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Review article

An overview of underutilized benefits derived from *Azolla* as a promising biofertilizer in lowland rice production

Said H. Marzouk [a,*], Hamis J. Tindwa [b], Nyambilila A. Amuri [b], Johnson M. Semoka [b]

[a] *Ministry of Education and Vocational Training, Zanzibar, Tanzania*
[b] *Department of Soil and Geological Science, Sokoine University of Agriculture, Tanzania*

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ABSTRACT

Currently, there is no doubt that *Azolla* can compensate for the nitrogen requirements of rice in different agroecological zones. Compared to synthetic N-fertilizers, Azolla has various positive impacts on lowland rice production, including improving soil fertility, minimizing weeds, increasing soil organic carbon, improving microbial biomass, and thus nutrient cycling and enhancing rice growth and yield. However, *Azolla* has not been accepted globally by rice farmers for field use and so far, farmers are relying on increasing rates of synthetic N fertilizers instead of taking advantage of *Azolla* which will improve long-term soil fertility and health. This systematic literature review and scientific evidence could help policymakers, scientists and researchers to understand the benefits, limitations, and innovative ways of utilizing *Azolla* as a cost-effective and eco-friendly amendment in rice production. The paper uses Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) method to review the hidden potential of *Azolla* as a biofertilizer in paddy and summarizes its benefits and problems by collecting information from different sources and presenting under different subheadings such as critical factors affecting *Azolla* growth and nitrogen fixation, nitrogen fixation of *Anabaena Azollae* and their contribution in soil health, release and availability of *Azolla*-N to rice, amounts and time of *Azolla* inoculation, influence of *Azolla* on ammonia volatilization, contribution of *Azolla* to yield and yield components of rice, and impact of *Azolla* on weed emergence in rice cropping system. Literature indicated that the use of *Azolla* as green manure incorporated before rice transplanting or grown together with rice and left until a few days of harvest alone or in combinations with other synthetic fertilizers in the lowland rice production saved the nitrogen requirement of rice up to 60 kg N ha^{-1}, it enhances the availability of nutrients, improves physiochemical properties of soils, minimizes soil salinity, reduces the soil pH, and minimize weed germination. However, it was observed that incorporating *Azolla* as green manure is labor-intensive, and maintaining the *Azolla* inocula and phosphorous requirement are major restrictions for farmers. Therefore, understanding mechanism of spore production, educating farmers on cheaper alternative ways of *Azolla* application, and testing different species of *Azolla* over different agroecological zones will help in maintaining *Azolla* biomass and applying it at low cost for further environmental conservation.

* Corresponding author.
E-mail address: binmarzouk@gmail.com (S.H. Marzouk).

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1. Introduction

The global trend of synthetic N-fertilizer consumption is expected to increase by 1.7 times in 2050 [1,2]. However, excessive use of chemical N-fertilizers leads to an increase in soil acidification [3,4], nutrient imbalance, and loss of organic matter [5,6], reduces soil cation exchange capacity (CEC), enhancing salt accumulation and a slightly low increase in crop production with further ecological damage if agronomic efficiency of applied N-fertilizer is not significantly improved [7]. Accordingly, the cost of purchasing fertilizers is estimated to increase in the next 40 years [8]. Thus, biological nitrogen fixation is an effective ecological strategy to improve N-use efficiency in lowland rice production systems [9]. For example, replacing synthetic fertilizers with *Azolla* in lowland rice production is beneficial to the rice crop and may increase rice growth, yield, and N-use efficiency [8].

Azolla is commonly known as duckweed, mosquito fern, or fairy moss and it belongs to a genus of aquatic ferns, mainly found in tropical regions. It is often distributed in ponds, canals, ditches, and rice fields. There are seven known species of Azolla: *A. caroliniana*, *A. filiculoides*, *A. microphylla*, *A. pinnata*, *A. Mexicana*, *A. rubra*, and *A. nilotica*. However, *A. pinnata* is the most common species found in tropical and sub-tropical ecosystems [10]. The symbiotic relationship between *Azolla* and Cyanobacteria is known as *Anabaena Azollae*, which provides resource complementarities between the two parts and the associate plants [11,12]. In this symbiosis, Cyanobacteria get shade beside *Azolla* fronds, and in turn, *Azolla* benefits from the nitrogen (N) fixed by bacteria. The *Azolla*-Cyanobacteria symbiosis hereinafter referred to simply as "Azolla" has the ability to accumulate high amount of fixed nitrogen which makes it a potential biofertilizer for a variety of crops, including rice (*Oryza sativa* L.) [13], soybeans (*Glycine max*) [14], wheat (*T. aestivum*) (1998; [11,15, 93], and banana (*Musa* sp.) [12]. *Azolla* biofertilizer extract is also used as foliar fertilizer in vegetables such as tomatoes [16] and cereal such as Maize (*Zea mays*) [17] and Roselle (*Hibiscus sabdariffa*) [18]. However, rice is a suitable crop for *Azolla* (*Oryza sativa* L.) application since both prefer flooded habitats [13]. *Azolla* as green manure and feed for animals has been adopted in many countries such as China [8], India [19], Bangladesh [20], Vietnam [21], Tanzania (Mvukiye and Msumali, 2000), Niger [22] and Kenya [23] as an important biofertilizer for improving N balance in rice fields. Previous literature shows that the most documented species of *Azolla* used as biofertilizers are *A. pinnata, A. filiculoides*, and *A. caroliniana* respectively, and they demonstrate global importance (see Table 1 [5,9,20,22,24–29]).

In paddy fields, *Azolla* is grown as monocrop or as an intercrop with rice. Mono-cropping is done before rice cultivation, while intercropping is practiced by growing *Azolla* together with rice followed by incorporation into the soil or harvesting when required elsewhere [10]. It is inoculated in the farm soon after rice transplanting and either incorporated or allowed to die naturally and decompose [30]. Incorporation of *Azolla* in the soil either before or after transplanting is better for improving soil fertility and minimizing the need for synthetic fertilizer [31,32]. Similarly, significant yield increase due to *Azolla* has been reported by many authors [8,22,33]. Unlike other biofertilizers, *Azolla* was reported to have vast other benefits in paddy fields including recovering a variety of both macro and micronutrients like Ca^{2+}, Mg^{2+}, P, Fe, S and K^{+} to a greater extent than rice alone does [34,35]. Another potential significance is lowering flooded water pH and temperature and hence reducing NH_3-volatilization in flooded habitats [36]. However, global utilization of *Azolla* is still lacking social, economical, and scientific support which has led farmers to lose confidence in the technology and end up on relying more on using chemical fertilizers. Therefore, we have highlighted the current status, gaps, limitations, and way forward of using *Azolla* in paddy production.

2. Methods deployed in literature search

This review explores overall information on *Azolla* as a beneficial amendment in the rice cropping system based on the information collected through the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) followed by other researchers (e. g., Refs. [37,38]). The PRISMA method provides checklists of important items to be covered and ensures adequate coverage of the literature. We developed a search strategy to identify relevant literature that was within the scope of the study. The information was collected from three databases: Scopus, Pubmed, and worldwide science. The search terms used were *Azolla* in rice production, factors affecting *Azolla* growth, nitrogen fixed by Azolla, impacts of *Azolla* in weeds, and constraints of using *Azolla*. The search spanned for database until 2022, including journal articles, review articles, and research reports published in English. Screening of articles was done to maintain the quality of the review, and all duplications were excluded. Articles included were selected by checking the abstracts and results that contained variables focusing on the search titles, and valid experimental design and statistical analysis (Fig. 1 [37,38]).

Table 1
Global distribution and uses of *Azolla* sp. in rice fields.

Azolla species	Distributions	References
Azolla pinnata	Bangladesh, China, India, Indonesia, Japan, Korea, Malaysia, Niger, Pakistan, Philippines, Sri Lanka, Taiwan, Thailand, Vietnam, Tanzania	[20,22,24, 25]
Azolla filiculoides	Iran, China, USA, Canada, Sab-Sahara Africa and Japan	[5,26–29]
Azolla caroliniana	Eastern parts of America, Wisconsin, Mexico, Uruguay, Argentina, Canton	[9]

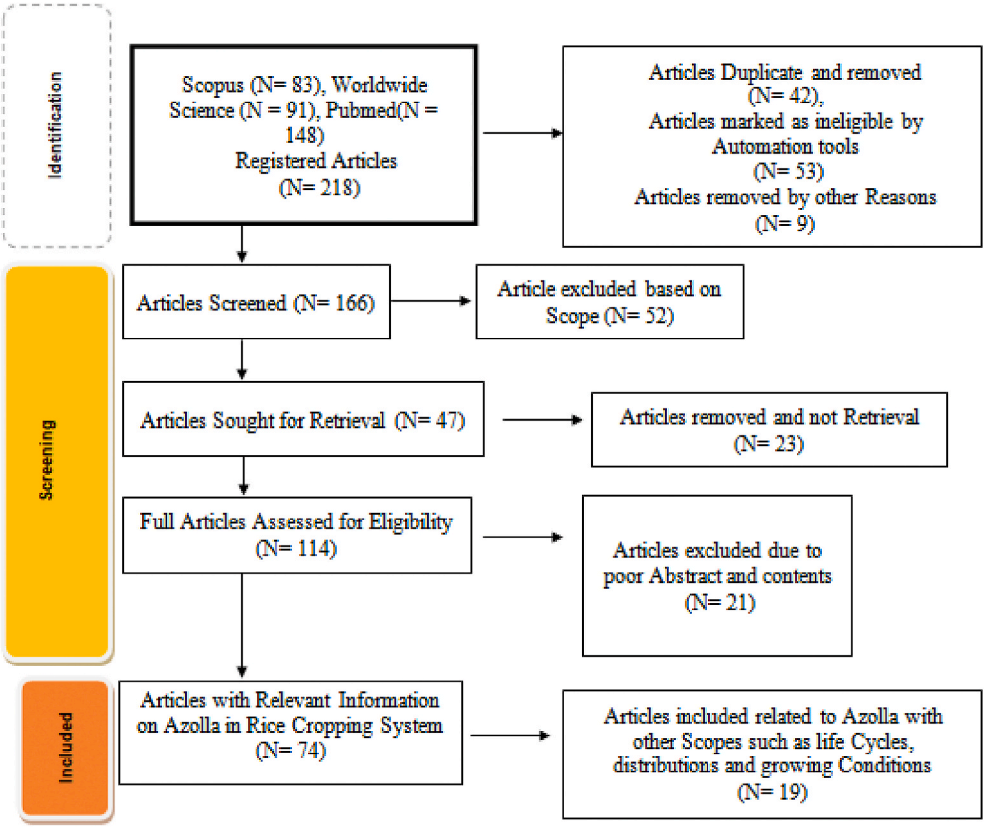

Fig. 1. Representation of literature search based on the PRISMA flow diagram, modified from other researchers [37,38].

Table 2
Some literature that recorded the growth conditions of different species of Azolla.

Summary	Growth factors						References
	Light intensity	Temperature	pH	Applied N	Applied P	Humidity	
Effect of photoperiod on the growth of *A. pinnata*	Artificial light; 7500 l×	Maintained at 20–30 °C	Not controlled	Urea	Absent	Not measured	[41]
Effect of temperature on Azolla growth	Artificial light 30 Klux	Maintained at 18–33 °C	Not controlled	Urea	Absent	Greenhouse conditions 75%	[29]
Factors Affecting Nitrogen Fixation of *A. caroliniana*	Controlled 100, 67, 43, 27, 10, 3.0 and 1.7% full solar irradiance	Maintained from 0 °C to 45 °C	5.0 and 6.0	KNO_3, NH_4Cl, and urea	Absent	Not measured	[42]
Growth analysis of *A. pinnata*	Natural light; not Quantified	21–28 °C	adjusted to 6.0	Nitrogen-free Hoagland's solution	KH_2PO_4	Greenhouse conditions 60%–70%	[43]
Phytoremediation of dairy wastewater using *A. pinnata*	Artificial light; 2000 lx	20 °C	Adjusted to 7.0	TN 4.13 mg/L)	TP 2.5 mg/L	55–70%	[44]
Effects of temperature on growth of *A. pinnata*	Natural light; not Quantified	18–38 °C	7.8	Farmyard manure	Rock phosphate	Field condition 85.71–90.00%	[45]
Influence of light and pH on the growth of temperate Azolla	82 Klux	17–27 °C	4–10			Not measured	[46]
Growth and essential nutrient requirements of *A. pinnata* and *A. filiculoides*	Natural light 30–70%	20–25 °C	Adjusted 4 to 8	NH_4NO_3	40 mg H_2PO-P	Not measured 44	[47]

3. Findings of the review

3.1. Critical factors affecting Azolla growth and nutrient composition

Understanding optimum conditions for *Azolla* growth and nitrogen fixation are vital for maximizing biomass and activity of nitrogenase in lowland rice production. In this review, some structural habitat, physical and chemical factors were reported to meet the habitat necessities of the fern. Through its name, the word *Azolla* derived from two Greek words, *azo*, and *ollyo*, which stand for killed by drought. In this view, *Azolla* growth requires flooded conditions throughout its life cycle [9]. *Azolla* grows well in free-floating water from 5 to 12 cm [39]. On the other hand, excessive water in the growing medium cause *Azolla* to migrate from the growing medium, and water fluctuation in the early-stage growth of rice causes *Azolla* to attack the rice and eventually rot and die [40]. *Azolla* growth is rapid in the first two weeks and thereafter growth tends to decline as the plant reaches maturity [29].

The conditions of *Azolla* growth such as light (type and intensity), presence of nitrogen (N), phosphorous (P), effects of pH, and humidity levels are presented in Table 2 [29,41–47]. Light (both natural and artificial) is reported in the literature, but many studies report the growth of *Azolla* under natural light. Light intensity affects *Azolla* growth, photosynthetic activity, nitrogen fixation, and Azolla-Cyanobacteria symbiosis [48]. The intensity of light also regulates the interaction of photoperiod, temperature, asexual reproduction and other factors such as N, P-supply and pH [10]. The optimum light intensity for the growth of *Azolla* is 20,000 lux [44]. Azolla growth decreases quickly under shaded conditions (below 1500 lux) [49].

Temperature is one of the main factors affecting Azolla growth and nitrogen fixation [29]. There have been many studies about the difference in temperature on *Azolla* species that indicate that high temperature (above 30 °C) or low (below 4 °C) inhibits *Azolla* growth. The optimum temperature for *Azolla* growth is between 18 and 28 °C [48]. Additionally, nitrogen fixation and the activity of Nitrogenase enzyme decline from 35 to 40 °C [42]. However, different species of *Azolla* have different ranges of temperature sensitivities. As regards relative humidity many authors have reported the range of 70–75% to be optimal for *Azolla* growth (e.g., Refs. [43,49]).

The effects of pH on *Azolla* growth and nitrogen fixation depend on other factors such as temperature, essential nutrients and light intensity [46]. The optimum growth of *Azolla* occurs at pH range from 4.5 to 7.5 (e.g. Ref. [46]). However, the ideal pH for *Azolla* growth is wide ranging from 3.5 to 10.0 provided that all essential nutrients are adequate [47]. This finding suggests that despite pH dependency of *Azolla,* its growth is also affected by other environmental and physiochemical factors.

Like other photoautotrophic aquatic organisms, phosphorous (P) is an important nutrient required for Azolla growth [47]. The effect of Phosphorous (P) on growth of *Azolla* is widely reported [43,45,48]. It was noted that, if adequate P is applied to the growing medium, *Azolla* will be able to grow without nitrogen fertilizer [49]. When enough P is applied in the growing medium, *Azolla* could multiply 5 to 7 times after inoculation until it becomes P-deficient [45]. Phosphorus deficiency results in the formation of reddish-brown colouration spread from the centre of the fronds and results in root death [50]. A high P concentration (above 0.1 mg kg^{-1}) is needed for proper growth and N fixation of *Azolla* [51]. In paddy fields, P is applied as superphosphate (TSP) or diammonium phosphate (DAP) 10–15 kg ha^{-1} in split doses at an interval of 4 days, or as a basal application of 30 kg P ha^{-1} [51]. Phosphorous application is directly correlated with nitrogen contents in *Azolla* dry matter (Fig. 2 [51]). Split application of P enabled *Azolla* to maintain N higher than 3%, and when P content in *Azolla* biomass decreased, the N content also decreased [51].

Other essential nutrients for *Azolla* growth and stimulation of nitrogenase activity include potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe) [52]. The thresholds levels of macronutrients nutrients (P, K^{+}, Mg^{2+}, and Ca^{2+}) and micronutrients (Mn, Zn, Fe, Co, Cu, Mo, and B) for Azolla growth and activity of nitrogenase range from 0.03 to 0.5 mmol/L and 0.3–30 μg/L respectively [53]. Below these values, the decrease in growth was very sharp with Ca-deficiency. The effects of Mo, Fe, and S on nodulation and nitrogen fixation of legumes are well documented (e.g., Ref. [54]). However, studies that report the effects of these nutrients (Mo, Fe, and S) on *Azolla* growth and nitrogen fixation are rarely documented.

The performance of *Azolla* under salinity stress is also widely documented [55,56]. Arora and Singh [57] compared six different

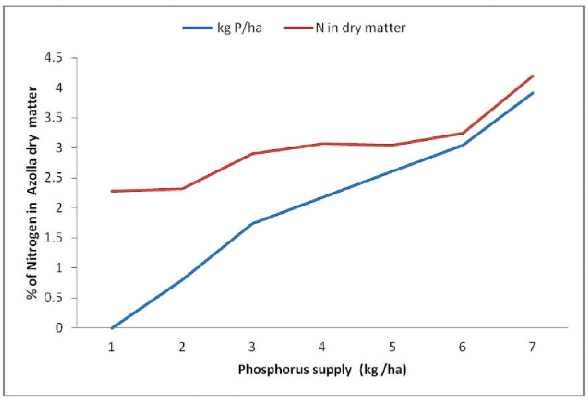

Fig. 2. Relationship between P supply and N contents in *Azolla* dry matter, data adopted from [51].

species of Azolla in a 0.32% sodium chloride medium and noted that *A. microphylla* produces significant biomass of 43% (4.8 g fresh wt) compared to other species and *A. pinnata* did not show any pattern of survival in saline conditions (Fig. 2). Additionally, there is no significant difference in biomass production and doubling times of *A. mexicana, A. rubra, A. flculoides* and *A. caroliniana*. The growth and biomass dry weight of *A. pinnata* and *A. filiculoides* were decreased when the concentration of sodium chloride increased in floodwater [58]. Although, *Azolla* is relatively sensitive to salt, when used in paddy fields in a saline environment for two consecutive years it decreased salt content from 0.35 to 0.15, and desalination rate of 71.4% which was 1.8 times faster than through leaching; also minimizes soil pH, electrical conductivity, and increased Ca^{2+} in soil [10]. The study by Ref. [55] investigates the responses of antioxidant enzyme status of *Azolla microphylla* in saline conditions and observed that salinity exposure decreases growth and nitrogenase activity of the fern. However, there is an increase in peroxide content and activity of the antioxidant enzyme with salinity and thus, *A. microphylla* could be used as a biological source of minimizing toxic effects of salinity.

Compared with other nutrients, reports on nitrogen requirements for *Azolla* growth vary. Lumpkin and Plucknett [59] suggested that nitrogen is not considered a limiting factor for *Azolla* growth and N-fixation of *Azolla*, and if added to the growing medium increases competition with other organisms whose growth is stimulated by nitrogen. Setiawati et al. [9] however, observed that only high N levels can hinder *Azolla* growth and N-fixation. At low N level, (below 30 kg N ha^{-1}) *Azolla* growth is not affected and is reduced when the N level increases to 60 kg N ha^{-1} due to increasing nitrogen concentration in *Azolla* tissue. *Azolla* is an aquatic plant that absorbs nutrients through roots and fronds, when NH_4^+ and NO_3^- are present in the water source, *Azolla* absorbs N in form of NH_4^+ rather than NO_3^-, and the presence of N in the water sources enables *Azolla* to take up N rather than fixing [60]. Similar observation was made by Ref. [42] demonstrating that *Azolla* is sensitive to higher NH_4^+ deficiency than NO_3^-, as NH_4^+ may prevent the growth of *Azolla*. Additionally, high NO_3^- concentration (greater than 10.0 mg N/L) reduces the activity of nitrogenase.

DAT = day after transplanting, DBT = day before transplanting, Dual inoculation = inoculating *Azolla* 2 times after rice transplanting, Dual incorporation = incorporating *Azolla* 2 times after rice transplanting between the rows, Basal inoculation = inoculating *Azolla* before rice transplanting, Basal incorporation = incorporating *Azolla* before rice transplanting.

Many researchers do not report all the necessary information about *Azolla* coming from their studies (Table 3 [7,9,13,23,31–33,61–65]). Available literature does not exhaustively quantify amounts of nitrogen contents in *Azolla* dry matter, and it does not estimate nitrogen fixed by *Azolla*. This information would be important as it can enable readers to understand nitrogen supply capacity of *Azolla* and other synthetic fertilizers.

Table 3
Description of some studies that report the use of *Azolla* in lowland rice production.

Azolla species used	Amount of *Azolla* inoculated	N content (% in DM)	Time of *Azolla* application	Inorganic N	Estimate N-fixed	References
Azolla filiculoides	12.2 t dry matter ha^{-1}	4.0	Basal (1 day before transplanting)	Applied N with biochar	33.80 kg N/ha	[31,32]
Azolla filiculoides	20 t ha^{-1}	Not quantified	Applied at 10 DAT	330 kg N ha^{-1} reduced to 15% + *Azolla*	120 kg N ha^{-1}	[7]
Azolla filiculoides	Applied at 1.5, 3.5 and 5 t DM ha^{-1}	3.17	Applied at 7 DAT	165 kg N ha^{-1}, reduced to 50% + Azolla	Not quantified	[33]
Azolla pinnata	Fresh Azolla at 10 and 20 t ha^{-1} while powder applied at 2.5 and 5 t ha^{-1}	Not quantified	Fresh *Azolla* applied at 8 DAT, and powder applied with half recommended N rate at 10 DAT	Half recommended 100 kg ha^{-1}	40 kg N ha^{-1}	[9]
Azolla filiculoides	2 t ha^{-1}	2.0	Dual at 25 and 40 DAT	80 kg N t ha^{-1}, 40 kg N t ha^{-1}	Not identified	[61]
Azolla pinnata	16.5–17.5 t fresh weight ha^{-1} during the wet and 21.0–22.0 t ha^{-1} during the dry season	4.4 and 3.5% N, wet and dry seasons respectively.	Basal (15 DBT) and dual (10 and 30 DAT)	15 and 30 kg N t ha^{-1}	fixed 52.5–55.1 kg N ha^{-1} during wet and dry seasons respectively	[62,63]
Azolla pinnata and *Azolla filiculoides*	2 t ha^{-1}	Not identified	Basal (1 DBT)	30, 60 and 90 kg N ha^{-1}	Not identified	[13]
Azolla pinnata	7.5 t ha^{-1}	4.0	*Azolla* (applied 7.5 t ha^{-1}) 1 DBT compared with 7.5 t ha^{-1} at 21 DAT	*Azolla* applied with half recommended rate of N (30 kg N ha^{-1})	Not identified	[23]
Azolla pinnata	Fresh Azolla 300 kg ha^{-1}		Either incorporated at 40 DAT or used un-incorporated	Not applied	Not quantified	[64]
A. filiculoides	50 g fresh wt ha^{-1} equivalent to 22.8 kg dry wt ha^{-1}	3.85	Incorporated at 46 DAT	Half and full recommended rate (90 and 180 kg N ha^{-1})	40 kg N ha^{-1}	[65]

3.2. Nitrogen fixation of Anabaena azollae and their contribution to soil health

The symbiotic association of *Anabaena azollae* plays an important role in fulfilling nitrogen requirements in lowland rice production [39]. This association has gained attention, as it may represent a feasible alternative to industrial nitrogenous fertilizers. The symbiont occurs in un-branched filamentous cells which consist of vegetative and heterocyst cells. Larger egg-shaped heterocysts cells specialize in fixing atmospheric nitrogen while more numerous smaller vegetative cells harvest sunlight and photosynthesize [66]. *Azolla* provides symbiotic organs that in nature are obligatorily infected by filamentous cyanobacteria held within a mucilaginous sheath. *Azolla* plants supply cyanobiont with fixed carbon, while cyanobiont in return supplies plants with N needed via N fixation [40]. *Azolla* can double its weight every 3–5 days and fix atmospheric N_2 at a rate exceeding legumes under suitable field conditions [26]. The nitrogen-fixing capacity of *Azolla* has been estimated to be 1.1 kg N per hectare per day which is sufficient to meet entire N requirement of rice [59]. *Azolla* is of great agronomic value for rice crops where it is used as dual crop with rice and contributes 40–60 kg N ha^{-1} per rice crop [19]. Studies indicate that inoculating *Azolla* 16.5–17.5-ton fresh weight ha^{-1} fixes up to 52.5 and 55.1 kg N ha^{-1} [63]. Similarly, the incorporation of 12.2 t dry matter ha^{-1} adds up to 33.80 kg N ha^{-1} [31,32] (Table 3). It was observed that nitrogen fixation depends, among others, on climate, nutrients in floodwater, *Azolla* species, management and stages of rice growth [34,62,63]. Table 4 [67,68], shows the estimated nutrient composition in *Azolla* biomass.

Nitrogen and potassium contents of *Azolla* biomass ranged from 3 to 5% and 3–6% on dry-weight bases, respectively, which are higher compared to other green manures [69]. It was also reported that *Azolla* has various positive long-term effects such as improving soil organic matter, increasing total N, P, K^+, Ca^{2+}, and Mg^{2+} [70], increasing activity of soil urease and phosphatase [19], and other biological properties [13]. *Azolla* decomposes easily and forms humus that increases water-holding capacity, improves soil porosity (3.7–4.2%), and decreases specific gravity [71]. This indicated that *Azolla* incorporation is most effective for improving microbial activity thereby enhancing nutrient cycling and thus increasing soil fertility [5,72].

3.3. Amount and time of Azolla inoculation

Azolla is inoculated and incorporated before or after rice transplanting [23]. The amount of *Azolla* inoculums required varies from 300 to 500 kg fresh biomass ha^{-1} [64] to 20 t fresh biomass ha^{-1} [7]. The most notable recommended rate of *Azolla* inoculation is between 1.5 and 3 t fresh biomass ha^{-1} as reported by other researchers [9,13,33,61], which can cover the field within 15–20 days after inoculation and yield up to 40 kg N ha^{-1} [9]. Studies indicated that *Azolla* is either incorporated before rice transplanting (basal incorporation) [13,31,32], or after rice transplanting between the rows [7,33,65]. However, many studies have adopted both dual and basal incorporations [9,23]. The study by Oyange et al. [23] incorporated Azolla at the time of transplanting and 21 DAT and observed that Azolla incorporation 1 day before rice transplanting significantly increase spikelet per panicle and grain weight. Nyalemegbe et al. [73] reported that a single application of fresh *Azolla* at 2 t ha^{-1} basal or dual *Azolla* between 20 and 25 days yields 30–40 kg N ha^{-1}. This is estimated to be 1.1 kg N ha^{-1} day^{-1}, and a range of 0.4–3.4 kg N ha^{-1} day^{-1}. It was also reported that growing *Azolla* twice and incorporating at 35 and 50 days after transplanting produces biomass of 26–39 t ha^{-1} which fixes 44–61 kg N ha^{-1} and when grown as basal DBT and dual after transplanting can fix 76–94 kg N ha^{-1} [74]. A higher rate attains full cover after a short period and therefore it is recommended that *Azolla* be applied 7 DAT to avoid rice damage. However, at low density, *Azolla* may be outcompeted by other plants such as algae and weeds [13].

Literature indicates that some constraints such as maintaining *Azolla* biomass, insect damage, constantly flooded water, phosphorous requirements and labor intensity hinder the utilization of *Azolla* in lowland rice production [75]. Based on the literature it was observed that the incorporation of *Azolla* is only based on biomass and not the actual amount of nitrogen within the biomass. Also, the time of incorporation varies with researchers, which creates the question that does *Azolla* dry weight N remains constant with time or varies. Considering this, upcoming research has to focus on evaluating how N contents in *Azolla* biomass change with time, and at which time *Azolla* biomass retains maximum fixed N. This information will provide a clear view of the right time for *Azolla* to be inoculated or incorporated into the soils.

3.4. Release and availability of Azolla-N to rice crop

Azolla had been used as manure in lowland rice production for centuries [24]. *Anabaena azollae* present in the leaf cavity of Azolla fixes atmospheric nitrogen that becomes abundant in *Azolla* tissue [13]. The *Azolla*-N becomes available to rice when either incorporated as green manure or leached into the soil from standing water (Fig. 3 [57]) [76]. The amount of N fixed in the field depends among others on climate, nutrients, and *Azolla* species [62,63]. Approximately 56–75% of *Azolla*-N is released after 3–6 weeks or 6–8 weeks of inoculation, respectively [30,51]. The C:N ratio is the main indicator of its quick decomposition [77]. *Azolla* decomposition is high at C: N ratio of 10. It was noted that nitrogen mineralization from dried Azolla is lower than from fresh *Azolla* [39]. The rate of increasing biomass is 2.5 kg N ha^{-1} day^{-1} between 15 and 25 days after inoculation, and only 3–4% of the total nitrogen fixed by *Azolla*

Table 4
Estimate nutrients composition in Azolla dry matter.

Nutrients	N	P	K	Crude protein	Ca	Mg	Mn	Fe
Percentage (%)	4–6	0.5–0.9	2–6	14.0–30	0.4–1.0	0.5–0.65	0.11–0.16	0.06–016

Source [67,68].

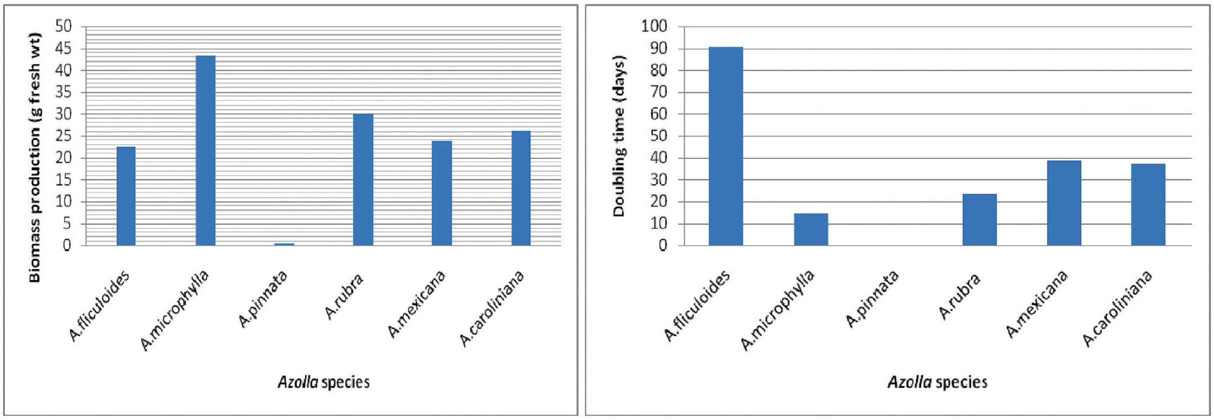

Fig. 3. Doubling time and biomass production of six selected species of Azolla in saline conditions. Data adopted from [57].

is excreted into the water medium during its growth. Most of the nitrogen fixed becomes available to rice only after the *Azolla* decomposition (Fig. 4) [65].

3.5. Influence of Azolla on ammonia volatilization

Nitrogen is one of the most important nutrients in rice growth and production [9]. However, nitrogen use efficiency (NUE) in lowland rice production is low (30%) due to N-loss (20 to 80%) through NH_3-volatilization [1,8]. This loss is ascribed to high pH of the

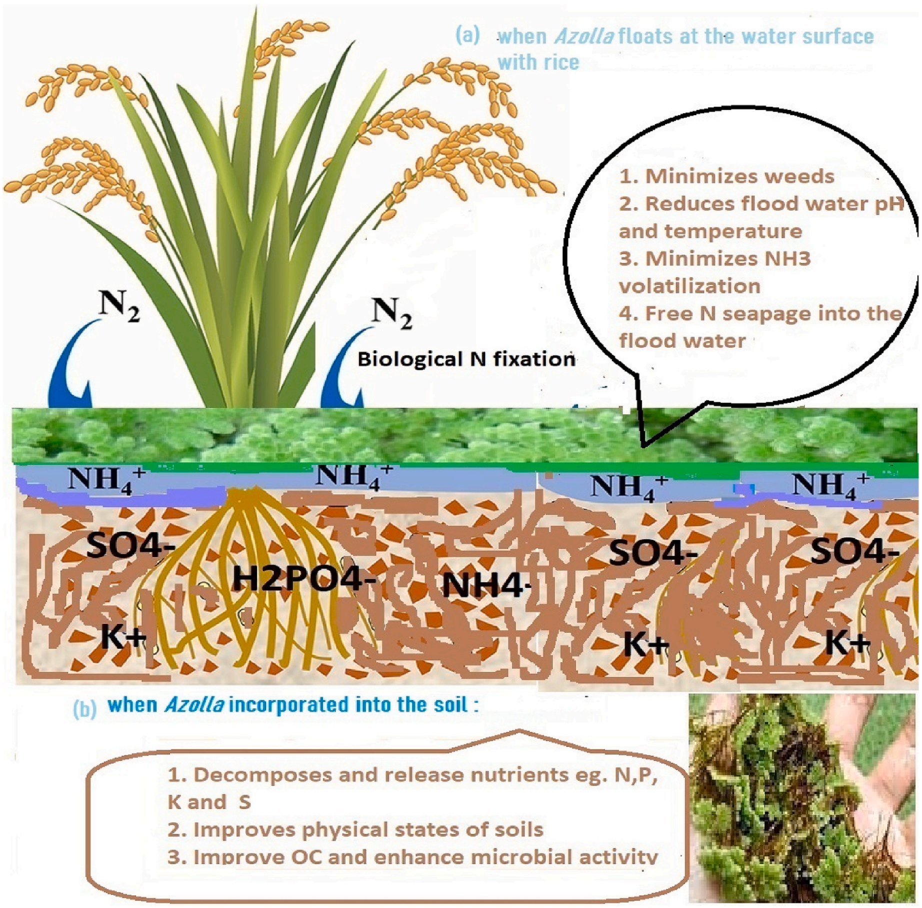

Fig. 4. Illustration showing some benefits of *Azolla* biofertilizer when either left flooded (a) or incorporated in the soil (b).

submerged environment. Furthermore, NH_3-volatilization and NO_3^- leaching in wet soil have been observed to pollute water sources and cause environmental concerns [78]. NH_3-volatilization is a function of the NH_3-partial pressure (P_{NH3}) in the floodwater determined by the total (NH_3 + NH_4)-Nitrogen level, temperature, and pH of the floodwater [79]. It was found that NH_3-partial pressure (P_{NH3}) increases ten-fold for every unit increase in pH and with pH below 7.5 the NH_3 losses are negligible [36].

Azolla grown together with rice and left in the floodwater covers the surface water within 15–20 days, which then has an impact on the absorption of NH_4^+, decreases pH and temperature of the water, thus reducing ammonia volatilization [11]. Reduction of N with other organic sources is more efficient in improving nitrogen use efficiency (NUE) and increases rice yield compared to the use of inorganic nitrogen reduction alone [7,80]. *Azolla* continuously kept in floodwater pH (below 8.0) for 14 days after applying urea decreases floodwater pH by up to 1.2 units on the sixth day, which represents a 16.5% reduction compared to control [8]. It also reported that *Azolla* decreases flood water temperature by up to 4.7 °C which then decreases ammonia volatilization as well as methane (CH_4) emission.

3.6. Contribution of Azolla to yield and yield components of rice

Generally, incorporation of *Azolla* in paddy fields or its use as dual cropping left floating on the floodwater surface or applied with other synthetic fertilizers significantly increases number of tillers and effective tillers [5], which are vital morpho-physiological traits of rice [8]. The high tillering capacity (number of tillers and effective tillers) is a desirable attribute in rice production and is directly related to the panicles number, grain yield, straw yield, and dry matter [81,82]. Out of 11 reviewed literatures that compare yield differences between *Azolla* and non-*Azolla* plots (Table 5 [7–9,22,23,31–33,61,81,83,84]), all the papers reported significant grain increase and other yield attributes by Azolla treatments over control. The yield increase depends on the amount of *Azolla* incorporated [8,81], modes of *Azolla* application [23,81] synthetic fertilizer used [7]; and management practices [23]. The increase in grain yield with *Azolla* application might be due to efficient supply capacity of *Azolla*-N after decomposition and possibly other released nutrients

Table 5
Description of some studies that compare yield differences between Azolla and non-Azolla plots.

Summary	Grain yield (t ha^{-1})					Yield differences over control and non-Azolla plot	References
	Control plot	Full N	Half N + Azolla	Full N + Azolla	Azolla alone		
Application of biochar and Azolla in rice production	8.0	Not measured	Not measured	Not measured	11.0	32.5–36.3% grain yield increase at Azolla and NPK + Azolla respectively compared to NPK alone	[31,32]
Azolla cover reducing NH3 volatilization	56.81 g pot^{-1}	81.6 g pot^{-1}	85.17 g pot^{-1}	62.65 g pot^{-1}	83.63 g pot^{-1}	Grain yield increased 2.9% and 6.3 when using Azolla with full N, half N rate compared with CT	[7]
Azolla in enhancing rice production	Not measured	10.76	11.32	Not measured	Not measured	40% of NPK rate with Azolla yields similar results (10.76 t ha-1) as the full NPK dose	[33]
Azolla in fresh and powder form in improving soil and rice yield	49.76 g pot^{-1}	Not measured	Not measured	Not measured	64.9 g pot^{-1}	yield from Azolla powder and fresh Azolla significantly different from CT, however no significant difference between powder and fresh-form treatments	[9]
Azolla in rice production	2.64	3.5	Not measured	Not measured	3.95	Grain yield of Azolla plot (3.9 t ha^{-1}) significantly different from CT plot (2.64 t ha^{-1})	[61]
Comparing the time of Azolla incorporation and synthetic fertilizer on the growth and yield of rice	0.51	6.3	Not measured	Not measured	6.21	Grain yield of Azolla treatments differs significantly with CT, however, incorporating Azolla before transplanting gave higher yield than incorporating at 40 DAT	[23]
Effect of P on Azolla growth	1.9	3.3	2.9	2.5	2.4	Azolla increased grain yield by 27% over the CT	[22]
Use of Azolla for improving low N-use efficiency	4.58	8.99	9.84	10.45	Not measured	14–20% yield increase in Azolla + half N and Azolla + full N over CT	[8]
2015–2016	4.91	8.75	9.69	9.24	Not measured		
Impact of Azolla on CH4 Emission	3.58	4.58	4.38	Not measured	4.38	Azolla treatments gave significantly higher yields than CT	[81]
Use of Azolla in moderating the dose of N fertilizer and mitigating GHG	426 g m^{-1}	518 g m^{-1}	515 g m^{-1}	Not measured	Not measured	Application of Azolla + Low N from urea gave significantly higher yield than full N dose	[83]
Use of Azolla in conserving urea-N in rice production	1.1	6.0	Not measured	14.0	5.1	Grain yield and N recovery were significantly influenced by Azolla compared to the split application of N-urea	[84]
	0.9	5.6	4.0	9.5	1.7		

RNA Azolla increased grain yield by 3.9, 6.4, and 3.9% in 40 (N4), grain yield by 3.9, 6.4, and 3.9% in 40 (N4).

[5], reducing N-loss and increasing nutrient use efficiency which promotes assimilates from source to sink [8,84,85]. Interestingly, literature indicated that applying *Azolla* with reduced nitrogen levels yields comparable results with farmers' full nitrogen doses (e.g., Refs. [7,22,33,81,83]). Indicating that if *Azolla* is well managed and used effectively it has potential to safeguard the sustainability in lowland rice production and to minimize the use of synthetic N-fertilizer without significant reduction in the growth and grain productivity of rice [33, 94].

The highest grain yield of 3.9 t ha^{-1} was obtained when inoculating 2 t ha^{-1} of *Azolla* at 25 and 40 DAT combined with a low N rate (40 kg N ha^{-1}) from urea at 15 and 60 DAT [61]. The use of *Azolla pinnata* as green manure significantly increases grain yields by 7.7% more compared to control [31,32]. The study carried out by Ref. [22] in Niger showed that when *A. pinnata* was applied as an intercrop at 5 DAT and incorporating it and re-inoculating at 27 DAT increases grain yield by 27% compared to urea when both were applied at 30 kg N ha^{-1}. In the same study, the author reported that there is a positive effect of *Azolla* cover on grain yield at half the recommended N rate than at farmers full recommended N rate. It was also reported that the use of 60 kg N ha^{-1} through *Azolla* with 30 kg N-urea ha^{-1} gave a yield comparable to a full dose of 60 kg N from urea ha^{-1} [81]. Setiawati et al. [9] compared the effects of *Azolla* in fresh and powder form in rice production, noted that, there yield is not significant when *Azolla* used as either powder or fresh form.

3.7. Effects of Azolla on weed emergence in a rice cropping system

In agricultural practices, the control of weeds in the rice cropping system is often accomplished through chemical herbicides such as 2,4-D, Glyphosate, fenoxaprop-p-ethyl, propanil, and pyrazosulfuron ethyl [86,87]. Weed control is vital for optimum rice production [88]. Weeds compete with rice for light, nutrients, water, and space [89]. Without weed control, yield losses have been estimated to range from 16 to 86%, or even 100% [90]. The yield decline depends not only on the infestation but also on the composition of weed flora. Yet, these herbicides are active substances that affect non-target organisms, and also have negative impacts on both soil and water [91]. Besides, the ability of *Azolla* to suppress weeds has been mentioned in literature across the world since 1927 but with little attention being given to smallholder rice farming systems in the rest of the developing countries [92]. *Azolla* cover was able to inhibit six species of weeds completely namely, *Scirpus juncoides* Roxb. var. *Hotarui* Ohwi, *Cyperus serotinus* Rottb, *Echinochla oryzicola* Vasing, *Eclipta prostrate* L. and *Monochoria vaginalis* Burm. f. Presl var. *Plantaginea* (Roxb.) *Solms-Laub* [92]. There are two mechanisms for this suppression, the most effective being the light-starvation of young weed seedlings by the blockage of sunlight, the other mechanism is the physical resistance to weed seedling emergence created by a thick, interlocking *Azolla* mat, which does not affect the growth of rice [59,92]. A study by Ref. [90] compared the efficiency of *Azolla* and herbicides in controlling weeds and noted that *Azolla* completely reduced grasses (*Echinochloa colona, Digitaria sanguinalis, Echinochloa crus-galli*, and *Panicum repens*) and broad-leaved weeds (Fimbristylis miliacea, Digera arvensis, Commelina benghalensis, Cyperus rotundus, Convolvulus arvensis, and Cyperus esculentus) as well as total weeds density.

4. Conclusions and way forward

For centuries *Azolla* has been recognized as a potential biofertilizer in lowland rice production. *Azolla* utilization is a low-cost option with significant long-term environmental conservation and great potential in supplying fixed N and other nutrients such as K^{+}, S, Mg^{2+} and P in lowland rice production systems. If *Azolla* is adopted and used properly will increase N use efficiency thereby minimizing N-loss and reducing the need for synthetic N fertilization. Additionally, due to its rapid growth rate, *Azolla* was found beneficial in improving Organic carbon, increasing microbial biomass, and enhancing nutrient cycling. Although it seems that incorporating *Azolla* needs extra manpower and maintaining *Azolla* biomass is doubtful, however, for the current increasing demands of N fertilizers with continuous deterioration of soil health. The use of *Azolla* is a great opportunity in rice production, accordingly strategic initiatives could focus on, but not limited to:

1. Training farmers and agronomists on the overall benefit of using *Azolla* in lowland rice production.
2. Selection of different species of *Azolla* and evaluate the effectiveness and performance of each species in the field, under different agroecological zone.
3. Extensive research on enhanced sporulation and stress adaptation to maintaining *Azolla* biomass over different ecology.
4. Developing a cheaper way of using *Azolla* in the rice field. For example, growing Azolla as either monocrop or as an intercrop and incorporating it at a time of farm preparation (plowing, harrowing, or leveling) to reduce the need for extra manpower. Also applying P while growing *Azolla* enables rapid multiplication that later will be released with other nutrients upon decay.
5. Establishing a project with different partners involving ministries, scientists, policymakers, local institutions, research organizations, and other private sectors that can develop an effective model of promoting *Azolla* in rice production.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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