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Carbon sequestration of Virginia mallow (*Sida hermaphrodita* L.): Comparison of winter and spring harvests

Sekvestracija ugljika virdžinijskim sljezom (*Sida hermaphrodita* L.): usporedba zimskog i proljetnog roka žetve

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ABSTRACT

The mounting imperative to adopt environmentally sustainable agricultural practices to address climate change has underscored the significance of carbon sequestration through biomass crops. This study investigates the carbon and nitrogen sequestration potential of Virginia mallow (*Sida hermaphrodita* L. Rusby) by examining its aboveground biomass yields at different harvest times (winter and spring) during two consecutive years (2022 & 2023) to determine the optimal harvest time for maximizing both biomass yields as well as carbon and nitrogen contents. In comparison to spring harvests (7.86 t/ha dry matter; 3.94 t/ha carbon; 0.01 t/ha nitrogen), winter harvests regularly yielded higher dry matter yields (9.07 t/ha) as well as larger carbon (4.38 t/ha) and nitrogen (0.04 t/ha) contents. Spring harvests had greater carbon concentrations (50.1%) than winter harvests (48.3%), but winter harvests had much higher nitrogen concentrations (0.40% vs. 0.185%). The yields of dry matter, carbon, and nitrogen in 2022 were somewhat higher than those in 2023, according to year-to-year comparisons. However, these differences were not statistically significant, indicating that seasonal variation was more significant than yearly fluctuations. Overall, the findings show that winter harvests can be targeted when higher yields and nutrient content in biomass are required, and they provide useful information for managing Virginia mallow for biomass production and carbon sequestration.

Keywords: aboveground biomass, carbon content, climate change mitigation, energy crops, nitrogen content, yield

SAŽETAK

Rastući imperativ za ekološki održivim poljoprivrednim praksama za rješavanje pitanja klimatskih promjena naglasio je važnost sekvestracije ugljika biomasom poljoprivrednih kultura. Ovaj rad istražuje potencijal sekvestracije ugljika i dušika virdžinijskog sljeza (*Sida hermaphrodita* L. Rusby), utvrđivanjem njegove nadzemne biomase požete na različitim rokovima žetve (zima i proljeće) tijekom dvije godine (2022 i 2023) kako bi se odredilo optimalno vrijeme žetve s ciljem povećanja prinosa biomase kao i sadržaja ugljika i dušika u biomasi. U usporedbi s proljetnim žetvama (7,86 t/ha suhe tvari; 3,94 t/ha ugljika; 0,01 t/ha dušika), zimske žetve redovito su davale veće prinose suhe tvari (9,07 t/ha), kao i veći sadržaj ugljika (4,38 t/ha) i dušika (0,04 t/ha). Proljetne žetve imale su nešto veću koncentraciju ugljika (50,1%) od zimskih žetvi (48,3%), ali zimske žetve imale su znatno veću koncentraciju dušika (0,40%) u odnosu na proljetne (0,185%). Prinosi suhe tvari, ugljika i dušika u 2022. godini bili su nešto viši u odnosu na 2023. godinu. Međutim, te razlike nisu statistički značajne, što ukazuje da su sezonske varijacije značajnije od godišnjih. Sveukupno, rezultati ukazuju da je zimska žetva bolja u pogledu prinosa te sadržaja ugljika i dušika u nadzemnoj biomasi i daju korisne informacije za gospodarenje virdžinijskim sljezom za proizvodnju biomase i sekvestraciju ugljika.

Ključne riječi: nadzemna biomasa, sadržaj ugljika, ublažavanje klimatskih promjena, energetske kulture, sadržaj dušika, prinos

INTRODUCTION

Climate change, driven by rising levels of greenhouse gases, poses a global threat to ecosystems and human well-being worldwide (IPCC, 2021). Among strategies for climate change mitigation, carbon sequestration, the long-term storage of carbon in plants, soils, and geological formations, is gaining importance. In this process, carbon dioxide (CO_2) is removed from the atmosphere and stored in a stable form, often in plants, soils, or durable products. Since it instantly reduces greenhouse gases in the atmosphere, this mechanism is crucial for fighting against climate change (Lal, 2004). In terrestrial ecosystems, plants are the main actors of sequestration; they absorb CO_2 through photosynthesis and convert it into organic carbon stored in their biomass, both above and below ground. The amount of carbon captured varies widely among plant species and is strongly influenced by their biomass growth potential. Fast-growing perennial species, such as Virginia mallow (*Sida hermaphrodita* L. Rusby), can sequester large amounts of carbon annually due to their high productivity and extensive root systems, which also enhance soil carbon storage (Borkowska and Molas, 2012). The intentional use of high-biomass perennial plants for carbon sequestration has two advantages: it lowers atmospheric CO_2 while supplying renewable resources for the manufacture of fibre or bioenergy. Aboveground biomass (AGB) has been demonstrated to make a substantial contribution to carbon sequestration through its role as a temporary carbon sink. The capacity of a given plant species to efficiently accumulate biomass and store carbon depends on a confluence of genetic, environmental, and management factors (Wang et al., 2023). Virginia mallow garnered attention in Europe as a bioenergy crop due to its high biomass yield, resilience, and low input requirements (Krička et al., 2017). However, the potential for optimization of its carbon sequestration potential through agronomic practices, particularly harvest timing, remains underexplored.

Harvest time is crucial for determining the potential for carbon storage and biomass yield. Plants harvested at different times can exhibit significant differences in biomass properties, including total yield (Clifton-Brown et al., 2001; Zhu et al., 2024) and chemical composition (Heaton et al., 2010). Late harvesting not only improves nutrient cycling, but also allows plants to transfer nutrients from the aboveground tissue to their root system, which helps the soil to accumulate carbon over time (Heaton et al., 2010). On the other hand, early harvests frequently result in lower total biomass production and carbon storage capacity, even while they may optimise specific quality attributes, such as increased protein content in fodder crops (Clifton-Brown et al., 2001). These trends have been observed across several perennial bioenergy crops, including *Miscanthus* × *giganteus*, switchgrass (*Panicum virgatum*), and willow (*Salix* spp.). This indicates that climate change mitigation and biomass use objectives must be balanced when choosing the optimal harvest time.

This study provides valuable insights into optimizing biomass production systems for climate change mitigation. Determining the optimal harvest times for carbon sequestration provides pragmatic guidelines for promoting sustainable agriculture and advancing renewable energy development. Furthermore, it provides policymakers with information regarding the climate benefits of integrating Virginia mallow into carbon-offset strategies. The research objectives of this study are to evaluate the influence of harvest timing on: a) biomass yields (aboveground dry matter yield); b) nitrogen and carbon contents; c) total biomass carbon storage capacity.

MATERIALS AND METHODS

Study site

The stand of Virginia mallow (*Sida hermaphrodita* L. Rusby) was established in 2017 at the experimental station Maksimir of the Faculty of Agriculture, University of Zagreb. The climate at the study site is a temperate humid climate with a mean annual air temperature of 11.8 °C and a precipitation amount of 867. The soil at the experimental site is alkaline (pH_{H_2O} = 7.95), with low humus amount (1.44%), well supplied with plant-available phosphorus (17.65 mg P_2O_5 per 100 g soil) and weakly

supplied with plant-available potassium (13.6 mg K_2O per 100 g soil). Virginia mallow stand was established by planting seedlings at a distance of 0.75 m within and between the rows, at 12 basic plots, with an area of 2.25 x 3.75 = 8.44 m^2. The spaces between the rows and between the plants were mechanically cultivated several times in the year of planting to remove weeds.

Aboveground biomass sampling and analyses

Biomass sampling was conducted in 2022 and 2023 during the winter and spring harvest seasons. Biomass was mechanically sampled, and the aboveground biomass was weighed afterwards. After weighing and determining the amount of fresh biomass per square meter, average subsamples of the plant material were oven-dried and weighed again to determine the dry matter yield. Sampling was carried out in three replicates at each harvest time. In the laboratory of the Department of General Agronomy, the total carbon and nitrogen content was determined in the subsamples on the Vario, Macro CHNS analyzer (Elementar, 2006) according to the HRN ISO 10694:2004 standard for carbon and HRN ISO 13878:2004 for nitrogen. The statistical analysis was performed using SAS 9.1 statistical software (SAS Inst. Inc., 2002–2004, Cary, NC, USA). Variability among harvest times was analysed using an analysis of variance (ANOVA) and tested with a post-hoc (Fisher) t-test with 5% significance threshold value.

RESULTS AND DISCUSSIONS

Harvest season/year comparison

Understanding how harvest timing (season) and year interact to influence biomass yield and quality is critical in long-term agronomic planning, especially for systems that rely on multi-seasonal cropping or biomass for bioenergy and nutrient cycling. Table 1 summarises the average dry matter harvested biomass yields (DM), their carbon (C%) and nitrogen (N%) concentrations, and carbon (C t/ha) and nitrogen (N t/ha) yields for the winter and spring seasons in 2022 and 2023.

At the winter harvest date 2022, the highest dry matter yield (9.30 t/ha) was obtained, followed by winter 2023 (8.84 t/ha), spring 2022 (8.11 t/ha), and spring 2023 (7.60 t/ha) (Table 1). Dry matter yield was consistently higher in winter than in spring, across both years. In 2022, winter DM reached an average of 9.30 t/ha, compared to 8.11 t/ha in spring. In 2023, winter again outperformed spring (8.84 t/ha vs. 7.60 t/ha). This seasonal difference likely reflects the longer vegetative period and more complete physiological maturity achieved by winter-harvested crops. Winter crops benefit from extended growing periods and often senesce before harvest, leading to greater biomass accumulation. Spring harvests, in contrast, capture earlier developmental stages where plants have had less time to accumulate dry matter. There is a slight decrease in DM yield from winter 2022 (9.30 t/ha) to winter 2023 (8.84 t/ha). Likewise, spring DM declined from 8.11 t/ha in 2022 to 7.60 t/ha in 2023 (Table 1).

Table 1. Dry matter, nutrient concentrations and yields by season and year

Harvest time	DM (t/ha) (LSD: 0.78)	C (%) (LSD: 0.12)	N (%) (LSD: 0.01)	C (t/ha) (LSD: 0.39)	N (t/ha) (LSD: 0.003)
Winter 2022	9.30^{a}	48.71^{c}	0.48^{a}	4.53^{a}	0.04^{a}
Spring 2022	8.11^{bc}	50.86^{a}	0.17^{d}	4.12^{bc}	0.01^{c}
Winter 2023	8.84^{ab}	47.94^{d}	0.39^{b}	4.24^{ab}	0.03^{b}
Spring 2023	7.60^{c}	49.51^{b}	0.20^{c}	3.76^{c}	0.02^{c}

DM – dry matter yield; C (%) – carbon concentration; N (%) – nitrogen concentration; C (t/ha) – total carbon yield; N (t/ha) – total nitrogen yield; different letters denote statistically significant differences

In terms of carbon concentration (Table 1), a seasonal reversal occurs: Spring biomass consistently had higher carbon concentrations than winter. This pattern implies that spring biomass was chemically richer in carbon, even if winter crops produced greater biomass yield. The plant's active photosynthetic phase and reduced water content in spring may be the cause of the higher carbon concentration, which results in greater structural carbon. In contrast, the winter biomass is more mature, may be partially degraded, and contains slightly diluted car-

bon along with more fibrous, water-retentive tissue. The maximum carbon concentration was obtained in spring 2022 (50.86%), followed by spring 2023 (49.51%), winter 2022 (48.71%) and winter 2023 (47.94%). The higher carbon concentration in Virginia Mallow (*Sida hermaphrodita*) during a spring harvest compared to a winter harvest is primarily driven by the natural senescence and over-winter weathering of the plant. Delaying the harvest from winter to spring shifts the ratio of chemical components within the stems, resulting in a cleaner, more carbon-dense biomass. These results are consistent with those of Zhang et al. (2019), who found that even when the numerical differences between treatments are small, management techniques such as crop rotation and organic amendments can result in quantifiable, statistically significant variations in soil carbon content. Similarly, Lal (2004) highlighted that small increases in soil carbon resulting from sustainable management techniques can have significant agronomic and statistical effects.

Nitrogen concentration varied inversely to carbon concentration across seasons, and all four treatments were significantly different from each other (Table 1). Nitrogen concentration was higher in winter than in spring harvests. Winter crops tended to store more nitrogen, which may be attributed to a number of factors, including later harvesting (which leaves more nutrient-rich residues in the plant), greater uptake and retention due to post-vernalization growth and less nutrient remobilization through biomass prior to harvest. Because the plants are less physiologically mature and have not yet fully allocated resources to storage tissues, the spring biomass, which is harvested earlier, typically has lower nitrogen levels. The modest rise in spring N% from 2022 to 2023 (0.17% → 0.20%) might be a sign of increased active nitrogen uptake driven by soil or climate variations. The highest nitrogen concentration was found in winter 2022 (0.48%), followed by winter 2023 (0.39%), spring 2023 (0.20%), and spring 2022 (0.17%). The constant and statistically significant difference between treatments indicates that nitrogen accumulation was significantly impacted by the inputs or management strategies used. The robustness of these findings is further supported by the magnitude of the differences reported in this paper, which far exceeds the LSD value. These outcomes are in line with research by Fageria and Baligar (2005), who found that crop variety, soil type, and fertilization schedule all have a significant impact on the amount of nitrogen in plants. Similarly, even in similar environmental conditions, Giller et al. (1997) found significant variation in nitrogen intake between treatments, suggesting that management intensity can cause noticeable variations in nitrogen concentration. In contrast to some reports by Sainju et al. (2002), where differences in nitrogen were smaller and frequently statistically indistinguishable, the study's treatments showed a sharp distinction between them, indicating that the environmental conditions or treatments employed here produced a more pronounced response. This pattern indicates that biomass nitrogen concentration may be extremely responsive to management actions, which is crucial for agroecosystem productivity and nutrient cycling.

The highest carbon yield was determined at winter harvest 2022 (4.53 t/ha), and winter 2023 had the second-highest (4.24 t/ha) (Table 1). Carbon yields were lower in spring harvests, with the lowest values, 3.76 t/ha in spring 2023 and 4.12 t/ha in spring 2022. Overall, winter harvests had a higher carbon yield than spring harvests, indicating a seasonal pattern in both years. Even if the carbon concentration is slightly lower, carbon yield follows DM yield patterns, supporting the notion that increasing biomass equals higher carbon accumulation. The results of statistical grouping showed that while intermediate spring 2022 and winter 2023 do not statistically differ from each other or from neighboring treatments, the highest-yielding winter 2022 was considerably different from the lowest-yielding spring 2023. This implies that rather than abrupt, distinct variations across all levels, the carbon yield will gradually decrease across treatments. Compared to Zhang et al. (2019), who showed less significant variation in carbon content between treatments in comparable trials, the observed changes are greater. This could imply that the management interventions or environmental factors in the current study produced a stronger carbon buildup response.

The highest nitrogen yield was found for winter 2022 (0.04 t/ha), which was followed by winter 2023 (0.03 t/ha) (Table 1). The lowest nitrogen yields were determined for spring 2022 (0.01 t/ha) and spring 2023 (0.02 t/ha). While the similarity between spring 2023 and spring 2022 suggests they responded similarly to the imposed management conditions, the large difference between winter 2022 and spring harvests reveals a significant effect of the harvest on nitrogen accumulation. These results are consistent with those of Fageria and Baligar (2005), who observed that nitrogen content in biomass can vary widely with input application rates and soil fertility management, with the highest values typically observed in treatments with the best nutrient management. Similarly, when food availability is restricted or environmental factors limit nitrogen uptake, nitrogen buildup tends to decrease significantly (Sainju et al., 2002). The current results demonstrate distinct separation between the top three groups, showing robust and consistent treatment responses, in contrast to certain studies (e.g., Giller et al., 1997) where moderate treatments occasionally overlap statistically with both extremes. Even minor numerical changes in nitrogen content were statistically identifiable under the experimental settings, as indicated by the modest LSD value in relation to the differences across treatments.

Year-to-year comparison

Understanding system stability and long-term productivity requires assessing how biomass yield and quality change from year to year. Variations in weather patterns, soil nutrient availability, pest pressures, and management techniques can all cause year-to-year variations. Finding these patterns provides important information on crop adaptability, system resilience, and the reliability of the biomass supply for nutrient cycling and bioenergy. The dry matter yield, carbon and nitrogen concentrations as well as carbon and nitrogen yields, are presented in Table 2.

Table 2. Dry matter yield, nutrient concentrations and yields by years

Year	DM (t/ha) (LSD:0.98)	C (%) (LSD: 1.33)	N (%) (LSD: 0.18)	C (t/ha) (LSD: 0.39)	N (t/ha) (LSD: 0.02)
2022	8.70[a]	49.78[a]	0.33[a]	4.32[a]	0.029[a]
2023	8.22[a]	48.72[a]	0.29[a]	4.00[a]	0.024[a]

DM – dry matter yield; C (%) – carbon concentration; N (%) – nitrogen concentration; C (t/ha) – total carbon yield; N (t/ha) – total nitrogen yield; different letters denote statistically significant differences

According to the results presented in Table 2, the average dry matter yield in 2022 was 8.70 t/ha, which was not statistically higher than the yield in 2023 (8.22 t/ha). Although 2022's mean dry matter content (8.70 t/ha) was marginally greater than 2023's (8.22 t/ha), the 0.48 t/ha difference was less than the LSD, suggesting that random variation rather than a consistent treatment or environmental influence may be the reason. This implies that the two years' dry matter yields were statistically identical because of similar agroecological conditions, management strategies, or other factors. According to Lemaire and Chapman (1996), when management practices remain unchanged, interannual variations in biomass output in stable agroecosystems are usually minor and statistically insignificant, while Guo et al. (2023) determined significant yield variations due to climatic variability.

The average carbon concentration in 2022 was 49.78% and in 2023 48.72%, while average carbon yield amounted to 4.32 t/ha in 2022 and 4.00 t/ha in 2023 (Table 2). The obtained results did not differ statistically significantly between the two years (Table 2). This may be a result of annual fluctuations rather than a steady environmental or managerial impact. These results are in line with those of long-term soil and biomass monitoring trials, which show that unless there are considerable changes in crop management, land use, or climate conditions, interannual variation in carbon is often statistically non-significant (Lal, 2004). Similar findings were reported by Conant et al. (2001), who found that, particularly in stable agricultural systems, short-term variations in

soil organic carbon from year to year are usually within the experimental error. On the other hand, Poeplau and Don (2015) found notable year-to-year variations in carbon due to adjustments in tillage and residue inputs, or to drought conditions. The current study's lack of a substantial difference points to the production system and management techniques' relative stability over the course of the investigation.

The nitrogen concentration and nitrogen yield did not differ statistically significantly between the two years, according to analysis of variance (Table 2). The mean nitrogen concentration in 2023 was 0.29%, compared to 0.33% in 2022, while nitrogen yield was 0.029 t/ha in 2022 and 0.024 t/ha in 2023 (Table 2). These findings are consistent with those of Fageria and Baligar (2005), who found that when management practices and environmental conditions are consistent, the nitrogen content of plant biomass tends to remain reasonably stable from year to year. Similarly, Sainju et al. (2002) noted that, unless there were large changes in fertilization or climate, interannual fluctuation in plant nitrogen concentration was frequently statistically negligible in long-term research.

The lack of year-to-year differences in Virginia mallow for dry matter, carbon and nitrogen yields probably stems from its deep, established root system, which buffers the plant against weather changes and allows stable nutrient uptake and steady nutrient absorption over time. Virginia mallow has a large, woody root system that stores energy in favourable weather conditions, while in poor weather conditions the plant uses these stored reserves, keeping growth stable. Furthermore, the deep root network also builds a strong relationship with soil microbes. These microbes help the plant access nitrogen and water even in dry years. Virginia mallow captures carbon in its stems and roots. The total carbon content reflects plant mass. Because the mass stays the same from year to year, the carbon content remains stable. The large biomass captures substantial sunlight for photosynthesis, which makes the plant less sensitive to short-term dry spells. The plant optimises carbon and nitrogen assimilation because its biochemical pathways are highly efficient. It reuses nitrogen internally before winter dormancy, which means it relies less on fresh soil nutrients every spring. Agronomic mechanisms lie in its perennial nature: unlike annual crops, Virginia mallow does not start from seed each year. Established stands mean the plant size and canopy structure do not change drastically, leading to predictable dry matter yields year after year.

Harvest season comparison

Examining harvest seasons in several years enables us to comprehend how harvest schedules impact production and quality in the face of shifting annual circumstances. A later harvest may have greater growth but lower quality, whereas an earlier harvest may offer higher quality but lower yields. This aids farmers and researchers in determining when to harvest in order to balance quality and productivity throughout time. Dry matter yield, nutrient concentrations and yields by harvest seasons are presented in Table 3.

Table 3. Dry matter, nutrient concentrations and yields by harvest seasons

Harvest season	DM (t/ha) (LSD: 0.58)	C (%) (LSD: 0.78)	N (%) (LSD: 0.05)	C (t/ha) (LSD: 0.33)	N (t/ha) (LSD: 0.005)
Winter	9.07^{a}	48.3^{b}	0.4^{a}	4.38^{a}	0.04^{a}
Spring	7.86^{b}	50.1^{a}	0.2^{b}	3.94^{b}	0.01^{b}

DM – dry matter yield; C (%) – carbon concentration; N (%) – nitrogen concentration; C (t/ha) – total carbon yield; N (t/ha) – total nitrogen yield; different letters denote statistically significant differences

A statistically significant difference in dry matter yield (t/ha) between the two harvest seasons was found in this study by analysis of variance (Table 3). The significantly higher average dry matter yield was obtained on winter harvest (9.07 t/ha) compared to the spring harvest (7.86 t/ha). The findings show that the dry matter content significantly decreased from winter to spring. This shift is unlikely to be the result of random fluctuation because the difference of 1.21 t/ha is much greater than the LSD

threshold of 0.58. This decrease in dry matter content can be attributed to the decrease of moisture content and leaf biomass. Other researchers have obtained similar findings. For example, Zhou et al. (2016) found that variations in temperature and radiation led to considerable interannual variability in maize dry matter yield. Similarly, Singh et al. (2014) observed that phenological events (leaf fall, leaf renewal, flowering and fruiting) of Virginia mallow varied considerably with the fluctuations in temperature and relative humidity. When comparing harvest seasons, it's crucial to keep in mind that as crops age, their dry matter content typically increases due to greater biomass accumulation and moisture loss. The observed variance may be somewhat explained with different weather conditions at harvest dates in the studied years. Overall, the notable variation in dry matter content across years indicates the influence of environmental conditions on crop development and biomass accumulation.

The ANOVA analysis demonstrated a statistically significant variation in carbon concentration between the two harvesting periods in this study (Table 3). The spring's mean carbon concentration amounted 50.1%, while winter's mean amounted 48.3%. With a difference of 1.8% between spring and winter harvests, the data clearly show a higher carbon in the latter, surpassing the LSD value of 0.7753%. This implies that there is a genuine difference in the carbon concentration between the two harvesting times in both years, and that the observed change is not the result of chance. Numerous factors, such as soil conditions or variations in climatic factors like temperature and moisture availability over the growing season, can affect carbon concentration. Spring's higher carbon concentration could result from improved biomass quality or altered plant physiology driven by more favourable growing conditions. Measurements of carbon concentration can potentially be affected by the timing of harvest. As plants age, structural carbohydrates accumulate, and their moisture content decreases, which usually results in an increase in carbon concentration. The greater carbon values could be explained in part by the harvest taking place later in the winter. Bilandžija et al. (2018) published similar results, showing that harvest timing is associated with significant seasonal variability in biomass carbon concentrations, with the highest concentrations in spring harvest (50%), followed by autumn harvest (47%) and the lowest determined value at winter harvest (32%). These results underscore the importance of considering both environmental conditions and harvest timing when evaluating changes in carbon content over time, contributing to a better understanding of carbon dynamics in agricultural systems.

The nitrogen concentration was significantly higher at the winter harvest (0.4%) compared to the spring harvest (0.2%) (Table 3). The difference of 0.2% is well above the LSD threshold of 0.05%, confirming that the variation is statistically significant and likely reflects real differences in nitrogen accumulation between the harvests. The higher nitrogen concentration observed in the winter harvest suggests that earlier harvesting captures plants with higher nutrient concentration, while the lower nitrogen concentration in the spring harvest reflects more advanced maturity and biomass accumulation. Harvest timing plays a crucial role in nitrogen concentration because nitrogen concentration typically declines as plants mature and grow due to dilution effects from increasing biomass (Zhu et al., 2024). Contrary results to ours have also been reported by Huisman et al. (1997), who indicated that delayed harvesting significantly affects the nitrogen content in Miscanthus sinensis and Miscanthus sacchariflorus; it was observed that the nitrogen content in autumn-harvested biomass was significantly higher than in winter harvest. Ameen et al. (2019) and Kusvuran et al. (2022) also observed that delayed harvesting significantly reduced the content of mineral elements in biomass, thereby enhancing biomass quality, as well as Bilandžija et al. (2018).

The time of harvest has a significant impact on total carbon and nitrogen yields according to the ANOVA analysis (Table 3). Winter harvest had a mean carbon yield of 4.38 t/ha, and spring harvest had a mean of 3.94 t/ha. Similar results were obtained for total nitrogen yield that on winter harvest amounted 0.04 t/ha, while spring harvest had a mean nitrogen yield of 0.01 t/ha.

Plants harvested in the winter have a higher moisture content, which affects the dry matter yield and carbon and nitrogen concentration which have main influence on total carbon and nitrogen yields. Early harvests capture plant tissue at a stage when nitrogen concentration is relatively high, while later harvests tend to have lower nitrogen levels due to increased biomass accumulation and nitrogen redistribution within the plant. This pattern of nitrogen dilution in plants as they mature explains the higher nitrogen levels in winter-harvested crops. The harvest date also directly affects crop quality and nutrient content, according to Fageria and Baligar (2005). Seasonal and developmental stage variations frequently have a greater impact on nitrogen yield than inter-annual variability in systems that are consistently managed. The significant disparity between the winter and spring harvests in this study emphasizes how crucial harvest schedule is when trying to optimize total carbon and nitrogen yields for biological sequestration and soil fertility.

Observed differences between harvest seasons could be due to nutrient translocation, lignocellulose accumulation, moisture reduction, nutrient return, carbon sequestration and habitat adaptability. During autumn, Virginia mallow reabsorbs nutrients and photosynthates from the stems and leaves, transferring them to the root system for winter storage. This causes a drop in nutrient concentrations as the plant dries. Another reason could be the increasing proportion of structural carbohydrates (cellulose, hemicellulose, lignin) over the winter, which creates a high-density, dry mass. Furthermore, over the winter, the mallow stalks dry naturally in the field, moisture falls significantly, and the winter crop becomes much easier to pelletise or burn directly, without high artificial drying costs. Also, since the plant sheds its leaves and drops them onto the soil before winter, most soil nutrients are retained. Removing the dried stalks in winter exports only carbon and trace elements, protecting the soil's nitrogen balance. As the deep root system of Virginia mallow stores large amounts of carbon underground year-round, by the time above-ground stalks are harvested in winter, the plant has already stored carbon in persistent, woody stems.

CONCLUSION

This study evaluated how harvest timing and year-to-year variation affect the biomass yield and nutrient composition of Virginia mallow cultivated in continental Croatia, with particular emphasis on its potential contribution to renewable energy production and carbon sequestration. These findings may provide useful information for biomass producers and researchers working with perennial energy crops in Croatia. The results indicated that harvest season (time) plays a more decisive role than harvest year in shaping both the quantity and quality of biomass produced, as winter harvests compared to spring harvests consistently delivered higher dry matter yields (9.07 vs 7.86 t/ha) and greater nitrogen (0.04 vs 0.01 t/ha) and carbon (4.38 vs 3.94 t/ha) outputs per hectare, while spring harvests compared to winter harvests provided a slight advantage in terms of carbon concentration (50.1 vs 48.3%), highlighting the necessity of coordinating harvest time with certain production objectives, such as increasing carbon content or optimizing biomass and nutrients. Even though yields were slightly higher in 2022 (8.70 t/ha) than in 2023 (8.22 t/ha), the year-to-year differences were not statistically significant. This suggests that seasonal timing has a stronger and more consistent influence than inter-annual fluctuations, with environmental factors like precipitation and soil moisture likely driving minor variations but not changing the overall robustness of winter harvests for consistent performance. Overall, the results support Virginia mallow as a sustainable energy crop with significant climate mitigation benefits, demonstrating that winter harvests maximise both biomass and nutrient outputs while spring harvests may serve niche purposes. The study also emphasises the need for further investigation into the ecological and economic trade-offs of various harvesting techniques, particularly across a range of climatic conditions, to better understand how these trends translate across management systems and ecosystems. To sum up, Virginia mallow harvest time optimisation offers a practical solution to boost the production of renewable energy while making a substantial contribution to carbon sequestration and climate change mitigation.

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