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Evaluating the Sustainability of *Azolla*-Rice Farming in Northern Senegal

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Doctor of Philosophy

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Abstract

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A sustainable agricultural system is essential for the creation of a secure, sufficient, and equitable global food supply. Farmers worldwide rely on expensive and synthetic fertilizers to produce food. Unfortunately, excess fertilized application can waste these expensive resources and cause nutrient pollution and degradation of aquatic habitats and drinking water sources. Therefore, strategies that recapture and recycle excess or lost nutrients could combat malnutrition while limiting economic and environmental costs. One promising strategy that captures nutrients involves an aquatic fern, *Azolla*. Farmers in Southeast Asia are using *Azolla* successfully to recover nitrogen in agricultural runoff and reuse it as input for rice and other crops. However, *Azolla's* use remains understudied compared to other farming practices, despite its ecological relevance to sustainable rice agriculture globally. Using diverse methodologies drawn from disciplines in ecology, sociology, and anthropology leveraged by the social-ecological systems framework, I characterized the potential of *Azolla*-rice farming as a sustainable practice in Senegal. First, I evaluated the potential of this sustainable practice by determining the suitability of *Azolla* in Africa using present and future climate models. I determined that most of Africa is suitable for *Azolla* growth and development in rice fields, with slight differences in regional suitability for different *Azolla* species. Then, I collaborated with Senegalese rice farmers to explore the effect of 1) adding *Azolla* to existing farmers' practices and 2) replacing urea, a dominant synthetic N source, with *Azolla* in irrigated rice fields in the Senegal River Valley. Lastly, I evaluated their attitudes and perceptions of the practice before and after the *Azolla* trials on their plots. I demonstrated that maintaining farmers' inputs while adding *Azolla* as an intercrop modestly increases rice yield. I also showed that omitting half of the urea input and substituting it with intercropped *Azolla* produces little change to the overall rice yield, signifying an opportunity for farmers to save on input costs. Overall, Senegalese rice growers had a positive perception of the *Azolla*-rice practice and have started adopting it in the next rice growing season. The results obtained from this experiment suggest that using *Azolla* in the Senegal River Valley has a potential as an organic fertilizer that can improve overall rice productivity and livelihoods.

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CHAPTER 1: Introduction

1.1. Overview

Building a sustainable world where the environment and its natural resources are preserved is a major concern. However, with the world's human population expected to reach 9.3 billion in 2050, there are growing concerns about the environment's ability to sustain this population with the limited resources on Earth (UNPD, 2001). The expanding population and increase in per capita food and energy consumption pose risks to food and water scarcity, human health, and large-scale deaths from overexploited ecosystems (Lindahl & Grace, 2015).

Sustainability requires equal consideration and effort in environmental sciences, sociology, and technology (Ho, 2005; Maria, 2015). While ecologists and environmentalists are developing best practices to aid in the protection of natural resources, there needs to be core and strong social institutions to complement the efforts. For example, the Atlantic cod fishery collapsed because of political infrastructures that failed to make the necessary conservation decisions to reduce fishing, despite rising annual catches (Hutchings, 1996; Lear, 1998; McGuire, 1997; R. A. Myers et al., 1997). Similarly, ecologists were aware of the decrease in age-specific abundance estimates of cod and their spring feeding migration patterns that made them vulnerable to fishing fleets, but continued to proceed without regarding the cultural, social, and behavioral systems in society (Lear, 1984; R. Myers et al., 1996; Parsons, 1971). Consideration of social factors is also important for introducing new technologies to new regions when advancing sustainability (Maria, 2015). A cross-sectional study in India investigated the factors that influences a farmer's choice of adopting organic farming and found that marketing, government policies, government support, economic viability, and social factors affect the adoption of organic farming (Azam & Shaheen, 2019). The challenge, however, remains building a sustainable environment that integrates multiple disciplines and knowledge sources to generate long-term beneficial impacts on sustainable development for both the environment and local stakeholders who depend on natural resources. Thus, it is imperative that we develop and implement strategies to robustly sustain natural resources while supporting human use and demand. Social-ecological systems (SES) research connects ecology to disciplines in the social sciences (e.g., sociology, economics) to provide an interdisciplinary approach to sustainable development to better meet the challenges presented in the Anthropocene (Reyers et al., 2018).

The social-ecological framework is a diagnostic tool used to systematically identify key system attributes from social characteristics (e.g., institutional, attitudinal, and behavioral) or biophysical dimension of an ecosystem, e.g., distribution of resources (Ostrom, 2009). To categorize the attributes in social-ecological systems and identify the variables that contribute to regime shifts, or large changes that effect ecosystem function (Folke et al., 2004). Ostrom (2007, 2009) developed a diagnostic framework for identifying, structuring, and organizing variables. The primary or first-level subsystems which are all socially, economically, and politically connected include resource systems (RS), resource units (RU), governance systems (GS), and actors (A). Nested within each primary subsystem are second-tier variables that all affect interactions and outcomes of the entire system. The framework was initially created to show how governance systems manage and arrange themselves when actors extract common-pooled resource units from a resource system (McGinnis & Ostrom, 2011). It was then adapted to the SES concept to understand governance challenges to best maintain cooperation in SES (Hinkel et al., 2015).

The Senegal River Valley was known for centuries as the breadbasket of the Sahel, the broad semi-arid southern border of the Sahara Desert (Koopman, 2009). Senegalese farmers relied on a traditional farming system, dependent on the annual flooding of the Senegal River, to cultivate two crops a year: millet in the rainy season (July-September) and sorghum in the dry season (October-June) (Koopman, 2009). However, beginning in the late 1960s, repetitive Sahelian droughts devastated the farming system, jeopardizing food security (J. E. Koopman, 2009). In response, the Senegalese government constructed two dams, and installed rice irrigation schemes in villages (Koopman, 2012; Koopman, 2009). Both irrigation and rice production were entirely new to farmers, who faced tremendous economic challenges adjusting to the changes (Adams, 1977). This problem is still prevalent among farmers in the Senegal River Valley (Dumas et al., 2010).

A sustainable agricultural system is essential for the creation of a secure, sufficient, and equitable food supply for Senegal (Tilman et al., 2001). Rice farmers in Senegal rely on expensive and synthetic fertilizers to produce food. Therefore, strategies that recapture and recycle excess or lost nutrients could combat malnutrition while limiting economic and environmental costs. One promising strategy that captures nutrients involves a tiny aquatic plant, *Azolla*. Farmers in Vietnam and China use *Azolla* to recover nitrogen and phosphorus in agricultural runoff and reuse it as input for rice and other crops (Dao & Tran, 1979; Liu, 1978; Pillai et al., 2002). Through a bacterial symbiont, *Azolla* also fixes nitrogen four times faster than legumes, the most widely-used nitrogen-fixing crops (Lumpkin & Plucknett, 1980). Greenhouse studies have shown that when co-cultivated with rice, *Azolla* suppresses weeds and fertilizes the soil for rice uptake (Lumpkin & Plucknett, 1980; Watanabe et al., 1977). Despite these applications and global distribution, *Azolla* is used agriculturally in only a few regions of the world and is rarely tested in field settings. This research uses sociological techniques to focus my biological research on solutions to urgent and relevant agricultural and ecological challenges faced by farmers in this region.

Senegal can greatly benefit from the usage of *Azolla*-rice farming for many reasons. First, Senegal is the second largest rice importer in the sub-Saharan region, with the goal of rice independence in the immediate future (Lancon & Benz, 2007; Van den Broeck et al., 2018). Resource recapture with *Azolla* could become a large-scale solution to this challenge. Second, *Azolla* grows natively and abundantly in water bodies in this region, and farmers are interested in its benefits (Carrapiço et al., 2000; de Waha Baillonville et al., 1991). Third, rice farmers in this region utilize large quantities (25.9kg/ha) of inorganic fertilizers and spend approximately ~400 USD per hectare/season for rice cultivation (Seck, 2016). Fourth, farmers often cooperate and work with government support groups to pool resources and increase their productivity. Altogether, these conditions suggest that successfully integrating a locally abundant native plant into their current rice system can be achieved through a close examination of the social-ecological contexts leveraged by the SES framework, a tool used to assess ecological and sociological variables that may be interacting and affecting outcomes in a system (Anderies et al., 2004; Ostrom, 2007, 2009)

In this dissertation, I contributed to our understanding of assessing the sustainability of new agricultural practices, *Azolla*-rice farming. To address this farming challenge, I applied an innovative mixed-methods approach leveraged by the social-ecological systems (SES) framework (Ostrom, 2009) to explore the social and ecological drivers of *Azolla*-rice farming and its potential as a sustainable practice based on a case study in Saint-Louis, Senegal. I addressed the following three questions:

1) How does temperature as an environmental factor affect the relative growth rate of *Azolla pinnata* in Africa currently and in the year 2050?
2) How does intercropping *Azolla* affect rice productivity in the Senegal River Valley?
3) What are rice farmers' current challenges and perceptions of *Azolla*-rice farming?

Using methods in ecology, sociology, and anthropology leveraged by the social-ecological systems framework, I evaluated the potential of this sustainable farming practice by determining the suitability of *Azolla* in Africa using present and future climate models. I determined that most of Africa is suitable for *Azolla* growth and development in rice fields, with slight differences in regional suitability for different *Azolla* species. Then, I collaborated with Senegalese rice farmers to explore the effect of 1) adding *Azolla* to existing farmers' practices and 2) replacing urea, a dominant synthetic N source, with *Azolla* in irrigated rice fields in the Senegal River Valley. Lastly, I assessed their attitudes and perspectives of the practice before and after the *Azolla* trials on their plots. I demonstrated that maintaining farmers' inputs while adding *Azolla* as an intercrop modestly increases rice yield. I also showed that omitting half of the urea input and substituting it with intercropped *Azolla* produces little change to the overall rice yield, signifying an opportunity for farmers to save on input costs. When asked about their experience with the *Azolla*-rice practice, Senegalese rice growers had a positive perception of the *Azolla*-rice practice and have started adopting it in the next rice growing season. Overall, this project is significant because it evaluates a sustainable agricultural practice that could accelerate economic growth, create more opportunities for young Senegalese, and drive Senegal towards rice self-sufficiency. *Azolla* is widely distributed, and rice is an important global crop that is jeopardized by climate change. Thus, if we can empower local farmers to use this practice, it can serve as a low-cost strategy spearheaded by smallholder farmers all over the world that both improves yield and reduces environmental degradation.

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Chapter 2: Mapping current and future habitat suitability of *Azolla* spp., a biofertilizer for small-scale rice farming in Africa

2.1. Introduction

The world population continues to grow rapidly, posing threats to global and local food security (Ehrlich et al., 1993). To meet this demand, farmers are pressured to use intensive farming practices such as pesticides, herbicides, and chemical fertilizers to improve crop yields (Carvalho, 2017; Kopittke et al., 2019). Specifically, the use of chemical nitrogen fertilizers can endanger soil health by reducing the abundance and activity of beneficial microbes (Ramirez et al., 2010), contribute to greenhouse gas emissions, and pollute the ground and surface waters which can have negative effects on aquatic ecosystems, human health, and the economy (Camargo & Alonso, 2006; Kumar et al., 2019). Moreover, most farmers in developing countries operate under a small-scale agriculture model and have difficulties accessing resources, such as mineral fertilizers, capital, information and technology, making it difficult for them to enter and compete in world markets (Jouzi et al., 2017). Thus, it is necessary to examine efficient use and alternative sources of nitrogen to meet crop demand with strategies that are equitable, sustainable, and involve social-ecological systems thinking to address complex sustainability challenges.

Rice is a staple crop in West Africa and Madagascar, and is increasingly becoming an important food source throughout Africa (Balasubramanian et al., 2007). However, rice production poses many challenges for African farmers (Balasubramanian et al., 2007), particularly those in the Sahel region where rice production is concentrated (Ibrahim et al., 2021). For example, lack of nutrient-rich soils and the availability and affordability of nitrogen fertilizers, a key input for high yielding rice varieties, make it difficult for African farmers to meet their total nitrogen demand required to produce a successful yield (Tsujimoto et al., 2019). Nutrient recapture/recycle systems, in which a biological agent captures or fixes nutrients from the environment into usable product (Baker et al., 2015), are a potential solution. One notable example of this process occurs within legumes and the soil bacteria, Rhizobia (Remigi et al., 2016). Rhizobia fix nitrogen after establishing inside the root nodules of legumes (Mia & Shamsuddin, 2010; Remigi et al., 2016) and improve soil fertility (Mia & Shamsuddin, 2010). Recently, the aquatic fern, *Azolla* spp., has gained increasing interest as an effective tool for nutrient recapture/recycle in sustainable agricultural development. The most distinguishing characteristic of *Azolla* is its symbiotic relationship with the nitrogen-fixing cyanobacterium, *Anabaena azollae* (Lumpkin & Plucknett, 1980). The cyanobacterium is capable of fixing atmospheric nitrogen to ammonium in excess of the plant's needs (Lumpkin & Plucknett, 1980; Watanabe, 1982). The *Azolla-anabaena* pair can fix ~30-100kg N/ha/month under optimal conditions (Watanabe, 1982; Yao et al., 2018), an estimate ~6-fold as large as legumes, which fix ~5-15kg N/ha/month (Guldan et al., 1996). Fresh *Azolla* growth increases nitrogen concentration in water by 3% (Van Hove, 1989) and when *Azolla* decomposes, it releases nutrients (e.g., nitrogen, phosphorus, potassium, etc.) into the water (Raja et al., 2012). Though *Azolla* can lead to eutrophication when mismanaged in water bodies (Hill, 1977), it can serve tremendous benefits when grown with irrigated agricultural crops (Watanabe, 1982). When co-cultivated with crops, *Azolla* can also suppress common weeds (Lumpkin & Plucknett, 1980). This occurs once *Azolla* forms a thick mat, starving weed seedlings of sunlight and prohibiting their emergence (Lumpkin & Plucknett, 1980).

Historically, farmers in Asia have already integrated the aquatic fern into low-input sustainable farming systems to fertilize their rice paddies (Dao & Tran, 1979; Liu, 1978). In

China, *Azolla* increased rice growth and also mediated CH_4 transport by evaporation from flooded rice soil into the atmosphere (Ying et al., 2000). In India, *Azolla* increased rice height and tiller number (Bhuvaneshwari & Singh, 2015). A small-scale experiment in the United States also demonstrated the effectiveness of *Azolla* as a biofertilizer on rice (Rains & Talley, 1979). For example, in California, *A. filiculoides* increased rice yields by 112%, 23%, 216% when incorporated as a basal manure, grown alongside rice, and applied using both techniques, respectively (Peters, 1978). Despite these advantages and the global distribution of the *Azolla* genus, it has not been co-opted widely outside of Asia and most of the research exploring *Azolla* adoption has been done before the 2000s. There is a lack of awareness of the potential of *Azolla*-rice farming in Africa that could be addressed with further study and science communication. Based on participant observations and interviews with Sahel rice farmers in the Saint-Louis River Valley in northern Senegal, there is a renewed interest for *Azolla* due to increasing demand for affordable sources of nitrogen. Senegalese farmers revealed that they would consider using *Azolla* if there were environmental and economic benefits to rice production, their main priority and goal (unpublished interview data, XSO). Therefore, a critical gap is to evaluate if *Azolla* could provide comparable benefits for African rice farming as it does elsewhere despite differences in regional and local environmental factors in the face of complex futures due to climate change. For example, *Azolla* is known to be highly sensitive to temperature, exhibiting slow growth at low temperature and die-offs in summer months or in response to heat waves (Lu, 1963; Lumpkin, 1987; Tran, 1973) (Fig 1). Therefore, predicting the performance of *Azolla* under current and future temperatures in rice-farming regions in Africa is a critical step towards equitable implementation of this practice.

Fig 1. Healthy green *Azolla* spp. (on the left) and decomposing red *Azolla* spp. (on the right) induced by high temperatures found in a canal in Saint-Louis region of Senegal. Photo taken by author XSO in 2019.

In a broader context, mapping *Azolla* suitability is a preliminary step to understanding whether *Azolla*-rice farming can be a valuable agricultural practice in Africa and can be leveraged by the Social-Ecological Systems (SES) framework. The SES framework was developed as a diagnostic tool for assessing sustainability, and recognizes the complex and interdependent relationship between biophysical and social systems (Ostrom, 2007, 2009). A key strength of the framework is understanding many dimensions of system functioning and seeks to understand all aspects related to the development, implementation, and transformation towards normative societal goals that are also sustainable (Abson et al., 2014; Gibson, 2006). In order to understand the feasibility of *Azolla*-rice system as a nutrient/recapture system and promising biofertilizer in Africa, it is important to first address whether Africa's climate is suitable for *Azolla* using two of the variables of the SES framework that focus on the resource unit (RU, *Azolla* in this application) (1) growth and replacement rate and (2) spatial and temporal distribution. Our aims are to integrate published estimates of *Azolla* growth across thermal gradients to establish thermal performance curves for two well studied species in the genus. Next, we will synthesize these performance curves with current and future climate scenarios (RCP 4.5 and 8.5, intermediate and pessimistic emission scenarios, respectively) to map current and future suitability for *Azolla* in Africa. We predicted that *Azolla* suitability in the Sahel region will be most impacted by climate change because monthly mean temperatures in that region are more frequently at or above temperatures typically cited as optimal for *Azolla*. Understanding the temperature-dependent growth rate and the temporal and spatial suitability, as well as adaptability to climate change, will be important in accurately communicating risks and benefits to African farmers. Additionally, these mapping efforts will help establish equitable implementation plans in places and times when *Azolla*-rice has the greatest potential to succeed.

2.2. Materials and Methods

2.2.1. Mapping Habitat Suitability

We assessed habitat suitability of *Azolla* for continental Africa using thermal performance curves (TPCs), annual temperature changes, and relative growth rate. To map the spatial and temporal suitability of *Azolla* spp. using the relative growth rate parameter, we compiled empirical data from the literature that examined the effects of temperature on the growth rates of various *Azolla* species using the scholarly databases, Web of Science and Google. We used the key words, "*Azolla*", "*pinnata*", "*filiculoides*", "*nilotica*", "*microphylla*", "*mexicana*", "*caroliniana*", "temperature", "relative growth rate", and "biomass", to search for all relevant empirical data. To satisfy our inclusion criteria, a study had to report the growth rate or biomass production of at least one species of *Azolla* in at least two temperature schemes. For each included study, we recorded the species, strain, temperature, initial and final time, relative growth rate, standard deviation, and other environmental conditions tested with temperature when applicable (i.e., added phosphorus, elevated CO_2, changes in pH, various light intensities) (data in S1). When relative growth rate (RGR) was not given, we manually calculated RGR if the initial mass, final mass, and time was provided using the definition of RGR:

$$RGR = \frac{ln(Mass_2 - Mass_1)}{(Time_2 - Time_1)} \tag{1}$$

Where: RGR = relative growth rate from initial time to final time expressed as g/g per day

$Mass_2$ = mass in grams at the end of growth period

$Mass_1$ = mass in grams at the beginning of growth period

$Time_2$ – $Time_1$= time interval of the growth period expressed as days.

When doubling time (DT) was only given, we used the following equation based on the definition of doubling time equation to calculate RGR:

$$DT = \frac{ln(2)}{RGR} \tag{2}$$

Where: DT = time it takes for a population to double in size expressed in days

RGR = relative growth rate from initial time to final time expressed as g/g per day

When data were not recorded in tables and only in graphs, we used Plot digitizer (http://plotdigitizer.sourceforge.net), an open-source software to manually extract estimated information (i.e., temperature and biomass, etc.). In total, we had 282 data points between six species of *Azolla*: *A. caroliniana* Willd., *A. filiculoides* Lam., *A. mexicana* C. Presl, *A. microphylla* Kaulf., *A. nilotica* Decne. ex Mett., and *A. pinnata* R. Br. The final data consisted of a total of 189 records from *A. pinnata* and *A. filiculoides* (data in S1). We only used data from *A. pinnata* because it is native to Africa and *A. filiculoides* because it is globally distributed and used in rice cultivation.

The focus of our literature review was to estimate how temperature changes caused relative differences in *Azolla* RGR by fitting TPCs for *A. pinnata* and *A. filiculoides* using maximum likelihood estimation strategies, using a similar approach to ongoing efforts to project changes in the distribution of vector-borne diseases, pests, invasive species, and other applications (Cornelissen et al., 2019; Ryan, 2020). A diversity of equations to represent TPCs exist. We chose to fit the Room model (Room, 1986) because it is capable of representing unimodal, asymmetric curves, which are extremely common. It has additional advantage over several other TPC equations in that it has only four parameters which have direct interpretation, e.g., a parameter for the optimal temperature and another for peak performance (maximum growth rate). Lastly, growth rate is defined at all temperatures, an important benefit that is not true for all TPC equations. While the Room model does not have a mechanistic or biochemical interpretation, this drawback is not critical, because we are mapping distribution based on performance, not assessing mechanism:

$$P\ (T) = \left\{ \begin{matrix} P_{max} exp\left[-a\ \left(T - T_{opt}\right)^2, T \leq Topt\ \right] \\ P_{max} exp\left[-b\ \left(T - T_{opt}\right)^2, T \geq Topt\ \right] \end{matrix} \right\} \qquad (3)$$

Where: $a > 0$ and $b > 0$ are in units of °C $^{-1/2}$

P_{max} = peak height of the curve

T_{opt} = Optimal temperature

a = slope of the rise of curve at temperatures below the optimal temperature

b = slope of fall of curve at temperatures above the optimal temperature

We then used the datasets for *A. pinnata* and *A. filiculoides* to estimate the parameters of the Room model for each species using maximum likelihood estimation (B. M. Bolker, 2008). We assumed a normal error distribution for the model. We also acknowledged that there could be pronounced differences in the maximum growth rate of *Azolla* from study to study. These differences could be attributable to variation in strains or genotypes, environmental conditions other than temperature, and the skill or decisions of experimenters. To account for this variation, we incorporated a random effect of study on the peak height parameter, *a*, using an integrated likelihood approach (Berger et al., 1999). We conducted our model fitting analysis using the R package bbmle (B. Bolker & Bolker, 2020).

2.2.2. Mapping Current and Projected Suitability for Azolla

We mapped the current and projected suitability of *Azolla* by inputting selected climatic data into the thermal performance curves that we parameterized for *A. pinnata* and *A. filiculoides*. We used current global annual temperatures (bioclimatic variable 1) for the climatic period of 1970–2000 at the spatial resolution of 5 minutes (~18.5 km at the equator) taken from the WorldClim Database (Fick & Hijmans, 2017). For future climate predictions, we also used the annual temperatures (bioclimatic variable 1) from downscaled IPPC5 Coupled Model Intercomparison Project Phase 5 (CMIP5) (Navarro-Racines et al., 2020) by the World Climate Research Programme, provided by WorldClim for 2050 (averaged for 2041–2060). The data available used the global circulation model, GISS-E2-R, and included four Representative concentration pathways (RCPs): RCP 2.6, 4.5, 6.0, and 8.5. For this project we selected an intermediate projection, RCP 4.5, and a pessimistic scenario, RCP 8.5, to model both possibilities (Rodríguez-Merino et al., 2019). For both sets of current and future data, we clipped the region to the African continent and computed the relative growth rate (RGR) of each *Azolla* species from the fitted thermal performance curves and the estimated temperature at each pixel for all scenarios. We then generated maps to visualize suitability across Africa.

2.3. Results

2.3.1. Thermal Performance Curves

We used thermal performance curves to estimate how the growth rate of *Azolla spp*. depends on temperature. The two species had similar optimum temperature (parameter *Topt* = 24.5°C), but they differed in the shapes of their TPCs (Fig 2). Specifically, *A. pinnata* displayed steeper increases in performance below its optimal temperature (parameter *a* = 0.0155 for *A. pinnata* vs. 0.005 for *A. filiculoides*) and shallower declines in performance above its optimal temperature (Fig 2A) than *A. filiculoides* (parameter *b* = 0.00344 for *A. pinnata* vs. 0.00495 for *A. filiculoides*) (Fig 2B). These parameterizations resulted in curves that are consistent with the generally recognized pattern that *A. pinnata* is more heat-tolerant than *A. filiculoides* (Fig 2).

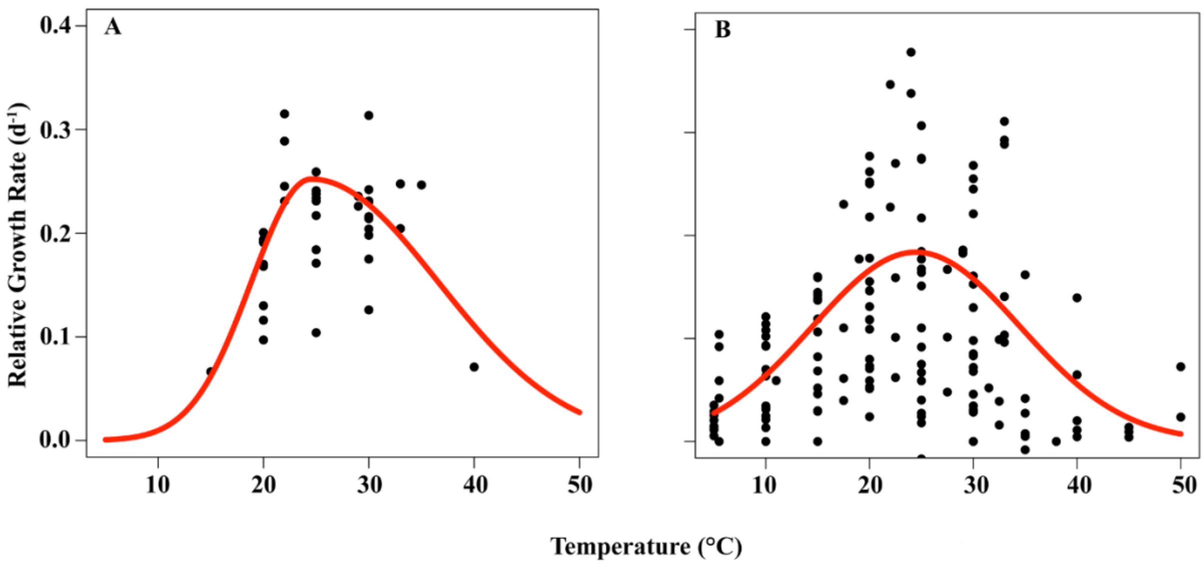

Fig 2. Overall mean thermal performance curves (lines) for (A) *A. pinnata* and (B) *A. filiculoides* estimated from experiments conducted over temperatures of 5°C - 50°C. Each point represents the mean estimated relative growth rate from a single temperature treatment in a primary study. Points represent 40 studies for *A. pinnata* and 149 studies for *A. filiculoides* showing T_{opt} = 24.5°C for both species, average maximum growth rate, P_{max} = 0.25 d^{-1} for *A. pinnata* and P_{max} = 0.18 d^{-1} for *A. filiculoides.*

2.3.2. Current and Future Projections of Azolla spp. in Africa

To predict *Azolla* spp. current and future spatial and temporal distribution in Africa, we used the thermal performance curve parameters to estimate the suitability for *Azolla* spp. across Africa. Our current prediction map shows that most of Africa has temperatures suitable for the production of both *Azolla* species (S2 Fig 1). The average relative growth rate across Africa for *A. pinnata* was 0.220 d^{-1} (S2 Fig 1B) and 0.172 d^{-1} for *A. filiculoides* (S2 Fig 1C) for current temperatures. We found that countries in central Africa have the highest suitability for both *Azolla* spp. compared to countries in the north, east and south of Africa (S2 Fig 1). We found that *A. pinnata* has a higher growth rate than *A. filiculoides* in the Sahel region (S2 Fig 1). *Azolla filiculoides* was better suitable for countries in the north, east, and south of Africa compared to *A. pinnata*. Our future prediction map for RCP 4.5 showed that the overall average relative growth rate of *A. pinnata* was 0.228 d^{-1} and 0.171 d^{-1} for *A. filiculoides* across Africa. For the pessimistic scenario (RCP 8.5), the relative growth rate of *A. pinnata* was 0.229 d^{-1} and 0.171 d^{-1} for *A. filiculoides*. Based on future climate predictions (S2 Fig 2A), *A. pinnata* is expected to perform better under RCP 4.5 (S2 Fig 2B) than RCP 8.5 in the Sahel region (S2 Fig 2D). *A. filiculoides* is also expected to have greater suitability under RCP 4.5 (S2 Fig 2C) than RCP 8.5 (S2 Fig 2E).

2.3.3. Visualizing the Difference Between Current and Future Climate Projections of Africa

We found that the mean difference between the current and future maps of *A. pinnata* for RCP 4.5 was 0.0074 d^{-1} and 0.0084 d^{-1} for RCP 8.5 (Figs 3A and 3B). The mean difference between the current and future maps of *A. filiculoides* for RCP 4.5 was –0.00066 d^{-1} and –0.00119 d^{-1} for RCP 8.5 (Figs 3C and 3D). Overall, we found a 3.38% and 3.82% increase for RCP 4.5 and 8.5 respectively in relative growth rate for *A. pinnata* and a 0.38% and 0.69% decrease for RCP 4.5 and 8.5 respectively in relative growth rate for *A. filiculoides* in the year 2050. On average between both *Azolla* species and both RCPs, the model predicted that productivity would increase in north, east, and south Africa. We also found that on average *Azolla* suitability decreased in the Sahel region as expected since growth rate declines at high temperatures and the Sahel region is the hottest region in Africa (Fig 3). This decline was more pronounced with *Azolla filiculoide*s. All future relative growth rate figures for *A. pinnata* and *A. filiculoides* can be found in S2 Fig 2.

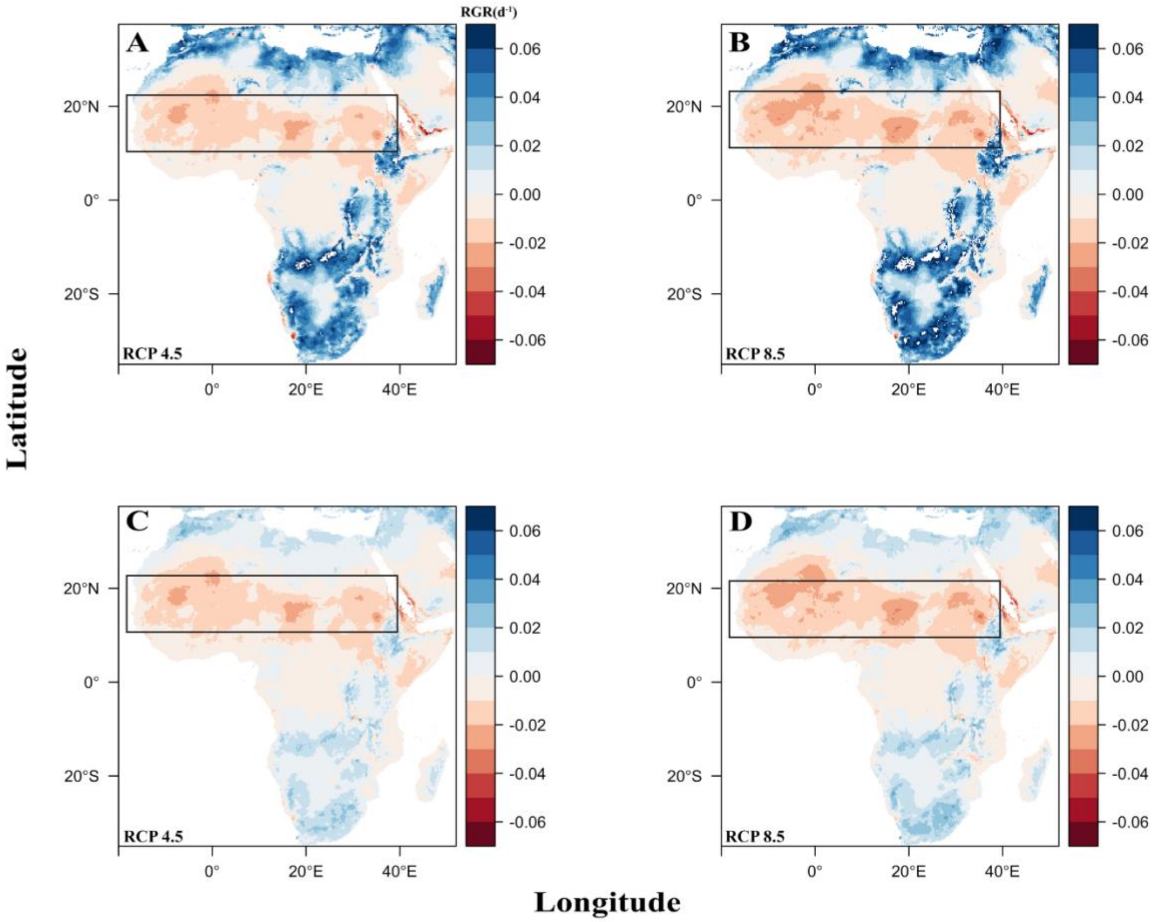

Fig 3. Suitability maps highlighting change in relative growth (RGR) and potential habitats of *A. pinnata* (A and B) *and A. filiculoides* (C and D) in continental Africa according to two RCPs. Regions expected to decline is shown in red, while regions expected to increase in suitability is highlighted in blue. For both RCP scenarios, the Sahel region (denoted in the black box) is expected to decrease in *Azolla* spp. suitability, whereas north, east, and south Africa is expected to increase in suitability. The suitability of *Azolla* for many countries in central Africa is projected to be relatively unchanged.

2.4. Discussion

Mapping tools for agriculture can show areas that are suitable for crops, symbionts, or other important co-cultivating species across space and time. Our synthesis estimated that the optimal temperature for *A. pinnata* and *A. filiculoides* is 24.5°C, and that *A. pinnata* is more tolerant to high temperatures and that *A. filiculoides* is more cold-tolerant than *A. pinnata*. Given these thermal performance curves, most of Africa is currently suitable for *Azolla*, consistent with its widespread distribution (Rodríguez-Merino et al., 2019). These results suggest that farmers in rice growing regions in current Africa can potentially adopt *Azolla*-rice farming as a low-cost and low-input alternative biofertilizer to traditional fertilizers depending on which *Azolla* species are locally available. However, we found that *Azolla* suitability in the Sahel region, where rice agriculture is currently highly concentrated, is likely to decrease while other areas in Africa are predicted to increase with *A. pinnata*. Moreover, we found that *A. filiculoides* might be a better long-term candidate as a biofertilizer for regions in central Africa by the year 2050 (Fig 3C and 3D).

2.4.1. Comparing Performance Curves

The finding that *A. pinnata* is more heat-tolerant while *A. filiculoides* is more cold-tolerant is concordant with narrative reviews of the literature on *Azolla* spp. The optimal temperature for *Azolla* spp. is between 18°C and 28°C (Tuan & Thuyet, 1979), although some species have a wide temperature range between –5°C and 35°C (Lumpkin, 1987). The optimal temperature for *A. pinnata* and several other *Azolla* spp. is 30°C (Watanabe, 1982). Growth rate begins to decrease above 35°C (Lumpkin, 1987) and fronds begin to die above 45°C and below 5°C (Lu, 1963; Tran, 1973). Although *A. pinnata* is widely distributed in the tropics, it grows better in cooler seasons (Watanabe, 1982). For example, in India, *A. pinnata* grew from July to December but was absent from ponds in the hot summer (April to June) (Gopal, 1967). In the Philippines, *A. pinnata* growth drastically declines in April and May when monthly average temperatures exceed 32°C (Watanabe et al., 1980). In contrast, *A. filiculoides* prefers lower temperatures of 25°C than *A. pinnata* (Watanabe, 1982). *A. filiculoides* could withstand temperatures as low as –5°C but was less tolerant than other *Azolla* species to high temperatures (Talley et al., 1977). Temperature is known to affect nitrogenase activity important for N-fixation and *Azolla* reproduction. When comparing nitrogenase activity of temperatures ranging from 10°C to 42°C, *A. filiculoides* prefers lower temperatures than *A. pinnata* (Becking, 1979). Because both *Azolla* species have wide thermal performance curves, suitability based on growth rate is not very different between current and future climate data. Integrating more data from extremely low and high temperatures for *A. pinnata* will help clarify *Azolla* performance in the extremes of the thermal range. Likewise, continuing to evaluate local *Azolla* strains and additional species such as *A. nilotica*, *A. mexicana*, *A. caroliniana*, and *A.microphylla*, across temperatures at many sites will help understand which species is most suitable for a given habitat. Although these finding highlight habitat suitability of *Azolla* across several countries, very few studies highlight suitability in African countries. This study highlights places where *Azolla*-rice has the greatest potential to succeed in Africa, representing most regions apart from the Sahel region. Since most of Africa is suitable for the growth and development of *Azolla*, examining how *Azolla* effects the stages of development of rice using field studies will be important in understanding if this practice biologically works in different country case studies.

2.4.2. Future projections for A. pinnata

The models under both future projections suggest that *A. pinnata* will have an overall higher relative growth rate and habitat suitability in 2050. On average across Africa, *A. pinnata* is predicted to have a higher relative growth rate with RCP 8.5, a worse-case climate change scenario, than RCP 4.5, an intermediate scenario. However, we project regional changes in suitability for this species. Specifically, distribution will increase in the northern, southern, and eastern regions of Africa, areas that are generally cooler signifying more suitable areas for *Azolla* production (Figs 3A and 3B). Conversely, the future models predict that the countries in the Sahel region will decrease in *Azolla* habitat suitability, especially with RCP 8.5. If these shifts in suitability are large enough, they could jeopardize west African farmers who are currently interested in *Azolla*-rice cultivation. Although, co-cultivating rice and *Azolla* may be feasible now, it may only be a viable strategy in the intermediate term, if climate change decreases *Azolla* habitat suitability in rice growing regions. For example, Senegal has two main rice growing areas: the irrigated Senegal River Valley and the rainfed Casamance regions (Faye et al., 2020). Senegal is likely a candidate for *Azolla*-rice farming because *Azolla* grows natively in their water bodies (Nguyen, 1996), Senegal highly depends on rice imports, and acquiring fertilizer is a major constraint for rice farmers (Diagne et al., 2013). However, by 2050, using *Azolla* as a biofertilizer for small-scale farming may not be recommended because it may be difficult to grow *A. pinnata* in nursery fields or in rice paddies under warmer climate. This anticipated challenge could be overcome through the identification or cultivation of heat-tolerant or otherwise locally adapted lineages of *Azolla*.

2.4.3. Future projections for A. filiculoides

Overall relative growth rate for *A. filiculoides* for both climate change scenarios show a decrease in RGR and habitat suitability over time. Similarly, the countries in the Sahel region will be more affected than areas in north, south and east Africa, and this effect is more pronounced in the worst-case scenario, RCP 8.5. One reason why *A. filiculoides* is predicted to decrease in RGR compared to *A. pinnata* is because it is not as heat-tolerant. The generally reported optimal temperature of *A. filiculoides* is 25°C compared to 30°C for other *Azolla* species (Watanabe, 1982). *A. filiculoides* is the only species found to withstand temperatures as low as –5°C (Talley et al., 1977). The effects of climate change will likely disrupt nitrogenase activity important for N-fixation and *Azolla* reproduction (Becking, 1979). Many countries in central Africa will experience little to no change of relative growth rate of *A. filiculoides*.

2.4.4. Comparing Methods to Evaluate Azolla Suitability

Multiple approaches to model global habitat suitability of a species exist, each differing in the data inputs and prediction algorithms. Here, we conducted an extensive literature review to identify studies that reported the productivity of *Azolla* spp. at different temperatures and used it to model the physiological thermal response of *A. pinnata* and *A. filiculoides* and to estimate the optimal temperature for the relative growth rate of *Azolla*. We then used this prediction to model change in habitat suitability across Africa based on current and future global annual temperatures (bioclimatic variable 1). Another recent study used correlative ecological niche models based on presence-only reports of *Azolla* spp. to predict areas in Africa suitable for *A. pinnata* and *A. filiculoides* identified similar results as ours (Karichu et al., 2022). They found that under current climate conditions, using 12 Bioclimatic variables and elevation, the potential habitat range was larger than recorded and that temperature was an important climate variable that affected *Azolla* species' distribution (Karichu et al., 2022). While we share similar maps that predict that Senegal, Ghana, Togo, Benin, are highly suitable areas currently for *A. pinnata* (S2 Fig 1B), our projected maps using only bioclimatic variable 1 provide further insight that Senegal is projected to experience a decrease in habitat suitability in the future based on relative growth rate. Their projections for future habitat suitability for RCP 4.5 and 8.5 predicted that *A. pinnata* would have the largest stable habitat followed by *A. filiculoides*, likely due to lower heat-tolerance as we also found in our study (Fig 3). They predict a greater loss in habitat suitability for *Azolla nilotica* across the Sahel (Karichu et al., 2022), but we found that *A. filiculoides* and *A. pinnata* will also experience a loss in habitat suitability in the Sahel region (Fig 3). Additionally, while our study predicts *Azolla* productivity, the other similar study, predicts the probability of species occurrence within a given pixel (Karichu et al., 2022). The general agreement of both approaches suggests that *Azolla*-rice farming can be pursued with confidence in many regions. On the ground results of such trials could also help evaluate and refine these current and predicted distributions.

2.4.5. Recommendations

The utilization of *Azolla* spp. in rice production would be beneficial to small-scale farmers, especially those who are resource constrained. Our projections suggest a decrease in *Azolla* productivity in the Sahel region of Africa, therefore it could be a priority to identify more heat-tolerant lineages that can withstand future climate temperatures. Additionally, countries in the Sahel region typically have one to two rice growing seasons (Laborte et al., 2017), therefore *Azolla*-rice farming could work better in one season even if it does not work in both (Gopal, 1967; Watanabe, 1982). The timing of *Azolla* usage may also be important when considering *Azolla*-farming in the Sahel. For example, if the best time to grow *Azolla* spp. is during the off season (December – May) when temperatures are cooler, it may be a better time to cultivate *Azolla* in large abundances in preparation for the regular rice season, when it is too hot for the survival of *Azolla* spp. In this case, farmers can take advantage of high temperatures by letting the ferns die and decompose in summer, thus releasing nutrients that are important for rice plants. Lastly, Sahel rice farmers can explore different strategies of incorporating *Azolla* into the rice paddies. For example, making *Azolla* compost during seasons when production is at its highest for the usage in later use. Although our projections predict that Sahel rice farmers might eventually find it difficult to exploit the full potential of *Azolla*, smaller scale climatic variation (site-to-site) could still show that *Azolla*-rice farming can work. To understand this, field experiments are needed to evaluate the best strain and farming strategy for a given habitat.

The Social-Ecological Systems framework allows for the integration of data from the natural and social sciences, which allow scientists and practitioners to tests hypotheses regarding the dynamics and functionality of food systems (Leslie et al., 2015). Previous work has highlighted that biological, institutional, and social factors increases the likelihood of sustainable systems (Cinner et al., 2012; Gutiérrez et al., 2011). If *Azolla*-rice farming is climatically suitable and improves rice production through field experimentation, using the social-ecological systems framework should be the way to facilitate broad adoption. To do so, it is recommended that researchers and practitioners involve local stakeholders to first identify variables relevant to the *Azolla*-rice system. For instance, understanding the economic value of *Azolla* in *Azolla*-rice farming as a resource unit can be an important indicator of adoption for farmers. For example, a higher net economic benefit was found when replacing urea with *Azolla* over a 3-year period

(Yao et al., 2018). Investigating other biological variables defined within SES, such as "Interactions Among Resource units", may be important when dealing with systems that contain invasive weed species or animals (i.e., fish, ducks, snails). Also, exploring the government and nongovernment organizations involved in *Azolla*-rice farming will help in understanding whether farmers who adopt the practice will also receive institutional support. Additionally, understanding the network structure of the Governance System subsystem will help clarify which farmers are part of unions and how information travels within and between groups. Researchers, practitioners, and stakeholders should also operationalize the SES framework by disentangling the Actor subsystem. For example, understanding how the actors use their current farming technology as opposed to the *Azolla*-rice practice will help determine the available resources and if they are resourced constrained. In general, researchers, practitioners and stakeholders should prioritize working together and combining experiences and perspectives to fill in the missing pieces of *Azolla*-rice farming as a social-ecological system to explore the potential on the biophysical and social side of *Azolla*-rice farming.

2.5. Conclusion

Agriculture is important to Africa's economy and accounts for the majority of livelihood and wellbeing across the continent (Diao et al., 2010). Africa is therefore a "hot spot" for the impacts of climate variability and change due to potentially devastating effects on crop production and food security (Parry et al., 2007). Our results provide useful insights to anticipate the presence and productivity of *A. pinnata* and *A. filiculoides* in Africa for the application of *Azolla*-rice farming as a sustainable agricultural practice under current and future climate change. The use of continental suitability maps can serve as a powerful resource to help local stakeholders establish areas of high and low *Azolla* suitability for regions considering *Azolla* as a biofertilizer for rice cultivation. Further studies should consider collaborating with local stakeholders for bidirectional learning to understand how societies can adopt new agricultural practices based on their goals and priorities. This type of work could be mobilized using the social-ecological systems framework by implementing interview data to understand how attitudes, customs, and social institutions influence *Azolla*-rice uptake. This would get us closer to understanding how to build a more sustainable world by understanding key interactions between humanity and nature. Moreover, this study can be applied to other aquatic species across the globe that are potential biofertilizer candidates.

2.6. References

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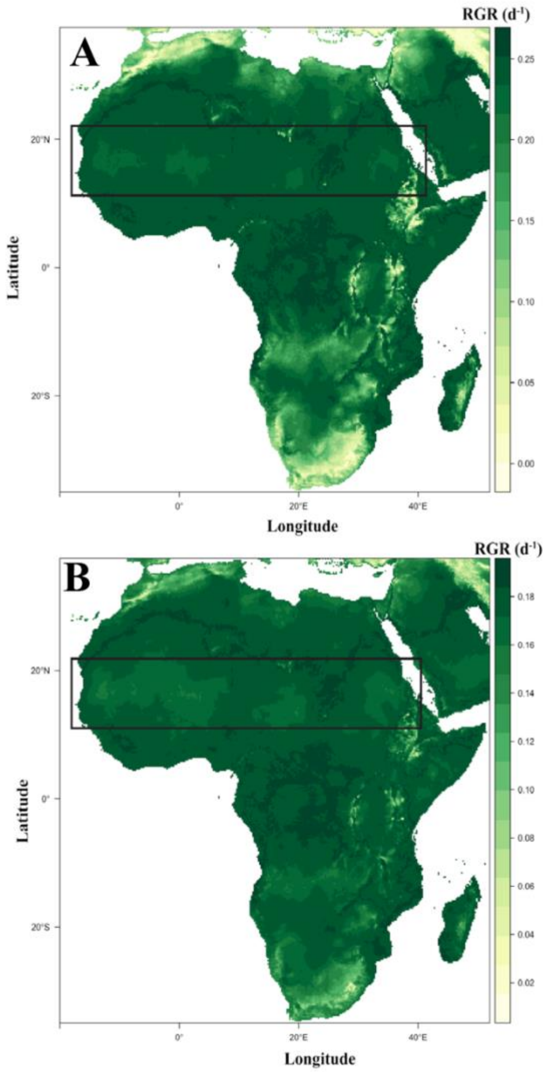
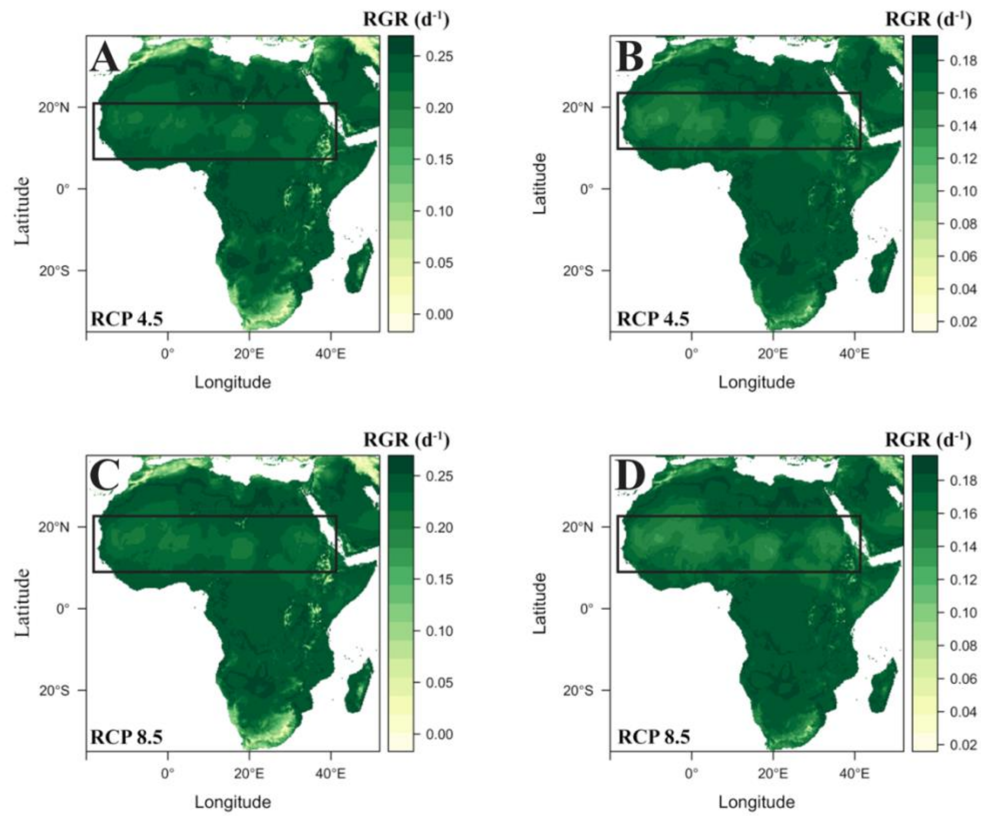
2.7. Chapter 2 Supplementary Material



S2 Fig 1. Estimated relative growth rate based on mean annual temperatures for *Azolla pinnata* (A) and *A. filiculoides* (B) computed from the parameterized thermal performance curves of relative growth rate on a white-green scale. Lower suitability habitats are denoted by yellow while highly suitable habitats are denoted by dark green. Most regions in Africa can support the growth of both *Azolla* spp. We predicted that the Sahel region (denoted in the black box) will decrease in habitat suitability for *Azolla* spp. because *Azolla* growth declines at high temperatures and this is the hottest region in Africa.



S2 Fig 2. *Azolla* spp. suitability shown under two Representative Concentration Pathways (RCPs): RCP 4.5 and RCP 8.5 averaged for year 2050. *A. pinnata* is expected to perform better under RCP 4.5 (A) than RCP 8.5 (C) in the Sahel region (denoted in the black box). *A. filiculoides* is also expected to have greater suitability under RCP 4.5 (B) than RCP 8.5 (D). Areas in dark green describe more suitable habitats for *Azolla* spp.

Chapter 3: Urea-ka: Replacing inorganic nitrogen fertilizer with *Azolla pinnata* maintains rice yields with reduced input costs in the Senegal River Valley

3.1. Introduction

Sub-Saharan Africa is the only developing region where food security continues to decline in recent decades (Van Ittersum et al., 2016). Agricultural development has the potential to ameliorate this problem since > 70% of the population participates in farming related activities (Balasubramanian et al., 2007). Sub-Saharan Africa's abundant natural resources could potentially support this development and expansion in agriculture, specifically for rice production (Balasubramanian et al., 2007). Demand for rice has been growing more than any other crop due to population growth and the inclusion of rice in many African traditional foods (Kubo & Purevdorj, 2004). Unfortunately, growing rice is labor intensive, expensive, and difficult to maintain due to limited resources such as nitrogen (N). Importing rice into Sub-Saharan Africa is also expensive, averaging a cost of more than US $1.5 billion per year (Balasubramanian et al., 2007; Lancon & Benz, 2007). Therefore, many African governments have made it a priority to develop their local rice sector as an important component of national food security, economic growth, and poverty alleviation.

Approximately two-thirds of the Senegalese population are farmers and heavily rely on the production of food to sustain a healthy lifestyle (McClintock & Diop, 2005). The Government of Senegal (GOS)-Société D'aménagement et D'exploitation des Terres du Delta (SAED) established irrigation canals in the Senegal River Valley to support rice and other crop cultivation in response to the World Food Crisis (Adams, 1977; Diagne et al., 2013). Unfortunately, farmers in this region still face challenges that limit rice yields (Cabral, 2010; Ceuppens et al., 1997). Soils in the region are extremely sandy and low in organic matter, making it difficult to retain nutrients and moisture necessary for rice uptake (Diop, 1999;

McClintock & Diop, 2005). Moreover, rice farmers in this region use large quantities (25.9kg/ha) of relatively expensive inorganic fertilizers high in N and phosphorus (P) and spend approximately US $200 (126,000 FCFA or 0.60% of total inputs) per hectare per season on urea, a dominant synthetic N source for rice crop cultivation (Seck, 2016). As a result, Senegal has become the second largest importer of rice in the sub-Saharan region (Lancon & Benz, 2007), producing 0.4 million tons of rice and importing 1.1 million tons (Van den Broeck et al., 2018). Investigating farming strategies that are equitable, cost-efficient, and sustainable for nutrient recapture and recycling is important for reducing the need for expensive inputs in high intensity farming.

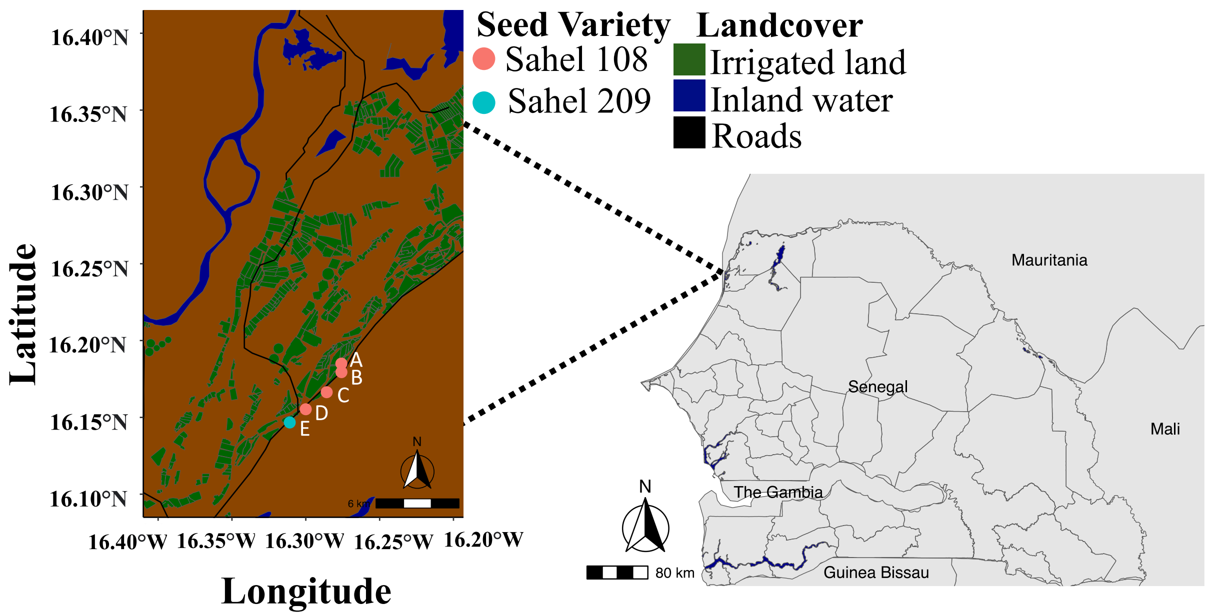
Nitrogen is a primary limiting nutrient of rice production (Safriyani et al., 2020). Unfortunately, N is prone to loss via runoff, volatilization, denitrification, and uptake by weeds, resulting in insufficiencies for rice growth (Ghosh & Bhat, 1998). Moreover, continuous application of N fertilizer can lead to long-term environmental degradation and health consequences (Yang et al., 2021). The incorporation of *Azolla* as a free organic N fertilizer, and therefore a urea replacement, in rice fields has the potential to address these challenges and improve soil health and yield sustainability (Akhtar et al., 2021). This substitution is possible because of the most distinguishing characteristic of *Azolla*, its symbiotic relationship with the N-fixing cyanobacterium, *Anabaena azollae* (Lumpkin & Plucknett, 1980). The cyanobacterium can fix atmospheric N (N_2) to ammonium (NH_4^+) in excess of the plant's needs (Lumpkin & Plucknett, 1980; Watanabe, 1982). The *Azolla-anabaena* pair can fix ~30-100kg N ha^{-1} $month^{-1}$ under optimal conditions (Watanabe et al., 1977; Yao et al., 2018), an estimate ~6-fold as large as legumes, which fix ~5-15kg N ha^{-1} $month^{-1}$ (Guldan et al., 1996). When co-cultivated with rice (termed "intercropping"), *Azolla* can benefit the crop by increasing N availability, inhibiting germination and growth of weeds, and decreasing N volatilization (Watanabe, 1982), increasing yield by 14-23% (Peters, 1978; Yao et al., 2018). Alternatively, cultivating *Azolla* and then tilling it into soil prior to growing rice (termed "monocropping"), can increase rice yield more than double (Peters, 1978). Combining these practices can increase rice yield >3-fold (Peters, 1978). Thus, there are several beneficial ways to incorporate *Azolla* in the cropping system based on local needs and abilities.

The objective of this study was to assess the feasibility of *Azolla*-rice intercropping as a nutrient/recapture system and promising organic N fertilizer in Saint-Louis, Senegal using the social-ecological systems (SES) framework. The SES framework was developed as a diagnostic tool for assessing sustainability, and it recognizes the complex and interdependent relationship between two main components: biophysical and social systems (Ostrom, 2007, 2007). Using components in the SES framework to investigate the spatial and temporal distribution of *Azolla*, Ocloo et al. (in review) found that Africa's current climate is suitable for *Azolla* productivity. However, the Sahel region, where Senegal is located, may be at risk for a slight decline in *Azolla* suitability by 2050. These assessments mirror those from another recent study, which used correlative ecological niche models based on presence-only reports of *Azolla* spp. to predict areas in Africa suitable for *A. pinnata* and *A. filiculoides* (Karichu et al., 2022). Here, we build on past work by assessing another biological component (Interactions between *Azolla* and rice) of the SES framework to assess the feasibility of *Azolla*-rice farming in the Senegal River Valley. We explored the effect of 1) adding *Azolla* to existing farmers' practice and 2) replacing urea, a dominant synthetic N source, with *Azolla* in irrigated rice fields in the Senegal River Valley. We hypothesize that *Azolla* will increase yield and market value, and the effect might be more pronounced under a reduced input of N fertilizer (urea). Understanding the effect of *Azolla* on rice productivity in this region will be important for accurately communicating the risks and benefits of *Azolla*-rice farming to Senegalese farmers. Furthermore, if *Azolla* has the potential to increase yields or maintain yields while reducing input costs, these efforts could provide an avenue by which Senegal can reach its goal of rice-sufficiency.

3.2. Materials and Methods

3.2.1. Field experiment

We conducted the field experiment in five sites across four villages in the Senegal River Valley: Bari Diam (2 sites physically separated and at 0.1 ha each) (16°10’46N, 16°16’33W), Mboltogne (1 site at 0.2ha) (16°09’19N, 16°17’57W), Ndelle Boye (1 site at 0.2ha) (16°09’59N, 16°17’09W), and Ndiole Maure (1 site at 0.2ha) (16°08’48N,16°18’39 W) (Fig. 1). The Senegal River Valley is characterized as having a semi-arid climate with two seasons: a hot and rainy season (June to October) and a dry cool season (November to May) (Maïga et al., 2020). In this region, rice is grown in two seasons: the dry season and rainy season. The field experiment was conducted during the dry rice season (March-August) of 2022 under irrigated conditions. The average annual temperatures fall between 25°C and 35°C with rainfalls averaging 215.9 mm (Maïga et al., 2020). Typical soils in this region are characterized as sandy soil (80-90% sand) and vertisols soil (55% clay content) (Maïga et al., 2020). We plowed, puddled, leveled, and subdivided each field into either 64 or 32 (2 m x 6 m) subplots depending on the size of the field (i.e., 0.1 ha = 32 subplots and 0.2 ha = 64 subplots). We separated each subplot with dirt levees at ~0.5 m high.



We used a randomized complete block design with four treatments (Table 1), each with 8 or 16 replicates (i.e., 0.1 ha = 8 replicates and 0.2 ha = 16). Because we were interested in the effect of adding *Azolla* to existing farmers' practice and replacing urea with *Azolla* in rice fields, we focus here only on the results comparing T1 vs T2 and T1 vs T4 (Table 1). For treatments that included *Azolla* sp., we inoculated each subplot with 535 g of fresh *Azolla pinnata* approximately 50 days after seed sowing and allowed it to grow to 100% *Azolla* coverage (i.e., coverage until no water was seen; Fig. 2). We inoculated each appropriate subplot with locally collected *A. pinnata* as it is the species native to Africa and Senegal (Kannaiyan & Kumar, 2006). We used the short-duration rice variety, Sahel 108, at village sites Bari Diam, Mboltogne, and Ndelle Boye, because this is the typical variety used for the dry season by farmers. We used the longer-duration rice variety, Sahel 209, in Ndiole Maure because farmers in this village use this variety during this season. Both Sahel varieties were soaked and incubated in water for 24 hours before sowing at a density of 120 kg ha^{-1}. All Farmer's practice and Farmer's practice +

100% *Azolla* coverage subplots in all five sites were treated with the farmer's recommended full dosage of Diammonium Phosphate (DAP) 18-46-0 fertilizer and N fertilizer 46-0-0 as urea twice throughout the rice season except for a third time at Ndiole Maure (Table 2). Subplots that were either Farmer's practice – 50% of Urea or Farmer's practice – 50% Urea input + 100% *Azolla* coverage were treated with the farmer's recommended DAP dosage but half of the recommended urea fertilizer dosage (Table 2). All subplots were also treated with propanyl, weedon and londax herbicides at their recommended dosage (Table 2). We selected these chemicals because they are used and recommended by farmers in the Senegal River Valley. Additionally, we manually weeded the plots, especially during the vegetative stage. All subplots were kept continuously flooded during the growth season until the maturation stage. Farmers and the research team also worked every three days to remove *Azolla* that blew or washed into subplots that were designated as *Azolla*-free. We manually harvested rice between July/August.

Table 1. Summary table of all experimental treatments with focal treatments highlighted in bold.

Treatment	**Description**
T1	**Farmer's practice**
T2	**Farmer's practice + 100% *Azolla* coverage**
T3	Farmer's practice – 50% of Urea input
T4	**Farmer's practice – 50% Urea input + 100% *Azolla* coverage**

Table 2. Each farmer's input application per rice season

	Bari Diam		**Mboltogne**		**Ndelle Boye**		**Ndiole Maure**	
Farmer	**F 1**	**F 2**	**F 1**	**F 2**	**F 1**	**F 2**	**F 1**	**F 2**
Seed Variety	Sahel 108	Sahel 108	Sahel 108	Sahel 108	Sahel 108	Sahel 108	Sahel 209	Sahel 209
Seeds (kg ha^{-1})	120	120	120	120	120	120	120	120
DAP (kg ha^{-1})	100	200	100	100	150	150	100	100
Urea (kg ha^{-1})	300	350	350	350	350	350	350	350
# of Urea applications	2	2	2	2	2	2	3	3
Propanyl (l ha^{-1})	5	5	8	8	8	8	8	8
Weedon (l ha^{-1})	1	1	1	1	1	1	1	1
Londax (sacs ha^{-1})	4	4	4	4	6	6	4	4

3.2.2. Measurement of rice yield and its components

Grain yield depends on various growth and yield component traits such as panicle number per plant, height, and weight per grain, etc. (Andrew et al., 2014). Before maturity, we selected ten random plants from the middle of each subplot and measured number of tillers (Simarmata et al., 2021). After maturity, we again selected ten random plants and measured plant height (cm) and number of panicles from five random plants. After harvest, ten random panicles were used to measure panicle length (cm) and panicle weight (g), and we also measured 100-grain weight (g) (Simarmata et al., 2021). An area of 1 m^2 from the middle of each subplot was harvested and dried to estimate grain yield based on 14% moisture content (kg ha^{-1}) (Seleiman et al., 2022) using an AMTAST USA INC grain moisture meter.

3.2.3. Statistical analysis

We conducted all analyses in the R statistical language (R Core Team, 2022). We calculated the percent change in rice grain yield per village within subplots with and without *Azolla* additions (T1 vs T2; see Table 1) and used a generalized linear mixed model (GLMM) using the glmmTMB function in the glmmTMB package (Magnusson et al., 2017) to analyze percent change in grain yield with treatment as a fixed effect and site as a random effect. We then repeated this procedure for the low urea subplots to evaluate *Azolla* as a substitution for urea (T1 vs T4; see Table 1).

We then conducted an exploratory analysis to determine which, if any, of the yield components best explained overall yield across all subplots. We defined a full model as a multiple linear regression containing each of the seven yield components using the lm() function. Then, we also fit all combinations of models containing 0-6 of the yield components using the dredge() function in the MuMIn package (Barton & Barton, 2015). After fitting all models, we ranked them by Akaike Information Criterion (AIC) and calculated unconditional model-averaged parameter coefficients for each yield component across all models with the mod.avg() function in the MuMIn package.

3.3. RESULTS

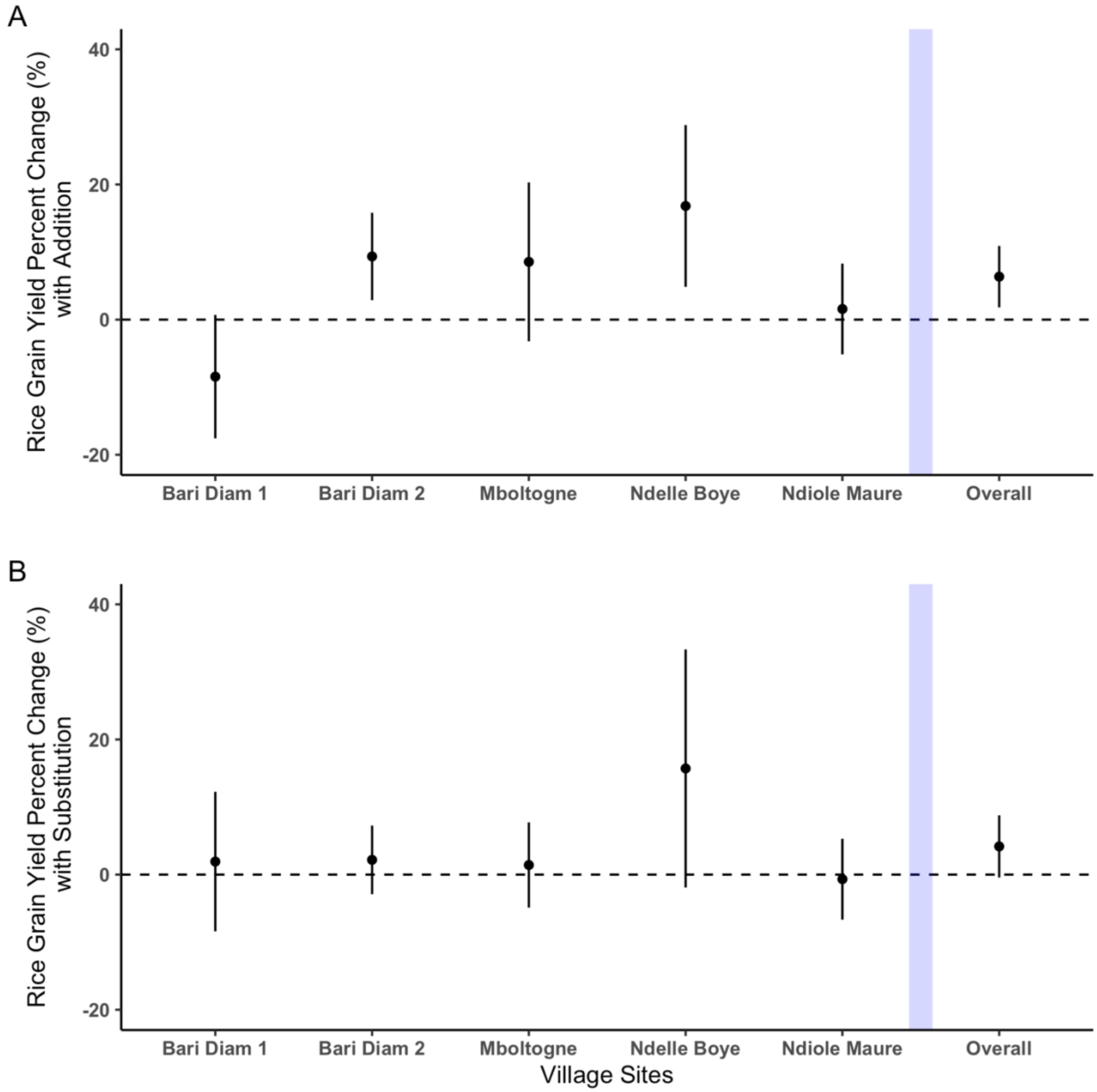
3.3.1. Investigating percent change in rice grain yield with Azolla additions

We found that in four out of five of the village sites (Bari Diam 2, Mboltogne, Ndelle Boye, and Ndiole Maure), *Azolla* addition increased rice grain yield. Overall, there was a significant mean percent change of 7.57 % ± 4.62% (*p-value* = 0.05) in rice grain yield with

Azolla additions. Bari Diam 1 experienced a -8.44% ± 9.14% change (*p-value* = 0.16), Bari Diam 2 experienced a 9.35% ± 6.47% change (*p-value* = 0.06), Mboltogne experienced a 13.99% ± 12.78% change (*p-value* = 0.13), Ndelle Boye experienced a 16.82% ± 11.97% change (*p-value* = 0.07), and Ndiole Maure experienced a 1.57% ± 6.72% change (*p-value* = 0.40) when *Azolla* was added to farmer's subplots (Fig. 3A).

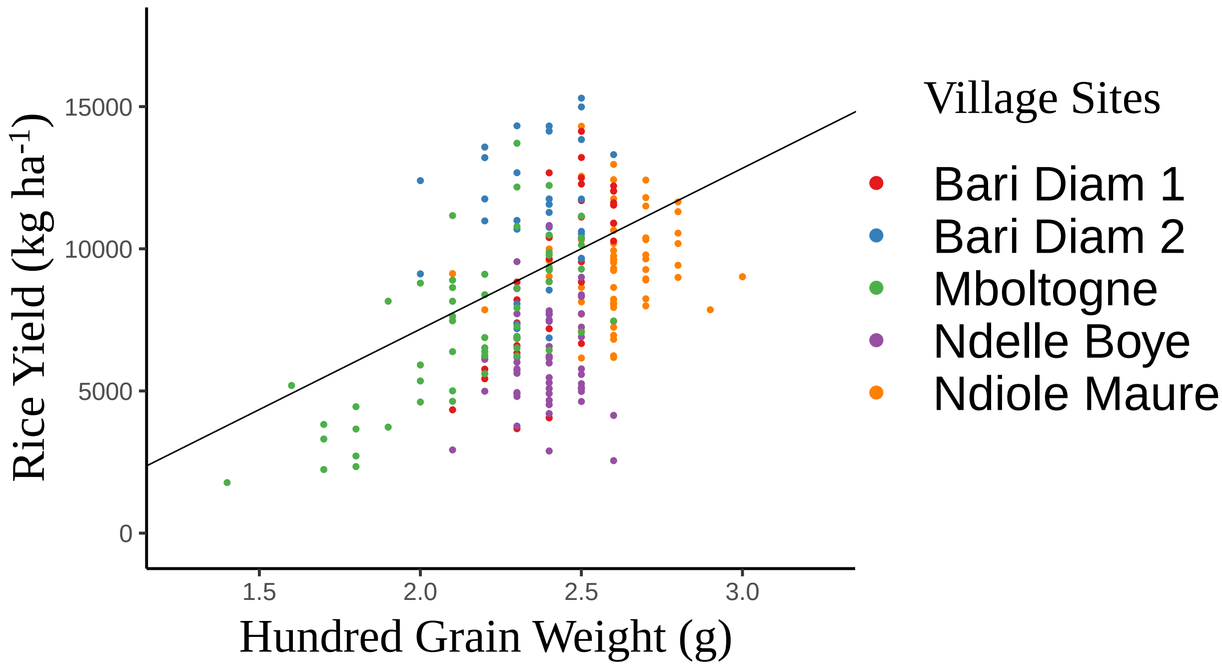
3.3.2. Investigating percent change in rice grain yield with Azolla substitutions

We found that in 4 out of 5 of the village sites (Bari Diam 1, Bari Diam 2, Mboltogne, and Ndelle Boye), urea replacement with *Azolla* had a positive percent change in rice grain. Overall, there was a mean percent change of 4.17% ± 4.61% (*p-value* = 0.18) in rice grain yield with *Azolla* substitutions. Bari Diam 1 had a 1.93% ± 10.33% change (*p-value* = 0.42), Bari Diam 2 had a 1.18% ± 5.08% change (*p-value* = 0.323), Mboltogne had a 1.42% ± 6.30% change (*p-value* = 0.408), Ndelle Boye had a 15.71% ± 17.62% change (*p-value* = 0.18), and Ndiole Maure had a -0.67% ± 5.99% change (*p-value* = 0.45) when *Azolla* was substituted for -50% of the urea input in each of the farmer's subplots (Fig. 3B).



3.3.3. Explaining variation in yield

We found that the best model to describe rice yield included the predictor variables: village, 100-grain weight, tiller number, plant length, and panicle length ($R^2 = 0.53$). The AIC statistic indicated that several other models performed comparably (22 models were within 4 AIC units, indicating good performance). We found that 100-grain weight was the only yield component with a significant model-averaged coefficient (*p-value* = $< 2 \times 10^{-16}$). Rice grain yield increased as 100-grain weight increased (Fig. 4).



3.4. DISCUSSION

This research presents one of the first documented experimental evaluations of the feasibility of *Azolla*-rice farming in the Senegal River Valley with farmer participants in a small-scale field setting. We show that maintaining the farmer's inputs while adding *Azolla* as an intercrop modestly increases rice yield. We also show that omitting half of the urea input and substituting it with intercropped *Azolla* produces little change to the overall rice yield, signifying an opportunity for farmers to save on input costs. Additionally, we found that bigger and heavier rice grains were a good predictor for high rice yield. Together, the results obtained from this small-scale experiment suggest that using *Azolla* in the Senegal River Valley shows potential positive benefits to overall rice productivity.

Farmers in China and Vietnam have historically integrated *Azolla* spp. into their low-input sustainable farming systems as biofertilizer in their rice paddies (Dao & Tran, 1979; Liu, 1978). Here, we found that in Senegal, intercropping *Azolla* in addition to standard inputs produced significantly higher rice grain yield by 7.57% averaged across all five sites with four of five sites exhibiting increases in yield and maximum increases approaching 20% in two villages. (Fig. 2A). Singh et al. (1988) found that intercropping *Azolla* in rice paddies produced a 32% high rice yield in the dry season and a 17% higher rice yield in the wet season. Other studies have also reported a 23% change (Peters, 1978), 8% change (El-Bassel & Ghazi, 1996), 0.95% change (Gutbrod, 1986), 14% increase (Yao et al., 2018) when *Azolla* was solely intercropped with rice. Our results showed a positive percent increase as supported by the literature. Several mechanisms can explain the observed increase in rice grain yield. Including *Azolla* as an intercrop can increase soil N content (inoculation at a rate of 500 kg ha^{-1} can increase 50 kg N ha^{-1}; Roy et al., 2016), *Azolla* reduces volatilization by 12-42% (Yao et al., 2018), and *Azolla* improves soil physical structure by supplying organic matter (Subedi & Shrestha, 2015). Other mechanisms include reduction of evaporation (Esiobu & Van Hove, 1992), suppression of weeds (Biswas et al., 2005), and increase plant establishment and survival at seedling or transplanting (Monajjem & Hajipour, 2010).

Our study's observed effect fell within the 0.95% - 32% range observed in other field studies conducted throughout the world. While we did not investigate "monocropping", the practice of tilling *Azolla* into the soil prior to planting rice, other studies have found combining these practices can increase rice grain yield by 16-31% (Kumar & Shahi, 2016) and 216% (Peters, 1978). Thus, future studies should examine the feasibility of *Azolla* rice farming on a larger multi-year scale in more village sites as well as applying *Azolla* as a monocrop or intercrop. Additionally, using other Sahel rice varieties or applying other *Azolla* species, such as *A. filiculoides*, at different seasons may also improve rice yield particularly in village sites such as Bari Diam 1 or Ndiole Maure that did not perform as well as the other village sites in this study. Using other *Azolla* species or hybrids may work better because different species have a higher heat tolerances or salt tolerances (Yadav et al., 2023) which is important when exploring how salinity affects rice production.

3.4.1. Azolla as a substituted intercrop

Using N efficiently in rice production is critical for meeting the challenges of food security while protecting the planet. Using free organic fertilizer, such as *Azolla* which have the capabilities of biological N fixation could be a promising solution to achieve better N use efficiency (Yang et al., 2020). We found that substituting urea for 50% *Azolla* produced an overall 4.11% increase in yield (Fig. 3B). In 4 out of the 5 village sites, there was very little change in rice grain yield. This suggests that farmers can save money on their fertilizer inputs by substituting urea with *Azolla* while still producing comparable yields. Each season, farmers in the Senegal River Valley spend approximately US $200 (126,000 FCFA) per hectare on urea. By using this substitution strategy, farmers in the Senegal River Valley can decrease their total input costs by 100 USD or 63,000 FCFA per hectare, which is an important factor considered by farmers before adopting *Azolla*-rice practice (Personal communication from farmer participants). Other studies have also reported that *Azolla* can be used as a partial substitution for N in rice production. For example, Yao et al. (2018) found that after three rice growing seasons, replacing 25% of urea for *Azolla* produced an 8% increase in rice grain yield. Malyan et al. (2021) found the highest grain yield observed in an *Azolla* treatment substitution of 25% Urea (30 kg N ha^{-1}), producing an overall 14.8% increase in grain yield. Together, this supports the idea that in an irrigated rice production, the application of *Azolla* as a substitute for partial urea can be an effective option for saving money on synthetic fertilizers while maintaining or increasing yields in the Senegal River Valley. Future work should address the amount of N per hectare replaced by *A. pinnata* to populate a more accurate percentage of urea that should be replaced to reach a net yield increase either at 0% or above.

3.4.2. 100-grain weight as a proxy for yield

We found a positive correlation between heavier grains and higher grain yields (Fig. 2). Simarmata et al. (2021) also found that 100-grain weight was positively correlated with grain yield (i.e., the higher the weight per plant, the higher the grain yield (Rostini et al., 2020)). The weight of 100 grains indicates the ability of rice plants to allocate available nutrients as food reserves (Simarmata et al., 2021). Other components, such as a larger number of productive tillers, are important in high rice productivity. This can occur when the nutrient supply in the soil is high and can be absorbed by the rice plant (Simarmata et al., 2021). However, productive tillers do not guarantee high yield because they could result in small or empty grains. Thus, grain filling determines the potential yield of rice plants (Rostini et al., 2020). This suggests that very small-scale experiments that test *Azolla*-based treatments and measure grain weight could be used to predict which interventions improve yield the most at the farm plot scale. Important factors to test in such experiments include the timing of adding *Azolla* spp. as well as how *Azolla* affects soil fertility. It will also be important to understand how Applying *Azolla* spp. During the rainy season and/or several days before sowing could increase tillers, plant height, number of panicles, panicle length, panicle weight, and 100-grain weight.

Additionally, because of the small-scale design of this experiment and *Azolla*'s high biomass productivity (Lumpkin & Plucknett, 1980), it was labor-intensive in some cases to contain *Azolla* in each subplot, given their small size. While this complicated the experiment, participant farmers noted that this would not be problematic for large-scale adoption because inoculation would occur over an entire plot.

3.5. Conclusion

The goal of this project was to investigate the feasibility of *Azolla*-rice farming in the Senegal River Valley during the dry season of 2022. We showed promising and positive effects of adding *Azolla* as a free organic fertilizer in farmers' rice fields and urea replacement. Next steps should continue incorporating the social-ecological systems framework to investigate the social dimension of this practice in this region. Though this has promising results ecologically, investigating the farmers' experience and attitudes after trialing *Azolla*-rice practice is an important next step to indicate the longevity and sustainability of the practice.

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Chapter 4: An In-depth Examination of Senegalese Farmers' Experience and Perception of *Azolla*-Rice Farming

4.1. Introduction

Achieving global food security while meeting the demands of a growing population calls for transforming current agricultural production systems towards more sustainable models. In agriculture, environmental sustainability depends on innovations in agroforestry practices (Sobola et al., 2015), rotating crops (Shah et al., 2021), integrating livestock and crops (Lal, 2020), nutrient recapture and recycling (Baker et al., 2015), and other practices that are environmentally important and beneficial. These sustainable agricultural practices not only protect the ecosystems by efficiently using natural resources, but they also increase the capacity to respond to climate change and variability (Wheaton & Kulshreshtha, 2017). Therefore, the adoption of sustainable practices by farmers may have significant benefits for the environment and help achieve more resilient and productive food systems to advance food security.

The adoption of sustainable practices requires incentives (e.g., farm income and improved yields), significant effort from farmers, and support from government and public-private partnerships at local and national levels (Piñeiro et al., 2020). The decision to adopt is often a difficult one where a farmer must consider their own values, environmental preferences, the incentives, economies, and cultural characteristics (Barnes et al., 2019). Farmers have a higher chance of adopting a new practice if it helps them achieve their personal goals (Pannell et al., 2006). As a result, adoption of a practice is based on the subjective perception which depends on three categories: the process of learning and experience, the landholder's social environment, and the characteristics of the practice in question (Pannell et al., 2006).

Here, we investigate a potential sustainable practice, *Azolla*-rice farming, in the Senegal River Valley (SRV). Recently, there has been a growing interest in using the floating aquatic fern, *Azolla* spp., as an effective tool for nutrient recapture/recycle to aid sustainable agricultural development. Currently, there are seven recognized species of *Azolla*: *A. nilotica*, *A. caroliniana*, *A. microphylla*, *A. mexicana*, *A filiculoides*, *A. pinnata*, *and A. rubra* (Watanabe et al., 1977). *Azolla* has a unique combination of traits that make it extremely useful in nutrient recapture from aquatic habitats: the capacity to fix atmospheric nitrogen and a fast reproductive rate (Lumpkin & Plucknett, 1980). Together, these qualities make *Azolla* a valuable resource in food cultivation. The irrigated farmland in northern Senegal is a prime candidate for benefitting from *Azolla*-rice farming for several reasons. *Azolla pinnata* grows natively and abundantly in drainage ditches, ponds, and irrigation canals in this region (personal observation) (Carrapiço et al., 2000; de Waha Baillonville et al., 214 1991). Rice is the primary crop in this region, and the demand to grow and import large quantities is a high priority (MAER, 2014). In 2013, Senegal produced 0.4 million tons of rice and imported 1.1 million tons (Van den Broeck et al., 2018), making Senegal the second largest importer of rice in the sub-Saharan region (Lancon & Benz, 2007). Further, rice farmers in this region utilize large quantities of relatively expensive inorganic fertilizers (personal communication).

Farmer engagement and understanding is necessary for the potential adoption of *Azolla*-rice farming in the Senegal River Valley. Much remains under studied about the viability of *Azolla* and its fit in the Senegal River farming system. A few studies have assessed the waterfern's potential in improving soil fertility, minimizing weeds, increasing soil organic carbon, further enhancing rice growth and yield (Kollah et al., 2016), but no documented studies have incorporated farmers' perspectives and priorities, and their interests and reactions in co-cultivating rice and *Azolla*. Farmers' engagement and experiences co-cultivating rice and *Azolla* could significantly shape the viability for the expansion of *Azolla*-rice into the future for other African nations. This research thus seeks to compile and synthesize practical information about the farm-based practices of growing rice in the Sene

gal River Valley, as expressed by the farmers themselves. Therefore, this research asked what the most significant constraints to growing rice as perceived by farmers, how *Azolla* can address these challenges, and assess the success potential of *Azolla*-rice farming as an adopted practice by evaluating farmers' perception.

4.2. Materials and Methods

4.2.1. Perceptions before the Azolla-rice trial

We conducted *Azolla*-rice trials in five village sites in the Senegal River Valley: Bari Diam (2 sites physically separated and at 0.1 ha each) (16°10'46N, 16°16'33W), Mboltogne (1 site at 0.2ha) (16°09'19N, 16°17'57W), Ndelle Boye (1 site at 0.2ha) (16°09'59N, 16°17'09W), and Ndiole Maure (1 site at 0.2ha) (16°08'48N,16°18'39 W). Using convenience sampling (Etikan, 2016), each village's chief selected two farmers to participate in the experimental trial. Because participants chose to join the study after negotiating land, labor, and other fees, this created self-selection bias. This possible overestimated the adoptability of the practice or lead to the underreporting of problems or issues. The eight farmers from each village were classified as focal farmers. Each village's chief also selected an additional farmer as a non-focal farmer (i.e., not directly part of the experiment trial). Before the start of the *Azolla*-rice trial, we conducted twelve semi-structured interviews. The interview guide was divided into four main categories: (1) farmer demographics and farm description, (2) farmer motivations, goals, and challenges, and

(3) farm management and innovation, and (4) perceptions of *Azolla* spp. Each interview lasted about 1-2 hours and included addition village visits to collect observational data of each farm's physical characteristics and village member dynamics. All interviews took place in-person, at the rice farmer's home, in March 2022. All interviews were conducted in Wolof and translated to English. All interviews were also Institutional Review Board (IRB00109615) approved.

4.2.2. Azolla-rice trials

We used a randomized complete block design to compare the yield of "Sahel 108" and "Sahel 209" variety rice between four treatments: control plots (using the existing practices of the farmer), *Azolla* plots (using existing practices and adding fresh *Azolla*, i.e., "intercropping"), low input plots (using reduced fertilizers, e.g., 50% reduction of urea), and low input + *Azolla* plots (using reduced fertilizers + *Azolla*) in farmers' plots located in the Senegal River Valley. Plots labelled "*Azolla*" were inoculated with 535 g of fresh *Azolla pinnata* 50 days after seed sowing. The *Azolla* located in these plots were allowed to grow to 100% *Azolla* coverage which took approximately 3 weeks.

4.2.3. Perceptions after the Azolla-rice trial

We conducted twelve semi-structured in-person follow up interviews with rice farmers after the *Azolla*-rice trial experiment in July/August 2022. The interview guide included questions based on the focal vs. non-focal experience and perceptions of *Azolla*-rice farming. We used a grounded theory qualitative approach to analyze the semi-structured interviews (Blesh & Wolf, 2014; Charmaz, 2000; Orne & Bell, 2015; Strauss & Corbin, 1998). After all interviews were recorded, translated, and transcribed, they were coded to identify main themes. We used an iterative coding scheme, until the point of saturation (i.e., no new themes emerged) (Charmaz, 2000; Orne & Bell, 2015). We used a combination of frequency and direct farmer participant quotes (Prokopy, 2011) to highlight the following results.

4.3. Results and Discussion

4.3.1. Farmer demographics and farm description

We interviewed twelve farmers who were geographically located in the five village sites in the Senegal River Valley: two locations in Bari Diam, Mboltogne, Ndelle Boye and Ndiole Maure. All farmers were male between the ages of 22-70 years old. Statistically, rice production is male dominated in this region (Krupnik et al., 2012), whereas in the Casamance region, rice production is female and male led (Sagna & Holmes, 1998). Of our twelve farmer participants, 58% (7/12) of the farmers came from the Wolof tribe, 25% (3/12) from the Maure tribe (3/12), 8% (1/12) from the Serer tribe, and 8% (1/12) from the Walo Walo tribe. Nine farmers (75%) started farming during childhood under the supervision of their fathers and three farmers (25%) who started during their adult years (19, 24, and 36 years old). Traditionally, casual farm labor is carried out by family members (e.g., women and children) (Brosseau et al., 2021). Farmers owned between 0.4-7 hectares of land for farming and used most of the land to grow rice, onion, and tomatoes, as well as other minority crops such as maize, watermelon, peanut, cabbage, okra, eggplant, millet, beans, peanuts, and peppers.

4.3.2. Farmer motivations, goals, and challenges

We found four main themes driving farmers' motivation in becoming rice farmers. Farmers in the Senegal River Valley mostly became rice farmers to continue the family business, as evident in direct farmers' quotes:

"Rice is our natural crop. I grew up and saw elders of the village doing it, so I do the same."

"I found my parents growing rice. Such activities helped the family a lot because it has several benefits."

Farmers were also motivated to become rice farmers because of the financial security. Many farmers cited that by becoming rice farmers, they were able to earn money, feed their animals, family, and community members. Becoming rice farmers meant that they never had to buy rice. We also observed environmental awareness/crop-habitat compatibility as another common theme driving the motivation to become rice farmers. Farmers cited:

"When we came to this new village the soil was clay. So growing cereal is not suitable for this kind of soil. That is why we become rice farmers."

Lastly, we noted that farmers had limited choices in becoming rice farmers as quoted by some farmers:

"The soil belongs to SAED [Société d'Aménagement et d'Exploitation des Terres du Delta du Fleuve Sénégal]. So, they want us to grow rice and other crops."

"Historically an agent from SAED introduced rice farming in the land. They say that he was a white man. This is why we grow rice here in collaboration with SAED which finances us."

Farmer's expressed that they had less autonomy over the land usage and that government extension agencies introduced the practice to the region. This agrees with the modernization of rice irrigation in the Senegal River Valley. Due to droughts in the 1970s, donors from the north and the Senegalese government replaced the flood dependent farming system that supported millet and sorghum production with labor-intensive irrigated rice farming (Koopman, 2009). Though farmers had different reasons for becoming rice farmers, all farmer participants expressed that their main goal in rice farming was to earn money and feed their family.

Farmers shared that it was challenging being a rice farmer in the SRV for various reasons. Reasons included seed viability, inefficient fertilizers and herbicides, high cost of inputs, help purchasing machinery, weed infestation, animal predation, drainage issues, insufficient road infrastructure to the feeds, field slope, and community planning and cooperation (i.e., sowing and irrigation planning).

One farmer summarized:

"We have many challenges about fertilizer, seeds, and herbicides. The problem with herbicide is that they are efficient, and the fertilizer is not a good quality. Years ago, one sack of DAP [a fertilizer] could be enough but now I am obligated to use 2 sacks to have a result."

Another farmer also quoted:

"The main issue is related to drainage. If you have a salted plot and no drainage canal, you will face many problems because rice does not support salinity. The second problem is the road to join fields. This is what happened last year. Water goes over the canal and floods the road. The fields were not joinable. At that time, we were obligated to pay for transport. This problem has been present for years. We told it to SAED but until now, nothing is done."

Another rice farmer elaborated:

"The government should help farmers with machines for plowing and harvest, and a good quality of seed and fertilizer. It also must reduce the price of inputs. If the inputs are low, then our local rice can be competitive with exported rice."

A rice farmer added:

"The main challenge is birds. They come in the field and eat grain at the maturity stage and early on sowing period. At that time rice does not start to germinate and birds can pick it up."

This is concordant with previous studies that identified production constraints. For example, Dingkuhn & Sow (1997) and Wopereis et al. (1998) found that delayed cropping reduced rice yields and was a major challenge for farmers. Additionally, the application, timing and quantity of fertilizer has been identified as a major challenge in previous studies (Becker et al., 2003; Haefele et al., 2000, 2001; Wopereis et al., 1998). Bird damage is also a well-known constraint to rice production in the SRV (Rodenburg et al., 2014). Unleveled fields have also resulted in poor crop establishment, uneven water level, and salinity problems, as reported in previous studies (Poussin, 1997; Sirisena et al., 2010).

In order for farmers to be successful and overcome these challenges, the main theme that arose was needing more support from government extensions (e.g., SAED, ISRA (Institut Sénégalais de Recherches Agricoles), and Africa Rice. A farmer elaborated by sharing their ideas of the supported needed to be successful:

"The government must increase the number of fields, make water available for irrigation and build new roads. They must also establish a factory for rice processing."

4.3.3. Farm management and innovation

With many Senegalese depending on farming activities, a deep understanding of the decision-making processes at the household level is important in understanding how they manage their farms to sustain their livelihoods. Majority of the rice farmers make the decision concerning their rice plots individually. Some farmers cited that they make the decision after discussing with the family members (e.g., sons, wives). Other farmers cited that they make decisions based on what is decided during farmer union meetings or based on what field technicians (i.e., SAED) tell them. All twelve rice farmers shared information regarding their rice fields with relatives and nonrelatives within and outside of their villages. All twelve rice farmers shared labor duties with relatives within their village. Seven out of the twelve rice farmers shared labor duties with nonrelatives within their village, and 5/12 rice farmers shared labor duties with nonrelatives outside of their village. Ten out of twelve rice farmers shared financial resources with relatives and non-relatives within their village, and 9/12 rice farmers shared financial resources with non-relatives outside of their village. Lutz et al. (2017) found that cooperation between farmers fosters shared infrastructure, food production, processing methods, transport and other needs that help optimize local farming and food systems. Therefore, shared resources are ways of empowering local small-scale farmers by giving them control of their own operations where they are key members of the local food supply chain.

In the Senegal River Valley, rice farmers' production management practices are labor intensive and expensive to attain high yields per season. We found cooperation between all community members as a main theme when growing rice. Before each rice season, all farmers have union meetings to discuss the upcoming growing season and what inputs (i.e., seeds, fertilizers, pesticides, etc.) each farmer needs to be successful. Each farmer puts in an order of what they need which is then paid back to the union after harvest. If the union president does not have the inputs, farmers must independently ask the bank or other government extensions like SAED for the inputs which is also paid back after harvest. Though farmers usually get all the inputs they need from the union or the bank, many farmers use previously grown rice grains as seeds for the next season. One farmer explained:

"If SAED does not give it to me, I get it in my own way. I mean a part of the production is stored

and used as a seed for the next season. For the fertilizers I buy it with my own money." Without support from union leaders and a reimbursement system from the bank, many farmers would not have the revenue to grow rice and become rice farmers.

Farmers use a combination of heavy machinery that they must pay for, tools that they sometimes borrow from community members, and manual labor to grow rice. For example, one farmer elaborated:

"The management of the field is related to leveling soil, taking out all impurity and cleaning any kind of things which can destroy rice. Field must have a good level of water and be clean without any impurity. This is the first management thing to do before sowing. Then after sowing I protect the field from birds day and night for 6 days until the beginning of the germination."

Farmers pay close attention when preparing their fields by leveling their plot, manually removing or chemically treating weeds, and applying fertilizers such as DAP and urea several times during the growing season. Because of the initial high investment in the field, farmers make sure to protect their seedlings from predacious night and day birds. We found that birds were a nuisance for farmers as a main theme when managing their fields. One farmer explained:

"To maintain rice farming I fight against night birds. They come at night to pick up rice grains. And the more the plot is full of water the more they pick a lot. They are over 50 and swim together to destroy sown seeds. If you reduce the level of water, the problem can be fixed temporarily. On this day we also have another kind of bird which attacks rice with low levels of water. I fight against that kind of birds too for 8 nights. So after the grain starts germination birds go away because they cannot pick it up."

Treca (1985) reported that late sowing during the wet season encourages bird damage because the maturation stage coincides with the arrival of migrating birds from Europe.

Lastly, we found that farmers will either manually harvest themselves, hire workers, or use machines. Once harvest, farmers will keep sacks of rice for the year, sell a portion, and freely give away rice to relatives and people outside of the village.

4.3.4. Innovation

When asked if farmers have ever changed their field management practices, all the farmers said they have made changes in the past. Some examples include adding different fertilizers, pesticides, and seed varieties. Our analysis shows that farmers are willing to experiment and optimize their management strategies to achieve high yields. One farmer remarked:

"The way of sowing has changed. I used to wet seeds for 24 hours and sometimes you see grains rotting. Then I knew that we did not need to wet the grain for 24h. Just 12h is enough. We made this kind of change. All seeds don't have the same wetting duration. Some can be wetted 24h and less for other varieties. Also, we used to incubate grains in the hole. Now I just put seeds in a sack without putting it in a hole for incubation. This year I used a new pesticide called 'Drameth'. I have never used it before. It kills some weeds such as Backet and Gallamoudou. Reasons why I made those changes are to avoid some destruction in my field and to have a good result in my farming."

When asked if farmers shared their new techniques with other farmers, all twelve farmers shared the new knowledge or technique with other farmers.

"Yes, I do. I taught it to relatives in the village and other people outside the village."

One farmer also mentioned:

"Some people tell me I am not educated, and they do not believe me"

This highlights that although most farmers will share the information to village members and it gets adopted, other times, they are not adopted due to a farmer's education level. Further, another farmer highlighted their experience when sharing new techniques to other farmers:

"They take time to think about the idea before adopting it. Farmers are not the same because sometimes you can tell some things to one and he believes that you want to destroy his farming. Then before using it they look for sure information next to other farmers. When the majority is with that new technology then he adopts it. If not, he rejects it. The other way is to observe what you are doing. So that they can go on if you get a good result."

4.3.5. Knowledge of Azolla

All twelve farmers were shown and asked about their initial experience and perceptions of *Azolla* that was found in their canals. Four farmers mentioned that they see it a lot in the canals in their village, three farmers said *Azolla* inhibits rice germination, three farmers consider *Azolla* as a weed, and two farmers also mentioned they were aware of *Azolla* since our first arrival in 2019. Although all farmers have seen *Azolla* before, none of the farmers have ever added it to their rice fields. When asked if farmers would be willing to trial the *Azolla*-rice project, two farmers stated:

"Yes. Whatever the time or labor, if it gives a good result, I will adopt it."

"Yes. If I can use Azolla cheaper than chemical fertilizer, I will adopt it despite labor or time. All our time we focus on farming."

Despite some negative perceptions of *Azolla* (i.e., considering it a weed), 12/12 farmers said they would experiment with the new technique even if it took more time and labor.

4.3.6. Focal vs. non focal experience and perceptions of Azolla-rice farming

4.3.6.1. Learning and experimenting

Adoption is based on the subjective perception rather than the objective truth (Pannell et al., 2006). Perception is based on three main categories: the process of learning and experience, farmer's social environment, and the characteristics of the practice/innovation. For farmers to make an informed decision regarding a potentially adopted practice, farmers need to be able to collect, integrate, and evaluate new information about the practice (Pannell et al., 2006). We interviewed them before and after the experiment to assess their experience and the potential of adopting *Azolla*-rice. Eight focal farmers participated in the *Azolla*-rice field trial experiment. The results from the field experiment revealed that intercropping *Azolla* showed a modest increase in rice grain yield, while an intercropped *Azolla* replacement for urea also showed a modest increase in yield while substantially reducing 1/3 of input costs. These results suggest that farmers in the Senegal River Valley can use *Azolla* to sustain rice productivity and potentially save 100 USD or 63,000 FCFA in urea cost per season/per hectare by replacing urea with *Azolla*. We interviewed the eight focal farmers after the *Azolla*-rice trials to understand their experience with the practice and how they make decisions about adoption.

All eight focal farmers said that they had a good rice yield this season. When asked if they observed differences in plots with *Azolla*, one farmer said:

"I feel great satisfaction with Azolla. The reason is in my plot I put some Azolla in. And I compared yields between two places with and without Azolla. Then I noticed that the place with Azolla recorded a good yield in comparison with the other place. In the Azolla place I got 0.75 kg/m² and 0.5 kg/m² for the other square. Even though I did the project with you I was also making solely some observations."

Another farmer mentioned:

"If you observe rice plots, rice with Azolla developed more than other rice plots. Even the quantity of seed was low, but Azolla really affected the growth."

When asked what their perceptions of the *Azolla*-rice trials were, 6/8 of the focal farmers had a positive perception. Two farmers said:

"Next season I am going to collect Azolla everywhere it can be because I see how it improves yield. I even told my father to keep a quantity of rice with Azolla inoculation to use it as seed for this coming season. This rice has a heavy panicle charge. I told the president of the union. I said during the meeting that we conducted a trial with an American team on Azolla and rice. We see that we can use Azolla for free and it improves yield more than chemical fertilizer."

Another farmer elaborated:

"This is the first time I use Azolla and I will keep using it. Even if I use chemical fertilizer, I will also add Azolla in the plot. Maybe results you presented are due to the sensitivity of the analysis you have ran but I am convinced that Azolla can give me a good result in the eyes of the farmer."

4.3.6.2. Farmers' social environment influence

Decision making is also characterized as a social process in which the decision maker involves others, such as family, friends, and neighbors in the decision-making process (Pannell et al., 2006). Seven out of eight of the focal farmers said that they would adopt the practice. When asked who they would have to consult in other to adopt the *Azolla*-practice, they said a combination of farmers in the union, farmers adjacent to their fields, and family members. Seven out of eight of the focal farmers shared that they would encourage others within and outside of their village about their experience with *Azolla*-rice farming. Additionally, we interviewed four non-focal farmers before and after the experiment to explore what they thought about the research results and whether they would trial the experiment after hearing the results. All four of the non-focal farmers mentioned that they would trial the *Azolla*-rice practice on their rice fields. This is likely due to farmer membership in union and trust (Lutz et al., 2017). Because a farmer in their village trialed the experiment and had a positive result, they are more likely to also trial the practice. One farmer mentioned:

"Yes. I see that Azolla improves growth, but I wanted to know more with an experiment. The little bit I know I learned from [name omitted] who is part of the project. I would prefer to work with you next time."

We found that farmers are more likely to trial a new practice if people in their trusted social networks have also trialed the experiment and had a positive experience. As perception becomes more positive, adoption is more likely to occur (Pannell et al., 2006).

4.3.6.3. Characteristics of the practice

4.3.6.3.1. Relative advantage

Seven out of eight farmers found the benefits of using *Azolla* in their rice fields. Because of their positive experience, two farmers already started implementing *Azolla* in another plot during the same season that we executed the experiment. The same farmers also have plans to implement for the next season:

"Yes. I said to [name omitted] we must go to the canal and collect all Azolla in. Because of the grain quality and the yield Azolla must be used next season.

These results agree with other cases on sustainable practices adoption showing that engaging farmers in the research process is critical for the success and expansion of novel agricultural practices (Dolinska et al., 2020; Hauser et al., 2016; Jowett et al., 2022).

4.3.6.3.2. Trialability

Agricultural practices that are relatively complex and difficult to trial, reduces the practice's relative advantage for the farmer (Pannell et al., 2006). In general, incorporating *Azolla* into a rice plot does not require a major time, labor, or financial investment. However, excluding *Azolla* from places where it may not be desired, e.g., drainage canals or animal watering sites, can be labor intensive. Farmer participants in the trial experienced this difficulty. Because we wanted to observe the yield difference between plots with vs. without *Azolla*, we set up $12m^2$ plots which were small and hard to manage because *Azolla* proliferates and can spill over to neighboring plots. To avoid that, farmers had to manually remove *Azolla* from non-*Azolla* plots. This challenge greatly increased the risk of technical failure, and required additional effort and time from farmers that took away from their learning experience. Farmers who saw the benefits of *Azolla*, stated:

"One problem with Azolla is the difficulty to contain it in place. It is very easy to move. If I knew it, I would suggest using a net in the irrigation hole in order to block it in the plot."

"The challenge is to control the growth of Azolla. That is why you have to be determined and committed to succeed."

"I did not see an issue in using Azolla. The problem regarding Azolla escaping from the plot was caused by the fact that plots were not well leveled."

"Azolla needs a lot of water. I had to keep the plots wet all the time."

In Bari Diam, rice yields were lower than the other three villages. Two farmers described their experience as:

"I do not see a difference because they are similar for me. But in terms of yield, Azolla is better than chemical fertilizer."

"If I focus on the results there are some parameters where Azolla is not good for. In those cases, I am not convinced to use it because we wish to have advantages such as grain weight, panicle weight etc. Thus, I would prefer to use chemical fertilizer."

One farmer from Bari Diam said they would not adopt the practice. Reasons for the negative perception could be due to the characteristics of the practice. Pannell et al. (2006) states that perception is driven by two categories of the characteristics of a farming practice: its relative advantage and its trialability. After sharing the results of yield on the experiment, the farmers at Bari Diam determined that there was not a significant difference when using *Azolla*. As noted above, trialability (i.e., the feasibility of testing the innovation) was decreased because farmers had to actively remove *Azolla* that were in non-*Azolla* plots. Ideally, if this practice was carried out on a larger scale, farmers would just have to inoculate their entire field once in the growing season and far less effort would be required to contain the floating plant. Although, this innovation was complex by design, it was as easy as running trials to test new insecticides or fertilizers. The *Azolla*-rice trial was also relatively easier to trial than paying high upfront costs in a rice processing facility, as suggested by one farmer. However, farmers still had to use excessive effort to maintain their *Azolla* plots which took away from the experience.

4.4. Conclusion

This assessment does not attempt to encourage uptake, but to attempt to better understand farmer's priorities when growing rice and their experiences and perceptions of *Azolla*-rice farming before and after a small-scale experiment on their plots. The aim of this study was to begin filling the gap in research on the agronomic and economic advantage of incorporating *Azolla* into their rice fields. Senegalese farmers' experiences are key in investigating the potential of *Azolla*-rice farming as a sustainable practice. We found that Senegalese farmers are motivated by their environmental awareness, the need to continue the family business, have financial security, and continue the practice because of limited choice. Their main farming challenges included the cost of fertilizers, poor seed quality, salinity, and bird attacks. We also found that farmers work together but make their own decision concerning their farmland with consultations from family and members in their farmer union. Farmers are likely to experiment on their farm to achieve the best result and yield. Lastly, we found that adoption is heavily influenced by trialing out experiments, farmer's social environment, and the characteristics of the trialed practice. Future studies should continue incorporating Senegalese rice growers' experiences and perspectives in other to promote dialogue between rice growers and researchers.

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Chapter 5: Conclusions and future directions

5.1. Future Directions and Broader Implications

In this dissertation, I contributed to our understanding of assessing the sustainability of a new agricultural practice, *Azolla*-rice farming, in Saint-Louis, Senegal. To address this farming challenge, I applied an innovative mixed-methods approach leveraged by the social-ecological systems (SES) framework to explore the social and ecological drivers of *Azolla*-rice farming and its potential as a sustainable practice based on a case study in Saint-Louis, Senegal. I addressed the following three questions:

1) How does temperature as an environmental factor affect the relative growth rate of *Azolla pinnata* in Africa currently and in the year 2050?
2) How does intercropping *Azolla* affect rice productivity in the Senegal River Valley?
3) What are rice farmers' current challenges and perceptions of *Azolla*-rice farming?

The results provide useful insights to anticipate the presence and productivity of *A. pinnata* and *A. filiculoides* in Africa for the introduction of *Azolla*-rice farming as a sustainable agricultural practice under current and future climate change. As the earth's temperature continues to rise and affect crop productivity, more robust strategies and agricultural practices need to be used to combat climate change and feed the world. I showed promising and positive effects of adding *Azolla* as a free organic fertilizer in Senegalese rice farmers' fields and when urea was replaced. Future studies should increase the scales of the intervention. For example, we should consider replicating this project over a longer period and on the same plots in order to see how *Azolla* could change the soil's nutrient profile. Replicating this experiment in different villages could also show insight on how other farmers respond to *Azolla*-rice farming, determine if local factors, e.g., soil or irrigation characteristics, influence the relative success of the farming practice, and test other important social variables within the SES framework. Moreover, it would be important to help build connections between government agencies such as ISRA, who have conducted *Azolla*-rice studies in the lab, with farmers. ISRA could provide technical support to farmers who are interested in incorporating *Azolla* in their fields. Future studies should also continue increasing farmer's input in the design of these experiments and consider alternative ways of assessing the success of the project. Including the perceptions of women, who mostly support rice farmers during the harvest, should also be a next step in determining if there is a difference in *Azolla*-rice uptake between sexes. Lastly, I found that Senegalese farmers' experiences are key in investigating the potential of *Azolla*-rice farming as a sustainable practice. Though rice growers are met with many challenges when growing rice, they are optimistic about new practices that can help improve their rice yields. Future studies should consider collaborating with local government agencies that can better support rice farmers who chose to adopt the *Azolla*-rice farming practice. This dissertation strives to better understand how to build a more sustainable world by dissecting key interactions between humanity and nature.

In this dissertation, I emphasized the importance of participatory research methods. More research that involves people in their environment should consider their voices and opinions in the creation of research projects. Assessing the sustainability or adoption of new agricultural practices should first start with identifying the community's priorities and then working with key members to address their concerns on the ground. Though many sustainability projects can be simulated in the greenhouse at a US academic institution, the results will not be as widely accepted by the communities impacted. Researchers need to be able to build rapport with community members and be able to conduct longer term projects to respond to conditions on the ground. Moreover, many community members want to be able to conduct experiments with researchers so that they can understand all facets of the project and assess the results themselves.

The field of social-ecological systems research is still relatively new and there are efforts to improve the utility of the framework. However, it is difficult to do so because of the lack of standard methodologies strictly for social-ecological systems research. This dissertation was inspired by the framework and evolved into using methods in social sciences and ecology/environmental sciences to investigate a potential adoptable practice. The few published social-ecological systems studies have explored only one dimension of the framework. Here, I integrate both dimensions by using techniques from both fields. This dissertation demonstrates how to do challenging research that addresses social-ecological issues and builds on the framework of working with farmers to create mutual goals that improves their daily lives.