



Five-year impacts of biomass crop monoculture on soil enzyme activity, nitrogen pools, and other soil health indicators

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ABSTRACT

Soil health and nitrogen cycling are critical for sustainable biomass production, yet the long-term effects of monoculture biomass cropping on these factors remain underexplored. This study examines the five-year monoculture cultivation effects of giant reed (*Arundo donax* L.), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita*) on soil biochemical properties, nitrogen species, and biomass yield stability under field conditions. Soil samples collected in autumn 2014 and spring 2015 revealed significant seasonal and crop-specific impacts on soil health indicators. Giant reed cultivation resulted in the highest urease activity in autumn ($346 \mu\text{g NH}_4^+ \text{g}^{-1} \text{soil h}^{-1}$), supporting enhanced nitrogen cycling, while miscanthus increased soil organic matter (SOM) to 4.77 % in spring, aiding carbon sequestration. Sida contributed to the highest dehydrogenase activity, indicating robust microbial activity, alongside increased organic nitrogen (4.92 mg kg^{-1}) and total nitrogen levels (17.0 mg kg^{-1}) in spring. Soil respiration and electrical conductivity varied by season, with pH values slightly higher in spring. Across all crops, nitrogen forms (nitrate, ammonium, and organic nitrogen) were generally higher in spring than autumn, highlighting seasonal dynamics. Yield stability was greatest in giant reed (2.01 kg m^{-2} on average), while miscanthus and sida averaged 1.62 and 0.86 kg m^{-2} , respectively. The findings underscore these crops' potential for sustainable biomass production and improved soil health in low-input systems.

1. Introduction

There is indeed a growing interest in the cultivation of biomass plants around the world. This interest is driven by several factors, including the need for renewable and sustainable energy sources, the desire to mitigate climate change, and the development of bio-based products and materials [1]. Biomass crops include giant reed (*Arundo donax* L.), miscanthus (*Miscanthus x giganteus*), sida (*Sida hermaphrodita* Rusby), switchgrass (*Panicum virgatum*), and fast-growing trees [2]. Biomass plants as a source of renewable energy can be converted into various forms of energy, including biofuels (such as biodiesel and bio-ethanol) and biogas, which can be used for electricity generation, heating, and transportation [3]. Biomass plants capture carbon dioxide (CO_2) from the atmosphere during their growth through photosynthesis.

This, known as the carbon cycle, can result in a net reduction of greenhouse gas emissions compared to fossil fuels, helping mitigate climate change [4]. Cultivating biomass plants for energy production can enhance energy security by diversifying energy sources. It reduces reliance on imported fossil fuels and helps stabilize energy prices, increasing energy independence and national security [5]. Biomass can also serve as a feedstock for the production of various bio-based chemicals and materials, reducing the reliance on petrochemicals and supporting the development of a bioeconomy [6].

Cultivation of biomass crops can be integrated into sustainable land management practices, such as agroforestry and crop rotation, which can improve soil health and reduce the need for synthetic fertilizers and pesticides [7]. The cultivation of biomass plants can have significant effects on soil enzyme activities and soil physicochemical properties due

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to the changes in organic matter input, root exudates, nutrient cycling, and other factors associated with plant growth and management practices [8]. Biomass plants contribute substantial amounts of organic matter to the soil through their aboveground biomass and root systems. As these plants grow and senesce, their residues are incorporated into the soil. This organic matter serves as a substrate for soil microorganisms and influences soil enzyme activities [9], including dehydrogenase (E.C. 1.1.1.1) – an intracellular enzyme involved in microbial respiration, serving as an indicator of overall microbial activity and oxidative processes in soil [10], urease (E.C. 3.5.1.5) – catalyzes the hydrolysis of urea into ammonia and carbon dioxide, playing a key role in nitrogen cycling and nutrient availability [11], phosphatase (E.C. 3.1.3.1) – hydrolyzes organic phosphate compounds, facilitating phosphorus mineralization and availability for plant uptake [12], and catalase (E.C. 1.11.1.6) – decomposes hydrogen peroxide into water and oxygen [13], helping mitigate oxidative stress in soil microbial communities. Enzymes produced by microorganisms break down organic matter, releasing nutrients and contributing to soil fertility [14]. Different types of biomass plants release specific root exudates that can stimulate the growth and activity of soil microorganisms. These exudates contain sugars, organic acids, and other compounds that fuel microbial metabolism and influence enzyme production. Some exudates may promote the production of enzymes that break down complex organic compounds, further influencing nutrient cycling [15]. Giant reed cultivation increased soil enzyme activities (e.g., dehydrogenase and urease) by 20–30 % compared to control soils [8]. This was associated with enhanced microbial activity and nutrient cycling.

Biomass plants take up nutrients from the soil as they grow. When these plants are harvested or their residues are incorporated into the soil, the nutrients are returned to the soil, impacting nutrient availability and cycling. Decomposition of biomass residues can release nutrients and affect the activity of enzymes involved in nutrient transformation [16]. The cultivation of biomass plants can affect soil pH through the release of organic acids and other compounds from plant residues and root exudates. Changes in pH can influence the activity of soil enzymes, as many enzymes have pH optima for their function [17]. Different types of biomass plants can influence the composition of soil microbial communities through their root exudates and organic matter inputs. The microbial community composition, in turn, can affect the types and quantities of enzymes produced in the soil [18]. Cultivating biomass plants can help prevent soil erosion by providing ground cover and stabilizing the soil with their root systems. This can lead to improved soil structure, which affects water infiltration, aeration, and nutrient availability. Enhanced soil structure can indirectly influence soil enzyme activities by creating more favorable conditions for microbial growth and enzyme function [19]. Some biomass crops are grown specifically for carbon sequestration purposes. These crops contribute to soil organic carbon accumulation, which can impact soil enzyme activities and microbial processes related to carbon decomposition and cycling [20]. The cultivation of biomass plants often involves specific management practices, such as tillage, fertilization, and irrigation. These practices can influence soil physicochemical properties, as well as microbial activities. For example, excessive tillage can disrupt soil structure and affect microbial habitats, potentially impacting enzyme-producing microorganisms [21].

The cultivation of biomass plants can have various effects on soil nitrogen species, including changes in nitrogen pools. These effects can be influenced by several factors, including the type of biomass crop, agricultural practices, and soil management. Biomass plants typically have high nitrogen requirements for growth. As they grow and accumulate biomass, they can take up significant amounts of nitrogen from the soil. This can lead to a decrease in the concentration of nitrate (NO_3^-) and ammonium (NH_4^+) ions in the soil, as these forms of nitrogen are taken up by the plants [14]. However, after biomass plants are harvested, their crop residues are often returned to the soil as organic matter. These residues can undergo decomposition, a process in which

soil microorganisms break down organic materials. During decomposition, nitrogen is released back into the soil in the form of NH_4^+ , contributing to the soil's NH_4^+ pool [22]. The decomposition of biomass crop residues and organic matter can also lead to a process called nitrogen mineralization. During this process, organic nitrogen compounds in crop residues are converted into NH_4^+ , which can then be further transformed into NO_3^- . This can increase the availability of these nitrogen forms in the soil [23]. Some biomass crops have the ability to form symbiotic relationships with nitrogen-fixing bacteria. These bacteria can convert atmospheric nitrogen gas (N_2) into NH_4^+ , making additional nitrogen available to the plant and potentially increasing soil nitrogen levels [24]. Miscanthus had high nitrogen use efficiency, with seasonal nitrogen uptake ranging from 100 to 150 kg N/ha [25]. However, this study did not quantify long-term changes in soil nitrogen pools. Giant reed cultivation reported a 15–20 % increase in soil nitrogen pools, likely due to root exudates and litter decomposition [8].

The choice of biomass crop and management practices can influence soil pH. Certain crops and management practices can either increase or decrease soil pH levels, which, in turn, can affect the chemical forms and availability of nitrogen in the soil [26]. The addition of organic materials from biomass crops can enhance soil organic matter (SOM) content. Increased SOM can help stabilize nitrogen and reduce the risk of nitrogen losses through leaching or denitrification [27]. Sustainable biomass cultivation practices often aim to optimize nitrogen use efficiency to minimize environmental impacts while maximizing crop yields. Proper nutrient management and soil testing are essential to ensure that nitrogen levels are maintained within an appropriate range for both crop growth and environmental conservation. Miscanthus cultivation increased soil organic carbon (SOC) by 0.2–0.3 % in the topsoil (0–30 cm) over a 4-year period [17]. This was attributed to the high biomass production and root turnover of miscanthus, which contributed to organic matter accumulation. Similarly, an increase of 12–15 % in SOC after miscanthus cultivation was reported compared to conventional crops [28]. This was linked to the recalcitrance of miscanthus-derived carbon in soil.

However, long-term monoculture of biomass crops can lead to nutrient depletion, particularly if residues are continuously removed for bioenergy production. For example, miscanthus has been reported to reduce soil nitrogen levels over time due to high biomass removal and limited nitrogen inputs [22,25]. Some biomass crops, such as miscanthus, may release allelopathic compounds that inhibit the growth of other plants and soil microorganisms, potentially reducing biodiversity and soil health [16]. Intensive cultivation of biomass crops can lead to soil acidification, particularly in soils with low buffering capacity. This can negatively affect microbial communities and nutrient availability [26].

While previous research has explored the short-term effects of biomass crops on soil properties [29], there is limited understanding of the long-term impacts of monoculture systems, particularly for crops like giant reed, miscanthus, and sida. Most studies focus on individual crops or short-term effects, leaving a gap in understanding how these crops influence soil biochemical properties, enzyme activities, and nutrient cycling over extended periods under monoculture conditions. This study uniquely addresses this gap by providing a comprehensive five-year field experiment that evaluates the seasonal and species-specific effects of these biomass crops on soil health indicators, including enzyme activities, nitrogen pools, and organic matter accumulation. The novelty of this study lies in its long-term perspective and the comparative analysis of three distinct biomass crops, which have not been extensively studied together in a monoculture system. By integrating seasonal dynamics and species-specific responses, this research offers new insights into sustainable biomass cultivation on marginal lands. The objective of this study is to investigate the long-term effects of monoculture biomass cropping on soil health indicators, including soil enzyme activities, nitrogen cycling, and biomass yield stability, under field conditions. We hypothesize that different biomass crops (giant

reed, miscanthus, and sida) exert distinct influences on soil biochemical properties, nitrogen cycling, and enzyme activities, which in turn affect soil health and biomass yield stability over a long-term monoculture system. Specifically, we anticipate that each crop will promote specific microbial and enzymatic activities, influencing nutrient availability and seasonal soil dynamics. This study uniquely integrates a five-year field experiment to assess seasonal soil health variations and nitrogen transformations under monoculture conditions, filling a critical gap in understanding sustainable biomass cultivation on marginal lands.

2. Materials and methods

2.1. Propagation of plant materials

2.1.1. Giant reed and miscanthus

The plant specimens used in this research were somatic embryo-derived plantlets of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus × giganteus*), originally sourced from the University of South Carolina [30,31] and later cultivated in the Ottó Orsós Laboratory at the Department of Applied Plant Biology, University of Debrecen, Hungary.

2.1.2. Sida

The seeds of *Sida hermaphrodita* were obtained from the Fruit and Ornamental Plant Breeding Research Institute (Budatétény, Hungary), from the seed collection of Hungarian plant breeder Prof. Zoltán Kovács. Before germination, the seeds were soaked in distilled water at room temperature for 30 min. They were then selected based on density and water absorption, with any shriveled seeds removed. To promote germination, the chosen seeds were surface sterilized and placed in sterile Petri dishes with sterile filter paper. As an additional germination-boosting step, the seeds underwent a pretreatment in an 80 °C water bath for 2 min.

After germination, the seedlings of giant reed, miscanthus, and sida were acclimated to greenhouse conditions before being transplanted into an open field at the Demonstration Garden of the University of Debrecen, Hungary (47° 32' 0" N; 21° 38' 0" E) (Fig. 2).

2.2. Experimental layout and growth conditions

The experiment followed a split-plot design with three replicates, resulting in a total of nine experimental plots. The main plots were assigned to different biomass crops (*Arundo donax*, *Miscanthus × giganteus*, and *Sida hermaphrodita*), while the sub-plots represented seasonal sampling times (autumn and spring) for soil and plant measurements. Each main plot measured 40 m² (4 m wide × 10 m long), with four rows of plants spaced 1 m apart. The sub-plots were designated within each main plot for seasonal soil sampling and biochemical assessments. This arrangement yielded a plant density of 10,000 plants per hectare. A 4-m buffer zone separated each plot. Soil tillage was performed on previously uncultivated grassland in autumn 2009 using deep plowing (40–50 cm). The soil's physicochemical properties are detailed in Table 1. Seedbed preparation was conducted in spring 2010, just before transplanting, using a rotary cultivator.

In our study, the aboveground biomass was harvested for heat and bioethanol productions, leaving only residual plant parts—such as fallen leaf blades, shoot tips, root remains, and root exudates—to return to the soil. Giant Reed has a chemical composition of 32–45 % cellulose, 20–25 % hemicellulose, 15–20 % lignin, and 5–10 % ash [32]. Miscanthus consists of 43.2–55.5 % cellulose, 17.1–25.1 % hemicellulose, 17.9–22.9 % lignin, and 0.9–2.95 % ash [33]. Sida is composed of 35–45 % cellulose, 25–30 % hemicellulose, 15–20 % lignin, and 5–10 % ash [34].

The growing cycle of the biomass crops in the study area followed a distinct seasonal pattern influenced by local meteorological conditions. Based on field observations, giant reed, miscanthus, and sida began shoot emergence in early spring (March–April), following winter

Table 1

Physicochemical characteristics of the experimental soil before cultivation of biomass plants in autumn 2010.

Character	Value
pH (1:2.5 soil:water suspension)	7.63 ± 0.03 ^a
Electrical conductivity (EC, dS m ⁻¹) ^b	0.45 ± 0.02
Soil organic matter (%)	2.29 ± 0.05
Saturation percentage (%)	30.0 ± 0.32
Texture grade	clay loam
USDA Soil Taxonomy	Mollisol
Available macronutrients (mg kg⁻¹)	
NO ₃ ⁻ -N	0.81 ± 0.01
NH ₄ ⁺ -N	1.52 ± 0.05
Org-N	1.41 ± 0.04
Total-N	6.40 ± 0.10
Extractable-P	247.6 ± 15.4
Extractable-K	465 ± 24
Soil enzyme activities	
Dehydrogenase (μg TPF g ⁻¹ soil h ⁻¹)	30.2 ± 0.05
Phosphatase (μg PNP g soil ⁻¹ h ⁻¹)	54.0 ± 0.09
Urease (mg NH ₄ 100 g ⁻¹ soil 24 h ⁻¹)	363 ± 26
Catalase (mL O ₂ 2 min ⁻¹)	13.0 ± 0.09

^a Standard deviation.

^b Measured in soil paste extract.

dormancy. This timing aligned with rising temperatures and increased soil moisture from early spring rainfall. The active vegetative growth phase spanned from late spring (May) to early autumn (September), with peak biomass accumulation occurring in summer. During this period, the crops benefited from longer daylight hours (~2300 h/year) and mean temperatures of 20–25 °C. As temperatures dropped in late autumn (November–December), plants entered dormancy, marked by the senescence of aboveground biomass. In winter (December–February), mean temperatures reached a minimum of −5 °C to −10 °C, which halted physiological activity, particularly in miscanthus and giant reed, which showed reduced soil enzyme activity and microbial respiration during this time. The biomass crops were harvested in February, before the next cycle of shoot emergence. Meteorological data at the experimental site between 2010 and 2016 are presented in Fig. 1 (Hungarian Meteorological Service, 2016; Hungarian Central Statistical Office, 2018).

No irrigation or fertilization was applied after the experiment was established. Weed control was conducted manually twice during the first two years, with no mechanical cultivation except for maintaining weed-free buffer zones using a rotary hoe in the first two years. The authors confirm that all experiments adhered to relevant national guidelines and regulations.

2.3. Soil sampling

Soil samples were collected 25 cm from the main stem of plants within the 0–20 cm soil layer. Sampling occurred four years after the experiment's initiation, during two seasons: autumn 2014 and spring 2015. Each sampling was conducted in triplicate, yielding a total of nine subsamples per biomass plant. The soil samples were carefully placed in polyethylene bags and transported on ice to preserve their integrity. In the laboratory, plant debris and gravel were removed to ensure sample purity. To preserve biological activity, soil samples intended for the analysis of biological parameters were initially sieved through a 5-mm mesh to remove gravel, stones, and large aggregates. These samples were then further sieved using a 2-mm mesh to ensure homogeneity and stored at 4 °C to maintain their integrity. In contrast, samples intended for chemical analysis were air-dried to prevent alterations in physicochemical properties, homogenized using a stainless steel crusher for consistency, and sieved through a 2-mm mesh before being stored at room temperature.

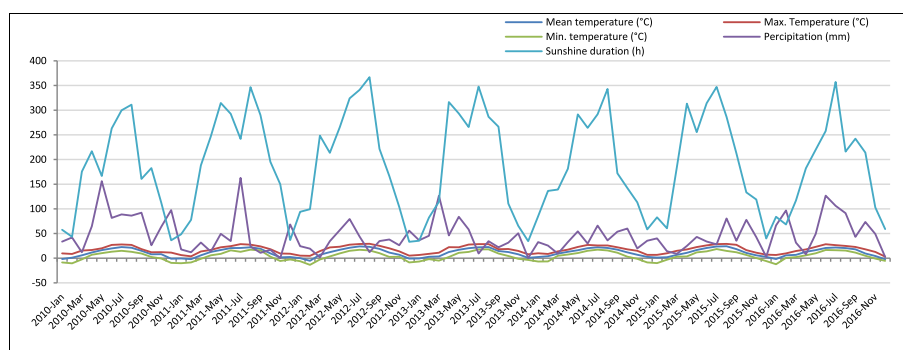


Fig. 1. Meteorological data collected at the experimental site between 2010 and 2016.

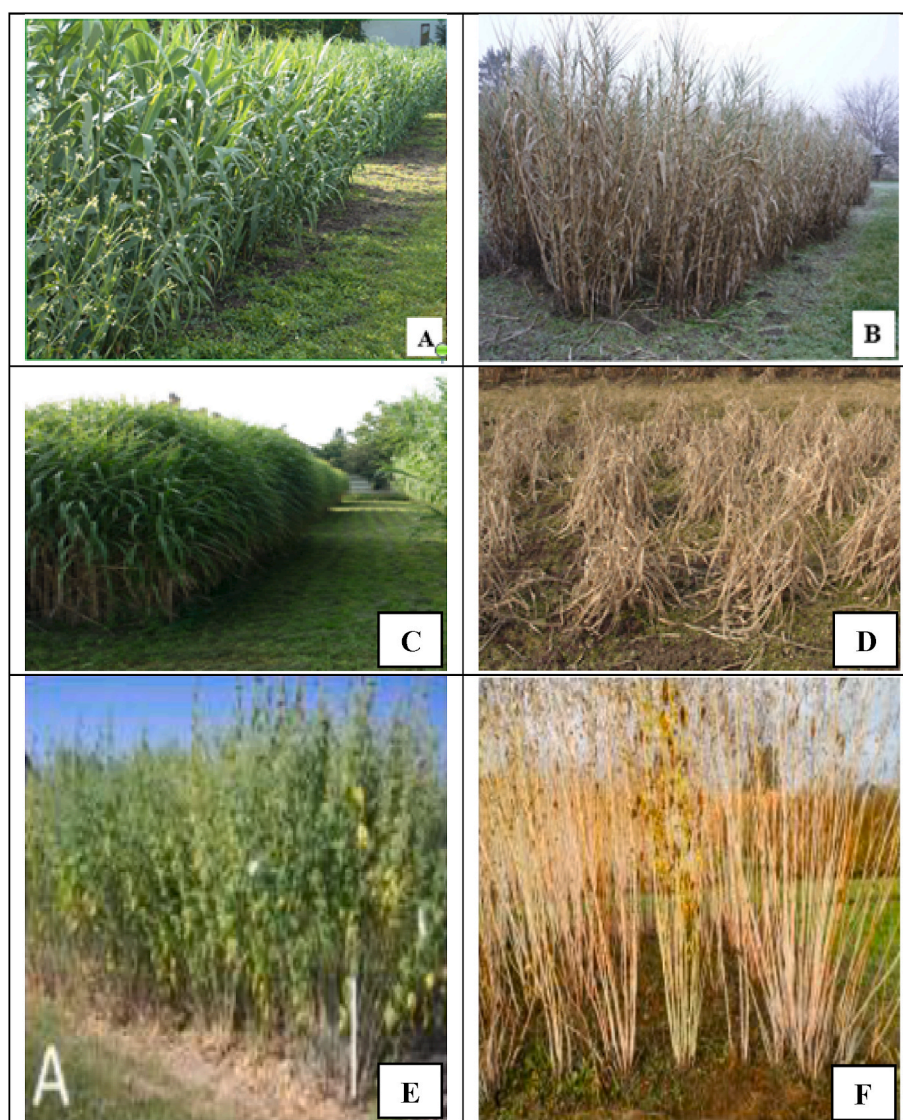


Fig. 2. Cultivated biomass crops: A and B) giant reed (*Arundo donax*), C and D) miscanthus (*Miscanthus x giganteus*), E and F) sida (*Sida hermaphrodita* Rusby) within 5-year of open field experiments during spring season (Left) and winter season (Right). Photos are taken by Dr. Gabreilla Antal.

2.4. Determination of soil enzyme activities

Before assessing soil enzyme activity, frozen soil samples were thawed at room temperature for 24 h. Glucose was then added at a rate of 80 mg per gram of soil, and moisture content was adjusted to 60 % of the soil's total water-holding capacity (WHC) to encourage microbial

activity after dormancy. Dehydrogenase activity was measured using the 2,3,5-triphenyl tetrazolium chloride (TTC) reduction method (3 % w/v), as described by Ref. [10]. Absorbance was recorded at 485 nm, with a standard curve generated using triphenyl formazan (TPF). Phosphatase activity was assessed following [12], by incubating soil samples with p-nitrophenyl phosphate and measuring color intensity at 440 nm.

Urease activity was determined according to Ref. [35], by incubating samples with a 2 % aqueous urea solution and quantifying remaining urea colorimetrically at 527 nm. Catalase activity was measured using the method of [13]. All measurements were performed with an Amersham Biosciences Ultrospec 2100 Pro UV/Visible spectrophotometer.

2.5. Soil respiration measurement

CO₂ emissions were used as an indicator of soil microbial activity. CO₂ production was measured after a 10-day incubation at 35 °C, using NaOH trapping as outlined by Ref. [36]. Before measuring soil respiration, soil samples were thawed for 24 h, enriched with glucose at 80 mg g⁻¹ soil, and adjusted to reach a moisture level of 60 % of the total WHC to promote microbial growth following dormancy.

2.6. Determination of soil physicochemical traits

The soil saturation percentage (%) was determined using the procedure specified by Ref. [37]. Soil pH was measured by preparing a 1:2.5 (w/w) soil-to-distilled water suspension, also following [37]. Soil electrical conductivity (EC_e), in dS m⁻¹, was assessed using a soil paste extract at 25 °C, as outlined by Ref. [37]. The Walkley-Black chromic acid wet oxidation method, detailed by Ref. [37], was used to determine soil organic matter (SOM) content.

Total nitrogen (N) content in the soil, including its forms as NO₃⁻-N, NH₄⁺-N, and organic N (Org-N), was analyzed using the Kjeldahl method as outlined by Ref. [37]. Extractable phosphorus was obtained using 0.5 N sodium bicarbonate following [38] and measured spectrophotometrically with the ascorbic acid method on the Amersham Biosciences Ultrospec 2100 Pro UV/Visible instrument, following [39]. Extractable potassium was determined using the ammonium acetate extraction method [37] and measured with a Sherwood Model 410 flame photometer [40].

2.7. Biomass harvest and yield stability

Plants were harvest by cutting the stems at 10 cm above the soil surface with a pruning shears during the month of February, spanning from 2010 to 2016. Following the harvest, the fresh weight was measured, and the plants were allowed to naturally dry under open field conditions to determine their dry weight (measured in kg m⁻²). Yield stability (referred to as S_i) serves as a metric to assess the consistency of yields across various cropping systems, as detailed in studies by Refs. [41,42]. S_i is calculated by taking the reciprocal of the coefficient of variation, according to the formula:

$$S_i = \frac{\text{Average biomass yield}}{\text{SD}}$$

In this formula, SD represents the standard deviation and the average biomass yield of each cropping system over the years 2010–2016 (denoted by $\bar{y}$). The S_i value is indicative of yield stability, with higher values indicating greater stability, and conversely, lower values indicating less stability.

2.8. Statistical analysis

The assessment of dependent variables included examinations for normal distribution and homogeneity of variances, with transformations carried out when deemed necessary. Data analysis was conducted utilizing Microsoft Excel 2010 (representing mean values with their respective standard deviations) and the SPSS 22.0 software package by SPSS Inc. based in Chicago, IL. To assess variations in plant biomass across different sampling times, a one-way analysis of variance (ANOVA) was applied. Also, two-way ANOVA was applied to evaluate the interaction between plant species and seasonal variations. Post hoc

analysis was then performed using Tukey's test to distinguish between means, with statistical significance established at a significance level of $p \leq 0.05$.

3. Results

3.1. Alterations in soil enzyme activities

Activities of soil enzymes responded significantly to the type of biomass crops grown and the growing season (Fig. 3). Overall, dehydrogenase and urease enzymes displayed their highest activities in autumn, while in spring, phosphatase and catalase activities were higher than in autumn. Dehydrogenase activity (μg TPF g⁻¹ soil h⁻¹) was 2–3 times higher in autumn than in spring. Dehydrogenase activity did not differ among species either in autumn or spring. The highest dehydrogenase activity in autumn (307) corresponded to sida plant, followed by miscanthus (273), whereas in spring, giant reed resulted in the highest dehydrogenase activity (110), followed by sida (102) and miscanthus (87). Activity of urease (μg NH₄⁺ g⁻¹ soil h⁻¹) was about 4–8 times higher in autumn than in spring. In autumn, the highest urease activity (346) corresponded to giant reed, followed by miscanthus (285), and sida displayed the lowest activity (193). In spring, however, the three biomass plants revealed almost the same urease activity, ranging between 42 and 50. Phosphatase activity (μg PNP/g soil/h) was significantly higher in spring than in autumn for all species. However, there is no significant difference between species in either autumn or spring. Phosphatase activity was at its peak in spring (81.3) and corresponded to miscanthus, while giant reed revealed the lowest activity of 66.8. In autumn, the highest phosphatase activity was 54.1 and linked to sida cultivation, while giant reed had the weakest phosphatase activity (46.7). Similarly, catalase activity (mL O₂ 2 min⁻¹) was 4–5 folds higher in spring than in autumn. In spring, giant reed and sida displayed almost the same catalase activity (ranging from 35.5 to 37.9), with a slight advantage for giant reed, while miscanthus resulted in the lowest catalase activity (27.2). In autumn, however, all three biomass crops revealed approximately the same catalase activity, ranging from 6.2 to 8.5 without significant differences.

3.2. Response of soil microbial activity and organic matter accumulation to cultivation of biomass crops

Soil respiration is indeed a valuable indicator of soil microbial activity. It refers to the process by which microorganisms in the soil break down organic matter, such as plant roots, leaves, and other organic materials, and release CO₂ into the atmosphere. This CO₂ release is a fundamental part of the carbon cycle and is a direct result of microbial metabolism. In the present study, the three biomass crops showed almost the same soil respiration in the autumn and spring seasons, except for the giant reed, which recorded higher soil respiration – as indicated by the amount of emitted CO₂ – than sida in the autumn (Fig. 4A). Moreover, soil samples collected in autumn revealed higher soil respiration than in spring. Giant reed resulted in the highest soil respiration activity in spring and autumn, recording a CO₂ of 16.1 and 18.0 mg 100 g⁻¹ soil 10 days⁻¹, respectively, followed by miscanthus and sida exhibited the lowest CO₂.

The SOM content was significantly higher in spring compared to autumn (Fig. 4B). Among the crops, miscanthus exhibited the highest SOM content in spring at 3.77 %, followed by sida (3.68 %) and giant reed (3.25 %). In autumn, both giant reed and miscanthus recorded the same SOM content (2.57 %), while sida had a slightly lower SOM content of 2.43 %. The variation in SOM content was more pronounced in spring than in autumn.

3.3. Variations of nitrogen pools under different biomass crops

Soil nitrogen species, i.e., NO₃⁻-N, NH₄⁺-N, Org-N, and Total-N,

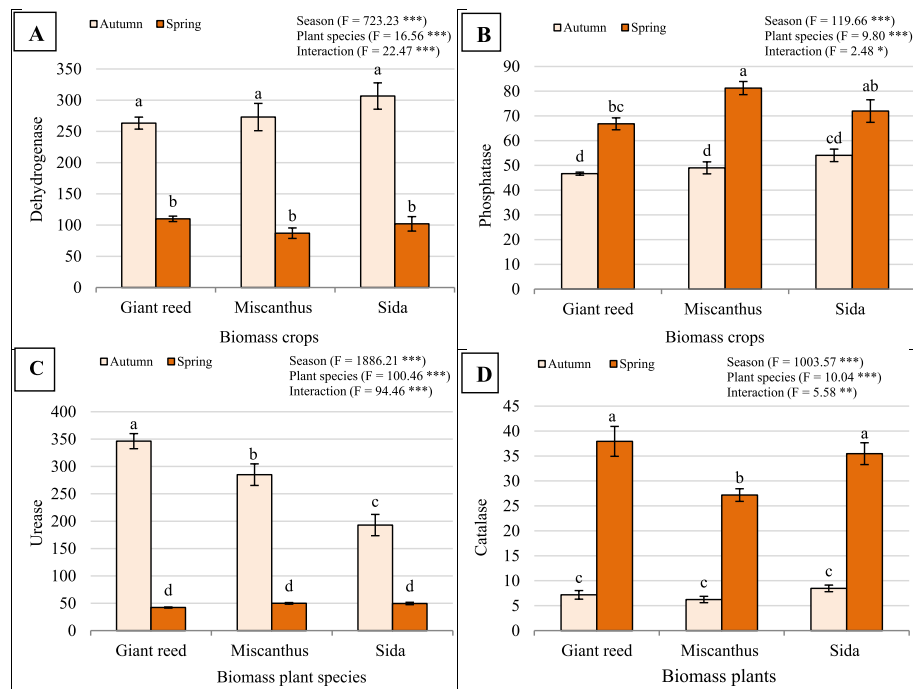


Fig. 3. Alterations in soil enzyme activities in autumn and spring seasons after the cultivation of three biomass crops, i.e., giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby). A) dehydrogenase ($\mu\text{g TPF g}^{-1} \text{ soil h}^{-1}$), B) phosphatase ($\mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$), C) urease ($\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ soil h}^{-1}$), and D) catalase ($\text{mL O}_2/2 \text{ min}$). Different letters on bars of the one sampling time are significant according to the Tukey's test at $P \leq 0.05$ ($n = 9$). ** and *** denote significance at levels of 0.01 and 0.001, respectively.

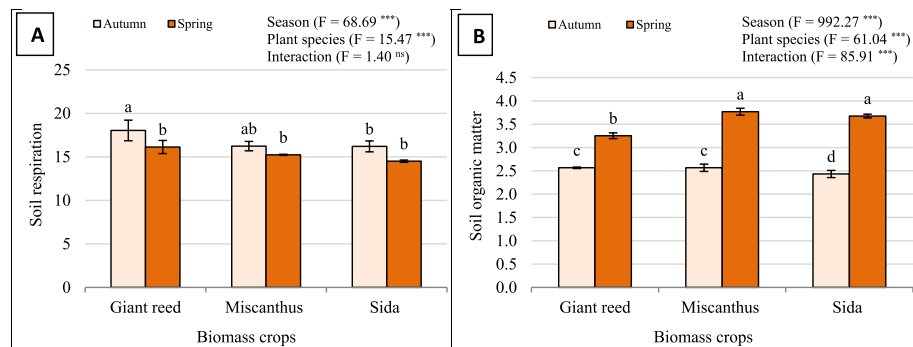


Fig. 4. Response of A) soil respiration ($\text{mg CO}_2 100 \text{ g}^{-1} \text{ soil 10days}^{-1}$) and B) soil organic matter (%) to the cultivation of three biomass crops, i.e., giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby) during two sampling times, i.e., autumn and spring. Different letters on bars of the one sampling time are significant according to the Tukey's test at $P \leq 0.05$ ($n = 9$). *** denotes significance at levels 0.001, while ns denotes non-significance.

significantly varied among the cultivated biomass crops in both sampling seasons (Table 2). Overall, nitrogen species displayed higher contents in spring samples than in autumn. In spring, samples showed an increase of 2.5, 5.3, 4.7, and 3.6 % in the NO_3^- -N, NH_4^+ -N, Org-N, and Total-N contents, respectively, than in autumn, regardless of the type of the biomass crop. Moreover, the NO_3^- -N was the predominant nitrogen form in the soil, followed by the Org-N, and NH_4^+ -N was the lowest. The highest NO_3^- -N contents in autumn and spring were 8.35 and 8.57 mg kg^{-1} , respectively, and corresponded to giant reed, while miscanthus resulted in the highest NH_4^+ -N contents in the autumn and spring, recording 3.61 and 3.76 mg kg^{-1} , respectively. On the other hand, the highest Org-N contents in autumn and spring were 4.81 and 4.92 mg kg^{-1} , respectively, and linked to sida cultivation. Sida plant displayed the highest Total-N contents in autumn and spring, recording 16.6 and 17.0 mg kg^{-1} , respectively. The effect of different biomass crops on soil nitrogen species had the same tendency in autumn and spring. The variations in soil NO_3^- -N contents in autumn and spring were as follows:

giant reed > sida > miscanthus, while changes in the NH_4^+ -N contents were as follows: miscanthus > sida > giant reed. The Org-N and Total-N contents were in the following order: sida > giant reed > miscanthus.

3.4. Alterations of soil physicochemical properties under different biomass crops

Cultivation of soil with different biomass crops significantly affected most of the soil's physicochemical properties (Table 3). Soil saturation percentages were slightly higher in spring than in autumn under the three biomass crops. Overall, the sida plant showed the highest soil saturation percentages in autumn and spring (39.0 % and 39.4 %, respectively). Giant reed displayed the second-highest soil saturation percentages, recording 37.2 % and 38.1 % in autumn and spring, respectively. Likewise, spring samples revealed higher pH values than those collected in autumn for all the biomass crops. In autumn, miscanthus exhibited the highest soil pH (7.20), while giant reed and sida

Table 2

Variations in soil nitrogen pools (mg kg⁻¹) under three different biomass crops, i.e., giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby) during two sampling times, i.e., autumn and spring.

		NO ₃ -N	NH ₄ ⁺ -N	Org-N	Total-N
Sampling time					
Autumn		7.33 ± 0.09 b	3.37 ± 0.07 b	3.65 ± 0.07 b	14.37 ± 0.07 b
Spring		7.51 ± 0.05 a	3.55 ± 0.05 a	3.82 ± 0.06 a	14.89 ± 0.05 a
Plant species					
Giant reed		8.46 ± 0.05 a	2.63 ± 0.06 c	3.51 ± 0.04 b	14.67 ± 0.04 b
Miscanthus		7.09 ± 0.08 b	3.70 ± 0.07 a	3.34 ± 0.09 c	14.13 ± 0.07 c
Sida		8.37 ± 0.05 a	3.55 ± 0.04 b	4.87 ± 0.05 a	16.78 ± 0.10 a
Interaction					
Autumn	Giant reed	8.35 ± 0.07 b	2.51 ± 0.05 d	3.45 ± 0.05 b	14.47 ± 0.04 d
	Miscanthus	6.99 ± 0.10 d	3.61 ± 0.10 b	3.23 ± 0.10 c	13.83 ± 0.10 e
	Sida	8.28 ± 0.07 b	3.49 ± 0.07 b	4.81 ± 0.07 a	16.57 ± 0.12 b
Spring	Giant reed	8.57 ± 0.04 a	2.75 ± 0.08 c	3.56 ± 0.04 b	14.87 ± 0.04 c
	Miscanthus	7.18 ± 0.06 c	3.79 ± 0.05 a	3.45 ± 0.07 b	14.43 ± 0.03 d
	Sida	8.46 ± 0.04 ab	3.61 ± 0.02 ab	4.92 ± 0.03 a	17.00 ± 0.08 a
Two-way ANOVA					
Season		***	***	***	***
Plant species		***	***	***	***
Interaction		*	ns	ns	ns

Means in the same column followed by different letters are significant according to the Tukey's test ($P \leq 0.05$). Data are mean ± SD and n = 9. *** and * denote significance at $P \leq 0.001$ and $P \leq 0.05$, respectively, ns denotes non-significance.

showed the same soil pH (7.12). In spring, the highest soil pH (7.32) corresponded to sida, while giant reed recorded the second-highest soil pH of 7.30. Miscanthus, in spring, revealed the lowest soil pH value of 7.24. In autumn, EC_e values were higher than those measured in spring under biomass crops, except for the giant reed. Sida displayed the highest EC_e (0.579 dS m⁻¹) in autumn, followed by giant reed (0.533 dS

m⁻¹), and miscanthus had the lowest EC_e value (0.518 dS m⁻¹). In spring, the order of EC_e was as follows: giant reed (0.539 dS m⁻¹) > miscanthus (0.487 dS m⁻¹) > sida (0.468 dS/m). Soil extractable phosphorus contents largely depended on the type of cultivated biomass plants, while to a lesser extent, it varied according to the sampling time. Miscanthus exhibited the highest soil extractable phosphorus contents in autumn and spring (102 and 108 mg 100 g⁻¹ soil, respectively), followed by sida, which recorded 92 and 76 mg 100 g⁻¹ soil in autumn and spring, respectively. Cultivated soil plots with giant reed had the lowest soil extractable phosphorus contents in autumn and spring (79 and 66 mg 100 g⁻¹ soil, respectively). Similarly, the highest soil extractable K contents in autumn and spring were 783 and 946 mg kg⁻¹ soil and corresponded to miscanthus, while sida resulted in the second-highest soil extractable K contents in autumn and spring. Giant reed resulted in the lowest soil extractable K contents in both sampling times.

3.5. Biomass yield and its stability of cultivated crops from 2011 to 2015

Plants were harvested at the end of the winter season (namely in February) between 2011 and 2015 when the aboveground parts were almost dry, and then dry biomass was measured (Fig. 5). Results of the average dry biomass yield between 2011 and 2015 showed that giant reed recorded the highest biomass yield of 2.01 kg m⁻². The average biomass yields of miscanthus and sida in the same period were 1.62 and 0.86 kg m⁻², respectively. The biomass yield of giant reed was 3.05 kg m⁻² in 2011 and decreased to 0.25 kg m⁻² after exposure to frost conditions in 2012 (it is highlighted more in the discussion section). However, from 2013, the dry biomass of giant reed started to increase again, recording 2.56 kg m⁻² in 2014. On the contrary, the biomass yield of miscanthus increased from 1.07 kg m⁻² in 2011 to 2.44 kg m⁻² in 2014 and then decreased to 1.22 kg m⁻² in 2015. Likewise, sida dry biomass yield changed from 0.30 kg m⁻² in 2011 to 1.13 kg m⁻² in 2013 and gradually decreased from 0.90 kg m⁻² in 2014 to 0.86 kg m⁻² in 2015.

Giant reed displayed an increased yield stability index, where yield stability increased from 4.07 in 2011 to 12.24 in 2013 and then started to decrease from 7.85 in 2014 to 3.49 in 2015. Similarly, yield stability index of miscanthus increased from 3.96 in 2011 to 13.26 in 2013, and then a decrease was noticed in 2014 and 2015, recording 3.58 and 3.18, respectively. In contrast, sida revealed its highest yield stability index 5.36 in 2011 and then gradually decreased, recording 1.40 in 2015.

Table 3

Alterations in the soil's physicochemical properties under three different biomass crops, i.e., giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby) during two sampling times, i.e., autumn and spring.

		Saturation %	Electrical conductivity (dS m ⁻¹)	pH	Extractable P (mg 100g ⁻¹ soil)	Extractable K (mg kg ⁻¹ soil)
Sampling time						
Autumn		36.1 ± 0.76 a	0.513 ± 0.01 a	7.11 ± 0.05 b	94.3 ± 2.41 a	591 ± 11.6 b
Spring		36.4 ± 0.43 a	0.472 ± 0.01 b	7.26 ± 0.01 a	80.3 ± 1.00 b	748 ± 15.1 a
Plant species						
Giant reed		37.6 ± 0.67 b	0.536 ± 0.01 a	7.21 ± 0.01 a	72.1 ± 0.45 c	505 ± 4.3 c
Miscanthus		35.3 ± 0.62 c	0.503 ± 0.01 b	7.22 ± 0.07 a	105.2 ± 0.15 a	864 ± 12.4 a
Sida		39.2 ± 0.49 a	0.523 ± 0.01 a	7.22 ± 0.03 a	83.8 ± 3.09 b	757 ± 16.7 b
Interaction						
Autumn	Giant reed	37.2 ± 0.95 b	0.533 ± 0.01 b	7.12 ± 0.01 b	78.7 ± 0.39 c	423 ± 5.7 f
	Miscanthus	34.2 ± 0.71 c	0.518 ± 0.01 b	7.20 ± 0.13 ab	102.0 ± 0.30 a	783 ± 13.1 c
	Sida	39.0 ± 0.55 a	0.579 ± 0.01 a	7.12 ± 0.05 b	91.7 ± 5.67 b	677 ± 13.1 d
Spring	Giant reed	38.1 ± 0.38 ab	0.539 ± 0.01 b	7.30 ± 0.00 a	65.6 ± 0.51 d	586 ± 2.9 e
	Miscanthus	36.5 ± 0.53 b	0.487 ± 0.01 c	7.24 ± 0.01 ab	108.4 ± 0.00 a	946 ± 11.7 a
	Sida	39.4 ± 0.44 a	0.468 ± 0.01 c	7.32 ± 0.01 a	75.9 ± 0.50 c	837 ± 20.3 b
Two-way ANOVA						
Season		ns	***	***	***	***
Plant species		***	***	***	***	***
Interaction		***	***	**	***	*

Means in the same column followed by different letters are significant according to the Tukey's test ($P \leq 0.05$). Data are mean ± SD and n = 3. ***, **, and * denote significance at $P \leq 0.001$, $P \leq 0.01$, and $P \leq 0.05$, respectively, ns denotes non-significance.

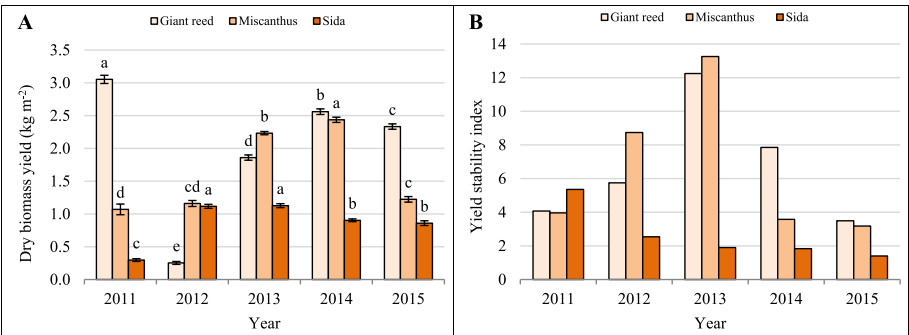


Fig. 5. A) Dry biomass yield and B) yield stability index of three biomass crops, i.e., giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby) within 5-year of open field experiments. Different letters on bars of the one biomass crop are significant according to the Tukey's test at $P \leq 0.05$.

3.6. Pearson correlation

The Pearson's correlation analysis of the measured traits revealed several significant relationships (Fig. 6). For example, EC_e showed a strong positive correlation with phosphatase activity and saturation percentage, while pH was positively correlated with catalase activity and SOM content. SOM had notable positive associations with dehydrogenase and urease enzyme activities. Nitrogen forms, such as NO_3^- -N and NH_4^+ -N, demonstrated significant correlations with total-N levels. Additionally, P levels were closely related to extractable K, indicating nutrient co-availability trends in the soil. These correlations underscore potential interactions among soil chemical and biological properties, reflecting an interconnected system where nutrient levels and enzyme activities can influence and reflect broader soil health indicators.

3.7. Heat map of soil properties

The heat map analysis of soil parameters measured after the cultivation of giant reed, miscanthus, and sida over five years revealed significant differences in soil health indicators among the three species (Fig. 7). Among the soil enzyme activities, sida exhibited the highest dehydrogenase activity in both autumn and spring. Phosphatase levels, which are crucial for phosphorus cycling, were also elevated under sida, especially in autumn, indicating a more active phosphorus turnover. Urease activity, a key indicator of nitrogen cycling, was significantly higher under giant reed, particularly in autumn, followed by miscanthus, while sida showed the lowest urease values. For catalase activity, an important antioxidant enzyme, both sida and giant reed demonstrated enhanced values. Miscanthus showed the highest SOM content, especially in spring, indicating a substantial contribution to organic carbon storage. Nitrogen forms varied across the crops, with sida yielding the highest total nitrogen and organic nitrogen levels in both seasons, supporting a more nutrient-rich soil profile under its cultivation. Soil saturation percentage was highest for sida, reflecting improved soil water retention capacity under this crop. In terms of EC_e

and pH, all species presented similar ranges, although EC_e was slightly higher for sida in autumn, pointing to potential differences in ionic composition. Considering these indicators, sida appears to promote the best overall soil health due to its influence on microbial activity, nutrient cycling, and water retention, which collectively enhance soil fertility and sustainability in a long-term cultivation system.

4. Discussion

The cultivation of biomass crops has garnered increasing attention globally due to the pressing need for sustainable and renewable energy sources that contribute to climate change mitigation, energy security, and soil conservation [1,3]. Biomass crops such as giant reed, miscanthus, and sida offer significant environmental and agronomic benefits, especially when cultivated on marginal lands, where they avoid competition with food crops while contributing to soil improvement [7]. The findings of this study emphasize the potential of these biomass crops to enhance soil health through improvements in nutrient cycling, SOM, and microbial activity. Furthermore, the yield stability index of these crops provides valuable insights into the resilience of biomass production systems, supporting the successful establishment and maintenance of biomass farms.

4.1. Importance of biomass crops for sustainable agriculture and soil health

The role of biomass crops in renewable energy production is well-established, but their positive impacts on soil health are equally significant. Biomass crops offer multiple ecological benefits, including carbon sequestration, improved soil structure, and nutrient cycling [20,43]. In particular, giant reed, miscanthus, and sida are perennial species with extensive root systems that contribute to soil organic carbon pools, improving soil porosity, enhancing soil's capacity to retain water and nutrients, enhancing soil structure, and reducing erosion. These root systems, along with aboveground biomass residues, continuously return

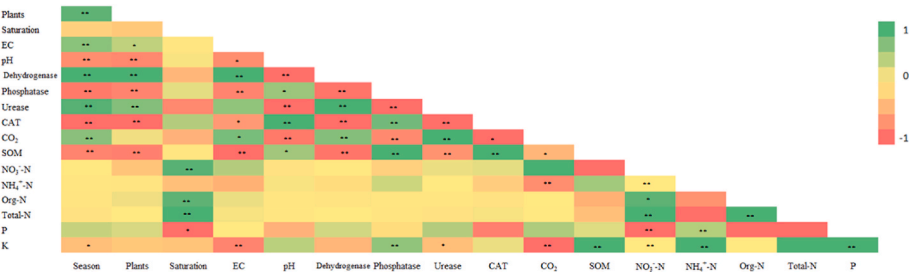


Fig. 6. Results of Pearson's correlation among different soil properties after growing three different biomass crops, i.e., giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby) within 5-year of open field experiments.

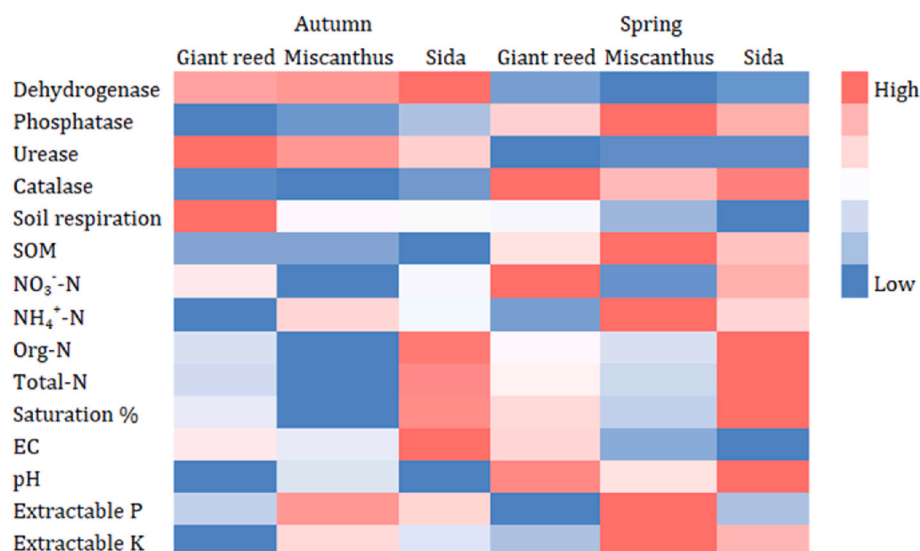


Fig. 7. Heat map of soil parameters following 5-year cultivation of giant reed (*Arundo donax*), miscanthus (*Miscanthus x giganteus*), and sida (*Sida hermaphrodita* Rusby) in open-field conditions. The figure highlights species-specific impacts on microbial activity (dehydrogenase, phosphatase, urease, catalase), soil organic matter, nutrient levels (N, P, K), and soil health indicators (saturation percentage, EC_e, pH) across autumn and spring seasons, with sida showing the greatest enhancement of overall soil health."

organic matter to the soil, which supports microbial activity and nutrient availability.

Our results showed that each biomass crop had species-specific effects on soil properties, particularly in relation to SOM and nutrient cycling. The SOM content was particularly high under miscanthus cultivation, reaching 3.77 % in spring and 2.57 % in autumn. High SOM levels are critical for improving soil structure, nutrient retention, and water-holding capacity, which collectively support soil fertility and long-term productivity [44]. The contribution of biomass crops to SOM is largely due to their aboveground biomass and root exudates, which are incorporated into the soil as they decompose. In our study, giant reed achieved the highest biomass yield, averaging 2.01 kg m⁻² over five years, thereby contributing substantial organic matter to the soil. This process fosters microbial activity and nutrient availability, ultimately enhancing soil health and fertility in the long term [17]. The observation that giant reed produced the highest biomass yield, while miscanthus contributed the most to SOM, is not entirely unexpected but warrants further exploration. Biomass yield and SOM accumulation do not always correlate directly because different species exhibit distinct root growth patterns, residue decomposition rates, and carbon sequestration capacities. Miscanthus has a dense, fibrous root system and high lignin content in its residues, which are known to contribute significantly to SOM over time through slower decomposition, as noted by Ref. [17]. Additionally, the SOM increase observed in spring under miscanthus may reflect seasonal factors such as residue accumulation from leaf and shoot shedding during the dormancy period [45]. The seasonal increase in SOM is likely influenced by the shedding of leaves and shoot tips during winter dormancy, which enriches the soil with organic residues that decompose during the warmer spring months. As highlighted by Chimento et al. [46], biomass crops like miscanthus deposit substantial litter during dormancy, contributing to soil carbon storage. This aligns with the findings of our study, which show higher SOM levels in spring than in autumn across all biomass crops, particularly under miscanthus. In addition to organic matter contributions, biomass crops play a significant role in nutrient cycling. For instance, nitrogen, a vital nutrient for crop productivity, was more available under biomass crops [47]. Sida, which showed the highest organic and total nitrogen content in both seasons, supports soil fertility by fostering nitrogen availability, potentially through symbiotic relationships with nitrogen-fixing bacteria [14]. The ability of biomass crops to maintain or enhance nitrogen

levels in the soil could help reduce dependence on synthetic nitrogen fertilizers, thus minimizing environmental pollution associated with nitrogen runoff and volatilization [27].

4.2. Seasonal and species-specific impacts of biomass crops on soil enzyme activities

The cultivation of biomass crops induced notable alterations in soil enzyme activities, which were also affected by sampling time, reflecting the dynamic interaction between crop type and seasonal changes. Soil enzymes, such as dehydrogenase, urease, phosphatase, and catalase, play vital roles in nutrient cycling, organic matter decomposition, and soil health [48]. In this study, enzyme activities varied significantly between autumn and spring, with each biomass crop exerting species-specific effects. For instance, dehydrogenase activity, an indicator of microbial respiration and organic matter decomposition, was highest in autumn under sida cultivation, reaching 307 µg TPF g⁻¹ soil h⁻¹, followed by miscanthus at 273 µg TPF g⁻¹ soil h⁻¹, indicating enhanced microbial respiration and organic matter decomposition. Dehydrogenase activity is strongly linked to microbial electron transport and is a reliable indicator of overall microbial metabolic activity [49]. The high activity observed under sida suggests a plant-microbe interaction that supports microbial energy metabolism, possibly through high carbon inputs from root exudates. This seasonal increase aligns with findings by Nassi O Di Nasso et al. [15], who noted that higher microbial respiration in autumn is often due to increased organic inputs and favorable conditions for microbial growth. Conversely, in spring, dehydrogenase activity was highest under giant reed, measuring 110 µg TPF g⁻¹ soil h⁻¹, suggesting that this crop supports microbial activity even after winter dormancy. Urease, which was particularly active in giant reed soils (346 µg NH₄⁺ g⁻¹ soil h⁻¹ in autumn), plays a crucial role in nitrogen cycling by converting urea into ammonium, a form of nitrogen that plants can readily assimilate. This conversion is essential for enhancing nitrogen availability, which supports plant growth and productivity. This high urease activity may result from nitrogen release from decomposing crop residues, aligning with findings from Refs. [8, 14] that biomass crops can increase soil nitrogen availability through residue decomposition. The elevated urease activity in giant reed soils suggests that this crop may improve nitrogen cycling, potentially reducing the need for synthetic nitrogen fertilizers and mitigating

environmental pollution from nitrogen runoff and volatilization [27]. Giant reed exhibited the highest urease activity ($346 \text{ mg NH}_4^+ \text{ g}^{-1} \text{ soil h}^{-1}$ in autumn), suggesting that its root exudates contain high levels of nitrogen-rich compounds, stimulating microbial production of urease, which facilitates nitrogen mineralization. This finding aligns with observations by Meena and Rao [50], who reported that nitrogen-fixing plant species often enhance urease activity through increased microbial nitrogen cycling. On the other hand, sida cultivation resulted in the highest dehydrogenase activity ($307 \text{ } \mu\text{g TPF g}^{-1} \text{ soil h}^{-1}$ in autumn), indicating enhanced microbial respiration and organic matter decomposition. Similarly, phosphatase activity reflects the soil's ability to cycle phosphorus, another vital nutrient for plant growth. In our study, phosphatase activity was particularly high under miscanthus in spring ($81.3 \text{ } \mu\text{g PNP g}^{-1} \text{ soil h}^{-1}$), suggesting that this crop may facilitate phosphorus cycling and availability. This is likely due to the plant's ability to release organic acids that increase phosphorus availability, as reported in similar perennial grass systems [51]. Phosphorus is often a limiting nutrient in soils, particularly on marginal lands, and its efficient cycling is crucial for maintaining soil fertility. By enhancing phosphatase activity, miscanthus could help maintain phosphorus availability in the soil, supporting sustainable crop production without the need for excessive phosphorus inputs [52]. Lastly, catalase activity, an indicator of oxidative stress management, was more stable but slightly higher in autumn under all crops, ranging from 6.2 to $8.5 \text{ mL O}_2 \text{ 2 min}^{-1}$. Catalase activity, an indicator of soil oxidative stress, was also positively influenced by biomass crops. Catalase activity was highest under giant reed and sida, particularly in spring, reflecting the capacity of these crops to manage soil oxidative stress. High catalase activity suggests that biomass crops can improve soil resilience by mitigating the effects of environmental stressors on soil health [13]. Enhanced catalase activity supports soil microbial communities, which are critical for organic matter decomposition and nutrient cycling, and contributes to a more resilient and productive soil ecosystem. Seasonal dynamics play a critical role in shaping soil enzymatic activities, nitrogen pools, and biomass yield. In our study, the highest urease activity was observed in autumn under giant reed cultivation ($346 \text{ mg NH}_4^+ \text{ g}^{-1} \text{ soil h}^{-1}$), while urease activity was notably lower but consistent across all biomass crops in spring. This seasonal variation may be attributed to higher organic matter decomposition rates in autumn, releasing more substrate for enzymatic activity [53]. Similarly, catalase activity was higher in spring compared to autumn for giant reed and sida, reflecting a seasonal increase in oxidative stress management during active plant growth phases. Catalase plays a vital role in decomposing hydrogen peroxide, a by-product of aerobic respiration, and is often upregulated during periods of high microbial and root activity [54].

4.2.1. Mechanisms of enzyme activity enhancement

Both giant reed and miscanthus are known for their extensive root systems, which release significant amounts of **root exudates** into the rhizosphere. These exudates, including sugars, amino acids, and organic acids, serve as energy sources for soil microbes, stimulating microbial activity and enzyme production [55]. For example, the release of labile carbon compounds by giant reed roots can enhance the activity of **dehydrogenase**, an enzyme involved in microbial respiration and organic matter decomposition [8]. Additionally, the rhizosphere of these crops creates a microenvironment rich in nutrients and microbial biomass, which further promotes enzyme synthesis. The close association between plant roots and microbes facilitates the production of enzymes such as **urease** and **phosphatase**, which are critical for nitrogen and phosphorus cycling, respectively [17].

The high biomass production of giant reed and miscanthus results in substantial inputs of plant litter into the soil. As this litter decomposes, it releases organic compounds that serve as substrates for soil enzymes. For instance, the breakdown of cellulose and hemicellulose in plant litter enhances the activity of **β -glucosidase**, an enzyme involved in carbon cycling [28]. The quality and quantity of litter also play a role in enzyme

dynamics. Miscanthus litter, with its high lignin content, decomposes more slowly, leading to a gradual release of substrates and sustained enzyme activity over time. In contrast, giant reed litter, which is relatively more labile, may result in a rapid but short-lived increase in enzyme activities [22].

The composition and activity of soil microbial communities are key drivers of enzyme production. Giant reed and miscanthus cultivation can alter microbial community structure by providing diverse carbon sources and creating favorable soil conditions (e.g., improved water retention and aeration). These changes, in turn, influence the synthesis of specific enzymes. For example, the presence of nitrogen-fixing bacteria in the rhizosphere of miscanthus may enhance **urease** activity, while phosphorus-solubilizing microbes associated with giant reed roots can increase **phosphatase** activity [16].

Seasonal changes in enzyme activities are closely linked to plant growth stages and environmental conditions. During the growing season, the combination of warm temperatures, optimal moisture, and high root exudation creates ideal conditions for microbial activity and enzyme production. For example, the peak activity of **urease** and **phosphatase** during the growing season reflects the increased demand for nitrogen and phosphorus by actively growing plants [25]. In contrast, enzyme activities decline during the dormant season due to reduced microbial activity and substrate availability. However, the residual organic matter from previous growing seasons can sustain baseline enzyme activities, ensuring nutrient availability for the next growth cycle [25].

4.3. Nitrogen dynamics and biomass crop effects on nitrogen pools

Biomass crops also influence soil nitrogen dynamics, which are essential for soil fertility and crop productivity. This study revealed species-specific effects on nitrogen pools, with significant variations observed in soil nitrate (NO_3^- -N), ammonium (NH_4^+ -N), and organic nitrogen levels [8]. Sida demonstrated the highest organic nitrogen and total nitrogen contents in both autumn and spring, with values of 4.81 mg kg^{-1} and 4.92 mg kg^{-1} , respectively, for organic nitrogen and 16.6 mg kg^{-1} and 17.0 mg kg^{-1} for total nitrogen. These elevated nitrogen levels suggest that sida may support nitrogen accumulation in the soil, potentially through symbiotic relationships with nitrogen-fixing bacteria [14]. While the current study suggests that sida contributes significantly to soil nitrogen pools, more direct evidence for its ability to engage in symbiotic nitrogen fixation should be provided. Previous studies have indicated that certain perennial biomass crops, such as sida, may host nitrogen-fixing bacteria within their rhizosphere, facilitating biological nitrogen fixation [56]. This process can enhance nitrogen availability in the soil and improve the crop's nutrient cycling potential. For instance, sida has been observed to improve total soil nitrogen levels, which may indirectly suggest nitrogen fixation processes, as supported by the elevated organic nitrogen (4.92 mg kg^{-1}) and total nitrogen (17.0 mg kg^{-1}) levels in our study during spring. To strengthen this argument, further investigation into the presence of nitrogen-fixing bacteria in the sida rhizosphere, similar to studies on other biomass crops like miscanthus, giant reed, and switchgrass [28], is recommended. This capacity for nitrogen accumulation is advantageous for reducing the need for synthetic nitrogen fertilizers, as nitrogen becomes available through natural cycling processes. The ability of biomass crops to influence nitrogen cycling may also be attributed to the decomposition of their residues [57]. As biomass crops senesce, their aboveground and root biomass are incorporated into the soil, where they undergo decomposition. During decomposition, nitrogen from plant residues is released in forms that plants can assimilate, thus enriching soil nitrogen pools and supporting nutrient cycling. The seasonal variations in nitrogen pools observed in this study, with higher nitrogen content in spring compared to autumn, highlight the influence of biomass crop residue decomposition on soil nutrient availability across seasons [25]. Nitrogen pools also exhibited seasonal trends, with higher levels of

NO_3^- -N, NH_4^+ -N, and Org-N in spring. This aligns with previous findings that seasonal temperature and moisture changes influence microbial nitrogen mineralization and organic matter decomposition, leading to enhanced nitrogen availability during spring [58]. These results indicate that nitrogen availability is closely tied to soil enzymatic activities and seasonal environmental conditions. The observed differences in nitrogen forms among crops indicate species-dependent impacts on nitrogen cycling pathways. Giant reed showed the highest NO_3^- -N accumulation (8.57 mg kg^{-1} in spring), likely due to its high nitrogen uptake efficiency and root-microbe interactions facilitating nitrification [59]. In contrast, the significantly higher Org-N content in sida (4.92 mg kg^{-1} in spring) suggests a slower nitrogen mineralization process, potentially driven by microbial immobilization and lignin-bound nitrogen stabilization [60].

4.4. Biomass yield stability and its importance in biomass farming systems

A crucial consideration in evaluating biomass crops for sustainable production is yield stability, as consistent biomass yields are necessary for reliable bioenergy production. In this study, the yield stability index (Si) was used to measure the consistency of biomass yields over the five-year cultivation period [27]. Giant reed exhibited the highest average biomass yield of 2.01 kg m^{-2} and demonstrated considerable yield stability, with the stability index increasing from 4.07 in 2011 to 12.24 in 2013 before gradually declining. Miscanthus and sida, while showing lower overall yields of 1.62 kg m^{-2} and 0.86 kg m^{-2} , respectively, displayed similar trends in yield stability. The decline in yield stability of the three biomass crops maybe attributed to the extreme climatic conditions during winter of 2012, which was the coldest in the last 10 years. High yield stability is an essential trait for biomass crops cultivated on marginal lands, where environmental conditions can be variable and may impact crop performance. Yield stability reflects the ability of a crop to maintain productivity despite environmental fluctuations, such as changes in temperature or precipitation [41,42]. In regions prone to environmental stressors, selecting crops with high yield stability, like giant reed, may improve the economic feasibility of biomass farming and ensure a stable supply of bioenergy resources. Biomass yield also varied seasonally, with peak harvests in late winter to early spring. Giant reed demonstrated the highest yield stability (2.01 kg m^{-2}), followed by *miscanthus* (1.62 kg m^{-2}) and *sida* (0.86 kg m^{-2}). The lower yield stability of *sida* might be attributed to its less vigorous growth under certain seasonal conditions, emphasizing the need for optimized management practices to harness its full potential as a biomass crop.

4.5. Cultivation on marginal lands to mitigate competition with food crops

A critical advantage of biomass crops is their suitability for cultivation on marginal lands, where soil conditions are often suboptimal for food production. By utilizing lands that are otherwise unsuitable for conventional crops, biomass crops provide an opportunity to produce renewable energy without impacting food production [21]. Marginal lands are characterized by poor soil fertility, limited water availability, or adverse climatic conditions; however, biomass crops such as giant reed, miscanthus, and sida can tolerate these constraints due to their resilience and adaptability [2]. As shown in this study, these crops achieved stable yields without the need for additional inputs, such as fertilizers or irrigation, highlighting their potential for sustainable bioenergy production with minimal resource use. The cultivation of biomass crops on marginal lands can also contribute to environmental resilience by reducing soil degradation and erosion. The root systems of these crops, particularly giant reed and miscanthus, stabilize the soil, reducing erosion and improving soil structure. This is especially important in regions with high erosion risk, where soil loss can threaten the productivity of agricultural land. Moreover, the ability of these crops to enhance soil nutrient cycling and organic matter content further supports soil recovery and productivity, making them suitable candidates for long-term land management strategies.

4.6. Future directions for biomass crop research and applications

The results of this study highlight the potential of biomass crops to enhance soil health and provide sustainable bioenergy; however, further research is necessary to optimize biomass crop cultivation. Studies examining the effects of targeted fertilization and irrigation on biomass crops grown on marginal lands could yield valuable insights into sustainable practices that maximize productivity with minimal environmental impact. Additionally, long-term studies extending beyond five years are recommended to assess the cumulative effects of biomass cultivation on soil health indicators, such as carbon sequestration, nitrogen dynamics, and microbial community composition. Further research into the economic and environmental implications of integrating biomass crops into existing agricultural systems would also be beneficial. Developing models that quantify the trade-offs between biomass production, soil health, and ecosystem services could provide decision-makers with valuable information for advancing sustainable bioenergy policies and land management practices. By balancing energy needs with environmental conservation, biomass crops can play a central role in the transition to sustainable agricultural and energy systems.

5. Conclusion

This study demonstrates that the long-term monoculture cultivation of giant reed, miscanthus, and sida has distinct effects on soil biochemical properties, enzyme activities, and nitrogen cycling, which are essential indicators of soil health. Giant reed significantly enhanced urease activity and nitrogen cycling, supporting soil fertility, while miscanthus contributed to higher soil organic matter and phosphatase activity, promoting carbon sequestration and phosphorus availability. Sida exhibited the highest dehydrogenase activity and total nitrogen content, suggesting improved microbial-driven nutrient cycling. These findings indicate that biomass crops not only serve as viable bioenergy feedstocks but also contribute to soil quality enhancement in low-input agricultural systems, particularly on marginal lands. From a practical perspective, integrating these crops into sustainable land management strategies could enhance soil fertility, reduce dependence on synthetic fertilizers, and promote carbon sequestration, making them valuable alternatives in climate-smart agriculture. However, the long-term sustainability of biomass crop cultivation requires further investigation. Future research should focus on extended monitoring beyond five years to assess long-term soil health trends, optimization of agronomic practices such as irrigation, fertilization, and crop rotation to maximize soil benefits while maintaining high biomass yields, economic and environmental evaluations to determine the feasibility of large-scale implementation in bioenergy production, and comparative studies incorporating different soil types and climatic conditions to assess the broader applicability of these findings. By addressing these aspects, future research can provide a more comprehensive framework for integrating biomass crops into sustainable bioenergy and agricultural systems, ensuring both environmental and economic benefits.

CRediT authorship contribution statement

Neven Elhawat: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Éva Domokos-Szabolcsy:** Resources, Methodology, Conceptualization. **Szilvia Veres:** Validation, Supervision, Conceptualization. **Miklós G. Fári:** Writing – original draft, Supervision, Formal analysis. **Tarek Alshaal:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization.

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Conflicts of Interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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