Floating Fern, Reducing Emissions: *Azolla's* Role in GHG Reduction

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Abstract

The study examines *Azolla microphylla*, an aquatic fern known for its rapid growth, nitrogen fixation, and carbon sequestration, as a sustainable tool for mitigating greenhouse gas (GHG) emissions. Conducted in the Sirakol region, West Bengal, the research quantified carbon and nitrogen stored in *Azolla* biomass across four ponds, with Pond 4 showing the highest sequestration capacity (3.09 t/ha carbon, 0.24 t/ha nitrogen). *Azolla*'s symbiosis with *Anabaena azollae* enhances its nitrogen fixation and resilience in nutrient-poor environments. Highlighting *Azolla*'s ecological benefits and potential for climate finance through carbon credits, the present study underscores its utility in climate mitigation, sustainable agriculture, and environmental remediation.

Keywords: Azolla microphylla, greenhouse gas mitigation, carbon sequestration, nitrogen fixation, Anabaena, climate finance, sustainable agriculture, aquatic ecosystems, biomass quantification, COP29.

Introduction

Azolla sp. is a genus that encompasses a group of aquatic floating plants, namely ferns, which are native to warmer climatic zones such as the tropics and sub-tropics as well as to the warm temperate regions of Africa, Asia, and USA. They have very high growth rates compared to other aquatic macrophytes with a doubling time ranging between 2-5 days, if the ambient hydrological parameters are congenial for their growth (Sood et al., 2012; Hamdan et al., 2022; Wagner,1997; Handajani, 2012; Kour et al., 2020; Sabetraftar et al., 2013; Bujak et al., 2024). Because of such rapid growth, *Azolla* spp. have the potential to sequester significant amount of atmospheric carbon dioxide and nitrogen dioxide in their biomass (Hamdan et al., 2022; Kumar et al., 2024; Kour et al., 2024; Sarkar et al., 2023; Kollah et al., 2016; Jama et al., 2023). The genus is a unique example of 3R's (reduce, reuse, and recycle), which is in alignment with the SDG 14. After attaining critical biomass, these aquatic ferns are used as animal feed, biofertilizers and biofuel production (Prabakaran et al., 2022; Korsa et al., 2024; Miranda et al., 2016; Vijayan et al., 2024; Bujak et al., 2022; Ahmad et al., 2021; Nasir et al., 2022; Alagawany et al., 2024). Given some of the growing characteristics of *Azolla* spp., such as its fast growth and tolerance to pollution, *Azolla*, in many cases, is considered a weed that can sometimes turn into an invasive plant in certain ecosystems, especially in wetlands, although it offers extensive benefits, such as being used as a biofertilizer for crops or a feed for cattle and even as a feed in aquaculture (Bocchi and Malgioglio, 2010; Katole et al., 2017; Mosha 2018; Mvandaba et al., 2019; Pinero-Rodríguez et al., 2019).

The symbiotic relationship of *Azolla* spp. with *Anabaena azollae*, which is an endophytic blue-green alga that lives within cavities in the leaves of the aquatic fern (Bocchi and Malgioglio 2010; Gresshoff 2018; Hove 1989) promotes the species to scrub atmospheric nitrogen. This cyanobacterium can get sufficient nitrogen for itself as well as for *Azolla* spp. allowing for optimal growth of the aquatic fern under nitrogen limiting condition. Thus, *Azolla* spp. can serve as an effective bio-tool to minimize potential greenhouse gases preferably atmospheric carbon dioxide and nitrogen dioxide.

There are six living species of *Azolla* comprising two sub-genera, with the sub-genus *Euazolla*, containing *Azolla filiculoides* Lam., *Azolla caroliniana* Willd, *Azolla mexicana* Schltdl. & Cham. ex C. Presl, and *Azolla microphylla* Kaulf, and the sub-genus *Rhizosperma* containing *Azolla pinnata* R. Br. and *Azolla nilotica* Decne. ex Mett. (Sadeghi and Zarkami 2013; USDA 2021).

In the Lower Gangetic Delta region, *Azolla microphylla* grows luxuriantly (Fig.1). The species can withstand direct sunlight and proliferates in slow moving water bodies and in almost stagnant water. The species is also called mosquito fern. This study explores the potential of *A. microphylla* as an effective sink for carbon and nitrogen, contributing to the global agenda of climate change mitigation. In alignment with the objectives of COP 29 in Baku, which emphasizes innovative solutions to reduce greenhouse gas emissions and promote sustainable practices, this research investigates the feasibility of leveraging this species for carbon credit opportunities through the lens of financial domain (https://flow.db.com/more/esg/cop29-four-key-takeaways-from-baku#!). By quantifying its capacity to sequester carbon and nitrogen, the findings of the current research aim to support global efforts to accelerate carbon neutrality, enhance ecosystem resilience, and address climate change challenges through nature-based solutions.

Fig. 1 Quantification of *Azolla* in the pond by quadrat method

Material and methods

Selection of sampling sites

The present research carried out at four ponds in the Sirakol region of South 24 Parganas (Table 1, and Fig. 2), West Bengal aims to focus the stored carbon and nitrogen in the leafy parts of the species.

The work was carried out during November, 2024 (postmonsoon season in the Lower Gangetic Delta) to monitor the level of stored carbon and nitrogen per unit area in the four ponds.

Table 1. Selected ponds for the present study

Ponds	Latitude-longitude
Pond 1	22°17'54″N; 88°16'15″E
Pond 2	22°17'52″N; 88°16'18″E
Pond 3	22°17'51″N; 88°16'18″E
Pond 4	22°17'49″N; 88°16'18″E

Fig. 2 Selected four ponds in the study area

Biomass Estimation

The biomass of *Azolla* was determined by weighing the dried biomass of fresh samples collected from a defined 1 m² area and expressed as kg/m² dry weight. This value was then converted to tonnes per hectare (tonnes/ha) for the waterbodies. To ensure quality assurance, the average from five such quadrats was considered for further analysis.

Analysis of stored carbon and nitrogen in the *Azolla* biomass

The stored carbon and nitrogen in dried *Azolla* samples were analysed using a CHN analyser, which operates based on the principle of combustion. The dried samples were combusted in an oxygen-rich environment at high temperatures, causing the organic matter to oxidize and release carbon as CO_2 and nitrogen as NO_x gases. These gases were separated and detected using thermal conductivity detectors, enabling the quantification of carbon and nitrogen percentages. The instrument was calibrated with standard reference materials such as acetanilide and atropine to ensure accuracy. The measured values were expressed as a percentage of the total dry weight of the samples, providing insights into the elemental composition of *Azolla* biomass. Quality control measures, including repeated analyses of the standards, were followed to validate the results.

To convert the measured carbon (C%) and nitrogen (N%) in dried *Azolla* samples to kg/m² and subsequently to tonnes/ha, the dry biomass in kg/m² was first determined. The carbon and nitrogen percentages were then multiplied by the dry biomass to calculate the mass of carbon and nitrogen in kg/m². These values were further converted to tonnes per hectare (tonnes/ha) by multiplying the kg/m² results by a factor of 10, as 1 hectare is equivalent to 10,000 m². This process ensures accurate estimation of carbon and nitrogen storage in the *Azolla* biomass of the selected four ponds.

Result

The analysis of stored carbon and nitrogen in the four ponds highlights their potential contribution in mitigating greenhouse gas (GHG) emissions, particularly carbon dioxide (CO_2) and nitrogen dioxide (NO_2), under climate change scenarios. Pond 4 demonstrates the highest carbon storage (3.09 t ha^{-1}) and nitrogen storage (0.24 t ha^{-1}), suggesting its superior role in sequestering these elements and reducing atmospheric GHGs. Pond 1 and Pond 3 also exhibit moderate storage capacities, whereas Pond 2, with the lowest carbon (1.28 t ha^{-1}) and nitrogen (0.09 t ha^{-1}) stocks, indicates a need for improved management practices to enhance its sequestration potential (Table 2).

These findings emphasize the importance of optimizing pond management to maximize carbon and nitrogen storage, contributing to the mitigation of climate change. Enhancing the organic matter and nutrient retention in ponds leading to *Azolla* growth, especially those underperforming like Pond 2, could significantly increase the level of atmospheric CO_2 and NO_2 in the near surface atmosphere at the local scale.

Table 2. Stored carbon and nitrogen related parameters of *Azolla* across four ponds

Parameter	Pond 1	Pond 2	Pond 3	Pond 4
Weight (kgm^{-2})	0. 571	0. 305	0. 508	0. 670
C%	44.5	42.0	43.8	46.1
N%	3.01	2.85	2.92	3.64
C per unit area (tha^{-1})	2.54	1.28	2.23	3.09
N per unit area (tha^{-1})	0.17	0.09	0.15	0.24

Discussion

Azolla, a genus of aquatic floating ferns, presents a unique opportunity for combating greenhouse gases (GHGs) such as carbon dioxide (CO_2) and oxides of nitrogen (NO_x) through its remarkable biological and ecological characteristics (Malyan et al., 2019; Malyan et al., 2021; Kimani et al., 2020; Korsa et al., 2024; Kimani et al., 2018; Kour et al., 2024; Kollah et al., 2016). *Azolla's* potential as a natural carbon and nitrogen sink lies in its symbiotic relationship with *Anabaena azollae*, an endophytic cyanobacterium capable of fixing atmospheric nitrogen. This partnership enables *Azolla* to thrive even in nutrient-limiting conditions, making it a sustainable and efficient tool for mitigating GHGs emissions while promoting environmental resilience.

Azolla's role in carbon sequestration is intrinsically linked to its ability to grow rapidly under suitable hydrological conditions, with a doubling time of just 2-5 days. This growth rate significantly surpasses that of many other aquatic plants, allowing it to amass substantial biomass in a short period. The high carbon content in its biomass, often exceeding 40%, highlights its effectiveness in capturing atmospheric CO_2 and storing it in organic form. In the Lower Gangetic Delta region, for instance, *Azolla microphylla* has demonstrated its capacity to sequester carbon at levels of up to 3.09 tonnes per hectare (value of pond 4 in the present study). The species also has the potential to sequester nitrogen up to 0.24 tonnes per hectare as revealed from the Table 2. This impressive performance underscores its suitability as a climate change mitigation agent in fresh water aquatic ecosystem.

The symbiotic association with *Anabaena azollae* is a cornerstone of *Azolla*'s ecological significance. *Anabaena*, a blue-green alga, resides within cavities in *Azolla* leaves, where it fixes atmospheric nitrogen and supplies it to the plant. This nitrogen fixation process not only supports the growth of *Azolla* but also contributes to the overall reduction of nitrogen oxides in the atmosphere, which are potent greenhouse gases. Nitrogen fixation by *Anabaena* is a highly efficient process, enabling *Azolla* to flourish in environments where nitrogen is otherwise a limiting factor. This symbiotic mechanism exemplifies nature's capacity to regulate and recycle essential nutrients while contributing to the mitigation of climate change.

The potential of *Azolla* extends beyond its biological capabilities. As a highly adaptable plant, it can proliferate in diverse aquatic habitats, including ponds, wetlands, and slow-moving water bodies. Its ability to thrive in polluted environments further enhances its value as a nature-based solution for environmental remediation. However, the management of *Azolla* in natural ecosystems requires careful consideration. While it provides extensive benefits, including carbon and nitrogen sequestration, unchecked growth can lead to invasive tendencies that disrupt native biodiversity and aquatic habitats.

The quantification of stored carbon and nitrogen in *Azolla* biomass offers valuable insights into its effectiveness as a GHG mitigation tool. Using advanced analytical techniques like CHN-based analysis, researchers have identified significant variations in the storage capacities of *Azolla* across different ponds. These variations are influenced by ecological factors such as nutrient availability, water quality, and sunlight exposure. For example, in the study of four ponds in South 24 Parganas, West Bengal, Pond 4 exhibited the highest carbon and nitrogen storage capacities, attributed to its optimal ecological conditions. In contrast, Pond 2, with suboptimal conditions, recorded the lowest values, highlighting the need for targeted interventions to enhance its performance.

The integration of *Azolla*-based strategies into broader climate change mitigation efforts aligns with global sustainability goals, such as the United Nations' Sustainable Development Goals (SDGs). By leveraging its capacity to sequester carbon and nitrogen, *Azolla* contributes to reducing GHG concentrations, improving air quality, and enhancing local ecosystem resilience. Moreover, the harvested biomass of *Azolla* can be repurposed for various applications, including as biofertilizers, animal feed, or biofuel, creating a circular economy that supports both environmental and economic sustainability.

Anabaena's role in nitrogen scrubbing is particularly noteworthy. As a cyanobacterium capable of fixing atmospheric nitrogen, *Anabaena* transforms nitrogen gas into forms that are usable by *Azolla* and other organisms. This process reduces the need for synthetic fertilizers, which are often associated with GHG emissions during production and application. By supporting *Azolla's* growth, *Anabaena* not only enhances nitrogen sequestration but also contributes to reducing nitrogen-related emissions, such as nitrous oxide, which has a global warming potential significantly higher than that of CO_2.

Despite its numerous advantages, the use of *Azolla* as a GHG mitigation agent is not without challenges. Its rapid growth and adaptability can sometimes lead to invasive behaviour, particularly in ecosystems with limited natural controls. Effective management strategies, such as periodic harvesting and biomass utilization, are essential to balance its ecological benefits with potential risks. The development of best practices for *Azolla* cultivation and management will ensure its sustainable integration into climate change mitigation frameworks.

The potential of *Azolla* to combat GHGs like CO_2 and NO_x is further enhanced by its ability to interact with other components of aquatic ecosystems. For instance, the presence of *Azolla* in water bodies can influence microbial communities, sediment dynamics, and nutrient cycling, all of which contribute to the overall reduction of GHG emissions. By fostering these interactions, *Azolla* can create a synergistic effect that amplifies its role as a natural climate solution.

Thus, *Azolla* represents a powerful and sustainable bio-tool for addressing the dual challenges of climate change and environmental degradation. Its capacity to sequester carbon and nitrogen, supported by its symbiotic relationship with *Anabaena*, positions it as a key player in nature-based solutions for GHG mitigation. The spatial variability observed in its performance across different aquatic habitats underscores the importance of tailored management practices to optimize its benefits. By integrating *Azolla* into global and local climate strategies, we can harness its potential to accelerate carbon neutrality, enhance ecosystem resilience, and promote sustainable development. As research and innovation continue to explore the full spectrum of *Azolla*'s capabilities, this unassuming aquatic fern may well become a cornerstone of our efforts to combat climate change and protect the planet for future generations.

Azolla can also act as a promising bio-tool for climate finance sector due to its rapid growth, high carbon sequestration, and nitrogen-fixing capabilities through its symbiotic relationship with *Anabaena* cyanobacteria. Considering the agenda of COP 29 in Baku, *Azolla* can be linked to climate finance mechanisms by showcasing its potential for reducing greenhouse gas emissions and improving water quality. Projects focused on large-scale *Azolla* cultivation can generate carbon credits and attract investments under carbon markets and funds like the Green Climate Fund (Table 3). Its role in sustainable agriculture as a biofertilizer and animal feed further enhances its climate and economic benefits, aligning with global goals for climate resilience and mitigation.

Table 3. Biomass production, forecasted yield, and revenue potential of *Azolla* across four ponds

No. of Ponds	Biomass (t/ha) Real Time Data (Vide Table 2)	Biomass (t/ha/yr) Forecasted Data (Considering the doubling times of the species as 5-days)	Revenue Earned (1 tonne=20,000 INR)*
1	5.71	416.83	83,36,600
2	3.05	222.65	44,53,000
3	5.08	370.84	74,16,800

4	6.70	489.10	97,82,000

* https://www.indiamart.com/proddetail/azolla-plant-26147571473.html

Conclusion

Azolla, with its rapid growth and symbiotic relationship with *Anabaena*, emerges as a promising nature-based solution for mitigating greenhouse gases like carbon dioxide and nitrogen oxides. Its ability to sequester significant amounts of carbon and fix atmospheric nitrogen underscores its role in addressing challenges related to climate change. By thriving in diverse aquatic habitats, *Azolla* not only enhances ecosystem resilience but also reduces reliance on synthetic fertilizers, contributing to sustainable agricultural practices. However, effective management, such as periodic harvesting and controlled cultivation, is essential to mitigate its invasive potential. The harvested biomass further adds value as biofertilizers, animal feed, or biofuel, fostering a circular economy. Spatial variability in its carbon and nitrogen storage highlights the need for tailored interventions to optimize its benefits. By integrating *Azolla* into global climate strategies, we can accelerate carbon neutrality and enhance environmental sustainability. This aquatic fern exemplifies the potential of leveraging natural processes for a greener future. Its adoption offers a scalable, cost-effective approach in combating climate change while supporting biodiversity and ecosystem health.

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