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RESEARCH ARTICLE

Nitrogen fertilization strategies to reduce the risk of nitrate leaching in open field cultivation of spinach (*Spinacia oleracea* L.)[#]

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Abstract

Background: Spinach is a nitrogen (N) demanding crop with a weekly N uptake of up to 60 kg ha^{-1}. Consequently, a high N supply is required, which can temporarily lead to high quantities of nitrate (NO_3^-) being at risk of leaching.

Aims: The objective of this study was to develop a N fertilization approach to reduce the risk of NO_3^- leaching in field-grown spinach production without adversely affecting crop yield and quality at an early and late harvest stage.

Methods: Ten fertilization trials were conducted to compare different base fertilization rates and splits of top dressings. For top dressings, granulated fertilizers or foliar sprays were used. In a further treatment, N supply was reduced by withholding the second top dressing of 50–70 kg ha^{-1}.

Results: Nitrate concentration at risk of leaching was considerably reduced by decreasing the base fertilizer rate as well as by splitting the top dressing. However, at an early harvest stage, total aboveground dry mass was reduced by, on average, 6% by these measures across all seasons. In contrast, at a later harvest stage, spinach was less affected by the fertilizer schedule. Urea foliar sprays proved to be insufficient in promoting plant growth and caused leaf necrosis. A reduced N supply led to impaired plant growth and yellowish leaves in both spring and winter.

Conclusions: Base N fertilization of spinach is only required in spring, but not in other seasons. Despite slight yield reduction, the top dressing should be split to reduce the risk of NO_3^- leaching after an early harvest.

KEYWORDS
base fertilization rate, frozen spinach, harvest stages, nitrate content, reduced N supply, soil mineral N-residue, split top dressing, urea foliar spray

1 | INTRODUCTION

Nitrate pollution in groundwater bodies is still a concern in many European regions. Despite the fact that it takes up a small proportion of the total land area, intensive field vegetable production in Europe is a major contributor to this pollution (Tei et al., 2020). In particular, on sandy sites under humid climatic conditions, the maximum permitted concentration of 50 mg NO_3^- L^{-1} groundwater (Council of the European Communities, 1991; European Commission, 2000) is often exceeded (De Haan et al., 2009; De Neve, 2017). As a consequence, further legal restrictions on the amount and timing of N-fertilization were issued (German Fertilizer Ordinance, 2020). Nitrate leaching in vegetable production is related to the short growing cycles, shallow root systems, and high N demand of many crop species (Tei et al., 2020). This requires a sufficiently high level of soil mineral N (N_{min}) in the root zone to ensure the demand of the market in terms of the quantity and quality of the produce (D'Haene et al., 2018).

Spinach grown for the processing industry requires approximately 200 kg N ha^{-1} within a cultivation period of 5–10 weeks in the spring, summer, and autumn seasons. In the winter season, this time span is extended to about 7 months in the temperate European climate. During the last 5–13 days before harvest, about half of the N amount taken up by the crop is acquired. This leads to weekly uptake rates of up to 60 kg N ha^{-1} (Feller et al., 2011; Niers, 1994). Total N supply in spinach cultivation is usually divided into a base fertilizer application at sowing and a top dressing before intensive N uptake starts. However, in the first weeks after sowing, rooting depth is limited to the upper centimeter of the soil and N uptake of plants is low (Schenk et al., 1991). Furthermore, spinach is typically grown on sandy sites to make field management easier and achieve harvests in spite of variable weather conditions (Massa et al., 2018; Nett et al., 2015). This is crucial for crops such as spinach that need to be harvested within a short time frame to avoid bolting and flowering, which would reduce the quality of the produce (Grevsen & Kaack, 1997). A drawback of these sites is, however, that mobile nutrients like NO_3^- can be easily leached below the root zone of young spinach plants (Niers, 1994). In order to reduce N losses due to unpredictable weather events, the base fertilizer rate should be reduced to the minimum required for growth until top dressing (Tremblay & Bélec, 2006).

The top-dressing rate of field-vegetable crops can be calculated based on measured N_{min} concentration and expected N mineralization as well as N losses in the rooted soil zone. The plant available N determined in this way has to be deducted from the crop N demand remaining until harvest (Tei et al., 2020). Optimum yield is expected when using this approach (Tremblay & Bélec, 2006). However, calculated N uptake at the time of fertilization can be overestimated because crop yield at harvest depends on weather conditions, diseases, and the requirements of the market (Vandecasteele et al., 2016). For these reasons, the vegetables are often harvested earlier than initially planned. As a result, higher quantities of NO_3^- remain in the soil.

In order to reduce N_{min} residue at harvest as well as flatten NO_3^- peaks after fertilization, N availability should be matched to actual uptake of the spinach crop by dividing N supply into several applications (Massa et al., 2018). Using this strategy, N losses due to unpredictable weather events can be reduced (Canali et al., 2014). Furthermore, later top dressings can be withheld when spinach is harvested before expected yield was achieved. On the other hand, N applied within the fast plant growth stage run the risk of temporary N deficiency that can adversely affect spinach growth and quality (Biemond et al., 1996). To maintain a sufficient N availability in the root zone, NO_3^--based fertilizers should be applied in combination with adequate irrigation (Quemada et al., 2013). In contrast to granulated fertilizers, nutrients applied by foliar sprays can be directly taken up by plant leaves without passing through the soil. In times of restricted nutrient uptake from soil, foliar fertilization results in a higher N uptake efficiency compared to soil-applied fertilizers (Krishnasree et al., 2021; Singh et al., 2013). Even small quantities of aerially applied urea or ammonium (NH_4^+) may promote growth and green coloration of spinach leaves as indicated by the results of a greenhouse fertilization trial (Borowski & Michalek, 2008). On the other hand, foliar sprays can lead to necrosis on leafy vegetables (Krishnasree et al., 2021; Singh et al., 2013).

A reduced total N application is often discussed as a measure to mitigate the risk of NO_3^- leaching in field-grown vegetable crops (D'Haene et al., 2018). In nitrate vulnerable zones, the recently revised German Fertilizer Ordinance (2020) restricts fertilizer N supply to 80% of the actual crop fertilization demand. Spinach, however, has a low N uptake efficiency and thus requires a relatively high minimum N_{min} of 40 kg ha^{-1} in the top soil (0–30 cm) from sowing to harvest (Feller et al., 2011; Schenk, 1996). If N availability at the root is limited, aboveground biomass production is impaired after a few days and deficiency symptoms such as yellowing on the older leaves become visible (Buysse et al., 1996; Hochmuth et al., 2018). This affects crop yield and marketability of the produce on the fresh market, but also its suitability for industrial processing. When processed into frozen food, high dry mass yield and intensive green color as well as a high leaf blade/stalk ratio are important spinach traits (Brandenberger et al., 2004; Grevsen & Kaack, 1997). A suboptimal N supply leads to a deterioration in all these properties. On the other hand, a reduced N fertilization rate can improve the nutritional quality of spinach by lowering the NO_3^- content of the produce (Breimer, 1982). For frozen spinach, a maximum nitrate content of 2,000 mg kg^{-1} (fresh matter basis) is permitted in the European Union (European Commission, 2011).

The objective of this study was to investigate different fertilization approaches to reduce the risk of NO_3^- leaching in field-grown spinach production from sowing to harvest. Furthermore, it was examined how these measures affect the yield of the crop and the quality of the produce for the frozen food processing industry. For this purpose, two N base fertilizer rates, several splits of N top dressing, and two levels of total N supply have been compared at an early and late harvest stage across all four seasons.

2 | MATERIALS AND METHODS

2.1 | Sites and experimental set-up

Ten fertilization trials were carried out in the years 2018–2020 during all four seasons at different sites in Borken, North Rhine-Westphalia, Germany (Table 1). The spinach varieties were selected for achieving maximum yield as well as quality traits such as delayed bolting in summer. In the spring season, spinach was grown as the first crop following winter catch crops. After spring-grown spinach was harvested, crop residues were harrowed and ploughed to 30 cm depth and a further trial (summer-grown spinach) was carried out at the same site, but at another position in the field. Autumn-grown spinach followed winter cereals. Winter-grown spinach was sown in autumn following potatoes or winter barley and was harvested in spring. When growing potatoes

TABLE 1 Growing season, sowing and harvest dates of the fertilization trials with the spinach variety and the previous grown crop grown at each site

Season	Previous crop	Site	Sowing date	Spinach variety (Breeding companies)	Harvest dates Early[a]	Harvest dates Late[b]
Spring	Green rye	1	March 20, 2018	Hudson (PV)	May 09, 2018	May 22, 2018
	Green rye	2	May 09, 2020	Santa Cruz (PV)	June 13, 2020	June 23, 2020
	Mustard	3	June 04, 2019	Ballet (Se)	July 05, 2019	July 15, 2019
Summer	Spinach	1	June 14, 2018	SV5591 (Se)	July 15, 2018	July 25, 2018
	Spinach	2	July 07, 2020	Rhino (RZ)	August 07, 2020	August 15, 2020
	Spinach	3	July 30, 2019	La Paz (PV)	September 01, 2019	September 12, 2019
Autumn	Barley	4	August 14, 2018	Solomon (Se)	September 24, 2018	October 09, 2018
	Triticale	5	August 18, 2020	Sonoma (PV)	September 19, 2020	October 05, 2020
Winter	Potatoes	6	September 28, 2018	Gorilla (RZ)	April 07, 2019	April 24, 2019
	Barley	7	October 02, 2019	Sonora (RZ)	April 12, 2020	April 25, 2020

[a] At a fresh mass yield of approx. 15–20 t ha^{-1}.
[b] At a fresh mass yield of approx. 25–30 t ha^{-1}.
Abbreviations: PV = Pop Vriend; Se = Semenis; RZ = Rijk Zwan.

or cereals as a precrop, 170 kg N_{tot} ha^{-1} liquid manure was applied in early spring. At harvest, cereals were cut a few centimeters above the soil surface and straw was removed from the fields. At all locations, spinach was sown with 250 or 300 seeds m^{-2} in summer/autumn and spring/winter seasons, respectively. In terms of texture, the soils were characterized as loamy sand with 73–88% (w/w) sand, 6–17% (w/w) silt, and 5–10% (w/w) clay. Soil organic carbon (C) content was usually in the range of 1.1–1.3% (w/w). Experimental site number 5 was an exception in this respect and had a content of 1.9% (w/w) organic C. Soil pH was maintained between 5.7 and 6.2 (0.01 M $CaCl_2$) by liming the fields with calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) before sowing. The fertilization experiments were performed in a randomized complete block design with three replications. Plot size varied between 56 and 105 m^{-2} depending on the working width of the agricultural machinery used at each site.

In practice, spinach is harvested once at an early or late harvest stage in each season. Early harvested spinach has a higher proportion of leaf blades. In the frozen food industry, it is processed without crushing into “leaf spinach.” In comparison, spinach harvested later has a higher proportion of leaf stalks and is processed into “chopped spinach” (Frerichs & Daum, 2021). In this study, total aboveground biomass was measured at both harvest stages. The early and late harvest dates were reached after achieving a fresh mass yield of approximately 15–20 and 25–30 t ha^{-1}, respectively. These calculations were based on a harvest index of 0.63 (Feller et al., 2011), meaning a total aboveground biomass of 24–32 and 40–48 t ha^{-1}, respectively.

2.2 | Nitrogen fertilization treatments

Total N fertilization rates for spinach grown in spring, summer, and autumn were calculated using the software N-Expert, versions 4.4.2 and 4.5.2 (IGZ Großbeeren/Erfurt, Germany). These calculations, conducted for each plot, were based on the N demand of spinach plants at a total aboveground fresh mass of 24 and 40 t ha^{-1} for the early and late harvest stage, respectively. Furthermore, the N_{min} concentration in the upper 30 cm, determined in each plot by taking soil samples before applying the base fertilization and a first top dressing, as well as the expected apparent net N mineralization and N losses, were considered in these calculations. The apparent net N mineralization and N losses were based on fertilization trials described by Fink and Scharpf (2000). When using N-Expert, these quantities were derived by the soil texture, the quantity of organic fertilizers applied to the previous crop as well as their crop residues, the expected soil temperature, and the time period from sowing to harvest of the spinach crop. The expected soil temperature was based on data provided by N-Expert depending on the soil texture and region. With the N-Expert versions used in this study (4.4.2 and 4.5.2) it was not yet possible to calculate the total N supply for winter-grown spinach, sown in autumn and harvested in the following spring. Therefore, a total of 160 kg N ha^{-1} was applied based on the calculations of Feller et al. (2011). In all four seasons, the required total N fertilization rate was divided into a base fertilizer application and one or more top dressings depending on the treatment (Table 2).

Treatments 1 and 2 reflected the standard base fertilization practice in spinach production in the Borken region. At sowing, 70–72 kg N ha^{-1} were applied to spring-grown spinach, 36 kg N ha^{-1} to summer-grown spinach, and 48–54 kg N ha^{-1} to autumn-grown spinach. In treatments 3–6, the base fertilizer rate was applied according to N-Expert. Compared to standard practice, the base fertilizer rate was reduced by 24–72 kg N ha^{-1}. In all treatments, liquid urea ammonium nitrate (UAN; 14.0% urea-N + 7.0% $NH_4{}^{+}$-N + 7.0% $NO_3{}^{-}$-N) was sprayed at base fertilization. In order to avoid N losses by volatilization of ammonia (NH_3) calcium ammonium nitrate granules (CAN; 13.5%

TABLE 2 Mean total N fertilization rates determined by N-Expert calculations and its division into several dressings as well as mean crop potentially available N up to the late harvest stage in the top soil (N_{min} concentration at sowing plus supply by mineralization, irrigation and fertilization) depending on the trial

				N dressings (kg ha^{-1})					
Season (sowing date)	Trt.	Base fertilization	Top dressings	Base fertilizer (UAN/CAN)	First top dressing (CAN)	Second top dressing (CN)[a]	Foliar spray (Urea)[b]	Total	Total crop available N (kg ha^{-1})
Early spring	1	Standard	Single	70	100	0	0	170	225
(March 20, 2018)	2	Standard	Split dose	70	45	55	0	170	225
	3	Reduced	Single	35	131	0	0	166	222
	4	Reduced	Split dose	35	80	52	0	167	222
	6	Reduced	Reduced	35	77	0	0	112	167
Mid-spring	1	Standard	Single	72	83	0	0	155	235
(May 09, 2020)	2	Standard	Split dose	72	18	51	0	141	221
	3	Reduced	Single	0	113	0	0	113	194
	4	Reduced	Split dose	0	64	59	0	126	207
	5	Reduced	Foliar spray	0	58	0	59	117	198
	6	Standard	Reduced	0	47	0	0	47	128
Late spring	1	Standard	Single	72	59	0	0	125	247
(June 04, 2019)	2	Standard	Split dose	72	0	51	0	123	231
	3	Reduced	Single	0	132	0	0	132	248
	4	Reduced	Split dose	0	59	72	0	131	239
	6	Reduced	Reduced	0	60	0	0	60	174
Early summer	1	Standard	Single	36	75	0	0	110	225
(June 14, 2018)	2	Standard	Split dose	36	20	55	0	110	225
	3	Reduced	Single	0	101	0	0	102	216
	4	Reduced	Split dose	0	51	57	0	107	222
	6	Reduced	Reduced	0	42	0	0	42	158
Mid-summer	1	Standard	Single	36	52	0	0	88	196
(July 30, 2019)	2	Standard	Split dose	36	0	43	0	79	187
	3	Reduced	Single	0	92	0	0	92	200
	4	Reduced	Split dose	0	41	53	0	93	201
	5	Reduced	Foliar spray	0	38	0	55	93	201
	6	Standard	Reduced	0	32	0	0	32	139
Late-summer	1	Standard	Single	36	51	0	0	87	219
(July 30, 2019)	2	Standard	Split dose	36	0	52	0	88	220
	3	Reduced	Single	0	89	0	0	89	222
	4	Reduced	Split dose	0	35	55	0	90	222
	6	Reduced	Reduced	0	35	0	0	35	167
Autumn	1	Standard	Single	48	125	0	0	173	235
(August 14, 2018)	2	Standard	Split dose	48	87	46	0	180	242
	3	Reduced	Single	24	145	0	0	169	231
	4	Reduced	Split dose	24	86	55	0	165	226
	6	Reduced	Reduced	24	88	0	0	112	173

(Continues)

TABLE 2 (Continued)

				N dressings (kg ha^{-1})					
Season (sowing date)	**Trt.**	**Base fertilization**	**Top dressings**	**Base fertilizer (UAN/CAN)**	**First top dressing (CAN)**	**Second top dressing (CN)[a]**	**Foliar spray (Urea)[b]**	**Total**	**Total crop available N (kg ha^{-1})**
Autumn	1	Standard	Single	54	88	0	0	143	222
(August 18, 2020)	2	Standard	Split dose	54	37	58	0	149	228
	3	Reduced	Single	0	147	0	0	147	226
	4	Reduced	Split dose	0	87	58	0	145	224
	5	Reduced	Foliar spray	0	86	0	58	144	223
	6	Standard	Reduced	0	88	0	0	89	168
Winter	3	n.a.	Single	105[c]	55	0	0	160	237
(September 28, 2018)	4	n.a.	Split dose	105[c]	0	55	0	160	237
	5	n.a.	Foliar spray	105[c]	0	0	55	160	238
	6	n.a.	Reduced	105[c]	0	0	0	105	183
Winter	3	n.a.	Single	105[c]	55	0	0	160	199
(October 02, 2019)	4	n.a.	Split dose	105[c]	0	55	0	160	199
	5	n.a.	Foliar spray	105[c]	0	0	55	160	200
	6	n.a.	Reduced	105[c]	0	0	0	105	144

[a] Applied at the early harvest stage.

[b] Divided into 5–6 foliar applications between the early and late harvest stages.

[c] Applied in early March as top dressing.

Abbreviations: CAN, Calcium ammonium nitrate; CN, Calcium nitrate (Tropicote®); n.a., not available; Trt., Treatment; UAN, Urea ammonium nitrate.

$NH_4{}^+$-N + 13.5% $NO_3{}^-$-N) were used in case of hot and dry weather. Winter-grown spinach, sown in autumn, generally does not receive a base fertilization. For these crops, a first dressing of 105 kg N ha^{-1} was applied to all treatments on the 08th and 04th of March in the calendar years 2019 and 2020, respectively. After the winter leaching period, sulfate concentration (S_{min}) in the root zone was at a low level of 5–13 kg ha^{-1} (0–30 cm) and supply by soil mineralization was also expected to be low in early spring. Therefore, in winter-grown spinach, sulfurous CAN granules (YaraBela Sulfan®, Yara International ASA, Oslo, Norway; 12.0% $NH_4{}^+$-N + 12.0% $NO_3{}^-$-N + 6.0% sulfur) were applied at first dressing to meet both N and sulfur demand of spinach.

In order to compensate for the reduced base fertilizer rates in treatments 3–6 of spring-, summer-, and autumn-grown spinach, the first top dressing was increased based on measured N_{min} (0–30 cm). In standard practice, a single top dressing is applied after the first true leaves become unfurl (BBCH 11–13 according to Feller et al., 1995). This procedure was followed in treatments 1 and 3 using a single CAN top dressing sufficient to achieve a fresh mass yield of 25 t ha^{-1}. In treatments 2 and 4, the top dressing was split into a first dose able to achieve a fresh mass yield of 15 t ha^{-1} (early harvest stage) and a second dose to achieve a fresh mass yield of 25 t ha^{-1} (late harvest stage) calculated by N-Expert. After reaching the early harvest stage, the second dose of 43–72 kg N ha^{-1} was applied, using calcium nitrate granules (CN; Tropicote®, Yara International ASA, Oslo, Norway; 15.5% $NO_3{}^-$-N). Tropicote® granules are coated with paraffin to avoid leaf necrosis when the fertilizer makes contact with the plant. In order to maintain a sufficient $NO_3{}^-$-N concentration in the root zone for plant growth, fields were irrigated within 2 days of CN application. Depending on the estimated soil moisture and expected water requirement of the plants, 11–45 L m^{-2} water was applied to all treatments using a hose reel irrigation system. In treatment 5, the second top dressing was further divided by frequent foliar urea sprays. This treatment was only realized in winter-grown spinach as well as in 2020 over the entire growing season. In order to avoid leaf damage caused by urea, its concentration was limited to a maximum of 3.0% (w/v) according to Krogmeier et al. (1989). To guarantee an adequate N supply, urea (46% N, 0.57% Biuret) was sprayed 5–6 times between the early and late harvest dates. In this way, 9–10 kg N ha^{-1} was applied almost daily during the corresponding period. The urea solution was sprayed before sunrise with the addition of 0.02% (v/v) of the nonionic organosilicon spray-adjuvant Break-Thru® S 240 (Alzchem AG, Trostberg, Germany) to improve the wetting of the leaves. In treatment 6 the second top dressing was withheld and therefore the total N supply reduced by approximately 50–70 kg ha^{-1}.

Spinach demand for potassium (K) and phosphorus (P) was calculated based on measured soil concentrations in 0–30 cm depth and soil fertility classes derived from them (Feller et al., 2011). Due to frequent manuring of the sites during the previous crop rotations, no P fertilization was required. Depending on the season and the previous crop, 30–130 kg K ha^{-1} was applied at sowing, using Korn-Kali® (K+S Minerals and Agriculture GmbH, Kassel, Germany). In addition, to prevent micronutrient deficiencies, ESPO Microtop® (K+S Minerals and Agriculture GmbH, Kassel, Germany) or EXELLO-331® (Jost GmbH, Iserlohn, Germany) were applied between the first top dressing and early harvest stage.

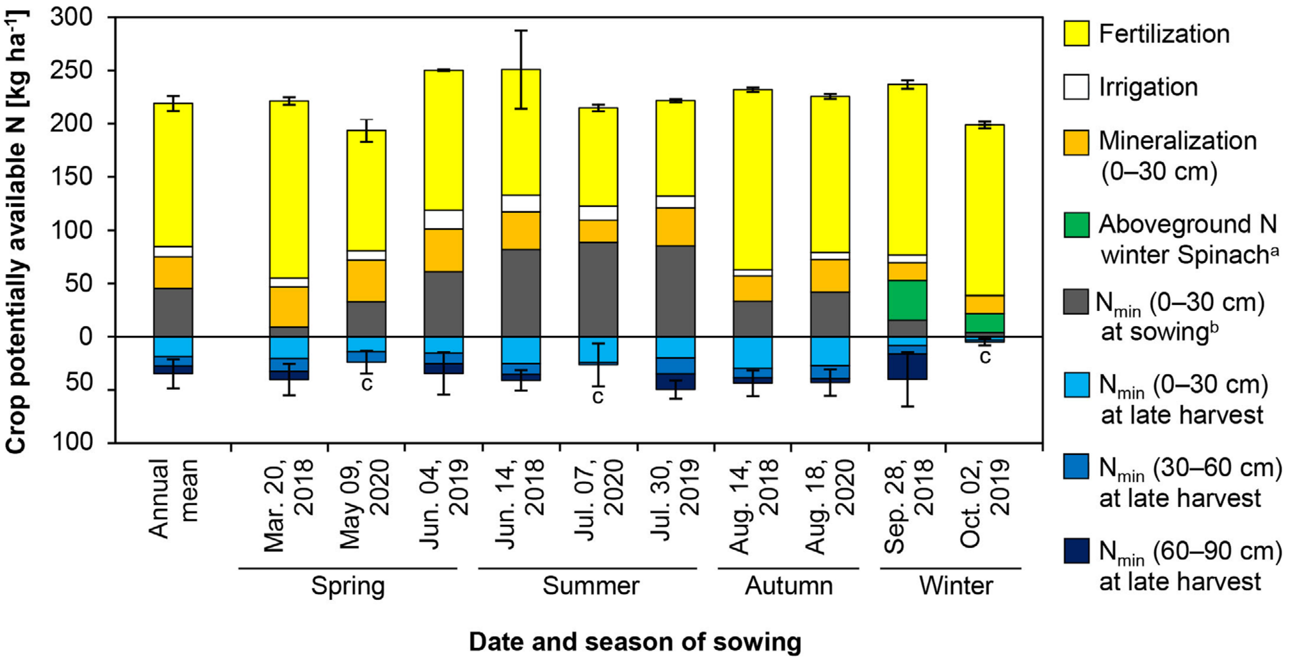


FIGURE 1 Sources of crop potentially available N in the top soil (0–30 cm) until late harvest as well as N_{min} residues at late harvest stage (0–90 cm) in treatment 3 along the ten field trials conducted between 2018–2020 (*n* = 3; Mean ± SD). *Note*: [a]Aboveground N of winter-grown spinach at early March; [b]Soil mineral N for winter-grown spinach at early March; [c]Soil depth 60–90 cm was not measured

2.3 | Crop potentially available N

In addition to the measured N_{min} concentration of the soil the crop potentially available N was calculated by summing the N_{min} (0–30 cm) at sowing and the N supply via mineralization (0–30 cm), irrigation water, and fertilizer applications until early or late harvest stage. In winter-grown spinach, sown in autumn, calculation started at the first fertilizer application at the beginning of the growing season in early March. The N uptake during the previous leaching period in autumn and winter was considered by the N content in the aboveground biomass, detected at early March, and added to the crop potentially available N (Figure 1).

Soil net N mineralization in the top soil (0–30 cm) was measured in situ using covered soil columns similar to those described by Heumann and Böttcher (2004). The columns are made of polyethylene and have a diameter of 20 cm and a length of 35 cm, of which 30 cm were driven vertically into the topsoil. On the day of sowing 24 soil columns were installed in a random design at the experimental sites. In winter-grown spinach, columns were installed at the beginning of the growing period on the 08th and 04th of March in the calendar years 2019 and 2020, respectively. In the event of the soil being dry at sowing, the soil surface inside the columns was watered with 5–15 L m^{-2} on the day of installation. In order to prevent leaching losses, the columns were loosely covered by a sunlight reflecting lid. Under the lid, temperature in the upper 2 cm soil differed by maximum ± 2.5°C from the soil temperature outside the columns. For calculating the net N mineralization, the initial N_{min} concentration was subtracted from the concentration measured in the columns at the time of the early or late harvest.

2.4 | Data collection and measurements

Air temperature and humidity were recorded by a local weather station near to the field sites (weather station Borken-Westphalia, Deutscher Wetterdienst, Germany). Also, the 30-year mean data were obtained from this weather station. Daily rainfall and irrigation were measured on site by a Hellmann gauge similar to that described by Hoffmann et al. (2016). The NO_3^- concentration of the irrigation water was determined with analytical test strips and a reflectometer (Reflectoquant® test strips and RQflex® plus 10, Merck KGaA, Darmstadt, Germany). Soil moisture and temperature were measured at approximately 5–10 cm soil depth by using UMP1-BT Plus sensors (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany). Soil mineral N (NH_4^+-N and NO_3^--N) within the soil layers 0–30, 30–60, and 60–90 cm was detected by taking soil samples, using a Pürckhauer boring rod. At sites 2 and 7, the soil sampling depth was limited to the upper two layers due to a field drainage in 80 cm depth.

Soil sampling and analysis of chemical soil parameters (N_{min}, S_{min}, total organic C, soil pH, plant available P, and K) as well as soil texture were performed according to the guidelines of the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2016).

At early and late harvests, the total aboveground biomass was determined by cutting the spinach plants at the apex of the hypocotyl. In each plot a bulk sample of three 0.25 m^{-2} subsamples were collected in the morning and forenoon and stored in plastic bags until the next day at 2–4°C in a fridge. In the laboratory, the plant samples were rinsed with tap water, spin dried and weighed. To obtain the dry mass, the material was freeze-dried (P22K-E-6, Dieter Piatkowski

Forschungsgeräte, Munich, Germany). The dried samples were ground in an ultra-centrifugal mill (Model ZM 200, RETSCH GmbH, Haan, Germany) to a particle size less than 0.5 mm. After this preparation, dry mass was used to analyze the total N content by dry combustion in an N-free oxygen atmosphere according to Dumas (Leco FP–628, LECO Instrumente GmbH, Mönchengladbach, Germany) as well as the NO_3^- content by ion chromatography (Compact IC plus 882, Deutsche Metrohm GmbH & Co. KG, Filderstadt, Germany) according to DIN EN 12014 (2017). However, the NO_3^- content in the plant biomass was only detected in the trial seasons 2019 and 2020.

The quality of the products was assessed regarding the green color of the foliage and the appearance of bolting shoots. The green coloration of the mid and upper leaves was visually assessed by using a three-level evaluation scheme (1 = yellowish; 2 = pale green; 3 = green). In addition, SPAD units were obtained by the chlorophyll meter SPAD-502 Plus (Konica Minolta Inc., Tokio, Japan) and used as a proxy to describe the intensity of the green of the youngest full developed leaves. The SPAD readings were carried out on 30 randomly selected leaves per plot. Finally, in the event of bolting, the number of bolting plants or the stem length from soil surface to the tip of the terminal was measured in three randomly chosen 0.25 m^{-2} subplots at the late harvest stage.

2.5 | Statistics

Treatments 1–6 were analyzed in a one-way design, whereas in further analysis the treatments 1–4 were treated as a two-way design considering the factors base fertilization rate and splits of top dressing. The data (total aboveground dry mass, SPAD units, plant N content, and plant NO_3^- content) were analyzed with analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$). Beforehand, assumptions of normality and homogeneity of variances were tested according to the Kolmogorov–Smirnov test and the F_{max} test (Köhler et al., 2012), respectively. If needed, data were logarithmically transformed to meet the requirements of ANOVA. In order to compare the trial factors over all 10 field experiments, the data were treated as a series of block trials as described in Gomez and Gomez (1984). In these data, model blocks are nested in the trials (Table S1). All statistical calculations were performed by using the software SPSS, version 26 (IBM Deutschland GmbH, Ehningen, Germany).

3 | RESULTS

3.1 | Weather data

The course of the mean daily air temperature as well as the daily rainfall and irrigation in each individual trial are depicted in Figure S1. Overall, the mean air temperature in spring-grown spinach was 12.5, 18.5, and 15.5°C in 2018–2020, respectively. However, in March 2018, air temperature was between 5 and 10°C and increased to the maximum of 21°C in mid-April. In summer, the mean daily air temperature ranged between 12 and 26°C. Also, in autumn-grown spinach air temperature was in the same range during the first weeks after sowing in late August and early September. However, in the last 2 weeks before late harvest average air temperature dropped to about 12°C. During the winter period, temperature ranged roughly between 0 and 10°C. Only a few days in January 2019, temperature dropped below 0°C. In April temperature rises again to a maximum of 16–18°C. Compared to the 30-year mean, the mean air temperature was increased by 0.4–2.4°C (averaged: 1.3°C) within the individual trial periods.

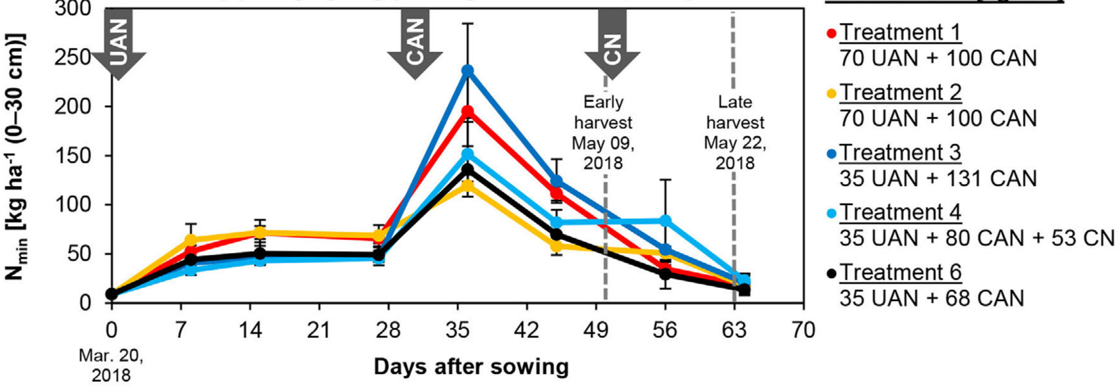
In contrast to the mean daily air temperature, the rainfall rate was on average 1.0 L m^{-2} d^{-1} lower than the 30-year mean. The fields had to be irrigated frequently in all 10 trials. On single days, rainfall or irrigation reached 45 L m^{-2}. However, based on visual observations during the soil sampling, soil in the upper 30 cm was moister compared to the deeper soil layers. Obviously, rainfall and irrigation did not reach the 30–60 cm layer during the cultivation period. Only in the winter season was the soil thoroughly moistened in all layers.

3.2 | Soil mineral N dynamics and risk of NO_3^- leaching

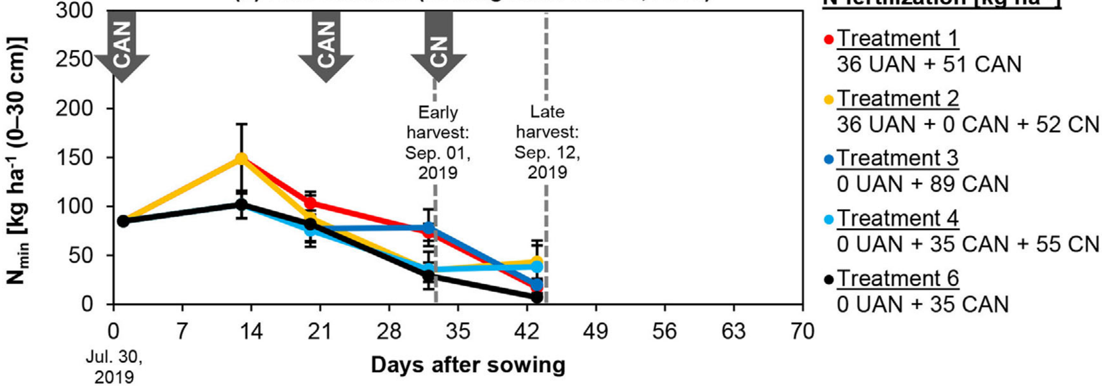
The crop potentially available N up until the late harvest stage ranged 187–248 kg ha^{-1} (in treatments 1–5) (Table 2). This is demonstrated by treatment 3 in Figure 1. In treatment 6, total N supply by fertilization was reduced by approximately 50–70 kg ha^{-1}. Apart from fertilization, N availability includes N_{min} concentration at sowing, net N mineralization, and NO_3^--N supply via irrigation. The contribution of these N sources were determined in all experiments. By irrigating with 29–96 L m^{-2} of well water, 9–17 kg N ha^{-1} and 0–7 kg N ha^{-1} was supplied to the spinach crop in the spring/summer and autumn/winter periods, respectively. The in situ soil columns showed that net N mineralization varied between 17–40 kg ha^{-1}. With the exception of lower mineralization in winter-grown spinach, no seasonal effect was observed. For winter-grown spinach, the crop available N during the winter leaching period was taken into account by the total aboveground N in early March of 38 and 18 kg ha^{-1} in the winter seasons 2018/2019 and 2019/2020, respectively. In early spring and generally in winter-grown spinach, N_{min} concentration at the beginning of the growing season was below 16 kg ha^{-1} (at 0–30 cm depth) and thus required high N fertilization input in order to meet plant demand. In contrast, in late spring and summer-grown spinach, initial N_{min} was higher and therefore N fertilization rate was reduced accordingly.

Soil mineral N residue at late harvest stage was always below 40 kg ha^{-1} in the upper 30 cm of the soil. However, at the early harvest, N_{min} level was often higher compared to the late harvest stage (Figure 2). This was observed even when N supply had been reduced until the early harvest stage, as was done in treatments 2, 4, 5, and 6. Soil N_{min} levels in the 30–60 cm and 60–90 cm layers remained quite low during the growing season (Figure 1).

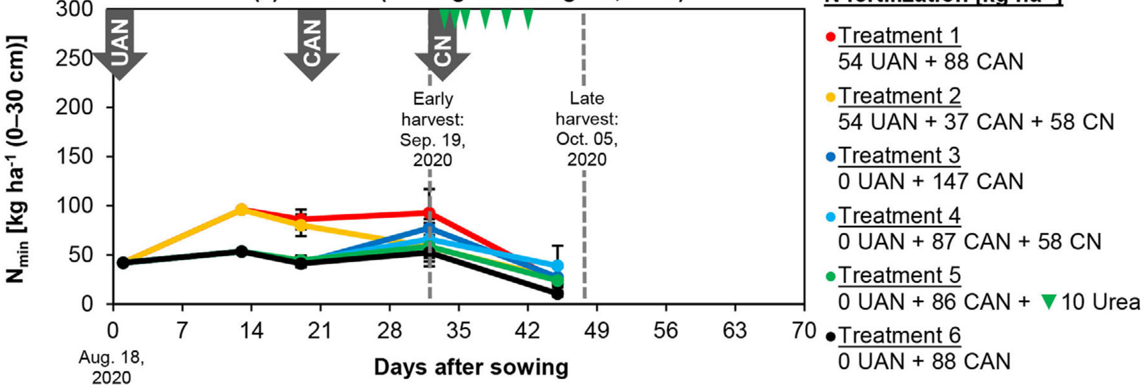
(a) Early spring (sowing date: Mar. 20, 2018)



(b) Late summer (sowing date: Jul. 30, 2019)



(c) Autumn (sowing date: Aug. 18, 2020)



(d) Winter (sowing date: Sep. 28, 2018)

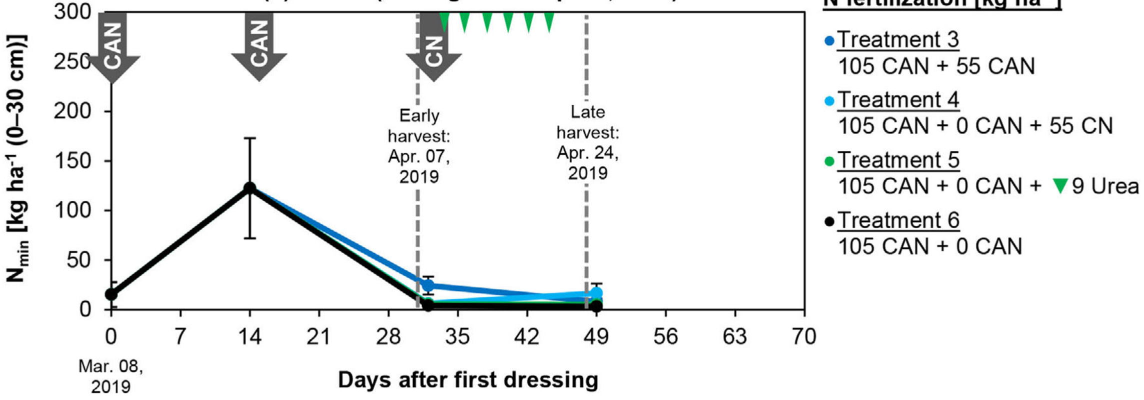


FIGURE 2 Soil mineral N concentration (NO_3^--N and NH_4^+-N) during the period of cultivation depending on the season (A–D) at different N base fertilization and top-dressing rates ($n = 3$; Mean ± SD). *Note*: CAN, Calcium ammonium nitrate; CN, Calcium nitrate (Tropicote®); UAN, Urea ammonium nitrate

Figure 2 shows the course of the N_{min} (0–30 cm) concentration of several spinach crops. The entire data for all trials are provided in Figures S2–S4. Within 1–4 weeks after the application of ammonium and urea containing fertilizers, the soil NH_4^+-N concentration decreased below 10 kg ha^{-1} (0–30 cm). Ammonium-N never exceeded 50% of the measured N_{min} concentration. When reaching the early harvest stage, generally no NH_4^+ was present in the soil. Therefore, during the cultivation period, most of the N_{min} was in the form of NO_3^- and thus at risk of leaching.

Initially, N_{min} level was low in the topsoil in early spring-grown spinach (Figure 2A). Therefore, a base fertilization of 70, or 35 kg N ha^{-1} was applied in treatments 1–2 and 3–6, respectively. In the following weeks N_{min} concentration ranged between 34–72 kg ha^{-1} depending on the base fertilization rate. In contrast, when summer spinach was grown following a spring-grown spinach crop, N_{min} at sowing was considerably higher (Figure 2B). After a base application of 36 kg N ha^{-1} (treatments 1 and 2), N_{min} concentration increased by 65 kg ha^{-1} within 2 weeks, indicating a high net N mineralization of soil organic matter and crop residues in this period. In autumn, when spinach was grown following cereals, the N_{min} concentration increased equivalent to the base fertilization rate of 0 or 54 kg N ha^{-1} (Figure 2C). In winter-grown spinach, sown in autumn 2018 and 2019, N_{min} concentration at sowing differed considerably by 112 and 18 kg ha^{-1} (at 0–30 cm depth), respectively (data not shown). However, soil N_{min} was low in both trials after the winter leaching period. Following the first fertilizer application in early March, N_{min} concentration rose according to the amount of N applied (Figure 2D).

The first top dressing was based on N_{min} soil samples taken 2–3 days before fertilizer application. Thereafter, the N_{min} concentration in the soil was temporarily increased until significant N uptake by the spinach began. However, this increase could only be monitored in the trial conducted in early spring 2018 (Figure 2A). In principle, no soil samples should be taken during the first 4 weeks after a mineral N application in order to avoid an over-, or underestimation of the N_{min} concentration by undissolved fertilizer granules in the soil samples or temporary immobilized N, respectively (Feller et al., 2011). However, following a rainfall of approximately 20 mm soon after the first top dressing dose, fertilizer grains appeared to be totally dissolved in the spring trial shown in Figure 2A.

In treatments 2 and 4–6 the first top dressing rate was reduced by approximately 50–70 kg N ha^{-1}. However, this reduced the N_{min} concentration at the early harvest stage by only 20–51 kg ha^{-1} compared to treatments 1 and 3, which had already received the total N supply required from the base fertilizer and first top-dressing dose. After reaching the early harvest stage, the second top dressing dose was applied in treatments 2, 4, and 5 in order to achieve a similar N_{min} concentration as in treatments 1 and 3.

The N_{min} residue at late harvest varied between 5–39 kg ha^{-1} (0–30 cm) in treatments 1–5. In treatment 6 (no second top dressing), a N_{min} residue of 3–15 kg ha^{-1} was observed at the late harvest stage. On average, N_{min} concentrations at late harvest were lowest in winter-grown spinach. In contrast, the highest concentrations at late harvest were observed in autumn-grown spinach.

TABLE 3 Table of ANOVA for total aboveground dry mass depending on the factors trial, base application rate, and split of top dressing at the early and late harvest stages

Source of variation	Early harvest stage	Late harvest stage
Trial	<0.001	<0.001
Base application rate (B)	0.002	n.s.
Split of top dressing (S)	0.022	0.006
Interactions:		
Trial × B	0.016	n.s.
Trial × S	n.s.	0.049
B × S	n.s.	n.s.
Trial × B × S	n.s.	n.s.

Note: n.s., not significant ($p > 0.05$).

3.3 | Aboveground dry mass

At the early harvest stage, the total aboveground dry mass was significantly reduced by both the reduced base fertilization rate as well as a split top dressing (Table 3). On average, this reduction was 6.5% and 6.0%, respectively. At the later harvest stage, the base fertilization rate did not affect the dry mass. However, in the split dose approach, the total aboveground dry mass was reduced by 2.4% on average. Overall, no significant interaction was observed between base fertilizer rate and split dose top dressings at both harvest stages. Hence, both factors are assessed independently from each other. However, the effects depended on the individual trial, as shown in Figures 3 and 4.

The largest biomass reduction of 21.1% was observed following a reduced base fertilization in early spring at the early harvest stage (Figure 3). However, plants sown from June to August showed no differences. At the late harvest stage, no significant yield reduction was observed following a reduced base fertilizer rate, in any of the trials. Thus, it appears spinach was able to compensate for any hindrances to growth observed in earlier development stages. Base fertilization was not considered as a factor in winter-grown spinach, as these crops received no N application at sowing. In these trials, a first application of 105 kg N ha^{-1} was applied to all treatments in early March (Table 2).

Up to the early harvest stage, total N supply using the split dose approach was reduced by approximately 50–70 kg ha^{-1}, compared to using a single top dressing. On average, this measure led to a statistically significant yield reduction at both harvest stages (Table 3). However, within the individual trials, no differences were observed between the top-dressing rates at either harvest stage (Figure 4). Overall, dry mass yield was highest in winter and lowest in autumn.

In Tables 4 and 5, the mean total aboveground dry mass and N content of spinach at the late harvest stage for all trials and treatments are depicted, respectively. This also includes treatment 5, which received a foliar urea spray 5–6 times between the early and late harvest stages. In contrast, calcium nitrate granules (Tropicote®) were

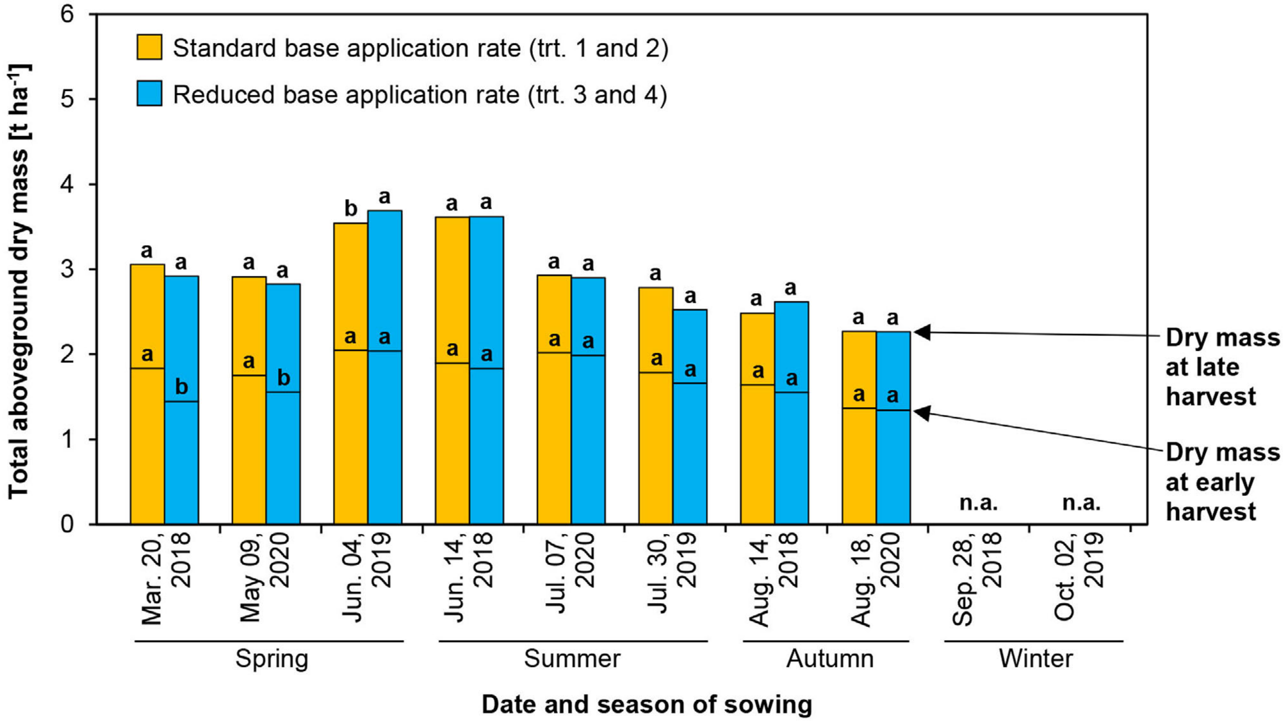


FIGURE 3 Mean total aboveground dry mass of spinach at different base fertilizer rates at the early and late harvest stages (within each trial and harvest stage, columns with different letters are significantly different according to Tukey's post hoc test, $p < 0.05$, $n = 6$). *Note*: n.a., not available; Trt., Treatment

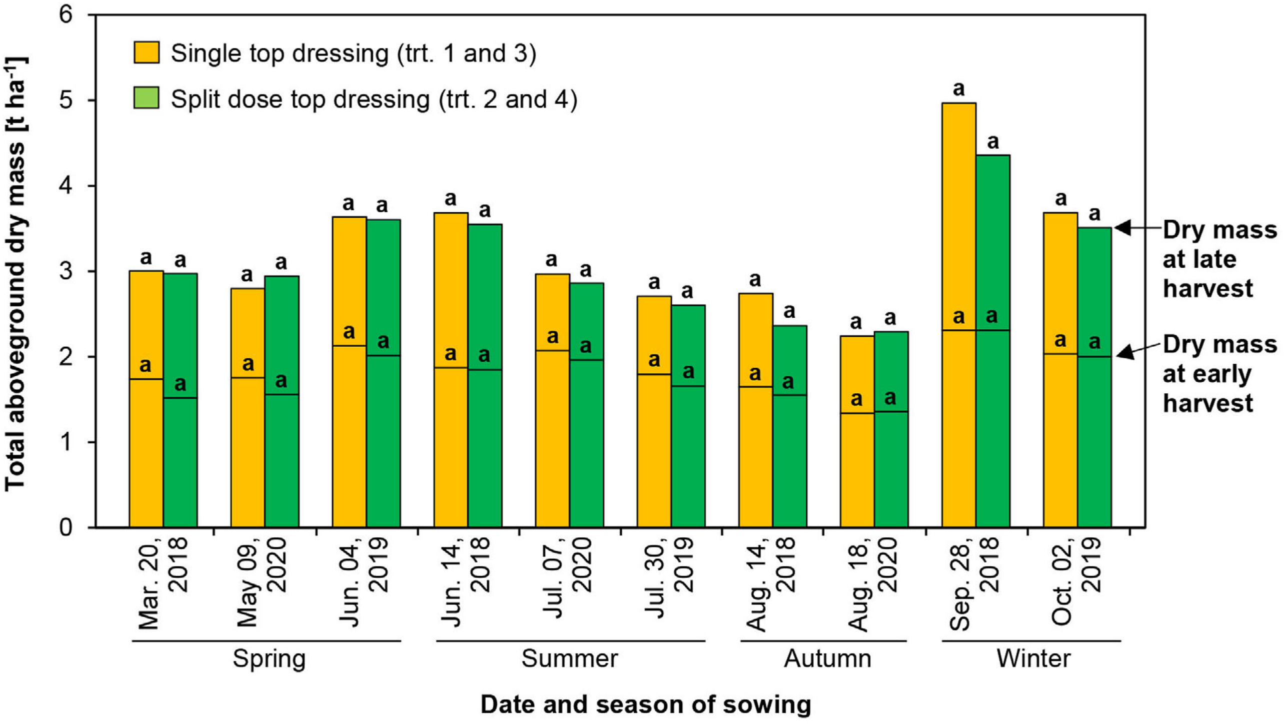


FIGURE 4 Mean total aboveground dry mass of spinach at a single and split dose top dressing at the early and late harvest stages (within each trial and harvest stage, columns with different letter are significantly different according to Tukey's post hoc test, $p < 0.05$, $n = 6$). Trt, Treatment; In winter-grown spinach only treatments 3 and 4 are considered

TABLE 4 Mean total aboveground dry mass at the late harvest stage. Means within the same line with different letters denote significant differences according to Tukey's post-hoc test ($p < 0.05$, $n = 3$)

		Total aboveground dry mass (t ha^{-1})					
Season	**Sowing date**	**Trt. 1**	**Trt. 2**	**Trt. 3**	**Trt. 4**	**Trt. 5**	**Trt. 6**
Spring	March 20, 2018	3.25a	2.87a	2.76a	3.08a	n.a.	2.73a
	May 09, 2020	2.82ab	3.01a	2.78ab	2.88ab	2.59b	2.56b
	June 04, 2019	3.58a	3.52a	3.70a	3.69a	n.a.	3.24a
Summer	June 14, 2018	3.65a	3.58a	3.72a	3.53a	n.a.	3.47a
	July 07, 2020	2.96a	2.90a	2.98a	2.82ab	2.53b	2.65ab
	July 30, 2019	2.84a	2.73a	2.58a	2.47a	n.a.	2.48a
Autumn	August 14, 2018	2.77a	2.20a	2.71a	2.53a	n.a.	2.47a
	August 18, 2020	2.27a	2.27a	2.22a	2.31a	2.12a	2.18a
Winter	September 28, 2018	n.a.	n.a.	4.97a	4.36ab	3.99b	4.30ab
	October 02, 2019	n.a.	n.a.	3.69a	3.51a	3.03b	3.39ab

Abbreviations: n.a., not available; Trt., Treatment.

TABLE 5 Mean aboveground N content at the late harvest stage. Means within the same line with different letters denote significant differences according to Tukey's post hoc test ($p < 0.05$, $n = 3$)

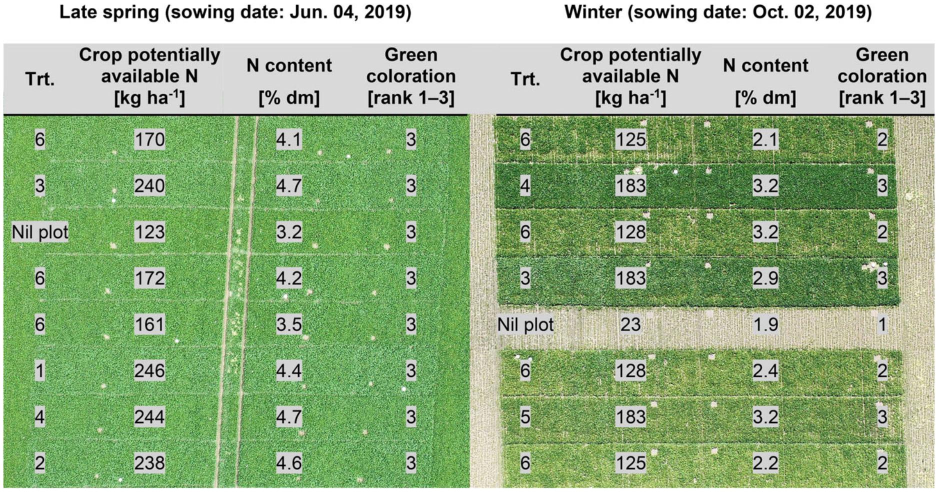
		Nitrogen content (% dm)					
Season	**Sowing date**	**Trt. 1**	**Trt. 2**	**Trt. 3**	**Trt. 4**	**Trt. 5**	**Trt. 6**
Spring	March 20, 2018	3.9a	4.0a	4.2a	4.0a	n.a.	3.4a
	May 09, 2020	4.3a	3.8ab	4.0a	4.1a	4.4a	2.8b
	June 04, 2019	4.4ab	4.4ab	4.8a	4.6a	n.a.	3.9b
Summer	June 14, 2018	3.9ab	4.0a	4.1a	4.2a	n.a.	3.5b
	July 07, 2020	3.9ab	4.1a	4.2a	4.4a	4.5a	3.2b
	July 30, 2019	4.9a	4.8a	5.1a	4.9a	n.a.	4.3b
Autumn	August 14, 2018	5.1a	4.9ab	5.1a	4.9b	n.a.	4.4c
	August 18, 2020	4.8b	4.9b	4.8b	5.2a	5.4a	4.2c
Winter	September 28, 2018	n.a.	n.a.	3.6ab	3.7a	3.5ab	3.1b
	October 02, 2019	n.a.	n.a.	2.7b	2.9a	3.1a	2.2c

Abbreviations: n.a., not available; Trt., Treatment; dm = g N (100 g dry matter)$^{-1}$.

used for the second top dressing dose in treatments 2 and 4, whereas in treatments 1 and 3 total N fertilization was completed by only one base fertilization and top dressing. In most of the trials, shoot N content at late harvest was independent from the fertilizer schedule and type of fertilizer used for top dressings. However, total aboveground dry mass was significantly reduced by 11.8% after frequent urea spray compared to treatments 1–4. Interestingly, omitting the second top dressing (treatment 6) resulted in a reduction of only 7.4% compared to the first four treatments. Also, in most of the trials, no significant yield reduction was observed when the fertilizer N supply was reduced by 50–70 kg ha^{-1}, in comparison to the first four treatments. However, the N content was generally at a lower level when the N supply was reduced.

3.4 | Quality of the produce

At the early harvest stage, no differences in the leaf color were observed between treatments 1–6. However, at the late harvest stage, coloration was affected by the crop potentially available N in the winter-grown spinach (Figure 5) as well as spinach grown in early and mid-spring. This was particularly evident in treatment 6 (reduced N supply), where leaves became "yellowish" at the late harvest stage. In contrast, in late spring and summer-grown spinach, hardly any differences in color were seen between the plots, even when the crop potentially available N differed by more than 100 kg ha^{-1} in the plots that received no N fertilization (nil plot) it is worth noting, however, that crop potentially available N as well as aboveground N content



Late spring (sowing date: Jun. 04, 2019)

Trt.	Crop potentially available N [kg ha^{-1}]	N content [% dm]	Green coloration [rank 1–3]
6	170	4.1	3
3	240	4.7	3
Nil plot	123	3.2	3
6	172	4.2	3
6	161	3.5	3
1	246	4.4	3
4	244	4.7	3
2	238	4.6	3

Winter (sowing date: Oct. 02, 2019)

Trt.	Crop potentially available N [kg ha^{-1}]	N content [% dm]	Green coloration [rank 1–3]
6	125	2.1	2
4	183	3.2	3
6	128	3.2	2
3	183	2.9	3
Nil plot	23	1.9	1
6	128	2.4	2
5	183	3.2	3
6	125	2.2	2

FIGURE 5 Experimental plots in the late spring and winter seasons 5 days before late harvest depending on the crop potentially available N in the top soil (N_{min} concentration at sowing plus N supply by mineralization, irrigation and fertilization) as well as the N content in the aboveground dry mass and the ranking of the green coloration at the late harvest stage. Nil plot received no N fertilization. Trt. = Treatment; % dm = g N (100 g dry matter)$^{-1}$

Summer-grown spinach

Winter-grown spinach

FIGURE 6 Necrosis at the leaf margin at the late harvest stage in summer-grown and winter-grown spinach after repeated (six times) foliar urea treatment [3.0% (w/v) urea]

was generally lower in winter. The different responses of the green color to the crop potentially available N were reflected in the SPAD measurements (Table S2). The fertilizer schedule and type of fertilizer used for the top dressings (calcium ammonium nitrate, calcium nitrate, urea spray) made no difference to leaf color. However 4–5 days after the first aerially applied urea, necrosis was observed at the leaf margin, being most pronounced in winter-grown spinach (Figure 6).

Generally, no bolting was observed until the early harvest stage. In the spring and summer crops plants started bolting between early and late harvest stages (Figure 7). In late spring and summer-grown spinach bolting was most pronounced and led to a uniform stem length of about 30 cm at the late harvest stage (Table S3). No bolting was observed in autumn or winter crops.

At the early harvest stage, NO_3^- content in the aboveground fresh mass was generally higher following a single N top dressing dose (treatments 1 and 3) (Table 6). Splitting the top dressing and thus reducing the total N supply until early harvest reduced the NO_3^- content in the biomass (treatments 2, 4–6). However, after using calcium nitrate granules for the second top dressing dose, NO_3^- content was increased in treatments 2 and 4 at the late harvest date. In contrast, frequent urea foliar sprays (treatment 5) as well as reducing the total N supply (treatment 6) kept the average NO_3^- content low. Overall, the NO_3^- content in winter-grown spinach was usually lower than in the other seasons. However, data varied considerably between individual trials.

4 | DISCUSSION

4.1 | Risk of NO_3^- leaching

In spring, when the first crops are grown after the winter period of leaching, N_{min} concentration and net N mineralization is low (D'Haene et al., 2018). Therefore, the quantity of NO_3^- at risk of leaching can be easily managed by adjusting application rates. However, in order to ensure sufficient N availability, approximately 70 kg N ha^{-1} are usually

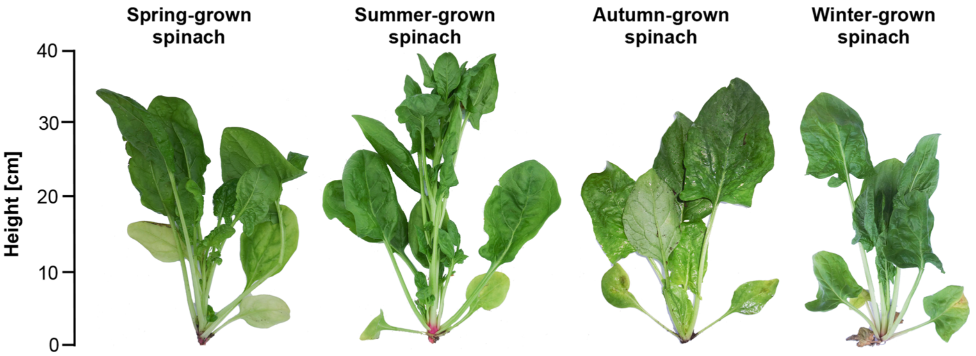


FIGURE 7 Spinach growth habit depending on the season at the late harvest stage

TABLE 6 Nitrate content in the total aboveground fresh mass at the early and late harvest stages. Means within a line and harvest stage with different letters are significantly different according to Tukey's post hoc test ($p < 0.05$, $n = 3–9$)

		Shoot nitrate content [mg (kg fm)$^{-1}$]					
		Early harvest stage		Late harvest stage			
Season	Sowing date	Single top dressing (Trt. 1, 3)	Split top dressing (Trt. 2, 4–6)	Single top dressing (Trt. 1, 3)	Split top dressing (Trt. 2, 4)	Urea top dressing (Trt. 5)	Reduced top dressing (Trt. 6)
Spring	May 09, 2020	2,723a	1,613a	1,506a	1,531a	773ab	381b
	June 04, 2019	3,993a	3,120a	2,556a	2,781a	n.a.	2,764a
Summer	July 07, 2020	2,219a	1,655a	1,800ab	2,400a	1,022ab	380b
	July 30, 2019	n.d.	n.d.	2,947a	2,707a	n.a.	1,542b
Autumn	August 14, 2018	2,477a	1,504b	1,068ab	2,249a	2,312a	327b
Winter	September 28, 2018	2,587a	1,121a	1,800ab	693a	205c	391bc
	October 02, 2019	758a	179a	100a	132a	44a	47a

Abbreviations: fm, fresh matter; n.a., not available; n.d., not determined, Trt., Treatment.

applied at sowing of spring-grown spinach (Figure 2A). The NH_4^+ and urea in the fertilizers used for N base fertilization were mostly nitrified within a few weeks after application, and consequently at risk of leaching. Applying urease or nitrification inhibitors as a way to reduce leaching is not recommended in spinach because a high soil urea or NH_4^+ concentration leads to a considerable decrease in biomass production of the crop (Canali et al., 2014; Conesa et al., 2009; Cruchaga et al., 2011; Hähndel & Wehrmann, 1986; Pasda et al., 2001).

In order to shorten the period with high soil NO_3^- levels, delaying the application of a base fertilizer might be an effective measure. However, whether this measure can be implemented without negative effects on plant growth greatly depends on the time of year. When summer-grown spinach directly followed spring-grown spinach, N_{min} increased to approximately 100 kg ha^{-1} (0–30 cm) until 2 weeks after sowing, even without a base fertilizer (Figures 2B and S3). This increase was probably due to mineralization of easily decomposable and N rich spinach crop residues and soil organic matter (De Neve et al., 1994).

In treatments where the base N fertilization was reduced, the top dressing rate was increased accordingly in order to meet plant N demand (Table 2, Figure 2). By the development stage, when the first top dressing was applied, spinach roots almost reach a depth of about 15 cm (Schenk et al., 1991). Thus, the risk of NO_3^- leaching at this stage is lower compared to the beginning of the cultivation period. Based on growing degree days and root measurements, this also applies to winter-grown spinach, which received its first dressing of 105 kg N ha^{-1} after the taproots penetrated below 15 cm soil (Smit & Groenewold, 2005). After juvenile vegetative growth, the taproot can penetrate more than 60 cm down into the soil profile (Kutschera et al., 2009). However, almost independently from the fertilizer placement, significant N uptake is restricted to the upper 30 cm soil (Heinrich et al., 2013; Schenk et al., 1991; Smit & Groenewold, 2005). Therefore, fertilization should be in synchronization with N uptake by spinach.

In the first weeks after sowing, and also after harvest, the spinach fields are bare. As a result, there is a high risk of NO_3^- leaching during these periods. Therefore, N_{min} residue at harvest should be reduced to a minimum and the following fallow period should be as short as possible. At the later harvest stage, after reaching a fresh mass yield of approximately 25–30 t ha^{-1}, N_{min} residue in the top soil was between 3 and 62 kg ha^{-1} (Figures 2, S2, S3, and S4). At a marketable yield of approximately 25 t ha^{-1}, D'Haene et al. (2018) found a minimum N_{min} residue of 7–12 kg ha^{-1} (0–30 cm), as long as fertilization corresponded to N uptake. However, at a higher total N supply, N_{min} residue continuously increased without affecting the mass of marketable yield (D'Haene et al., 2018). Therefore, most of the variation in N_{min} residue was due to the crop available N and the actual N uptake by plants. When comparing all field trials, also the crop potentially available N varied by more than 50 kg ha^{-1} (Table 2, Figure 1). This variation can partially be explained by the N supplied by irrigation which was not accounted for in the fertilizer calculation using N-Expert. In addition, the calculated N supply from mineralization can deviate from actual supply (Fink & Scharpf, 2000). Therefore, the total fertilizer N requirement can be over- as well as underestimated and consequently affect N_{min} residues. Particularly in autumn, when N mineralization is still high and N uptake by plants declines due to decreasing irradiation and temperatures, calculated N fertilization can be overestimated (Breimer, 1982; Gent, 2016; Proietti et al., 2004;Tei et al., 2020). These observations were also made in autumn-grown spinach epitomized by a reduced dry mass growth (Table 4, Figures 3 and 4) and a comparable higher N_{min} residue (Figures 2C and S4).

At the early harvest statge, N_{min} residue was higher compared to late harvest even when the first top dressing dose was reduced. The lower N use efficiency of field-grown spinach before early harvest stage might be due to a lower root density in the 15–30 cm soil layer (Schenk et al., 1991) as well as higher fertilizer input in treatments 1 and 3. Generally, plant N recovery is lower at high crop available N due to a decreased net mineralization as well as higher leaching losses and gaseous emissions (Canali et al., 2011; Fink & Scharpf, 2000). This was confirmed by an average N recovery rate (kg aboveground N per kg crop potentially available N × 100) of 49% and 58% at the early and late harvest stages, respectively (data not shown). Furthermore, the split dose approach is only effective at reducing N_{min} residue as long as the second dose is not applied. After the second dose, the crop potentially available N was similar to the treatments that received a single top dressing. In practice, spinach is often harvested at an intermediate stage between early and late harvest. Thus, N_{min} residue at actual harvest might be higher than at the late harvest stage depicted in Figures 1 and 2. Furthermore, the split dose approach can affect yield and quality of the produce, as discussed below.

4.2 | Aboveground biomass

Spinach biomass growth until the early harvest stage was significantly affected by the base fertilization and first top-dressing rate (Table 3, Figures 3 and 4). At the later harvest stage, initial hindrances to growth were partially compensated for by the top dressings. Obviously, a high N supply seemed to be more important for plant growth in the early compared to later development stages.

For field-grown spinach, the N_{min} concentration should be set to a minimum of 40 kg ha^{-1} (0–30 cm) from sowing to harvest (Feller et al., 2011). For spring-grown spinach, Lorenz et al. (1989) recommended setting the minimum at 60 kg N ha^{-1} (0–30 cm). As shown in Figure 2A, N_{min} in the spring-grown spinach was between 34–50 and 53–72 kg ha^{-1} (0–30 cm) until the first top dressings were applied at a reduced, and standard base fertilization rate, respectively. The initial lower N supply led to a significantly reduced dry mass yield at the early harvest stage in spring-grown spinach (Figure 3), but in autumn, 50 kg N ha^{-1} (0–30 cm) was sufficient for proper plant growth (Figures 2C and S4). This sesonal effect might be due to temperature. At low ambient temperatures transpiration and consequently the transport of NO_3^- in soil by mass flow towards plant roots is reduced (Barber, 1995). However, the lower the NO_3^- concentration in the soil solution, the more N is taken up by diffusion (Kage, 1997). At spinach harvest, approximately 10%–25% of the acquired N is taken up by mass flow at a total N supply of 100 and 175 kg ha^{-1} (0–60 cm), respectively (Heins, 1989). In addition to a reduced passive NO_3^- uptake via mass flow, low soil temperatures might inhibit the active absorption of NO_3^- by spinach roots (Chadirin et al., 2011; Schenk, 1996). Furthermore, N mineralization triggered by root exudates can be reduced by low soil temperatures (Zhang et al., 2016). These limitations due to low temperatures can be partially offset by a higher N availability in the soil (Laine et al., 1993), as observed in the spring-grown spinach (treatments 1 and 2). In summer, soil temperature was high and initial N_{min} concentration was about 80 kg ha^{-1} at sowing. Therefore, base fertilizer rate did not affect spinach growth in summer (Figures 2B, 3, and S3).

By using a split dose approach, the first top dressing was reduced by approximately 50–70 kg N ha^{-1}, which significantly affected dry mass yield at both harvest stages (Table 3). This is in line with the observations of Massa et al. (2018), who recommended an N_{min} concentration of 135 kg ha^{-1} (0–40 cm) for maximum spinach growth and 41 kg ha^{-1} (0–40 cm) required for minimum growth. However, N_{min} concentration at early harvest was temporarily below 40 kg ha^{-1} (0–30 cm) without affecting dry mass yield at late harvest (Table 4, Figures 2, S2, S3, and S4). Therefore, spinach seemed to be able to compensate for short periods with low N supply.

From the early to late harvest stage, the difference in dry mass due to the single and split dose top dressing decreased, on average, from 6.0% to 2.4% (Figure 4). Withholding the second top dressing resulted in a 7.4% reduction compared to the full N supply in treatments 1–4. This indicates that the N supplied with the second top dressing was sufficiently available for plants. It is likely that irrigating within the first two days after the second top dressing is crucial to N availability. However in practice, it might take several days to thoroughly irrigate entire fields by using a hose reel irrigation system. In addition, the actual harvest date is decided on short notice, which makes it difficult to determine the exact date when the second top dressing should be applied. On one hand, it should not be applied too late in order to maintain sufficient NO_3^--N availability in the soil (Biemond et al., 1996). On the other hand, it should not be applied too early, so that the second top dressing can be withheld in the case of an early harvest. Therefore, a nutrient management strategy would be helpful, so that an adequate N supply can be ensured independent of irrigation schedule and actual harvest date.

In order to adapt total N supply more precisely to the actual plant demand, foliar urea was frequently applied in treatment 5. Spinach requires a N fertilization rate of approximately 50–70 kg ha^{-1} between the early and late harvest stages. Therefore, an urea solution of 3.0% (w/v) (9–10 kg N ha^{-1}) was sprayed almost daily between the early and late harvest stages. However, this concentration resulted in necrosis at the leaf margin 4–5 days after its first application (Figure 6). This suggests that the full N requirement of spinach cannot be met by foliar applications alone. In addition, dry mass growth was often significantly reduced compared to all other treatments (Table 4). However, the N content in the aboveground biomass was similar across treatments 1–5 (Table 5). Therefore, a reduced N availability due to an inhibited N uptake as well as signigicant gaseous losses by NH_3 cannot explain the growth impairments. However, up to 11% of the foliar applied urea-N is often lost via NH_3 and can lead to necrosis in plant leaves even at low exposure levels (Schlossberg et al., 2018; Singh et al., 2013; Stiegler et al., 2011). Growth impairments and necrosis can also be due to plant stress caused by the accumulation of urea or biuret in plant leaves (Bremner, 1995; Cruchaga et al., 2011; Krogmeier et al., 1989). In order to avoid leaf damage, a maximum of 0.25%–2.00% biuret in foliar applied urea fertilizers and a urea concentration of maximum 3.0% is recommended depending on the crop and environmental conditions (Krishnasree et al., 2021). However, biuret is not easily metabolized in plant tissue. Therefore, repeated spray applications may have a cumulative effect (Mikkelsen, 1990). The urea fertilizer used in treatment 5 had a biuret concentration of 0.57% and was sprayed 5–6 times. Therefore, it seems likely that the observed leaf necrosis and diminished growth can be due to both NH_3 exposure as well as biuret accumulation in the leaf tissue.

In autumn-grown spinach, the total aboveground dry mass in treatments 1–4 formed until the late harvest stage was on average 24% lower than the average mean for all trials (Table 4). As mentioned in the chapter above, this might be due to decreasing irradiation and temperatures between early and late harvest stages in autumn. Contrastingly, in winter-grown spinach the total aboveground dry mass was 30% higher compared to the annual mean. The higher yield at similar crop potentially available N levels was reflected by a lower biomass N content (Tables 2 and 5). The higher N efficiency of winter-grown spinach might be due to the ontogenetic stage at the late harvest stage. In contrast to spring and summer crops, no bolting was observed in winter- and autumn-grown spinach (Figure 7). In general, dry mass growth rate is reduced after reaching the generative development stage due to, for example, a lower dry mass content of stem and stalk tissues formed while bolting (Biemond et al., 1996; Feller et al., 2011; Smolders and Merckx, 1992). As long as bolting is not initiated, a reduced fertilizer N supply can be compensated for by delaying the harvest date (Heins, 1989; Smolders & Merckx, 1992). Therefore, as observed, dry mass growth continued even under low N availability. Overall, spinach grown during the winter season would be preferable for achieving high processed spinach output at low NO_3^- leaching risk. However, the product quality was also affected by total N supply, as described below.

4.3 | Quality of the produce

Spinach traits relevant for processing into frozen goods were affected by the N fertilization approaches. However, high crop yield was not necessarily associated with high plant quality and vice versa.

Overall, the intensity of the leaf green color was affected by total N supply in winter as well as early and mid-spring. In the late spring and summer/autumn seasons, however, mostly no effects were observed (Table S2, Figure 5). Nitrogen deficiency symptoms in spinach become visible when N content in the youngest fully developed leaves drops below 3.0% (Hochmuth et al., 2018). In late spring, summer, and autumn as well as generally at the early harvest stage, the N content was always at least 3.0% even when total N supply was reduced (Table 5). Consequently, green coloration and SPAD units tended to be uniform between treatments (Table S2). In contrast, in winter-grown spinach, leaf blades became yellowish at a reduced N supply (treatment 6). In this treatment, the N content ranged between 2.2% and 3.1% compared to 2.7% and 3.7% under sufficient N availability. Similar observations were also made in spinach sown in March and May. Using visual ratings, D'Haene et al. (2018) also observed differences in leaf color at a total N availability of up to 200 kg ha^{-1}. Contrastingly, by using the SPAD meter, spinach seemed to be unaffected by a N dose of up to 225 kg ha^{-1} (Canali et al., 2014). However, SPAD units are correlated with the chlorophyll and N content, whereas green coloration depends on plant N status, carotenoid content, the variety chosen, the development stage, the site, weather conditions, the time of day, as well as pests and diseases (Martins et al., 2020; Padilla et al., 2020). Therefore, a reduced N supply is not necessarily going to lead to pale or even chlorotic spinach leaves.

When comparing spinach from various growing seasons, differences in phenotypic plant characteristics must also be taken into account. In contrast to winter- and autumn-grown spinach, spring- and summer-grown spinach began bolting after reaching the early harvest stage (Figure 7). Bolting is initiated by increased daylength, but occurs independently to N supply (Navarrete, 2016). This was also confirmed for late spring and summer-grown spinach by a uniform final stem length and number of bolting plants across all treatments (Table S3). When spinach starts bolting, stalk and stem tissues are almost exclusively formed, rather than leaf blade tissue (Biemond et al., 1996). However, spinach with a high proportion of leaf blades is preferred by the markets (Brandenberger et al., 2004; Grevsen & Kaack, 1997). Summer-grown spinach should therefore be harvested at an earlier stage to meet market demands.

Spinach tends to accumulate NO_3^- in the stalk and stem tissues (Beis et al., 2002). Therefore, bolting favors an increase in shoot NO_3^- content (Colla et al., 2018). Abiotic stress under summer weather conditions due to heat and drought can exacerbate this effect (Breimer, 1982; Kaiser & Förster, 1989). This might also explain why the European threshold of 2,000 mg NO_3^- (kg fm)$^{-1}$ for processed spinach (European Commission, 2011) was often exceeded in summer and late spring (Table 6, Figure S1). However, NO_3^- content was also elevated in autumn-grown spinach, even without bolting and with less temperature and water stress at both harvest stages. In autumn, this increase might be due to lower irradiation at the time of harvest and the subsequent lower NO_3^- reduction capacity in plant cells (Breimer, 1982; Colla et al., 2018; Gent, 2016; Proietti et al., 2004). Contrastingly, in winter-grown spinach NO_3^- content in the biomass was usually lower compared to the other seasons. Compared to autumn, this might be due to increasing light intensities as well as low N_{min} concentration at harvest. This suggest that NO_3^- reduction capacity at harvest was enhanced, leading to low NO_3^- content in the biomass (Breimer, 1982; Chadirin et al., 2011; Kaminishi and Kita, 2006). The split dose approach can be an effective measure to lower the NO_3^- content at the earlier harvest stage by lowering soil N_{min} concentration. However, at the later harvest stage the second top dressing led to a higher content, as studies by Biemond et al. (1996) have confirmed. Even under reduced N supply (treatment 6), NO_3^- content can be above the European threshold. Therefore, the NO_3^- content in spinach crops can only partially controlled by the N fertilization strategy (Breimer, 1982).

5 | CONCLUSIONS

Spinach is a fast-growing and N demanding crop. In order to maintain a sufficient N availability, high N doses are applied to the field leading to temporarily elevated quantities of NO_3^- at risk of leaching. Due to low N uptake in the first weeks after sowing, the risk of leaching is especially high after a base fertilizer is applied. The results of this study show that, except for spinach grown in early and mid-spring, N base fertilization can be omitted, without reducing dry mass yield. However, N top dressing rate then has to be increased to meet total plant N demand. The resulting N_{min} peak concentration after top dressing can be flattened by splitting the top-dressing dose. Furthermore, by using the split dose approach, growers are able to withhold the second top dressing in the case of an early harvest. Soil N_{min} residue and biomass NO_3^- content are considerably reduced in this way. However, due to a reduced first top dressing dose, dry mass yield can be negatively affected at the early harvest stage. After the second top dressing application, there was a comparable total N supply between the single and split top-dressing approaches, and plants were able to partially recover from early growth retardations. A further dividing of the N application by frequent urea foliar sprays proved to be insufficient and can cause leaf necrosis. In autumn, growth was diminished in the last weeks before late harvest, and a higher N supply did not compensate for it. Furthermore, in summer an early bolting deteriorates the quality of the spinach due to increased stalk and stem biomass as well as a higher NO_3^- content in the produce. Therefore, for summer and autumn-grown spinach, it is recommended that the total amount of N fertilization is reduced by withholding the second top dressing of 50–70 kg N ha^{-1} and harvesting the crop at an earlier stage. In this way, the risk of NO_3^- leaching can be reduced, and the quality of the produce improved.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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