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Late sowing of cover crops reduces potential for nitrate leaching reduction and carbon inputs

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ABSTRACT

Good establishment of cover crops early after harvest of the main crop is challenging and requires optimized sowing time to maximize ecosystem services in Northwestern Europe. We quantified the abilities of three cover crop species, fodder radish (*Raphanus sativus*), oats (*Avena sativa*), and phacelia (*Phacelia tanacetifolia*) to (i) take up nitrogen (N), reduce nitrate leaching when sown at four sowing times from 10 Aug to 7 Sep at two sandy loam sites in Denmark (Foulum and Flakkebjerg) during two percolation periods of 2022–23 and 2023–24, and (ii) provide carbon (C) input to the soil at two sowing times at Flakkebjerg in 2023. N uptake and nitrate leaching reduction declined with delayed sowing time. The decline followed different patterns for N uptake and nitrate leaching reduction and varied depending on interactions between species, percolation period, and site. Each day of delayed sowing from 10 Aug to 7 Sep decreased the ability of cover crops to reduce nitrate leaching by 0.5 kg N ha^{-1} day^{-1} at Flakkebjerg and 1.2 kg N ha^{-1} day^{-1} at Foulum. Overall, fodder radish and phacelia more efficiently reduced leaching at the early sowing times, while fodder radish was most effective at the later sowing times. At Flakkebjerg in 2023, fodder radish accumulated more C than oats and phacelia when sown on 10 Aug. Delaying sowing to 30 Aug reduced total C input from all cover crops by 60 %. These findings can potentially be used for enhancing environmental and climate benefits of cover crops in sustainable cropping system design.

1. Introduction

Cover crops are non-harvested crops generally grown to cover bare soil after the harvest of the main cash crop with the aim to reduce nitrate leaching and enhance the performance of the next main crop. Inclusion of cover crops into various cropping systems is well-recognized for providing many ecosystem services such as increasing soil fertility and soil carbon (C) inputs (Abdalla et al., 2019; Nouri et al., 2022). The role of cover crops in enhancing C input for climate change mitigation has been particularly gaining attention in recent years (Kaye and Quemada, 2017; Rodrigues et al., 2021). The effectiveness of cover crops in providing these ecosystem services depends on their successful establishment, length of their growing period, sufficient biomass accumulation, nitrogen (N) uptake, and root development (Thorup-Kristensen et al., 2003; Tonitto et al., 2006). In northern European countries such as Sweden, Denmark, and Finland, where radiation and temperature rapidly decline during the autumn after harvest of the main crop, achieving sufficient cover crop growth is challenging (Aronsson et al., 2016; Heller et al., 2024). Under these circumstances, delayed sowing of cover crops significantly restricts them to reach their full potential (Hashemi et al., 2013; Cottney et al., 2022).

To increase the degree days accumulation and growth period, cover crops can be undersown within the main crop (Thomsen and Hansen, 2014). However, undersowing of cover crops that have shallow roots and moderate growth rates can reduce their ability to take up N and reduce nitrate leaching with less effective biomass production due to resource competition from the main crops (Doltra and Olesen, 2013; Burger et al., 2017). Cover crops can also be sown after the main crop is harvested (Munkholm and Hansen, 2012; Thomsen and Hansen, 2014; Heller et al., 2024). Although optimal harvest date of the main crop can vary within a few hundred kilometers, early sowing of the cover crop is critical for their effective establishment and biomass accumulation in

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northern latitudes (Thorup-Kristensen et al., 2003; Pullens et al., 2021).

A recent study by Kumar et al. (2023) demonstrated that delaying the sowing time of cover crops can generate variations in N uptake based on the interaction between sowing time and cover crop species, and between sowing time and growth year. However, there was no such interaction observed for nitrate leaching reduction. The authors concluded that differences in growth, canopy, and root traits among cover crops likely contributed to the variations in N uptake, and frost tolerance, and residue decomposition rate during winter resulting in similar nitrate leaching reduction across different sowing times. The study of Kumar et al. (2023) was limited to a single site. The variation in N uptake and nitrate leaching reduction can be dependent on the interactions of sowing dates and pedoclimatic conditions (land use, soil type, and precipitation) (Hashemi et al., 2018; Hu et al., 2018; Jensen et al., 2021; McClelland et al., 2021; Teixeira et al., 2016). Additionally, the impact on these ecosystem services can be influenced by frost tolerance characteristics of the cover crop and their sensitivities to different pedoclimatic conditions (Thorup-Kristensen et al., 2003; De Notaris et al., 2018; Kumar et al., 2023). Therefore, further study is needed to assess the impact of sowing time on cover crop benefits across a broader range of soil characteristics and precipitation conditions.

Besides reducing nitrate leaching by taking up N after the main crop, the role of cover crops as a key measure to mitigate climate change, via increased soil organic carbon (SOC) input, is also well established (Blanco-Canqui et al., 2015; McClelland et al., 2021). The effectiveness of cover crops in promoting SOC is, as for nitrate leaching reduction, strongly influenced by the amount of biomass they produce, which is largely determined by the length of the growing period, i.e., the time between germination and termination (McClelland et al., 2021). For SOC, most studies have focused primarily on aboveground C input, often neglecting root contributions. Excluding root biomass from these assessments may overlook substantial variations in total C input. Similarly, shallow root sampling in the plough layer only (e.g. 0–25 cm) might lead to a biased understanding of C allocation patterns such as root-to-shoot ratios (Hodge, 2004; Poorter et al., 2012). Information on belowground C input is crucial as research has shown that these inputs contribute more to the stabilization of SOC than C input from aboveground biomass (Ghafoor et al., 2017; Kätterer et al., 2011; Poeplau et al., 2021; Rasse et al., 2005: Ruf and Emmerling, 2022). Hence, determining both the total C input and the C distribution between above and belowground biomass pools is crucial for understanding climate change mitigation potential of cover crops in relation to their sowing time, which has rarely been studied.

In this study, we evaluated three cover crop species, fodder radish (*Rafanus sativus*), phacelia (*Phacelia tanacetifolia*), and oats (*Avena sativa*) from Brassicaceae, Boraginaceae, and Poaceae plant families. These species are known for their varied characteristics of growth, N uptake, and nitrate leaching reduction (Justes et al., 1999; Munkholm and Hansen, 2012; Kumar et al., 2023). This study aimed to explore how delaying sowing times of above cover crop species affects: i) N uptake and the reduction of nitrate leaching, and ii) root and shoot biomass C input to the soil. The study was carried out over two years on two sandy loam sites with varying clay content and precipitation. The main hypotheses of this study were: (i) delayed sowing of cover crops decreases the ability to take up N and reduce nitrate leaching, and the effect may depend on growth characteristics of the cover crop species and pedoclimatic conditions; and (ii) delayed sowing of cover crops significantly reduce total biomass C inputs to the soil, with species-dependent effects on both above- and belowground C inputs.

2. Materials and methods

2.1. Field experiments

The field experiments were conducted over the growing season of 2022 and 2023 at Foulum (56.30 °N, 9.35 °E) and Flakkebjerg (55.30 °N, 11.23 °E) in Denmark. The soil at Foulum is a sandy loam classified as Luvic Umbrisol according to the World Reference Base (WRB) and FAO Unesco soil map system (IUSS Working Group WRB, 2022; Krogh and Greve, 1999) with 10 % clay (<2 μm), 12 % silt (2–20 μm), 42 % fine sand (20–200 μm) and 35 % coarse sand (200–2000 μm), and 1.5 % organic C at the top 25 cm. The Flakkebjerg soil is also a sandy loam classified as Glossic Phaeozem with 13 % clay, 15 % silt, 46 % fine sand, 24 % coarse sand, and with 1.6 % organic C at the top 25 cm. In all four trials, spring barley was grown as the main crop which was followed by different species and sowing times of cover crops. The main crop spring barley was fertilized with 157 kg N ha^{-1} in 2022 and 137 g N ha^{-1} in 2023 in Foulum. At Flakkebjerg, spring barley was fertilized with 144 kg N ha^{-1} in both years. Three cover crop species, fodder radish (cv. Brutus), oats (cv. Poseidon), and phacelia (cv. Angelia) were sown at four different sowing times after harvesting spring barley in 2022 and 2023, with seeding rates of 20 kg ha^{-1}, 105 kg ha^{-1}, and 6 kg ha^{-1}, respectively (Table 1). At Foulum the cover crops were sown using a Pöttinger disk coulter seeding machine fitted with a Pöttinger rotary harrow. At Flakkebjerg the sowing was carried out with a Kvernland disc colter seed drill.

In addition to the 12 combinations of cover crop species and sowing times, a control treatment with bare soil was included (treated with herbicide in autumn). The treatments were arranged in a four replicated complete block design with treatments randomized within blocks. In the second year, the experimental trial was conducted in a nearby field with the same crop history to prevent any carry-over effects from the first year. The size of the experimental plots was 15 m^2. Dates of the various field operations during the experiment are presented in Table 2.

2.2. Weather at experimental sites

Weather data were obtained from climate stations installed in Foulum and Flakkebjerg close to the experimental plots. Precipitation was recorded at 1.5 m height and corrected to the soil surface based on Allerup et al. (1998). Average temperatures during cover crops growth and percolation period from first sowing time in August to the last water sampling date in April (Table 1 and Table 2) were between 6.7 and 7.6 °C at both sites (Fig. 1a). The precipitation sum was 711 mm at Foulum and 575 mm at Flakkebjerg for the same period during the first percolation period of the experiment (2022–23) (Table 2, Fig. 1b). During the second percolation period (2023–24), precipitation sum was 898 mm at Flakkebjerg and 853 mm at Foulum for the respective period. As expected, daily average temperature and precipitation sums decreased with delayed sowing time (Figs. 1c, 1d). The decrease was approximately 40–43 % for the daily average temperature sum and 20–30 % for the precipitation sum from the first sowing to the last at both sites. The comparative climate perspective of the two studied percolation periods relative to the long-term climate normal is as follows: from August to April, the daily average temperature and precipitation sum for the 1991–2020 climate normal at Foulum were 6.4 °C and 820 mm, respectively. We do not have the long-term climate normal for Flakkebjerg.

2.3. Measurement of nitrate leaching

Water samples were collected at 14 days intervals (except in dry and frosty periods) from two permanently installed ceramic suction cups in each plot (length, 68 mm; diameter, 20 mm; and 0.1 MPa air entry

Table 1
Sowing times of cover crops.

Site	Sowing times in 2022 and 2023			
	1	2	3	4
Foulum	10-Aug	20–21 Aug	29–30 Aug	7-Sep
Flakkebjerg	10-Aug	19–21 Aug	30-Aug	7-Sep

Table 2
Overview of the field operations and water sample collection dates for nitrate-N concentration analysis during percolation periods of 2022–23 and 2023–24.

	Foulum	Flakkebjerg
Fertilizer application	5-Apr−2022 (157 kg N/ha).	21-Apr−2022 (144 kg N/ha)
Spring barley sowing	11-Apr−2022	21-Apr−2022
Harvest spring barley	5-Aug−2022	4-Aug−2022
Cover crop plant sampling	31-Oct−2022	25-Oct−2022
Ploughing	1-Apr−2023	5-Dec−2022
Fertilizer application	2-Apr−2023 (137 kg N/ha)	19-Apr−2023 (144 kg N/ha)
Spring barley sowing	2-Apr−2023	21-Apr−2023
Harvest spring barley	17-Jul−2023	4-Aug−2023
Glyphosate application#	2-Aug−2023	10-Aug−2023
Cover crop plant sampling	31-Oct−2023	25-Oct−2023 (separate sampling for above and below ground C and N input on 31-Oct−2023)
Ploughing	8-May−2024	15-Dec−2023
Percolation period	Water collection dates	
2022–23	from 13-Oct−2022 to 14-Apr−2023	from 10-Jan−2023 to 18-Apr−2023
2023–24	from 27-Sep−2023 to 4-Apr−2024	from 27-Nov−2023 to 25-Mar−2024

Applied for weed control after harvesting the main crop. There were little to no weeds in 2022, therefore glyphosate was not applied.

value, type K 100, UMS GmbH) placed at 1 m depth for nitrate-N concentration analysis. The water samples were extracted from the installed suction cups by imposing a suction of 60–70 kPa on the sampling device. Samples from the two suction cups in each plot were equally mixed in equal proportions to represent the plot and then analyzed for nitrate-N concentration using a Technicon Auto Analyzer (Tarrytown, NY, USA). The water sample collection dates are presented in Table 2. We estimated daily percolation using the EVACROP model (Olesen and Heidmann, 1990). EVACROP simulates daily percolation as a function of daily precipitation, temperature, and evapotranspiration. EVACROP accounts for ten crop types with varying leaf area indices and root characteristics, and seven soil types differing in texture and water-holding capacity, all of which influence evapotranspiration and water uptake from the root zone, and thus percolation. Percolation weighted interpolation between the sampling times was used to calculate daily nitrate leaching using the daily percolation values (Vogeler et al., 2023). For statistical analyses, we used cumulated nitrate leaching measured over each percolation period of 2022–23 and 2023–24 (hereafter referred to as nitrate leaching) and calculated nitrate leaching reduction by subtracting the nitrate leaching in cover crops treatments from nitrate leaching in herbicide-treated bare soil in corresponding blocks. EVACROP simulated percolation earlier than the first water sampling dates on 3 Aug 2023 at Foulum in 2023–24 and 25 Dec 2022 and 24 Oct 2023 at Flakkebjerg in 2022–23 and 2023–24 (Larsen et al., 2024). This suggests that a small proportion of the nitrate leached early in the percolation period may not have been included in the measurement.

2.4. Measurement of cover crop N uptake

Plant samplings were carried out from a randomly chosen predetermined area of 0.5 m^2 to determine the N uptake in aboveground biomass for all four sowing times at Foulum and Flakkebjerg (see the sampling dates in Table 2). Weed and volunteers, if present in the selected area, were dried, weighed separately, and analyzed for N content by dry combustion at 950 ∘C using Vario Max Cube. The statistical analysis of N uptake was conducted using the total N content, which includes the N contents of weeds and volunteers, and cover crops per plot. This is subsequently referred to as N uptake in cover crops.

2.5. Measurements of cover crop above- and belowground C and N at Flakkebjerg in 2023

To determine C and N inputs from roots and shoots of cover crops at different sowing times, plant samples were collected at the end of growing period on 31 Oct 2023 in all three cover crops for two sowing times of 10 Aug and 30 Aug in 2023 only at Flakkebjerg. A steel column (20 cm in diameter and 50 cm in height) was inserted in each plot to sample one plant of fodder radish and phacelia, and several plants of oats, based on general field plant density. The columns were pressed into the soil using a tractor-mounted press tool, then dug up and sealed with plastic covers at both ends. Above-ground shoot biomass was determined by cutting at the soil surface. In the lab, soil was pressed out of the columns and separated into two layers: 0–25 cm (topsoil) and 25–40 cm (subsoil) (Fig. S1). The soil from each layer was weighed separately, sieved through 1 cm sieve to homogenize the soil and to collect main roots. A representative subsample for each column of approximately 1 kg of moist soil was kept cool until the isolation of small roots: Two 200 g moist soil subsamples per column were taken to isolate small roots. The soil was repeatedly dispersed (10 times) with water and decanted over a 250 μm sieve and washed (Fig. S1). Non-root organic material was removed from the small root fractions with forceps, and the roots were oven-dried at 60 ° C for at least 48 h, weighed and scaled to total soil mass for each layer. Shoots and main roots were gently washed and dried in the oven in the same manner as the small roots.

Due to small sample sizes of small roots and subsoil main roots, C and N concentrations were only measured on topsoil main roots and assumed to reflect the concentrations in the smaller fractions. The C and N concentrations in shoot and root samples were analyzed using Vario Max Cube.

2.6. Statistical analysis

All analyses were conducted in R version 4.3.0 (R Core Team, 2023). We used a linear mixed effect model (function *lmer* in package lme4, Bates et al., 2015) to analyze N uptake and a generalized linear mixed effect model (GLMM) using Template Model Builder (TMB) in package glmmTMB (Brooks et al., 2017) to analyze nitrate leaching reduction. Models were constructed with sowing time, cover crop species, year, and site and their interactions as fixed effects. Each sowing time was converted to sowing time after 1 August and was used as a continuous variable (to estimate site and cover crop species dependent regression coefficients) and year as a categorical variable. The models were estimated with a random intercept that varied for unique block, year, and site combinations for N uptake. We applied a second-order polynomial function on sowing time for N uptake and nitrate leaching reduction analysis to address non-linearity in the measured data. Nitrate leaching reduction was log transformed and gaussian distribution was used with identity link to address non-normality. For root and shoot C and N at Flakkebjerg in 2023, a linear mixed model (LMM) was used to evaluate

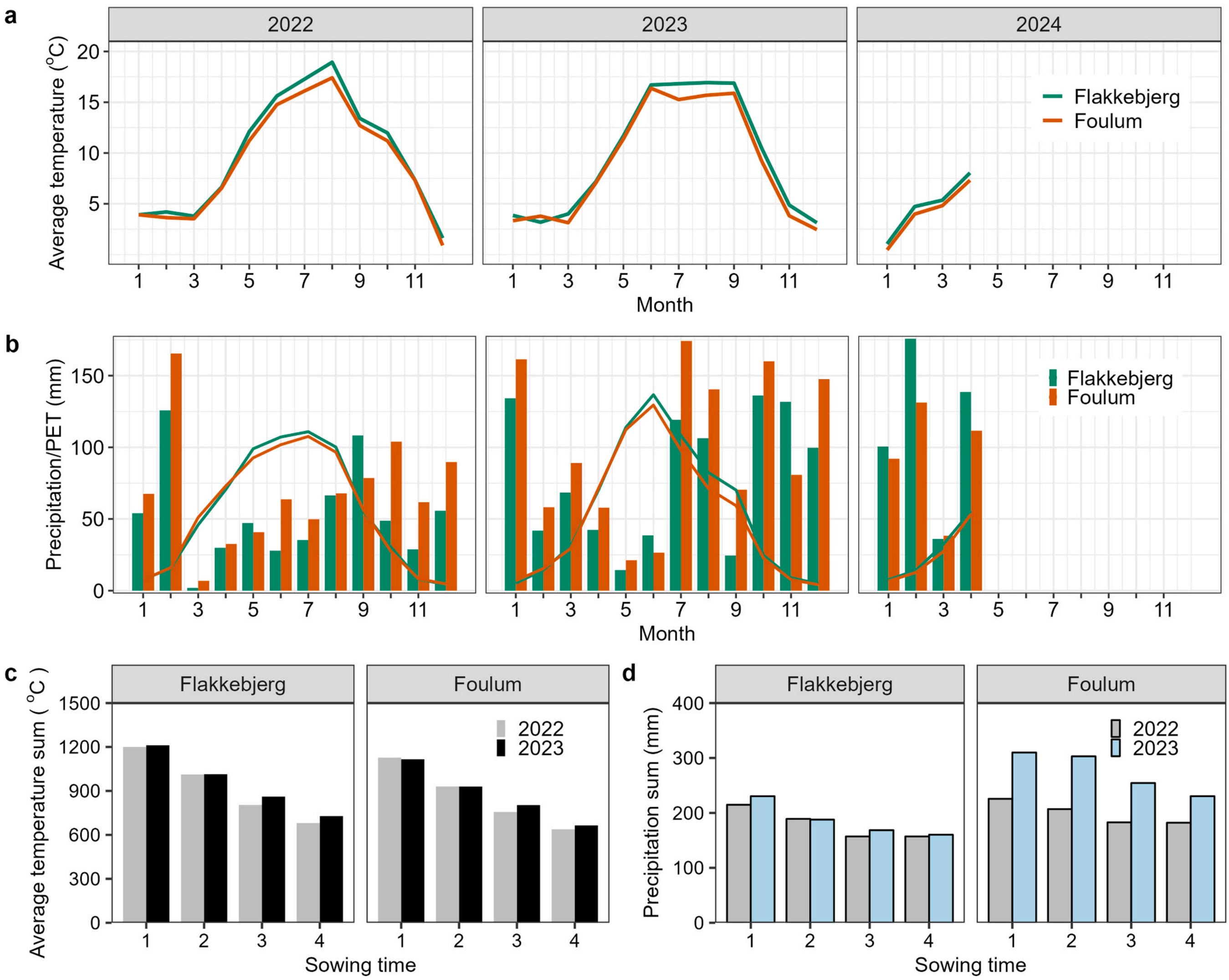

Fig. 1. Monthly average temperature (a), monthly precipitation sum (bars) and potential evapotranspiration (PET) sum (lines) (b) during the experimental years, and average temperature sum (c), and precipitation sum (d) from each sowing time to the cover crop plant sampling (31 October).

the effects of species and sowing time on each dependent variable, accounting for both fixed and random effects (field block effects). To meet model assumptions, variables were transformed using logarithmic or square root transformations. Various model formulations were evaluated, and the best models were selected based on the lowest Akaike information criterion (AIC, Akaike, 1974) and their ability to represent the observed data distribution. To assess the differences between species and years/percolation periods, we back transformed model estimates when relevant and applied post-hoc pairwise comparisons using the R package *emmeans* (Lenth, 2023), wherein we used Holm's method for *p* value adjustment.

Delaying sowing time is expected to reduce the temperature sum for the growth of cover crops, therefore, we pooled the data and analyzed the correlation between N uptake in the cover crops and temperature sum from each sowing time to the latest plant sampling on 31 October in any year for each site. We also examined the relationship between nitrate leaching reduction and temperature sum. To quantify the daily decline in the nitrate leaching reduction ability of the cover crops we applied simple linear regression using pooled data to model nitrate leaching reduction as a function of sowing time for each site. Codes that were used to perform statistical analyses are provided here: https://github.com/UKum540/Sowing_date_cover_crop_statistical_analysis.

3. Results

3.1. Above ground N uptake in cover crops

Distribution and trend in the measured N uptake for each block based on sowing time, cover crop species, sites, and years were best captured by the quadratic function, as estimated by the statistical model used for analysis (Fig. 2). For all cover crop species and both sites, N uptake decreased from the first to the last sowing time but with varying patterns. Sowing time, cover crop species, and site significantly affected N uptake in cover crops (Table 3). Based on test statistics of F values, sowing time was the factor explaining the largest part of the variation. Besides, N uptake was also depended on the interaction between sowing time, cover crop species, and site, and between sowing time, cover crop species, and years. The average N uptake was significantly higher at Foulum with 31.6 kg N ha^{-1} than at Flakkebjerg with 24.3 kg N ha^{-1}, and significantly higher in 2022 with 31.9 kg N ha^{-1} than in 2023 with 24.0 kg N ha^{-1} across the sowing time and cover crop species (Fig. 2, Table S1). On average, cover crops took up N between 19.1 and 29.1 kg N ha^{-1} at Flakkebjerg and between 25.8 and 36.0 kg N ha^{-1} at Foulum. Fodder radish took up significantly higher N than oats at Flakkebjerg across four sowing times. Fodder radish and phacelia took up significantly more N than oats at Foulum.

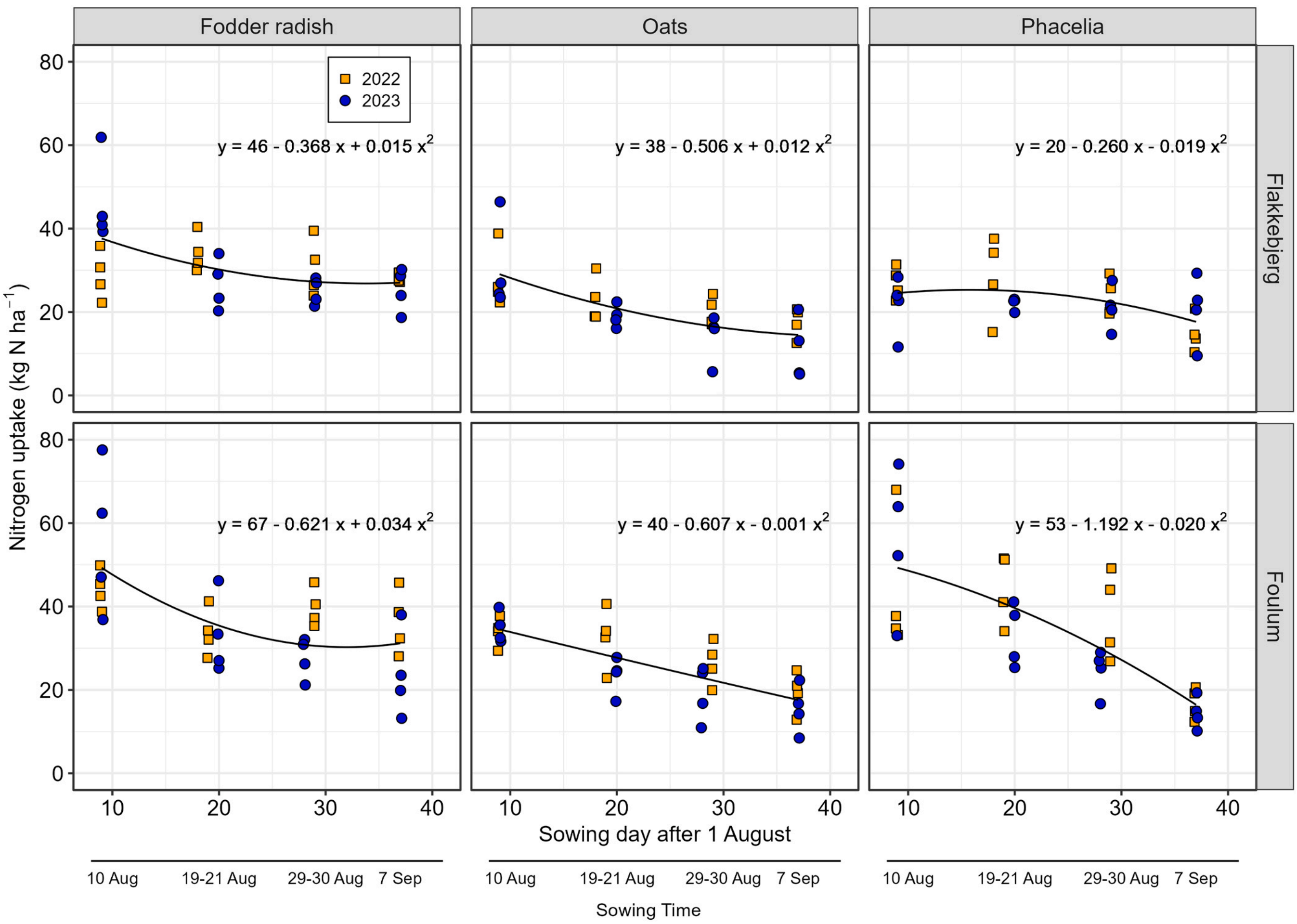

Fig. 2. Nitrogen uptake in aboveground biomass of three cover crop species as a function of sowing day after 1 August at Flakkebjerg and Foulum. Second x-axis indicates the sowing times. The yellow squares (for 2022) and blue circles (for 2023) represent observed data from each of the four blocks at each sowing time. Lines showing the average trend across percolation periods represent the fitted regression lines from the mixed effects model used for the analysis of variance described in Section 2.6.

Table 3
F and Chi-Square (X^2) statistics, with degrees of freedom (df) in the parenthesis, were presented for analysis of variance results for nitrogen (N) uptake and nitrate leaching reduction, respectively.

Response variable	N uptake			Leaching reduction[#]		
Effect	Test statistics		*p* value		Test statistics (X^2 (df))	*p* value
Sowing time[#]	$F_{(2152)}$	71.20	< 0.001	Sowing time[#]	115.00 (2)	< 0.001
Cover crop (CC) species	$F_{(2152)}$	35.00	< 0.001	CC species	5.39 (2)	0.067
Year*	$F_{(1,12)}$	4.52	0.055	Percolation period[Δ]	0.08 (1)	0.777
Site	$F_{(1,12)}$	33.50	< 0.001	Site	96.80 (1)	< 0.001
Sowing time:CC species	$F_{(4152)}$	2.87	< 0.050	Sowing time:CC species	28.60 (4)	< 0.001
Sowing time:Year	$F_{(2152)}$	14.50	< 0.001	Sowing time:Percolation period	7.35 (2)	< 0.050
CC species:Year	$F_{(2152)}$	0.55	0.579	CC species:Percolation period	1.92 (2)	0.382
Sowing time:Site	$F_{(2152)}$	9.27	< 0.001	Sowing time:Site	0.76 (2)	0.684
CC species:Site	$F_{(2152)}$	3.28	< 0.050	CC species:Site	2.08 (2)	0.353
Year:Site	$F_{(1,12)}$	0.74	0.406	Percolation period:Site	7.22 (1)	< 0.010
Sowing time:CC species:Year	$F_{(4152)}$	2.78	< 0.050	Sowing time:CC species: Percolation period	15.50 (4)	< 0.010
Sowing time:CC species:Site	$F_{(4152)}$	3.56	< 0.010	Sowing time:Percolation period:Site	10.90 (2)	< 0.010
				Sowing time:CC species:Site	22.20 (4)	< 0.001

applied with second order polynomial, * current year as sowing, Δ from sowing of CC in autumn to following spring.

At the first sowing time, 10 Aug, average N uptake in fodder radish, oats, and phacelia was in the range of 37.7–49.3, 29.0–34.4, and 24.4–49.2 kg N ha^{-1}, respectively across two years and both sites (Fig. 2, Table S2). At the last sowing time, 7 Sep, the N uptake was 26.9–31.0, 14.5–17.5, 16.3–17.6 kg N ha^{-1} for the respective cover crops species.

At Flakkebjerg in 2022, there was a tendency to higher N uptake at second and third sowing times compared to the first sowing time for fodder radish. In 2023, N uptake in fodder radish decreased from the first to second sowing time and began to plateau in the subsequent sowing times. For oats, the decline of N uptake with delayed sowing time across years was more of a linear manner. However, N uptake was similar at the first and second sowing times for phacelia and followed by a slight decrease with delayed sowing time for both years. At Foulum, N

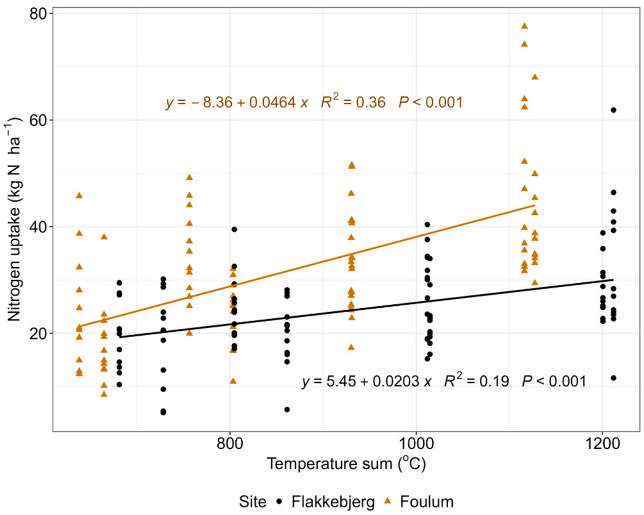

Fig. 3. Nitrogen uptake as a function of temperature sum (cumulated from each of the four sowing times to 31 October) across cover crop species and years for Foulum (orange triangles and regression line) and Flakkebjerg (black circles and regression line). Lower temperature sum indicates late sowing times, while higher temperature sum corresponds to early sowing times.

uptake in fodder radish followed the same trend as at Flakkebjerg. The N uptake in phacelia decreased from the first to the last sowing time at a faster rate than for oats. N uptake in phacelia and oats declined with delayed sowing at a faster rate at Foulum than at Flakkebjerg.

The N uptake was significantly and positively correlated with the temperature sum upon pooling the data for both years of 2022 and 2023 within both sites (Fig. 3). With increasing temperature sum, the N uptake increased at a more than double rate at Foulum than at Flakkebjerg (slope of 0.046 vs. slope of 0.020).

3.2. Nitrate leaching reduction

As for N uptake, the distribution and trend in the measured nitrate leaching reduction compared to bare soil for each block based on sowing time, cover crop species, sites, and years were best captured by the quadratic function (Fig. 4). The declining pattern of nitrate leaching reduction from the first to the last sowing time varied based on cover crop species and site. The highest variation was explained by the sowing time based on Chi-square values (Table 3). Furthermore, various three-way interactions were observed between sowing time, percolation period, cover crop species, and site. The average leaching reduction by cover crops was higher at Foulum with 80.4 kg N ha^{-1} than at Flakkebjerg with 28.3 kg N ha^{-1} across sowing times, cover crop species, and percolation periods (Fig. 4, Table S3) compared to 133.0 kg N and 47.2 kg N leaching from bare soil at respective sites (Fig. S2). Oats reduced nitrate leaching by the lowest (on average 25.3 kg N ha^{-1} at

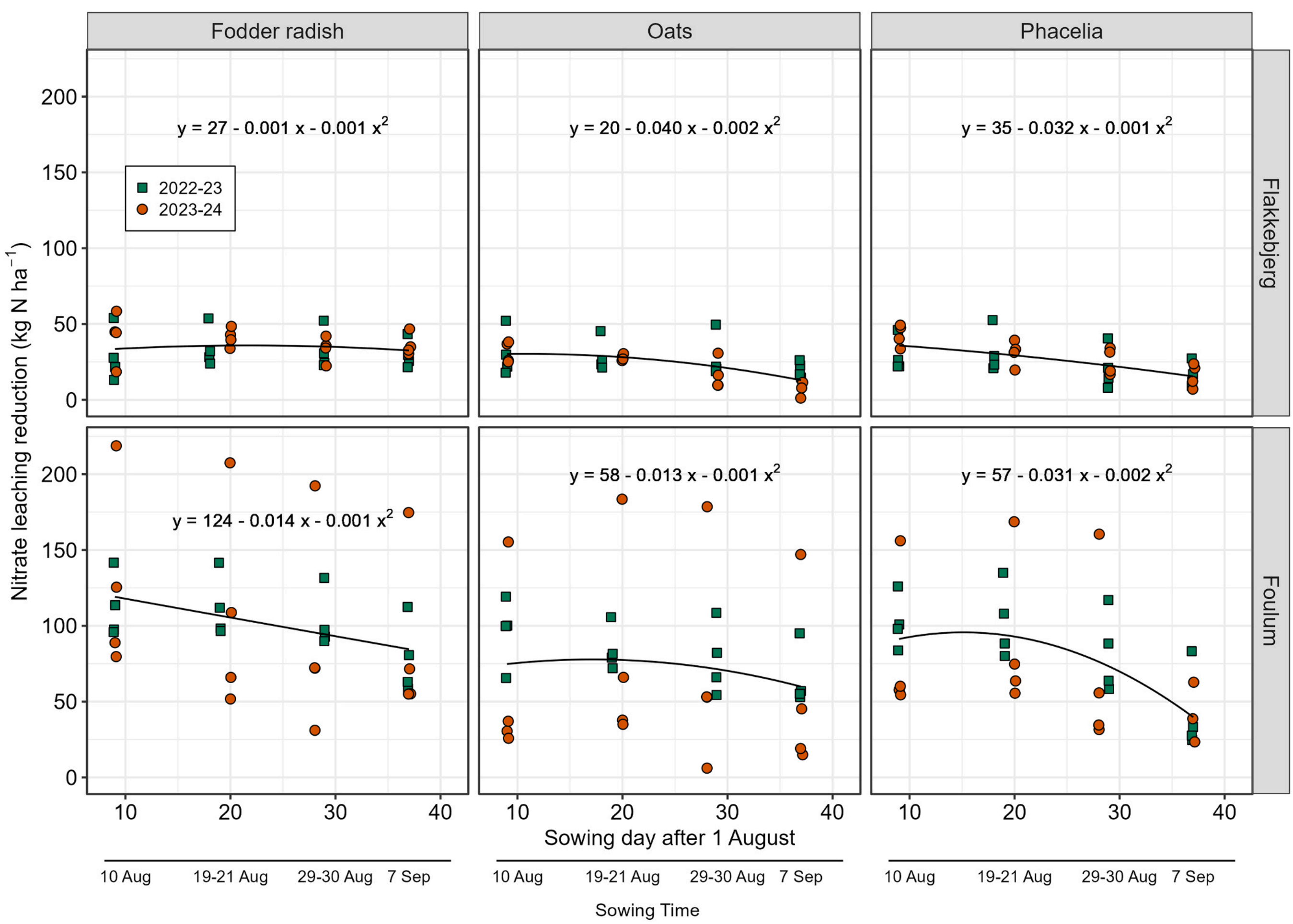

Fig. 4. Nitrate leaching reduction with three cover crop species compared to herbicide treated bare soil shown per block as a function of sowing day after 1 August at Flakkebjerg and Foulum. Second x-axis indicates the sowing times. The yellow squares (for 2022–23) and blue circles (for 2023–24) represent observed data from each of the four blocks at each sowing time. Lines showing the average trend across percolation periods represent the fitted regression lines from the mixed effects model used for the analysis of variance described in Section 2.6.

Flakkebjerg and 65.5 kg N ha^{-1} at Foulum) and fodder radish the highest (on average 34.8 kg N ha^{-1} at Flakkebjerg and 95.7 kg N ha^{-1} at Foulum) across sowing times and percolation periods, although the differences between the three cover crops only tended to be significantly different at $P = 0.067$.

At the first sowing, 10 Aug, nitrate leaching reduction was in the range of 29.0–67.0, 34.6–86.0, 31.9–114.5 kg N ha^{-1} by oats, phacelia, and fodder radish, respectively, at both sites (Fig. 4, Table S4) compared to the nitrate leaching range of 40.3–143.3 kg N ha^{-1} from the bare soil (Fig S2). At the last sowing, 7 Sep, the nitrate leaching reduction was in the range of 9.66–47.3, 14.5–35.8, and 31.2–77.1 kg N ha^{-1} for the respective cover crops species at both sites. At Flakkebjerg, the ability of cover crops to reduce nitrate leaching declined with delayed sowing times. The decline was more pronounced with oats and phacelia and less with fodder radish. At Foulum, the declining trend was more evident for all three cover crops. Additionally at Foulum, the reduction ability tended to be higher at the second sowing (20–21 Aug) compared to at the first sowing (10 Aug) followed by a lower reduction ability at subsequent sowings for oats and phacelia. With fodder radish, the trend of reduction ability with sowing time declined more linearly.

The variations in nitrate leaching reduction were higher at Foulum than at Flakkebjerg in both percolation periods (Fig. 4). At Foulum, the variation primarily came from the leaching from the bare soil control for the percolation period of 2023–24 (used in the calculation of leaching reduction), where nitrate leaching was much higher in one block with 232 kg N ha^{-1} compared to others which were in the range of 91–142 kg N ha^{-1} (see Fig. S2 for the values in each block of bare soil treatment) Such variations in different blocks have been observed in various past experiments (Personal communication (Elly Møller Hansen)). Block was included as a random effect in the statistical model (Section 2.6), ensuring that different levels of nitrate leaching reductions in comparison to that of bare soil in the blocks were properly accounted for in the global estimates.

Delaying sowing from 10 Aug to 7 Sep decreases the ability of cover crops to reduce nitrate leaching on average of 0.46 kg N ha^{-1} day^{-1} at

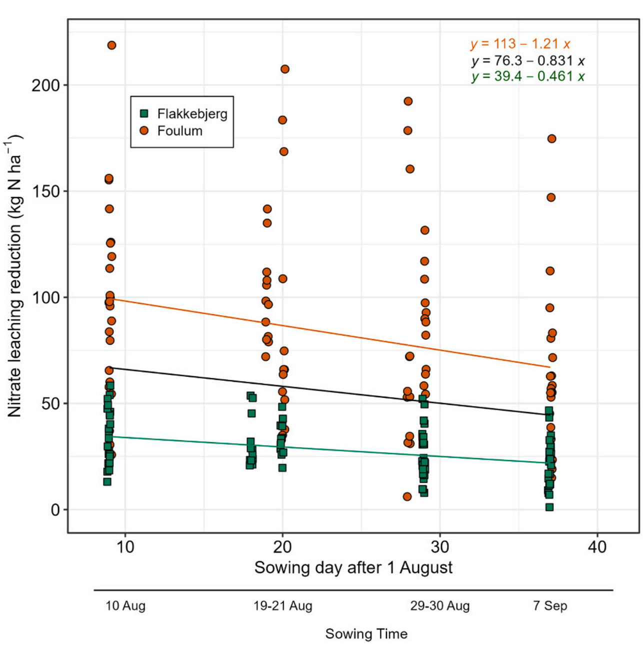

Fig. 5. Simple linear regression for nitrate leaching reduction for each block as a function of sowing day after 1 August for Foulum (orange circles and regression line) and Flakkebjerg (green circles and regression line) across all three cover crops and both percolation periods. Second x-axis indicates the sowing time. Black regression line represents the combined effect across cover crops, sites, and percolation periods. All regression lines are presented with the corresponding regression equations.

Flakkebjerg and 1.21 kg N ha^{-1} day^{-1} at Foulum (Fig. 5) across both percolation periods. The decline across both sites and percolation periods was 0.8 kg N ha^{-1} day^{-1}. The correlation between temperature sum and nitrate leaching reduction and between N uptake and nitrate leaching reduction was significant at both sites (Fig. 6). The reduction in nitrate leaching per unit increase in temperature sum or N uptake was greater at Foulum than at Flakkebjerg.

3.3. C and N inputs from cover crop shoot and root at Flakkebjerg in 2023

There was a significant effect of both cover crop species and sowing time on both shoot C, topsoil root C (0–25 cm) and total C, while subsoil root C (25–40 cm) were neither significantly affected by species nor sowing time (Fig. 7, Table 4). According to the F statistics (Table 4), representing the relative importance of the different predictor variables, total C was more dependent on sowing time than on species, while total N was more dependent on species rather than on sowing time.

With an average of 1296 kg ha^{-1} at 10 Aug sowing and 519 kg ha^{-1} at 30 Aug, early sowing gave rise to an average of 2.5 times higher total C input across all species (Fig. 7d), with the highest effect of early sowing being on topsoil root C of the fodder radish (Fig. 7b). Fodder radish accumulated significantly higher shoot C than phacelia and significantly higher topsoil root C than both phacelia and oats when sown early (10 Aug). When sown 20 days later (30 Aug), fodder radish showed similar low root C as for phacelia and oats at both soil depths, but significantly higher shoot C (Fig. 7a).

A relatively high proportion of the total C inputs (on average 43–62 %) was allocated to the belowground biomass (top+subsoil root C fraction). A relatively small proportion of the total C inputs (382–2042 kg C ha^{-1}) was allocated to the subsoil root C fraction (58–77 kg C ha^{-1}, i.e. 4–15 %) (Fig. 7c, d). However, when compared to the total root C, 5–15 % and 22–30 % C was located in the subsoil fraction for early-sown and late-sown cover crops, respectively.

Total N was in the range from 30 to 91 kg N ha^{-1} at early sowing on 10 Aug and from 21 to 69 kg N ha^{-1} at later sowing on 30 Aug. Compared to C, total cover crop biomass N showed a less pronounced, yet significant, effect of delayed sowing, with 2.0 times higher than total N at early sowing compared to later sowing for oats and with 1.5 times higher for fodder radish, while there was no differences between sowing times for phacelia (Fig. 7h). At both sowing times, fodder radish exhibited significantly higher total N compared to the other species. Interestingly, subsoil root N (25–40 cm) tended to be slightly higher for both phacelia and fodder radish when sown later (Fig. 7g).

For both shoots and topsoil roots, the C:N ratio significantly decreased with delayed sowing (Table S5), reflecting the characteristics of a younger plant, except in the case of oats roots. Interestingly, while phacelia's root:shoot ratio was unaffected by delayed sowing, oat tended to show a higher belowground investment, while fodder radish, in contrast, showed significantly lower belowground investment when sown late.

4. Discussion

4.1. Decline of N uptake and nitrate leaching reduction with delayed sowing time

The N uptake of the cover crops declined with delayed sowing as hypothesized. The significant correlation between temperature sum and N uptake (Fig. 3) implies that temperature sum was a limiting factor for growth and N uptake with the delayed sowing. Three-way interactions between sowing time, cover crop species, and site, as well as sowing time, cover crop species, and year, suggest that N uptake by cover crops was dependent on the conditions associated with sowing time, year, and site, leading to differences in the pattern of N uptake decline.

The range of N uptake between 29.1 and 36.0 kg N ha^{-1} at the first

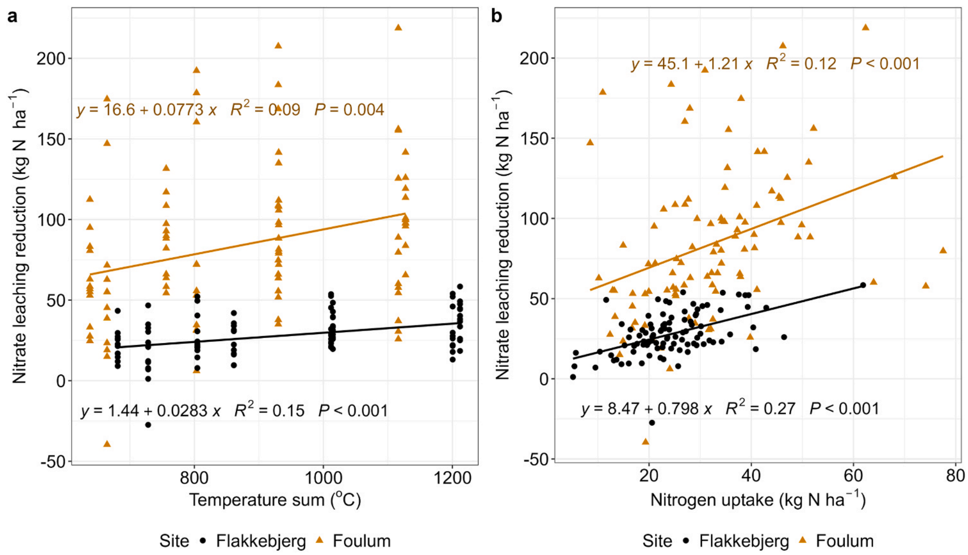

Fig. 6. Nitrate leaching reduction as functions of temperature sum (cumulated from each of the four sowing times to 31 October) (a) and N uptake (b) across cover crop species and percolation periods for Foulum (orange triangles and regression line) and Flakkebjerg (black circles and regression line).

sowing time (10 Aug) in this study was in the range of N uptake in earlier studies in Sweden, Finland, and Denmark (Kumar et al., 2023, Thomsen and Hansen, 2014; Aronsson et al., 2016). The plateauing pattern of N uptake in fodder radish from the second sowing time (19–21 Aug) onwards suggests that its N uptake was less influenced by the delayed sowing as compared to oats and phacelia at both sites. At Foulum, fodder radish and phacelia took up a similar amount of N at first sowing time (10 Aug). Phacelia showed, however, a faster and more linear decline with delayed sowing than fodder radish. The observed differences between the cover crops may be attributed to the differences in growth characteristics of canopy and root development (Teixeira et al., 2016; Thorup-Kristensen et al., 2003; Kumar et al., 2023).

Analysis of N concentrations in soil water at both sites, reported in a Danish policy report (Larsen et al., 2024), revealed earlier and higher levels at Foulum. Nitrate concentrations in the soil water were detected at Foulum as early as November 2022, compared to January 2023 at Flakkebjerg, during the 2022–23 percolation period. For 2023–24, nitrate concentrations remained higher at Foulum than at Flakkebjerg until mid-November 2023 (approx. 40–45 mg NO_3^- L^{-1} at Foulum vs. <20 mg NO_3^- L^{-1} at Flakkebjerg). These findings suggest that more N was available for cover crop growth during early autumn at Foulum than at Flakkebjerg, contributing to higher N uptake at sampling time in late October. This can be attributed to the higher N fertilizer application in 2022 compared to 2023 at Foulum.

As for N uptake, nitrate leaching reduction ability of cover crops decreased with delayed sowing time at both sites as hypothesized. With the delay of sowing from 10 Aug to 7 Sep, the ability of cover crops to reduce nitrate leaching was low at Flakkebjerg and high at Foulum. The observed daily decline in nitrate leaching reduction ability at Foulum with 1.2 kg N ha^{-1} day^{-1} is higher than the 0.8 kg N ha^{-1} day^{-1} reported by Kumar et al. (2023) for the same experiment, although for the period of 2019–2022. The higher daily decline in this study can be associated with higher precipitation in 2022–23 and 2023–24 than in the 2019–2022 percolation periods (i.e. 201–273 mm (Fig. 1) vs 136–219 mm (Fig. 1 in Kumar et al., 2023)). However, the daily decline across both sites in this study (0.83 kg N ha^{-1} day^{-1}) is similar to 0.85 kg N ha^{-1} day^{-1} as reported in a Danish policy report (Larsen et al., 2024), which presented the analyzed data from various experiments conducted between 2019 and 2023, including this study's data. The declining ability of cover crops to reduce nitrate leaching with delaying sowing time can be explained by a decreasing temperature sum from sowing to end of the growing season, limiting growth and N uptake (Figs. 3 and 6). Notable, the leaching reduction per unit increase in temperature sum and N uptake was significantly higher at Foulum than at Flakkebjerg suggests that Foulum provided better cover crop growth and N uptake conditions (such as more N availability), which resulted in higher nitrate leaching reduction.

Several studies have reported that fodder radish is highly efficient in reducing nitrate leaching (Justes et al., 1999; Munkholm and Hansen et al., 2012; Nouri et al., 2022). Here, we also found that fodder radish and phacelia tended to perform better than oats across sowing times at Foulum (Table S4). At Flakkebjerg, there was no significant difference between the three cover crops across sowing times and years, although fodder radish (34.8 kg ha^{-1}) tended to be higher than oats and phacelia (25 kg ha^{-1}). Fodder radish performed generally better than oats and phacelia at later sowing times. This confirms the findings of Kumar et al. (2023) that fodder radish performs better in terms of high N uptake and nitrate leaching reduction. The better performance of fodder radish under restricted growing conditions implies that it is the farmers' best choice considering the uncertainty about sowing time and growing conditions when selecting the cover crop before harvesting the main crop. Since previous research has highlighted the benefits of cover crop mixtures (Wendling et al., 2019; Heuermann et al., 2022), our results suggest that sowing a mix of phacelia and fodder radish by August 29–30 can further maximize the benefits of the cover crops for enhanced N uptake and reduced leaching. Specific for cover crop species, the results suggests that fodder radish may be a good choice at Flakkebjerg, while both fodder radish and phacelia worked well at Foulum to increase cover crop benefits. However, the success of the cover crop selection will also depend on the variability of climate in the year and time of sowing.

4.2. *Differences in the patterns of N uptake and nitrate leaching reduction with delayed sowing*

On average, N uptake was 30 % higher at Foulum than at Flakkebjerg across sowing times, cover crops, and years, however, the nitrate leaching reduction was 184 % higher. The significant correlation between N uptake and leaching reduction in this study was also found in earlier studies (Kumar et al., 2023; De Notaris et al., 2018). Despite the correlation, the effect of delayed sowing time differed on N uptake decline and nitrate leaching reduction patterns. For example, for fodder radish, the plateauing of N uptake from the second sowing onwards at both sites was not mirrored in the pattern of nitrate leaching reduction with sowing time. In general, the lower slopes for leaching reduction

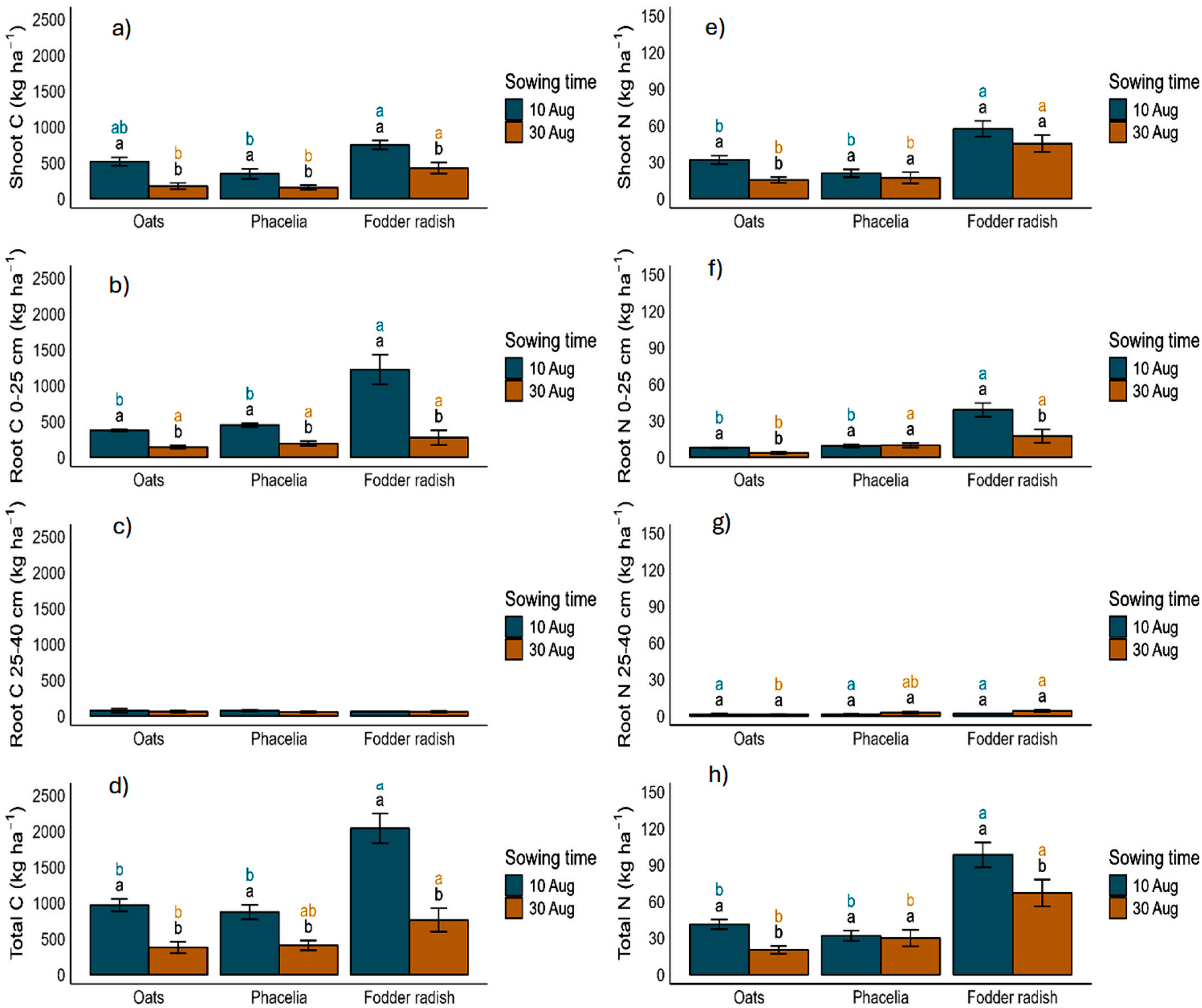

Fig. 7. Cover crop C (a-d) and N (e-h) (kg ha^{-1}) dependent on species and sowing time reported as mean and SE (measured only at Flakkebjerg in 2023). Green letters denote significant differences between species sown 10 Aug, yellow letters denote differences between species sown 30 Aug, and black letters denote differences between sowing times within each species.

Table 4
Results of the linear mixed effects model for total cover crop (CC) C and N (kg ha^{-1}). F statistics presented with their numerator (NumDF) and denominator (DenDF) degrees of freedom in brackets (estimated with Satterthwaite approximation) and p-values.

Response variable		Total C (kg ha^{-1})		Total N (kg ha^{-1})	
Effect	F (NumDF, DenDF)	F-value	p-value	F-value	p-value
CC species	$F_{(2,15)}$	21.49	< 0.001	33.14	< 0.001
Sowing Time	$F_{(1,15)}$	65.23	< 0.001	10.44	< 0.010
CC species: Sowing time	$F_{(2,15)}$	2.40	0.125	1.74	0.203

than N uptake suggest that leaching reduction was less sensitive to delayed sowing than N uptake (Figs. 2 and 4).

The inconsistent patterns of N uptake and nitrate leaching reduction with sowing time may to some extent be related to the different timing of the N uptake and nitrate leaching measurements. We measured N uptake by the end of October (25–31 October), while nitrate leaching was estimated for the entire autumn to early spring period. Plant sampling was conducted one time only in the last week of October for two reasons: (1) In regions with higher clay content, like Flakkebjerg, cover crop termination is permitted in late autumn. (2) Cover crops grow rapidly from sowing in August to October, reaching sufficient biomass to uptake N and reduce leaching. Beyond October, growth slows due to low temperatures, and frost kills them in the following weeks. Photos of plots were taken to monitor the growth of the cover crops in the autumn and winter months (Fig. S3 to Fig. S8). These photos show, in correspondence with N uptake and C input results, that biomass production in late autumn (November photos) was highest for the early sowing dates. Interestingly, the early sown crops were also the most sensitive to early frost, and the late sown cover crops remained greener after the first frost in December (late December and March photos). This indicates that the late sown cover crops continued to grow and accumulated N beyond the last week of October at Foulum, and thereby contributed to nitrate leaching reduction.

N mineralized from the frost-killed cover crops may also have influenced the leaching pattern. As fast decomposition of the cover crops is expected due to a relatively low C:N ratio in the aboveground plant parts, although not significant in this study, the lowest C:N ratio at early sowing can promote faster mineralization of frost-killed fodder radish than oats and phacelia (Trinsoutrot et al., 2000). Additionally, at Flakkebjerg, the plots were ploughed in early winter whereas at Foulum they were ploughed in the following spring (Table 2). The early ploughing can speed up the mineralization of dead cover crops (Miranda-Vélez et al., 2023), releasing N into the soil. As a result, this process may have influenced the N leaching in the latter part of the percolation periods. In the present study, we did not quantify the share of cover crop mineralization and its contribution to leaching. This can be another aspect to explore in future studies.

Root growth and N uptake in roots is another factor that may explain the inconsistency in the patterns of N uptake and leaching reduction. Contrary to our expectations, including both root and shoot N in the N uptake did not improve the relationship between leaching and N uptake for the Flakkebjerg 2023–24 results ($p = 0.038$ vs $p = 0.054$, Fig. S9). The insights discussed above on the factors driving the differences in the less-explored patterns of N uptake and nitrate leaching reduction across sowing dates can support the effective use of cover crops, whether for N uptake, C input, leaching reduction, or a combination of these benefits. For future studies, we recommend including the measurements of N mineralization, root N, and shoot N in the autumn and winter months together with leaching to help better understand the pattern in N uptake and leaching reduction during the entire cover crop growing season and percolation period, and the relationship between the two.

4.3. C input reduced with delayed sowing at Flakkebjerg in 2023

A twenty-day delay in sowing resulted in a 58–63 % reduction in total C input from cover crops (Fig. 7d). Such substantial reduction is in agreement with hypothesis II and the general findings in the literature (Thorup-Kristensen et al., 2003; McClelland et al., 2021; Cotteny et al., 2022; Seitz et al., 2022; Qin et al., 2023; Nilsson et al., 2024). The result shows that fodder radish was the cover crop species with both the highest total C input when sown early and the species most affected by delayed sowing, with the bulk taproot (0–25 cm) showing the most pronounced reduction in C input. In a similar experiment from Northern Ireland, the steepest decline in above- and belowground biomass was also shown for tillage fodder radish compared to phacelia and ryegrass with a three-week sowing delay in autumn (Cotteny et al., 2022). However, as we observed, the differences in C input from the cover crops may disappear with late sowing.

Our findings highlight the importance of early sowing as a critical management strategy to maximize the potential for soil organic carbon (SOC) input, thereby enhancing the climate change mitigation benefits of cover crops. Findings by Blanco-Canqui (2022) and Joshi et al. (2023) indicate that cover crop biomass below 2 Mg ha^{-1} yr^{-1} is unlikely to have a significant positive effect on SOC storage. Additionally, combined results from a long-term field trial and a mesocosm study on Danish soils suggest a critical transition point for a positive effect on SOC with a 0.7–1.1 Mg ha^{-1} of aboveground biomass production (Liang et al., 2023). In the present study, this threshold was met with early sowing (average of 1.3 Mg ha^{-1}) but not with late sowing (average of 0.5 Mg ha^{-1}), underscoring the need for timely management (and species choice) to optimize SOC input.

The total belowground C contributed 42–62 % of the total C input, highlighting the importance of quantifying root biomass. Large differences in subsoil root C allocation highlighted the potential for species-specific bias due to shallow root sampling. Accurate sampling methodology is particularly critical for capturing finer roots in the subsoil, where 91–98 % of root C was isolated in the small roots fraction (Fig. S1) through repeated decantation (explained in Section 2.5, data not shown). In the topsoil, the recovery of small roots was less for fodder radish (18–40 %) but high for phacelia and oat (96–84 %), emphasizing how differences in root morphology can skew root estimates without careful sampling. It should also be noted that these results are from limited plant population sampling (one plant for fodder radish and several plants for oats and phacelia) and there is a potential for small roots to be lost in the repeated decantation process.

Apart from C input in quantitative terms, qualitative characteristics such as cover crop C:N ratio can also affect to which extend and by which pathways, soil organic C is formed (Cotrufo et al., 2015). Following the framework of Microbial Efficiency-Matrix Stabilization (MEMS) suggested by Cotrufo et al. (2013), high substrate quality (i.e. low C:N ratio and low lignin content) for microbial decomposition promote high carbon use efficiency (CUE) and long-term C storage through the formation of more persistent mineral-associated organic matter (MAOM). The observed high substrate quality for later sown cover crops in this study is likely less influential on C storage, as C input from the early sowing was 2–3 times higher, an argument backed by recent studies evaluating quantitative and qualitative C inputs from cover crops (Engedal et al., 2023, 2025).

5. Conclusion

Over the two experimental years (2022–23 and 2023–24), the ability of cover crops to take up nitrogen and reduce nitrate leaching decreased with delayed sowing time, hence lowering environmental benefits of cover crops. The decrease was dependent on cover crop species, sowing times, percolation periods, and sites. Fodder radish and phacelia took up more N compared to oats at most sowing times and sites. Fodder radish and phacelia tended to reduce nitrate leaching more than oats at the early sowing times, while fodder radish was the most effective among the three cover crops at the latest sowing time. The average nitrate leaching reduction at the first sowing on 10 Aug at both sites ranged from 31.9 to 114.5 kg N ha^{-1} for fodder radish, 29.0–67.0 kg N ha^{-1} for oats, and 34.6–86.0 kg N ha^{-1} for phacelia. At the last sowing time on 7 Sep, the average reductions for the respective cover crops were from 31.2 to 77.1 kg N ha^{-1}, 9.7–47.3 kg N ha^{-1}, and 14.5–35.8 kg N ha^{-1}. At the site Foulum, the overall N uptake was 30 % higher and leaching reduction was 184 % higher than at Flakkebjerg. The pattern of the declining N uptake (measured in late October) and nitrate leaching reduction (measured until the following spring) with delayed sowing time differed between cover crop species, sites, and percolation period. At Flakkebjerg in 2023, the cover crop derived C input both from shoot and root to the soil decreased with delayed sowing. Differences in total C input among the cover crops were more distinct at the early sowing time. Fodder radish contributed more total C (2042 kg ha^{-1}) than phacelia (875 kg ha^{-1}) and oats (970 kg ha^{-1}) when sown early on 10 Aug. Our study demostrates the significant importance of early sowing of cover crops to increase their positive effects, but the results also highlight the need for further research to understand the complex and interacting effects of sowing time on the ability of different cover crops to take up N, provide C input to soil, and leaching reduction.

CRediT authorship contribution statement

Uttam Kumar: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis. **Tine Engedal:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Veronika Hansen:** Writing – review & editing, Data curation. **René Gislum:** Writing – review & editing. **Lars J Munkholm:** Writing – review & editing, Validation, Methodology, Conceptualization. **Ingrid K. Thomsen:** Writing – review & editing. **Søren Ugilt Larsen:** Writing – review & editing. **Elly Møller Hansen:** Writing – review & editing, Visualization, Project administration, 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.

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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.2025.109858.

Data availability

Data will be made available on request.

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