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Crop Residue Management Strategies to Reduce Nitrogen Losses during the Winter Leaching Period after Autumn Spinach Harvest

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Abstract: In open-field vegetable production, high quantities of soil mineral nitrogen (N_{min}) and N-rich crop residues often remain in the field at harvest. After the harvest of crops in autumn, this N can lead to considerable nitrate (NO_3^-) losses during the subsequent winter leaching period. In four field trials, different tillage depths (3–4, 10, 30 cm) and dates (early autumn, late autumn, early spring) were investigated to reduce N losses after growing spinach in the autumn. In a further treatment, the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) was directly applied to the crop residues. Potential N losses were calculated by a balance sheet approach based on N_{min} concentration (0–90 cm), measured N mineralization and N uptake by catch crops. By postponing the tillage date from early to late autumn or spring, resprouting spinach stubbles acted as a catch crop, reducing N losses by up to 61 kg ha^{-1}. However, if the spinach biomass collapsed, the N losses increased by up to 33 kg ha^{-1} even without tillage. The application of DMPP as well as the tillage depth were less effective. Overall, postponing tillage to spring seems to be the most promising approach for reducing N losses during the off-season.

Keywords: *Spinacia oleracea* L.; N_{min} residue; balance sheet; nitrate leaching; tillage depth; tillage date; nitrification inhibitor; 3,4-Dimethylpyrazole phosphate

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1. Introduction

In regions with intensive vegetable production, the maximum permissible nitrate (NO_3^-) concentration of 50 mg L^{-1} groundwater is often exceeded [1–3]. Nitrate leaching losses occur particularly when vegetables are grown on sandy sites, as is often the case with crops such as spinach [4–6]. In order to reduce NO_3^- leaching, much research has been conducted to increase the N uptake efficiency of a single crop rather than focusing on the system as a whole [7,8]. The off-season should specifically be considered in such a system approach [9].

Spinach (*Spinacia oleracea* L.), cultivated for the processing industries, is typically grown in frequent sowings and harvested from April to late October [10]. The crop is typically grown on sandy soils because this facilitates specific field management and reduces the impact of variable weather conditions on yield [4,5,11]. Spinach generally requires a mineral N buffer value of approximately 40 kg ha^{-1} in the upper 30–40 cm of the soil to obtain a product that fulfills market-quality requirements [4,12]. However, calculated N uptake at the time of fertilization is often overestimated because crop yield depends on weather conditions, diseases, and the requirements of the market [13,14]. Particularly at the end of the growing season, N uptake of spinach can be reduced due to decreased solar radiation [15,16]. On the other hand, soil N mineralization is still high in autumn because of relatively high soil temperatures, potentially leading to high soil mineral N (N_{min}) concentrations at harvest [17]. Therefore, depending on the actual harvest stage, 50–100 kg NO_3^--N ha^{-1} (0–30 cm) typically remains in the soil at the harvest of autumn-grown spinach [10,18].

Vegetable crop residues are usually incorporated into the soil shortly after the harvest in order to minimize the risk of infection by plant pathogens such as damping-off diseases and downy mildew in spinach crop rotations [9,19,20]. However, spinach crop residues are characterized by a low carbon (C) to N ratio which accelerates net mineralization and nitrification after incorporation into the soil. Consequently, the N_{min} concentration sharply increased after spinach harvest [21–23]. In order to reduce high post-harvest soil NO_3^- concentration, catch crops are usually grown during the winter leaching period. However, after incorporation of vegetable crop residues in autumn, the combined N_{min} residue and N mineralization often exceed the N uptake capacity of catch crops, depending on the catch-crop sowing time. As a result, high quantities of NO_3^- are susceptible to leaching in humid climates such as Central Europe [24]. The average N_{min} concentrations at the end of the spinach growing season were reported to be about 120 kg N ha^{-1} (0–90 cm) [25–31]. During the succeeding winter leaching period, the remaining NO_3^- is leached to below 120 cm in sandy soils [17]. Thus, even deep rooting crops may be unable to take up this NO_3^- sufficiently in the following growing season [32,33].

To achieve decreased mineralization after crops are grown in the autumn, strategies such as a shallower tillage depth [34] or a postponement of the tillage date from autumn to winter or even to spring may be appropriate [35–38]. During winter and early spring, the soil temperature is lower compared to the autumn season, which can considerably reduce the mineralization and nitrification of vegetable crop residues and native soil organic N [39,40]. Furthermore, spinach is able to re-sprout after harvest and thus continue to absorb nitrogen [41]. Another approach that has been suggested to reduce NO_3^- leaching is the co-incorporation of materials with a high C/N ratio and/or high polyphenol content, such as immature compost, straw, paper waste, or sawdust, which cause N immobilization and/or slow down N mineralization [24]. However, such materials have to be applied in large quantities and their effectiveness depends on soil microbial activity, which is largely dependent on soil temperature [42]. In contrast, if N immobilization continues after the winter season, it may have a negative impact on the yield of the following crop [9]. A further often-stated option to reduce post-harvest N losses is the removal of crop residues in combination with reapplication in the following season [43]. However, spinach crop residues often only contain around 30 kg N ha^{-1} [17], thus limiting the usefulness of this option in reducing N leaching losses. In addition, the removal of crop residues and the application of N-immobilizing materials are costly management options and thus, are often not economically feasible [44]. Another option to reduce the NO_3^- concentration at the end of the growing season is to delay nitrification by applying inhibitors directly to the plant debris. In an incubation experiment, nitrification after the incorporation of cauliflower leaves was inhibited by at least 95 days when using 3,4-Dimethylpyrazole phosphate (DMPP) [45]. This approach also proved successful in field experiments [46]. Compared to other nitrification as well as urease inhibitors, DMPP is effective at small concentrations and less prone to leaching [45–48]. Therefore, DMPP seems to be the most promising method for reducing nitrification in the post-harvest stage. However, the effectiveness of nitrification inhibitors has not yet been investigated in situ on spinach crop residues.

This study focuses on crop-residues management following autumn-grown spinach. The aim was to reduce N losses during the subsequent winter leaching period. It was hypothesized that mineralization and nitrification of spinach crop residues and native soil organic N can be reduced by (a) reducing the tillage depth, (b) postponing the tillage date from early to late autumn or early spring, and (c) via the application of DMPP, a nitrification inhibitor, to crop residues.

2. Materials and Methods

2.1. Sites and Experimental Set-Up

In total, four field trials were carried out in the winter seasons 2018/19, 2019/20, and 2020/21 at different sites in Borken, North Rhine-Westphalia, Germany. In Table 1, the trials are arranged according to the harvest date of the spinach crop (*Spinacia oleracea* L.; taxonomy ID: 3562), regardless of the individual year. All trials were established immediately after spinach harvest in mid-September (trial 1) or October (trials 2–4) and completed in the following March. Based on the soil samples obtained at spinach harvest, soils were characterized by a loamy sand texture (DIN 4220:2008 [49]) and 1.1–1.5% organic C. In trial 4, soil organic C content was 3.6% with a comparably higher C/N ratio of above 25. All four sites are classified as “Plaggenesch” [50]. Experimental sites 1, 2, and 3 were subject to arable cultivation even before the 20th century. In contrast, site 4 was originally a forest and has been subject to arable cultivation since the 1950s. Soil pH at spinach harvest was between 5.2 and 6.0 (0.01 M $CaCl_2$). Summer-grown spinach, carrots, or cereals were grown before autumn-grown spinach. Within these crop rotations, a total annual fertilizer-N of 162–296 kg ha^{-1} was applied. In the case of summer- and autumn-grown spinach, only the mineral fertilizers urea ammonium nitrate and calcium ammonium nitrate were applied. When cereals or carrots were grown as a pre-crop, liquid manure (170 kg N_{tot} ha^{-1}) was applied in early spring. At every cereal harvest, straw was removed from the fields. After spinach harvests in autumn, a quantity of 30–64 kg N ha^{-1} in aboveground crop residues remained on the field, with a C/N ratio ranging roughly between 6–9. All trials were performed in a randomized complete block design with three replications. Plot size varied from 192 to 346 m^{-2} depending on the working width of the agricultural machinery used at each site.

Table 1. Trial periods and soil parameters of the experimental sites as well as details on crop rotations and chemical characteristics of the spinach crop residues.

		Trial 1 (10 September 2019–6 March 2020)	Trial 2 (5 October 2020–1 March 2021)	Trial 3 (9 October 2018–13 March 2019)	Trial 4 (10 October 2019–6 March 2020)
Soil parameters (0–30 cm)	Sand [% (*w*/*w*)]	80.5	82.4	80.2	87.3
	Silt [% (*w*/*w*)]	12.1	11.3	13.1	06.8
	Clay [% (*w*/*w*)]	07.5	06.3	06.6	05.8
	Organic C [% (*w*/*w*)]	01.1	01.5	01.2	03.6
	C/N ratio	15.7	12.1	09.2	25.7
	Soil pH	06.0	05.6	05.7	05.2
Crop rotation details	Crop rotation	Spinach/Spinach	Triticale/Spinach	Barley/Spinach	Carrots/Spinach
	Liquid manure [kg N ha^{-1}]	0	170	170	170
	Mineral fertilization [kg N ha^{-1}]	162	126	101	122
	Marketable yield autumn-grown spinach [t ha^{-1}]	17.8	20.2	7.3	17.8
Aboveground crop residues	Total N [kg ha^{-1}]	64	30	44	45
	N content [% (*w*/*w*)]	5.0	4.0	3.7	5.5
	C/N ratio	6.6	9.0	9.0	5.9

2.2. Treatments

Within the four field trials conducted, different tillage depths and dates were investigated with the aim of reducing N losses after growing spinach in the autumn (Table 2). Harrowing (10 cm) and/or plowing (30 cm) immediately after spinach harvest and the subsequent drilling of a catch or cash crop are the standard procedures used for spinach crop residue management in the Borken region (treatments 1 and 3). A few days after tillage, the winter catch crop was sown by drilling into the upper 3–4 cm of soil. In treatment 4, direct drilling was conducted and the soil surface remained untreated for 11–17 days after spinach harvest. In treatments 5 and 6, tillage and subsequent drilling were postponed until the soil temperature dropped below 5 °C at a 5 cm depth. In treatment 7, no tillage or drilling was performed until the trials were completed in March.

Table 2. Tillage and nitrification inhibitor treatments (trt.) as well as subsequent catch crops sowing (drilling) dates after growing spinach in the autumn.

Trt.	Tillage Depth [cm] (Tillage Implement)	Tillage Season	Nitrification Inhibitor	Catch Crop Sowing Dates			
				Trial 1 (Harvest: 10 September 2019)	Trial 2 (Harvest: 5 October 2020)	Trial 3 (Harvest: 9 October 2018)	Trial 4 (Harvest: 10 October 2019)
1.	10 (Harrow)	Early autumn	n.a.	16 September 2019	16 October 2020	13 October 2018	19 October 2019
2.	10 (Harrow)	Early autumn	DMPP [1]	16 September 2019	16 October 2020	n.a.	19 October 2019
3.	30 (Plow + harrow)	Early autumn	n.a.	n.a.	n.a.	13 October 2018	n.a.
4.	3–4 (Direct drilling)	Early autumn	n.a.	n.a.	16 October 2020	26 October 2018	n.a.
5.	10 (Harrow)	Late autumn	n.a.	2 December 2019	n.a.	23 November 2018	16 November 2019
6.	10 (Harrow)	Late autumn	DMPP [1]	n.a.	n.a.	n.a.	16 November 2019
7.	n.a. [2]	Early spring [2]	n.a.	n.a.	n.a.	n.a.	n.a.

[1] 3,4-Dimethylpyrazole phosphate (3.0 L ha^{-1} VIZURA®); [2] Tillage after the trials have been completed in March; n.a. = not applicable.

In order to inhibit nitrification, 3,4-Dimethylpyrazole phosphate (DMPP) was applied before harrowing in early or late autumn in treatments 2 and 6, respectively. A total of 3.0 L ha^{-1} VIZURA® (SE BASF, Ludwigshafen, Germany) mixed with 0.1 L ha^{-1} nonionic organosilicon spray-adjuvant Break-Thru® S 240 (AlzChem Group AG, Trostberg, Germany) and diluted in 500 L ha^{-1} water was sprayed directly onto the crop residues. The inhibitor was applied in cloudy weather or before sunrise, no more than 3 h before harrowing.

Different catch crops were sown by drilling in treatments 1–6. In trial 1, a mixture of oil radish (*Raphanus sativus*; taxonomy ID: 3726), mustard (*Sinapis alba*; taxonomy ID: 3728), and rye (*Secale cereale*; taxonomy ID: 4550) was sown in mid-September (treatments 1–4). After the later tillage date, triticale (×*Triticosecale*; taxonomy ID: 49317) was sown (treatment 5). However, triticale seeds failed to germinate in late autumn, resulting in a bare soil during winter. In trials 2 and 3, after both the early and late tillage date, a mixture of 70% (*w*/*w*) rye and 30% (*w*/*w*) grass (*Lolium perenne*; taxonomy ID: 4522) was sown. In trial 4, triticale was sown after both tillage dates in October and November. No drilling was performed in treatment 7, i.e., the spinach crop residues were left intact and thus, were able to re-sprout.

2.3. Data Collection and Measurements

Soil and air temperatures were recorded by a nearby weather station (Borken Westphalia, Deutscher Wetterdienst, Germany). Precipitation was measured at the experimental sites using Hellmann gauges similar to those described by Hoffmann et al. [51]. The soil N_{min} concentration [ammonium (NH_4^+) + NO_3^-; 0.0125 M $CaCl_2$] in the soil layers 0–30, 30–60, and 60–90 cm was determined with the obtained soil samples, using a Pürckhauer auger. The soil-sampling procedure and laboratory analyses of soil N_{min}, soil total C, soil pH as well as the soil texture were based on the guidelines of the Association of German Agricultural Analytic and Research Institutes [49]. Soil total N content was analyzed according to DIN EN 16168:2012 [52].

The net mineralization of soil organic N and crop residues in the upper soil layer was estimated via in situ covered soil columns similar to those described by Heumann and Böttcher [53]. Columns (polyethylene) with a diameter of 20 cm and a length of 35 cm were driven vertically into the topsoil to a depth of 30 cm. On the day of drilling, 3–6 columns per treatment were installed using a random design and thus the amount of crop residue inside the columns was variable. However, when columns were installed without previous soil perturbations (treatments 5–7), columns were inserted between the rows, meaning that there were no plants inside the columns. After installation, in all treatments, the columns were loosely covered with a sun-reflecting lid, which permits the exchange of gas as well as the prevention of water logging and NO_3^- leaching losses. Soil temperature in a 2 cm soil depth varied by ±2.5 °C from the soil temperature in the adjacent open field. In order to derive the net N mineralization, the initial N_{min} in 0–30 cm of soil after spinach harvest was subtracted from the final concentration measured in the columns at the end of the field experiments in March. In treatments 5 and 6, the tillage was postponed from early to late autumn. Therefore, the columns were installed twice. A first installation took place soon after spinach harvest in autumn without previous soil preparations and a reinstallation was carried out after the postponed tillage and drilling in late autumn at another position in the plot. At this time, the N_{min} concentration in the soil columns was also measured and was taken into account in the calculation of the net mineralization.

The total aboveground crop residues were determined at spinach harvest as well as at the postponed tillage dates in late autumn (treatments 5 and 6). In early spring, the total aboveground biomass (including herbs) was determined in all treatments. For this purpose, a bulk sample of four 0.25 m^{-2} subsamples was collected in each treatment. Plants were cut at the soil surface and stored for one day in a fridge at 4 °C. In the laboratory, the plant material was rinsed with tap water, spin-dried and weighed. The material was then freeze-dried (P22K-E-6, Dieter Piatkowski Forschungsgeräte, Munich, Germany) and ground in an ultra-centrifugal mill (model ZM 200, RETSCH GmbH, Haan, Germany) to a particle size of less than 0.5 mm. The dry mass was used to analyze total N by combustion in an oxygen atmosphere according to Dumas (Leco FP-628, LECO Instrumente GmbH, Mönchengladbach, Germany) and total C (ELTRA CS 500, ELTRA GmbH, Haan, Germany) according to DIN EN 15936:2012 [54].

The DMPP content in soil was determined by taking soil samples in the 0–15 and 15–30 cm soil layers (treatments 2 and 6). Treatments 1 and 5 were used as non-treated controls. The first samples were taken immediately after DMPP application and harrowing. Subsequently, samples were frozen at −18 °C. The extraction procedure and analysis methods were performed as described by Doran et al. [55]. Deviating from this description, 15 g of soil was extracted and evaporated to a final volume of 200 µL methanol. With this procedure, a limit of 5 µg DMPP (kg soil)$^{-1}$ was reached with an extraction efficiency of 95%.

In order to estimate the risk of plant damages to a succeeding spinach crop due to disease infection, a pathogenicity test was conducted. For this purpose, in each treatment a bulk sample of >2 kg soil (0–20 cm) was taken at the end of trials 1, 2, and 4 in March 2019 and 2020. Soil samples were filled into pots (n = 4) and placed into a greenhouse. Afterwards, 15 spinach seeds were sown on the soil surface. A soil originating from a virgin field, where spinach had never been grown before, was used as a control. Three weeks after sowing, the disease severity index was calculated according to Larsson and Gerhardson [56]. For this purpose, the amount of damaged tissue in the range from 0%, without symptoms, to 100%, dead plants, was assessed visually for every single plant.

2.4. Nitrogen Balance Sheet Calculations

The potential N losses were estimated by using a balance sheet approach. The supply side consists of the N_{min} concentration (0–90 cm) at spinach harvest and the net N mineralization (0–30 cm) measured within the soil columns. To derive the potential N losses, the N taken up by the catch crops (treatments 1–6) or the resprouting spinach plants (treatment 7) as well as the final N_{min} concentration (0–90 cm) in March were subtracted from N supply by mineralization and the initial N_{min} at spinach harvest. Nitrogen fixation and N depositions were not taken into account, but the potential N losses between treatments could still be estimated, given that every trial was analyzed individually. Furthermore, symbiotic N fixation can be neglected in non-legume crops [57]. The different potential N losses (types of gaseous and leaching losses) were not measured directly and therefore, only the lumped N loss was calculated in the balance sheet approach.

To calculate the total N uptake, the aboveground as well as belowground N was considered. The root-N was derived from the measured aboveground biomass-N and the root-N/shoot-N ratio based on the literature data. The root N of spinach plants at harvest was assumed to be 20% of the aboveground plant N [18,58,59]. In order to obtain the root

N of the spinach crop residues at later tillage dates (late autumn and early spring), a ratio of 2 between aboveground and belowground N was assumed. The cereal and grass plants were at the tillering stage in early March, for which an equal distribution of aboveground and belowground N is described [60–63]. In contrast, the mixture of radish, mustard, and rye, grown in trial 1, was found to be at an advanced development stage in early March. For this mixture, a shoot-N to root-N ratio of 2 was assumed [61,63,64].

2.5. Statistical Analysis

The potential N losses were statistically analyzed within each individual trial using a one-way ANOVA followed by Tukey's post hoc test (alpha < 0.05). Beforehand, assumptions of normality and homogeneity of variances were tested according to the Kolmogorov–Smirnov test and the Fmax test, respectively. If needed, data were transformed logarithmically to meet the requirements of the ANOVA. All statistical calculations were performed using SPSS, version 26 (IBM Deutschland GmbH, Ehningen, Germany).

3. Results

3.1. Soil Temperature and Precipitation

From mid-September to mid-October, the temperature at a depth of 5 cm soil was between 10–20 °C (Figure 1). In November, the temperature dropped below 5 °C for the first time. During winter, soil temperature remained between 0 and 10 °C. Only in trial 3 (2018/19) was a temporary drop below 0 °C observed. Compared to the 30-year average mean, the air temperature was about 1.0 °C higher during the three trial periods.

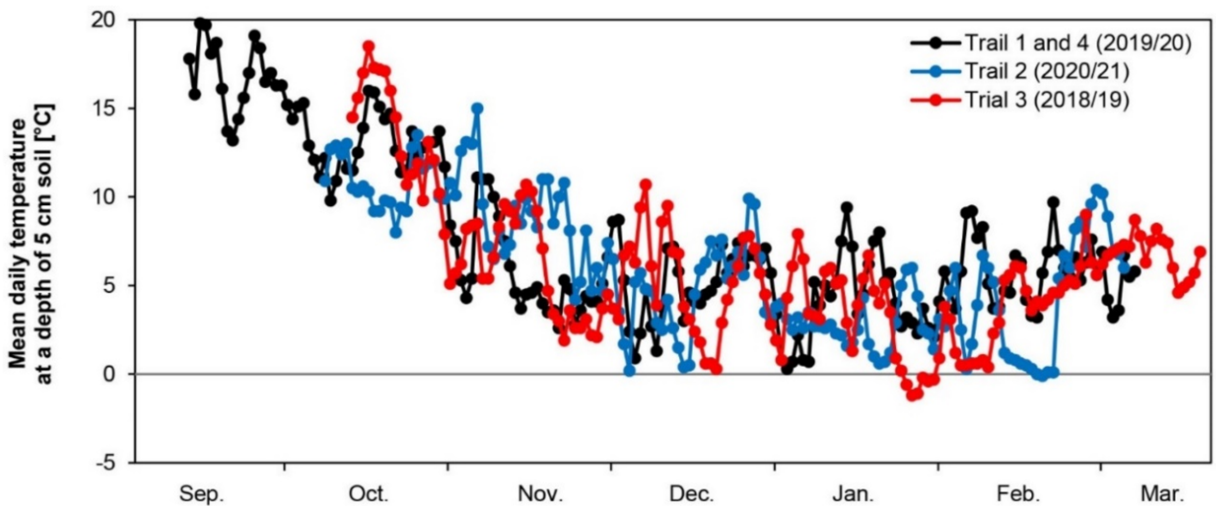

Figure 1. Mean daily temperature at a depth of 5 cm soil from spinach harvest until completion of the trials in March (weather station Borken-Westphalia, Deutscher Wetterdienst, Germany).

From October until the completion of trials in early or mid-March, the total precipitation was 349–384 L m^{-2} (Figure 2). Trial 1 was set up a few weeks earlier than the other trials, resulting in a total precipitation of 502 L m^{-2}. Autumn in 2018 (trial 3) was much drier than autumn in 2019 and 2020. Based on visual observations during the soil sampling at the start of each trial, the soil below 30 cm was always drier than the upper soil layer. As a result of the dry autumn in 2018, it took until December until the soil was moistened below 30 cm. In the other years, leaching water reached the 30–60 cm layer by October.

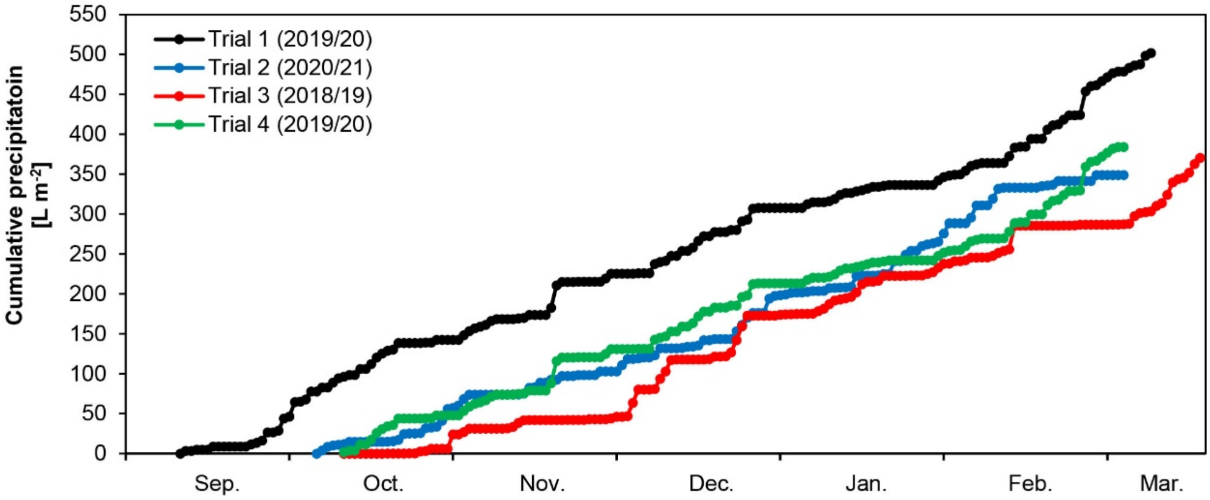

Figure 2. Cumulative precipitation from spinach harvest until completion of the trials in March (weather station Borken-Westphalia, Deutscher Wetterdienst, Germany).

3.2. Effects of the Maximum Tillage Depth and Frequency

Nitrate was the predominant mineral N form during the autumn and winter season. No NH_4^+ was detected in treatments 1, 3–5, and 7. Therefore, in Figures 3–5 only the NO_3^--N concentration is provided.

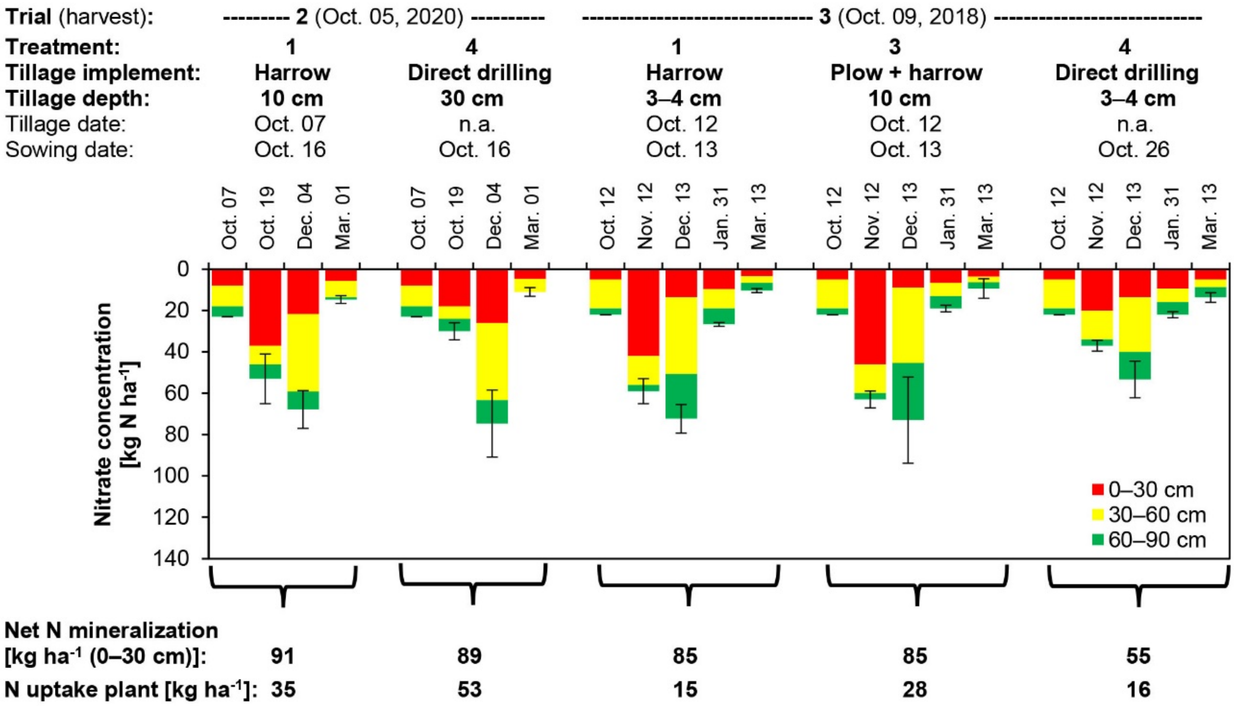

Figure 3. Soil mineral N concentration in the upper soil layers (0–30, 30–60, 60–90 cm) during autumn and winter seasons as well as net N mineralization (0–30 cm) and total N uptake by plants as a function of tillage depth after spinach harvest (n = 3; Mean ± SD). n.a. = not applicable.

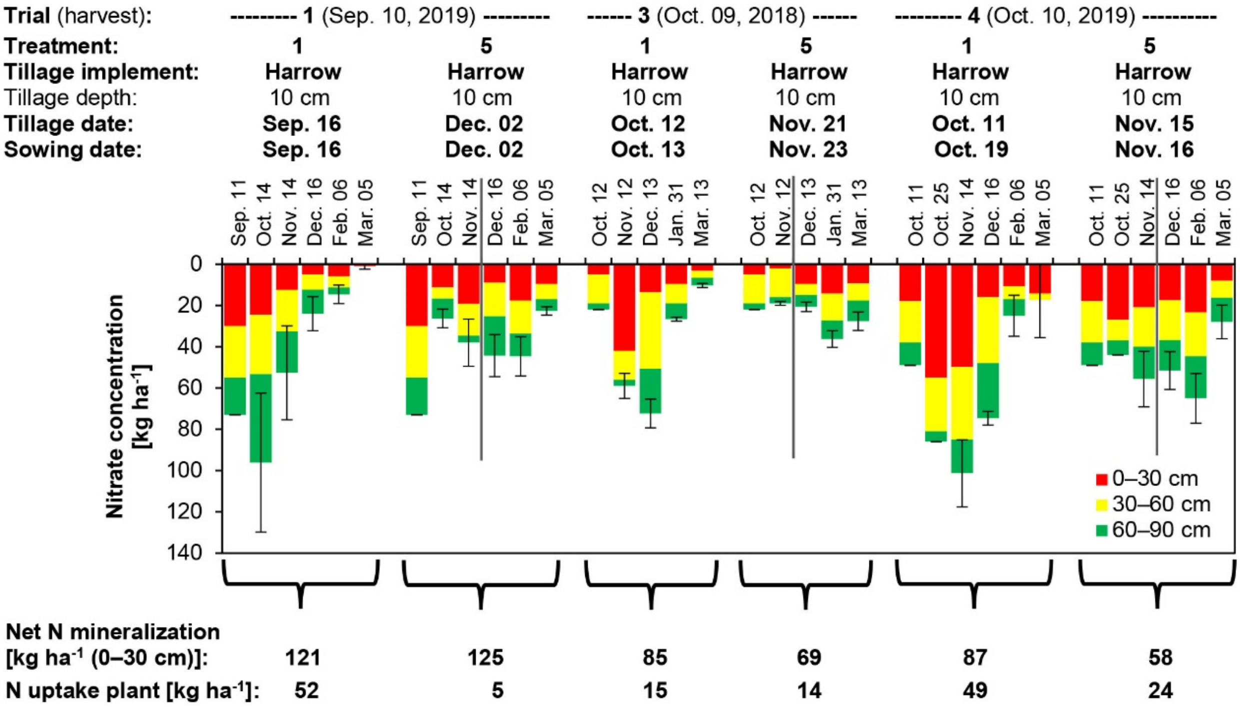

Figure 4. Soil mineral N concentration in the upper soil layers (0–30, 30–60, 60–90 cm) during autumn and winter seasons as well as net N mineralization (0–30 cm) and total N uptake by plants depending on the tillage date after spinach harvest. The gray vertical lines indicate the dates of the postponed tillage ($n = 3$; Mean ± SD). n.a. = not applicable.

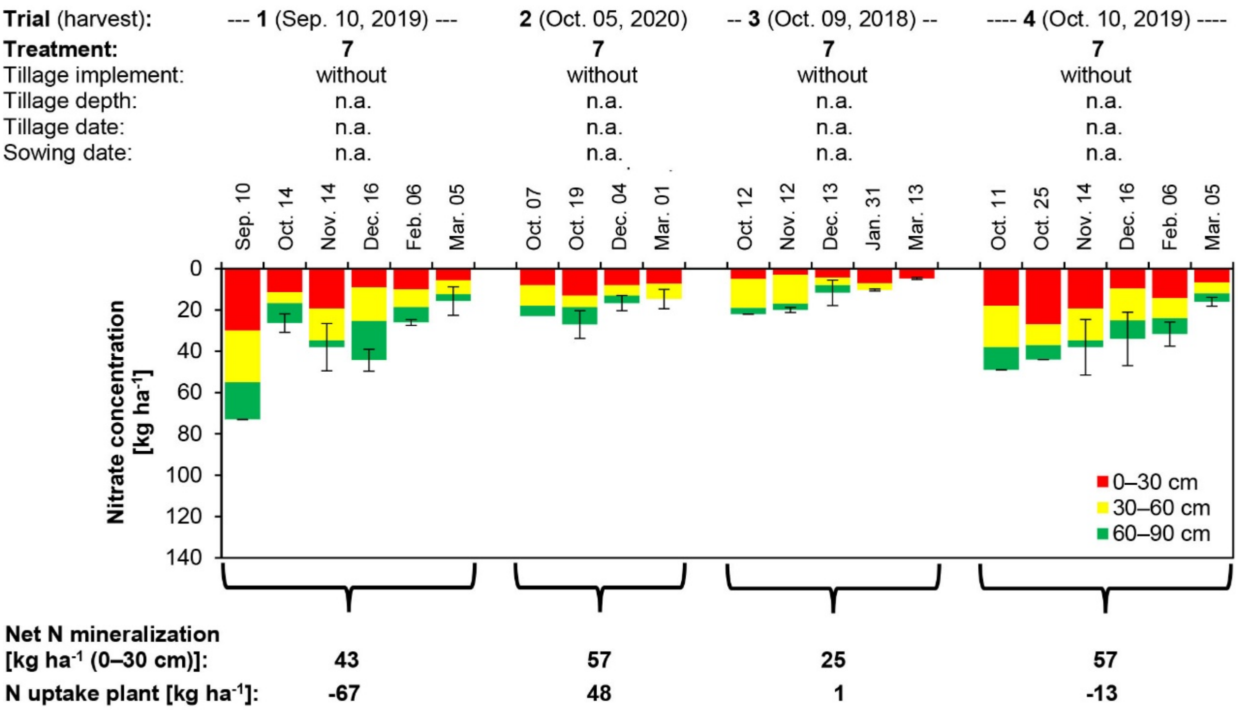

Figure 5. Soil mineral N concentration in the upper soil layers (0–30, 30–60, 60–90 cm) during autumn and winter seasons as well as net N mineralization (0–30 cm) and total N uptake by plants after spinach harvest without tillage until trials were completed in March ($n = 3$; Mean ± SD). n.a. = not applicable.

Both the harrow (10 cm) and plow (30 cm) plus harrow treatments seemed to be very similar in N_{min} concentrations (trial 3) (Figure 3). Even after direct drilling (3–4 cm) in trial 2, the peak NO_3^- concentration was at the same level as observed after harrowing. In contrast, in trial 3, the peak N_{min} concentration in the direct drilling treatment was lower compared to the harrow or plow plus harrow treatment. Finally, the N_{min} concentration in the upper 90 cm of soil dropped to a maximum of 15 kg ha^{-1} by early or mid-March, irrespective of the tillage depth.

The potential N losses after plowing the crop residues into 30 cm were slightly lower compared to harrowing into 0–10 cm soil depth (Table 3). According to the N balance sheet, this difference is due to a higher N uptake of the rye/grass mixture after plowing in autumn 2018 (Figure 3). The direct drilling (treatment 4) also resulted in a significant decrease in potential N losses. This was the result of a comparably lower mineralization or increased N uptake in trials 3 and 2, respectively. The higher N uptake after direct drilling was reflected by irregularly resprouting spinach plants, which increased averaged N uptake per plot. Furthermore, it should be noted that the first soil perturbation in treatment 4 was delayed by 9 to 14 days compared to treatments 1 and 3. This delay might also have affected N losses, as described below (Section 3.3).

Table 3. Potential N losses according to the N balance sheet [N_{min} (0–90 cm) at spinach harvest + net mineralization (0–30 cm) − total N uptake by plants − N_{min} (0–90 cm) in March] depending on the maximum tillage depth and tillage season. Means within the same trial that do not share a letter are significantly different according to Tukey's post hoc test (alpha < 0.05, *n* = 3).

Treatment	Tillage Implement	Tillage Depth [cm]	Tillage Season	Potential N Loss [kg ha^{-1}]			
				Trial 1	Trial 2	Trial 3	Trial 4
1.	Harrow	10	Early autumn	141 a	64 b	81 c	70 ab
3.	Plow + harrow	30	Early autumn	n.a.	n.a.	70 b	n.a.
4.	Direct drilling	3–4	Early autumn	n.a.	48 ab	48 a	n.a.
5.	Harrow	10	Late autumn	170 b	n.a.	49 a	55 a
7.	Without	n.a.	Early spring [1]	167 b	17 a	20 a	103 b

[1] Tillage after the trials have been completed in March; n.a. = not available.

3.3. Effects of the Season of Tillage

Postponing tillage from early to late autumn (Figure 4) or early spring (Figure 5) was effective in reducing the NO_3^- concentration that was exposed to leaching, but the N balance sheet was affected differently depending on the individual site and year (Table 3). In trials 3 and 4, the calculated N losses were reduced by 15–32 kg ha^{-1} when tillage was postponed from early to late autumn. This was mostly due to a lower net mineralization as well as a higher N_{min} concentration in March. However, biomass growth was diminished after late sowings, leading to a minor N uptake during winter. This was reflected by reduced soil cover in early spring compared to sowing soon after spinach harvest (Figure 6). Triticale, sown on 2 December 2019 (trial 1), completely failed to germinate, leading to a bare soil surface during winter. Weeds took up only 5 kg N ha^{-1} in this treatment and N mineralization was rather high. Consequently, potential N losses increased by 29 kg ha^{-1} compared to early tillage in mid-September.

By postponing tillage from early autumn to early spring, spinach stubbles were able to continue to grow (Figure 7). Nitrogen uptake by spinach and a low net mineralization reduced the potential N losses by 47 or 61 kg ha^{-1} in trials 2 and 3, respectively (Table 3). In contrast, in 2019/20 (trials 1 and 4) N losses were increased by up to 33 kg ha^{-1} due to postponing tillage from autumn to spring. In these trials, spinach biomass decomposed partially or fully during autumn and winter, resulting in lower biomass N in spring compared to autumn. This is shown by the negative N uptake in trials 1 and 4 (Figure 5). This means that the amount of N in the spinach plants decreased from spinach harvest in autumn until the trials were completed in early spring.

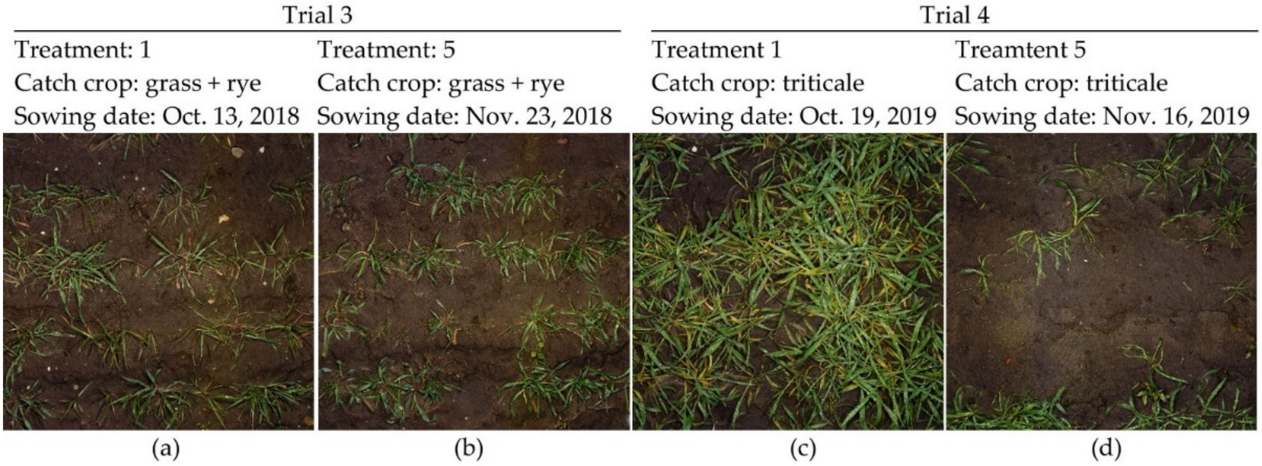

Figure 6. Aboveground biomass at the end of February depending on the catch crop species grass + rye (**a**,**b**) and triticale (**c**,**d**) as well as the sowing date in early (**a**,**c**) and late (**b**,**d**) autumn.

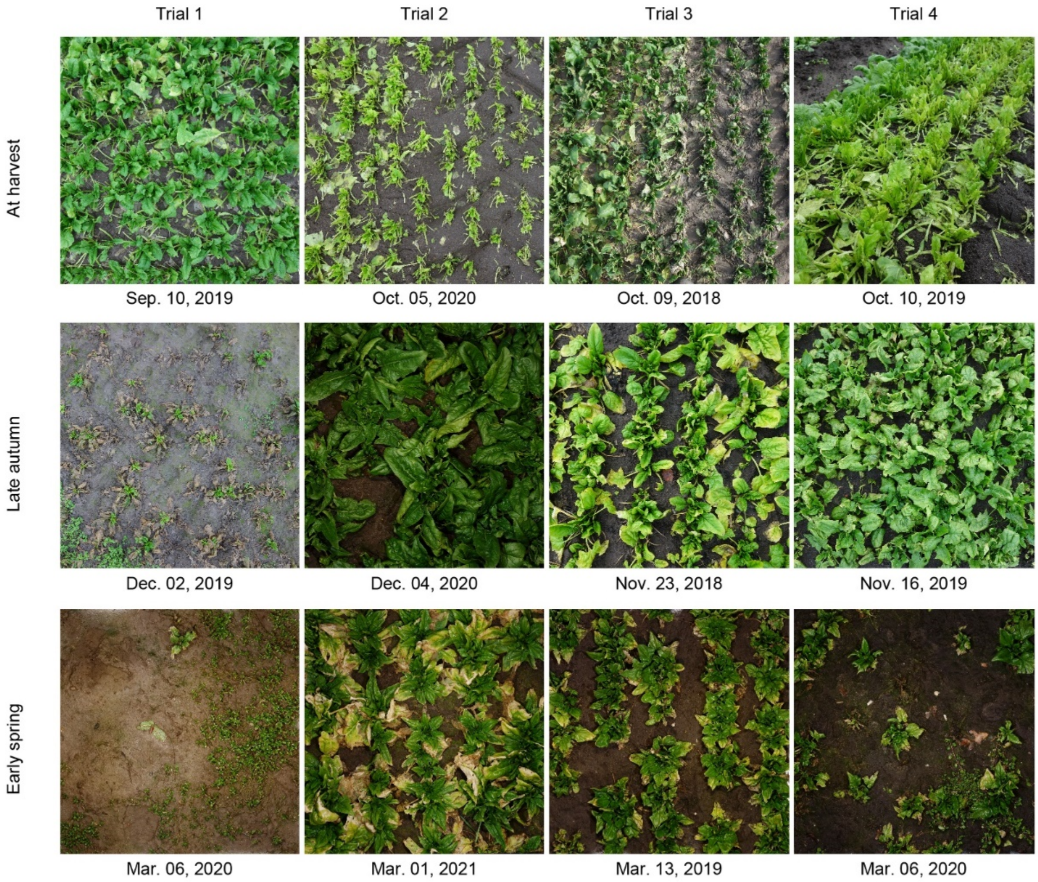

Figure 7. Spinach crop residues in treatment 7 depending on the season and trial.

Besides the growth of the catch crops and resprouting spinach crop residues, the disease severity index of spinach based on soil samples taken at the end of experiments 1, 2, and 4 was also calculated (Table 4). However, based on visual evaluations, no differences between the treatments was observed. Spinach seemed to be more affected by the individual site than by the previous tillage treatment.

Table 4. Disease severity index of spinach seedlings at the end of the trials (n = 4; Mean ± SD).

Treatment	Tillage Implement	Tillage Depth [cm]	Tillage Season	Disease Severity Index [%]		
				Trial 1	Trial 2	Trial 4
1.	Harrow	10	Early autumn	88 ± 8	57 ± 14	72 ± 10
3.	Plow + harrow	30	Early autumn	n.a.	n.a.	n.a.
4.	Direct drilling	3–4	Early autumn	n.a.	50 ± 6	n.a.
5.	Harrow	10	Late autumn	72 ± 13	n.a.	64 ± 4
7.	Without	n.a.	Early spring [1]	74 ± 6	40 ± 3	67 ± 9

[1] Tillage after the trials were completed in March; n.a. = not available.

3.4. Effects of the Nitrification Inhibitor DMPP

Ammonium was detectable for a maximum of 4 weeks after DMPP application in treatments 2 and 6 irrespective of the season of application (Tables S1 and S2). In contrast, no NH_4^+ was detectable in the other treatments without DMPP application. However, in treatments 2 and 6, the NH_4^+ concentration was consistently below 7 kg N ha^{-1} (0–30 cm). This means that there was still a high NO_3^-/NH_4^+ ratio after DMPP application. Consequently, no delay in NO_3^- leaching below 30 cm of soil was observed compared to the corresponding treatments 1 and 5 (Figure 4; Tables S1 and S2).

After DMPP application to crop residues and its subsequent incorporation into a layer of 10 cm soil, a DMPP content of 400 µg (kg soil)$^{-1}$ was assumed. Based on the sampling depth of 15 cm, this concentration was 260 µg DMPP (kg soil)$^{-1}$. In trials 1 and 2 and at the later tillage date in trial 4, less than half of the applied quantity was detectable immediately after application and harrowing (Figure 8 and Figure S1). By contrast, in trial 4, more than 300 µg DMPP (kg soil)$^{-1}$ was detected when applied immediately after spinach harvest. Within the first month after the DMPP application, a small quantity of the active ingredient leached into the 15–30 cm soil layer in trial 1, but no such leaching was observed in the other trials. Within the following weeks, the DMPP content decreased in all trials. By March, the content dropped below the detection limit of 5 µg (kg soil)$^{-1}$ DMPP.

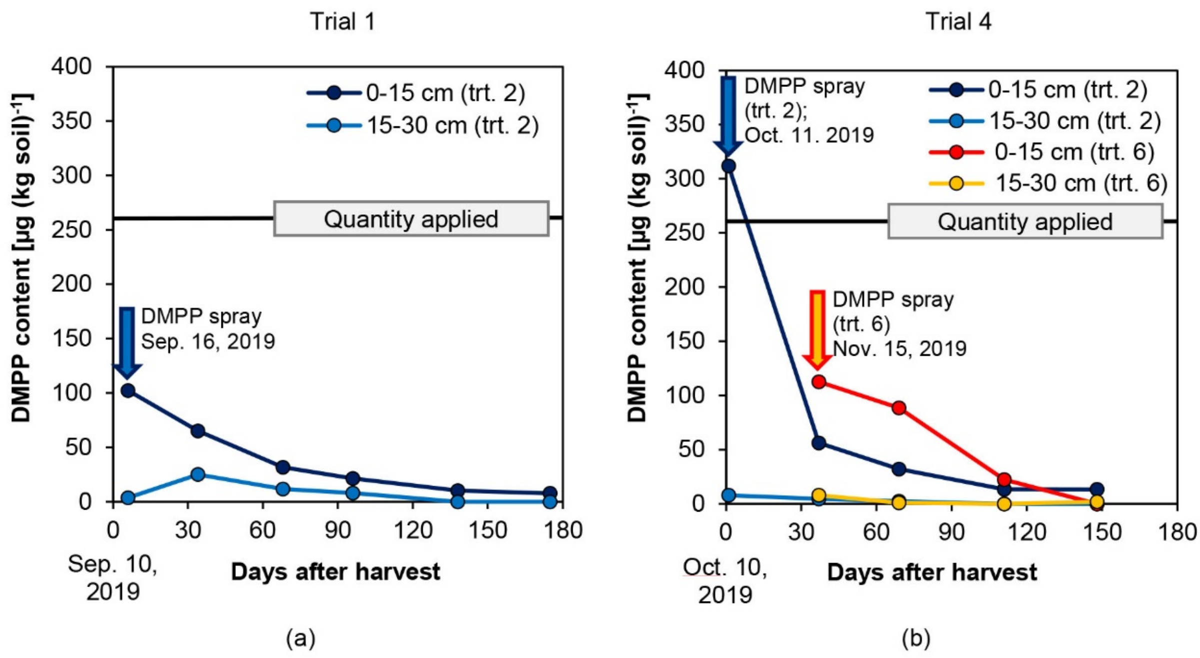

Figure 8. Content of the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) within the upper soil layers (0–15 and 15–30 cm) after application to the spinach crop residues and subsequent harrowing into the soil in (**a**) trial 1 and (**b**) trial 4 (n = 1). Trt = Treatment.

4. Discussion

At spinach harvest, 5 to 30 kg N ha^{-1} remained in the upper 30 cm of soil (Figures 3–5). D'Haene et al. [59] measured comparable N_{min} residues at spinach harvest as long as total N fertilization corresponded to actual plant demand. The authors derived a minimum N_{min} residue of 7 kg ha^{-1} (0–30 cm) at a marketable yield of 25 t ha^{-1}. However, when spinach is harvested at an earlier stage, this leads to higher N_{min} residues even if total N supply corresponds to plant demand [10].

Aboveground crop residues contained 30–64 kg N ha^{-1} (Table 1), similar to levels of 25–62 kg N ha^{-1} reported earlier [31,35,65,66]. The C/N ratio of spinach residues was in a range of roughly 6–9 (Table 1). This range was also provided by Agneessens et al. [44] and Whitmore [22]. After incorporating the aboveground autumn-grown spinach crop residues characterized by a C/N ratio of 9.6, De Neve et al. [23] detected a net mineralization of approximately 45% of the plant biomass-N within two weeks. In contrast to the aboveground residues, the root biomass of most vegetable crops was characterized by a higher C/N ratio as well as a higher lignin content, leading to a reduced net mineralization or even its immobilization after its incorporation [67,68]. Therefore, by considering the root mass, the net mineralization of total plant debris during autumn and winter can be expected to be lower than the reference data that is based on only aboveground crop residues. Nevertheless, between 85 and 121 kg N ha^{-1} (0–30 cm) were mineralized following tillage soon after harvest until early spring (Figures 3 and 4). This is also reflected by the peak N_{min} concentrations of 53–101 kg ha^{-1} (0–90 cm) within the first two months after harvest. The presence of high N_{min} concentrations after a growing season of spinach are in line with other studies [25–31]. Based on the N content of the crop residues, post-harvest N losses can only partially be explained by the residual fertilizer N and mineralization of plant debris. In addition, the mineralization of native soil organic N must be considered in this context [9]. The total amount of N mineralization from native soil organic matter with a similar texture is mainly determined by the combination of organic C content and the C/N ratio, i.e., the total N content [17]. However, the recalcitrance of the organic matter also plays a role, in particular in soils with a non-agricultural history. In sandy soils that were part of forests at least 100 years before the introduction of arable cultivation, the biochemical resistance against N mineralization is often enhanced compared to historically arable soils, even though their organic N and C content is high [69,70]. This was probably also the case in trial 4, which was conducted on a field that was turned from forest into arable cultivation in the 1950s, and in which the high organic C and N content did not lead to an excessive N mineralization (Table 1; Figure 4).

4.1. Effects of the Tillage Depth and Frequency on Potential N Losses

In common practice, spinach crop residues are incorporated soon after harvest by plowing (30 cm) and/or harrowing (10 cm). Here, we observed similar N_{min} concentrations for both tillage depths (Figure 3). In contrast, the potential losses calculated by using the N balance sheet were reduced following a tillage depth of 30 cm (Table 3). It is possible that in the dry autumn of 2018 (Figure 2), germination and subsequent N uptake of the catch crop increased by mixing in more humid layers from a depth of 30 cm into the top centimeters of soil by plowing. In contrast, after harrowing, the upper soil remained dry until December, which delayed the germination of the grass/rye mixture. The reduced mineral N concentration and potential N losses after direct drilling with a shallow tillage depth of 3–4 cm (Figure 3, Table 3) can be attributed to two factors. Firstly, direct drilling was performed 9–14 days later than tillage in treatments 1 and 3, which allowed for the spinach plants to continue growing after harvest. Secondly, after direct drilling, an irregular resprouting of the spinach plants was observed in trial 2, further increasing the total N uptake in these plots and thus decreasing the potential N losses. Overall, N_{min} concentrations and potential N losses appeared to be affected by weather conditions and the date of first tillage rather than by the tillage depth and frequency.

According to van den Bossche et al. [34] a reduced tillage needs to be continued for many years to affect annual NO_3^- leaching losses. Furthermore, due to the less stable soil aggregates, mineralization in sandy textured soils is less affected by tillage practices compared to loamy textured soils [71,72]. After the mixing (rotary-tillage), plowing, or mulching of cauliflower residues, an almost uniform N_{min} concentration increase was observed in loamy sand. In contrast, in heavier textured soils, mineralization after mulching was reduced compared to mixing or plowing [73]. This means that NO_3^- leaching after harvest seemed to be independent of post-harvest tillage intensity in sandy soils.

4.2. Effects of the Tillage Season on Potential N Losses

By postponing the tillage date, the N_{min} concentration remained at a constant level or decreased within the first weeks following harvest (Figures 4 and 5). Even after tillage in late autumn when the soil temperature temporarily dropped below 5 °C, the N_{min} concentration remained constant (treatments 5 and 6). In general, at soil temperatures below 10 °C mineralization and the nitrification of native soil organic N, vegetable crop residues, and catch crops were found to be strongly reduced [39,40,74]. However, the temperature-dependence of N mineralization is affected by the degradability of the plant material. For easily degradable plant material, 20–40% of the biomass-N can be nitrified within 5–10 weeks after incorporation even at temperatures below 5 °C [75,76]. Furthermore, the N turnover rate can be increased, especially at fluctuating temperatures compared to constant incubation temperatures [74]. This was confirmed by a high N mineralization and nitrification after tillage of spinach crop residues in late autumn, (Figure 4). In contrast, without tillage (treatment 7) N mineralization was much lower (Figure 5). Consequently, based on the low N_{min} concentration after late tillage, high N losses occurred during the winter season. Therefore, the incorporation of easily decomposable crop residues high in N should be postponed until spring to minimize the risk of, e.g., NO_3^- leaching during the winter season [37,74,75]. However, this strategy highly depends on the N uptake capacity and growth performance of the spinach crop residues, as discussed below.

In order to compare the overall N losses, the N balance sheet was calculated. According to the N balance sheet, postponing the tillage date to late autumn or early spring resulted in either reduced (trials 2 and 3) or increased (trials 1 and 4) potential N losses (Table 3). These contrasting results were due to the N_{min} residue at spinach harvest, the net mineralization during autumn and winter, and the growth performance of the resprouting spinach plants (Figures 4 and 5). Resprouting crop residues can reduce NO_3^- losses considerably, especially at high precipitation in autumn by conserving N in the plant biomass, and thus effectively acting as a catch crop [8,77,78]. In trials 2 and 3, spinach crop residues successfully acted as a catch crop, resulting in low potential N losses (Figure 7; Table 3). By contrast, in trial 1, the wet and cold weather led to a complete dying off by December, resulting in considerable N losses even without tillage. In field trials of Myrbeck et al. [79] the degradation of the plant biomass by the application of herbicides affected the N_{min} concentration in a similar way to tillage. However, even without frost kill, catch crops can lose N during the winter season [80–82]. Therefore, the removal of the aboveground crop residues before its decomposition begins is considered an effective measure to reduce winter N losses [83]. However, removing crop residues in the winter season may lead to soil compaction. In addition, collecting and processing these residues, e.g., by composting or digestion, is laborious and costly, thus limiting the practical potential of these options [24].

Besides leaching, N can also be lost from the soil–plant system via the gaseous emission of nitrous oxide (N_2O) and dinitrogen (N_2) and it can also be volatilized as ammonia (NH_3). For example, up to 15% of the biomass N of *Brassica* species, sugar beet, or leek crop residues was lost by N_2O and N_2 during winter after incorporation into sandy soils [84,85]. However, Whitmore [22] calculated that after the incorporation of spinach leaves in August or September, N_2O losses due to denitrification were negligible compared to losses from *Brassica* or leek crop residues, despite their equally low C/N ratio. In general, gaseous N emissions are subject to considerable variability. Even after the cultivation of similar crops (cauliflower, broccoli) at the same site, with a similar crop residues management, the emission factor for N_2O varied between 1.3% and 7.7% of the applied crop residues N [86,87]. Most of this variation is due to the actual soil moisture content, as well as the N and C fractions in the soil [88–90]. However, differences due to tillage practice seemed to be insignificant following the incorporation of cauliflower or lettuce crop residues into sandy soils [73,91]. In contrast, the NH_3 volatilization of crop residues is often considerably reduced by their incorporation into the soil before their decomposition begins [5,73,92]. However, if plant biomass decays on the soil surface, up to 5–16% of plant-N can be volatilized during winter [92]. Based on these observations, in the no-till treatment (treatment 7) the comparable high N losses in trials 1 and 4 might be partially explained by NH_3 volatilization during the decay of the spinach crop residues (Table 3).

By using the soil columns, the net mineralization of the crop residues as well as soil organic N was determined. However, in treatment 7, the columns were inserted between the rows. Thus, no crop residues were inside the columns and the mineralization resulting from their decay in trials 1 and 4 was not detectable using this approach. Therefore, the calculated negative N uptake (Figure 5) was assumed to be lost from the upper 90 cm of soil. However, a certain part of this N was probably still bound in the soil in different organic N fractions. For a better assessment of the actual N losses, soil N turnover in arable soils should be measured over several years [93].

The total N uptake of the catch crops depended highly on the sowing date (Figures 3–5). At sowing dates in early autumn, N uptake until March ranged between 15 and 53 kg ha^{-1}. Sowing in late autumn reduced the catch crop N uptake to 5–15 kg ha^{-1}. However, both sowing dates were insufficient to compensate for the high soil NO_3^- concentration (Figure 4). The negligibly small N uptake of late-sown catch crops as compared to a fallow control has been reported a number of times [63,78,94,95]. Beside the sowing date, the variability of N uptake was also due to varying growth conditions within the first weeks after sowing. For example, emergence in trial 3 was delayed due to dry soil conditions caused by minor precipitation during autumn (Figure 2), whereas the cloudy and very wet weather conditions in autumn 2019 may explain the reduced N uptake in trial 1. Generally, high precipitation rates and subsequent NO_3^- leaching within the first weeks after sowing reduce the effectiveness of catch crops in sandy soils [77]. This is true for even the deep rooting *Brassica* species [95]. Despite low N uptake, catch crops mixtures including winter hard species are recommended after late harvest dates to ensure a soil cover during winter [8], thus reducing the risk of erosion, weed growth, and the survival of obligate diseases [24,96]. Agneessens et al. [44] recommended the completion of the catch crop sowing by the end of August to ensure a sufficient N uptake after a spinach crop rotation. However, this implies a shorter growing season, which would reduce farmers' income considerably.

Beside N turnover, the crop residues management can also affect the population and activity of obligate plant diseases in the following growing seasons [19]. Therefore, spinach is usually only grown every four years on the same site in the region Borken [97]. However, as shown by the disease severity test, spinach was not affected by the tillage treatment compared to the control (treatment 1) (Table 4). Overall, the disease severity index was rather high, between 40% and 88%. Based on the results of Larsson and Gerhardson [98], comparable disease indices have been observed in cases where spinach was grown in monoculture. In contrast, when spinach was grown in rotation with other crops, the degree of plant damages was reduced. However, from the data provided in Table 4, no estimates can be made with regard to how long the cultivation of a spinach crop should be avoided depending on the treatment.

4.3. Effects of DMPP on Soil N Dynamics

In order to delay nitrification and subsequently NO_3^- leaching, DMPP was sprayed on crop residues immediately before tillage in treatments 2 and 6 in early and late autumn, respectively. As an equal distribution within the upper 10 cm of soil after harrowing was expected, a content of 0.40 mg (kg soil)$^{-1}$ DMPP was applied. Within the soil sampling layer of 15 cm, its content was 0.26 mg (kg soil)$^{-1}$ DMPP. However, the application of DMPP in early or late autumn delayed nitrification at best for only a few weeks (Tables S1 and S2). Similar observations were made in a glasshouse pot study at an air temperature of 16–24 °C after the application of 0.70 mg (kg soil)$^{-1}$ DMPP to cauliflower residues [99]. In contrast, in an incubation experiment, the application of 0.90–1.80 mg (kg soil)$^{-1}$ DMPP to cauliflower crop residues delayed nitrification for at least 95 days at a fluctuating soil temperature of 2–14 °C (mean: 7 °C) [45].

The effectiveness of a nitrification inhibitor depends on the immobilization and decomposition of its active ingredient by soil microorganisms as well as leaching and soil adsorption kinetics [100]. Likewise, in trial 1, some DMPP leaching at below 15 cm was observed (Figure 8a). However, most of the non-extractable DMPP was probably due to the adsorption, immobilization, and mineralization of the active ingredient. In general, the DMPP half-life ranged from a few days to several weeks within the upper centimeters of soil at 20–25 °C [101]. In addition, DMPP only has an inhibitory effect on ammonium oxidizing *Bacteria* rather than on ammonium oxidizing *Archaea* or comammox *Nitrospira* [102,103]. Especially at low soil pH, nitrification by *Archaea* can be considerable [104,105]. Thus, they can at least partially compensate for the reduced bacterial activity [106], and this may also have been the case here, given the low soil pH (Table 1).

Overall, a higher DMPP content in the bulk soil might be more effective in reducing the nitrification of both the crop residues as well as the soil organic N. This can be realized by a shallower tillage depth after DMPP application. However, based on research of Nett et al. [73], a higher NH_3 volatilization can also be expected by this approach. Therefore, further research is required to facilitate the efficient use of nitrification inhibitors to reduce N losses after the incorporation of vegetable crop residues.

5. Conclusions

This study aimed to determine whether the N losses during the off-season following autumn-grown spinach can be reduced by (a) flatter tillage depth, (b) postponing the tillage date from early to late autumn or early spring, or (c) the application of the nitrification inhibitor DMPP to crop residues. Averaged over the four field trials, postponing the tillage date from early autumn to spring seemed to be the most promising management option to reduce total N losses after growing spinach in the autumn. This strategy led to low net mineralization and allowed the spinach crop residues to resprout, effectively turning them into a catch crop. However, the N uptake of spinach and catch crops strongly depended on the actual weather conditions. The other two approaches, a shallow tillage depth or the application of DMPP, proved to be less effective in reducing N losses from spinach crop residues during autumn and winter.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12030653/s1, Figure S1: Content of the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) within the upper soil layers (0–15 and 15–30 cm) after application to the spinach crop residues and subsequent harrowing into the soil in trial 2 (n = 1). Trt = Treatment; Table S1: Soil ammonium N and nitrate N concentrations (0–30 cm) following application of 3,4-Dimethylpyrazole phosphate (DMPP) to spinach crop residues in trials 1 and 4 from autumn to early spring (n = 3); Table S2: Soil ammonium N and nitrate N concentration (0–30 cm) after application of the nitrification inhibitor 3,4-Dimethylpyrazole phosphate (DMPP) to spinach crop residues in trial 2 from autumn to early spring (n = 3).

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