1989; Erkman, 1997). Whereas prior studies seeking to assess the environmental impacts of different FPSs mainly draw on MFA, ex-post LCA, qualitative methods, or a mix of these (e.g., Jouan et al., 2020; Lathuillière et al., 2017; Eriksson et al., 2005; Clark and Tilman, 2017), the current study demonstrates the effectiveness of ex-ante LCA as a methodology for assessing the future impacts of emerging FPSs. As far as the researcher is aware, it is among the first to combine scenario development and data parametrization practices in the new AB modelling tool. Thereby, it has appeared especially relevant to translate the life cycle of environmental innovations and customer adoption curves into concrete, quantifiable narratives of product diffusion. Also, data parametrization was unveiled as a relatively simple and straightforward way to interlace future pathways with hypothesized technological improvements.

6.3 Barriers and opportunities for scaling up *Azolla*-based FPSs

The implementation of a nationwide *Azolla*-based FPS may be hampered by various spatial barriers. In the Netherlands, there are approximately 270,000 ha of, predominantly drained, wetlands (see *Table B15A-C* of Appendix B). In the LFP and RSC scenarios, *Azolla* would occupy about 120,000 ha of these by 2030 and about 240,000 ha by 2050. This amounts to about a third of the area reserved for feed production, and one ninth of the total agricultural land in the Netherlands (CLO, 2020). In such a monocultural cropping system, little space would be left for alternative paludiculture crops (e.g., canary grass, reed, and sphagnum moss), or for yet other purposes (e.g., nature conservation, and recreation) (Jurasinki et al., 2020). Especially in a densely populated country like the Netherlands, careful spatial planning of the scarcely available land is essential. The environmental benefits of *Azolla* in the Dutch agricultural landscape may be questioned considering that *Azolla* is an invasive, exotic species, which through its proliferation may suppress sensitive, endemic species and irreversibly impair vulnerable ecosystems. Plus, the roughage that is currently retrieved from wetland-based pastures (i.e., grass, hay) would have to be cultivated elsewhere, displacing environmental burdens abroad rather than reducing these. Overall, despite the suspected advantages, it may be scrutinized whether scaling up *Azolla*-based FPSs is the most desirable option in the transition to a CAS.

Chapter 7. Conclusion and recommendations

Throughout this study, it has been attempted to answer the following RQ: *To what extent does the large-scale implementation of* Azolla-*based feed production affect the future environmental performance of the Dutch livestock feed sector, in the context of the transition to a circular agricultural system?*

To conclude, under the optimistic assumptions used for estimating key technology parameters, the LFP and RSC scenarios exhibit a considerably better environmental performance than the BAU scenario across input- and output-related impact categories. Taken into account the "what-if" nature of the examined, normative feed production trajectories, differences were particularly pronounced for land use-, nutrients-, and pollutants-related impacts. These effects can be explained by the high protein yield/ha of *Azolla* cultivation, the high nutrient-efficiency of *Azolla* biomass, the low biogenic C losses from crop residues, and the short transport distances along the *Azolla* life cycle. In the abiotic resource-, water use-, and toxicity-related categories, improvements in LFP and RSC relative to BAU were smaller, yet substantial. The low demand for artificial fertilisers and pesticides, the absence of irrigation and, again, the short transport distances in the *Azolla*-based FPSs, are mainly responsible for these outcomes.

In other words, based on the hypothetical scenario results, the large-scale implementation of *Azolla*-based FPSs may indeed contribute substantially to the objective of establishing a CAS by 2050. Nevertheless, some shortcomings have been identified that require special attention in follow-up research. To begin with, the original LCA model appears sensitive to an adjustment of biogenic C emissions and inputs of pesticides in the *Azolla* cultivation process. In order to reduce the uncertainty of parameters within this foreground process, there is a need for high quality, primary data on GHGs and heavy metal fluxes, recorded in an operational *Azolla* paludiculture setting. Such experimental research efforts could also aid in confirming or adjusting crucial assumptions that underpin the model's performance (f.i., with respect to the quantities of wetland-based *Azolla* yield). Besides that, it is suggested to revise the currently flawed LCA methods for biogenic C accounting, and develop a robust approach to modelling the complexities of the biogeochemical C cycle, supported by state-of-the-art scientific insights. Furthermore, this study demonstrates a need for research on standardized methods suitable for quantifying relevant neglected environmental impacts, like biodiversity loss and ES, in order to detect a broader range of trade-offs between aquatic and terrestrial FPSs.

Simultaneously, this study points at the problem of mismatching fore- and background data in future-oriented LCA. It advocates for the renewal and diversification of background LCI datasets that cover a wider range of possible future scenarios, including one directed towards an increasing circularity of production processes (e.g., building on the sustainability-centred SSP1). Complementary knowledge from adjacent research fields (e.g., on sociotechnical transitions) could facilitate the development of more sophisticated, workable scenarios (Geels and Schot, 2007). On top, it is recommended to explore the opportunities of the AB beyond the LCA framework, for example by further developing the Sankey diagram functionality, building in allocation methods and a combined process-intervention contribution analysis, and by connecting the modelling tool to geographically-specific data.

On a final note, uncertainties on how the future, and the role of *Azolla*-based FPSs therein, will unfold, remain omnipresent as long as these systems of interest are at a low TRL. Failing to capture the indeterminacies inherent to novel FPSs, reduces the integrity and effectiveness of an LCA study and may mislead technology development (Moni et al., 2020). A narrow focus on *Azolla*-based feed as the “golden bullet” may result in a blind spot for other potential solutions to alleviate the environmental burdens of the livestock sector. Therefore, it is proposed that follow-up research continues to investigate the here demonstrated environmental potential of *Azolla* as a livestock feed, while simultaneously taking a broader view on feed production. For instance, it would be useful to identify the barriers for scaling up *Azolla*-based FPSs, but also to include yet other novel feed types (e.g., insects, yeasts, algae) in ex-ante LCAs. All in all, this study offers a starting point for a roadmap towards a more sustainable agro-industrial complex, one in which *Azolla* may turn out as a possible, and even highly valuable, piece of the puzzle.

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