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Addressing Nitrogen-rich Biomass Production Challenges in *Azolla microphylla* Cultivation from Varying Shading and Water Depth Dynamics

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ABSTRACT

Azolla microphylla, a rapidly growing aquatic fern with the unique ability to fix atmospheric nitrogen, presents significant potential for sustainable agriculture. Despite its nitrogen-fixing prowess, challenges persist in optimizing biomass production, prompting a detailed exploration of influential factors in this study. This paper addresses the persistent challenge of optimizing nitrogen-rich biomass production in *Azolla* cultivation. Employing a split-plot experimental design, the study investigates the influential factors of shading percentage (N) and water depth (G) in *Azolla* growth, systematically ranging from 0% (full sunlight/N1) to 75% (N3) shading percentages and 2.5 cm (G1), 5.0 cm (G2), and 7.5 cm (G3) water depths. In addition to assessing growth and production outcomes, this study explores the nitrogen content in *Azolla* under three different conditions: fresh, dried, and composted *Azolla*. Findings unveil the significant influence of shading percentage and water depth on *Azolla* growth, with the N1G2 treatment identified as the optimal condition for achieving maximum biomass production. Set against the backdrop of tropical agriculture, specifically within the high temperatures in Indonesia, our study underscores the resilience of *Azolla* to elevated temperatures, highlighting

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its potential as a nitrogen-fixing agent. Notably, fresh *Azolla* closely matches urea in nitrogen content, suggesting its potential as an organic fertilizer substitute for urea. This research sheds light on the critical challenges surrounding nitrogen-rich biomass production from fresh *Azolla*, emphasizing the necessity of temperature resilience and water depth optimization. The insights provided hold significance for tropical agriculture practices seeking to harness the potential of *Azolla* as a free-air nitrogen fixator.

Keywords: *Azolla microphylla*, nitrogen fixation, shading percentage, sustainable cultivation, tropical agriculture, water depth

INTRODUCTION

Azolla microphylla, commonly known as *Azolla*, represents a captivating aquatic fern within the Azollaceae family. Its distinct characteristics include a small stature, rapid proliferation, and a noteworthy symbiotic relationship with the nitrogen-fixing cyanobacteria *Anabaena azollae* (Abd El-Aal, 2022; Adhikari et al., 2020; Akhtar et al., 2021; G. Kumar & Chander, 2017). *Azolla*'s paramount attribute in agriculture lies in its proficiency as a biofertilizer. Its ability to fix atmospheric nitrogen provides a sustainable alternative to synthetic nitrogen fertilizers, thereby enhancing soil fertility and creating optimal conditions for crop cultivation (Adhikari et al., 2020; Ahmad & Tariq, 2021). This agricultural significance has positioned *Azolla* as a comprehensive subject for study and exploration. The distinctive nitrogen-fixing symbiosis of *Azolla*, where *Anabaena* resides within specialized dorsal hairs on the upper leaf surface, is a focal point for research into biological nitrogen fixation (Devaprakash et al., 2024). This symbiotic relationship is integral to the plant's role in promoting soil fertility and sustainable agricultural practices.

Beyond agriculture, *Azolla*, as a biomass source, has garnered attention for its potential in bioenergy production (Purnama et al., 2024). A study exemplified by Chupaza et al. (2021) has demonstrated the feasibility of utilizing *Azolla* biomass for bioethanol production. Its rapid growth rate and high biomass productivity make it an attractive candidate in the renewable energy sector. *Azolla*'s utility also extends to animal husbandry, where it serves as a valuable nutritional supplement in livestock diets. Rich in proteins, vitamins, and minerals, *Azolla* contributes to enhanced livestock health and productivity, as evidenced by the findings of G. Kumar and Chander (2017) as well as Nasir et al. (2022).

From an environmental sustainability perspective, *Azolla* cultivation seamlessly aligns with sustainable agricultural practices, alongside efforts to seek alternative pesticides that do not contaminate agricultural products (Purnama et al., 2023). Its ability to thrive in diverse water conditions and minimal environmental impact positions *Azolla* as an eco-friendly intervention in agricultural systems (Alam et al., 2023; Xu et al., 2017). Despite its numerous advantages, *Azolla* cultivation confronts several challenges that hinder its widespread adoption. Environmental factors, such as extreme temperatures or fluctuations, can impede *Azolla* growth (Costarelli et al., 2021). High temperatures may induce thermal stress, affecting nitrogen fixation efficiency, while insufficient sunlight due to shading or low light conditions may restrict photosynthesis, hampering overall growth potential. Sunlight intensity plays a multifaceted role in the growth dynamics of *Azolla*, profoundly impacting photosynthetic activity, transpiration rates, and overall plant physiology (Kakaeian & Mohammadi, 2022; Pouil et al., 2020). Water depth also emerges as a crucial factor affecting *Azolla* cultivation, which exerts a substantial influence on the nutrient absorption rates and evapotranspiration of *Azolla* (Adzman et al., 2022; Kimani, Kanno, et al., 2020).

Numerous research endeavors have delved into mitigating environmental challenges, specifically addressing issues such as high light intensity and water depth in the cultivation of *Azolla*. The dynamic interplay between these factors and *Azolla*'s growth dynamics has been a focal point in the quest for sustainable and optimized cultivation practices. One significant facet of prior investigations lies in understanding the impact of high light intensity on *Azolla* growth. Studies exemplified by the work of Kakaeian and Mohammadi (2022) have underscored the need for nuanced light management strategies to harness optimal growth conditions. Similarly, research by Pouil et al. (2014) contributes valuable insights into the multifaceted effects of light intensity on *Azolla*, emphasizing its role as a pivotal factor influencing key physiological processes.

Another critical environmental parameter explored in past research is water depth. The depth of water habitats inherently influences *Azolla* cultivation, and studies such as those conducted by Adzman et al. (2022) have delved into the consequences of varying water depths on *Azolla* biomass production. These investigations have provided essential insights into the correlation between water depth and nutrient absorption rates, offering a foundation for developing cultivation strategies tailored to specific water depth conditions. However, studies combining shading and its variations have not been extensively explored, even though the integrative approach of these two factors provides a holistic understanding of the environmental parameters influencing *Azolla* growth. Given the intricacies of these interactions, there exists an urgent need to thoroughly investigate and establish the optimal combination of water depth and shading percentage that is environmentally conducive to the biomass growth of *Azolla*.

This study, which aims to identify optimal combinations of shading percentages and water depths conducive to promoting maximum nitrogen-rich biomass production, distinguishes itself by including an analysis of nitrogen content in *Azolla* biomass under three conditions: fresh, dried, and composted. This comprehensive approach involves a meticulous examination of synergies between shading and water depth dynamics, highlighting the multifaceted nature of the study. The focus on addressing challenges related to nitrogen-rich biomass production in *Azolla* cultivation, along with including nitrogen content analysis from various biomass conditions, sets this research apart from previous studies. While prior research has provided valuable insights into individual factors, this study uniquely integrates them, considering their combined effects on biomass yield. The integrative approach contributes to a more holistic understanding of the cultivation dynamics and offers practical insights for optimizing *Azolla* cultivation practices in the context of nitrogen-rich biomass production.

MATERIALS AND METHODS

Experiment Design and Management

An eight-week field experiment aimed to assess *A. microphylla*'s response to various culture conditions, involving three water depth levels and three light level conditions. The split-plot experimental design included 27 ponds, oriented east-west, resulting in nine treatment conditions (N1G1, N1G2, N1G3, N2G1, N2G2, N2G3, N3G1, N3G2, N3G3) replicated thrice. The control condition exposed nine ponds to direct sunlight (0% shade net), while nine were covered by a 50% shade net and another nine by a 75% shade net, designated as N1, N2, and N3, respectively. Water depths (G) were 2.5 cm (G1), 5.0 cm (G2), and 7.5 cm (G3).

The experiment took place during the dry season in a designated artificial pond at Universitas Lancang Kuning's recycling center ("Waste Bank") in Pekanbaru, Indonesia. The pond (each consisting of 1 m^2 with a depth of 0.20 m), as shown in Figure 1, was positioned at coordinates 0°34'52.6"N 101°25'36.9"E and an altitude of 30 m. Continuous light intensity and air temperature monitoring were performed using a light/temperature meter (SMART SENSOR® AS803 Digital Lux Meter, China). Digital lux meters were strategically placed in three ponds per light condition at 5 cm above the water surface, capturing measurements during the morning (07:00-08:00 local time [UTC+0:700]), noon (12:00-13:00 local time [UTC+0:700]), and afternoon (16:00-17:00 local time [UTC+0:700]). The shading effect was achieved using locally available, cost-effective nylon materials, creating a shading gradient among the experimental conditions. The lux-recorded data for light intensity were transformed to μmol/m^2/s, following the methodology outlined by Pouil et al. (2022).

Plant Materials and Cultivation Procedures

Azolla specimens were sourced from the Faculty of Agriculture's small pond at Universitas Lancang Kuning, Pekanbaru, Indonesia (0°34'36"N 101°25'29"E, altitude 33 m). Several academic reasons underpin our decision to select *A. microphylla* as the focal species for this study over other *Azolla* species. Firstly, *A. microphylla* has been widely recognized for its suitability and adaptability to tropical environments. According to Sarah et al. (2023), *A.*

microphylla has demonstrated superior growth performance and environmental adaptability in tropical regions, making it a suitable candidate for cultivation in the Indonesian context. Additionally, *A. microphylla*'s ability to thrive in nutrient-rich aquatic habitats, as documented by Ting et al. (2022), renders it an ideal species for studying nitrogen-rich biomass production, a key focus of our investigation.

Azolla microphylla, inoculated at 100 g fresh weight (FW) per 1 m^2 pond (i.e., 100 g FW/m^2) followed previous research (Pouil et al., 2022), underwent cultivation. Each pond received 3 kg of cow manure (i.e., 30 tons/ha) one week before the experiment's initiation. Throughout the cultivation process, phosphorus fertilizer (superphosphate 36%) was applied three times at 20 g intervals (i.e., 200 kg/ha). The first application, 6 g per pond, occurred two weeks after the initial inoculation. The second application, 6 g per pond, followed the second harvest, with an additional 8 g per pond applied after the third harvest.

Figure 1. Experimental design overview with 1 m x 1 m artificial ponds

After each harvest, 100 g of *A. microphylla* per unit was redistributed, consistent with the initial inoculation treatment.

Total plant biomass assessments were conducted at the experiment's onset and three subsequent times after two weeks of phosphorus addition during the eight-week experiment by subtracting the initial seeding (total 300 g FW per pond). Samples were carefully rinsed and drained. Dry weight (DW) determination adhered to the procedures outlined in previous research (Kimani, Kanno, et al., 2020), wherein the samples were subjected to oven drying at 70°C for 48 hr and subsequently weighed (± 0.1 g). Productivity was calculated by dividing the total biomass of *Azolla* per square meter over the 60 days for each pond.

Nitrogen Analysis in *Azolla*

Nitrogen analysis in *A. microphylla* encompassed three distinct conditions: fresh, dried, and composted *Azolla*. Nine replicates for each condition were collected from experimental ponds, where fresh *Azolla* was directly harvested, dried *Azolla* was obtained after oven-dried, and composted *Azolla* was derived from the decomposition process based on previous research (Lestari et al., 2019). Two treatments were applied to composted *Azolla*: the first with cow manure and the second without. These samples were systematically taken from *Azolla*, which had been previously cultivated. Sample preparation involved using fresh *Azolla* without any pre-treatment, while dried and composted *Azolla* underwent grinding into a fine powder for homogeneity. The Kjeldahl method was then employed for nitrogen extraction based on previous research with several modifications (Shamsudin et al., 2021), with 1 g of fresh *Azolla* and 0.5 g of dried or composted *Azolla* subjected to digestion.

Data Analysis

Collected observational data from the experimental treatments underwent statistical analysis using SPSS software (version 21). The analysis began with a significance assessment by comparing the calculated *F*-value with the critical *F*-table value at 5%. This step determined whether the observed differences were statistically significant or inconsequential. Subsequent analysis involved using Duncan's multiple range test (DMRT) at a 5% significance level to identify specific treatment groups that differed significantly. This detailed post-hoc analysis ensured a thorough examination and validation of any observed variations among treatment levels.

RESULTS AND DISCUSSION

Effects of Shading and Water Depth on *Azolla* Production

In the effort to unravel the intricacies of *A. microphylla* cultivation, investigating the relationship between shading percentage and water depth is imperative in this study. The experimental results unveil that the combination of N1G2, representing the treatment without shading or fully exposed to sunlight with a water depth of 5.0 cm, exhibits no significant difference in *Azolla* biomass compared to shaded conditions. Statistically, there is no significant difference compared to combinations with 2.5 cm and 7.5 cm water depths. Nevertheless, a numerical trend indicates an increase in biomass for the combination without shading and a water depth of 5.0 cm (Table 1).

The findings of previous research, as elucidated by Adzman et al. (2022) and Kimani, Kanno, et al. (2020), underscore a consistent pattern of *Azolla* thriving in environments characterized by deeper water depths. The fundamental mechanism driving this is *Azolla*'s ability to access sufficient dissolved oxygen (DO), a crucial resource for its growth. While *Azolla*, like other plants, relies on carbon dioxide (CO_2) in photosynthesis, it also undergoes respiration to generate the energy needed for growth, nutrient absorption through its roots, and the uptake of active ions. In this context, the submerged roots of *Azolla* require a continuous supply of oxygen to trigger these vital processes (Amit et al., 2016; Xu et al., 2017). As a result, lower water levels imply reduced availability of DO, while higher water levels enhance the DO levels, creating a supportive environment for *Azolla* growth.

These findings clarify the relationship between shading percentage and water depth influencing *Azolla* growth. Water depth is associated with optimal DO supply for *Azolla*. This understanding has practical implications for *Azolla* cultivation, as it indicates that the N1G2 combination condition, characterized by the absence of shading and a water depth of 5.0 cm, can play a crucial role in enhancing *Azolla* biomass, especially *A. microphylla*. By

Table 1
Total fresh biomass of Azolla microphylla *(g) across shading percentage and water depth (n = 9)*

Treatments	Total fresh biomass (g)
N1G1	4,059.78bc
N1G2	4,686.89c
N1G3	4,439.89c
N2G1	3,547.56ab
N2G2	3,309.11a
N2G3	3,740.67ab
N3G1	3,595.89ab
N3G2	3,615.00ab
N3G3	3,767.11ab

Note. Full sunlight or 0 % shade net (N1), 50 % shade net (N2), 75 % shade net (N3), water depth of 2.5 cm (G1), water depth of 5 cm (G2), and water depth of 7.5 cm (G3). The numbers followed by the same letter do not significantly differ based on the post hoc Duncan's multiple range test at a 5% significance level

leveraging these findings, farmers and agricultural practitioners can optimize *A. microphylla* cultivation techniques, contributing to sustainable farming practices by generating a natural nitrogen source and potentially reducing or replacing reliance on synthetic fertilizers (Jama et al., 2023). Furthermore, this research reinforces the confidence that *Azolla* can thrive and be applied to agricultural lands under high-temperature and sunlight-intensity conditions, particularly in flooded rice fields with varying water depths.

In the field, temperature and light intensity are also crucial determinants of *Azolla* growth rate, as evidenced by studies (Akhtar et al, 2020; G. Kumar & Chander, 2017; Pouil et al, 2020). Water depth significantly influences air temperature, with higher ponds maintaining cooler air temperatures. Lower temperatures result in denser air molecules, increasing DO levels (Ali et al., 2016). Conversely, higher air temperatures decrease oxygen solubility, ultimately determining DO availability for *Azolla*. Additionally, light intensity plays a vital role in plant physiology, significantly influencing the fundamental process of photosynthesis (Kakaeian & Mohammadi, 2022; Pouil et al., 2020). It is well-established that reduced light intensity will inevitably lead to decreased photosynthetic efficiency (Liu & Van-Iersel, 2021), a phenomenon of paramount importance in plant biology.

However, our research has revealed intriguing and unexpected findings that challenge conventional expectations. Contrary to the anticipated results, shading did not consistently yield a proportional increase in *A. microphylla* biomass, as presented in Table 1. To delve deeper into this interesting phenomenon, Table 2 and Figure 2 comprehensively illustrate the observed variations in temperature and light intensity at different time intervals. Concerning temperature, it is evident that the shade net used failed to significantly reduce temperature. For each treatment, the average morning, noon, and afternoon temperatures were 27–34°C, 29–41°C, and 27–36°C, respectively. *Azolla*'s ability to thrive over a wide temperature range, from 18 to 28°C (Korsa et al., 2024), 6 to 30°C (Sadeghi et al., 2012), with optimal growth observed at 25°C and rapid reproduction occurring between 18–26°C (Veerabahu, 2015), is one of the advantages in *Azolla* cultivation. Temperature primarily influences photosynthesis when light is a limiting factor (Hussain et al., 2021; Moore et al., 2021). CO_2, coupled with limited light, can enhance photosynthesis. Furthermore, temperature has a significant impact on air absorption by plant roots, with increased absorption rates at higher temperatures (Cannavò et al., 2023). Changes in air viscosity, cell membrane permeability, and root cell activity cause this phenomenon. Temperature also affects nutrient absorption, with low temperatures inhibiting nutrient uptake due to reduced respiration activity or decreased cell membrane permeability (Bhattacharya, 2022).

Meanwhile, the sunlight intensity, particularly the treatment without shading (N1), consistently exhibited the highest light intensity throughout the observation period. Conversely, the treatment with 75% shade net consistently showed the lowest light intensity. This observation effectively underscores that shading restricts the quantity of incoming light (Timmermans et al., 2020). This phenomenon challenges our traditional understanding of the impact of shading percentages on plant growth, especially in the context of *A. microphylla* cultivation. It suggests that *A. microphylla* may have unique adaptations and responses to different light exposures compared to other plant species. Further investigation into the intricate interaction between *A. microphylla* and light intensity is required to unveil the fundamental mechanisms governing its growth dynamics and potentially optimize cultivation practices.

Effendi et al. (2019) reported that applying 30–50% shading during cultivation decreased biomass yield compared to the unshaded condition. Interestingly, shading percentages of 50–75% did not show a significant impact on the growth of *A. microphylla*, as supported by Pouil et al. (2020), reinforcing the findings in this study. Table 1 demonstrates an inverse relationship between shading percentage and *A. microphylla* biomass. Consistent with the research by Adzman et al. (2022), it is suggested that *A. microphylla* requires full sunlight exposure combined with a water depth of 20 cm, making field conditions such as fishponds ideal for *Azolla* cultivation (Pouil et al., 2020).

However, another critical factor influencing *Azolla* biomass production is the nutrient concentration within the pond. As commonly known, the water volume also expands with increasing water depth. Under such circumstances, the nutrient concentration will decrease if the same amount of fertilizer is applied to each treatment with varying water depths. Despite direct exposure to sunlight, *Azolla*'s growth and biomass production at a water depth of 7.5 cm is not as favorable as at a water depth of 5 cm. Therefore, future research should ensure uniform nutrient concentration by adjusting the amount of nutrients used according to the volume or depth of water treatment, as demonstrated by Adzman et al. (2022).

Table 2
Summary of the light intensity and temperature conditions measured throughout the experiment in the three shading conditions (n = 60)

Shading treatment	Light Intensity (μmol/m²/s)				Temperature (°C)			
	07:00–08:00 (UTC+0:700)	12:00–13:00 (UTC+0:700)	16:00–17:00 (UTC+0:700)	Day	07:00–08:00 (UTC+0:700)	12:00–13:00 (UTC+0:700)	16:00–17:00 (UTC+0:700)	Day
N1 (0% shade net)	74 ± 31	116 ± 29	66 ± 29	85 ± 37	29 ± 1.1	35 ± 2.8	32 ± 1.9	32 ± 3.0
N2 (50% shade net)	42 ± 13	42 ± 20	35 ± 24	51 ± 24	29 ± 1.3	36 ± 3.1	32 ± 1.9	32 ± 3.5
N3 (75% shade net)	34 ± 10	64 ± 22	42 ± 19	47 ± 22	29 ± 1.3	35 ± 2.8	32 ± 1.9	32 ± 3.2

Note. Data are means ± standard deviations

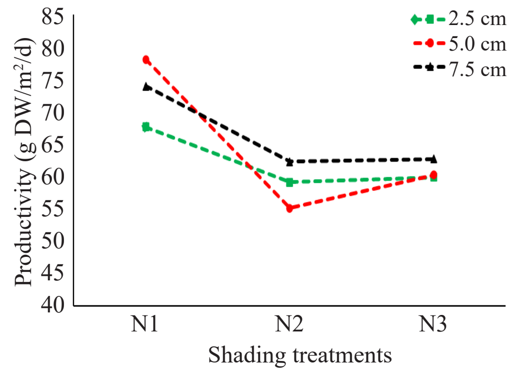

Figure 2. Biomass productivity due to the influence of shading and water depth

Note. N1 = 0% shade net; N2 = 50% shade net; N3 = 75% shade net

Nitrogen Content in *A. microphylla*

The exploration into the nitrogen content of *A. microphylla* has uncovered intricate dynamics, significantly contributing to our understanding of *Azolla*'s nutritional profile and its potential as an organic nitrogen source in agriculture. Fresh *A. microphylla* exhibited an exceptional nitrogen content of 40.3%, marking a pivotal discovery, as shown in Table 3. This high nitrogen concentration underscores the inherent capability of *Azolla* species in fixing atmospheric nitrogen, aligning with the seminal work of Yao et al. (2018), which emphasizes the nitrogen-fixing prowess of *Azolla*. Contrastingly, the dehydration process significantly influenced the nitrogen levels in dried *Azolla*, resulting in a substantial decrease of 3%. The impact of moisture loss on nitrogen concentration is well-documented, as reported by da Silva et al. (2022), further affirming the sensitivity of *Azolla*'s nitrogen content to processing methods.

Table 3
Total N from Azolla *in several conditions from this study (n = 9)*

No.	*Azolla* conditions	Total N (%)
1	Fresh	40.3 ± 1.76
2	Dried	3.00 ± 0.22
3	Composted without cow manure	2.76 ± 0.06
4	Composted with cow manure	1.94 ± 0.03

Note. Data are means ± standard deviations

Furthermore, the observed decrease in nitrogen content upon drying can be attributed to several factors. Firstly, water loss during dehydration leads to a concentration effect, where the nitrogen content becomes more concentrated in the remaining biomass, resulting in higher nitrogen content per unit weight. The dehydration process may also trigger biochemical reactions, such as enzymatic degradation or microbial activity, which could contribute to the breakdown or loss of nitrogen-containing compounds. Moreover, volatilization of ammonia, a common nitrogenous compound, may occur during drying, further diminishing the nitrogen content of the dried *Azolla* biomass (Bao et al., 2022). These mechanisms collectively contribute to the observed decline in nitrogen content following the dehydration process, highlighting the importance of considering processing methods when assessing the nitrogen content of *Azolla* biomass.

Composting dried *Azolla* introduced another dimension to the nitrogen dynamics, with composted *Azolla* displaying a nitrogen content of 2.76%. Incorporating cow manure as a composting agent further reduced the nitrogen content in the composite to 1.94%. The observed decrease in nitrogen content during composting can be attributed to several factors. On the one hand, microbial activity during composting leads to the breakdown of nitrogen-containing compounds present in *Azolla* biomass, resulting in the release of nitrogen in gaseous forms such as ammonia and nitrous oxide, as reported by Wong et al. (2017). On the other hand, incorporating cow manure, although rich in nitrogen, may promote microbial activity that accelerates the decomposition of organic matter, thereby leading to nitrogen loss through volatilization and leaching processes (Toledo et al., 2020). These processes cumulatively decrease nitrogen content observed during composting, underscoring the complex nitrogen transformation dynamics within *Azolla*-based composting systems.

The observed variations in nitrogen content across different *Azolla* conditions emphasize the critical role of processing methods and additives in shaping the nutritional composition of *Azolla* biomass. These nuanced findings are pivotal for optimizing the utilization of *Azolla* as an organic nitrogen source in agriculture, aligning seamlessly with the sustainable and eco-friendly farming paradigms advocated by Alam et al. (2023) and Xu et al. (2017). Moreover, the significance of the nitrogen content in fresh *Azolla* becomes even more pronounced as our investigation, based on an extensive literature review, reveals a lack of previous studies comparing the nitrogen analysis of fresh, dried, and composted *Azolla*. Existing research, if any, predominantly focuses on the utilization of *Azolla* in cultivating various crops (Jama et al., 2023; G. P. Kumar et al., 2020; Marzouk et al., 2023; Seleiman et al., 2022), comparing the outcomes of using fresh, dried, and composted *Azolla*. Notably, our study aligns with the findings of Muñoz et al. (2008), who compared nitrogen content in fresh and composted poultry (*Gallus domesticus*) manure (57% : 14%).

This comprehensive exploration of nitrogen content in *A. microphylla* enriches our understanding of *Azolla*'s nutritional dynamics. It provides a robust foundation for leveraging its potential as a nitrogen-rich organic fertilizer. Considering its diverse applications within sustainable agriculture, these findings underscore the imperative for tailored approaches in *Azolla* utilization.

Azolla is an Eco-friendly Fertilizer

The substantial nitrogen content inherent in *A. microphylla* positions it as a promising and sustainable alternative to synthetic fertilizers, especially urea. This attribute has the potential to significantly reduce our dependence on inorganic fertilizers and mitigate the environmental impacts associated with their production and application. The growth and decomposition rates exhibited by *A. microphylla* make it a highly valuable resource in green manure and biofertilizers, particularly in paddy fields, as highlighted by Roy et al. (2016). Previous studies have consistently highlighted *Azolla*'s nitrogen-fixing potential (Devaprakash et al., 2024; Yao et al., 2018). This symbiotic relationship allows *Azolla* to convert atmospheric nitrogen into a form readily available to plants, contributing significantly to nitrogen enrichment in the soil (Akhtar et al., 2021; Ssenku et al., 2022).

Based on findings from previous research, *Azolla* can be seamlessly integrated into agricultural practices through two primary approaches. The first approach involves directly incorporating *Azolla* into paddy fields. Studies indicate that this method effectively enhances nitrogen availability in the soil, resulting in improved plant growth. Simultaneously, it offers additional benefits, such as weed control and enhanced water management in agricultural fields (Borkar et al., 2023; Kimani, Bimantara, et al., 2020). On the other hand, the second approach entails a controlled fermentation process applied to *Azolla* before its introduction to agricultural fields. This fermentation process provides flexibility in modifying the composition of *Azolla* based on specific agricultural needs. Research outcomes suggest that this approach provides greater control over the nutritional properties of *Azolla*, offering options to include or exclude additional organic fertilizers as per diverse agricultural requirements (Seleiman et al., 2022). Integrating *Azolla* into agricultural practices through these two approaches provides farmers with flexible and adaptable options tailored to local conditions and specific crop needs. It underscores *Azolla*'s position as a reliable source of organic fertilizer that can be easily integrated into sustainable agricultural contexts.

Moreover, *A. microphylla* has demonstrated efficacy in improving crop yields. Studies by Ahmad et al. (2024) and G. P. Kumar et al. (2020) have shown positive effects on the growth and productivity of various crops when *Azolla* is integrated into agricultural practices. The nitrogen content in *A. microphylla*, especially in its fresh form, closely resembles that of urea, underscoring its potential as a sustainable nitrogen source (Jama et al., 2023). The use of *A. microphylla* as a fertilizer alternative aligns with sustainable agriculture principles. It addresses concerns related to the environmental consequences of conventional nitrogen-based fertilizers, such as groundwater contamination and greenhouse gas emissions (Adabembe et al., 2022; Akhtar et al., 2021). Furthermore, *Azolla* cultivation can be integrated into paddy fields, providing additional benefits such as weed suppression and enhanced water management (Ahmad et al., 2024; Hermawan et al., 2021).

Incorporating *A. microphylla* into agricultural systems as an environmentally friendly fertilizer alternative holds significant promise. The cumulative findings from various studies underscore its potential to contribute to sustainable agriculture, emphasizing the need for further research and practical applications to maximize its benefits.

CONCLUSION

This research contributes valuable insights to optimize *Azolla* cultivation, specifically *A. microphylla*, by revealing the intricate interplay between shading percentage and water depth. These factors influence temperature, light intensity, DO, and nutrient concentration, subsequently affecting *Azolla* growth. The findings not only advance our comprehension of *Azolla* cultivation but also foster ongoing research and the development of sustainable agricultural practices. Harnessing the nitrogen potential of *Azolla* presents an opportunity to contribute to an environmentally conscious and economically sustainable agricultural ecosystem, mitigating the environmental repercussions associated with synthetic fertilizers and reinforcing food security.

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