



# Diversifying maize rotation with other industrial crops improves biomass yield and nitrogen uptake while showing variable effects on nitrate leaching

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## ABSTRACT

Crop rotation and diversification can alleviate the high nitrate leaching associated with maize (*Zea mays* L.) monoculture, without reducing yields. Prior research focused on maize-legume/cereal rotations, with limited exploration of rotations with industrial crops destined for biorefining. In 2012, four-year rotation systems were established on sandy soil in Denmark, consisting of maize, hemp (*Cannabis sativa* L.), beet (*Beta vulgaris* L.), and triticale (*Triticosecale*), organized into four sequences to ensure the occurrence of each crop in each year. The fallow periods in the rotations were covered with “secondary crops”: winter rye (*Secale cereale* L.), winter rape (*Brassica napus* L.), or grass-clover (*Festuca rubra* L. – *Trifolium repens* L.). Over two rotation cycles (eight years), we assessed the aboveground biomass dry matter, biomass nitrogen (N) uptake, and their stabilities of maize in rotations versus monoculture, alongside quantifications of nitrate leaching, and soil carbon (C) and N stocks up to 100 cm deep. In the first cycle, the aboveground biomass of maize in rotation (15.5 Mg ha<sup>-1</sup>) was 7% significantly lower than that in monoculture (16.6 Mg ha<sup>-1</sup>), but this difference disappeared in the second cycle (17 versus 16.5 Mg ha<sup>-1</sup>). The maize biomass N uptake in rotation (194.5 kg ha<sup>-1</sup>) was similar to that in monoculture (196.6 kg ha<sup>-1</sup>) in the first cycle and was significantly higher by 8% in the second cycle (195.5 versus 165.7 kg ha<sup>-1</sup>). Nitrate leaching varied interannually with precipitation and secondary crops. Higher rainfall increased nitrate leaching for both systems in cold months, while rotations had more leaching after irrigation in dry periods. Initially, diverse rotation halved nitrate leaching compared to monoculture, but increased at the onset of the second cycle when the preceding winter rape did not survive in the winter. Winter rye following maize reduced nitrate leaching, except when the preceding crop was grass-clover or poorly thriving winter rape. During the whole period, the rotation system increased soil C and N stocks at 0–100 cm depth. This study highlights the environmental and yield benefits of diversifying maize rotations, and the crucial role of secondary crop management to optimize maize rotation strategies.

## 1. Introduction

Maize (*Zea mays* L.) is a widely utilized and versatile crop, cultivated globally on nearly 200 million ha (FAOStat, 2021). It is a vital staple for human consumption, livestock feed, and also acts as an industrial raw material for producing bioenergy and biomaterials (Poole et al., 2021; Zampieri et al., 2019) including bioethanol and biogas—possible alternatives to fossil fuels (Aghaei et al., 2022). However, intensive maize monoculture systems are often associated with excessive fertilization

and soil degradation (Robson et al., 2002; Soto-Gómez and Pérez-Rodríguez, 2022), with risks to both food security and soil ecosystem sustainability (Cox et al., 2006). To address these challenges, enhancing crop diversity, such as integrating rotation schemes into maize production, has emerged as an effective approach to bolster the sustainability of crop production systems worldwide (Bowles et al., 2020; Jungers et al., 2021; Renard and Tilman, 2019).

The existing research on maize rotations has predominantly focused on maize-legume or -cereal rotation systems (Smith et al., 2023;

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Gwenambira-Mwika et al., 2021). Within such systems, legumes enhance soil nitrogen (N) fertility for greater crop production due to their capacity to fix atmospheric N, thereby reducing the need for synthetic N inputs (Zhao et al., 2022), while maize-cereal rotations contribute to improved overall crop production by reducing pests and diseases, improving soil quality, and optimizing water usage (Beillouin et al., 2021a; Karlen et al., 1994; Zegada-Lizarazu and Monti, 2011). Concurrently, the rising global demand for industrial and energy crops to meet the requirements of bioenergy and bio-based product sectors is reshaping agricultural priorities in many regions (Zegada-Lizarazu and Monti, 2011). For example, hemp (*Cannabis sativa* L.) cultivation in the EU has increased substantially from 20,540 ha in 2015–33,020 ha in 2022 (European Commission, 2022). As a fast-growing crop known for its deep root system, hemp can help control weeds and pests and improve soil conditions (Struik et al., 2000). However, the rotational effects of industrial crops (including hemp) with maize are not yet well comprehended, with existing studies yielding contrasting results. For example, the rotation of maize with oilseed rape has been associated with enhanced biomass stability compared to monoculture practices, due to the rapid decomposition of oilseed rape residues improving soil nutrient availability (Łukowiak et al., 2016). Conversely, diversification of maize with legumes may exacerbate nitrate ( $\text{NO}_3$ ) leaching due to their  $\text{N}_2$ -fixing capabilities (Leimer et al., 2015; Martínez-Mena et al., 2021). These contrasting results indicate that the overall effects of diversified crop rotations might be strongly influenced by the specific crop types and crop compositions within rotation.

Nitrate ( $\text{NO}_3$ ) leaching is a major problem in monoculture systems on sandier soils with surplus of water from precipitation after harvesting main crops (Svoboda et al., 2013; Wey et al., 2021). To address this issue, farmers in many countries grow a secondary (catch) crop such as grass, rye or rape planted post-harvest the main crop to serve an additional sink for the residual  $\text{NO}_3$  (Manevski et al., 2015; Preissel et al., 2015; Vogeler et al., 2023). Despite this practice, there exists a paucity of knowledge concerning the impact of integrating secondary crops into rotations (i.e., double cropping - two harvests per year, main and secondary crop) on  $\text{NO}_3$  leaching compared to monoculture systems. Hence, an improved understanding of the influence of diverse rotations designed to produce biomass for biorefinery while balancing soil nutrients and  $\text{NO}_3$  leaching needs to be elucidated (Katakajwala and Mohan, 2021).

The temporal aspect is crucial in delineating the impact of diversified crop rotations on both yield and soil-related variables (Knapp et al., 2023; Rasmussen et al., 1998). This is because a complete rotation cycle spans several years and the impact on soil properties tends to accumulate gradually. For example, significant changes in soil carbon (C) and N may require a minimum of 8–10 years to become detectable (Smith, 2004; Smith et al., 2020; Shang et al., 2024). Moreover, while the benefits of diversified crop rotations on yield and yield stability are documented, it remains unclear whether the advantages in biomass and biomass stability in the rotation with industrial crops are sustained over time or are limited to certain rotation cycles. To address this knowledge gap comprehensively, a minimum of two rotation cycles within experimental frameworks are required to document and understand the temporal dynamics.

The main objective of this field study is to reveal if maize in rotation can improve maize biomass yield and N uptake, and to assess  $\text{NO}_3$  leaching and soil C and N stocks at the soil depth of 1 m, compared to maize monoculture. We used a 4-years crop rotation experiment with maize initiated in 2012 under Danish conditions of sandy soil and maritime climate, involving two cycles of maize in rotation compared to maize monoculture (Fig. S1). Specifically, we aimed to assess: 1) whether maize biomass and biomass N uptake, as well as their stability, are improved in the rotation system compared to monoculture due to improved nutrient availability from crop residues mineralization, 2) if the rotation system including secondary crops effectively reduces  $\text{NO}_3$  leaching, and 3) whether the rotation contributes to increased soil C and

N stocks due to higher crop residue inputs (especially via the additional root C inputs of the secondary crops).

## 2. Materials and methods

### 2.1. Study site, experimental design, and agronomic management

This study is part of an ongoing long-term field experiment initiated in 2012 at Foulum, Tjele, Denmark (9°35'E, 56°30'N, 48 m a.s.l., Fig. S2; Manevski et al., 2017). The soil type is Typic Hapludult, characterized as a sandy loam with a pH of 6.5 and comprising 78.2% sand, 10.7% silt, and 8% clay in the 0–25 cm soil layer (Manevski et al., 2017). The top 30 cm contains 4% organic matter, with a soil C/N ratio of 12, and a dry bulk density of  $1.3 \text{ g cm}^{-3}$ . The climate at Foulum is temperate, with a mean annual temperature of  $7.8 \text{ }^\circ\text{C}$  and an annual precipitation of 740 mm (Manevski et al., 2018). The potential annual evapotranspiration is approximately 600 mm. A detailed meteorological overview for each year from 2013 to 2020, corresponding to periods of maize growth, is provided in Fig. S3. Before 2012, the land was cultivated with annual crops, including winter wheat (*Triticum aestivum* L.), winter rye (*Secale cereale* L.), potato (*Solanum tuberosum* L.), and spring barley (*Hordeum vulgare* L.). These crops were provided with mineral and organic fertilizers, with an average N input of  $150 \text{ kg ha}^{-1}$  annually.

In 2012, the field experiment was initiated with the objective of investigating potential biomass productivity to support several purposes in the biobased economy through diverse agricultural cropping systems (Manevski et al., 2018). The experimental design was an incomplete split-plot design with four blocks i.e., replicates spaced 8 m apart, and the plot area within each block was designed based on the different crop types, ensuring that potential effects caused by proximity of crops with distinct heights were minimized. The experimental treatments in this study consisted of a maize monoculture and maize in rotation (Fig. 1). The rotation was organized into four sequences to ensure each crop appeared each year and included maize, beet (*Beta vulgaris* L.), hemp and triticale (*Triticosecale*) as main crops; the specific varieties are shown in Table S1. Hemp was changed to faba bean (*Vicia faba* L.) in the second rotation due to suboptimal performance (i.e., lower harvested aboveground biomass than expected) over the first rotation cycle. Ryegrass was grown for two months after faba bean harvest, until triticale sowing. Maize and hemp were sown in late April to early May and harvested in September or October, respectively. Beet was sown in May and harvested in November. Winter triticale was sown in late September and harvested in early July of the following year before full maturity. Winter rye (*Secale cereale* L.), winter rape (*Brassica napus* L.), grass-clover (*Festuca rubra* L. – *Trifolium repens* L.) or festulolium (*Festulolium braunii* L.) were grown as secondary crops after maize or in the triticale plots before the maize to maximize land utilization and to explore options to reduce  $\text{NO}_3$  leaching. The secondary crop after maize, winter rye, was sown in late October after maize harvest, and was harvested in April/May of the following year. Secondary crops grown after triticale varied during the experimental period, with the aim of exploring best performing crops under Danish winter conditions. Briefly, winter rye was grown from July or November 2013 to the following April; grass-clover was grown in the triticale from March to the following May and harvested twice in the years of 2014–2016; winter rape was grown from July to the following April in the year of 2018–2019. However, in the years 2016 and 2017, the regrowth after the first cut of winter rape in late autumn was so poor that it did not allow for a second cut the following spring. Festulolium was sown in the young triticale and developed after triticale harvest from August to the following April in the year of 2019–2020 (Fig. 1). Mineral composite fertilizer (NPK) was applied to all main crops according to the Danish agro-legislation, which is adjusted per crop on a yearly basis due to changing weather conditions and also depend on the previous crop. For maize, the average amount was  $174 \text{ kg N ha}^{-1}$ ,  $37 \text{ kg P ha}^{-1}$ , and  $177 \text{ kg K ha}^{-1}$ . Detailed management information of all crops, including

Year	2012												2013												2014												2015												2016														
Month	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12
Rotation 1	winter rye				maize				winter rye				beet				hemp				triticale				rape																																						
Rotation 2	triticale				winter rye				maize				winter rye				beet				hemp				triticale																																						
Rotation 3	hemp				triticale				grass-clover				maize				winter rye				beet				triticale																																						
Rotation 4	winter rye				beet				hemp				triticale				grass-clover				maize				winter rye																																						
Monoculture	maize				maize				maize				maize				maize				maize				maize																																						

Year	2017												2018												2019												2020												2021			
Month	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4
Rotation 1	rape				maize				winter rye				beet				faba bean				triticale				festulolium																											
Rotation 2	triticale				rape				maize				winter rye				beet				faba bean				triticale																											
Rotation 3	hemp				triticale				rape				maize				winter rye				beet				triticale																											
Rotation 4	rye				beet				faba bean				triticale				festulolium				maize				winter rye																											
Monoculture	maize				maize				maize				maize				maize				maize				maize																											

Fig. 1. Scheme of the cropping systems design comparing maize in rotation and monoculture from 2012 to 2021. The 4-yr rotations had main crops of maize, triticale, hemp, and beet, which appeared each year. The secondary crop winter rye always followed maize in the rotation, in addition to secondary crops winter rape, grass-clover, and festulolium following triticale before the maize in the rotation, but these were not present in the monoculture. The data in this study refer to the maize harvest year, thus include the secondary crops following triticale cultivated before the year before the maize.

the fertilizer rates, can be found in Table S1 in the Supplementary material. Pest and disease management involved application of common pre- and post-emergence herbicides. Irrigation was not applied, except to alleviate drought in May, June and July 2018, and June and July 2019, 2021 and 2022, with typically one irrigation of 30 mm per month.

## 2.2. Plant, soil, and soil leachate analyses

The aboveground biomass of all main and secondary crops (except beet, which included both above- and belowground biomass) was harvested annually from 2013 to 2021 as whole crop from the central area of each plot (1.5 m × 10 m, excluding the borders) using a plot harvester (Haldrup F-55, Germany). The stubbles of maize and secondary crops were left on the field at a height of 15–20 and 5–10 cm, respectively. Details regarding the harvest can be found in Table S1. Subsamples (~2 g) of plant biomass were dried at 60°C to a constant weight to determine the dry matter yield. Subsamples (2 g) were ground using a mill (Retsch Mill, Haan, Germany) for the analysis of N content using an elemental analyzer (Elementar Analysensysteme AG, Langensfeld, Germany).

Soil samplings were conducted in the spring of 2012 (before the establishment of the field trial), 2017, and 2021 in both maize monoculture and in the rotation systems. Prior to sampling, plant residues on the soil surface were cleared, and eight subsamples were collected diagonally using a soil auger with a 1 cm inner diameter. The soil samples were collected at three soil depths (i.e., 0–20 cm, 20–50 cm, and 50–100 cm), and samples from the same depth were pooled for homogenization. A total of 60 soil samples were collected for each of the three sampling years. Soil samples were air-dried and sieved through a 2 mm sieve to remove stones and visible plant residues, prior to total soil C and N measurements. The combustion method was used for analyzing soil C and N content with an element analyzer (Elementar Analysensysteme AG, Langensfeld, Germany). Soil bulk density was measured in 2017 using a split tube Eijkelkamp soil auger (inner diameter 4.7 cm).

Soil moisture levels (volumetric, cm<sup>3</sup>/cm<sup>3</sup>) were assessed using Time Domain Reflectometry (TDR) sensors and a calibration formula specifically designed for soils in Denmark (Jacobsen and Schjønning, 1993). The TDR sensors were positioned in pairs vertically, extending from the soil's surface to depths of 20, 50, and 100 cm. These moisture readings were taken three to six times per month from 2013 to 2021. Soil leachate samples were collected from the studied systems using porous ceramic cups (K100, UMS GmbH, Munich, Germany) installed in duplicates at the center of each plot at the beginning of the experiments. The cups were installed at 1 m depth, corresponding to the maximum rooting depth of the experimental soil. Sampling commenced in April 2013 and continued once or twice during each summer (May to September) and

bi-weekly from September to the following April each year, since the main leaching occurred during autumn and winter. Prior to sampling, the cups were subjected to nearly c. 80 kPa suction for two to three days. Nitrate (NO<sub>3</sub>-N) concentration in the soil leachate samples was measured by a continuous segmented flow auto-analyzer (SEAL AA500 AutoAnalyzer, Norderstedt, Germany).

## 2.3. Estimation of soil nitrate leaching

The water balance for the 0–100 cm soil columns of each treatment was simulated by Daisy (ver. 5.19), a fairly detailed and one-dimensional, deterministic and hydrological coupled heat-energy process-based model able to estimate water fluxes in a soil-plant-atmosphere system (Hansen et al., 2012; Salazar et al., 2013). In the model, the water balance comprises fluxes at the surface and in the soil, where the atmosphere and the groundwater constitute the system boundaries. The surface fluxes considered are precipitation (and irrigation, if any; gain), and evapotranspiration (and surface runoff, if any; losses), whereas soil fluxes are deep percolation (drainage; loss) or capillary rise (gain). Soil water dynamics, hence, drainage, are modelled by a numerical solution of the Richards's equation. Evapotranspiration is described by the potential evapotranspiration concept according to the FAO Penman-Monteith equation (Allen et al., 1998) and a crop coefficient. Further information about the model can be found in Hansen et al. (2012) and Manevski et al. (2018). The model was calibrated, and details are provided in Description S1 of the Supplementary material. The simulated daily percolation was used to calculate NO<sub>3</sub> leaching. The estimated NO<sub>3</sub> leaching (see next section) was accumulated from April to April of next year to annual values from 2013 to 2021 to facilitate comparison with the corresponding annual biomass N. The N loss through surface runoff was not considered due to the soil's sandy nature and flat surface.

## 2.4. Calculations and statistical analysis

The aboveground biomass of crops including straw and grain was reported on a dry matter (DM) basis (Mg DM ha<sup>-1</sup>) and biomass N uptake of crops was calculated as follows:

$$\text{Biomass N uptake (Kg N ha}^{-1}\text{)} = \text{N content (g 100g}^{-1}\text{)} \times \text{aboveground biomass (Kg ha}^{-1}\text{)} \times 0.01$$

Stability (unitless) of biomass and biomass N uptake were calculated as the inversed coefficient of variation according to Knapp and van der Heijden, (2018):

$$\text{Stability (Biomass or Biomass N uptake)} = \mu / \sigma$$

where  $\mu$  and  $\sigma$  represent the mean and standard deviation, respectively,

of the biomass production or biomass N uptake of each cropping system during the periods of 2013–2017 (first rotation cycle), 2017–2021 (second rotation cycle), or 2013–2021. Higher biomass stability indicates higher production consistency interannually under varying weather and management practices, which is important when designing feedstock supply to biorefinery.

Soil C and N stock was calculated as follows:

$$\text{Soil C (N) stock}_i (\text{Mg ha}^{-1}) = \text{content of soil C}_i (\text{N}_i) \times \text{BD}_i \times \text{D} \times 0.1,$$

where C (N) is the content of soil C or N ( $\text{g kg}^{-1}$ ), BD is soil bulk density ( $\text{g cm}^{-3}$ ) of the corresponding soil layer, D is the soil layer thickness (cm),  $i$  is the year of soil sampling and 0.1 is a conversion factor.

The soil  $\text{NO}_3$  concentrations ( $\text{mg L}^{-1} = \text{mg dm}^{-3}$ ) between measurement dates were interpolated to daily values using an improved version of the percolation-weighted concentrations method originally described by Lord and Shepherd, (1993) in order to calculate daily  $\text{NO}_3$  leaching:

$$\text{Daily nitrate leaching} (\text{kg ha}^{-1}) = \text{Soil nitrate concentration} \times \text{daily drainage} \times 0.1,$$

where soil nitrate concentration is the concentration of  $\text{NO}_3$  in the soil water ( $\text{mg dm}^{-3}$ ), daily drainage is the daily drainage amount simulated by the model (mm) and 0.1 is unit conversion factor.

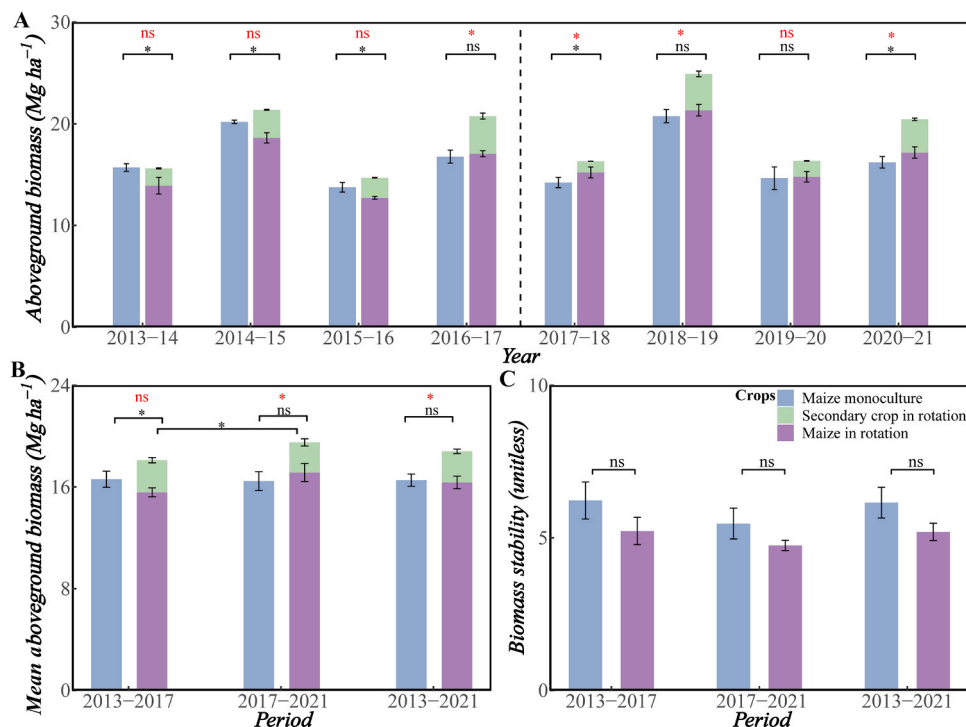
We used linear mixed effects models with restricted maximum likelihood estimation in the 'nlme' package of R to analyze the differences in aboveground biomass, biomass stability, biomass N uptake, biomass N uptake stability, soil C and N stocks and  $\text{NO}_3$  leaching (Pinheiro et al., 2018). For the rotation systems, soil C and N stocks from the year of sampling (2012) were initially averaged across the four different rotation systems. Then we calculated absolute changes of soil C, N stocks in different periods between maize monoculture and the maize rotation system. Cropping system, year, and their interactions were treated as

fixed effects, while block was considered a random factor. To examine the effects of different rotational cycles on the measured variables between these two systems, we grouped the years into different periods and set cropping system, period, and their interactions as fixed effects, with block as a random factor. To assess the impact of individual years or periods on the variables within each cropping system, we included year or period as fixed factor and block as the random factor. Similarly, we evaluated the effects of cropping systems on the variables within each year or period by using cropping system as a fixed factor and block as a random factor. All models were checked for normality and homogeneity of residuals using the Kolmogorov-Smirnov method. Data transformation (e.g., log) was applied when necessary to meet assumptions. Multiple comparisons were conducted for determining differences among treatments using the post hoc Tukey test at the significance level of 0.05 with the "emmeans" function in the emmeans package. All the significance of statistical results were reported as such ( $F_{\text{numerator DF, denominator DF, } P}$ ). All statistical analysis and data visualization were performed in R 4.0.5 (R Development Core Team, 2020).

### 3. Results

#### 3.1. Aboveground biomass and biomass stability

The aboveground biomass of maize was 13–20  $\text{Mg ha}^{-1} \text{ yr}^{-1}$  in monoculture and 12–21  $\text{Mg ha}^{-1} \text{ yr}^{-1}$  in rotation over the 8 years (Fig. 2A). Year effects on yields were significant (Fig. 2A,  $F_{7,45} = 74.45$ ,  $P < 0.001$ ), along with interactive effects of cropping system and year ( $F_{1,45} = 3.37$ ,  $P = 0.005$ ). Thus, initial maize aboveground biomass in rotation was up to 13% lower than in monoculture in earlier years of 2013–2016, but later years (2017–2018 and 2020–2021) saw increases in biomass by up to 6%. Overall, in the first cycle (2013–2017), the maize biomass in the rotation as mean of the first four years was



**Fig. 2.** Annual (A) and periodic (B) aboveground biomass (straw + grain) and maize biomass stability (C) in rotation (maize and winter rye secondary crop harvested the following year, see Fig. 1) and monoculture (maize) systems in the experimental period of 2013–2021. During the first cycle of 2013–2017, the last harvest of maize and winter rye secondary crop was in 2016 and 2017, respectively (Fig. 1). During the second cycle of 2017–2021, the last harvest of maize and winter rye secondary crops was in 2020 and 2021, respectively. In each panel, black and red symbols indicate significance of maize biomass or total biomass (the sum of maize and the second crops) between maize monoculture and rotation, respectively. \* $P < 0.05$ ; "ns", non-significant difference. Data are means and standard errors ( $n = 4$ ). The dashed line on panel A indicates the division between the two rotation cycles.

significantly lower than the respective mean value in the monoculture by 7% (Fig. 2B,  $t_{27} = 2.16$ ,  $P = 0.04$ ), yet with no significance in the second cycle ( $t_{27} = 0.65$ ,  $P = 0.52$ ). On average across the 8 years, there was no significant difference in maize biomass ( $t_3 = 0.26$ ,  $P = 0.79$ ) or biomass stability ( $t_3 = 0.62$ ,  $P = 0.58$ ) between monoculture and rotation systems (Fig. 2B, C). The secondary crops provided additional biomass to the rotation system (Fig. 2A), resulting in comparable and even higher total biomass in the rotation system than in the monoculture (Fig. 2A, B), ranging from 1.8 to 5.9 kg DM ha<sup>-1</sup> (Table S6).

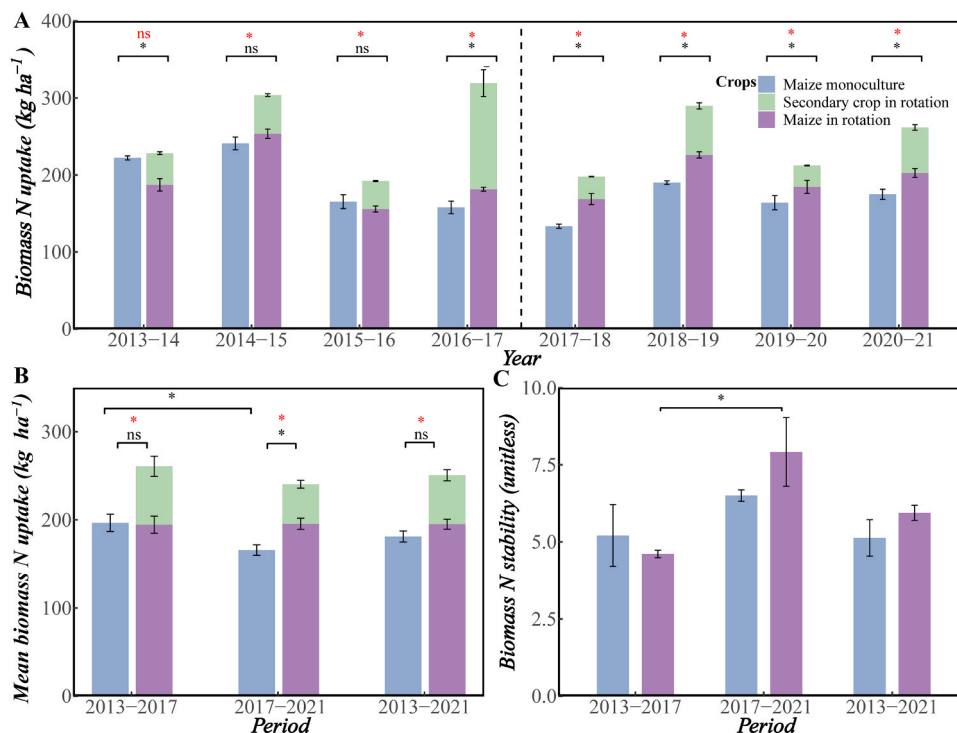
### 3.2. Biomass N uptake and N uptake stability

Experimental year, cropping system, and their interaction significantly affected the maize biomass N uptake (Fig. 3 A,  $F_{7,45} = 55.43$ ,  $P < 0.001$ ;  $F_{1,45} = 22.04$ ,  $P < 0.001$ ;  $F_{7,45} = 8.55$ ,  $P < 0.001$ ). During the first cycle (2013–2017), no significant difference in biomass N uptake was found between the two systems in the first cycle (Fig. 3B,  $t_{27} = 0.14$ ,  $P = 0.88$ ), but the biomass N uptake in the rotation maize was significantly higher by 8% than the monoculture during the second cycle (2017–2021, Fig. 3B,  $t_{27} = -3.46$ ,  $P = 0.001$ ). Maize biomass N stability did not significantly differ between systems across cultivation periods (Fig. 3 C,  $t_3 = 0.59$ ,  $P = 0.59$ ;  $t_3 = -1.42$ ,  $P = 0.25$ ;  $t_3 = -1.27$ ,  $P = 0.29$ ). However, maize N uptake stability in the second cycle was higher (2017–2021, by 9%) than that in the first cycle (2013–2017, Fig. 3 C,  $t_3 = -4.02$ ,  $P = 0.01$ ). The rotation system including maize and secondary crops, consistently increased biomass N uptake compared to monoculture (Fig. 3B,  $t_{27} = -3.7$ ,  $P = 0.001$ ;  $t_{27} = -6.3$ ,  $P < 0.001$ ,  $F_{1,59} = 40.92$ ,  $P < 0.001$ ). Biomass N uptake of secondary crops before maize in rotation ranged from 46 to 139 kg N ha<sup>-1</sup> (Table S6).

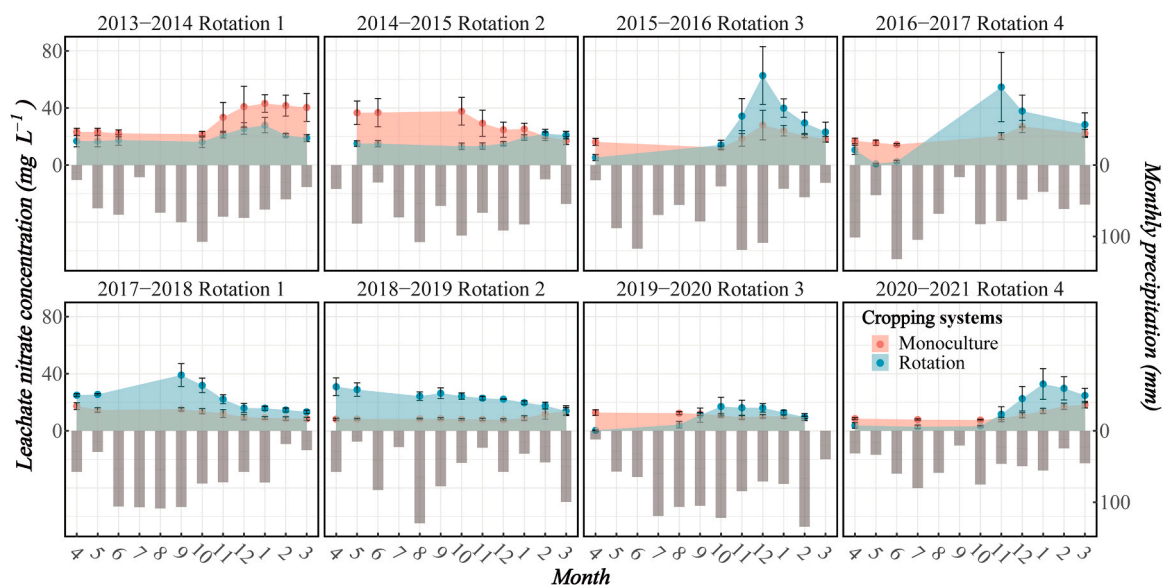
### 3.3. Soil NO<sub>3</sub> concentrations and leaching

In the first cycle (2013–2017), leachate NO<sub>3</sub> concentrations were 12–43 in maize monoculture and 1–63 mg L<sup>-1</sup> in rotation (Fig. 4). Initially, rotation system had lower leachate NO<sub>3</sub> concentrations than monoculture from November 2013 (after maize harvest) to November 2014 (before maize harvest). However, the opposite pattern was observed in subsequent years, with rotation showing higher concentrations after maize harvest in 2015–2016 and 2016–2017 (Fig. 4). The highest leachate NO<sub>3</sub> concentration (63 mg L<sup>-1</sup>) was found during winter rye growth. In the second cycle (2017–2021), NO<sub>3</sub> concentrations were generally lower than in the first cycle (8–28 and 0.4–39 mg L<sup>-1</sup> in the monoculture and rotations, respectively). Across this cycle, concentrations were consistently higher in rotation, except during maize growth in 2019–2020 and 2020–2021.

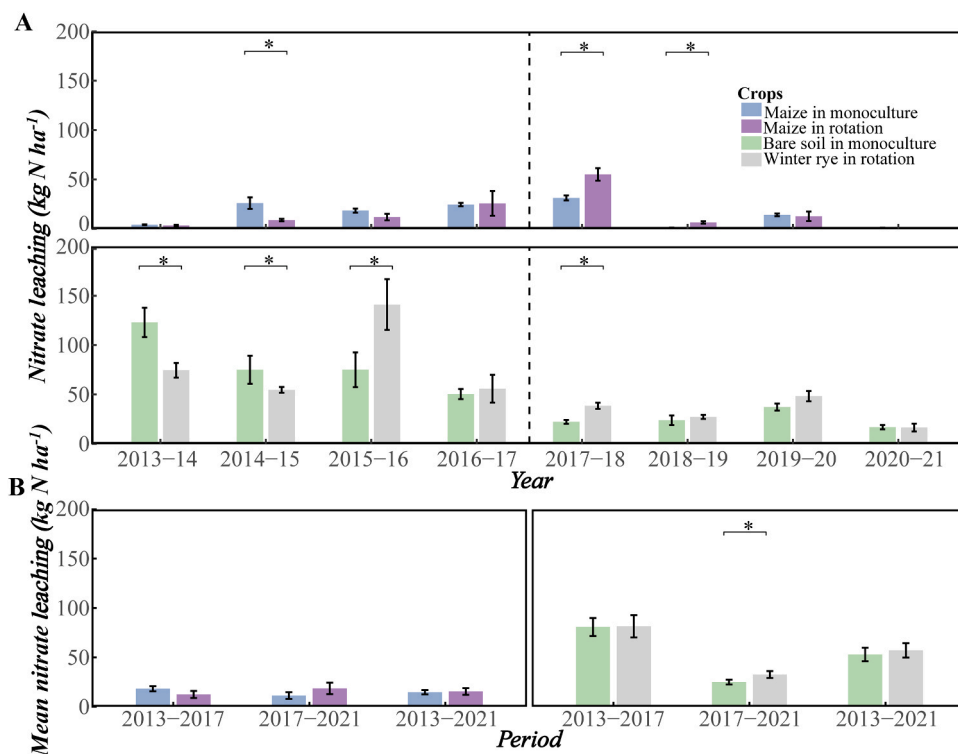
Seasonally, NO<sub>3</sub> leaching (Fig. 5A, B) for maize monoculture was 1–31 kg ha<sup>-1</sup> during maize growth (April to October) and 17–123 kg ha<sup>-1</sup> after harvest (November to following April). In the maize rotation, in-season NO<sub>3</sub> leaching ranged from 0.5 to 55 kg ha<sup>-1</sup> compared to the post-harvest 16–141 kg ha<sup>-1</sup> during winter rye cultivation. During the season, NO<sub>3</sub> leaching in rotational maize was 1.9 times lower than in maize monoculture in 2014–2015 (Fig. 5 A,  $F_{1,6} = 8.26$ ,  $P = 0.02$ ). However, in 2017–2018 and 2018–2019, NO<sub>3</sub> leaching during maize in the rotation system exceeded that in monoculture by 1.8 and 5 times, respectively (Fig. 5 A,  $F_{1,6} = 12.20$ ,  $P = 0.01$ ,  $F_{1,6} = 20.93$ ,  $P = 0.003$ ). After maize harvest, compared with bare soil in monoculture, winter rye in rotation had lower NO<sub>3</sub> leaching in 2013–2014 ( $F_{1,6} = 8.60$ ,  $P = 0.02$ ) and 2014–2015 ( $F_{1,6} = 7.95$ ,  $P = 0.03$ ) but higher in 2015–2016 ( $F_{1,6} = 12.20$ ,  $P = 0.04$ ), 2017–2018 (Fig. 5 A,  $F_{1,6} = 18.95$ ,  $P = 0.004$ ). Across rotation cycles, only the second cycle (2017–2021) showed 30% higher NO<sub>3</sub> leaching from winter rye in rotation compared to bare soil in monoculture (Fig. 5 C,  $F_{1,30} = 4.51$ ,  $P = 0.04$ ). Other



**Fig. 3.** Annual (A) and periodic (B) biomass nitrogen (N) uptake and biomass N uptake stability (C) of crops in rotation (maize and winter rye secondary crop harvested the following year, see Fig. 1) and monoculture (maize) systems in the experimental period of 2013–2021. During the first cycle of 2013–2017, the last harvest of maize and winter rye secondary crop was in 2016 and 2017, respectively (Fig. 1). During the second cycle of 2017–2021, the last harvest of maize and winter rye secondary crop was in 2020 and 2021, respectively. In each panel, black and red symbols indicate significance of maize biomass N uptake or total biomass N (the sum of maize and the second crops) between maize monoculture and rotation, respectively. \* $P < 0.05$ . Data are means and standard errors ( $n = 4$ ). The dashed line on panel A indicates the division between the two rotation cycles.



**Fig. 4.** Dynamics of nitrate concentrations in soil leachate at 1 m depth in maize in rotation and monoculture against monthly precipitation (grey bars) from 2013 to 2021. From April to October, both monoculture and rotation systems included maize. After maize harvest (November to next April), monoculture plots were left bare, while winter rye was the secondary crop after maize in rotation. The preceding crops of maize were as follows (also see Fig. 1): winter rye in 2013–2014 and 2014–2015, grass-clover in 2015–2016 and 2016–2017, winter rape in 2017–2018, 2018–2019, and 2019–2020, and festulolium in the last year. The height of the area plot denotes mean values and error bars are the standard error.



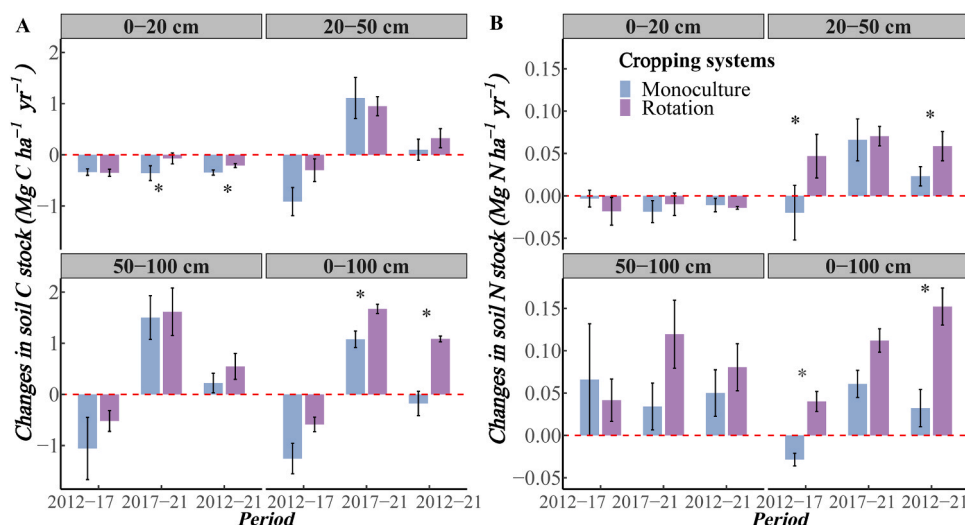
**Fig. 5.** Annual (A) and periodic (B) nitrate leaching between rotation (maize + winter rye) and monoculture (maize + bare soil) systems during the experimental period of 2013–2021. Panel A shows differences in nitrate leaching for maize from April to October and for bare soil or secondary crop winter rye from November to next April of each year. Panel B shows differences in mean nitrate leaching for each period. \* $P < 0.05$ . Bars denote means and error bars are the standard error ( $n = 4$ ).

cycles had no significant differences between maize rotation and monoculture.

### 3.4. Changes of soil C and N stocks

Soil C stocks in topsoil (0–20 cm) decreased in both cropping systems

over the whole period (2012–2021, Fig. 6). However, during the whole investigated period, and particularly the second cycle (2017–2021), such decline was significantly lower in the rotation systems than that of the monoculture (Fig. 6 A,  $t_3 = -4.15$ ,  $P = 0.03$ ;  $t_3 = -4.02$ ,  $P = 0.03$ ). Soil C stocks across the 0–100 cm soil depth decreased in both cropping systems in the first cycle (2012–2017) (Fig. 6A). However, during the



**Fig. 6.** Changes in soil carbon stock (A) and nitrogen stock (B) at different soil depths (0–20, 20–50, 50–100 and 0–100 cm) in maize monoculture and rotation systems at different periods (2012–2017, 2017–2021, 2012–2021). During the period of 2012–2021, the first soil sampling was in the spring of 2012 before the establishment of the field experiment. The second soil sampling was in the spring of 2017 (i.e., the end of the first crop rotation cycle). The last soil sampling was in the spring of 2021 (i.e. the second crop rotation cycle). \* $P < 0.05$ . Data are mean values and standard errors ( $n = 4$ ).

second cycle (2017–2021), both systems showed an increase in soil C stock, and such increase was significantly higher in the rotation system than in the monoculture (Fig. 6 A,  $t_3 = -3.23$ ,  $P = 0.04$ ). Across the experimental period, the rotation system significantly increased soil C stock compared to maize monoculture (Fig. 6 A,  $t_3 = -5.13$ ,  $P = 0.01$ ). Similarly, the maize rotation also showed a higher absolute change in soil N stocks across the 20–50 and 0–100 cm depth, during the first and whole period compared to the monoculture (Fig. 6B,  $t_3 = -3.25$ ,  $P = 0.04$ ;  $t_3 = -3.38$ ,  $P = 0.04$ ).

## 4. Discussion

### 4.1. Improved biomass and biomass N uptake of maize in diverse rotations is time-dependent

During the initial three years of the experimental period and in the first cycle (2013–2017) the results showed lower biomass of maize in the rotation than in monoculture (Fig. 2A), contrary to previous studies suggesting improved biomass performance in maize rotation with legumes (Huynh et al., 2019; Xia et al., 2023; Zegada-Lizarazu and Monti, 2011). One possible explanation is that these early years of cultivating maize and industrial crops in rotation might deplete soil nutrients (e.g., N but also P and K) via the cultivation and the harvest of main and secondary crops. Since it may take some years to increase soil organic matter (SOM), the potential benefits of diverse crop rotations on soil fertility were not realized in these initial years. Potential phytotoxic compounds from hemp (Russo et al., 1997; Zegada-Lizarazu and Monti, 2011) could negatively affect maize growth, however, such effect could be minor since maize was grown 1.5 years after harvesting hemp (Fig. 1). For the difference in biomass yield between the two systems in 2013, one explanation could be the reduced fertilization given to winter rye in the rotation compared to the monoculture in the establishment year of 2012 (Fig. S5). In the first cycle (2013–2017), the maize in both monoculture and rotation exhibited comparable biomass N uptake (Fig. 3A). Maize has a substantial N demand to support its growth and development (Sandhu et al., 2021). The similar biomass N uptake may be attributed to the adequate provision of soil N to maize plants in both systems during this phase, further supporting that the lower biomass in rotation may have been due to the depletion of other nutrients such as P and K. The available N in the soil, either from residual sources or introduced through fertilization, appeared sufficient to sustain the early

growth phases of maize in both systems, especially in the periods of 2014–2015 and 2015–2016 (Fig. 3A).

As time progressed, maize in rotation achieved comparable and even higher biomass in two out of the four years of the second cycle (2017–2021) (Fig. 2A) and displayed a notable advantage in maize biomass N uptake in comparison to maize monoculture (Fig. 3A). These results indicate that maize in rotation with other industrial crops may require a longer observation period to uncover its biomass potential and maintain N accumulation in maize biomass, compared to maize monoculture. The second cycle's biomass and biomass N uptake dynamics could be attributed to four mechanisms. First, compared to monoculture, the decomposition of diverse crop residues in the rotation liberates N to the soil at a later stage (Johnston et al., 2002; Robson et al., 2002). This controlled N release ensures a steady nutrient supply to maize throughout the rotation, and eventually contributed to comparable biomass and enhanced biomass N uptake in the later years (Figs. 2, 3B). For instance, winter rape has a relatively high C/N ratio, leading to slower decomposition when compared to leguminous residues (Kriaučiuniene et al., 2008), especially for the poor-thriving winter rape in 2016 and 2017 because it was ploughed into the soil next spring. Consequently, the N released during the decomposition of winter rape residues becomes available to subsequent crops over an extended period. In contrast, continuous maize monoculture depletes soil nutrients faster because of no extra C sources from diversified crops (Fujisao et al., 2020). Second, despite its potential phytotoxic effect on subsequent crops in rotation, hemp's presence can effectively suppress pests and diseases in the soil through unique bioactive secondary metabolites such as, cannabinoids, terpenoids and flavonoids, particularly targeting weeds, insects, and nematodes (Amaducci et al., 2008; Hazekamp et al., 2010; Mcpartland and Glass, 2001; Venendaal et al., 1997). Third, crop rotations can enhance soil structure and water retention because of diverse root systems (Zegada-Lizarazu and Monti, 2011). For example, hemp is a relatively deep-rooted crop thus can improve soil pore space, which provides a better soil environment for maize growth and N uptake (Emmerling et al., 2017; Peel, 1998). Fourth, diverse crop rotations support a varied microbial community (Liu et al., 2023; McDaniel et al., 2014). The inclusion of crops such as hemp, festulolium and faba bean can stimulate the growth of beneficial soil microorganisms such as mycorrhizal fungi and N-fixing bacteria, increasing N availability and absorption for maize in 2020 (Dellagi et al., 2020). Overall, when considering the future adoption of maize and industrial crops in

rotation, selecting suitable crop combinations becomes crucial for biomass production in the long term.

Our study shows that the introduction of winter rye secondary crops to maize in rotation significantly increased both biomass and biomass N uptake compared to maize monoculture (Figs. 2,3). This underscores the potential of secondary crops in enhancing sustainable energy and bio-based material production, especially given the current climate scenario with a substantial gap between available resources and growing demands (Gaffey et al., 2023). This improvement stems from the ability of the secondary crops such as grasses, winter rye, and winter rape to make full use of their extended growing season, thereby prolonging the period for photosynthesis and biomass accumulation and N uptake (Graß et al., 2013). Additionally, their inherent ability for rapid growth during the cool winter months further supported the potential for biomass production and N uptake (Thomashow, 1998).

We found no significant difference in the biomass stability and biomass N uptake of maize in the two systems across the experimental cycles (Figs. 2,3), contrary to previous studies observing enhanced maize biomass stability in rotation due to increased resilience to stressful weather conditions (Degani et al., 2019). However, during the experimental period with drought in 2018 and 2020 (Fig. S3), the management practices with targeted fertilizer and irrigation, may have mitigated the adverse effects of environmental variability on biomass stability in both systems (St-Martin et al., 2017). This is further supported by the absence of significant relationships between precipitation, temperature, and changes in biomass of these two cropping systems during the experimental period (Fig. S4). Accordingly, the potential advantages in biomass stability of diversified maize rotations with industrial crops seem to be more likely to be realized in rainfed areas under arid and semiarid conditions (Bowles et al., 2020; Zegada-Lizarazu and Monti, 2011).

#### 4.2. Nitrate leaching as influenced by precipitation and secondary crops in rotation

We observed significant fluctuations in NO<sub>3</sub> leachate from 2013 to 2021 (Figs. 4, 5). Precipitation is the primary transport factor influencing variability in NO<sub>3</sub> leaching from the studied soil as no or seldom irrigation was applied (Hess et al., 2020). More precipitation leads to greater movement of NO<sub>3</sub> and this was particularly noticeable in the first rotation cycle (2013–2017), with significantly higher levels of NO<sub>3</sub> and leaching compared to the second cycle (2017–2021; Fig. 5, S2). The increase in leaching during the cooler months, mainly autumn and winter (Fig. 5B), is due to precipitation surpassing evapotranspiration and low nutrients requirements of crops, which causes soil water percolation and movement of NO<sub>3</sub> mineralized and unused during the growth season (Dai et al., 2020; Krüger et al., 2021). Comparisons between maize monoculture and rotation systems reveal distinct NO<sub>3</sub> and leaching patterns (Figs. 4, 5). Initially, rotation systems showed lower NO<sub>3</sub> concentrations and leaching in 2013–2015, especially during periods of high rainfall when winter rye was growing. This supports findings from Manevski et al. (2015), Malone et al. (2017) and Vogeler et al. (2023), suggesting that secondary crops absorb excess N and reduce the impact of precipitation on bare soil, thereby lessening leaching (Fig. 5A). During periods with higher temperature and irrigation (e.g., 2018–2019), as well as the second rotation cycle (2017–2021) and compared to monoculture, higher NO<sub>3</sub> levels and leaching were observed in rotation systems. This may be due to enhanced soil porosity and drainage on the sandy soil from broader root systems and higher amount of plant residues returned (Talukder et al., 2023), along with additional N applications to secondary crops (Fig. S5).

Alongside the water balance as a transport factor, these disparities were also largely attributed to the management practices of the secondary crops. Despite the similar roles of grass-clover, winter rape, and winter rye as secondary crops to cover the post-harvest period of the main crop (maize or triticale) and thus increase biomass production on

an annual scale (Fig. 2; Manevski et al., 2017), and also to absorb unused mineral N during autumn (Manevski et al., 2018), its choice plays an important role in controlling NO<sub>3</sub> leaching. Notably higher NO<sub>3</sub> concentrations and NO<sub>3</sub> leaching were found in the rotation than in the monoculture in the year 2015–2016 during the growth of the secondary crop (Figs. 4, 5 B). This can be linked to the secondary crop species of this year involving legume (grass-clover), which can increase soil N levels via N fixation and fast mineralization of its N-rich residues (Kebede, 2021; Yang et al., 2023). Higher NO<sub>3</sub> leaching of maize in the rotation was also found in 2017–2018 and 2018–2019 (Figs. 4, 5A), although a new secondary crop (winter rape) was chosen. However, this could be explained by the fact that the winter rape was only harvested one time due to poor growth in late 2016 and 2017 and was ploughed into the soil in the next spring, with the subsequent slower mineralization of the crop residues contributing to the increase soil NO<sub>3</sub> concentrations due to lower precipitation. In contrast, during the following year (2019) winter rape was able to grow until May 2019, which could help reduce the amount of N in the soil through plant N uptake (Beilouin et al., 2021b). In summary, if an increase in biomass production was accompanied by an increase in NO<sub>3</sub> leaching (Fig. S6), this suggests that adequate management of the secondary crops in the rotation is vital to mitigate N losses from the cropping systems. When comparing the entire rotation cycle, NO<sub>3</sub> leaching levels between maize monoculture and maize rotation appear to be the same (Fig. 5C). This result challenges our hypothesis for the positive impact of crop rotation with secondary crops on NO<sub>3</sub> leaching (Manevski et al., 2018; Zegada-Lizarazu and Monti, 2011) and also calls for optimization of such rotations with industrial crops.

#### 4.3. Improved soil carbon and nitrogen stocks in rotation

The soil C and N stocks of 0–20 cm decreased in both monoculture and rotation systems over the experimental period (Fig. 6). which might be a general consequence of intensive agricultural production, where C inputs to the soil are limited due to the removal of aboveground biomass, while tillage (and soil disturbance) stimulates the microbial turnover of organic matter (Adkins et al., 2019; Kabiri et al., 2016; Manevski et al., 2018) and therefore C losses. However, the overall C loss of topsoil was negligible in the rotation system, particularly during the second cycle (2017–2021). This can be attributed to the improved biomass performance (Fig. 2) and thus stubble and root C inputs in the rotation during this period, offsetting to a greater extent the C effluxes (Chen et al., 2022). Significant increases in soil C were observed in both systems within the subsoil layers (20–50 cm and 50–100 cm) during the second cycle (2017–2021, Fig. 6), aligning with similar shifts reported by Shang et al. (2024) within annual and perennial cropping systems. Deep-rooted crops including maize in both systems contribute to SOM at depth over time. The period of observation also included high precipitation in the first cycle (2012–2017) and notable droughts in 2018 and 2020, affecting leaching and decomposition dynamics. Increased leaching during wet periods and decreased microbial activity during droughts likely led to SOM accumulation at deeper soil depths.

We observed a higher increase in soil C and N stocks in the rotation at 0–100 cm soil depth from 2012 to 2021, compared to monoculture (Fig. 6). Diversified rotations result in higher and more diverse crop residue inputs and decomposition into soil, promoting nutrient recycling and organic matter accumulation. This effect is particularly noticeable in sandy soils, such as those of our study (de Oliveira Ferreira et al., 2021; Keiblinger et al., 2023). Additionally, the N-rich crop residues, of legume species such as faba bean in the second cycle may strengthen and increase the organo-mineral associations in the soil and stabilize SOM (Kopittke et al., 2020). Furthermore, more extensive root systems and root exudates from various plants (Zegada-Lizarazu and Monti, 2011), such as hemp, can contribute to higher soil C and N stocks in the subsoil. Notably, during the first cycle, compared to monoculture, we observed inconsistency in soil C and N stocks in rotation, with soil N stocks



increasing and soil C stocks decreasing (Fig. 6). This may be linked to higher use of mineral fertilizers in rotation to meet the needs of more crops. The increased use of mineral fertilizers initially led to higher soil N stock in the first cycle. However, as the rotation duration extended, inputs of plant-derived organic matter and soil nutrients began to accumulate (Kaiyong et al., 2011), leading to an increase in both soil C and N stocks for the rotation system in the second cycle (2017–2021). This aligns with a meta-analysis indicating that only soil N stocks tend to increase within the first five years after fertilizer application, but over time, both soil C and N stocks consistently increase (Bohoussou et al., 2022).

#### 4.4. Implications and limitations of the study

This study demonstrates that integrating industrial crops into maize rotations not only enhances maize biomass and soil C and N levels but can also reduce NO<sub>3</sub> leaching. Such improvements underscore the positive impacts of crop diversity on agricultural productivity and environmental health. The effectiveness of these benefits depends on the choice and management of secondary crops. For instance, introducing legumes as a secondary crop can increase NO<sub>3</sub> leaching the following season with maize, indicating a need for targeted research to identify crop combinations that optimize both biomass increase and environmental pollution. Future studies should address the limitations of our experiment. One key limitation is the reliance on bulk density data solely from 2017 for assessing soil C and N stocks. Although short-term changes in bulk density are typically minor and concentrated in the topsoil (Meurer et al., 2018), and consistent tillage practices across our cropping systems suggest similar bulk density changes across treatments, this approach might overlook subtle variations. Future studies should incorporate more frequent bulk density measurements to enhance the accuracy of soil C and N stock assessments. The variation in many of the measured variables across different years underscores the need to understand dynamic interactions to tailor agricultural practices to specific local conditions, such as soil type and climate, thereby fine-tuning the application of rotational systems to curb the environmental impacts of agriculture. Also, noteworthy is that winter rye secondary crop consistently followed maize in the diversified rotation, while the preceding secondary crop to the triticale main crop changed (Fig. 1), producing their own legacy effects on the maize in relation to organic matter turnover and soil NO<sub>3</sub> release. This effect could not be isolated in the current analytical approach, which neither was the aim of the study; instead, the comparison focused on the period covering maize + secondary crop (in rotation) compared to maize + bare soil (monoculture), while the effects of preceding secondary crops were explained by the available data related to these elements, i.e., biomass and N uptake of secondary crops (see Table S6 in the Supplementary material), as well as soil NO<sub>3</sub> concentrations (data not published).

## 5. Conclusion

We assessed the effects of a 4-year diversified maize rotation with other industrial crops on yield-related potential, NO<sub>3</sub> leaching, and soil C and N stocks across two rotational cycles. Maize monoculture yielded more biomass in the first rotation cycle (2013–2017), but maize in rotation caught up in the second cycle (2017–2021), suggesting long-term benefits of rotation may not be immediately apparent. Notably, maize biomass N uptake in rotation was higher in later years than that of monoculture, indicating improved nutrient availability and potential sustainability benefits. Stability of biomass and biomass N uptake did not differ significantly between the systems, because fertilization and irrigation were adequate to mitigate environmental variability. Increased rainfall in the colder months led to escalated NO<sub>3</sub> leaching, but maize in rotation initially showed lower NO<sub>3</sub> leaching than monoculture. However, the interplay of precipitation, preceding crops in terms of N quality of residues and management practices modified this

picture: in preceding warm periods, the rotations increased leaching after irrigation as introducing a grass-legume or short winter rape growth before the maize elevated NO<sub>3</sub> concentration. Therefore, careful crop selection and management practices are crucial for reducing NO<sub>3</sub> leaching in rotation systems, especially under variable climatic conditions. Rotation increased soil C and N stocks compared to monoculture throughout the study period. This study underscores the advantages and limitations of diversified maize rotations for enhancing agricultural sustainability compared to monoculture.

#### CRedit authorship contribution statement

**Poul Erik Lærke:** Writing – review & editing, Investigation. **Mohamed Jabloun:** Software. **Diego Abalos:** Writing – review & editing. **Zhi Liang:** Writing – review & editing. **Uffe Jørgensen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Kiril Manevski:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Mingming Zong:** Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Data Availability

Data will be made available on request.

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#### Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.agee.2024.109091](https://doi.org/10.1016/j.agee.2024.109091).

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