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Optimizing carbon bio-sequestration and biomass yield of setaria grass for net-zero goals in karst ecosystems

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Abstract

Addressing the global climate crisis necessitates innovative carbon sequestration strategies, particularly in marginal ecosystems such as karst regions characterized by low fertility and limited organic carbon content. This study evaluates the potential of *Setaria splendida* grass to enhance carbon bio-sequestration under varying doses of organic liquid fertilizer. Above- and below-ground biomass measurements were used to quantify total carbon storage. Fertilizer application significantly increased dry below-ground biomass (BGB), which accounted for most carbon stored, highlighting the critical role of root systems in carbon sequestration and soil improvement through root exudates. Additionally, increased above-ground biomass (AGB) contributes to carbon storage and supports local livestock by enhancing forage availability. Total carbon storage ranged from 7.34 to 37.26 tons/ha, depending on fertilizer dosage. These findings demonstrate the effectiveness of organic liquid fertilizer in optimizing carbon storage and biomass productivity, providing a scalable approach for restoring degraded ecosystems. This approach could be scaled to similar marginal ecosystems globally, offering dual benefits for carbon sequestration and sustainable agriculture.

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Keywords

Carbon bio-sequestration, *Setaria splendida* grass, Karst ecosystems, Organic fertilizer, Net-zero goals

Introduction

Since the industrial revolution, atmospheric CO_2 levels have risen by over 40%, triggering a cascade of adverse environmental impacts [1], including rising global temperatures, melting ice caps, and extreme weather events such as floods and droughts. As one of the most significant contributors to global greenhouse gas emissions [2], Indonesia ranks fourth globally in emissions when adjusted by GDP. The multifaceted impacts of climate change extend to biodiversity loss, sea level rise, and disruptions to agricultural productivity. In particular, unpredictable rainfall patterns and soil degradation exacerbate the vulnerability of farming regions, leading to significant erosion and loss of arable land. Globally, over 33% of soils are now degraded due to erosion and loss of organic carbon, further diminishing their capacity for carbon sequestration and making restoration efforts increasingly complex [2]. Indonesia's ambitious commitment to achieving net-zero carbon emissions by 2060 represents a critical turning point in addressing the global climate crisis. To meet this target, it is imperative to develop innovative, scalable, and region-specific strategies for carbon sequestration.

Among these, marginal ecosystems such as karst regions offer a unique challenge. They are typically avoided for agricultural or ecological purposes due to low fertility and limited organic carbon reserves [3]. In Indonesia, karst landscapes span approximately 154,000 km^2, covering diverse regions from Sumatra to Papua, with significant areas in Java, Sulawesi, and Kalimantan. Globally, karst regions account for around 20% of the Earth's terrestrial surface [4]. These areas are typically avoided for agricultural or ecological purposes; however, their challenging characteristics also present an intriguing opportunity for bio sequestration efforts. Planting vegetation in these regions enhances carbon sequestration and improves soil health as a valuable side effect, addressing long-standing issues of soil degradation and fragility [5]. These potential highlights the importance of deeper investigations into adaptive interventions tailored to karst ecosystems' unique conditions.

Carbon sequestration—capturing and storing atmospheric CO_2—is fundamentally driven by photosynthesis. During this process, plants convert atmospheric CO_2 and water into glucose and oxygen using sunlight as an energy source. *Setaria* grass, particularly *Setaria splendida*, plays a vital role in carbon bio sequestration through its efficient C4 photosynthetic pathway. This mechanism separates carbon fixation in mesophyll cells from the Calvin cycle in bundle sheath cells, enhancing CO_2 utilization. The chloroplast NADP-malate enzyme (NADP-ME) ensures a high CO_2 concentration near Calvin cycle enzymes, reducing photorespiration and maximizing carbon fixation under high light and nutrient-poor conditions [6], [7]. These traits make *Setaria splendida* highly effective for carbon storage and biomass production in degraded karst ecosystems, highlighting its ecological and agricultural significance. The general reaction for photosynthesis can be summarized as follows:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (1)$$

This biochemical mechanism supports plant growth and facilitates carbon transfer into biomass, with grasses like *Setaria splendida* playing a pivotal role in carbon bio sequestration. These grasses exhibit distinct ecological advantages, including rapid biomass production, extensive root systems, and remarkable resilience in nutrient-deficient and marginal environments [8]. The physiological and morphological traits of *Setaria splendida*, particularly its capacity to thrive in degraded soils, underscore its potential to contribute to ecosystem improvement by producing high biomass and carbon storage. While the agronomic applications of *Setaria splendida* have been studied, its role in carbon bio sequestration and potential contributions to rehabilitating marginal ecosystems such as karst regions remain inadequately explored, leaving a substantial gap in the existing body of literature.

The research focuses on the impact of variable treatments, including organic fertilizer dosages and irrigation regimes, on biomass productivity and carbon content. By employing an integrated approach that combines growth monitoring with biomass carbon quantification, this study aims to optimize carbon sequestration strategies designed explicitly for karst soils. A particular emphasis is placed on below-ground biomass (BGB), which contributes to carbon storage and is pivotal in improving soil structure, fostering microbial activity, and enhancing long-term ecosystem resilience. While not measured directly in this study, these functions highlight the broader ecological benefits of increasing biomass in karst soils [9]. This study operates on a conceptual framework that posits the synergistic effects of optimal organic liquid fertilizer application and controlled irrigation in enhancing both biomass productivity and carbon sequestration. The framework emphasizes the physiological adaptability of *Setaria splendida*, particularly its C4 photosynthesis and extensive root systems, which contribute to significant carbon storage and soil improvement.

Beyond addressing the practical aspects of carbon sequestration, this research contributes to the theoretical understanding of plant growth dynamics in marginal ecosystems. Systematically exploring *Setaria splendida* under controlled treatment conditions provides valuable insights into the interplay between plant growth and carbon dynamics. By elucidating these interactions, this study offers a robust framework for advancing sustainable land management practices in karst regions. Furthermore, the emphasis on scalable, low-cost, and ecologically sustainable solutions positions this research as a critical contribution to global efforts to mitigate climate change through innovative biosequestration techniques. The study hypothesizes that optimal fertilizer dosages and irrigation regimes will maximize biomass production and carbon storage, with BGB playing a dominant role in below-ground carbon sequestration. Additionally, the study expects that increasing biomass production will foster soil organic carbon (SOC) levels through root exudates, enhancing microbial activity and improving soil quality in karst ecosystems.

The overarching aim of this research is twofold: first, to quantify the carbon sequestered in the biomass of *Setaria splendida* under varying treatment conditions, and second, to evaluate the growth performance of *Setaria splendida* in marginal karst ecosystems. While this study does not directly assess soil quality improvements, it highlights the ecological benefits of increased biomass in degraded landscapes.

Materials and Method

Experimental design

The experiments were conducted using reactors designed to simulate natural karst conditions, including planter bags with a diameter of 40 cm and polybags with a diameter of 30 cm. The study was carried out over a two-month planting period to observe the effects of treatments on *Setaria splendida*. The experimental design included two levels of watering intensity and six dosages of organic liquid fertilizer, following a factorial design to assess the interactions between these variables (Table 1).

Table 1. Experimental design detailing the variations in fertilizer doses and watering intensities applied to *Setaria splendida* during the two-month study period

Variations Code	Fertilizer Doses (ml)	Watering Intensity (ml/day)
P0	0	235
P11	1.5	235
P12	2	235
P13	2.5	235
P14	3	235
P15	3.5	235
P16	4	235
P21	1,5	700
P22	2	700
P23	2.5	700
P24	3	700
P25	3.5	700
P26	4	700

This study's novelty lies in using reactors to simulate karst conditions, allowing precise control over environmental variables such as watering and fertilizer application. This approach provides insights into the carbon sequestration potential of *Setaria splendida* under conditions that mimic degraded karst soils.

Materials

Uniform *Setaria splendida* seedlings were used, weighing 100 g and measuring 40 cm in length (Figure 1). The same type of organic liquid fertilizer was applied across all variables, ensuring consistency. The planting medium was standardized across all reactors, and karst rocks sourced from Gunung Kidul, Yogyakarta, were used to replicate natural conditions. Watering was performed daily using a measuring cup to maintain precision. Additional equipment included a measuring tape, analytical balances for accurate biomass measurements, drying ovens for processing plant samples, blenders for preparing biomass powder, soil samplers for collecting substrate samples, and soil moisture sensors to monitor reactor conditions. All equipment and methodologies were calibrated and validated to ensure the reliability and reproducibility of results.

Plant growth assessment

Plant growth parameters were monitored daily to evaluate development, including leaf length, width, clump diameter, and the emergence of new tillers. Among these, leaf length exhibited the most significant changes, which was emphasized in the results. Measurements were conducted using a measuring tape for accuracy. Data were systematically recorded daily to ensure consistent treatment monitoring and analyzed to observe growth trends.

Figure 1. Experimental setup simulating karst ecosystem conditions using *Setaria splendida* in controlled reactors

Biomass sampling and analysis

Above-ground biomass (AGB) and below-ground biomass (BGB) were collected separately to evaluate biomass production. AGB was dried in an oven at 70°C for 48 hours to determine moisture content and then weighed to calculate dry biomass. BGB was collected by excavating the root system within each reactor, washed to remove soil particles, dried similarly to AGB, and ground into a fine powder using a blender for subsequent carbon content analysis. The carbon content of dry BGB was measured using a gravimetric method involving the following steps: porcelain crucibles were first weighed empty, then filled with homogenized biomass powder, and dried at 105°C for 3 hours to reach a constant weight. Subsequently, the samples were heated in a furnace at 300°C for 1.5 hours and then at 550°C for 2.5 hours, with weights recorded after each stage. The carbon content was calculated based on weight loss at each temperature stage, following standard gravimetric equations for organic carbon determination.

Results and Discussion

Vegetative growth

Daily monitoring of *Setaria splendida* growth revealed significant differences in leaf length, leaf width, clump diameter, and the emergence of new tillers across treatments. Variations in organic liquid fertilizer dosage and watering intensity were observed to influence the vegetative growth of *Setaria splendida*. Among the parameters measured, leaf length showed the most dynamic and prominent changes. For instance, Treatment P14 exhibited the highest total leaf length increase of 71 cm over the 8 weeks (Table 2). Interestingly, the most significant leaf length increase did not come from the treatment with the highest fertilizer dosage, highlighting the intricate balance required in nutrient management to optimize plant growth. Table 2 illustrates the total leaf length increase across treatments, highlighting the variations observed over the eight weeks.

Table 2. Total leaf length increase across treatments

Variations Code	Total Leaf Length Increase (cm)
P0	52.6
P11	54.3
P12	61.3
P13	68.2
P14	71
P15	56.4
P16	59.8
P21	35.7
P22	58.8
P23	43.4
P24	45.2
P25	55
P26	55.9

The increased leaf length and clump diameter observed in Treatment P14 can be attributed to the balance between moderate fertilizer application and optimal watering intensity, which supported enhanced photosynthesis and nutrient uptake [10], [11]. However, excessive organic liquid fertilizer application can disrupt the nutrient balance, reducing plant growth [12]. The influence of watering intensity further highlights the delicate interplay between soil oxygen availability and root health. Treatments simulating moderate rainfall supported better growth than those mimicking high rainfall conditions. This result can be linked to limited drainage within the reactors, as excess water trapped in the growing medium reduced oxygen availability in the root zone, thereby impairing root respiration and microbial activity [13]. Conversely, balanced watering regimes, such as the 235 ml/day treatment, maintained healthy root systems by ensuring adequate oxygen levels, promoting efficient nutrient absorption, and enhancing vegetative growth.

Biomass yield and distribution

The variations applied to the 13 plants in this study directly influenced the biomass produced. After 8 weeks, the plants were harvested simultaneously by uprooting them. This process yielded two categories of biomass: above-ground biomass (AGB), which includes leaves, and below-ground biomass (BGB), which provides for roots within the growing medium and extending into karst rocks. The harvested biomass was separated into these categories and oven-dried to determine moisture content and dry weight. Table 3 provides a detailed breakdown of dry biomass distribution, emphasizing the dominance of BGB in specific treatments.

For *Setaria splendida*, the moisture content of AGB was found to be 59%, while BGB had a moisture content of 27%. The dry biomass was calculated per unit area, with all clumps having an approximate area of 0.01 m^2. The biomass yield showed a trend similar to leaf length growth, with Treatment P14 producing the highest biomass among all treatments. This indicates that the growth rate of the plants correlates directly with biomass yield [14]. A notable finding was the dominance of BGB, which accounted for up to 60%-80% of the total biomass weight in some cases. The findings highlight the role of BGB as a critical component of carbon sequestration in karst ecosystems. Dominant BGB supports plant stability and contributes significantly to underground carbon storage [15]. As roots decompose, they enhance soil organic carbon (SOC) levels, a process supported by root exudates that stimulate microbial activity [16]. These microbial interactions play a vital role in nutrient cycling, releasing essential nutrients such as nitrogen and phosphorus in plant-available forms [17], [18], [19]. The extensive root system observed in Treatment P14 further indicates the plant's potential to mitigate the challenges of karst ecosystems, offering a sustainable strategy for biomass production and carbon sequestration in marginal soils.

Table 3. Dry Biomass Distribution Across Treatments.

Variation Code	AGB (gr/area)	BGB (gr/area)	BGB-karst (gr/area)	Total Biomass (gr/area)
P0	25.20	30.00	29.40	84.60
P11	26.00	37.00	33.20	96.20
P12	29.93	29.20	35.69	94.82
P13	30.00	38.00	37.80	105.80
P14	35.70	41.25	105.00	181.95
P15	28.00	24.50	58.80	111.30
P16	31.20	31.50	93.50	156.20
P21	29.24	49.00	17.00	95.24
P22	31.50	29.60	38.25	99.35
P23	34.40	36.50	54.60	125.50
P24	29.64	37.00	59.50	126.14
P25	32.00	30.00	42.50	104.50
P26	32.25	40.70	42.00	114.95

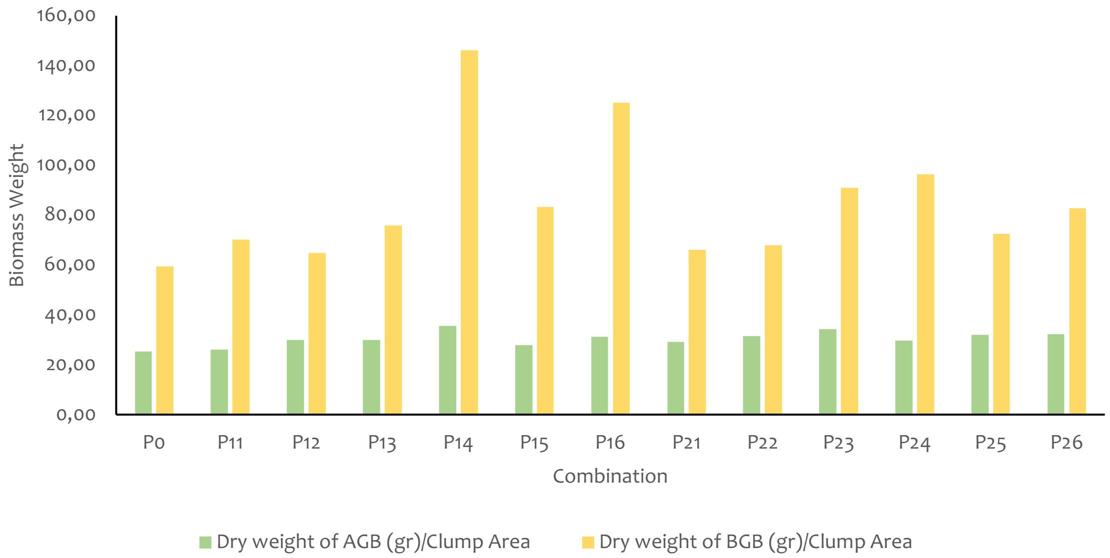

Figure 2. Biomass Production

The highest biomass production (see Figure 2) was not associated with the highest organic liquid fertilizer dosage, highlighting the importance of maintaining a nutrient balance. Excessive organic liquid fertilizer application can disrupt nutrient equilibrium [20], where high nitrogen levels may lead to ammonium toxicity and reduced microbial efficiency.

Carbon sequestration potential

Measuring carbon in biomass is a critical initial step in determining the carbon sequestration capacity of plants [21]. Carbon fractions in above-ground biomass (AGB) and below-ground biomass (BGB) vary across different plant species and parts [22]. In this study, dried AGB and BGB of *Setaria splendida* were processed into powder and analyzed using the gravimetric method, revealing carbon fractions of 32.03% for AGB and 30.58% for BGB. These fractions were then used to calculate the amount of carbon sequestered by multiplying the carbon fraction with the dry biomass weight. The molecular mechanisms underlying carbon absorption and storage in *Setaria splendida* are reflected in the experimental results. The dominance of BGB, contributing up to 60–80% of the total biomass weight in several treatments, underscores its critical role in carbon storage. The efficient C4 photosynthesis pathway ensures high carbon fixation rates, with significant carbon deposition into the root system. Root exudates further enhance microbial activity, stabilizing soil organic carbon (SOC) and supporting long-term carbon retention in karst soils. The porous structure of karst allows deeper root penetration, as observed in treatments like P14, where optimal fertilizer and irrigation facilitated maximum biomass and carbon storage

The initial biomass of *Setaria splendida* seedlings used in the experiment weighed 100 g, with an AGB carbon content of 32.03%, corresponding to an initial carbon stock of 18.90 g. The carbon sequestered in biomass correlates directly with the biomass produced during the experiment. The results also highlight that the sequestered carbon can be projected for larger-scale applications. For instance, converting the clump area of approximately 0.01 m^2 to a hectare. Table 4 summarizes the estimation of the carbon sequestration potential of *Setaria splendida* biomass, showcasing its applicability for large-scale implementation.

Table 4. Estimation of Biomass Carbon Sequestration

Variation Code	AGB Carbon (Ton/ha)	BGB Carbon (Ton/ha)	Total Carbon (Ton/ha)	Carbon Sequestered (Ton/ha)
P0	8.07	18.16	26.24	7.34
P11	8.33	21.47	29.79	10.90
P12	9.59	19.84	29.43	10.53
P13	9.61	23.18	32.79	13.89
P14	11.43	44.72	56.16	37.26
P15	8.97	25.47	34.44	15.54
P16	9.99	38.23	48.22	29.32
P21	9.37	20.18	29.55	10.65
P22	10.09	20.75	30.84	11.94
P23	11.02	27.86	38.88	19.98
P24	9.49	29.51	39.00	20.11
P25	10.25	22.17	32.42	13.52
P26	10.33	25.29	35.62	16.72

The carbon sequestration potential of *Setaria splendida* aligns directly with its biomass yield, highlighting the critical role of BGB in long-term carbon storage and its importance as a stable carbon reservoir in ecosystems with high porosity and low nutrient availability (Figure 3). The dominance of BGB not only enhances underground carbon reservoirs but also mitigates carbon loss through utilization as forage and decomposition compared to AGB. Projecting the carbon sequestration potential to a hectare scale reveals promising implications for large-scale applications. The ability of *Setaria splendida* to sequester significant amounts of carbon in marginal karst ecosystems, coupled with its adaptability and rapid growth, underscores its role as a viable solution for enhancing ecosystem resilience and contributing to global carbon management efforts.

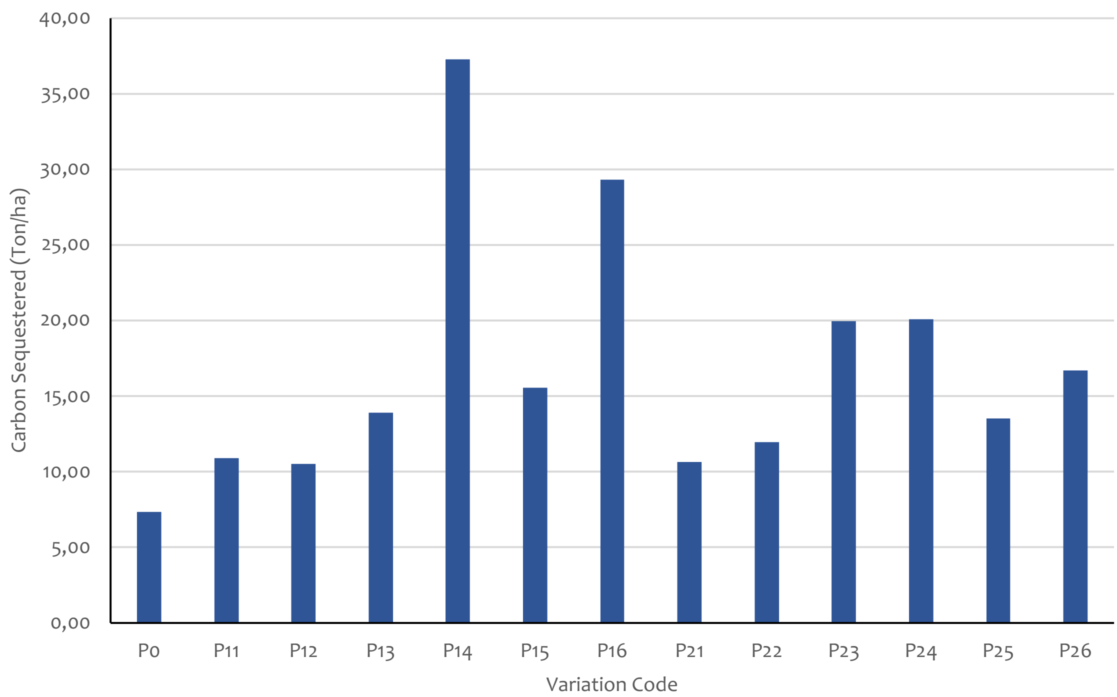

Figure 3. Estimated carbon sequestered on biomass

Water use efficiency (WUE)

Water use efficiency (WUE) is a critical measure in agriculture, particularly for biomass grasses, as it indicates the amount of biomass produced per unit of water used [23]. The WUE for different types of biomass grasses can vary significantly based on species, environmental conditions, and management practices [24]. Water Use Efficiency (WUE) was calculated to determine how effectively *Setaria splendida* converted water into biomass under different treatments. The WUE was derived by dividing the total biomass yield (dry weight) by the total water applied over the 8 weeks. For example, treatments with moderate irrigation, such as P1 (235 ml/day), used 14.10 liters over the experimental duration. In contrast, treatments with higher irrigation intensity, such as P2 (700 ml/day), used 42 liters. The results indicated that moderate irrigation (235 ml/day) coupled with moderate fertilizer dosage (3 ml/L, P14) achieved the highest WUE of 12.90 g/L (Table 5). Conversely, treatments with excessive irrigation (e.g., 700 ml/day) showed lower WUE, likely due to waterlogging effects that reduced biomass production efficiency. Table 5 demonstrates the WUE values across treatments, linking water availability to biomass production.

Table 5. Water Use Efficiency

Variations Code	Water Use Efficiency (g/L)
P0	6.00
P11	6.82
P12	6.72
P13	7.50
P14	12.90
P15	7.89
P16	11.08
P21	2.27
P22	2.37
P23	2.99
P24	3.00
P25	2.49
P26	2.74

Moderate irrigation supported optimal root respiration and microbial activity, facilitating nutrient uptake and biomass growth [25]. In karst soils, where porosity leads to rapid water drainage, moderate irrigation balances water availability with soil oxygenation, ensuring sustainable growth conditions—the importance of balanced water availability for maximizing plant productivity in porous soils. Excessive irrigation reduced WUE, underscoring the risks of waterlogging, which impairs oxygen availability in the root zone. In contrast, moderate irrigation balanced water supply and root aeration, enhancing plant growth and carbon allocation efficiency.

Root and Karst interaction

Root systems of *Setaria splendida* were observed to penetrate deeply into the karst rocks through polybag openings. This extensive root architecture facilitated water and nutrient absorption from deeper soil layers, compensating for the limited fertility of the growing medium. Figure 4 visually represents the root system extending into the karst substrate.

Figure 4. Root system extending into karst

The dominance of BGB observed in this study reflects the plant's adaptive strategy to thrive in nutrient-poor, high-porosity soils [26]. Karst ecosystems, characterized by their limited organic content and rapid nutrient leaching, create a challenging environment where root systems must penetrate deeply to access water and nutrients. Root interactions with karst substrates promote microbial activity, facilitating organic matter decomposition and releasing essential nutrients such as nitrogen and phosphorus [27]. These interactions are significant in karst soils, where microbial communities maintain soil fertility despite harsh environmental conditions. This interaction underscores the plant's role in biomass production and ecosystem restoration.

Conclusion

This study demonstrates the adaptability of *Setaria splendida* in marginal karst ecosystems, showing its dual role in enhancing biomass production and carbon sequestration. The most effective treatment, combining moderate irrigation (235 ml/day) and organic fertilizer dosage (3 ml/L, Treatment P14), achieved the highest biomass yield, the most significant water uses efficiency (9.68 g/L), and the maximum carbon sequestration potential of 37.26 tons of carbon per hectare. The presence of *Setaria splendida* in karst soils offers the possibility to enhance soil fertility over time. The extensive below-ground biomass (BGB) stabilizes the porous karst structure, contributes organic matter, and promotes nutrient cycling, creating opportunities for improving the overall soil quality in these marginal ecosystems. For future research, it is recommended to include the measurement of soil organic carbon (SOC) to provide a more comprehensive understanding of the plant's impact on soil health and biomass carbon sequestration.

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