



M.Sc. Stefan Hügel

## RUVIVAL Book Series

**Productive Ponds as Part of Rainwater Harvesting  
Systems in the Context of the Slope Farming Project  
Arba Minch, Ethiopia**



# Impressum

RUVIVAL Book Series: Productive Ponds as Part of  
Rainwater Harvesting Systems in the Context of  
the Slope Farming Project Arba Minch, Ethiopia

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Please consider the environment before printing.

# Editorial by Prof. Dr.-Ing. Ralf Otterpohl

In restoration engineering there should be a preference for integrated rural development that we call rurvival – short for rural revival. There are two major principles:

1. Solve a number of major problems together
2. Each part of the system must have several synergistic connections to other parts

This book by Stefan Hügel gives an excellent example of how to make reservoirs highly beneficial on several levels. It is part of the development of the project ‘Slope Farming Arba Minch’ in Ethiopia ([www.slopefarming.org](http://www.slopefarming.org)), which is run by the Arba Minch Municipality, the Arba Minch University and the Institute of Wastewater Management and Water Protection of Hamburg University of Technology.

Reservoirs are a crucial part of rainwater harvesting systems in regions with seasonal rainfall patterns. Our project is following the Keyline Design published by P.A. Yeomans [Yeomans, 1993]. This allows for placing reservoirs in the most efficient places. At the same time, water is directed away from the valley, which is otherwise turned into a gully in eroded areas. Groundwater recharge and a thorough feed of rainwater runoff into the shoulders of the area will reduce dependency on the reservoirs in the first place. Reservoirs can be smaller and in the long run, with massive humus building in bio-intensive forest gardening and reforestation of the upper ranges, become part of a water system that flows all year long. Implementing such systems on a vast scale will cause a shift from the devastating drought-flooding pattern of degraded areas towards a more balanced distribution of rain. Nevertheless, reservoirs are an important life-line when restoration starts and are needed to create perennial productive vegetation covers. However, this does require capable people and capacity building. And this is one of the major feedback loops of the systems design that Stefan Hügel presents: Very high quality of food that will enable people to develop to their full potential.

When I read the first draft of Stefan’s book, I was absolutely shocked! How can it be that all these great methods and elements, all proven in practice, are not implemented everywhere already? How stupid can mankind be with having access to all the great scientific findings and knowing about the few wonderful applications and not do this all over the world? Just imagine a multifunctional reservoir or pond, as suggested in this book: Floating plants in a mix of duckweed and Azolla, a world champion in productivity and capturing nitrogen from the air, reducing evaporation and avoiding infestation by mosquitoes. Despite its phenomenal productivity and multi-functionality, it is hardly attracting any attention in agriculture and is only found in some 2% of global wet rice production. The floating plants can feed fish, ducks and chicken – duckweeds and Azolla are both very high in protein and contain high proportions of Omega 3 fatty acids. Azolla has even been shown to contain the most important and usually hardly available in plants DHA/EPA fatty acids that are crucial for human brain development and maintaining its function. Floating plants as animal feedstock can play an essential role in the production of high quality foods, promoting both animal and human health.

The high amounts of biomass will also provide high quality fodder for other animals and can, indirectly through manure, but also directly, provide fodder for the soil to build humus and consequently feed diverse crops. With well-fed soil and proper intercropping, including Moringa trees, there will be an abundance of high quality food and fodder.

Floating plants actively decrease the evaporation rates in reservoirs to improve the availability of irrigation water in the dry season, while increasing biodiversity. Stefan has gone a step further and this is what makes the system even more productive. Local bio-waste and the fresh waste from fish, chicken and duck processing can be put into a black soldier fly (BSF) system. Instead of a high risk slaughter waste, BSF larvae will convert it to a high protein fodder for fish and chicken, once again enriched in DHA/EPA, while the seepage from the BSF system provides a fertilizer concentrate that can either feed the floating plants again or be used in the permaculture gardens.

As the reservoir receives nutrients to support the floating plants, it provides a combination of irrigation and fertilization of the crops. Therefore, reservoirs have to be designed with the spillway for releasing overloads starting from the inlet, to avoid flushing valuable nutrients out of the reservoir – which, besides the needed capacity building for people running such systems is more difficult than a simple overflow. So I end with a word of caution: Such systems have an enormous potential, they can be highly profitable. However, proper system design and maintenance is crucial for the safety and functioning of the productive ponds and this is not a simple task. In order to cope with the overall Rainwater Harvesting structures and manage the reservoirs properly, I am very much in favor of clusters of independent family farms that own their land (experience shows that only then they work with care and build humus in the long run) and work in co-operatives. A lot of knowledge is needed on all levels and TUHH of the City of Hamburg, Germany works hard on providing the knowledge through the interactive online teaching tool *ruvival* (see [www.ruvival.de](http://www.ruvival.de)). Development of small scale versions of this system, also with greenhouses for colder climates is the actual research work of Stefan Hugel, see [www.tuhh.de/aww](http://www.tuhh.de/aww) for details. This book is part of the teaching materials and I hope you will enjoy reading it just as much as I did – and I hope that you can support implementation, too.

# Zusammenfassung

In Gebieten mit langen Trockenzeiten sind Rainwater Harvesting Systeme von entscheidender Bedeutung für die Anpassung an den Klimawandel, besonders im Regenfeldbau. Wasserspeichernde Erdteiche können in ländlichen Bereichen einfach angelegt werden und in ihrer Funktionalität erweitert werden, um nicht nur Wasser zu speichern, sondern auch um aquatische Pflanzen und Tiere zu züchten.

In diesem Zusammenhang sind besonders *Azolla spp.* und Teichlinsen hervorzuheben, die beide zu den produktivsten Pflanzen gehören und Potential zur Verwendung als Tierfutter, Gründünger und zur Energieproduktion haben. Darüberhinaus dämmen sie die Ausbreitung von Malaria übertragenden Moskitos ein, indem sie Teich vollständig bedecken und dadurch zusätzlich die Verdunstung des Wassers verringern. In produktiven Teichen können eine Vielzahl an Pflanzen zum Einsatz kommen, um eine große Auswahl an landwirtschaftlichen Erzeugnissen zu ermöglichen, die Artenvielfalt in landwirtschaftlichen Systemen zu erhöhen und um zu stabilen und widerstandsfähigen Ökosystemen beizutragen. Die hohe Ertragsdichte einiger bestimmter Wasserpflanzen ermöglicht einen schnelleren Humusaufbau, indem Gründünger und Mulch für landwirtschaftlich genutzte Flächen in Mengen produziert werden können, die in Boden-basierten Systemen kaum möglich sind.

## Abstract

Rainwater harvesting systems are crucial for climate change adaption in climates with a pronounced dry season and especially under rainfed agricultural systems. Earthen storage ponds can be easily constructed in rural areas and expanded in their functionality to not only store water, but also to produce aquatic plants and animals.

Particularly important plants in this context are *Azolla spp.* and duckweeds that are both among the most productive plants providing potential sources of animal fodder, bio-fertilizer and energy, while acting as biological control against malaria spreading mosquitoes by covering the pond surface and even reducing the evaporation rate of the pond. A variety of different aquatic plants can be grown inside productive ponds to provide a great variety of agricultural products, boost biodiversity of agricultural systems and contribute to more stable and more resilient ecosystems. The high biomass productivity of certain aquatic macrophytes enables an accelerated soil building rate by supplying bio-fertilizer and mulch to agricultural fields in amounts that are hardly achievable in soil-based environments.

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# 1 Introduction

The world is upside down. By 2050, an estimated 0.5 - 3.1 billion people will be exposed to water scarcity due to climate change [Gosling and Arnell, 2016]. At the same time, climate change brings an estimated increase in global flood risk of 187 % [Arnell and Gosling, 2016].

Global hunger is affecting two billion people today, more than ever before [Hickel, 2016]. At the same time worldwide obesity has more than doubled since 1980, with 1.9 billion obese people in 2014 [WHO fact sheets, 2016].

In 1981 there were 3.3 billion people that earned less than \$5 per day. In 1990 there were 3.8 billion and today there are 4.3 billion. Nearly 80 % of the world population is earning less than \$10 per day [Hickel, 2016]. On the other hand the rich are getting richer. At the beginning of 2017 it was found that the eight richest men share the same amount of wealth as the poorest half of the globe [Hardoon, 2017].

The current global population of 7.5 billion is expected to reach 9.6 billion in 2050 and 10.9 billion in 2100 [Gerland et al., 2014]. The majority of the current population is depending on an agricultural system that still relies heavily on fossil fuels and phosphate rock, two resources that are non-renewable and expected to be exhausted in the foreseeable future [Tirado and Allsopp, 2012]. Conventional agriculture is still causing soil erosion at a rate of 1 - 2 orders higher than the rate of natural soil formation, including erosion under natural conditions. The current loss of fertile soil is unsustainable and is threatening global food security [Montgomery, 2007].

Short-term thinking and the inability of today's society to manage the distribution and utilization of resources on a large scale is creating an unprecedented multitude and magnitude of problems. A transition towards sustainable land management systems that depend on local resources only could decrease poverty and environmental pollution, create meaningful jobs, resilience and food security.

The Slope Farming Project in Arba Minch, Ethiopia has the goal of putting together a holistic land management system that integrates rehabilitation and conservation agriculture in order to improve food security and water availability in the rural area. In this way, local jobs can be created that contribute to the protection against erosion and provide a sustainable and productive land management system in the interest of the local population.

This work deals with the integration of productive systems that are also part of rainwater harvesting systems. By strategically constructing water storage ponds that collect runoff during the rainy season, irrigation water is provided during the dry season in order to improve crop yields and the building of humus in the soil. At the same time, the storage ponds are productive themselves and serve to grow high-yielding aquatic plants that can be used as supplemental animal fodder and bio-fertilizer to further improve crop yields with locally produced materials.

## 2 Ponds in Rainwater Harvesting Systems

Agriculture is the largest consumer of water and global evapotranspiration rates from agricultural land are expected to double by 2050 [Sharma et al., 2015].

There is a general consensus that climatic variability will increase on a global scale, resulting in more droughts and floods. Degradation of ecosystems and reductions in agricultural productivity are direct consequences of climate change [Vohland and Barry, 2009].

Changes in climate can be devastating in regions that are already prone to droughts. In the tropics and subtropics, changes in the onset of the rainy season have a significant impact on agriculture and food security. In this regard, the whole continent of Africa is especially vulnerable. Africa was the only major region that had a decline in food production per capita during the years 1980 - 2000. From 1970 to 1994, there was a 180 % reduction in human water supply in Africa, while in Europe there was a reduction of 16 %. Drought-induced losses in crops and livestock in north-eastern Ethiopia between 1998 - 2000 were estimated at US\$ 266 per household, which is more than the average annual household income for more than 75 % of the households in the region [Biazin et al., 2012]. Not only is Africa struggling with water availability, but also the agricultural productivity per unit of water ("crop per drop") in the semi-arid zones is the lowest worldwide [Vohland and Barry, 2009]. This is due to soil evaporation and surface runoff, which prevent plants from taking up the rainwater. More than 50 % of the rainfall in dryland agriculture may be lost non-productively. In sub-Saharan Africa, the percentage of terrestrial rainfall used for plant transpiration might only reach 15 % [Biazin et al., 2012]. Currently, only about 2 % of cultivable land in sub-Saharan Africa is irrigated. Rainfed agriculture is by far the predominant land management type, which illustrates the strong dependency on regular rainfall patterns to ensure food security [Vohland and Barry, 2009]. Rainwater harvesting practices are aimed at the accumulation and storage of rainwater for later use. They have great potential to increase agricultural productivity, especially in arid and semi-arid regions, as well as domestic water supply and environmental measures, such as the refilling of aquifers. By improving rainwater infiltration and reducing runoff, rainwater harvesting practices are effective against erosion and consequently an integral component of sustainable land management systems in general [Becher, 2012].

The different methods of rainwater harvesting exert a wide variety of functions, such as groundwater recharge, maintenance of aquatic and wetland ecosystems, nutrient cycling, biomass production, biodiversity conservation, food security, water availability and income generation [Vohland and Barry, 2009].

Rainwater harvesting technologies can be divided into in-situ and ex-situ types, which are also referred to as micro- and macro catchment systems (or internal and external). The in-situ technologies are soil management strategies that enhance the infiltration of rainwater and reduce run-off. They can also function to reduce soil evaporation and to increase soil moisture during dry spells. The distance covered between the point of water intake to the point of infiltration is usually less than 5 - 10 m. In most cases, the storage medium is the soil itself. In-situ technologies include terracing, pitting (as shown in Figure 1), conservation tillage, swales and mulching.

Ex-situ technologies accumulate the water from the catchment area and lead it to the point of water storage, as illustrated in Figure 2. The catchment areas usually have a low infiltration capacity and include rooftops, roads, pavements, rocky areas and sloping land. The collected water is stored in wells, sand dams, dams, ponds or cisterns and can be used for irrigation and domestic uses [Becher, 2012].

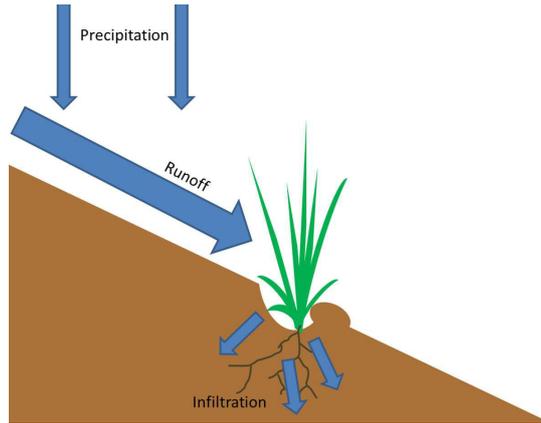


Figure 1: Pitting as an example of in-situ rainwater harvesting techniques. Small holes next to the plants help to increase infiltration of rainwater [Biazin et al., 2012].

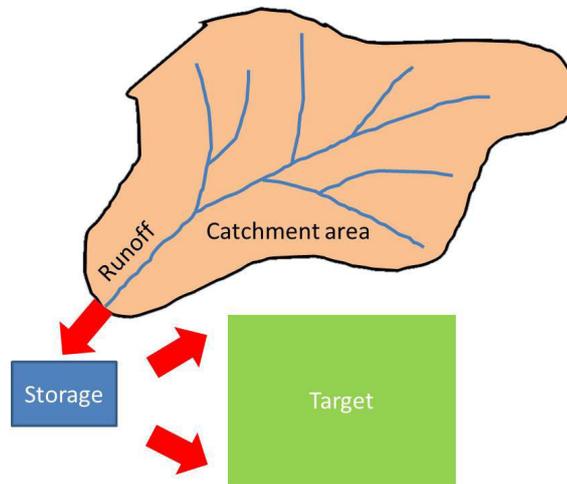


Figure 2: A water storage, its catchment area and the target for irrigation (an agricultural field) are shown [Biazin et al., 2012].

Another definition describes in-situ rainwater harvesting as capturing local rainfall on the farmland and ex-situ as capturing rainfall that falls outside of the farmland [Moges et al., 2011]. The most common types of ex situ household rainwater harvesting reservoirs in Ethiopia are unlined excavations, excavations compacted with clay, excavations lined with plastic and excavations lined with cement [Moges et al., 2011]. The catchment area of ex-situ systems is usually smaller than 2 ha, but can also span over an area of 50 km<sup>2</sup> [Biazin et al., 2012]. In-situ rainwater harvesting methods are currently more common than ex-situ systems in sub-Saharan Africa [Biazin et al., 2012].

## 2.1 Functions of Ponds in Rainwater Harvesting Systems

Ponds have several functions in the context of rainwater harvesting and even beyond. Domestic water supply is among the most important functions, as it sets the basis for human habitation in arid and semi-arid rural areas with limited access to drinking water supply. Hence, rainwater harvesting is a key element in the sustainable improvement of livelihoods in rural areas at any scale. The storage volume of the pond should be large

enough to retain water through the whole dry season. The required pond volume depends on the rainfall amount and pattern, receiving runoff volume of the catchment area and the seepage and evaporation losses of the pond.

One group of researchers investigated the water supply of one family with an average size animal herd using a pond in the lowlands of Makanya, Tanzania. The annual water demand for livestock watering and domestic use was given at 1000 to 1100  $m^3$  per annum. The annual precipitation was often below 500 mm and the catchment area of the pond was 5 ha with a 5-10 % slope. It was concluded that in normal years, a pond of 150  $m^3$  would be enough, while in dry years, a pond of 320  $m^3$  was necessary, so that it would not dry out at any time. The ponds were about 1 to 3 m deep and had a clay bottom layer, which minimized seepage losses [Pachpute et al., 2009].

Besides domestic supply, ponds can be used to store water for irrigation of agricultural fields. Essentially, excessive runoff is caught in the ponds during the rainy season and used for irrigation during the dry season. Cereal yields under rainfed agriculture in sub-Saharan Africa can be increased from 1 to 2 t/ha only by the provision of 100 mm supplemental irrigation during dry spells. Rainwater harvesting has enormous potential to increase food security in areas prone to droughts. More than 30 million rural poor in sub-Saharan Africa can be reached on 15,2 million ha by applying supplemental irrigation. The ratio of storage volume of a rainwater tank to farm plot surface area is about 1 to 10 for supplemental irrigation, meaning a 50  $m^3$  storage tank can be used to irrigate 500  $m^2$  or more of agricultural fields. If open ponds are used, losses through evaporation and seepage have to be considered, which depend on climate and soil types (infiltration) [Biazin et al., 2012]. In the best case, the pond lies on a higher level than the field that has to be irrigated, so that the water is gravity fed onto the field via tubes. Low-cost drip systems were shown to achieve water savings of more than 50 %, compared to conventional surface irrigation during an on-farm study in a semi-arid region in Zimbabwe [Biazin et al., 2012].

Another function of a pond that goes beyond the basic idea of rainwater harvesting is the prevention of nutrient loss through runoff. By catching runoff in a pond, leached nutrients can be kept in place and used for aquaculture, for instance. This is especially important for runoff from agricultural fields, where the soil is rich in soluble nutrients. Not only does this preserve plant nutrients, but it actively prevents the eutrophication of natural ecosystems, especially with nitrates and phosphates. The slope of the agricultural field, fertilizer applications of mineral fertilizer and manure, plowing and erratic rainfall patterns all contribute to runoff that is potentially high in nutrients.

When ponds are dug out, the excavated soil can directly be used to produce loam bricks, for the construction of housing. Using loam bricks reduces the need for cutting trees and provides a much longer lasting material. Combining pond construction with brick production can effectively cut labor requirements.

## 2.2 Control of Water Loss

The construction of earthen ponds is among the cheapest ways to store rainwater for irrigation, however, they come with serious downsides. Water losses due to evaporation and especially seepage can be significant, undermining their potential in rainwater harvesting systems.

Losses of the harvested water at an average of 12 % from evaporation and an average of 57 % from seepage have been reported for earth dams in semi-arid Kenya. An on-farm study in Burkina Faso revealed losses of 5 % due to evaporation and 75 % due to seepage from hand-dug earth dams [Biazin et al., 2012].

### 2.2.1 Reduction of Seepage

Daily water losses due to seepage in unlined ponds range from 24 - 27 mm and 6 - 10 mm in sandy and loamy soils respectively [Moges et al., 2011].

The seepage rate in lake Abaya, which lies east of Arba Minch, results in a water loss of slightly less than 1 mm per day. On the other hand, the lake has a size of 1162 km<sup>2</sup> and therefore doesn't quite compare to a constructed irrigation pond in this regard. However, it demonstrates the capability of natural water bodies to seal themselves [Asrat and Azeb, 2012].

There are several ways to decrease seepage in constructed earthen ponds. Compaction of the soil, application of a clay layer, bentonite, dispersants and liners are all used to minimize seepage in ponds. In the rural areas of developing countries, however, these methods tend to be too costly or need sophisticated machinery to be employed [Stone, 1999].

A more promising method is gleying, which originated from Russia and only needs manure or green waste to work. It is therefore quite cheap and the resources are readily available in rural setups. In order to seal a pond by gleying, several layers of manure or other organic materials, such as straw, crop residuals etc. are deposited in the drained pond at the bottom and the sides. These layers are then topped off with soil in order to compact it. Gleying is a biological process in which bacteria that break down organic matter under anaerobic conditions are excreting a substance that is supposed to form a plastic-like membrane that acts like a liner to seal the pond permanently. This process takes from 1 to 3 weeks, after which the pond can be filled. The method is almost absent from scientific literature, but can be found in several permaculture resources [Brown, 2010].

The layer of straw or manure-straw-mixture should be about 15 cm thick. Alternatively, a future pond basin area can be fenced in and pigs, cattle or other livestock will distribute manure and help to compact the soil with their trampling [Stone, 1999].

In addition to the biological process of gleying, manure seems to have certain physical characteristics that enable pond sealing by clogging the pores of the soil. For this kind of treatment, the manure is simply mixed into the pond water. One study that investigated several sealing agents for farm ponds in Missouri, concluded that manure as a sealing agent had a profound effect in only 1 to 2 weeks after application. Organic gums and acrylic polymers gave disappointing results, while suspended clay and ground hay improved sealing, but to a lesser degree than manure. However, the study authors advised against the use of manure in highly permeable soils [Jamison and Thorton, 1963].

A group of researches in Quebec examined the self-sealing of dairy manure storage ponds in order to assess the risk of infiltration and contamination of soil and groundwater in intensive livestock operations. The storage ponds were 2.4 m deep and were filled with liquid dairy manure containing 10 % total solids. Even in coarse sand, the manure infiltration rate decreased from 2.07 - 2.51 mm per day in the first 2 weeks to 0 - 1.56 mm per day after one year. They attributed this effect mainly to physical effects, as the temperature was less than 10°C. Biological and chemical reactions were reportedly only secondary. Due to "excellent surface sealing" the reservoirs were easily passing the required environmental safety restrictions. It is notable that even the very first measurements of 2.07 - 2.51 mm seepage per day are already extremely low, compared to the 24 - 27 mm given for constructed irrigation ponds in sandy soil, even compared to 6 -10 mm for loamy soil. This suggests that physical effects might play a much greater role in pond sealing than the biological process of gleying [Barrington et al., 1987].

A very similar study found even lower seepage of water of manure-laden ponds with less than 1 mm per day. Their findings applied to both sand and clay soils. A decrease of seepage over time was observed with marked anaerobic activity at the bottom of the reservoir and significant stratification, which could probably have affected the sealing process

by additional gleying formation [Meyer et al., 1972].

Another study investigated the effect of chicken litter application at 125, 250, 500 and 1000 kg/ha total solids per week on the seepage rate of fish ponds in Panama. Before the application, the mean seepage for all ponds ranged from 27 to 37 mm/day. After application it ranged from 8 to 17 mm/day. The higher the application rates, the faster a limit was reached where no further improvement could be observed. The authors therefore suggest a rate of at least 250 kg/ha per week for about a month to rapidly decrease seepage [Teichert-Coddington et al., 1989].

While this study could clearly observe an improvement in the seepage losses with very low amounts of manure, it might still pay off to fill a pond completely with liquid manure for a certain time period before putting it into operation. A reduction from 27 -37 mm to 8 - 17 mm daily seepage is remarkable, however, the self sealing of liquid manure ponds to close or even less than 1 mm would dramatically improve the applicability of water storage ponds in the rural area, if this practice is transferable at all.

### 2.2.2 Reduction of Evaporation

Besides lowering seepage, several methods exist to reduce evaporation as well. One very effective measure is to reduce the wind velocity by planting trees around the pond. Wind barriers have been shown to decrease evaporation by 17 to 34 % [Pachpute et al., 2009]. The average daily water loss of lake Abaya by evaporation is 4.74 mm. Due to its large size of 1162 km<sup>2</sup>, wind speeds can be expected to be rather high, which increases evaporation considerably [Asrat and Azeb, 2012].

Trees can be planted with a certain distance to the pond, but also planting right inside the pond is possible with trees that are tolerant to permanent water-logging, for instance, several species of the genus *Sesbania* (see Chapter 5.3.3). However, the interactions of tree roots and gleying layers in ponds are not known and might possibly lead to increased seepage.

While most plants that grow in ponds or on the shore will increase the water loss from the pond by transpiration needed for photosynthesis, there appear to be some very few plants that are able to decrease water loss from the pond. In one study, 14 plants were investigated for their impact on evapotranspiration rates in ponds. Of those 14, three were lowering water losses, while all the other plants were increasing them. The three plants were *Lemna minor*, *Wolffia columbiana* and *Spirodela polyrhiza*, which are all genera of the sub-family *Lemnoideae*, which are also referred to as duckweeds or water lens of the family of *Araceae* (see Chapter 5.2). They are freely floating plants, just several millimeters in size. The evapotranspiration rates were between 85 % and 90 % respectively of the control without any plants. These plants are highly prolific in terms of biomass production and can be suitable as livestock fodder or for many other uses (see Chapter 5.2). It is therefore surprising to see that a highly productive pond growing animal fodder is more effective in storing water than an ordinary water storage pond without any further productive value [Boyd, 1987].

Another study conducted in Florida compared the evapotranspiration rates of the aquatic plants common duckweed (*Lemna minor*), water hyacinth (*Eichhornia crassipes*) and many-flower (*Hydrocotyle umbellata*) and found yearly mean values of 90, 170 and 180 % relative to the control without plants. *Lemna minor* was reducing the average daily water loss of 4.5 mm down to 4.1 mm. The authors noted that the water loss in the tanks containing *Lemna minor* was constant even with varying plant cover densities. It was also observed that increasing wind velocities resulted in a greater water loss from open water than from tanks with *Lemna minor* [Debusk et al., 1983].

Another plant that holds potential in evapotranspiration reduction is *Azolla spp.*, a small floating fern with great biomass production capacity that can be used for green manure and livestock fodder. A study conducted in Argentina compared the evapotranspiration rates of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), watermoss (*Salvinia herzogii*) and water fern (*Azolla caroliniana*). The average values during four months of cultivation during summer were respectively 267, 114, 120 and 96 % relative to the control. *A. caroliniana* was reducing the average daily water loss from 7.4 mm to 7.1 mm [Lallana et al., 1987].

In another study, only *Azolla filliculoides* and *A. pinnata* were analyzed under climatic conditions giving rise to an annual evaporation on open water of 1343 mm. The respective mean values of evapotranspiration were 79.7 and 76.9 % relative to the control. This would equate to annual water savings of 3102 m<sup>3</sup> per hectare of *Azolla pinnata* covered water surface or a daily water loss reduction from 3.68 mm to 2.83 mm [Diara and van Hove, 1984].

### 2.3 Biological Malaria Control in Ponds

While rainwater harvesting practices have the potential to mitigate the effects of erratic rainfall patterns, the creation of open water surfaces, like cisterns, wells, barrels and ponds can lead to the transmission of several water-related diseases. Hence, it is crucial for the planning of any structure that involves even temporary storage of open water to employ proper risk evaluation beforehand. The associated benefits of increased water availability might otherwise be canceled out by unnecessary health risks for the local population [Boelee et al., 2013].

Water borne diseases (e.g., guinea worm and schistosomiasis) and water-related insect-borne diseases (e.g., malaria, dengue fever, river blindness, yellow fever, and filariasis) are transmitted by vectors that spend at least part of their life cycle in water [Boelee et al., 2013]. Several different health risks can be directly linked to drinking water, sanitation, hygiene and water resource management, as can be seen in Table 1.

Of all water-related diseases in Ethiopia, besides diarrhea, malaria is the most prevalent one. In 2015, an estimated 212 million cases occurred globally, 429000 of which were fatal. For sub-Saharan Africa, 114 million cases were reported in 2015, while there were 131 million in 2010. In Ethiopia in 2015, having about 99 million inhabitants, there were 2.8 million cases, of which 4900 were fatal [World Health Organization, 2016].

It was found that the incidence of malaria cases in the Rift Valley, central Ethiopia is negatively correlated to the distance between the villages and the Koka Dam, a large water reservoir. It could also be shown that in the Tigray Region, households with a pond had at least five times more malaria incidents than households without ponds [Boelee et al., 2013].

Malaria is caused by parasitic protozoans, while the *Anopheles* mosquito serves as a vector. The adult mosquito lays its eggs on the surface of standing water. The eggs float on the surface until the larvae hatch. They live in the water for a week or longer before they turn into adults and leave the water. Therefore, malaria prevention in ponds works by either inhibiting egg deposition on the surface or by killing the larvae. Several control techniques exist that make use of pesticides, biocides (such as *Bacillus thuringiensis var. israelensis* and *B. sphaericus*), oil film layers or other chemicals to prevent the spreading of mosquitoes [Ghosh et al., 2012].

Table 1: Water related health risks. Annual DALYs (in thousands) lost globally for health risks associated with drinking water, sanitation, hygiene, and water resource management [Boelee et al., 2013].

Health risk	Global	Africa*
Diarrheal diseases	72,777	32,203
Malnutrition	17,462	7,095
Intestinal nematode infections	4,013	1,528
Lymphatic filariasis	5,941	2,263
Trachoma	1,334	601
Schistosomiasis	1,707	1,502
Malaria	33,976	30,928
Drowning	10,728	1,824
Other (dengue fever, Japanese encephalitis, onchocerciasis and combinations)	1,740	384
Total water-related	149,678	78,328
Total DALYs**	1,523,259	376,525
% Water-related	9.8	20.8

\*Low- and middle-income countries only.

\*\*The disability adjusted life year (DALY) can be thought of as one lost year of "healthy" life and the burden of disease can be thought of as a measurement of the gap between current health status and an ideal situation where everyone lives into old age, free of disease and disability.

Those methods will not be taken into account as either their environmental safety is questionable or they constantly need to be imported from outside sources, both of which is not acceptable in the context of local resilience building.

One suitable method to drastically decrease the multiplication of *Anopheles* mosquitoes in open water sources is to grow *Azolla spp.* (see Chapter 5.1). Several species exist throughout the tropics and sub-tropics and some are also native to Africa. This aquatic floating fern, also known as mosquito fern, grows on the surface of still or slow moving waters and can quickly form dense mats that cover the whole surface area. It has been shown that this layer is quite effective against several species of mosquitoes. Larval productivity was observed to be lowest in ponds that had the highest coverage of *Azolla* [Mwingira et al., 2009].

A study done on ponds with *Azolla pinnata* and *A. microphylla* in Tanzania demonstrated that several mosquito species of the genus *Culex* stop depositing their eggs in ponds with a layer of *Azolla* in it. However, under the same conditions, species of the genus *Anopheles* will still lay their eggs inside, but it was observed that their larval development was retarded and the successful emergence of the adults was blocked [Mwingira et al., 2009]. *Azolla pinnata* was shown to increase the mortality rate of the larvae of *Anopheles* under laboratory conditions. The emergence rate of *Anopheles* pupae was 15,3 % in a container completely covered with *Azolla*, while it was 95,8 % in the control ones, without any *Azolla* [Bao-lin, 1988].

The common duckweed, *Lemna minor* and the large duckweed *Spirodela spp.* are both floating plants similar to *Azolla spp.* In one study these plants are claimed to act as promising biological control against mosquitoes as well. However, they were only tested in conjunction with the guppy fish, *Poecilia reticulata*, a larvivorous fish [Tariq et al., 2009]. Besides species of *Azolla* and *Lemna/Spirodela*, the two carnivorous plants *Aldrovanda vesiculosa* and *Utricularia reflexa* have been found to be effective in eradicating malaria

spreading mosquitoes. However, this was under laboratory conditions. *Aldrovanda vesiculosa* uses traps similar to that of the venus flytrap, but underwater, to catch small invertebrates. *Utricularia reflexa* has bladders that, when triggered, suck in their prey. Both plants are floating right below the water surface and are native to Africa. They prefer water that is low in nutrients, which makes them more suitable for domestic water storage than high output integrated farming systems [Ogwal-Okeng et al., 2013].

Another option for malaria vector control is the use of larvivorous fish. Apart from raising fish in aquaculture systems, a variety of fish are being used successfully, mostly in rice paddies, to prevent the spreading of mosquitoes. The most popular fish for mosquito control is the mosquitofish, *Gambusia affinis*. It is in fact the most disseminated biological control agent in the world and has been used extensively for eradicating mosquitoes, mainly for malaria control. Several success stories claiming the local eradication of malaria are attributed to the application of the mosquitofish, however, some experts also argue that the introduction of the mosquitofish into new ecosystems can put local fish and other invertebrate predators at risk. There are even cases stating an increase of mosquito populations since the introduction of the mosquitofish due to outcompeting other predators [Rupp, 1996].

Others claim that the mosquitofish has no effect on local fish, not even when released in aquaculture ponds together with other fish. It is advised for ponds with underwater vegetation to combine the mosquitofish with the grass carp *Ctenopharyngodon idella*, an edible species to clear the vegetation in which the mosquito larvae hide.

The guppy *Poecilia reticulata*, a popular ornamental aquarium fish, is also used extensively to eradicate mosquitoes, sometimes together with the mosquitofish. The guppy, however, is used more in closed environments, such as barrels and wells, while the mosquitofish gets released into open ecosystems. Both species are highly adaptable and can flourish in a wide range of ecosystems [Ghosh et al., 2012].

The Sabaki Tilapia, *Oreochromis spilurus spilurus*, which naturally occurs in Ethiopia, was studied for its potential use as biological vector control. A field study was conducted in several small villages in Northern Somalia, where cement lined water tanks that were the only sources of water were inoculated with about 0.4 Sabaki Tilapia per m<sup>2</sup>. After a few months the difference between the infection rates of malaria in the local people that were supplied with the fish and the respective control was highly significant. This fish reaches a size of about 15 - 20 cm and is edible and can therefore additionally contribute to the local livelihood [Alio et al., 1985].

Another larvivorous fish that occurs naturally in Ethiopia is the Arabian toothcarp, *Aphanius dispar*. This fish is just a few centimeters long and can even be used in domestic water storage tanks. One study found that when these fish were put in cisterns, wells and barrels, the proportion of sites having mosquito larvae fell from 34 % to 1,6 %. Monthly restocking of the fish, where necessary, was sufficient for effective mosquito control [Fletcher et al., 1992].

### **3 Uptake of Ex-Situ Rainwater Harvesting Systems in Ethiopia**

The major obstacle to reducing poverty in Ethiopia is the poor performance of rainfed agriculture. In large parts of sub-Saharan Africa, agricultural productivity is among the lowest worldwide, mainly due to the lack of available water, or to be more precise, the inhomogeneous rainfall patterns that above all are becoming increasingly unpredictable due to climate change [Moges et al., 2011].

Agricultural water scarcity in the rainfed agricultural fields in sub-Saharan Africa is

more related to the variability of the rainfall than to the amount of annual precipitation [Biazin et al., 2012].

Increasing crop yields has been shown to be an effective method against poverty worldwide, especially because the majority of the poor are farmers themselves [Ray et al., 2013]. It therefore makes sense to capture runoff in storage ponds that would be lost from the land anyway during the rainy season and use it during the dry season to boost agricultural productivity. The construction of storage ponds can be realized in a rural setup without the use of sophisticated machinery and it contributes to increased yields from the first season on.

However, the construction of ponds and rainwater harvesting systems in general has its obstacles. In Ethiopia, the uptake of rainwater harvesting systems by smallholders has been limited. The main reasons for this are reported to be poor planning and implementation, water availability, operation and maintenance, socio-economics and the farmer's lack of skill to operate those systems properly.

Poor planning is associated with a lack of technical skills of implementing agencies, like low quality of design and construction and unsuitable site selection. As a consequence, many reservoirs have been constructed with high seepage losses and insufficient inflow of catchment runoff, resulting in domestic water shortages.

In the past, the Federal Ministry of Agriculture and Rural Development (MoARD) had initiated several rainwater harvesting projects in the whole country, while further planning and implementation was carried out by the regional authorities. Various authorities were often involved during different phases of the projects, which resulted in poor coordination and lack of accountability. As the government was actively trying to have an impact over large areas of the country, location-specific social and economic factors were largely ignored in favor of acting fast.

The lack of maintenance, including tasks, such as the repairing of embankments and cleaning runoff drains and reservoirs led to compromised system's functionality or failure. Beneficiaries are often not able or willing to carry out the maintenance tasks that are required for proper functioning of the technology, as many consider the government or the NGO who built the systems also responsible for their maintenance.

Only one experimental research site for the testing and developing of rainwater harvesting systems was available for the whole country, which led to shortcomings in location-specificity. Education of farmers about best practices was lacking as well, resulting in low participation and uptake of the rainwater harvesting technologies.

Other issues were health hazards, such as increased incidence of malaria (see Chapter 2.3), risk of drowning of children and animals and increase of rodents near ponds [Moges et al., 2011].

## 4 Transforming Storage Ponds into Productive Ponds

Strategically placed ponds could also take up farmland runoff that is rich in nutrients, where it can be used for aquatic plant production. This would extend the function of a water storage pond to not only capture and store water, but nutrients as well. The biomass of the aquatic plants could be used as mulch on the agricultural fields to bring back the nutrients that were lost by leaching and erosion and provide organic matter to increase the microbial life and water retention of the soil (see Chapter 5.1.3).

The construction of productive ponds also dramatically increases the variety of plants and animals that can be produced in an area (see Chapter 5), leading to a greater diversity of food items contributing to higher self-sufficiency and food security.

Productive ponds can boost biomass productivity in three ways basically: They capture

runoff, which would otherwise lead to water erosion and soil loss, they supply agricultural fields with water during dry spells and they act as a productive system by themselves. Usually, irrigation ponds only have the function of accumulating and storing water. However, their functionality can be extended by using these ponds as productive areas without compromising their storage function. The integration of productive ponds can have several benefits.

Studies performed on the cultivation of the aquatic plants *Azolla spp.* and *Lemna minor* suggest that it is possible to produce far more biomass in a pond than on soil (see Chapters 5.1.1 and 5.2.1). Especially in dry climates, where water availability is the main limiting factor for agricultural productivity, floating plants that are immersed in water will easily outcompete plants in the soil in terms of biomass production.

This is not to be understood as the suggestion to replace soil agriculture by superior pond agriculture. The idea is to rather complement soil-based agriculture with productive ponds in synergistic ways in order to create a holistic system that is more resilient and better capable of coping with the effects of a changing climate.

A healthy soil with vegetation is of paramount importance for a proper functioning water cycle. Without a vegetation cover, soil degrades quickly, especially in drier climates, so that soil will be flushed away by water erosion and finally fill up reservoirs with sediments. Healthy soil, forests and conservation agriculture all play a crucial role to provide a controllable inflow to keep ponds filled up instead of disastrous mudslides rushing down the hills. However, the more erratic rainfall patterns are in an area, the more ponds should be constructed, as an insurance for the agricultural fields.

The construction of productive ponds does not compete for land area in Ethiopia. Ethiopia's agricultural potential is largely unexploited, as less than 40 % of the arable land is currently under cultivation. The majority of agriculture takes place in the highlands, mostly due to cooler temperatures and higher precipitation. Productive ponds could rather have the potential to make highly productive agriculture possible in lower areas, where there is currently only little cultivation in place [Boelee et al., 2013]. Constructed ponds and wetlands in general have many important functions, such as serving as wildlife habitats, flood mitigation, regulation of micro and macro climates, degradation of pollutants and erosion control [Kırım et al., 2014].

With the right selection of plants, the net water loss from ponds can even be lowered, as these plants are able to decrease the evaporation to a greater amount than their own transpiration (see Chapter 2.2.2). In this regard, a productive pond is better suited for storing water than a water storage pond.

Considering a pond with a floating plant cover, like *Azolla sp.* for instance, several labor tasks that are typical for traditional soil-based agriculture become less time consuming or even unnecessary. For example, plowing and weeding will hardly be necessary in a pond growing *Azolla sp.* The spreading of manure or other nutrient sources is a lot less labor intensive, as it does not have to be evenly spread throughout the whole surface. In a pond, the manure or other organic matter will simply decompose under water and release the nutrients that are solubilized and transported by diffusion and currents to the plants.

In most cases, there is also less nutrient pollution from ponds than from agricultural fields, provided possible overflow is adequately managed and appropriate seepage control techniques have been employed (see Chapter 2.2.1).

At difficult terrains with temporal high runoff rates, such as gullies and steep slopes, it is crucial to provide a spillway around the pond. This is necessary to prevent the overflow at the downhill side in order to protect the berms and keep the floating plants and nutrient rich pond water in place, as illustrated in Figure 3. The spillway should lie on a higher level than the inflow point, so that water only flows into the spillway, when the pond is completely filled.

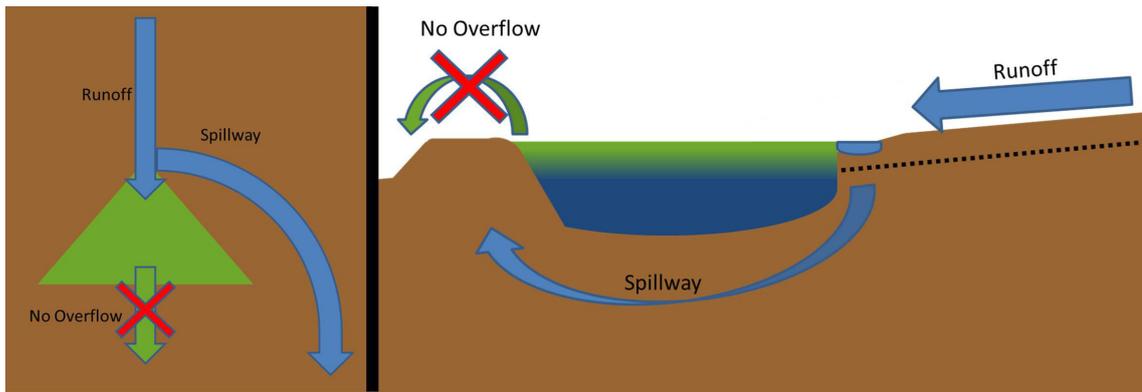


Figure 3: A spillway at the inflow point of the pond diverts runoff water from the pond when it is filled up to prevent the overflow of the nutrient rich water at the downhill berms. As viewed from above (left) and side profile (right).

Especially when mineral fertilizers have been applied or when fields are located on a steep slope, runoff can contain high amounts of nutrients, such as nitrate and phosphates that are not only lost from the agricultural fields, but potentially harmful to the environment as they can cause eutrophication, especially in natural aquatic ecosystems.

In the USA, only about 18 % of the nitrogen that is applied to the fields in the form of fertilizers is eventually found in the produce. The rest volatilizes into the atmosphere, gets flushed away with the runoff or infiltrates into the groundwater, as both ammonia and nitrate are highly water soluble.

Phosphate is less mobile than the common nitrogen compounds found in common mineral fertilizers and it doesn't volatilize. Net phosphorus storage in soil and freshwater ecosystems is now estimated to be 75 % higher than in pre-industrial times.

The projected increase in global fertilizer consumption is predominantly expected to take place in the developing world [Nasir and Firoz, 2014].

## 5 Suitable Plants and their Uses for Agricultural Production in Ponds

Aquaculture is the production of aquatic organisms, such as fish, shrimp and other seafood. Aquatic plants are included as well, however, they make up a rather small portion. The total value of aquatic plants from aquaculture in 2014 worldwide was US\$ 5.6 billion, while the total farm gate value of all aquaculture products was US\$ 165.8 billion.

Moreover, of those aquatic plants, at least 98 % are seaweeds and microalgae, which are actually not belonging to the plant kingdom, meaning, technically they do not represent plants in a taxonomical sense. The fraction of actual aquatic plants is either so small or not reported to the FAO that it doesn't show up in the statistics. Paddy rice, which is grown in standing water, is still counted as a terrestrial crop, as are possibly some other plants of the species presented in this Chapter. Most of them, however, do not have any relevant economic value in the reported crop statistics [FAO, 2016].

On the contrary, many of the presented species have received a considerable amount of attention as invasive pests.

The main reason for these species to be considered invasive is their ability to adapt to a

wide range of climatic and environmental factors and in most cases their extreme productivity. They can become a threat to ecosystems where they are introduced by outcompeting the native plants. Considering their cultivation, this is a risk factor, but on the other hand, some invasive aquatic plants have the potential to mitigate many of today’s most pressing issues, such as food security, erosion control, climate change, pollution, energy demand, etc.

Current global crop yields would have to be doubled until 2050 to sustain a rising world population, diet shifts and increasing biofuel consumption. Today’s yield trends are not sufficient to reach that goal. In order to double crop yields, the four key global crops, maize, rice, wheat and soybean, that together account for nearly two-thirds of global agricultural calories, would need to have an annual production increase of 2.4 %. However, present increase rates are 1.6, 1.0, 0.9 and 1.3 % respectively, far below what is needed to meet projected demands in 2050 [Ray et al., 2013].

In Table 2, the five most important crops in terms of production amount are presented for the world and for Ethiopia. Their cropping area, yield and annual production of calories and protein per area are shown in order to give an orientation for the production potential of the presented aquatic plants.

Table 2: Cultivated area, grain yield, calories per area and protein per area for the five most important crops of the world and Ethiopia. Calories were only counted for crude protein, starch and sugars with 4 kcal/g and ether extract (fat) with 9 kcal/g. Differences in the average nutritional make up between grains of the world and Ethiopia were not taken into account. All values are based on the grains only [FAO, 2014], \*[Feedipedia, 2016], \*\*[Francom and Tefera, 2015].

Crop	Cultivated Area [million ha]	Grain Yield 2014 [t/ha/a]	Calories per Area [million kcal/ha/a]*	Protein per Area [kg/ha/a]*
World				
Maize	184.8	5.62	21.5	523
Rice, paddy	162.7	4.56	14.1	379
Wheat	220.4	3.31	11.8	417
Soybeans	117.6	2.61	10.7	1033
Barley	49.4	2.92	9.2	345
Ethiopia				
Maize	2.1	3.42	13.1	318
Teff **	3.7	1.20	4.5	158
Sorghum	1.8	2.37	8.9	256
Wheat	1.7	2.54	9.0	320
Barley	1.0	1.97	6.2	233

In order to get a rough estimate of the total biomass for the grain crops, studies undertaken in Romania suggest a ratio of straw to grain in wheat of about 0.6 and for maize ca. 1.3 [Antohe, 2007].

The values for the following aquatic plants for yield, calories and protein were taken from [Feedipedia, 2016] if no other references are given. Calories were calculated in the same way as for Table 2. The values for the yield per surface area only serve as orientation. Especially the higher end values often come from values observed over a short time period

under laboratory conditions that are then extrapolated to one year and might be overestimated because of that. Particularly the maximum yield values for *Azolla* and duckweed are rather unrealistic under field conditions, but they serve to indicate what is potentially possible. The values for protein content and calories only act as orientation as well, as they are highly dependent on the conditions of cultivation, especially the concentration of available nitrogen and phosphates.

The following plants are categorized as different aquatic plant types, see Figure 4. This aims to help choose the right aquatic plants for a given pond. For instance, a pond with strong fluctuations in its water level throughout the year should be planted preferably with freely floating plants, while other plant types require a certain water depth range, as they do not tolerate strong fluctuations.

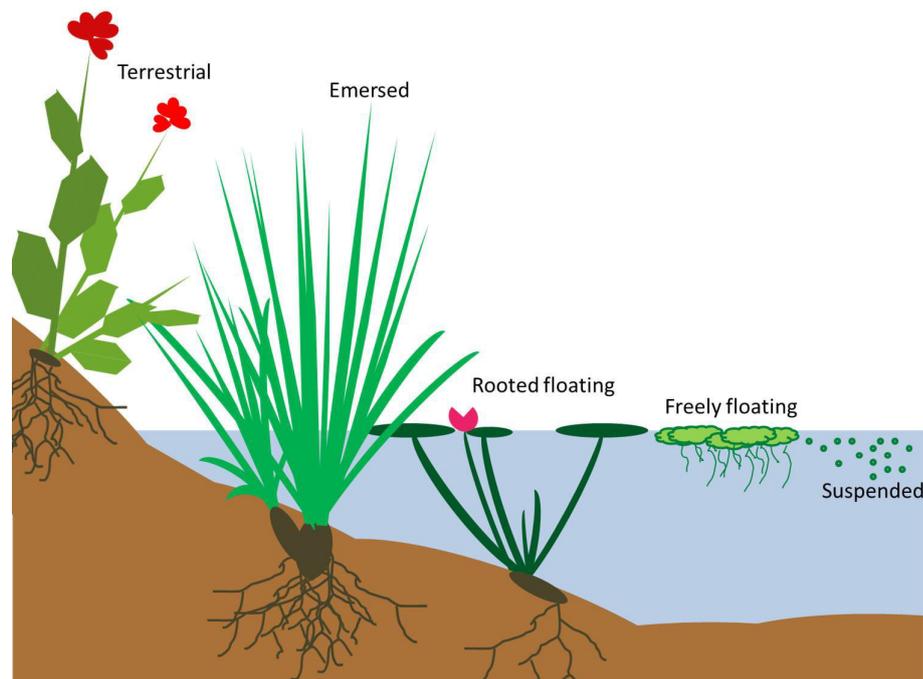


Figure 4: Different plant types in a pond.

The different aquatic plant types used here are:

- Terrestrial (dry): able to grow outside of standing water, tolerates dry soil
- Terrestrial (wet): able to grow outside of standing water, does not tolerate dry soil
- Emersed: roots are in the sediment under water, stems and leaves stand out of the water
- Rooted floating: roots in the sediment under water, leaves floating on the surface
- Freely floating: roots have no contact to the bottom, entire plant floating on the surface
- Suspended: suspended in the water at any depth, applies to microalgae only

## 5.1 *Azolla* spp.

*Azolla* is distributed worldwide in the tropics and subtropics, but also in temperate climates. For instance, *Azolla filiculoides* (see Figure 5) that originates from the American continent can even be found in Ireland, where it was introduced over 100 years ago, supposedly as an ornamental pond plant [Baars, 2012].

Table 3: Basic information on *Azolla* spp. [Feedipedia, 2016], \*[Hasan and Chakrabarti, 2009].

Common names	water fern, mosquito fern
Botanical name	<i>Azolla</i> spp.
Plant family	Azollaceae
Other species of interest	<i>A. filiculoides</i> , <i>A. caroliniana</i> , <i>A. mexicana</i> , <i>A. microphylla</i> , <i>A. pinnata</i>
Aquatic plant type	terrestrial (wet), freely floating
Water depth range	any depth
Harvested plant part	whole plant
Dry mass yield [t/ha/a]	10 - 390*
Calories per area [million kcal/ha]	11.6 - 451.2
Protein per area [kg/ha]	2150 - 83850



Figure 5: *Azolla filiculoides*

It was introduced in many areas, where it was not indigenous, mainly to be used in rice cultivation. At least four species can be found widespread on the African continent. *A. nilotica* and *A. pinnata* subsp. *africana* are native to Africa. One unidentified species could also be found near Arba Minch [Carrapiço et al., 2000].

This plant was already mentioned for its role as a biological control agent against mosquitoes and its ability to reduce evaporation from water bodies (see Chapters 2.3 and 2.2.2).

*Azolla* is a genus that comprises five species, that are all aquatic floating ferns. They grow in fronds of up to 15 cm in size, with branched stems that horizontally float on the water

surface and downward growing freely suspended roots. As the fronds grow bigger, they separate into smaller fronds. This fragmentation is the vegetative reproduction of *Azolla*. Sexual reproduction usually only occurs under stressful conditions.

The taxonomy of *Azolla* is controversial, as most species are very hard to be distinguished from each other. Hence, there are different suggestions of how many species actually exist. *Azolla* is an association of a plant and the cyanobacteria *Anabaena azollae*, also referred to as the *Azolla-Anabaena* system. This type of cyanobacteria is able to fix large amounts of atmospheric nitrogen and is only found in *Azolla*. This symbiotic relationship has been in agricultural use for several centuries. It was and is still utilized as green manure in the cultivation of wet rice in Vietnam and China. The high rate of nitrogen fixation contributed to higher rice yields by incorporating the *Azolla* biomass into the soil. This practice was largely replaced by synthetic nitrogen fertilizers, however, as the awareness of the negative impacts of synthetic fertilizers is rising, *Azolla* is gaining new interest for its use as green manure and several other uses as well [Carrapiço et al., 2000].

### 5.1.1 Cultivation and Biomass Production

The potential for biomass production with *Azolla* is enormous. Additionally, it can grow under a variety of conditions. It withstands pH levels between 3.5 and 10 with an optimum between 4.5 - 7.0. Optimal temperatures are around 25°C for all species except for *A. mexicana*, which prefers 30°C. Several species can also grow in brackish water. The tolerable salt concentration was reported to be up to 1 % [Hasan and Chakrabarti, 2009]. *A. microphylla* was found to be the most salt tolerant species in one experiment [Arora, 2003]. This allows the use of sea salt up to a certain concentration for the cultivation of *Azolla* to provide several trace elements that might be in short supply in the soil. *Azolla* would then be integrated in form of animal fodder or green manure in order to bring the trace elements from the sea onto the land.

A small-scale method of cultivating *Azolla* has been introduced in India, where it is used by smallholders as cattle fodder to increase the milk yield. Therefore, *Azolla* is grown in plastic lined pits of 4 m<sup>2</sup> surface area and a water level of 10 cm. At the beginning of the cultivation, the pit is supplied with 10 - 15 kg of sieved soil, 2 kg of cow dung and 30 g of super phosphate. About 0,5 - 1 kg of fresh *Azolla* culture is spread on the surface to start the process. After 10 - 15 days, it will have spread over the whole surface area of the pit and from then on should be harvested daily for optimal growth.

The daily yield is reported to be 500 - 600 grams of fresh weight. With an estimated dry matter content of 6.0 %, this is equal to about 27.4 - 32.9 t/ha of dry mass per year. Every five days, 20 g of super phosphate and 1 kg of cow dung should be supplied during the daily harvest. Every six months, the whole pit should be emptied completely, cleaned and the process can be repeated [Pillai et al., 2005].

Another very similar method for this process exists, also for smallholders in India. Here, the pit has a surface area of about 2,2 m<sup>2</sup> and is also set up with soil and cow manure or biogas slurry. One kg of fresh *Azolla* is used for the inoculation at the beginning. After 2 - 3 weeks, daily harvest can be started. During the harvest time, 1 kg of cow dung and 100 g of super phosphate should be applied every two weeks. Here, the expected average yield is specified at 800 - 900 g of fresh weight per day. Assuming a dry matter content of 6.0 %, this correlates to an annual dry mass yield of 78.6 - 88.4 t/ha. Also, in this description, one growing cycle lasts six months, then the pond should be emptied and the whole cycle is to be repeated with fresh *Azolla*, soil, cow dung and super phosphate. [Giridhar et al., 2013].

One team of researchers compared the biomass productivity of all *Azolla* species in an artificial medium for 14 days and found that *A. microphylla* had a significantly higher

growth rate than all other species. *A. pinnata* showed poor growth, while the other species were somewhere between those two and did not differ significantly from each other, see Table 4.

Table 4: Biomass, doubling time, relative growth rate (RGR) and nitrogenase activity of *Azolla spp.* after 14 days growth in a polyhouse at 30+/-2°C [Arora, 2003].

<i>Azolla spp.</i>	Biomass (fresh wt.) [g]	Doubling time (days)	RGR [g <sup>-1</sup> d <sup>-1</sup> ]	Nitrogenase nmol [g <sup>-1</sup> h <sup>-1</sup> ]
<i>A. filiculoides</i>	4.65	6.3	0.11	240
<i>A. microphylla</i>	5.86	5.4	0.13	336
<i>A. pinnata</i>	2.41	11.1	0.06	232
<i>A. rubra</i>	4.93	6.1	0.11	219
<i>A. mexicana</i>	4.29	6.6	0.10	327
<i>A. caroliniana</i>	4.89	6.1	0.11	445
Standard error	0.43	-	-	-
Critical difference	1.19	-	-	-

The specified biomass yields of *Azolla* found in scientific literature are differing to a great extent. Yields of 39 - 390 t/ha dry matter in crop cycles of 40 - 365 days have been reported [Hasan and Chakrabarti, 2009].

There is no record so far of any plant or other photosynthetic organism, like microalgae that can produce such an amount of biomass per surface area.

Under optimal conditions, *Azolla* can double its biomass in three to five days. [Liu et al., 2008b]. However, there are also considerably lower yields reported. An annual yield of 10.6 t/ha was obtained for, according to the authors "nutrient non-limiting waters" in central Florida, USA

[Hasan and Chakrabarti, 2009]. However, considering the increasing awareness of the importance of up to 80 trace elements possibly being involved in the plants' metabolism, the certainty of declaring nutrient non-limiting conditions is rather vague.

For optimal growth, *Azolla* needs shading of about 25 - 50 %, especially in tropical areas [Liu et al., 2008b]. Too much sunlight leads to a red coloring of the *Azolla* fronds, which is a clear sign of stress and comprised growth performance. The red color stems from the increased production of anthocyanin. It can also be caused by a lack of phosphorus or too much heat [Carrapiço, 2010].

The shading should ideally be provided by useful trees. That way, the biomass production potential per surface area can be further increased. By using leguminous trees, the nitrogen fixation per area is increased as well. *Sesbania rostrata* is a leguminous fast growing fodder/green manure tree that tolerates both dry conditions and permanent standing water as well, enabling it to grow directly in shallow ponds. Tree rows in the ponds could further decrease wind speeds on the water surface to lower evaporation, while the canopy of the trees are giving ideal shading conditions for *Azolla*. Both *Sesbania rostrata* and *Azolla* are used already as green manure crops between crop cycles of rice, but just for short periods and usually not in combination [Ventura and Watanabe, 1993].

*Azolla* is said to have caused a massive reduction of atmospheric carbon dioxide 50 million years ago, called the Arctic *Azolla* Event. Back then, *Azolla arctica* supposedly floated on a freshwater layer on top of the Arctic Ocean and completely covered it. Consequently, the ocean became anoxic and *Azolla* plants that died off and sank to the bottom were not decomposed, but their organic matter was preserved, so that vast amounts of carbon were sequestered from the atmosphere and stored at the bottom of the Arctic Ocean. This

event lasted for almost 1 million years.

Drillings at the Arctic Ocean provided evidence of this as the 50 million years old sediments were almost completely composed of *Azolla* fossils. The Arctic *Azolla* Event is considered to have had a dramatic impact on the global climate. This clearly demonstrates the enormous biomass production potential of *Azolla* [The Azolla Foundation, 2010].

### 5.1.2 Nitrogen Fixation and Uptake

Besides possessing the highest biomass production potential, *Azolla*, or to be more precise, the *Azolla-Anabaena*-System, also has the most efficient nitrogen fixation rate known. For this reason, *Azolla* is often referred to as a superorganism. Each leaf of *Azolla* has a cavity inside the dorsal lobe, which is the emerged part of the leaf that is photosynthetically active. The cavity, an extracellular compartment, has a size of 0.15 by 0.30 mm and is filled with gas or liquid and holds several immobilized bacteria inside, see Figure 6.

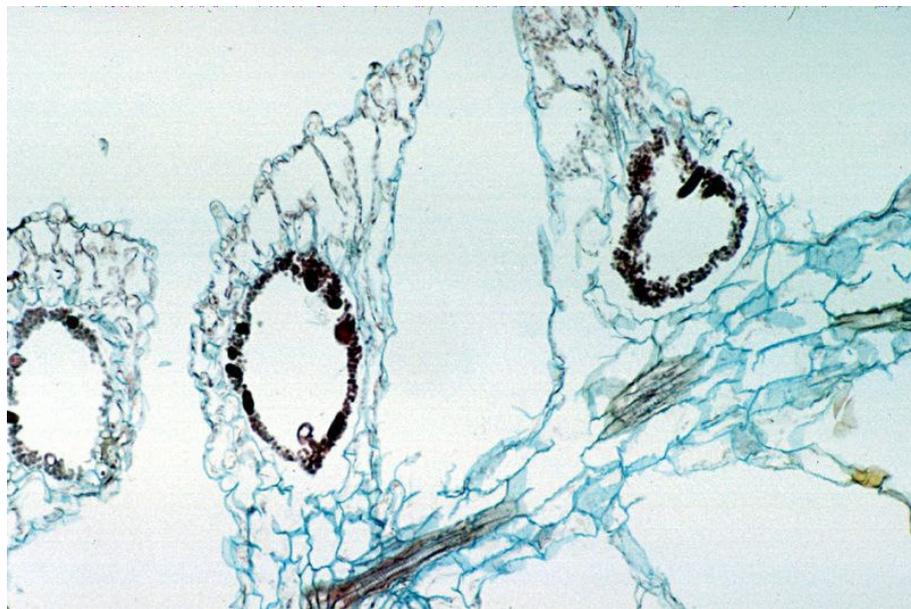


Figure 6: Light micrograph of a longitudinal section of *Azolla*, showing the cyanobacterium *Anabaena* in leaf pockets.

The most important one is *Anabaena azollae*, which is a cyanobacterium or blue-green algae that is responsible for the high nitrogen fixation rate. This cyanobacterium is only found in association with *Azolla*. Besides, there are also other bacteria found inside the cavity, some of which also have nitrogenase activity. Most of them are from the genera *Arthrobacter*, *Corynebacterium* and *Agrobacterium*. This symbiosis of a plant and bacteria is unique, as the association is found in every life cycle of *Azolla*. There is no plant part of *Azolla*, not even during sexual reproduction, where the symbiotic bacteria are not also found inside the typical cavities, suggesting a phylogenetic co-evolution of both partners [Carrapiço, 2010].

Other nitrogen fixing plants, like the ones of the family of *Fabaceae*, that form symbiotic relationships with *Rhizobia* always have to find each other first. For example, an alfalfa seed has no nitrogen fixing bacteria attached to it. After germination, the roots have to connect with the right type of bacteria to be inoculated and form nodules (see Figure 7. Only then can atmospheric nitrogen be fixed by the bacteria and used by the plant. If the specific bacteria are not present in the soil, the symbiotic relationship can not be formed.

Some legumes accept a wide range of possible *Rhizobia* strains as a symbiont, others, however, rely on very specific strains. This can be problematic, especially in heavily degraded soils that have poor bacterial diversity.

For other nitrogen fixing plants that do not associate with *Rhizobia*, but with *Frankia* for instance, the situation is similar. *Azolla* therefore holds great potential for nitrogen input in agricultural systems, as it not only fixes far greater amounts of nitrogen, but is also independent from the local microbial soil life. This makes *Azolla* a more efficient and reliable choice for biological nitrogen fixation, especially for poor soils that need to be restored for agricultural production [Wagner, 2011].



Figure 7: Root nodules of alfalfa inoculated with nitrogen fixing rhizobia.

Historically, legumes had a very important role in agriculture to sustain soil fertility. They were often alternated with cereal crops, which have a high nitrogen demand.

”Without fertilizers, periodically cultivating legumes is the only way to retain soil nitrogen and still harvest crops over the long run. Native cultures around the world independently discovered this basic agricultural truth” [Montgomery, 2008, p. 185].

Only seven years after the discovery of biological nitrogen fixation, the Haber-Bosch process was patented in 1908. This process enables the transformation of atmospheric nitrogen into ammonia. About 80 % of the synthesized ammonia are used for the production of agricultural fertilizers. In the last 100 years, the Haber-Bosch process has had a tremendous impact on modern agriculture through the supply of synthetic fertilizers that boosted crop productivity. It is estimated that about half of the nitrogen atoms in human tissue on average originate from the Haber-Bosch process. The reliance on biological nitrogen fixation, as a natural cooperative system, was largely replaced by synthetic nitrogen fertilizers, such as urea.

However, this shift has resulted in serious environmental problems globally. The main problem with synthetic fertilizers lies in the fact that most of what is applied is not taken up by the plants, which results in the formation of nitric gasses, such as nitrous oxide, soil degradation and pollution of water courses and storages. Globally, in 2005, of 100 million tons applied nitrogen from the Haber-Bosch process, only 17 million tons were consumed by humans through crop, dairy and meat products.

The global nitrogen use efficiency in cereal crops has declined from 80 % in 1960 to 30 % in 2000. The extremely low nitrogen use efficiency that is still decreasing is causing a rise in the application rates of nitrogen fertilizer to keep up the crop productivity for an increasing global population. The decline in soil fertility is fueling a vicious cycle [Erisman et al., 2008].

Furthermore, the Haber-Bosch process consumes large amounts of fossil fuels. For crop production, it amounts to 36 % of the total fossil input. For a sustainable agriculture, alternatives to the Haber-Bosch process have to be employed [Bardi et al., 2013].

The overall biological nitrogen fixation in agricultural systems is estimated to be 50 - 70 million tons annually. Legumes, together with their symbiotic partners, the *Rhizobium* bacteria, make up the largest percentage with 33 - 46 million tons, including crop legumes and fodder legumes/pasture. The average nitrogen fixation rate of the legume-rhizobia-associations within agricultural systems lies between 110 - 227 kg N/ha/year. The most important crop in terms of total global fixed nitrogen is the soybean, with 16.4 million tons of fixed nitrogen per year globally and a fixation rate of 176 kg N/ha/year. The percentage of fixed nitrogen as part of the total nitrogen found in the soybean plant is on average in the range of 58 - 68 % based on various experiments [Herridge et al., 2008].

*Leucaena leucocephala*, a leguminous tree, is the most popular choice for producing green manure and fodder in agroforestry systems in the tropics. It is one of the most efficient nitrogen fixers among the legumes, with rates of 100 - 500 kg N/ha/year [Youkhana and Idol, 2008].

The *Azolla*-*Anabaena*-system represents the most efficient biological nitrogen fixation agent, with an annual fixation of 1100 kg N/ha under optimal conditions [Wagner, 1997]. Another source reports a range of 53 - 1000 kg N/ha/year [Hasan and Chakrabarti, 2009]. The total annual amount of fixed nitrogen of free-living cyanobacteria and cyanobacteria in symbiosis with *Azolla* amounts to 4 - 6 million tons globally. The *Azolla*-*Anabaena*-system is at least 2 - 3 times more efficient at fixing nitrogen than the free-living cyanobacteria. However, almost all rice paddies contain free-living cyanobacteria, whereas only about 2 % have *Azolla* growing in them. Hence, even though *Azolla* has the biggest potential for biological nitrogen fixation, it takes up a very small percentage of the total global amount of biologically fixed nitrogen, as it has little relevance in today's agricultural systems [Herridge et al., 2008].

In order for the nitrogen fixation in *Azolla* to take place at high rates, sufficient amounts of the macronutrients phosphorus, potassium, calcium and magnesium, as well as the micronutrients iron, molybdenum and cobalt are essential [Hasan and Chakrabarti, 2009].

In an experiment where all species of *Azolla* were compared in terms of nitrogenase activity, *A. caroliniana* showed the highest value, followed by *A. microphylla* and *A. mexicana*, see Table 4.

Besides having by far the highest nitrogen fixation rate, *Azolla* also has a high capacity to take up nitrogen compounds solubilized in the water. *Azolla* is considered as a biological water purification agent for space travel. For this purpose, *Azolla* is grown in a urine solution as part of a purification process to turn urine into drinking water. In experiments, *Azolla* was grown in a urine solution with 25.25  $\text{NH}_4^+$ -N mg/l and reduced the ammonium concentration to 1.80  $\text{NH}_4^+$ -N mg/l in five days. Several plants were evaluated for the described purification process and *Azolla* was found to be the most effective one [Liu et al., 2008a].

In another study, *A. filiculoides* was grown in domestic wastewater with an ammonium concentration of 34.1  $\text{NH}_4^+$ -N mg/l and reduced it to 10.6  $\text{NH}_4^+$ -N mg/l in three days. The nitrogen uptake rate was 194.1 mg N/m<sup>2</sup>/day. The nitrogen fixation rate was reduced by 42 % compared to a culture growing in an artificial medium without any nitrogen

[Costa et al., 2009].

One group of researchers compared the nutrient removal performances of *A. filiculoides* and duckweed, *Lemna minor* in different growing media for the cultivation duration of 2 weeks. In a medium containing 600 mg/l of  $\text{KNO}_3$ , which correlates to 83.1  $\text{NO}_3^-$ -N mg/l, the removal rate of nitrate was 78 % for *Azolla* and 49 % for *Lemna*. In another medium with 600 mg/l of  $\text{NH}_4\text{Cl}$ , correlating to 157.1  $\text{NH}_4^+$ -N mg/l, *Azolla* removed 67 % and *Lemna* 23 % of the ammonium. A third medium was prepared with 600 mg/l of  $\text{NH}_4\text{NO}_3$ , which is 105.0  $\text{NH}_4^+$ -N mg/l and 105.0  $\text{NO}_3^-$ -N mg/l. *Azolla* removed 98 % of  $\text{NH}_4^+$  and 51 % of  $\text{NO}_3^-$  and *Lemna* 2 % of  $\text{NH}_4^+$  and 30 % of  $\text{NO}_3^-$ . The nitrogenase activity indicating nitrogen fixation rate in *Azolla* was significantly reduced in the media containing nitrogen, compared to a nitrogen free medium control [Forni et al., 2001].

This indicates a higher nitrogen removal capacity for *Azolla filiculoides* compared to *Lemna minor*, for ammonium, nitrate and the combination of both. However, another study found much better nutrient removal capacity for *Lemna punctata* compared to *A. filiculoides*. While *Lemna* showed good growth and nutrient removal from anaerobically digested swine wastewater at a concentration of 10 %, it had to be diluted to 2.5 % for *Azolla* to be able to grow, while it absorbed significantly less nutrients [Muradov et al., 2014].

### 5.1.3 Phosphate Uptake and Green Manure Production

The problems associated with the application of mineral nitrogen fertilizers are in some regards very similar to those of mineral phosphate fertilizers. Globally, five times more phosphorus is being mined than consumed by humans, whereas about 10 % of the agricultural phosphorus input is finally consumed by humans. Just like with mineral nitrogen fertilizer, mineral phosphate fertilizer has rather low uptake rates in crops. This results in ever increasing application rates, degrading soil fertility, while vast amounts of phosphate are introduced into the environment by run-off, leakage and erosion. Phosphate is the most limiting nutrient in aquatic ecosystems and increasing phosphate concentrations can lead to eutrophication, anoxic dead zones, loss of biodiversity and so on. On top of that, rock phosphate is non-renewable, with peak-phosphorus being expected in the next 100 - 300 years [Tirado and Allsopp, 2012].

A general problem of mineral phosphate fertilizers is their plant availability. Only a few days after application, soils fix the water-soluble phosphate into insoluble forms. In acidic soils, phosphate is fixed by ions of iron, aluminum and manganese, while in alkaline soils, calcium and magnesium are responsible for the fixation. The optimal pH for phosphate availability in soils is 5.5 - 7.0. As the organic matter content of the soil increases, the availability of phosphate increases as well. Both plant roots and the decomposition of organic matter in the soil release organic acids that transform insoluble forms of phosphate into soluble ones. This is especially important in alkaline soils. With a low organic matter content, phosphorus availability will be very low [Sekhar and Aery, 2001].

To increase phosphate ability in agricultural systems, green manure can be applied, as it both contains phosphate and organic matter. The phosphate is incorporated into the cellular structure of the green manure, so it gets released by the decomposition process, which results in a more evenly distributed nutrient supply. *Azolla* might be the perfect candidate for the production of green manure, due to its ability to grow in nitrogen-free water. Phosphorus is the main limiting nutrient for *Azolla* [Carrapiço, 2010].

So, the mineral phosphate fertilizer might be used to grow large amounts of *Azolla* that fix nitrogen and take up other macro- and micronutrients as well. Provided the pond where *Azolla* is grown doesn't leak or overflow, there would be a minimal loss of the phosphate fertilizer. A lot more labor would be needed to grow, harvest and distribute the green manure instead of just applying the mineral fertilizer. However, it would save tremendous

amounts of nitrogen fertilizer, increase the availability of phosphate, minimize nutrient losses into the environment, supply other important plant nutrients and feed the soil microbes with organic matter. This applies also for liquid livestock manure. Leakage from manure on farmlands is problematic as a well. Bad odor is a sign of anaerobic conditions that lead to losses of nitrogen in form of ammonia. The cultivation of *Azolla* as green manure could be part of a nutrient management system that aims at reducing nutrient losses and increases nutrient uptake efficiency in crops, while building humus.

The nitrogen to phosphorus ratio (N:P ratio) in *Azolla* depends on several conditions, but is mainly influenced by the available nutrients of N and P. Phosphorus uptake is most efficient without any nitrogen present in the media. In one experiment, for artificial media without any nitrogen, *Azolla* had a N:P ratio of 5.7:1, while in the same medium with 40 NO<sub>3</sub><sup>-</sup>-N mg/l it had a N:P ratio of 10.2:1 [Costa et al., 2009]. The NPK values for several types of animal manures can be seen in Table 5.

Table 5: NPK values of several different manures (% per dry matter) [Harrison, n.d.].

	Nitrogen (N) %	Phosphorus (P) %	Potassium (K) %
Cow manure	0.6	0.4	0.5
Horse manure	0.7	0.3	0.6
Pig manure	0.8	0.7	0.5
Chicken manure	1.1	0.8	0.5
Sheep manure	0.7	0.3	0.9
Rabbit manure	2.4	1.4	0.6

These values only serve as an example, as they can vary greatly due to diet, age etc. Even horse and sheep manure, that have the highest N:P ratio of 2.3:1, are at least 2 times below that of *Azolla*. Hence, the production of *Azolla* green manure with animal manure has the potential to at least double the nitrogen content, if full assimilation of the phosphorus is assumed. Another advantage of this type of fertilizing is the prevention of bringing in seeds into the crop fields. For example, in Arba Minch, goat manure always had a large number of *Leucaena* seeds in it, so every time it was used for fertilization on the field, additional weeding had to be done in the next weeks. By using goat manure to grow *Azolla*, the seeds would simply sink to the ground and decompose.

In addition to animal manure, other sources that are especially high in phosphorus should be included as well, as it constitutes the main limiting nutrient for the growth of *Azolla*. In some countries, farmers are obligated to buy phosphate fertilizer, such as diammonium phosphate (DAP), to ensure higher yields and food security. However, in Arba Minch for instance, several farmers refuse to use it on their fields, even though they bought it, as they were lawfully required to do so. Reasons for this are mainly environmental concerns. As a result, the demand for phosphate fertilizers is oversaturated. This might be an opportunity to use it for the production of *Azolla* green manure to mitigate environmental risks, such as eutrophication and increase the availability of phosphorus to crops.

However, phosphate fertilizers contain trace amounts of cadmium, uranium and fluoride, which can be problematic depending on the concentrations. For instance, in western countries, 54 - 58 % of the cadmium found in the environment is estimated to originate from the application of mineral phosphate fertilizers in agriculture. In this regard, the use of these fertilizers for the production of *Azolla* is still problematic due to contamination. For proper risk evaluation, further investigations about the accumulation and availability of cadmium in soils using *Azolla* fertilized with mineral phosphates as green manure would be needed [Tirado and Allsopp, 2012].

An alternative to mineral phosphate fertilizers would be bone ash. In many rural areas, the bones from slaughtered livestock are simply buried in the ground. However, bone ash contains about 18.5 % phosphorus and would present an alternative to DAP, which has 20.1 % phosphorus [Hansen, 2010].

Provided all other essential nutrients are available, 1 kg of bone ash could produce about 30.3 kg of *Azolla* dry mass. The collection of bones from slaughter houses and the processing of bone ash could become a small business integrated into the production of charcoal to support the cultivation of *Azolla* out of renewable resources to produce green manure that is also rich in nitrogen [Feedipedia, 2016].

The main application of *Azolla* in terms of surface area is its use as green manure in rice paddies. It is estimated to be present in about 2 % of all rice paddies, which is about 3 million hectares [Herridge et al., 2008].

There are two main methods of how *Azolla* is used as a green manure in rice paddy cultivation. The first is to grow *Azolla* on the flooded rice field for 2 - 3 weeks, then the field gets ploughed and *Azolla* is incorporated in the soil about 2 weeks before the rice plants are transplanted. The second method is to grow *Azolla* and rice simultaneously and when *Azolla* has covered the surface, the whole field is drained, *Azolla* worked into the soil and then the field gets flooded again. Both methods are very labor intensive, but save about half of the nitrogen fertilizer input. Molybdate can be used to increase the nitrogen fixation rate of *Azolla*.

An experiment to evaluate the suitability of *Azolla* as a bio-fertilizer for paddy rice was conducted in the Republic of Guinea-Bissau in West Africa. Several fertilization schemes with either *Azolla* compost, nitrogen fertilizer (urea) or both were compared in terms of rice yield. The results are summarized in Table 6.

Table 6: *Azolla* as a biofertilizer for rice. Average yield (compared to control assay) of BG 90-2 rice variety relative to different fertilizer mixtures [Carrapiço et al., 2000].

Treatment [per hectare]	Rice yield [kg/ha] (percentage of control)
No treatment (control)	1637 (100 %)
87 kg N (urea)	3158 (193 %)
7 t <i>Azolla</i> compost	2355 (144 %)
14 t <i>Azolla</i> compost	2440 (149 %)
7 t <i>Azolla</i> compost + 43.5 kg N (urea)	2835 (173 %)
14 t <i>Azolla</i> compost + 43.5 kg N (urea)	2946 (180 %)

The treatment with 7 t of *Azolla* compost and 43.5 kg N of urea fertilizer was determined to be the most suitable one for the region, as 50 % of the nitrogen fertilizer could be saved with only a slight loss of rice yield [Carrapiço et al., 2000].

Another study conducted in Jamaica was investigating the effect of *Azolla* as a mulch for pepper plants. Fresh *Azolla*, dried *Azolla*, 100 kg N/ha of ammonium sulfate fertilizer were compared with a control plot without any fertilizer. Both the dried *Azolla* and the nitrogen fertilizer increased yields by 80 %, the fresh *Azolla* by 60 % relative to the control. The dried *Azolla* was decreasing the period until harvest by three days compared to the fertilizer treatment and helped in keeping the soil moist. The authors concluded that for small farmers, the cost of preparing *Azolla* to be used as a fertilizer is about 10 - 15 % of the cost for commercial fertilizer [Prasad and Potluri, 2000].

Another study carried out experiments on tomatoes that were fertilized with ammonium sulphate and *Azolla* biomass in varied amounts and combinations. The authors con-

cluded that *Azolla* biomass could fully substitute inorganic nitrogen without affecting crop productivity and quality. Moreover, they witnessed a ripening of the fruits 10 days earlier and an 8 % reduction in damaged fruits when *Azolla* was used as bio-fertilizer [Milicia and Favili, 1992].

#### 5.1.4 Livestock Feed

*Azolla* is interesting as a potential feed, or rather supplemental feed, because it is very cheap to produce. Its enormous biomass production potential together with the very high nitrogen fixation rate makes *Azolla* potentially the cheapest source of protein available. Even very low harvests of 10 t/ha of dry mass will provide at least twice as much protein as one hectare of soybean on a global average, see Table 2. The protein content of *Azolla* in the dry matter lies between 20 - 30 %, however, new breeds can contain up to 35 % [Liu et al., 2008b].

The dry matter content of fresh *Azolla* lies between 5 - 7 %, crude lipid levels are around 3 - 6 % and the ash content is quite high with 14 - 20 %, all based on dry matter [Hasan and Chakrabarti, 2009].

Compared with other plants, the ratio of omega 3 to omega 6 fatty acids is quite high in *Azolla* with 1.4 - 2.3:1. *Azolla* even contains the long chain omega 3 fatty acids EPA and DHA that are usually not found in plants [Bhaskaran and Kannapan, 2015].

These fatty acids play a major role in human health and especially cognitive function. The main source for EPA and DHA is fish, fish oil and other seafood, while they are only found in very little amounts in terrestrial animal products. However, the incorporation of *Azolla* in the diet of livestock might enable the accumulation of these valuable fatty acids in terrestrial animal products as well. This could benefit the health of millions of people that have no access to seafood and hence are likely to be deficient in EPA and DHA.

Moreover, the ratio of omega 3 to omega 6 fatty acids in the diet has a profound effect on human health in general. While humans evolved with a ratio of about 1:1, today's typical values in the western nations reach ratios of 15:1 or higher. This imbalance promotes the onset of many diseases, such as cardiovascular disease, cancer, osteoporosis, and inflammatory diseases. Increased levels of omega 3 fatty acids exert suppressive effects. The replacement of grains with *Azolla* in feed for livestock production could benefit the animal's health, as well as human health, by promoting a shift to a healthier better balanced fatty acid profile, as *Azolla* has a much higher ratio of omega 3 to omega 6 fatty acids than cereal grains and especially soy [Simopoulos, 2006].

*Azolla* is also rich in amino acids, with 7 - 10 % in the dry matter. It also contains high levels of beta-carotenes and even vitamin B12, which is very unusual to be found in a plant. Its fat and carbohydrate content is rather low, which makes it mainly interesting for the supply of proteins, minerals and vitamins.

Experiments have shown an increase in milk yield of about 15 % when 1.5 - 2 kg of fresh *Azolla* was fed to the cows together with their regular feed. This is surprising as the increase in milk was higher than could be expected based on the amount of nutrients provided by *Azolla*. Hence, it is assumed that several micronutrients in *Azolla* promote the increase in milk production by increasing the feed conversion ratio. The *Azolla* biomass should be washed before feeding it to the cows and mixed with concentrate 1:1 until the cows get accustomed to the new taste. After that it can be fed on its own [Pillai et al., 2005].

A study on cultivating *Azolla* as a supplemental cattle feed in India proposed that the construction of a pond for the cultivation of *Azolla* for one cow would cost a farmer Rs. 500, while he would realize an income increase of Rs. 4000 per year, due to the increased milk yield [Giridhar et al., 2013].

Maity and Patra (2008) investigated the use of *Azolla pinnata* meal as a partial substitute for fishmeal in the diet of carp fingerlings. Increasing dietary levels of *Azolla* increased the depression of growth in the fish. The main reason for this was considered to be trypsin inhibitors present in the *Azolla* meal. These are antinutritional factors that inhibit digestive enzymes in the fish and retard their nutrient uptake. However, a 25 % substitution of fishmeal with heat treated *Azolla* meal resulted in only about 3 % less weight gain, with a major cost reduction. Heat treatment (90°C for 10 min) was found to decrease trypsin inhibitors in *Azolla* meal and therefore resulted in better growth performance of the carps [Maity and Patra, 2008].

Poultry under rural semi-range conditions in India were fed fresh *Azolla pinnata* ad libitum and compared to a standard diet. The *Azolla* ponds were integrated into the poultry enclosure, so they could pick them directly out of the pond. Birds with access to *Azolla* gained more than 40 % more weight in 14 weeks and laid more than 40 % more eggs during a 72 week period. The inclusion of *Azolla* into the diets of poultries holds promise to alleviate poverty in rural areas, as it was found to cut feed costs by over 80 % [Rai et al., 2012]. *Azolla* that was grown for the experiment (Chapter 8.2) in Arba Minch was fed to chickens and cattle, see Figure 8 and Figure 9. 25 chickens were eating about 500 g of fresh *Azolla* in less than an hour at the first time they saw it. The cows, however, would only eat it, when it was mixed with concentrate at equal parts.



Figure 8: Chickens feeding on fresh *Azolla* in Arba Minch



Figure 9: Cattle feeding on fresh *Azolla* mixed with concentrate in Arba Minch

In most feeding trials with *Azolla*, it was found that the inclusion of low levels in the range of 10 - 15 % resulted in equal or slightly better livestock performance. However, higher levels almost always resulted in significantly reduced growth rates, which is supposedly due to antinutritional compounds that at a certain level disrupt the metabolism of livestock. The inclusion of *Azolla* as a feed supplement usually reduced total feed costs substantially. Chapter 6 deals with methods to increase the inclusion rates of *Azolla* and other aquatic plants without the harmful effects of excessive antinutritional factors.

#### 5.1.5 Other Applications

*Azolla* can be used for the production of biofuels and petrochemicals. This is of special interest when *Azolla* is used as part of a wastewater treatment system, as in this scenario it does not compete with arable land, in contrast to the plants currently used for biofuels, such as corn, sugarcane, rapeseed etc. Pyrolysis products of *Azolla* fall in the same range as bio-oils derived from algae that contain alkanes that can be used directly as a diesel fuel supplement. *Azolla* can be used together with duckweeds (*Lemna spp.*) and microalgae for wastewater treatment at different stages and harvested for the production of renewable fuels [Muradov et al., 2014].

*Azolla* has been proposed as part of a space agriculture system for the habitation on planet Mars. The whole system is designed towards as much nutrient recycling and as little waste as possible in order to reduce the launch mass from Earth for manned missions on Mars that are assumed to last for 20 years on Mars with a population size of 100 people. This life support system is engineered by synthesizing an artificial ecosystem that consists of plants, microorganisms and animals.

Beside *Azolla*, rice, soybean, sweet potato and mustard greens are the main components of the space diet. Moreover, fish (loach), silkworms and trees are included as well. Mulberry trees serve to raise the silkworms and *Azolla* is grown in rice paddy fields, where the loaches will be grown in an integrated system. *Azolla* was chosen for its rapid growth

and high nitrogen fixation rate to be used as a bio-fertilizer for the whole system. It can be eaten cooked and is also converted to animal meat by the loaches that feed on it. The production of animal products is important to the diet of the Mars inhabitants to provide them with vitamin D3 and B12, certain fatty acids, such as EPA and DHA, cholesterol and high quality animal protein [Katayama et al., 2008].

*Azolla*, or to be more precise *Azolla's* chloroplasts, have a higher oxygen-releasing capacity than those of other plants, such as rice, corn cauliflower and chives. Even on a wet weight basis, *Azolla* produces more oxygen than all the other tested plants, although it has a high water content of about 95 %. In an experiment lasting 72 h, it could be shown that 16 m<sup>2</sup> of *Azolla* culture was enough to provide sufficient amounts of oxygen for two persons inside a closed chamber. This makes *Azolla* an integral part of the oxygen supply inside the artificial atmosphere on Mars. *Azolla* also showed the most favorable properties for the treatment of urine in space station operations in terms of nutrient uptake capacity [Liu et al., 2008b].

Space agriculture is designed towards a materials recycle loop as close as possible due to severe constraints of resources. This is exactly what makes its concepts interesting for the design of sustainable agricultural systems on planet Earth as well.

*Azolla* is a known hyper-accumulator and can remove heavy metals and other contaminants from wastewater. It can remove strontium, copper, cadmium, zinc, chromium, nickel, lead, gold, platinum and even radioactive uranium. If *Azolla* is specifically used to remove heavy metals, it should of course not be used as green manure or fodder [Bennicelli et al., 2004].

One study investigated the use of *Azolla* as an insecticide against the root-knot nematode in okra plants. When dried *Azolla* was incorporated into the soil at 3 %, maximum reduction in egg masses and the nematode population were observed. The underlying mechanism was not specified [Ramakrishnan et al., 1996].

*Azolla* can also be used as a mosquito control agent and to reduce evaporation losses from open water bodies, as was already discussed in Chapter 2.2.2 and 2.3.

## 5.2 *Lemna minor*

Duckweeds were already mentioned as potential biological control agents against the spreading of malaria, as well as reducing evaporation rates of ponds. *Lemna minor* is the most well-known duckweed, however, several species also from different genera exist (see Figure 10) that have very similar properties and potential applications to *Lemna minor*, the common duckweed. Duckweeds include the five genera *Lemna*, *Landoltia*, *Spirodela*, *Wolffia* and *Wolffiella*. They comprise the sub-family *Lemnoidea* of the family *Araceae* and are the smallest known flowering plants. The plants have a very simple structure, lacking stems and leaves. Each plant is organized in a frond structure that grows by division. Each frond is flat and round or ellipsoid with or without small roots attached and a diameter of between 1 mm to 1 cm.

Table 7: Basic information on *Lemna minor* [Feedipedia, 2016], \*[Leng, 1999].

Common names	common duckweed, common waterlens
Botanical name	<i>Lemna minor</i>
Plant family	Araceae
Other species of interest	<i>Landoltia punctata</i> , <i>Lemna gibba</i> , <i>Spirodela polyrhiza</i> , <i>Wolffia arrhiza</i>
Aquatic plant type	terrestrial (wet), freely floating
Water depth range	any depth
Harvested plant part	whole plant
Dry mass yield [t/ha/a]	10 - 182.5*
Calories per area [million kcal/ha]	17.1 - 312.6
Protein per area [kg/ha]	2910 - 53108

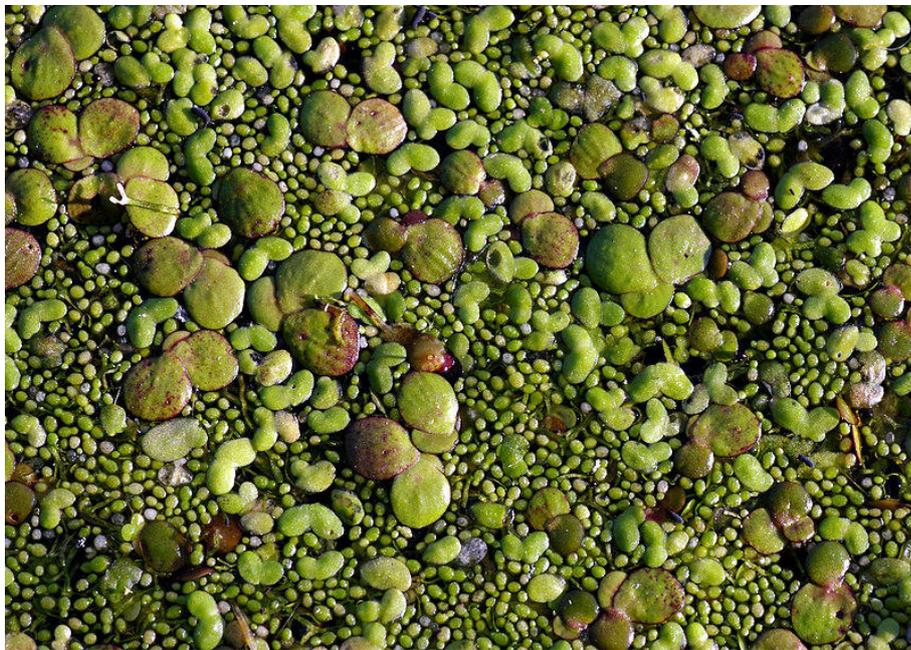


Figure 10: Close-up of different duckweeds on the surface of a pond: *Spirodela polyrhiza* (large fronds), *Lemna minor* (medium), and *Wolffia arrhiza* (very small).

They have a lot of similarities with *Azolla spp.*: They are both very small free floating aquatic plants, have the capacity to grow at an extraordinary speed and grow best under very high nutrient conditions. They reproduce mainly asexually and usually flower or sporulate only under certain conditions often unpredictable. The differentiation and identification of species is difficult for both *Azolla* and duckweeds. Both are distributed worldwide, but most species are found in tropical and subtropical areas.

The main difference between duckweeds and *Azolla* is that duckweeds do not fix nitrogen (or do so in much smaller amounts), do not need any shading and can have a much higher protein content than *Azolla*.

Historically, duckweeds have been used for several generations in small farming systems in South Asia as feed for ducks, poultry, pigs, fish and even as human food [Leng, 1999].

### 5.2.1 Cultivation and Biomass Production

Duckweeds grow at temperatures from 6 to 33°C and have their optimum at around 30°C. Similar to *Azolla*, duckweeds tolerate salinity to about 0.5 - 2.5 % of sodium chloride. They survive at a pH level between 5 and 9, but prefer 6.5 to 7. At a higher pH ammonium ions are converted to ammonia, which is at a certain concentration toxic to duckweeds. Nitrogen sources are crucial for duckweeds, as they do not have the ability to fix nitrogen from the air. Nitrate and ammonium can both be taken up, while ammonium is always taken up first. The cultivation methods of duckweeds are quite similar to *Azolla*: shallow earthen or lined ponds that are supplied with animal manure, biogas digester effluent, agricultural runoffs or human waste. Daily harvest and regular fertilizing is necessary for optimal yields. In contrast to *Azolla*, most duckweeds seem to prefer full sunlight, without any shading necessary. Some species can form turions, which are plant parts that are high in starch and sink to the ground to overwinter in cold climates. When temperatures are rising again, the turions are floating up and begin to grow.

The biomass production potential of some duckweeds is enormous, but reported maximum values are lower than those for *Azolla*. Under sub-optimal growing conditions yields of 5 - 20 t/ha/year of dry mass are common, see Table 8. Under optimal conditions, values of up to 182.5 t/ha/year of dry mass are recorded, but are commonly more in the range of 30 - 50 t/ha [Leng, 1999].

Table 8: Some reported yields of duckweed dry matter under a variety of growth conditions [Leng et al., 1995].

Location	DM Yield [t/ha/a]
Sub optimum environment	
Thailand	10 - 11
Israel	10 - 17
Russia	7 - 8
Uzbekistan	7 - 15
Germany	16 - 22
India	22
Egypt	10
Southern States - USA	2 - 23
Near optimum environment	
Southern States - USA	27 - 79
Israel	36 - 51

### 5.2.2 Senescence and Rejuvenation Cycles

In *Lemna minor* and probably other duckweeds as well, a senescence and rejuvenation cycle appears to exist. Every frond has a fixed age of about 40 days for the biggest fronds. With every daughter frond that is produced, the size of the subsequent daughter fronds decreases. The daughter frond that is produced first is the biggest, lives the longest and produces the most daughter fronds. The last daughter frond produced is the smallest, lives the shortest and produces the fewest daughter fronds. However, the first daughter frond can be substantially bigger than the mother frond and the first daughter frond from the second generation can be bigger than its mother frond as well. The biggest fronds can only produce fronds of their own size, which then rapidly declines with every following daughter frond, which represents the senescence. The smallest frond, however,

produces daughter fronds of bigger size than its own, which represents the rejuvenation cycle [Wangermann and Ashby, 1950].

If a colony is started from a few plants, they will be synchronized in their senescence and rejuvenation cycles. This can have a dramatic effect on their growth rate by a factor of three. Hence, fluctuations in observed biomass production are not only depending on environmental factors, but on an intrinsic sequence of cycles that are more pronounced the more synchronized they are in one colony. This observation implies that values for extrapolated biomass productivity have a great chance of being overestimated, especially if data has only been collected for a few weeks. *Azolla* is speculated to behave in a similar way. This might also be one reason for the great variance in reported biomass productivity [Leng, 1999].

One strategy to overcome low productivity due to synchronized senescence could be to create systems with a great diversity or mix plants that come from different plant orders, for instance, *Azolla* and *Lemna*.



Figure 11: *Azolla fliculoides* and *Lemna minor* can produce more biomass together than in separate cultivation.

Mixing them might lead to great periodic fluctuations in the ratio of the amount of each species in one shared system, but could hold the potential to keep high combined productivity stable over long periods. For instance, considering daily harvest, when *Azolla* is grown together with *Lemna* and *Azolla* enters a phase of low productivity, *Lemna* experiences less competition and can grow faster, which would off-set periods of low productivity. The more species are combined in one system, the more stable the combined growth rate should become.

There were currently no studies found that evaluate the long term effect of mixing aquatic macrophytes on combined productivity by overcoming the synchronization of senescence cycles. Typically, one species of interest is isolated and then cultivated, often only for some weeks. Further research on mixed aquatic species systems might uncover much higher production rates over long term than it is possible to obtain with single species systems.

Another reason to grow *Azolla* and *Lemna* together is to enhance the growth rate of *Azolla* by reducing self-shading. One study found that when *Azolla filiculoides* and *Lemna minor/gibba* were grown together, with each species covering 50 % of the water surface, the growth of *L. minor/gibba* was reduced by about 23 %, while the growth rate of *A. filiculoides* was raised by about 46 %. As the increase in growth rate is much bigger than the decrease, this combination results in enhanced total productivity. However, this finding is probably not connected to senescence and rejuvenation cycles as the cultivation period was only 2 weeks. The effect of different ratios of *A. filiculoides* and *L. minor/gibba* on their growth rates was not investigated [Peeters et al., 2016].

Further research investigating the effects on growth rates in mixed species aquatic macrophyte systems is absolutely necessary, as several mechanisms exist that facilitate higher combined species productivity. Extreme biomass yields that were only achieved under laboratory conditions might thereby become realistic under field conditions and over long term periods.

### 5.2.3 Nutrient Uptake

The nitrogen content of the medium has a great influence on the protein content of duckweed. Trace levels of nitrogen of 1 - 4 mg N/l resulted in a crude protein content of the duckweed of 15 %, while 10 - 15 mg N/l produced a crude protein content of 37 %. Values of up to 45 % crude protein have been reported for enriched cultures. Above 60 mg N/l had a toxic effect on duckweed, however, this depends on the pH and on whether the nitrogen is mainly provided by ammonium or nitrate. The crude protein is closely correlated to the root length of duckweed. The longer the root, the lower the protein content. This can be used to determine the optimal nitrogen concentration for growing duckweed as livestock feed. Optimal nitrogen levels are reported to lie between 20 - 60 mg N/l. In this concentration, duckweed will not only acquire higher protein contents, but also grow faster and have a lower fiber content. All of these properties are crucial for its usage as livestock feed.

Phosphorus concentrations in duckweed dry matter reach a maximum of 10 - 14 mg/kg, when phosphorus concentrations in the medium are 1.0 mg/l or higher. Some duckweeds are also able to take up sugars from the medium. They can be used to increase the growth rate and at sufficient amounts, the sugars can serve as the exclusive energy source, enabling the duckweed to grow in complete darkness [Leng, 1999].

Unlike *Azolla*, duckweeds need nitrogen in their medium. Depending on the medium, either nitrogen or phosphate is the main limiting nutrient for common nutrient sources, such as biogas slurry, animal manures or wastewaters.

In one experiment, *Landoltia punctata* was grown in anaerobically digested swine wastewater. When the wastewater was diluted down to 2.5 %, the duckweed took up 100 % of ammonium, but only 54.3 % of phosphate. Clearly, nitrogen was the limiting nutrient. Further uptake of phosphorus would have required more nitrogen in the medium. In order to improve nitrogen and phosphate uptake in wastewater treatment operations, the combination of duckweed with *Azolla* is of potential interest for complete removal of both nutrients, as *Azolla* can take up phosphates in the absence of nitrogen in the medium [Muradov et al., 2014].

It would therefore make sense to treat the wastewater first by the cultivation of duckweed to remove most of the nitrogen compounds and then use *Azolla* subsequently for the removal of phosphates. *Azolla* has significantly higher rates of phosphate uptake for lower levels or even no nitrogen in the medium. Additionally, the growth of *Azolla* is reduced with high levels of nitrogen in the media. In one experiment using artificial cul-

ture medium, *Azolla* had a growth rate of 0.122/day without nitrogen, 0.126/day with 5  $\text{NO}_3^-$ -N mg/l and 0.119 with 40  $\text{NO}_3^-$ -N mg/l, indicating a preference for low levels of nitrogen. The nitrogenase activity of *Azolla* is also higher with lower levels of nitrogen, leading to a higher total amount of harvestable protein, which also suggests a foregoing cultivation of duckweeds [Costa et al., 2009].

The production of duckweeds for animal fodder might be one of the best options to make use of mineral nitrogen fertilizer in order to mitigate adverse effects on the soil and the environment. Just like with mineral phosphate fertilizers, in many areas farmers are required by law to buy nitrogen fertilizers, such as urea, even though they do not want to use them. A duckweed pond with proper leakage control minimizes the risk of environmental contamination and provides very high uptake rates of nitrogen, when mineral nitrogen fertilizers are used. By closely monitoring pH and ammonium levels during the cultivation, large amounts of high quality protein can be produced.

One Brazilian study examined the nutrient uptake of *Landoltia punctata* from swine waste and its potential for cheap protein production during the period of one year. The treatment of the swine waste consisted of an anaerobic digester, a storage pond and two duckweed ponds, all in a linear sequence. The digester was receiving swine waste from 300 animals at about 3  $\text{m}^3$  per day. The effluent was going into the storage pond from where 2  $\text{m}^3$  were taken to fertilize the fields and 1  $\text{m}^3$  was going into the duckweed ponds. Together the duckweed ponds had a surface area of 143  $\text{m}^2$ . The whole system achieved a nitrogen removal of 98.0 % (Total Kjeldahl Nitrogen) and 98.8 % of total phosphorus. The nitrogen removal rate by the duckweeds was one of the highest recorded, with 4.4 g N/ $\text{m}^2$ /day and the phosphorus uptake was also high, with 470 mg P/ $\text{m}^2$ /day. The first duckweed pond had a higher concentration of ammonium than the second pond, so that the duckweed from the first pond had an average crude protein content of 35 %, while it was 28 % for the duckweed from the second pond. The ammonium concentration reached up to 182 mg  $\text{NH}_4^+$ -N mg/l in the first pond without any toxic effects. *Landoltia punctata* was reported to have good growth even with ammonium concentration as high as 240 mg  $\text{NH}_4^+$ -N mg/l. The crude protein content in the duckweed sometimes went over 40 %. Nitrification and denitrification processes accounted for 72 % of nitrogen removal in the first pond and only for 4 % in the second pond. This wide difference is due to different nitrogen concentrations, but might be also due to the fact that the first pond had a depth of 0.8 m and the second one only 0.4 m. Both ponds together produced 68 t/ha/year of biomass (dry weight). The authors concluded that this setup produces 20 times more protein than soy in Brazil. At the same time, it has the potential to grow this amount without external input of fertilizers and provides a high degree of environmental protection by avoiding eutrophication from animal manure leakage [Mohedano et al., 2012].

Even though duckweeds do not fix nitrogen, there is some evidence that they can associate with nitrogen fixing heterotrophic bacteria and cyanobacteria. It was reported that 15 - 20 % of the nitrogen requirements of duckweeds can be supplied by certain bacteria that are attached to the duckweed mats [Xu et al., 2011].

#### 5.2.4 Livestock Feed

Several duckweed species have very favorable properties for their usage as livestock feed. Compared to *Azolla*, several duckweeds have a considerably higher protein content, are higher in lipids and starch (depending on species and growing conditions) and have a lower lignin content. However, high nitrogen concentrations in the growing medium are crucial to grow duckweeds with a suitable nutritional value. The whole nutritional composition of duckweeds is highly depending on the culture medium. Nutrient poor conditions can lead to a high mineral content and crude fiber, while the protein content can be as low as 7 %.

Obviously, this has a great effect on livestock performance when fed [Feedipedia, 2016]. Researches from Brazil concluded that their experimental setup with *Landoltia punctata* that yielded 68 t/ha/year with an average protein content between 28 and 35 % amounting to 24 t/ha crude protein would produce 20 times more protein than soy [Mohedano et al., 2012]. The yearly protein demand in pig production can be estimated for 100 pigs with a bodyweight of 8 kg requiring about 9 t/a of protein, while 100 pigs with a bodyweight of 116 kg require about 18 t/a [Williams et al., 2012]. Theoretically, depending on the size distribution of the pigs, the amount of protein produced with *Landoltia punctata* in the described setup using the manure of the 100 pigs could generate enough protein to meet the protein demand of the pig production in the range of about 3.2 - 6.5 %. The duckweed ponds had a combined surface area of 243 m<sup>2</sup> or 2.43 m<sup>2</sup> per pig. However, the swine waste was anaerobically digested at first, so that the great majority of the nitrogen was not taken up by the duckweeds, but lost from the system through ammonia volatilization, nitrogen sedimentation, nitrification and denitrification processes before entering the ponds. Considering the provision of protein usually being among the most expensive factors in livestock rearing, the on-farm cultivation of duckweeds using manure could realize cost reductions for both small and large scale operations, while minimizing environmental hazards.

However, yields of up to 182.5 t/ha/year have been reported. Furthermore, the crude protein content can be as high as 45 %, which suggests a far greater potential protein productivity [Leng, 1999].

The starch content of duckweeds can vary from 3 - 75 %. Starch accumulation can be triggered artificially by inducing stress, for instance, by transferring fast growing duckweed from high nutrient media into well water that is poor in nutrients. Increasing salinity and decreasing temperature also encourages starch accumulation. *Spirodela polyrrhiza* has been researched for this application for bio-ethanol production. However, it might be interesting for livestock fodder production as well. In the experiment the duckweed had a final starch content of 31 % and an average biomass productivity of 12.4 g/m<sup>2</sup>/day in a four week period, which can be extrapolated to 45.3 t/ha/year of dry mass. This would produce more than three times as much starch as maize, when calculated for the world average yield and assuming 75 % starch content [Xu et al., 2011].

Duckweeds constitute a valuable fish feed and can be integrated at much higher inclusion rates than *Azolla*. Under one farming system in Bangladesh tilapia were only fed duckweed. Both fish and duckweed were grown in two separate lagoons. Fresh duckweed was transferred to the fish lagoon twice a week, so the fish would only eat duckweed and what would naturally occur in the lagoon. The fish were of mixed size, so every time they were harvested only the biggest ones were sold and the rest put back. This system could produce 10 tons of tilapia per hectare in one year with minimal input [Leng et al., 1995]. Grass carp are herbivorous fish that prefer duckweed over most other plants in their natural diet. They are the most important fish species in global aquaculture and their production is especially popular in China, where they are often fed on duckweed alone. Grass carp fed exclusively with *Spirodela polyrrhiza* ad libitum had a specific growth rate of 3.41 % per day [Cui et al., 1994].

In one experiment, poultry were fed with 0, 25 and 40 % dried *Lemna gibba* of their standard diet. While the number of eggs per hen per week did not differ much in the three groups, the live weight gain changed considerably. The group that did not receive any duckweed gained 40 g, while the group receiving 25 % gained 114 g. The group with 40 %, however, lost 118 g [Leng et al., 1995].

Duckweed can be an important source for minerals and protein in the diet of cattle. In one feeding trial, bulls were fed with mixed duckweed *Lemna sp.*, *Spirodela sp.* and *Wolffia sp.* at 10 % of the their live weight. It was concluded that duckweed can be a component

of the concentrate mixture [Huque et al., 1996].

In general, duckweeds can be incorporated at slightly higher levels in the diet of various livestock, compared to *Azolla*. In herbivorous and omnivorous fish duckweed is much more suitable as part of their diets than *Azolla* is. Several duckweeds are very high in oxalic acid, an antinutritional factor that might be the reason for its detrimental effects on most livestock when fed at very high levels [Feedipedia, 2016].

*Azolla* might therefore be combined with duckweeds for livestock feed, as both have different compositions of antinutritional compounds that are usually harmless up until a certain threshold. Chapter 6 describes methods to increase the utilization of aquatic plants for livestock feed.

### 5.2.5 Other Applications

*Spirodela polyrrhiza* can be used to produce great amounts of starch for the production of bioethanol. The concentration of starch can be greatly increased by stressing the plants through sudden transfer into nutrient poor media, as described in Chapter 5.2.4. The ethanol yield of manipulated *Spirodela polyrrhiza* was 6420 l/ha, which is about 50 % higher than the ethanol yield of maize based production. Globally, maize is the one plant that causes the most erosion. Ethanol production from maize and other field crops is controversial, as it competes for arable land with food crops. Duckweeds, such as *Spirodela polyrrhiza* could be grown in sewage treatment systems, with much less competition for arable land and with apparently higher yields for bioethanol as well [Xu et al., 2011].

The high starch content also makes it a potential feedstock for the production of biogas via anaerobic digestion.

Duckweed biomass can also be pyrolysed to yield a range of bio-oil components that can be used for green gasoline and diesel fuel using techniques, such as catalytic dehydrooxygenation [Muradov et al., 2014].

Duckweeds are used to purify contaminated water bodies, as they have the ability to hyperaccumulate pollutants, such as various heavy metals, similar to *Azolla* [Hou et al., 2007]. The role of duckweeds as mosquito control and in the reduction of evaporation of water bodies has already been described in Chapter 2.2.2 and 2.3.

## 5.3 Other Plants

Species of *Azolla* and duckweed are the most important plants for productive ponds, due their inherent properties and abilities, like biomass production, nitrogen fixation, biological mosquito control, reduction of evaporation losses, usage as green manure, livestock feed, wastewater treatment and energy production.

However, other aquatic plants can play an important role, too, for instance, for increased species diversity, more complex ecosystems, more diverse products and human food, such as herbs, vegetables and starchy tubers that can even be a staple crop of the human diet. When grown together with *Azolla* or duckweed, they might increase total production by overcoming the synchronized senescence period (see Chapter 5.2.2).

Many of them also provide flowers, which is an important aspect in holistic land management systems to supply the local bee populations and other insects. *Azolla* is a fern, so it doesn't produce flowers and duckweed produces the smallest flowers in the world in a quite irregular fashion, so these two contribute little to nothing to pollinating insects. Other plants than *Azolla* and duckweed also enhance the local scenery by providing a more natural display.

Some of the following plants grow only up to a certain water depth, which can be realized by growing them at the bank of the ponds or creating shallow ponds that can be recharged with water from the storage ponds, rivers or greywater as part of a household microfarm system.

### 5.3.1 *Colocasia esculenta*

The taro is a high-yielding tropical perennial plant that grows to a height of about 2 m (see Figure 12) and produces edible tubers underground of about 1 kg in fresh weight, see Figure 13. They are usually grown as annual plants, meaning that they are uprooted, the aerial part is discarded, the biggest tubers are harvested for sale and the small tubers are planted again and grown until they are harvested, and so on. The tubers are very high in starch and have been used as a staple food by many cultures for thousands of years. It is a popular crop in the highlands of Ethiopia.

Table 9: Basic information on *Colocasia esculenta* [Feedipedia, 2016].

Common names	taro, elephant's ear
Botanical name	<i>Colocasia esculenta</i>
Plant family	Araceae
Other species of interest	<i>Cyrtosperma merkusii</i> , <i>Xanthosoma sagittifolium</i>
Aquatic plant type	terrestrial (dry and wet), emersed
Water depth range	up to 30 cm
Harvested plant part	tubers, leaves and stems
Dry mass yield [t/ha/a]	1.4 - 10.6 (only tubers)
Calories per area [million kcal/ha]	5.1 - 38.7 (only tubers)
Protein per area [kg/ha]	76 - 572 (only tubers)

This plant can easily compete with the popular cereal crops in terms of providing calorie-dense food. Hundreds of different cultivars exist, with some preferring drier conditions and some preferring standing in water. The ones that prefer standing in water have the advantage of having less weeds that have to be removed and can be easily integrated into a multi-species aquaculture system. The big leaves of taro are shading the water, so that *Azolla* can be grown underneath.

One study compared the yields of taro plants that were either fertilized with mineral fertilizer, with *Azolla* biomass incorporated into the soil or simply intercropped with *Azolla*. Surprisingly, the intercropping showed the highest yields. This suggests a polyculture system that does not require chemical fertilization and little labor input. Fish could also be part of this integrated system [Tekle-Haimanot and Doku, 1995].

In traditional East Asian rice cultivation with *Azolla* (see Chapter 7.1), *Azolla* is worked into the soil, so that its decomposition releases nutrients for the rice plants. The fact that in this study, taro would grow better if *Azolla* was just left growing beneath it, without working it into the soil, suggests a new type of minimum input polyculture system that is self-fertilizing in terms of nitrogen. This needs further research.

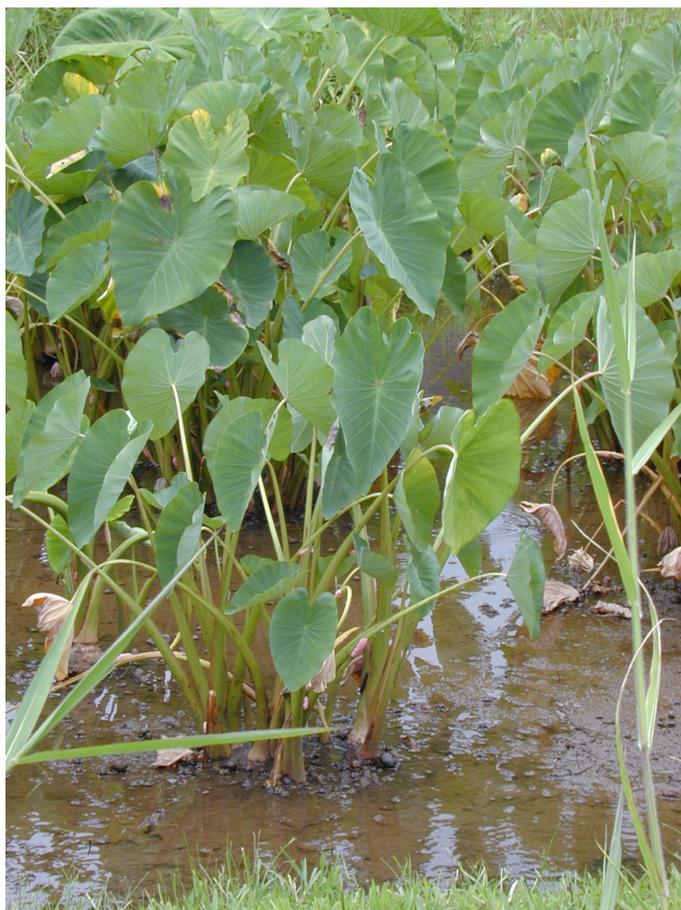


Figure 12: Cultivated taro plants in standing water.



Figure 13: The harvested corms or tubers of taro.

The leaves and stalks of taro are also used for animal fodder and also human food. They contain high amounts of calcium oxalate crystals and need to be cooked, dried or ensiled to be used. Apart from that, they make very good fodder and have high protein levels of 20 % or more [Feedipedia, 2016].

### 5.3.2 *Ipomoea aquatica*

Water spinach is a tropical perennial that grows on wet soil or on open water. It can be found growing wild at the banana farms near Arba Minch, see Figure 14. It is closely related to the sweet potato, but does not produce tubers. It is grown for its stems and leaves as animal fodder mainly for pigs and cattle, but its young shoots are very popular as a human food in South-East-Asia. With a crude protein content of about 34 % they are a very protein-rich vegetable. In several Asian countries it is among the most important vegetables. Plants are usually cultivated on flooded fields, where plants are floating on small bamboo rafts and the shoots are harvested on a monthly basis [Westphal et al., 2016].

Table 10: Basic information on *Ipomoea aquatica* [Feedipedia, 2016].

Common names	water spinach, kangkong
Botanical name	<i>Ipomoea aquatica</i>
Plant family	Convolvulaceae
Other species of interest	-
Aquatic plant type	terrestrial (wet), emersed, rooted floating, freely floating
Water depth range	any depth
Harvested plant part	leaves and shoots
Dry mass yield [t/ha/a]	2.2 - 36.8 (leaves and shoots)*
Calories per area [million kcal/ha]	3.8 - 63.4 (leaves and shoots)
Protein per area [kg/ha]	755 - 12622 (leaves and shoots)



Figure 14: Wild water spinach in a river near Arba Minch

### 5.3.3 *Sesbania rostrata*

*Sesbania rostrata* is a leguminous short-lived perennial reaching a height of 1 - 3 m. It can grow in water-logged soils and when it is mature it is able to grow in 30 cm deep water permanently. Stem cuttings work well at a length of 30 cm, as do seeds, but both need to be planted in moist soil and not under water. The foliage is used as green manure, often for the cultivation of paddy rice and as animal fodder [Cook et al., 2005]. Its use as green manure in paddy rice is comparable to *Azolla*. They are both only grown for a short period and then incorporated into the soil. It might be possible to integrate them as permanent elements into a perennial polyculture system including fish and ducks. The foliage of *Sesbania rostrata* would be ideal to provide shade for the *Azolla* [Ventura and Watanabe, 1993].

Table 11: Basic information on *Sesbania rostrata* [Feedipedia, 2016], \*[Cook et al., 2005].

Common names	sesbania, rostrate sesbania
Botanical name	<i>Sesbania rostrata</i>
Plant family	Fabaceae
Other species of interest	<i>Sesbania grandiflora</i> , <i>Sesbania sesban</i>
Aquatic plant type	terrestrial (dry and wet), emersed
Water depth range	up to 30 cm
Harvested plant part	twigs with foliage
Dry mass yield [t/ha/a]	18 - 42*
Calories per area [million kcal/ha]	24.9 - 58.1 (assuming 4.7 % crude lipids)*
Protein per area [kg/ha]	4320 - 10080*

In one experiment, several plants of *Sesbania rostrata* were directly grown in fish ponds to supply them with nitrogen. The foliage was put in the ponds, so they would decompose and release ammonium that triggers the growth of microalgae, an important feed for many herbivorous fish. The authors concluded that this practice could substantially reduce the amount of fertilizer needed for the fish ponds [Mazumder et al., 2009].

### 5.3.4 Microalgae

Microalgae are microscopic unicellular algae that do not belong to the plant kingdom. Microalgae are part of every pond system; the less coverage by floating plants, the more light will penetrate into the pond and microalgae will flourish. They also respond quickly to high levels of nitrogen and phosphates and grow rapidly. In order to grow microalgae in high densities and produce them in great amounts, they have to be grown in shallow ponds at a depth of about 30 cm with permanent stirring to keep the algae suspended and supply them with atmospheric carbon dioxide, see Figure 15. The harvest requires sophisticated techniques, but can be in part achieved by settlement in another pond without stirring. Microalgae have the advantage to supply the water with great amounts of oxygen, which is not achieved with floating plants. The oxygenation is particularly valuable for wastewater treatment, when the biological oxygen demand (BOD) needs to be reduced to a certain limit, before it can be discharged [Campbell et al., 2011].

Table 12: Basic information on Microalgae [Feedipedia, 2016], \*[Campbell et al., 2011], \*\*[Brown, 2002].

Common names	microalgae, microphytes
Botanical name	-
Plant family	-
Other species of interest	-
Aquatic plant type	suspended
Water depth range	any depth
Harvested plant part	whole organism
Dry mass yield [t/ha/a]	54.8 - 109.6*
Calories per area [million kcal/ha]	167.1 - 334.3**
Protein per area [kg/ha]	19180 - 38360**



Figure 15: Microalgae grown in outdoor raceway ponds, constantly being stirred.

Microalgae constitute the basis of the aquatic food chain and are essential for the nutrition of fish and other aquatic animals. On average, microalgae consist of about 35 % proteins, 15 % lipids and 7.5 % carbohydrates. They contain large amounts of the omega 3 fatty acids EPA and DHA that are practically not found in plants, which are vital for all aquatic animals, especially in their early growth stages [Brown, 2002].

### 5.3.5 *Eleocharis dulcis*

The water chestnut is a perennial plant growing up to 1 m in height, see Figure 16. It is not to be confused with *Trapa sp.*, also called water chestnut. It prefers growing in standing water and produces edible corms underground that are very high in starch, see Figure 17. The high yielding cultivars come from China, where the water chestnut is an important part of many dishes. One plant can yield 2.3 kg of tubers in one season [Morton et al., 1988]. The water chestnut can produce an impressive amount of calories per surface areas that easily competes with conventional grains.

Table 13: Basic information on *Eleocharis dulcis* [Feedipedia, 2016], \*[Morton et al., 1988].

Common names	Chinese water chestnut, water chestnut
Botanical name	<i>Eleocharis dulcis</i>
Plant family	Cyperaceae
Other species of interest	-
Aquatic plant type	emersed
Water depth range	10 - 20 cm
Harvested plant part	tubers
Dry mass yield [t/ha/a]	12.5 - 22.6 (only corms)*
Calories per area [million kcal/ha]	53.7 - 97.0 (only corms)*
Protein per area [kg/ha]	659 - 1192 (only corms)*



Figure 16: Cultivated water chestnut together with *Azolla* plants that suppress weeds and bring nitrogen into the system, while they are shaded by the water chestnut foliage. Two plants that benefit from each other.



Figure 17: The harvested corm of the water chestnut.

### 5.3.6 *Trapa bispinosa*

The water caltrop is a floating plant with a root that extends to the bottom through the water column. It grows in flat rosettes with a diameter of about 20 cm, see Figure 18. The seeds have a size of 3 cm and possess a very odd looking shape with 2 - 4 big thorns, see Figure 19. The seeds have been eaten by humans for thousands of years and contain high amounts of starch. They are produced under water and if not harvested, they will sink to the ground and germinate to give rise to the next generation [Tulyathan et al., 2005].

Table 14: Basic information on *Trapa bispinosa* [Feedipedia, 2016], \*[Tulyathan et al., 2005].

Common names	water caltrop, water chestnut
Botanical name	<i>Trapa bispinosa</i>
Plant family	Trapaceae
Other species of interest	<i>Trapa natans</i>
Aquatic plant type	rooted floating
Water depth range	0.5 - 3 m
Harvested plant part	seeds
Dry mass yield [t/ha/a]	5.6 (only seeds)*
Calories per area [million kcal/ha]	21.8 (only seeds)*
Protein per area [kg/ha]	629 (only seeds)*



Figure 18: *Trapa bispinosa*.



Figure 19: Edible seeds of *Trapa natans*.

### 5.3.7 *Oryza glaberrima*

African rice is one of the two cultivated rice species, the other one being Asian rice, *Oryza sativa*. African rice is an annual cereal grain and quite similar to Asian rice, see Figure 20. It is mainly cultivated in West Africa in dryland cultivation, in paddy fields similar to Asian rice and floating in water more than 3 m deep. African rice has the ability to handle different water levels and still produce grains, which makes it applicable in areas prone to flooding, where other cereal crops would simply drown. In theory, African rice has about the same grain yield as Asian rice, but usually up to one half of the rice grain is lost due to the shattering of the seeds before harvest [National Research Council, 1996].

Table 15: Basic information on *Oryza glaberrima* [Feedipedia, 2016], \*[National Research Council, 1996].

Common names	African rice
Botanical name	<i>Oryza glaberrima</i>
Plant family	Poaceae
Other species of interest	<i>Oryza sativa</i>
Aquatic plant type	terrestrial (dry and wet), emersed, rooted floating
Water depth range	up to 3 m
Harvested plant part	seeds
Dry mass yield [t/ha/a]	0.45 - 3.0 (only seeds)*
Calories per area [million kcal/ha]	1.7 - 11.1 (only seeds)*
Protein per area [kg/ha]	34.2 - 228 (only seeds)*



Figure 20: Seeds of African rice.

### 5.3.8 *Eichhornia crassipes*

The water hyacinth is a floating plant widespread in the tropics. It has been distributed as people were using it as an ornamental pond plant because of its beautiful flowers. Today it is among the most invasive plants. Under favorable conditions, the plants grow in thick mats that can get over 1 m in height above the water surface. Its uses include green manure, soil conditioner, wastewater treatment, animal fodder, biogas feedstock and fiber production [Lindsey and Hirt, 2000]. It is usually not cultivated, but rather collected from wild streams, where it can be gathered in large quantities in little time. Its value as livestock fodder is limited, as the fresh plant contains microscopic sharp calcium oxalate crystals that can cause irritation and low palatability in livestock. It can be cooked, dried, ensiled or composted to improve its acceptance. It is fed to all kinds of livestock, but mostly as a supplement, as it contains little energy, but very high amounts of minerals.

Table 16: Basic information on *Eichhornia crassipes* [Feedipedia, 2016].

Common names	water hyacinth, common water hycinth
Botanical name	<i>Eichhornia crassipes</i>
Plant family	Poaceae
Other species of interest	-
Aquatic plant type	freely floating
Water depth range	any depth
Harvested plant part	leaves and stems or whole plant
Dry mass yield [t/ha/a]	11.6 - 38.1
Calories per area [million kcal/ha]	10.6 - 34.8
Protein per area [kg/ha]	2123 - 6972

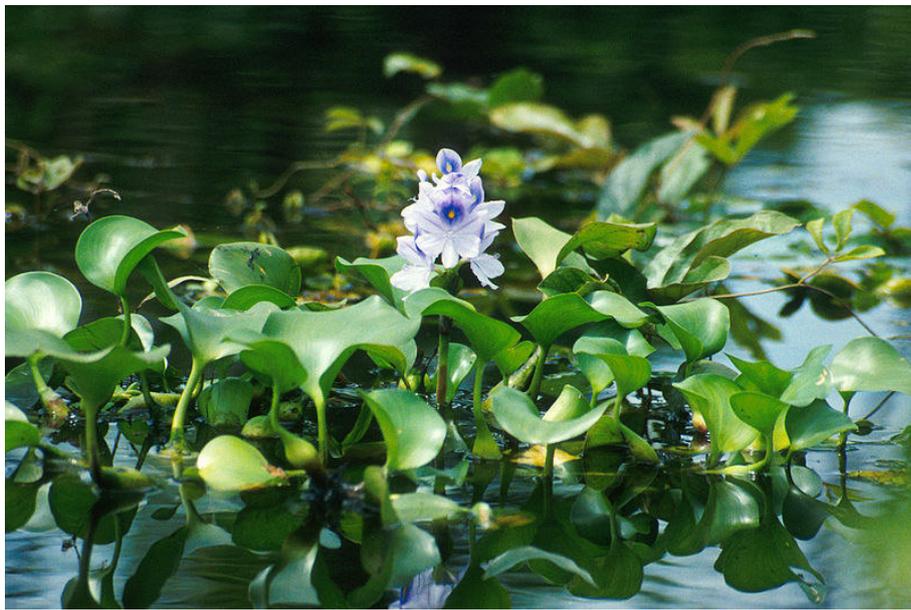


Figure 21: Water hyacinth.

The water hyacinth increases water loss by evapotranspiration of open water bodies by a factor of 1.4 - 3.7 when the whole surface is covered. This might be useful for the volume reduction of wastewater, especially if it is highly diluted with rainwater [Debusk et al., 1983]. It could be combined with *Azolla*, so that *Azolla* can be grown without further shading, as the shade is provided by the water hyacinth. However, it is not known if this combination provides adequate mosquito control.

### 5.3.9 *Canna edulis*

Achira is a perennial edible plant that grows up to 3 m high and is also a very popular ornamental plant, see Figure 22. It originates from the Andes where it has been cultivated for its edible rhizomes that are rich in starch. Surprisingly high rhizome yields have been achieved that are equal or even higher than that of common root crops, such as cassava or potatoes. The stalks and leaves are produced in great amounts as well and are used as livestock fodder.

However, it is unclear if this plant is fully suitable for aquatic conditions. Several ornamental species thrive with their roots permanently under standing water, see Figure 23, but whether satisfactory rhizome yields can be achieved this way with edible cultivars remains to be investigated. Achira can definitely be planted at the bank of a pond to serve as an ornamental and productive windbreak [Ker-Gawl., 2007].

Table 17: Basic information on *Canna edulis* [Feedipedia, 2016], \*[Ker-Gawl., 2007].

Common names	Achira, Indian shoot
Botanical name	<i>Canna edulis</i>
Plant family	Cannaceae
Other species of interest	<i>Canna indica</i> , <i>Canna coccinea</i> , <i>Canna flaccida</i>
Aquatic plant type	terrestrial (dry and wet), emersed
Water depth range	0 - 10 cm, possibly more
Harvested plant part	rhizomes
Dry mass yield [t/ha/a]	4.1 - 27.4 (rhizomes)*
Calories per area [million kcal/ha]	14.6 - 97.5 (rhizomes)*
Protein per area [kg/ha]	61.5 - 411 (rhizomes)*



Figure 22: *Canna edulis*, an edible ornamental plant next to a maize field in North Vietnam.



Figure 23: An unidentified *Canna* species growing in a constructed pond in Arba Minch, together with *Azolla*.

### 5.3.10 Further Species of Interest

Further species of interest include:

- *Pistia stratiotes*: water lettuce, a fast growing freely floating plant, related to duckweeds, also naturalized in the lakes around Arba Minch
- *Aeschynomene afraspera*: a floating legume native to Africa that fixes nitrogen and is increasingly being used as green manure for paddy rice, comparable to *Azolla*
- *Euryale ferox*: a giant water lily covered with thorns, grown in India and East Asia for its edible flower pods and seeds, rather low-yielding, but high value products
- *Rorippa nasturtium-aquaticum*: watercress, an aquatic spicy herb eaten all over the world
- *Oenanthe javanica*: an aquatic type of celery, leaves and shoots are eaten
- *Nelumbo nucifera*: Sacred lotus, a very ornamental plant, seeds and the rhizomes are popular in Asian cooking
- *Salvinia molesta*: a fast growing freely floating fern, related to *Azolla* naturalized in Africa
- *Cyperus esculentus*: tiger nut, a small grass-like plant producing tubers underground, grows in flat water, popular food in West Africa

## 6 Processing of Aquatic Plant Biomass to Enhance its Nutritional Value

The main limitation of *Azolla*, duckweed and most other aquatic plants as feed for livestock is due to the fact that these plants contain a variety of antinutritional compounds in sometimes high concentrations. The effect of these compounds is dose dependent, so while a diet containing 10 % *Azolla* for an animal can result in better health and growth performance, 20 % can already be detrimental to the animal's health and even make it lose weight. Antinutritional compounds or antinutrients are substances found in feedstuff that reduce nutrient intake, digestion, absorption and utilization or result in other adverse effects on the animal. Common antinutrients include toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, oxalates, goitrogens, lectins and protease inhibitors.

Antinutrients are found in the seeds and foliage of virtually every plant. The effect of one antinutrient is not the same for every animal, but depends mainly on its digestion. For instance, trypsin inhibitors are antinutritional compounds for monogastric animals, while they have no adverse effects on ruminants, as they can degrade those substances in their rumen [Akande et al., 2010].

The concentration of antinutrients in a plant depends a lot on environmental factors, particularly the ones that exert a certain type of stress on the plant. Many plants produce antinutrients to deter herbivores or at least restrict their appetite to protect themselves from being eaten. One example is *Azolla spp.*, which contains deoxyanthocyanins. This compound is responsible for the red color of the fronds when they are stressed by too much sunlight, cold or lack of nutrients, see Figure 24.



Figure 24: The very same strain of *Azolla filiculoides*, the green one was cultivated indoors, while the red one was collected from an outside pond in Germany in March, with an apparent stress-induced color-shift

In an experiment, *Azolla pinnata* had 85 % less of the concentration of deoxyanthocyanins in summer, when it was green than it had during winter, when it had a red color. When *Azolla pinnata* was cultivated together with tadpoles that were feeding on it, its concentration of deoxyanthocyanin was 260 % higher than in a culture without tadpoles. When given the choice, both tadpoles and snails had a significant preference for *Azolla filiculoides*, which had about 20 times less deoxyanthocyanin in its tissues than *A. pinnata*. [Cohen et al., 2002].

Antinutrient concentrations in aquatic plant tissues can also be influenced by certain minerals in the water. The calcium oxalate content of *Lemna minor* was shown to increase with increasing calcium ion concentration in the water. The formation of calcium oxalate crystals is thought to be part of a mechanism to remove excess biologically active calcium when other mechanisms have become saturated. Calcium oxalate is a highly insoluble salt that is no longer osmotically or physiologically active and might be the most limiting factor in duckweeds to be used as feedstuff [Mazen et al., 2003]. Several techniques exist to decrease the content of antinutrient compounds in plant matter in order to improve their nutritional qualities. The plant biomass can be dried, heated, cooked, soaked or fermented with yeasts and/or lacto-bacteria to reduce several antinutrients with varying success. The biomass can also be fed to other animals, like insects, snails or zooplankton and those animals can then be used as feed for fish, chicken and so on in order to create a food web within a highly productive ecosystem.

The incorporation of *Azolla*, duckweeds and other aquatic weeds in the diets of livestock always has the effect of decreasing the total cost of feedstuff, as they can replace high protein sources that usually constitute the most expensive part of the diet. By increasing the amount of aquatic plant biomass that can be fed to animals, the whole system can be made more profitable. However, animal welfare should still be ensured and reasonable inclusion rates should be applied. This can be realized by letting the animals choose their food rather than mixing it under their regular formula.

## 6.1 Reduction of Anti-Nutrients through Lacto-Fermentation

One study looked into the nutritional properties of some aquatic macrophytes before and after anaerobic fermentation. Their focus was on the reduction of antinutritional compounds through the fermentation process. The examined macrophytes were *Lemna minor*, *Spirodela polyrrhiza*, *Azolla sp.* and *Eichhornia crassipes*.

They were partially dried to achieve a dry mass content of 350 - 450 g/kg and mixed with molasses at a final concentration of 150 g/kg, as the bacteria need a source of water soluble carbohydrates for proper fermentation. The mixtures were inoculated with two commercial strains of *Lactobacillus plantarum*, vacuum packed into gas tight plastic bags and fermented for 60 days at 25°C.

After the fermentation process, the crude fiber content was reduced significantly, while the ash content stayed the same. The crude protein content was reduced for *Eichhornia crassipes* and *Azolla sp.*, but increased for *Lemna minor* and *Spirodela polyrrhiza*. The concentrations of the antinutritional compounds that were analyzed were significantly reduced for the most part, while some decreased only slightly, see Table 18.

Table 18: Concentration of anti-nutritional substances in raw and fermented aquatic macrophytes, *Lemna minor*, *Spirodela polyrhiza*, *Azolla filiculoides* and *Eichhornia crassipes* (leaves). Data from [Cruz et al., 2011].

Anti-nutritional substances	<i>L. minor</i>		<i>S. polyrhiza</i>		<i>A. filiculoides</i>		<i>E. crassipes</i>	
	Raw	Ferm.	Raw	Ferm.	Raw	Ferm.	Raw	Ferm.
Trypsin inhibitor [ppt]	2.31	0.5	0.8	0.17	1.86	1.37	1.05	0.6
Phytates [% phytic ac.]	0.32	0.12	0.25	0.11	0.15	0.15	0.15	0.12
Soluble tannins [%]	0.3	N.d.	1.31	N.d.	0.44	N.d.	0.7	N.d.
Condensed tannins [%]	N.d.	N.d.	3.87	N.d.	N.d.	N.d.	0.99	N.d.
Oxalates [%]	2.02	0.04	0.1	N.d.	1.67	0.19	0.52	0.1

Both soluble and condensed tannins were reduced to non-detectable levels in all plants. Oxalic acid went down by at least 88 % in all plants. The change in phytates gave mixed results, as they decreased by 63 % in *Lemna minor*, but stayed the same in *Azolla sp.* The trypsin inhibitors were reduced by 78 % in both *Lemna* and *Spirodela*, by 26 % for *Azolla* and by 43 % for *Eichhornia*. In total, the antinutrients were lowered significantly by the fermentation process, so that the nutritional value was greatly improved.

It is very likely that fermented aquatic plants can be included into the diets of livestock at much higher rates, but feeding trials to investigate this have to be conducted first [Cruz et al., 2011].

The lacto-fermentation of aquatic weeds allows for a significant improvement in its nutritional value with very little energy input. It also preserves it for at least some weeks, so that supply shortfalls in animal fodder can be compensated. However, the need for the addition of water soluble carbohydrates is substantial at 150 g/kg of molasses. The special treatment of *Spirodela polyrrhiza* to increase its starch content to 31 % might have the potential to cut back on molasses, when a significant proportion of starch rich duckweed is included in the fermentation process [Xu et al., 2011].

## 6.2 Breeding of Black Soldier Flies as Livestock Feed

Another way to make better use of large amounts of aquatic plants is the breeding of black soldier flies. They can be fed with aquatic weeds among other things and produce larvae that can be fed to fish and chicken. The larvae would actively dewater the aquatic weeds and reduce their volume, while the majority of the nutrients would be converted into nutrient-dense larvae.

This bioconversion process is somewhat comparable to vermi-composting and requires no input of energy, chemicals or water. The larvae are high in protein and fat and constitute a high quality feedstuff that could fully replace fishmeal, while being sustainable and easy to produce, even in developing countries.

Black soldier fly, *Hermetia illucens* originates from the American continent, but has already spread to Europe, Asia and Australia. It is increasingly being used to recycle food waste. The larvae can be fed a variety of waste products, such as manure, human excreta, meat and dairy products, oils, green garden waste and all kinds of food waste. The black soldier fly is said to possess the most robust digestive system in nature [ESR International, 2008].

### 6.2.1 Breeding and Harvesting

The black soldier flies are bred to harvest the pre-pupae, the last larval stage before they turn into pupae. Under optimal conditions, i.e. 35°C in the colony and sufficient food, the development starting from the hatched egg to the pre-pupae takes 2 weeks. However, the larvae are able to extend this period up to six months, when temperatures drop or they do not have enough food. The larvae generate substantial amounts of heat, so by providing sufficient insulation, they can easily be bred in cooler air temperatures, even close to the freezing point. When the white larvae have turned into the black pre-pupae after five instars, they instinctively want to climb up any structures, so they are safe from predators on the ground and can morph into pupae that are immobile. After some days the pupae hatch into flies that only live for five to eight days. In that time they select their mates and the females lay up to 900 eggs during their last days. The female prefers to lay its eggs near waste, but not in the waste, so that the hatching larvae need to crawl a short distance to their food source.

In order to mass-produce black soldier pre-pupae, the egg production is usually secluded from the rearing of the larvae that grow into pre-pupae. For the egg production, some of the harvested pre-pupae are taken into a sort of cage (see Figure 25), where they can develop into flies, mate and produce eggs. Therefore, the flies need an air temperature in the range of 24 - 40°C or higher.



Figure 25: Black soldier fly egg production cage, made from wood and metal mosquito screen in Arba Minch. Pupae were hatching, but did not manage to produce eggs due to great variance in hatching time and hardly more than two flies present at the same time.

The eggs are usually laid into small pieces of cardboard that are strategically placed above the food source to facilitate efficient harvesting of the eggs. The eggs are transferred to the larvae rearing bins, where they hatch after four days. The bins are typically operated in a continuous mode, so larvae of all stages are together and are being fed every day. As soon as larvae turn into pre-pupae after a minimum of 2 weeks, they instinctively crawl up ramps that are implemented in the bin. When the pre-pupae follow the ramps upwards, they eventually fall in a hole and land in a harvesting bucket. The harvest of the pre-pupae is taking advantage of their natural behavior, so that they are harvesting themselves at the best possible time, when they have the biggest size. At this time one pre-pupae weighs about 0.2 g and has a length of 25 mm.

Under normal circumstances the larvae are fed every day, restocked with eggs regularly

and also harvested every day. The harvested pre-pupae are still alive and can either be used as feedstuff or put into the egg production cages where they turn into flies.

Alternatively, the larvae bins are supplied by the black soldier flies directly, either by wild populations or by releasing some of the pre-pupae. This makes the caged egg production obsolete. Black soldier flies are attracted to the smell of waste that is processed by their larvae and stimulates them to position their eggs inside of the bins.

Unlike the common housefly, the black soldier flies are not associated with the transmission of diseases. The flies only live up to eight days and do not take in any food, as they do not even have functional mouth parts, but only drink water. They do not bite nor sting or bother humans in any way and usually do not enter houses. They also do not land on human food or feces, as they lay their eggs next to the food source and not into it [ESR International, 2008].

Black soldier flies are even reported to decrease transmission of diseases by outcompeting the common house fly. The larvae of black soldier flies were shown to reduce the house fly population on pig or poultry manure by 94 - 100 %. The house fly is a major vector of diseases, hence the black soldier fly can actively contribute to the prevention of human disease, especially under poor hygienic conditions [Feedipedia, 2016].

### 6.2.2 Bioconversion of Waste

The larvae of the black soldier fly (see Figure 26) are among the most efficient and fastest biomass converters known. As soon as they hatch from their eggs they start feeding. Under optimal conditions, one egg that weighs 28  $\mu\text{g}$  develops into a pre-pupae that weighs 0.2 g, multiplying its weight by a factor of over 7000 in 2 weeks. The larvae excrete digestive enzymes as soon as waste is put into the bin, so the waste is digested before it can start rotting and smelling.

A surface area of 1  $\text{m}^2$  of larvae are able to completely convert 15 kg of food waste per day, while 3 kg of pre-pupae can be harvested daily, see Figure 27. A weight and volume reduction of food waste of 95 % can be realized in a matter of hours. On a dry weight basis, the conversion of food waste into pre-pupae is at almost 24 %. The residue is turned into carbon dioxide and other gases, solid excrements and liquids, that have to be drained or otherwise it can lead to anaerobic conditions and reduce the efficiency of the bioconversion.

However, the larvae are extremely robust, surviving all kinds of adverse conditions that would kill most other organisms. They tolerate high concentrations of salts, alcohols, ammonia and food toxins. They can even survive for two hours submerged in rubbing alcohol and be centrifuged at an acceleration 1000 times the force of gravity without any harm. They have been reported to survive the human digestive tract, if they are swallowed in one piece, however, this does not pose any health risk.

These properties should enable them to digest aquatic plants with high amounts of anti-nutrients without any problems, especially if they are mixed with other substances, like slaughter waste and animal manure.



Figure 26: Black soldier fly larvae feeding on watermelon peels and *Azolla filiculoides* as part of a pre-experiment in Germany. The white larvae are in their last instar, the black ones have already developed into pre-pupae.

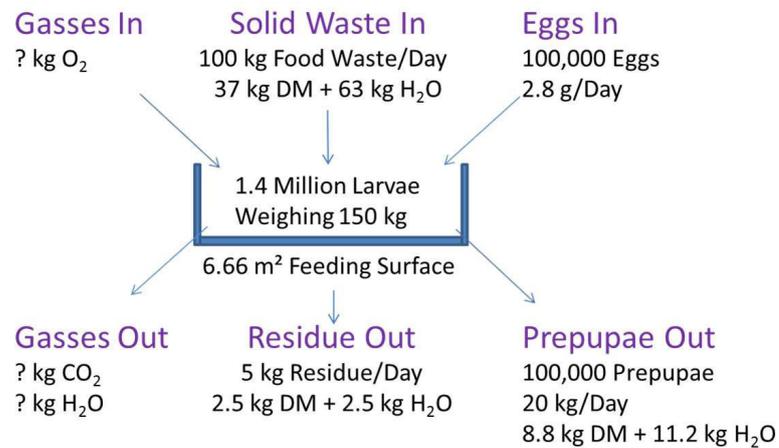


Figure 27: Flow diagram of the bioconversion with black soldier fly larvae feeding on food waste [ESR International, 2008].

The dry matter content of food waste is reported at 370 g/kg, while most freshly harvested aquatic plants are around 60 g/kg. A high content of aquatic plants in the larvae bin might lead to excess moisture and anaerobic conditions. It might be necessary to provide some structure material, like dried cow manure, that soaks up excess liquids or the standard design of the larvae bins has to be customized in a way that it provides better drainage and aeration. Partially drying the aquatic macrophytes is also an option.

Experiments to investigate the bioconversion of high moisture materials via black soldier fly larvae have to be conducted first [ESR International, 2008].

One study bred black soldier fly larvae with 100 % chicken manure from laying hens and found a conversion of manure into pre-pupae of 7.8 % on a dry matter basis. The volume of the manure could be cut in half. The manure of one hen produced about 530 g of pre-pupae per year. The natural reproduction of the black soldier flies was sufficient and did not need to be managed. Savings in chicken fodder, manure handling and house fly abatement could be realized [Sheppard et al., 1994].

### 6.2.3 Black Soldier Fly Pre-Pupae as Livestock Feedstuff

An analysis of dried pre-pupae gave the following results: 42.1 % crude protein, 34.8 % lipids, 7.0 % crude fiber, 7.9 % moisture and 14.6 % ash, of which 5 % is calcium [ESR International, 2008].

Another source states the following ranges of pre-pupae dry mass: protein 40 - 44 %, 15 - 49 % fat and ash 11 - 28 %. The fatty acid content and composition is extremely variable and depends on the type of waste used. For pre-pupae that were fed with 100 % cow manure, the omega 3 fatty acid content was 0.2 %, when they were fed 50 % cow manure and 50 % fish offal, it went up to 3.0 %. Hence, valuable nutrients can be concentrated in the larvae, both by feeding them slaughter waste and aquatic plant biomass, such as *Azolla* that is high in omega 3 fatty acids [Feedipedia, 2016].

One study investigated the effect of replacing different proportions of fishmeal with black soldier fly maggot meal in the diet of quali. Inclusion levels of 25 and 50 % maggot meal had beneficial effects on feed consumption, egg weight, egg production and feed conversion. A replacement of 75 % resulted in very little changes from the control diet, while 100 % showed negative effects in some parameters. It was concluded that black soldier fly maggot meal was able to replace 50 % of fishmeal, while maintaining good results of the quali production and substantially lowering the cost for feed [Widjastuti et al., 2014].

In an experiment, catfish were fed a diet containing up to 30 % of black soldier fly maggot meal. The weight gain in the fish did not change with 30 % replacement. With 7.5 % replacement, weight gain and protein efficiency ratio both increased by 13 % and 14 % respectively. The authors suspect it would be possible to completely eliminate fishmeal from the diet of commercial catfish without a decline in performance by using black soldier fly maggot meal [Burtle et al., 2012].

## 7 Examples of Integrated Aquaculture Systems

Integrated aquatic systems are to be understood as specifically designed productive elements that are part of agricultural landscapes to improve the most crucial parameters for long term productivity, such as availability of water, building humus, total biomass production, nitrogen fixation, biodiversity and efficient nutrient recycling. The underlying idea is that these systems are not only productive for themselves, but increase the productivity of the original farming system as well. The polyculture of different plants and animals together is an important aspect, as it mimics natural ecosystems.

### 7.1 Co-Culture of Plants and Animals

In the year 2014, for the first time ever, more fish was produced in aquaculture systems than was caught from the wild [FAO, 2016]. Aquaculture is gaining more popularity as the demand for sea food is increasing, while natural fish populations are threatened by overfishing. Large scale operations of aquaculture systems need sophisticated technology

to be able to keep fish in high densities, such as filters and aerators. By combining fish with the production of plants, fish have to be kept in lower densities, however, the whole system is not depending on electricity and is largely self-regulated, enabling these systems to be employed in rural areas. The combination of growing fish or other aquatic animals together with aquatic crops can have a number of synergistic effects.

Additionally, lower densities of fish are better for their health and closer resemble their natural habitats.

One study in India compared the yield of four different types of fish, two types of carp and two types of catfish, and two different types of aquatic plants, water caltrop (*Trapa bispinosa*) and foxnut (*Euryale ferox*), each under isolated cultivation and several combinations with fish and plants together. The yield for a single species of fish or plant was always highest in isolated cultivation, however, every combined cultivation yielded between 50 - 196 % more income compared to any isolated cultivation, as the combined yields in total would be significantly higher per pond surface area. In every combined treatment, the income generation was higher for fish than for crops [Puste et al., 2004].

Rice-fish-*Azolla* integration is described as one of the most successful uses of *Azolla* as a fertilizer and fish feed. *Azolla* is seeded in and grown in the paddies until it covers the surface area. Then the rice plants are transplanted and the floating *Azolla* biomass is incorporated at the same time. Some of the *Azolla* plants are left floating and fish are introduced. Trenches and channels of 0.5 - 1.0 m depth that are connected are integrated into the paddy field to provide shelter for the fish, while they also have access to the whole paddy field with a water depth of 10 - 20 cm. The provision of the channels and trenches compromises the rice crop area, but the income generated by the fish is overcompensating the reduction in crop area. The fish should include planktophages, macrophytophages and polyphages, so they will feed on microalgae, *Azolla* and insects and snails. The fish provide a weed and insect control for the rice plants, as well as fertilization. Some recommended species are Nile tilapia, common carp, Indian major carp and Java barb. Grass carp are advised against, as they can damage the rice crop.

An experiment on different fertilizer regimes was conducted to study its effect on fish and rice yield in integrated systems. The three different regimes were *Azolla microphylla* biomass only, inorganic fertilizer only and *Azolla* biomass and half of the inorganic fertilizer. The results are summarized in Table 19. The use of *Azolla* only and inorganic fertilizer only resulted in the same yield of fish and a higher rice yield for the inorganic fertilizer. Both combined, however, gave considerably higher yields in both fish and rice [Hasan and Chakrabarti, 2009].

Table 19: Use of *A. microphylla* as fertilizer in rice-fish culture system - fish species: *O. niloticus* [Hasan and Chakrabarti, 2009].

Initial weight [g]	Fish density [Numbers/ha]	Duration [days]	Fertilizer regimes	Fertilizer rate [kg/ha]	Quantity of N [kg/ha]	Fish yield [kg/ha]	Rice yield [kg/ha]
8.9 - 9.4	5000	75	Azolla only	3750	5.63	45.1	2567
			Inorganic fertilizer	150	38.5	45.0	3096
			Azolla + Inorganic fertilizer	3750 75	5.6 19.3	79.0	3524

Another experiment was aimed at establishing the profitability of the cultivation of rice alone, rice with fish and rice with fish and *Azolla*, see Table 20. While the co-cultivation of rice and fish resulted in a lower net income than rice alone due to a lower rice yield and only a very small yield of fish, rice with fish and *Azolla* had the highest fish and rice yields and hence the highest net income [Hasan and Chakrabarti, 2009].

Table 20: Economics of rice-fish-*Azolla* integration in India [Hasan and Chakrabarti, 2009].

Treatment	Rice yield [kg/ha]	Fish yield [kg/ha]	Gross return [US\$/ha]	Net return [US\$/ha]	Benefit cost ratio
Rice alone	8765	-	822	353	1.75
Rice-fish	7813	98.5	812	297	1.57
Rice-fish- <i>Azolla</i>	9226	154.0	985	463	1.88

The income from fish grown in rice paddies can sometimes even surpass that of the rice harvest [FAO, 2016].

The rice-*Azolla*-fish system is even part of a proposed space agriculture for the colonisation of planet Mars, due to its integration of several species in one area. The authors advised the use of loach, a fish species that is capable of living under low-dissolved oxygen conditions. It is able to gulp air from above the water surface into its digestive tube and expel air bubbles from its anus, while making air exchange along the gut. Loaches are traditionally cultured in rice paddies in East Asia. This might also be a good choice for hot climates, as both a dense *Azolla* mat on the water surface and high water temperatures can lead to low dissolved oxygen levels, which might become dangerous to other fish species [Katayama et al., 2008].

Integrated rice-duck cultivation is a common practice in East Asia, where ducks are being raised in sheds near the rice fields and used as a biological control for weeds and snails, after the rice harvest. However, this practice has a lot potential for improvement by the incorporation of *Azolla* and fish, as one study in the Phillipines has proven. *Azolla* is used as a bio-fertilizer for the rice, suppresses weeds and provides feed for the ducks and fish. The ducks produce meat and eggs, help to fertilize the rice and help with pest control, especially snails. The fish also fertilize the rice and feed on algae and insects, see Figure 28.

The study analysed 10 different rice cultivation systems including different amounts of integrated elements and their effects on the yield of rice, fish (Nile tilapia) and eggs from the ducks. For instance, the mean final weight of Nile tilapia after 83 days of cultivation was compared in different systems, see Figure 29.

In the conventional rice-fish system that made use of herbicides and molluscicides the mean weight of the fish was 31 g, while the fish in the same rice-fish system without pesticides weighed 30 g. Rice-fish cultivation with *Azolla* resulted in 40 g, rice-fish cultivation with ducks had 73 g, while rice-fish cultivation with *Azolla* and ducks combined yielded fish with a mean weight of 80 g.

The rice-fish-*Azolla* system had an increase of 8 % in rice yield in comparison to the rice-fish-duck system, however, it had no duck eggs, see Table 21.

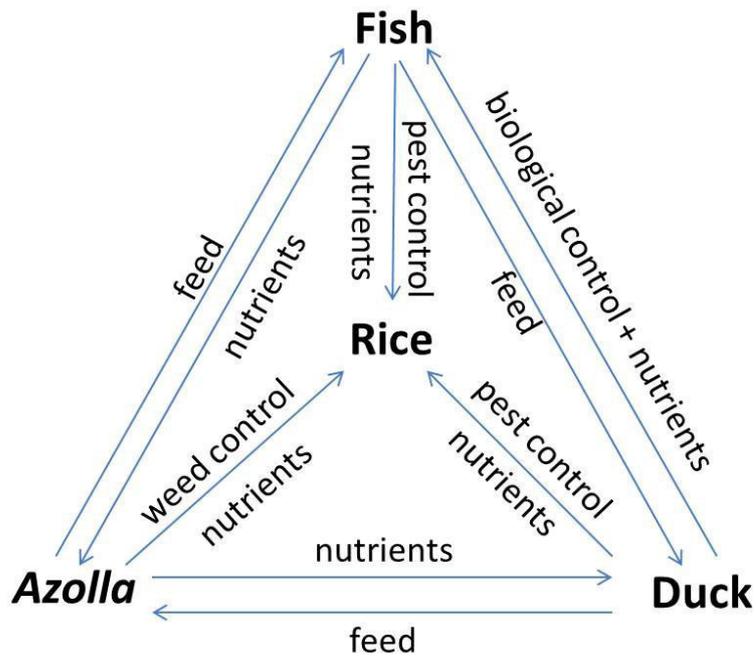


Figure 28: Relations of rice, fish, *Azolla* and ducks in integrated systems [Cagauan et al., 2000].

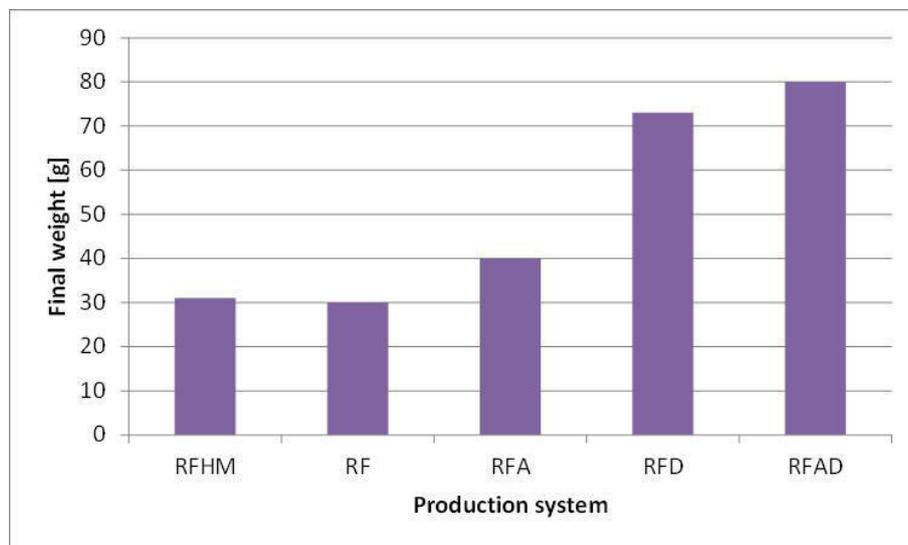


Figure 29: Average final weight of Nile tilapia after 83 days in different paddy rice cultivation systems. R=rice, F=fish, H=herbicides, M=molluscicides, A=*Azolla*, D=ducks [Cagauan et al., 2000].

The rice-*Azolla*-duck system had a 29 % higher rice yield and 4 % more duck eggs than the rice-fish-duck system, but had no fish. The rice-fish-*Azolla*-duck system had a 2 % lower rice yield and a 4 % lower duck egg yield than the rice-*Azolla*-duck system, but it had fish; the highest total yield and mean weight of fish of all tested systems.

Table 21: Yields of rice, fish and duck eggs in different rice-based systems [Cagauan et al., 2000].

Production system	Mean Rice yield [kg/ha]	Mean Nile tilapia yield [kg/ha]	Mean egg production [number of eggs/ha]
Conventional* rice	2743	-	-
Conventional* rice-fish	3023	195	-
Rice	2661	-	-
Rice-fish	2161	148	-
Rice- <i>Azolla</i>	3180	-	-
Rice-duck	2908	-	35905
Rice-fish- <i>Azolla</i>	3691	259	-
Rice-fish-duck	3424	559	37551
Rice- <i>Azolla</i> -duck	4409	-	37481
Rice-fish- <i>Azolla</i> -duck	4343	618	35955

\*Conventional rice and rice-fish systems applied with pesticide. Other systems not applied with herbicide and molluscicide.

The rice-fish-*Azolla*-duck system had higher costs for additional feed for the fish and ducks, had higher initial costs for the duck shelter and the animals and more labor was needed to incorporate the *Azolla*, feed the animals, collect the eggs, catch the fish etc. On the other hand, fertilizer was cut in half and pesticides were not needed. In the first cropping cycle, this system was among the least profitable systems, however, over time gross returns were growing fast and total costs were decreasing, so that in the third cropping cycle already it was the second most profitable of the 10 different systems [Cagauan et al., 2000].

The rice-fish-*Azolla*-duck system has proven to be a more profitable and sustainable system through the combination of several plants and animals in one system. Yet, some problems remain:

First, the nitrogen fertilizer demand is not eliminated, but only cut in half. A possible solution would be the integration of black soldier fly bins into the duck shelters. They could be fed with duckweed, manure and slaughter waste from outside the system. The nutrient-rich leachate that is produced by the larvae would directly drop into the fish pond inside of the paddy field and act as a readily available nutrient source. The pre-pupae would serve as additional feed for the ducks and fish, so more animals could be sustained within the system to produce more feces and provide further nutrients for the plants. Fresh duckweed from outside sources could complement the animals' diet as well and would most likely be preferred over *Azolla*.

Another problem is that the ducks are not allowed on the rice paddy when the rice begins to flower until the rice harvest in order to keep the ducks from damaging the crop. During this time, snails can reproduce in great numbers and the ducks must be confined and supplied with additional feed. This decreases the egg production as the ducks have to adjust to the new circumstances. Additionally, feeding costs increase.

A solution could be to replace rice by other crops that are less likely to be damaged by the ducks, for example taro (*Colocasia esculenta*), see Figure 30. This plant forms starchy tubers underground of which the biggest are harvested and the smaller ones put back to let them grow for the next harvest.



Figure 30: Demonstration Farm at the Philippine Rice Research Institute, where taro plants are combined with several *Azolla* strains.

Other candidates would be Achira (*Canna edulis*) and the water chestnut (*Eleocharis dulcis*) that require a similar approach, as the harvestable part also grows below ground. It is assumed that the ducks do not dig out the tubers or rhizomes, however, this has to be confirmed under realistic circumstances first. *Sesbania rostrata*, a leguminous fodder tree, would be another choice. It does not produce human food, but provides forage for livestock, stem cuttings for reforestation, as it also grows under dry conditions and fixes high amounts of nitrogen, which could further cut the dependence on inorganic fertilizer. Those plants could also be combined to provide even more biodiversity, while the ducks could forage on the whole system year round.

Another solution would be to simply grow taller rice plants where the developing rice cannot be reached by the ducks. This can be achieved by switching to taller rice cultivars. An innovative method yet to be tested would be to employ a modified method for the system of rice intensification (SRI), "Mound-SRI", as suggested by the Rural Development Group at Hamburg University of Technology [Otterpohl, 2017].

In contrast to the conventional paddy rice system, where the rice plants grow on permanently flooded fields, in SRI, rice plants are grown in soil with regular irrigation, but no stagnant water. Additionally, the SRI employs a far lower plant density, resulting in bigger plants, less labor for transplanting and less seed input. Increasing the organic matter content of the soil by incorporating large amounts of compost is encouraged and can in many cases spare inorganic fertilizers entirely and realize higher rice yields than the conventional paddy systems [Thakur et al., 2011].

In order to combine the advantages of wet rice and SRI, the Mound-SRI can be implemented by constructing raised mounds for the individual rice plants in the paddy field, as shown in Figure 31. The rice-fish-*Azolla*-duck system could be combined with the SRI to provide for higher plants (mound plus stronger plant by the wider spacing), while the ducks can control the snails for the whole season and prolong egg production, too. Fish

and ducks can swim in the whole area around the mounds and *Azolla* can be grown as well. The flooded area is connected to some deeper channels and/or ponds to provide shelter for the fish. The *Azolla* could be regularly scooped out and placed on top of the raised mounds to fertilize the rice plants. Alternatively, a similar effect could be achieved by simply alternating drain and flood-cycles of the whole paddy field at specific intervals. This way *Azolla* (or *Azolla/Lemna*-mixtures) would decompose and fertilize the rice without the need to incorporate it into the soil, as is a common practice in paddy cultivation in a few regions. Mound stability requires a suitable soil composition, as it is found in many paddy fields.

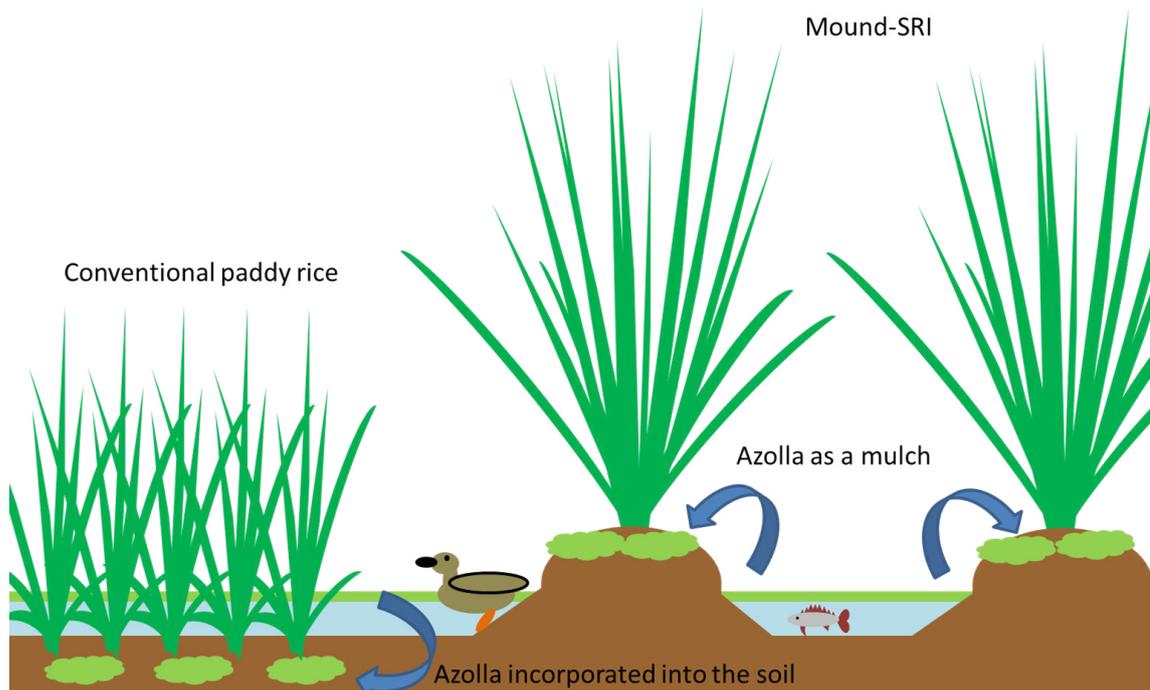


Figure 31: Conventional paddy cultivation on the left side with *Azolla* buried in the soil to fertilize the rice and modified SRI on the right side with *Azolla* applied as a mulch sparing labor. The higher plants protect the rice from the ducks.

## 7.2 Example of a Productive Pond for Irrigation

As an example, the dimensioning of an unlined irrigation pond is simulated for the irrigation of an agricultural field of the size of 2000 m<sup>2</sup> in Arba Minch. The ratio of the volume of a rainwater tank [m<sup>3</sup>] and the irrigated field surface area [m<sup>2</sup>] is about 1 to 10. However, a rainwater tank has practically no seepage, so an unlined pond should have a bigger volume to counterbalance its water losses [Biazin et al., 2012].

A study investigating ex-situ rainwater harvesting methods in Melikassa and Ziway, Ethiopia found an unlined pond of 180 m<sup>3</sup> would store enough water for the irrigation of 659 m<sup>2</sup> of onions or 257 m<sup>2</sup> of maize in Melikassa and 655 m<sup>2</sup> of onions and 195 m<sup>2</sup> of maize in Ziway for an average rainfall year. This results in a pond volume to irrigated field area ratio of 1 to 3.64 - 3.66 for onions and 1 to 1.08 - 1.43 for maize. The average annual rainfall in both areas is slightly below that of Arba Minch and the values are based on an average daily water loss of 30 mm from the pond [Moges et al., 2011].

The estimated ratio of pond volume and irrigated area shall be 1 to 5, considering water loss control in the pond. The goal could be to complement the precipitation during the dry season, so that the combination of rainfall and irrigation reaches at least 70 mm in every month of the year. According to Figure 32, that would mean supplemental irrigation in eight out of 12 months.

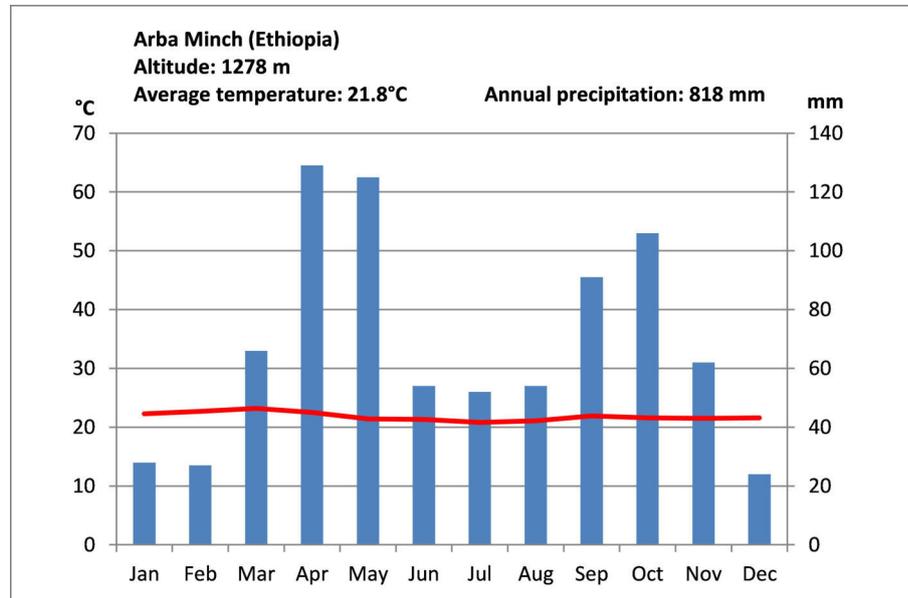


Figure 32: Climate graph for Arba Minch [Climate-Data.org, n.d.].

With a supposed average depth of 2 m, the surface area of the pond would be 200 m<sup>2</sup>. For a pond with proper control of seepage and evaporation losses, the daily water loss is estimated to be 18 mm per day, which is 6570 mm or 1314 m<sup>3</sup> per year. The amount of water used for irrigation for an average year according to Figure 32 would be 193 mm on the irrigated field or 386 m<sup>3</sup>. The water needed for irrigation, considering the amount lost due to evaporation and seepage and the rainfall falling into the pond, amounts to 1536 m<sup>3</sup>. This is the amount of inflow that the pond needs in one year to stay in balance. When assuming a runoff coefficient of 30 %, meaning the other 70 % of the rainfall are infiltrating in the catchment or are otherwise lost on the way into the pond, a total catchment area of 6261 m<sup>2</sup> would be needed.

To summarize, a 2000 m<sup>2</sup> agricultural field could be irrigated by a 200 m<sup>2</sup> storage pond, that receives runoff from a catchment area of 6261 m<sup>2</sup>. In the event of a complete absence of rain, the full pond would last 1.59 months before running dry, still considering irrigation of 70 mm on the field per month. Without irrigation the pond would then last 3.58 months. It should be emphasized that the actual amount of runoff from a catchment area is highly variable and depends on the respective physical characteristics of the soil, the slope of the land and the severity of the rainfall (amount per time).

One study that simulated the dimensioning of a rainwater harvesting pond in Rift Valley, Ethiopia, used an estimated runoff coefficient of 20 %, but had only taken into account rainfall events greater than 3 mm [Moges et al., 2011]. However, single heavy rain events can approach a runoff coefficient of 100 %, so it is much depending on the distribution and severity of rainfall events.

Alternatively, the agricultural field could be used as the catchment area to fill up the storage pond. This would have the benefit of capturing more nutrients, originating from the applied fertilizers on the field. Additionally, groundwater might be used for the irrigation as well.

Considering that the storage pond also serves as a productive pond to grow *Azolla*, bio-fertilizer and mulch could be produced for the agricultural field as well. According to the guidelines of the Natural Resources Development Project (NARDEP) on a 200 m<sup>2</sup> pond surface, 443 kg dry mass of *Azolla* could be produced per year, or almost 8 t of fresh biomass and up to 22 kg of atmospheric nitrogen could be fixed biologically. The pond would have to be supplied with some kind of manure to fertilize the *Azolla*. Additionally, the manure would seal the pond to reduce the seepage rate. The *Azolla* cover would provide biological mosquito control and reduce the evaporation rate of the pond [Pillai et al., 2005].

### 7.3 Proposed Aquatic Microfarm System

In the following, an example of a possible productive pond system that could easily be turned into a small business is outlined. At the center is the pond that receives water in form of runoff or greywater and has a dense mat of *Azolla* or duckweed on top to provide biological control of mosquitoes and reduce water losses, see Figure 33.

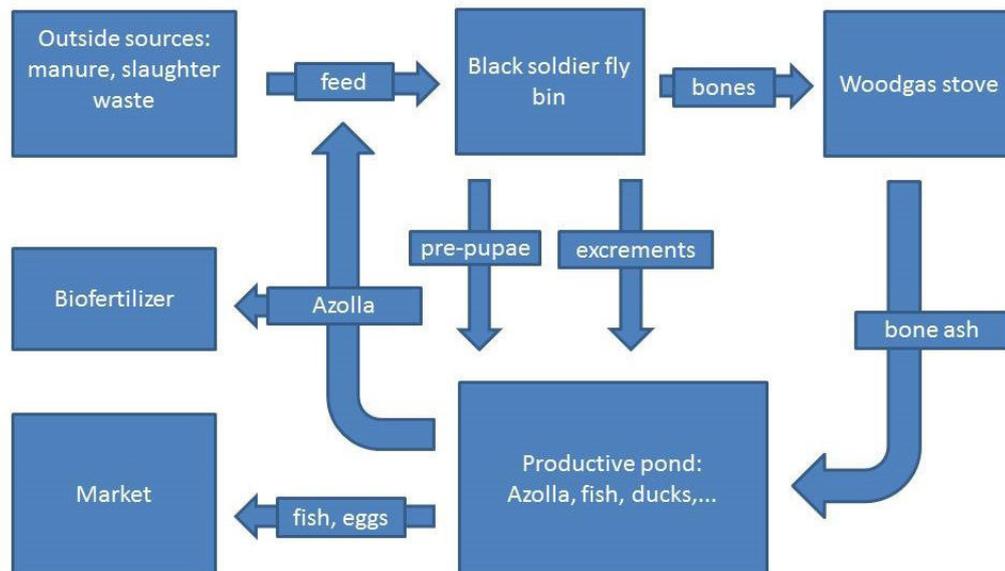


Figure 33: Flow diagram of an integrated productive pond system that allows full nutrient recycling and depends on local resources only.

In a sunny spot, duckweed should be grown, otherwise *Azolla* is preferable, however, a combination is possible as well. Freely floating plants allow for a fluctuating water level, as it is influenced by the rain. Ducks and fish are grown in the pond that feed on *Azolla*, duckweed, insects and snails and constitute the main income source. In the pond is a shelter for the ducks where they lay their eggs and a black soldier fly bin is integrated, so every day the eggs can be collected and the bin can be supplied with waste material. The black soldier fly larvae are fed with duckweed or *Azolla*, manure, slaughter waste and any organic waste material that can be obtained at the moment.

The leachate of the bins drops directly into the pond below and supplies it with nutrients continuously. The pre-pupae can either be collected in a bucket to be fed to the fish and ducks manually or they can simply drop into the pond on their own, as they climb up the harvesting ramp in the bin. Once the bin is full with bones and residues, it gets cleaned with water, so that everything except the bones gets flushed into the pond. The bones are collected and the bin is recolonized with larvae from another bin. The bones are pyrolyzed in a wood gas stove for hygienisation and to make them brittle, so they can be easily ground into a powder of bone ash. Parallel to the pyrolyzation of the bones, heat energy for cooking and charcoal for the production of terra preta can be provided. This process was tested in Arba Minch as can be seen in Figure 34 and Figure 35.



Figure 34: Bones that were pyrolyzed in a wood gas stove.

The obtained bone ash was later used in an experiment (see Chapter 8.2). This powder is rich in calcium and phosphorus, which constitutes the limiting nutrient for *Azolla*.

The cultivation of *Azolla* with recycled phosphorus is the main mechanism to obtain nitrogen through biological fixation in order to be independent of both phosphorus and nitrogen chemical fertilizers.

The *Azolla* biomass is used as a bio-fertilizer and an animal feed wherever possible to distribute the nutrients into the system. This is an example for a sustainable basic system that can be expanded as needed. The pond could be used to water the crops, while the *Azolla* can be used to mulch and fertilize them. It could also be used in conjunction with livestock rearing, where great amounts of manure could be processed into pre-pupae and *Azolla* that could both be pressed into pellets for commercial aquaculture, for instance.



Figure 35: Ground pyrolyzed bones in different grain sizes.

Even the sourcing of organic waste, especially slaughter waste, could create employment and recycle valuable materials that are often not used, but simply buried. The most important feature of this system is that its only inputs are local waste products. Phosphorus is recycled as much as possible, as is nitrogen, which is additionally biologically fixed by *Azolla* and possibly legume crops and leguminous trees.

Agroforestry systems can be an important part as well, as the trees take up minerals and micronutrients with their roots from deep soil layers. Important trees for this system include species of *Moringa*, *Leucaena*, *Gliricidia*, *Sesbania*, *Faidherbia* and *Albizia*. These trees provide fodder for livestock, so that the minerals including rare trace elements from the deeper soil layers are introduced into the cycle, see Figure 36.

The floating plants can easily be combined with other aquatic plants in the pond to increase the species diversity of the whole system. In Figure 37 can be seen a pond that was constructed in Arba Minch and stocked with *Azolla* and some other plants.

In Figure 38 the same pond can be seen about 4.5 months later. The plants grew well without any care and the pond attracted frogs that laid their eggs inside, so there are tadpoles now (Figure 39), which could be scooped up and used as a high quality chicken fodder. The pond provides both chicken fodder and human food, with very little labor involved.

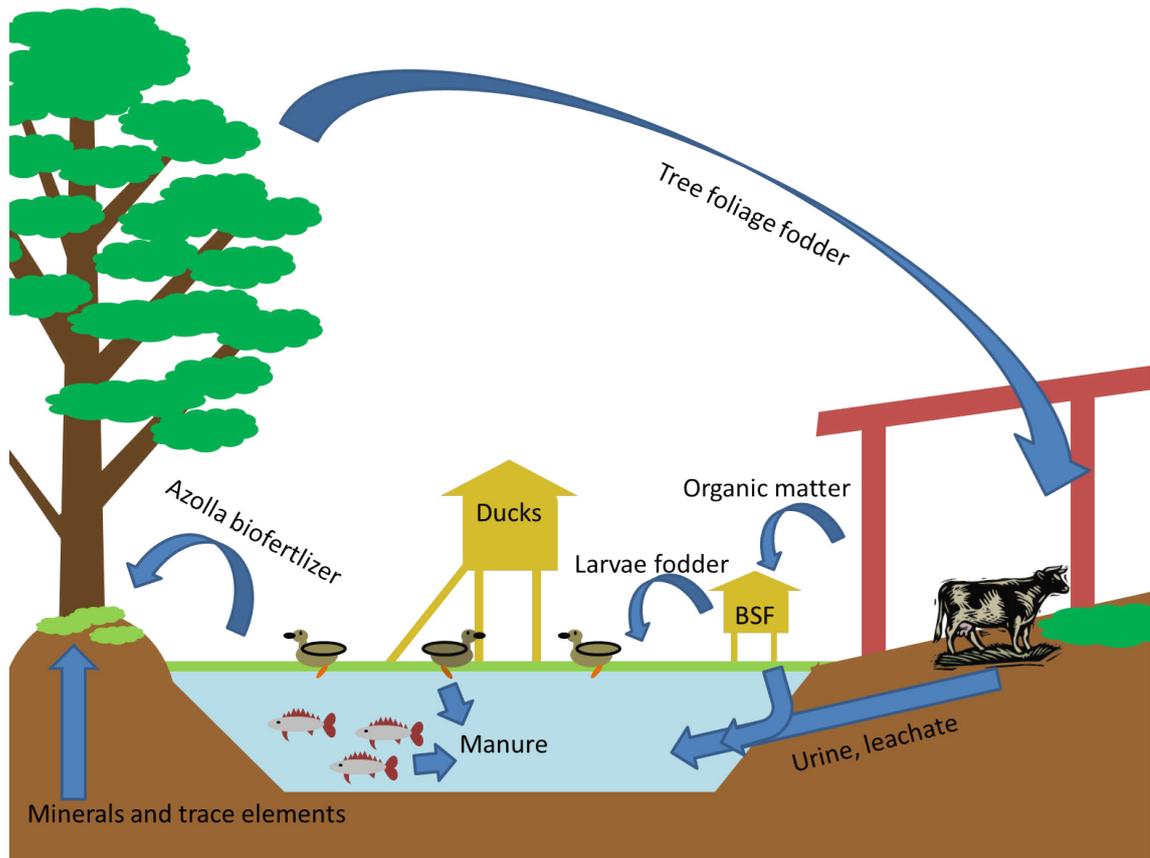


Figure 36: Nutrient cycles of an holistic food production system. Fodder trees in the context of agroforestry systems can provide fodder for livestock, where urine is diverted into the pond to grow aquatic plants and the manure used to produce black soldier fly (BSF) larvae to provide fodder for fish and ducks.



Figure 37: Newly constructed pond in Arba Minch on the 03.12.16. Besides *Azolla*, Taro (*Colocasia esculenta*), three different Achira cultivars (*Canna spp.*) and water spinach (*Ipomoea aquatica*) were planted.



Figure 38: The same pond in Arba Minch on the 23.04.17. All plants grew well, but *Azolla* needed additional shading with the bamboo mats. Later, the Moringa trees planted next to the pond will take over that task.



Figure 39: Tadpoles in the pond in Arba Minch, feeding on algae and *Azolla* they constitute valuable chicken fodder.

The black soldier fly bin in combination with *Azolla*/duckweed can also be an interesting feature for small scale systems, for example for households that raise some chickens in the backyard. Assuming one household in Ethiopia produces 200 l of greywater per day and has a roof of 30 m<sup>2</sup> surface area that collects water, a pond with a surface area of 17 m<sup>2</sup> could be built that would only receive the grey water and the rainwater from the roof, without the need of additional water when a daily water loss of 18 mm is assumed. When the greywater is used in food production systems, household chemicals, such as soaps should be restricted to natural and bio-degradable products only. Hazardous chemicals, such as mercury-soap must not under any circumstances enter the pond.

The manure of one hen produced 530 g of black soldier fly pre-pupae in one study [Sheppard et al. 1994]. If all food waste from one household is discarded into the black soldier fly bin together with the chicken manure, a good amount of supplemental feed can be harvested for the chickens.

The breeding of black soldier fly larvae obviously provides a more significant incentive for the active participation of recycling organic waste compared to composting. Composting achieves less volume reduction of waste and does not provide self-harvested fodder in the same way as a black soldier fly bin does.

Typically, in Ethiopian households organic waste is commonly not separated from non-degradable waste and thrown in a dug out pit that is regularly lit on fire. Encouraging people to recycle organic waste for their own interest solves multiple problems. The leaking fluids from the bin directly drop into the pond and fertilize the *Azolla*/duckweed that should easily yield about 680 kg of fresh weight per year. Both pre-pupae and *Azolla*/duckweed can improve the dietary status and productivity of the chickens, while the operation of the whole pond system involves a minimal amount of time, requires no external inputs and has virtually no maintenance costs. It could contribute to food security in rural areas, as a lot of organic matter could be recycled that is otherwise not used, especially slaughter waste. Proper recycling will also help to reduce the smell of organic waste and attract less house flies that can transmit diseases.

## 8 Practical Research in Arba Minch

In order to realize the idea of productive ponds, two experiments were conducted in Arba Minch about the sealing effect of gleying in ponds and the productivity of *Azolla sp.* under local conditions and different fertilizer regimes.

### 8.1 Gleying Experiment

Gleying is the process of bacterial anaerobic fermentation of organic matter whereby a plastic-like substance is produced and used to seal ponds (see Chapter 2.2.1). A gleyed pond is supposed to be completely water-tight just like a lined pond. This technique requires only local resources and would dramatically improve the effectiveness of water storage ponds as part of rainwater harvesting systems. In order to assess this method an experiment was conducted where little ponds were gleyed with varying thickness of the gleying layers.

#### 8.1.1 Materials and Methods

Four holes with a diameter of about 1 m and 0.5 m deep were dug out. Several layers of cow manure slurry were applied to the whole surface of the holes with a thickness of about 3 cm, see Figure 40. Every layer was dried in the sun for one day before the next layer was applied. In three holes, one, two and three layers were applied respectively,

while the fourth hole served as a control, with no layer applied. After all the layers of cow manure were applied, the three holes were covered with banana leaves, see Figure 41. Soil was shoveled on top of the banana leaves in order to press them down, so they would seal the cow manure to create anaerobic conditions. The holes were in the open rain and occasionally watered, so that the cow manure would not dry out. After 3 weeks, the soil on top of the banana leaves was removed and the holes were filled with water to check if they were properly sealed.



Figure 40: Several layers of cow manure slurry were applied.



Figure 41: Banana leaves were laid on top of the manure layers to encourage anaerobic conditions.

### 8.1.2 Results

Unfortunately, while removing the soil on top of the banana leaves, hundreds of termites were already present. They had fed on the banana leaves and the cow manure in all three holes and created small tunnels through the gleyed layers. Therefore, no hole was water tight, due to the termite infestations. During a heavy rainfall, however, it could be observed that the gleyed holes were almost filled to the top, while the control hole had almost no standing water inside. When the gleyed holes were filled with tap water, it would only take some hours before it would all seep into the ground, see Figure 42.



Figure 42: After three weeks the gleyed holes were filled with water after the soil on top of the banana leaves was removed.

### 8.1.3 Discussion

Apparently, this method does not work at all in areas with high incidents of termites, as they simply feed on the organic matter. Reducing the time period from 3 weeks to 2 or 1 might work, but a better approach would be to weigh down the banana leaves with stones or steel bars and fill the ponds directly with water. Another approach would be to simply fill the pond with liquid manure and refill any lost water until the pond is sufficiently sealed. Cow manure is an interesting sealing agent, as it not only seals the pond, but also fertilizes it, so *Azolla* or duckweed can be grown to further reduce water losses.

## 8.2 *Azolla* Cultivation

In order to assess the biomass production potential of *Azolla*, an experiment was conducted in the context of the Slope Farming Project in Arba Minch, Ethiopia. The Slope Farming Project aims to develop a holistic approach for the sustainable rehabilitation and conservation of degraded land and improvement of soil and water related resources. For the transplanting of trees in erosion gullies with impoverished soil, the cultivation of *Azolla* could benefit the whole operation tremendously by providing organic matter to build up the humus at a much quicker rate.

The productive ponds would store water to irrigate the trees and would start to produce high amounts of biomass in a very short time frame. While it takes time to build up the

soil and make it fertile again, a pond system fertilized with local animal manure would bypass the lack of soil fertility and jump-start the process of land restoration. The harvested biomass can be used as a bio-fertilizer and mulch for the trees, while the trees secure the soil with their root structure and shield the ground with their canopies. The combination of productive ponds and agroforestry might present one of the fastest and most sustainable ways to rehabilitate degraded land.

### 8.2.1 Materials and Methods

The *Azolla* plants that were used for the experiment were collected from a pond connected to a waterway near a small village 15 km south of Arba Minch, see Figure 43.



Figure 43: A naturalized strain of *Azolla* found near Arba Minch. No apparent red coloration of the fronds was detected, even though there was no shade.

The species was not identified. The plant material was brought to the compound of the Catholic Mission Arba Minch, where it was cultivated in a lined pond under the shade of trees, illustrated in Figure 44, for some days to produce enough biomass for the start of the experiment.

The aim of the experiment was to evaluate the biomass production potential of this local *Azolla* species in five ponds with different nutrient sources. The setup of the ponds and the cultivation was carried out according to the guidelines of the Natural Resources Development Project (NARDEP) that conducted research on the cultivation of *Azolla* for smallholders in India as supplemental feed for their milk cows [Pillai et al., 2005].



Figure 44: A lined pond was used for the propagation of the *Azolla* collected from the wild.

However, some of the guidelines were slightly adapted and changed. The ponds were dug out on the fenced in compound of the Catholic Mission Arba Minch and were lined with 2 x 3 m silpauline sheets that were bought from the local market. Each pond was filled with tap water and had a final water level of  $0.15 \pm 0.05$  m and a surface area of  $4.5 \pm 0.2$  m<sup>2</sup>. During the experiment, tap water was used to refill the evaporated water in the ponds about once a week. All ponds were receiving full sun. However, during the experiments, when signs of too much sunlight became obvious by the red color of the fronds, see Figure 45, bamboo mats were laid over the ponds permanently to protect the plants from the sun, see Figure 46.

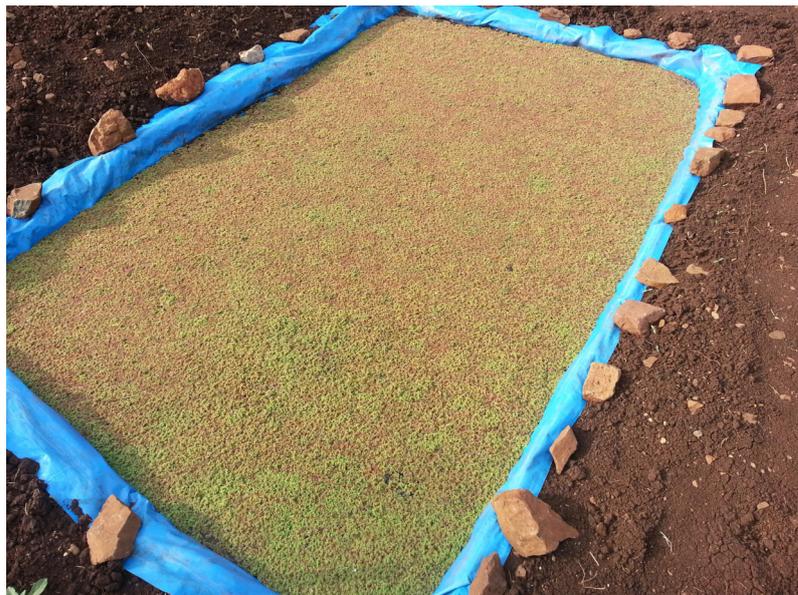


Figure 45: *Azolla* showing signs of stress through its red color caused by too much direct sunlight.



Figure 46: Bamboo mats were applied on all ponds to provide partial shade for *Azolla*.

NARDEP suggests to use soil, cow manure and Super Phosphate (SSP), an inorganic phosphorus fertilizer, as a source of nutrients for the cultivation. The five ponds were all supplied with soil and cow manure, but different sources of phosphorus were used. Pond 1 was supplied with bone ash, pond 2 with urine, pond 3 had no additional phosphorus source, pond 4 got additional cow manure and pond 5 received Diammonium phosphate (DAP). All additional phosphorus sources were used in amounts that would contain the same amount of phosphorus as in the original NARDEP guideline, see Table 22.

Table 22: Treatment schemes of the experimental ponds and the NARDEP guidelines

	Fertilizer application at inoculation	Fertilizer application at day 5	Additional P-Source	Assumed P % (w/w)
Pond 1	16.88 kg soil, 2.25 kg cow manure, 14.6 g bone ash	1.13 kg cow manure, 9.7 g bone ash	Bone ash	18.5
Pond 2	16.88 kg soil, 2.25 kg cow manure, 3.38 l urine	1.13 kg cow manure, 2.25 l urine	Urine	0.08
Pond 3	16.88 kg soil, 2.25 kg cow manure	1.13 kg cow manure	-	-
Pond 4	16.88 kg soil, 2.64 kg cow manure	1.76 kg cow manure	Additional cow manure	0.7
Pond 5	16.88 kg soil, 2.25 kg cow manure, 13.4 g DAP	1.13 kg cow manure, 8.93 g DAP	Diammonium phosphate (DAP)	20.1
NARDEP	11.25 - 16.88 kg soil, 2.25 kg cow manure, 33.75 g SSP	1.13 kg cow manure, 22.5 g SSP	Single Super Phosphate (SSP)	8

The phosphorus contents of the different materials were not measured, but only estimated. Differences in the ratios of soluble/insoluble or organic/inorganic phosphorus were not considered. The ponds received the nutrients only on the day of inoculation and on the 5th day of harvest.

On the 15th November 2016, the five ponds were filled with water, supplied with the nutrients according to Table 22 and inoculated with 2.0 kg of fresh *Azolla*, see Figure 47.



Figure 47: The five experimental ponds on the day of the inoculation (15.11.2017). Every pond was inoculated with 2 kg of fresh *Azolla*.

The plants were grown for 13 days until the whole surface area was evenly covered with a mat of *Azolla*. The first day of harvest (day 1) was on the 29th November 2016 and the last day of harvest (day 21) was on the 19th December. The harvest amount was different for every pond and day depending on how fast it would grow back. The goal was that before every harvest the *Azolla* mat would cover about 95 % of the water surface. This was based on visual assessment only. The purpose was to keep the mat from covering the pond by 100 %, which was expected to result in reduced growth due to crowding, but on the other hand only a minimal amount of light should pass through the mat, as this would encourage algal growth that would compete for nutrients. On the last day (day 21) the whole biomass in the ponds was taken out and weighed.

The harvested biomass for every pond and every day was put directly into a strainer and manual pressure was applied to remove the excess water. Directly afterwards, the biomass was weighed and discarded. One sample from every pond was also dried in an oven at 100°C for five hours and weighed to determine the percentage of dry matter.

## 8.2.2 Results

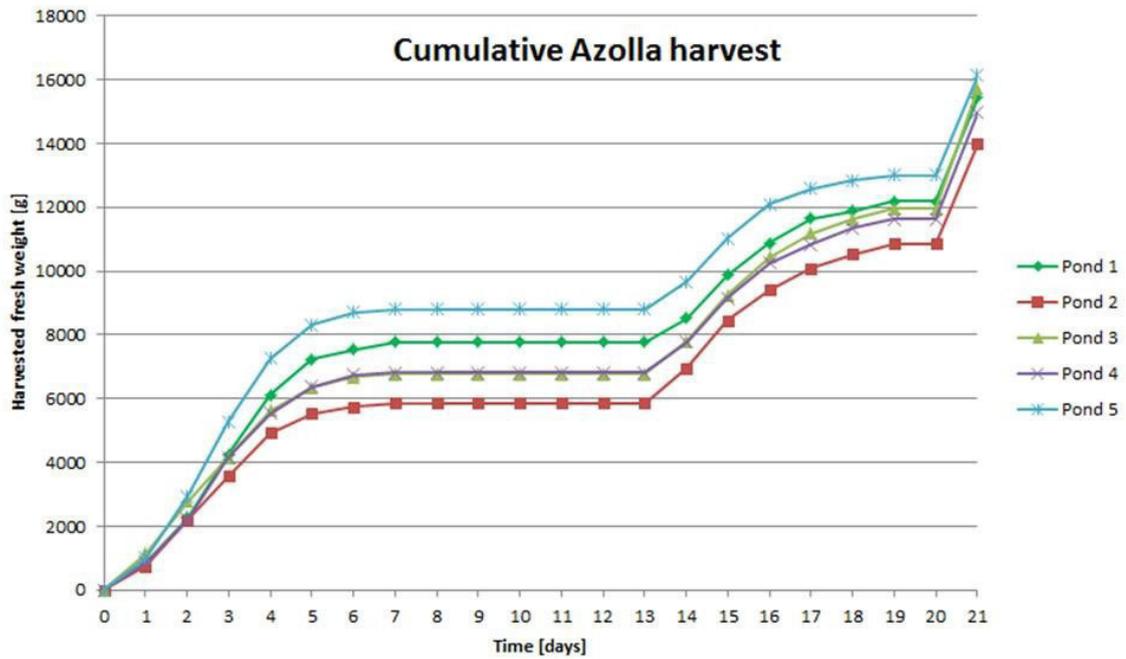


Figure 48: Cumulative *Azolla* harvest for the five experimental ponds, starting on the first day of harvest (day 1), 13 days after the inoculation with 2 kg of fresh *Azolla* fronds in each pond. At the last day (day 21) all ponds were harvested completely.

The cumulative harvest of *Azolla* biomass of each pond is shown in Figure 48. It becomes very obvious that the growth rate of *Azolla* had great fluctuations during the period of harvesting. Unfortunately, the NARDEP guidelines only state a daily average yield of 500 - 600 g of fresh weight per day per 4 m<sup>2</sup> of growing area, but no further information on harvest fluctuations during the 6 month growing cycle are given. The yield was highest during the first five days of harvest for all five ponds. Pond 5 that had DAP in it had reached an extrapolated dry mass yield of 75.20 t/ha/a in that time period, see Table 23.

Table 23: Extrapolated dry mass yields of the five ponds and the values given by the NARDEP. \*Inoculation biomass was subtracted in the last column

	Extrapolated dry mass yield [t/ha/a]			
	day 1 to day 5	day 13 to day 20	day 1 to end	from inoculation to end*
Pond 1	65.51	33.21	33.20	17.34
Pond 2	50.05	37.70	30.08	15.47
Pond 3	57.51	38.67	33.72	17.65
Pond 4	57.68	36.18	32.22	16.75
Pond 5	75.20	31.66	34.72	18.25
NARDEP	27.95	27.95	27.95	22.14

Pond 1 with bone ash came in second, followed by pond 4 with additional cow manure and pond 3 that served as control. Both were very similar and pond 2 that had urine in it provided the lowest yield. After the 5th harvest, fertilizer was applied again according to Table 22. After that, the harvest amounts dropped tremendously, so that on day 8 the daily harvest routine was stopped until day 14, as no apparent growth was noticed. On day 14, harvest was commenced again, as new growth was visible until day 20, when growth stopped again. However, this second growth phase was much less productive than the first one. On day 21, every pond was harvested completely.

The five different ponds all behaved in a very similar manner, the phases of growth and rest seemed very synchronized. While there were clear differences in yield during the first five days of harvest, the differences became less pronounced in the final yields. Pond 5 still provided the highest total yield, but pond 3 that got the smallest amount of nutrients overtook pond 4 and 1 and came in second. Pond 1 was the third best, then pond 4 and the smallest yield came from pond 2.

The extrapolated yields in Table 23 are compared to the yields given by NARDEP. The yields for the harvesting periods were all higher in the five experimental ponds, but when the time after inoculation until the first harvest was taken into account, the yield provided by NARDEP was higher than that of all five ponds. This stems from the fact that one cultivation period according to the NARDEP guidelines lasts for six months, while the experiment only lasted 34 days in total, including the starting phase before the harvest. Hence, the proportion of the starting phase was much bigger in the experiment. Additionally, the amount of inoculum used in the experiment was more than twice as much as in the NARDEP guidelines, which further reduced the total yield in the experimental ponds, as the inoculum was subtracted from the total harvest in the last column of Table 23.

### 8.2.3 Discussion

The growth conditions for the experiment were not optimal. The *Azolla* fronds were getting a red color a few days after the inoculation, see Figure 45 and Figure 49.



Figure 49: Red *Azolla* in pond 2 on the 27.11.2016, two days before the first harvest. The *Azolla* appeared to be most stressed in pond 2 that was fertilized with urine.

The *Azolla* plants in all ponds were heavily sporulating over the whole course of the experiment as can be seen in Figure 50, which is suggestive of stressful conditions.



Figure 50: Sporocarps of *Azolla* can be seen as the two yellow round capsules in the middle. Sporocarps are part of the sexual reproduction and might indicate stressful conditions in *Azolla*.

The sunlight sensitivity and reddening in this local *Azolla* strain was not expected. When they were collected from their natural habitat, they had no shade, but also showed no reddening. Why they turned red in the lined ponds remains unexplained. When the bamboo mats were applied on the 24th November 2016, five days before the harvest began, the red color reduced over the next days, but remained to a slight extent. The lined pond that was used to propagate the collected samples was in the shade under trees and produced *Azolla* plants that had a brighter color and a bigger frond size. The productivity of this pond was not assessed, but supposedly it had a better performance than the five experimental ponds, due to less stressful conditions.

In the first five days of harvesting, the plants had the greatest growth rate. Right after the second application of fertilizer, it dropped significantly and growth stopped after two days. It was assumed that the fertilizer application caused the shock in growth, however, this connection is not clear. According to the NARDEP guidelines, fertilizer should be applied every five days, but in the experiment the subsequent fertilizer applications after day 5 were left out to prevent further growth shocks.

The fact that pond 3 that received the lowest amount of nutrients produced more total biomass than pond 1, pond 2 and pond 4 suggests that there was no shortage of nutrients. Ammonia toxicity might be a possible explanation, but was not assessed. However, because of the addition of the local soil that is quite alkaline, the water in all ponds always had a pH of over 7, which would favor the formation of ammonia. Also, pond 2, which was fertilized with urine received the highest amount of nitrogen and was the least productive. The fact that the courses of the growth rate of *Azolla* behaved very similarly in all ponds suggests that this plant does not have a constant growth rate, but rather growth cycles.

As already discussed in Chapter 5.2.2, it is possible that the *Azolla* plants were synchronized with their cycles of senescence and rejuvenation, which resulted in phases with very high growth rates, but also phases with little growth.

The total yields of all ponds were comparable to those proposed by NARDEP, even though the growth conditions were not optimal from the beginning on. The fact that the least productive pond produced an extrapolated dry mass yield of 15.47 t/ha/a under adverse conditions is encouraging, as it is still considerably higher than that of conventional crops, especially under rainfed agriculture in the midlands of Ethiopia. The setup, cultivation and harvest management of these ponds is rather simple and can be done even on the most impoverished soils and still yield high amounts of biomass beginning just two weeks after the inoculation.

The fact that the least productive pond had an extrapolated yield that was just 15 % less than the most productive pond, but all ponds had seven days out of 21 days where they showed no growth at all, suggests that in this scenario the unstable growth patterns of *Azolla* that were similar in all ponds had a bigger influence on the total productivity than the different fertilization schemes. The difference in the extrapolated dry mass yield of pond 3 that served as control and had no additional source of phosphorus in it and pond 5 that received DAP was less than 3 %, indicating that in this setup, lack of phosphorus availability was hardly limiting growth in *Azolla*. However, this could be due to the short cultivation time of just 34 days. It would have been interesting to see how a pond with mixed species, for instance, *Azolla* and *Lemna* combined, would have performed, as *Lemna* could have produced more biomass during the days when *Azolla* did not grow. Further research towards mixed species aquatic macrophyte systems is needed to investigate possible synergistic effects for increased stability and enable higher yields under the same conditions.

Adhering to the NARDEP guidelines for the cultivation of *Azolla* comes with one major downside: The amount of cow manure exceeds the amount of produced *Azolla* by a factor of almost 8 on a dry matter basis. Hence, this type of cultivation is a big waste of organic matter, the most valuable asset of fertile soil. While the addition of organic matter to the soil is of paramount importance to develop its physical structure and water holding capacity, it will have little effect when sinking to the ground of a pond. How much of the organic matter can be retrieved after one 6 month cycle of growing *Azolla* is not clear, however, it is assumed that the majority is decomposed by bacteria, releasing carbon dioxide that is lost from the system. It is suggested that cow manure should either be applied to the field directly to build humus or fed to black soldier fly larvae, while using their nutrient rich leachate to grow *Azolla*, as it is described in Chapter 7.3.

Alternatively, other manure sources can be used instead of cow manure for the *Azolla* cultivation that have higher NPK values in relation to the organic matter content, such as chicken, poultry or rabbit manure. As a matter of fact, cow manure is among the worst choices in this regard, as it is rather low in nutrients relative to its organic matter content. Additionally, other sources with high nutrients to organic matter ratios include: urine, blood, bone ash, wood ash, all mineral/chemical fertilizers, digestate (from methane production) and any leachate (low solids content) from any organic sources. This does not only apply to the cultivation of *Azolla*, but all plants that are grown in an aquatic system. Systems that are only flooded occasionally or regularly alternated with aquatic and terrestrial crops might allow an exception. Building fertile soil must be the highest priority in an agricultural system that is supposed to be sustainable.

## 9 Conclusion

The underlying principle of rainwater harvesting is simple and comprehensible. The criticism it receives or rather the attention it does not receive in places where it is most needed is mainly due to improper planning, construction and management of these systems. The functionality of storage ponds can be greatly enhanced by methods as simple as supplying the pond with manure to minimize leakage and planting trees around it to reduce wind and evaporation.

However, the most important aspect is the acceptance of the farmers that are actually using storage ponds to water their crops. Most of them will see it from an economic point of view, evaluating the initial costs, maintenance and reliability to keep their crops watered through the dry season. When the functionality of a water storage pond is extended beyond its function to store water, the farmers' stance towards these structures could positively change. A productive pond can provide a substantial amount of bio-fertilizer, animal fodder, human food, such as tubers, herbs, fish and duck eggs.

*Azolla* and duckweed stand out in their ability to control malaria spreading mosquitoes and reducing evaporation of the ponds, while producing biomass in amounts that can not be achieved by any other crop. Increased overall productivity by implementing productive ponds provides a clear incentive from an economical, as well as ecological point of view. The large scale production of *Azolla* green manure as part of an integrated crop production system might very well be the fastest way to increase organic matter in impoverished soils, as this plant is potentially the most productive plant and has the most efficient biological nitrogen fixation rate at the same time.

The experiment has shown that considerable amounts can be produced even under problematic conditions with locally available inputs. Productive ponds can be integrated in existing land management systems in order to make them more sustainable by improving the natural soil fertility and increasing crop water use efficiency. This can raise crop yields without relying on external inputs, such as nitrogen fertilizers. In the Slope Farming Project, productive ponds can be constructed on top of the erosion gullies to supply the newly planted tree rows with bio-fertilizer and water to speed up the process of stabilizing the slope and stopping the soil erosion. Further research is needed for finding the ideal plant combinations to increase the long term productivity of mixed species aquatic systems.

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## 11 Appendix

Table 24: Harvested amount of fresh *Azolla*

	Harvested amount of fresh <i>Azolla</i> [g]				
Day	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5
0	0	0	0	0	0
1	877.4	760.3	1139.2	874.3	1008.8
2	1400.3	1428.8	1638.8	1349.3	1933.3
3	1964.8	1389.8	1382.3	1943.1	2326.1
4	1876.8	1350.3	1463.3	1362.3	1984.5
5	1131.3	609.7	741.1	854.3	1069.8
6	303.1	215.7	306.1	376.5	395
7	216.2	104.8	105.4	66.3	95.9
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	747.8	1094.3	1033.8	956.8	843.8
15	1364.8	1491.6	1438.3	1366.3	1356.8
16	1009.8	964.3	1194.8	1103.3	1073.3
17	753.3	667.7	737.9	586.7	506.7
18	243.9	456.9	443.5	499.7	249.9
19	290.5	331.5	287.1	292.3	173.5
20	0	0	0	0	0
21	3251.7	3118.1	3759.9	3343.6	3121.1

Table 25: Cumulative harvested amount of fresh *Azolla*

Cumulative harvested amount of fresh <i>Azolla</i> [g]					
Day	Pond 1	Pond 2	Pond 3	Pond 4	Pond 5
0	0	0	0	0	0
1	877.4	760.3	1139.2	874.3	1008.8
2	2277.7	2189.1	2778	2223.6	2942.1
3	4242.5	3578.9	4160.3	4166.7	5268.2
4	6119.3	4929.2	5623.6	5529	7252.7
5	7250.6	5538.9	6364.7	6383.3	8322.5
6	7553.7	5754.6	6670.8	6759.8	8717.5
7	7769.9	5859.4	6776.2	6826.1	8813.4
8	7769.9	5859.4	6776.2	6826.1	8813.4
9	7769.9	5859.4	6776.2	6826.1	8813.4
10	7769.9	5859.4	6776.2	6826.1	8813.4
11	7769.9	5859.4	6776.2	6826.1	8813.4
12	7769.9	5859.4	6776.2	6826.1	8813.4
13	7769.9	5859.4	6776.2	6826.1	8813.4
14	8517.7	6953.7	7810	7782.9	9657.2
15	9882.5	8445.3	9248.3	9149.2	11014
16	10892.3	9409.6	10443.1	10252.5	12087.3
17	11645.6	10077.3	11181	10839.2	12594
18	11889.5	10534.2	11624.5	11338.9	12843.9
19	12180	10865.7	11956	11631.2	13017.4
20	12180	10865.7	11956	11631.2	13017.4
21	15431.7	13983.8	15715.9	14974.8	16138.5

Table 26: Dry matter of *Azolla*

Pond	Dry mass [%] of fresh <i>Azolla</i>
1	6.66
2	4.21
3	5.37
4	5.96
5	5.65
Mean	5.57
Stdv	0.90