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Reduction of nitrogen losses in vegetable production by optimization of the fertilization and crop residues management

Dissertation
Universität Osnabrück
Hochschule Osnabrück

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Dissertation
zur Erlangung des Doktorgrades (Dr. rer. nat.)

Universität Osnabrück
Fachbereich Kultur- und Sozialwissenschaften
Institut für Geographie

in Kooperation mit der Hochschule Osnabrück
Fakultät Agrarwissenschaften und Landschaftsarchitektur

vorgelegt von
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aus Aurich
am 08.08.2022, Osnabrück

Disputation
am 24.01.2023, Osnabrück

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Abbreviations

AAF	Amino acid fertilizer
ANOVA	Analysis of variance
BBCH	Einteilung des morphologischen Entwicklungsstadiums von Pflanzen (Biologische Bundesanstalt für Land- und Forstwirtschaft, Bundessortenamt und Chemische Industrie)
CO_2	Carbon dioxide
CEC	Cation exchange capacity
DMPP	3,4-dimethylpyrazole phosphate
H^+	Proton
KNS	Kulturbegleitende N_{min}-Sollwerte
N	Nitrogen
N_2	Dinitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
N_{min}	Soil mineral nitrogen
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_x	Nitrogen oxides
Trt.	Treatment
VDLUFA	Association of German Agricultural Analytic and Research Institutes

Chapter 1
General introduction

1.1 Background and objectives

Nitrogen (N) is often the most limiting element for plant growth in arable and horticulture production. Therefore, N fertilizers are applied to fulfil plant demands. However, N applied on arable fields is prone to getting lost via leaching, drainage, runoff, erosion, and gaseous emissions. In addition, these N losses in the forms of e.g. ammonium (NH_4^+), ammonia (NH_3), nitrate (NO_3^-), and nitrous oxide (N_2O) can have direct and indirect adverse effects on ecosystems, water quality, atmosphere, and human health. Therefore, strategies are needed to reduce diffuse N inputs into the environment. The highest N losses from arable fields can be found in vegetable crop rotations on sandy textured soils (Cameira and Mota, 2017; Cameron et al., 2013). In contrast to arable crops, most vegetables like spinach, lettuce, or cauliflower have a short cultivation period, which allows the production of two or even three crops within a single growing season. Thus, high N fertilizer inputs are required in such crop rotations. Furthermore, many vegetable crops are characterized by only superficial rooting and require a high soil mineral N (N_{min}) concentration until harvest to ensure quality demands such as size and color of the produce (Armbruster et al., 2013; Smit and Groenwold, 2005). As a result, the N use efficiency of vegetable crops is usually low compared to arable crops (Tei et al., 2020).

The actual N uptake of a vegetable crop depends, among other things, on weather conditions, diseases, and the actual harvest date (Vandecasteele et al., 2016). In addition, the N supply via mineralization of various organic soil N fractions varies as well (De Neve, 2017). Thus, the fertilizer N demand cannot accurately be predicted in advance and N fertilizers are often applied in excess to ensure a high marketable yield. The synchronization between N supply and crop demands can be improved by dividing the fertilization into several dressings based on crop growth parameters and the soil N_{min} concentration (Tei et al., 2020). However, this strategy bears the risk of adversely affecting fast-growing leafy vegetables like spinach, which are sensitive to temporary N shortages (Buysse et al., 1996). Therefore, further efforts are needed to develop a staggered fertilization strategy appropriate to these field vegetable crops.

In addition to the cropping season, N losses during the off-season following a vegetable crop rotation can be considerable as well. These losses largely result from high N_{min} residues and the mineralization and nitrification of readily decomposable crop residues remaining in the field at the end of the growing season (Armbruster et al., 2013; Zemek et al., 2020). In sandy textured soils, most of the NO_3^- leaches below 120 cm of the soil during the winter leaching period in temperate humid climates (De Neve, 2017). Even deep-rooted crops struggle to take up this NO_3^- sufficiently when sown at the end of the growing season (Kage, 2000; Thorup-Kristensen, 2002). Overall, there appears to be no viable crop residues management that can reliably mitigate the risk of high off-season N losses in vegetable crop rotations (Abalos et al., 2022a; Agneessens et al., 2014a).

Unlike open-field cultivation, soilless cultivation systems can efficiently reduce NO_3^- leaching losses and thus increase N use efficiency in vegetable crop production (Massa et al., 2020; Pignata et al., 2017). On the other hand, N nutrition of organically grown crops is challenging in such systems since N supply is dependent on the mineralization of organic fertilizers in a small substrate volume. However, both an excess N supply as well as an insufficient one often caused growth impairments in organically pot-grown plants such as basil (Bergstrand, 2022; Burnett et al., 2016; Paillat et al., 2022). Different N fertilization strategies and substrate compositions have been developed to help growers to overcome these issues (Koller et al., 2014; Verhagen, 2021). However, there is still a paucity of research on the effect of optimized applications of organic fertilizers to provide a sufficient nutrient supply in soilless cultivation systems without adversely affecting crop growth (Lohr and Meinken, 2018; Zandvakili et al., 2019).

This thesis focused on the production of spinach and pot-grown basil cultivated in open field and greenhouses, respectively. The general objective was to study measures to reduce potential N losses during the cropping season as well as the off-season without adversely affecting the marketable yield. Therefore, several series of fertilization trials were performed with the aim of better synchronization of the N supply and actual crop N demand of these crops. In further field trials, crop residue management strategies were compared, aiming at reducing off-season N losses following autumn-grown spinach.

Structure of this thesis

This thesis is a cumulative work, divided into three parts. In **Chapter 1**, the research topics are introduced. This includes a brief review of potential N losses in vegetable production systems. Special attention is given to the production of field-grown spinach as well as pot-grown basil cultivated in greenhouses. At the end of this chapter, the research questions and corresponding hypotheses are formulated.

The subsequent **Chapter 2** comprises three articles published in international peer-reviewed journals, beginning with a study focused on N fertilization in field-grown spinach (**Section 2.1**). In these investigations, different base fertilization and top dressing strategies have been compared with the aim of reducing the NO_3^- concentration exposed to leaching without negatively affecting crop growth and quality attributes. The second article (**Section 2.2**) deals with the crop residue management following autumn-grown spinach. In a series of field trials, different tillage practices as well as the application of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) on the spinach crop residues were examined for their effectiveness in reducing potential off-season N losses. The third article (**Section 2.3**) focuses on organic N fertilization strategies of pot-grown basil cultivated in greenhouses. Special attention was paid to NH_3 and NH_4^+ exposures in the plant canopy environment and growing media, respectively. With the aim of minimizing harmful exposure to both ammonical N species, the effectiveness of the base fertilization rate, substrate pH, and substrate composition was examined.

Afterwards, the results of the experimental investigations are discussed in a broader context including other vegetable crops (**Chapter 3**). Furthermore, the remaining research gap is described in more detail. Finally, a conclusion summarizes the potential and challenges for optimizing the N fertilization strategy and crop residues management to mitigate both N losses and harmful effects on crop growth in the production of field-grown spinach and organically pot-grown basil.

1.2 Risk of N losses in vegetable production systems in temperate humid climates

Nitrogen is an essential and quantitatively dominant element for plant growth. In the form of dinitrogen gas (N_2), this element makes up 78% of the troposphere. However, only N_2 fixing microorganisms are able to metabolize this N species. In European climate zones, on average only about 7 kg N_2 ha^{-1} a^{-1} becomes available to plants via this fixation process depending on the N fixing microorganisms and their interaction with the plant roots. In addition, a similar quantity of atmospheric N is converted into plant available mineral N forms via electrostatic discharges in the troposphere and depositioned to the pedosphere (De Vries et al., 2021; García-Ruiz et al., 2022). Besides atmospheric N, the uppermost 15 cm of the soil commonly contains between 2,000 and 12,000 kg N ha^{-1}. However, most of this N budget is bound in various organic fractions originating from plant and animal residues as well as animal excreta (Cameron et al., 2013). These N fractions have to be mineralized to NH_4^+ and further nitrified to NO_3^- for them to be sufficiently absorbable by plant roots. Roughly, 1.0% of the soil organic N is mineralized within a calendar year depending on the microbial activity in the soil, the recalcitrance of the organic fractions, soil type, as well as weather conditions and climate. Besides being taken up by plant roots, mineral N can be immobilized back to organic N, adsorbed on soil particles, or denitrified to gaseous N_2. Other intermediate N species of the soil N cycle are NH_3, nitrite (NO_2^-), nitric oxide (NO), and N_2O (García-Ruiz et al., 2022).

Overall, N supply via microbial fixation of atmospheric N, natural deposition, and mineralization of soil organic N is usually low, making nitrogen often the most limiting element required for plant growth (Martínez-Dalmau et al., 2021). On the other hand, the entry of readily plant available N species into the environment can cause serious damage to the biodiversity and services of terrestrial and aquatic ecosystems since most ecosystems are adapted to N scarcity (Krupa, 2003; Walling and Vaneeckhaute, 2022). In Europe, N emissions are mainly caused by agricultural practices due to NO_3^- leaching, gaseous emission of N_2O, and surface runoff. In contrast to arable fields, in livestock farming NH_3 volatilization is dominant (De Vries et al., 2021). Besides affecting ecosystems, NO_3^- contamination of drinking water and pollution of the troposphere with nitrogen oxides (NO_x) and NH_3 can pose a direct threat to human health. Moreover, N_2O contributes to global warming and depletion of the ozone layer in the stratosphere (Walling and Vaneeckhaute, 2022). In many European regions, N emissions from agriculture are above acceptable environmental thresholds. Based on a study of Schulte-Uebbing and De Vries (2021) the field-level N use efficiency (defined as crop N removal divided by total N input including fertilization, N fixation, atmospheric depositions, and mineralization) of arable cropping systems must be increased from 64% up to 90% to meet European regulations such as the Water Framework Directive (European Commission, 2000), the Nitrates Directive (Council of the European Communities, 1991) and the recently declared goals of the European Green Deal (European Commission, 2021).

In the European Union, vegetables are only grown on 1.2% of the agricultural area (EUROSTAT, 2019). However, this segment significantly contributes to the total N pollution of the environment (Tei et al., 2020). Many vegetable crops are characterized by an N uptake efficiency of only 20 to 50%, which is explained by their shallow root system and high N_{min} concentration that needs to be available until harvest (Cameira and Mota, 2017). In addition to the often high N_{min} residues, many vegetable crops leave high quantities of N-rich and readily decomposable crop residues in the field at harvest. In *Brassica* species such as brussels sprouts and broccoli the aboveground crop residues usually contain above 200 kg N ha^{-1}

(Zemek et al., 2020). The mineralization of this biomass leads to a further increase of the soil N_{min} concentration. As a result, within a few weeks after harvest, the mean NO_3^- concentration in the upper 0–90 cm of the soil increases to above 100 kg N ha^{-1} (Noij and Ten Berge, 2019; Zemek et al., 2020). This N budget can be considered in the fertilization of the following crop. However, at the end of the growing season, N uptake by plants is low (Agneessens et al. 2014a). Hence, NO_3^- losses following a harvest in autumn are often considerable during the autumn and winter leaching period in humid climates (Smith et al., 2016; Spiess et al., 2021). In contrast, if a catch crop was already sown at the beginning of September, approximately 60 kg N ha^{-1} are usually taken up until the end of the growing season, thus reducing NO_3^- leaching losses (Agneessens et al. 2014a). However, the earlier the last vegetable crop in a growing season is harvested, the lower the farmer's revenue is. Therefore, strategies are needed to allow a long growing season with minimum risk of N losses.

Many vegetable crops are typically grown on sandy sites to ensure early warming in spring as well as a trafficability and workability that is largely independent of weather conditions and irrigation practices (Nett et al., 2015). However, in contrast to fine textured soils, NO_3^- can easily be leached in coarsely textured sandy soils (De Neve, 2017). Therefore, most of the N lost from vegetable cropped sites is due to NO_3^- leaching (Agneessens et al., 2014a; Armbruster et al., 2013). However, also gaseous emissions of N_2O and N_2 as well as NH_3 volatilization can be significant within the first weeks after decomposition of N-rich crop residues has begun (Abalos et al., 2022b; Janz et al., 2022; Nett et al., 2016). The final step of denitrification, where N_2O is converted to N_2, is the most sensitive step in this reaction. Especially at a high soil NO_3^- concentration the N_2O/N_2 ratio is often increased (Zhu et al., 2013). As mentioned above, this is crucial in the context of the depletion of the ozone layer and global warming since atmospheric N_2O is a greenhouse gas that is 298 times more potent than carbon dioxide (CO_2) (Guenet et al., 2021; Walling and Vaneeckhaute, 2022). In contrast to the post-harvest period, direct gaseous N emissions are usually low between sowing and harvest as long as N fertilizers are used in moderation and in synchronization with plant demand (Norton and Ouyang, 2019; Tei et al., 2020; Vico et al., 2020).

Nitrogen surplus (fertilizer-N minus crop export) is usually at 80 kg ha^{-1} a^{-1} in vegetable crop rotations. However, when also considering the soil N_{min} concentration and crop residues N, the surplus tends to be above 200 kg N ha^{-1} a^{-1} (Armbruster et al., 2013; Nett et al., 2011). The inclusion of long-standing and deep rooting cereal crops in vegetable crop rotations can significantly reduce this balance gap (Nendel, 2009). On the other hand, the cultivation of arable crops with a lower financial return is less attractive for growers (Vandecasteele et al., 2016). Another option is to turn from conventional production to organic cultivation, which is less intensive in terms of e.g. the quantity of fertilizer N applied and tillage practices. Thus, N losses on a hectare basis are usually reduced (Tei et al., 2020). On the other hand, organic production systems are often associated with a lower productivity per area unit than conventional systems, and challenges relating to a proper supply of nutrients are likely one of the main causes of this (Bergstrand, 2022). In organic cultivation the application of synthetically produced N fertilizers is not permitted. Instead, organic fertilizers of natural origin can be used (European Commission, 2018). However, N supply via organic fertilizers is dependent on their mineralization dynamics in the soil, which makes it difficult to synchronize the N supply with the N demand of the crop. This is typically also true for slow and controlled release fertilizers when applied to fast-growing and N demanding crops (Tei et al., 2020). Thus, there is a high risk of both an insufficient N supply in times of high N demand as well as N losses at times of negligible N uptake like after harvest. A further option to reduce potential N losses is to inhibit the oxidation of NH_4^+ to NO_3^- since NH_4^+ is less prone to leaching. However, to prevent diminished growth and plant damage, nitrification inhibitors should not be applied to NH_4^+-sensitive crops such as spinach or basil (Kiferle et al., 2013; Pasda et al., 2001).

In contrast to the open-field production, NO_3^- leaching can easily be minimized by cultivation in greenhouses and soilless closed-loop irrigation systems (Pignata et al., 2017; Shrestha et al., 2021). In temperate climates vegetables such as tomato, cucumber, pepper, and culinary herbs are grown almost exclusively in soilless cultivation systems in greenhouses. However, for many vegetables like spinach and cauliflower, these production systems are economically not feasible due to high energy and investment costs that are not compensated for by the consumers (Bergstrand, 2022; De Haan et al., 2014). On the other hand, greenhouse cultivation opens the possibility of optimizing growth factors such as temperature. Thus, the plant growth rate can be increased as well as the growing season be extended to 12 months. Consequently, a higher N supply is required to meet plant demands (Bergstrand, 2022; Morano et al., 2017). However, due to the higher productivity greenhouse gas emissions can be increased in comparison to cultivation in open fields (Breukers et al., 2014). In addition, closed-loop systems must occasionally be discharged to avoid salinization of the recirculating solution. However, this measure results in nutrient emissions to surface water (Gabriel and Quemada, 2017).

Overall, in both open field and greenhouse vegetable cultivation systems N losses can be high. The N fertilization strategy as well as the management of crop residues appear to be key factors in reducing N emissions to the environment.

1.3 Field-grown spinach production

Spinach (*Spinacia oleracea* L.) is a leafy vegetable grown for both the fresh market and food processing industry. In the following section, the market and cultivation practices of field-grown spinach are described. Special attention is given to the production in temperate humid climates in central Europe.

Spinach production and market requirements

On average, an annual quantity of 30 million tons of spinach was harvested worldwide in the years 2018 to 2020. About 92% of this tonnage was grown in China, followed by Europe with 2.3% and the USA with 1.4% (FAOSTAT, 2022). In Europe, each 10 to 15% of the spinach tonnage was harvested in Italy, France, Belgium, Germany, Spain, the Netherlands, and Greece (EUROSTAT, 2022). The annual production in Germany was about 87,000 t in 2021 with an annual increase of approximately 3% in recent years (Rogge, 2022). About 9% of this quantity was grown organically (Statistisches Bundesamt, 2022). In Germany, most of the spinach is grown for the frozen food industry (Rogge, 2022; Strohm et al., 2016). To ensure a high quality of the produce, the crop is usually grown close to processing plants. One such area is located near Borken (North Rhine-Westphalia, Germany), where spinach is grown on about 2,500 ha (Pretty et al., 2008). About one third of the total spinach quantity harvested in Germany is grown in this region (IT NRW, 2019; Strohm et al., 2016).

Generally, both the fresh market and processing industry require a high proportion of leaf blades. For the production of leaf and baby-leaf spinach almost exclusively leaf blades are harvested. However, in chopped spinach, a certain proportion of leaf stalks is required. The leaf blade to leaf stalk ratio can be partially regulated by the cutting height, the cultivar, and harvest stage (Grevsen and Kaack, 1997). Furthermore, markets require a dark green product (Biemond et al., 1996; Brandenberger et al., 2004; Grevsen and Kaack, 1997). Deficient symptoms such as yellowing and chlorosis are unacceptable in leafy vegetables. Until maximum N uptake is reached, the marketable yield correlates positively with the level of N supply (D'Haene et al., 2018; Massa et al., 2018). On the other hand, the contents of NO_3^- and oxalates also tend to increase with higher N supply (Cai et al., 2018; Conesa et al., 2009). Oxalates can cause kidney stones and, in addition, NO_3^- can be converted to NO_2^- and cause the formation of methemoglobinemia in infants and young children as well as carcinogen nitrosamines in adults (Koh et al., 2012; Ranasinghe and Marapana, 2018). Therefore, the maximum permitted NO_3^- content in fresh and frozen spinach is limited to 3,500 and 2,000 mg kg^{-1} (fresh matter basis), respectively (European Commission, 2011).

Field-grown spinach cultivation practices

Spinach is an herbaceous annual crop. It is a fast-growing leafy vegetable with a cropping cycle of only 5 to 10 weeks from sowing to harvest depending on the harvest stage and season. If spinach is grown during the winter season, the cropping cycle can last up to 7 months in European climates (Canali et al., 2014; Feller et al., 2011). These short periods of time allow the sowing of two or even three crops within a single growing season.

Depending on the variety and plant age spinach starts bolting at long-day conditions and higher temperatures (Abolghasemi et al., 2021; Niers, 1994). The more the harvest is delayed, the higher the proportion of leaf stalks and bolting shoots (Frerichs and Daum, 2021). However, leaf blade growth is reduced after the onset of bolting (Biemond et al., 1996). To ensure vegetative growth, a spinach crop is therefore only harvested once. Thus, crop residues are incorporated into the soil and a new crop is sown. It is only in the autumn and winter seasons that bolting does not usually occur, which opens the possibility of a second harvest of the same crop (Laber and Lattauschke, 2020; Suzuki et al., 2019).

In subtropical and Mediterranean climates spinach must be frequently irrigated to meet plant demands. For this purpose, circle irrigation trolleys or sprinklers are typically used. Due to heat stress in the summer season spinach is only cultivated in the winter half-year in these climates (Canali et al., 2014; Goreta and Leskovar, 2006). In contrast, in temperate climates, spinach is grown in all four seasons (Feller et al., 2011). However, irrigation is still essential to ensure an adequate moisture content in the soil (Schlering et al., 2020). In mid-Europe, usually hose reel irrigation systems are used for irrigation (Laber and Lattauschke, 2020). Maximum yield and quality of the produce can only be achieved within a time frame of just a few days (Grevsen and Kaack, 1997). Therefore, spinach need to be harvested mostly independent from weather conditions and irrigation schedule. To ensure trafficability of heavy harvest machines, spinach is usually grown in sandy soils (Massa et al., 2018; Nett et al., 2015).

Nitrogen nutrition management

Spinach is an N demanding crop, which requires a total N availability of approximately 200 kg ha^{-1} (0–30 cm) to achieve a fresh mass yield of 30 t ha^{-1}. Most of this N demand must be covered by NO_3^- since spinach is an NH_4^+ sensitive crop (Hähndel and Wehrmann, 1986; Xing et al., 2015). To ensure a sufficient N supply from sowing to harvest, the N_{min} concentration should be at least 40 kg ha^{-1} (0–30 cm) (Feller et al., 2011; Niers, 1994). However, within the first weeks after sowing N uptake by the crop is rather low. Therefore, the fertilizer N supply is usually divided into a base fertilization applied at sowing supplemented by a single top dressing applied after the first true leaves become unfurled at the end of the seedling stage (Feller et al., 1995; Feller et al., 2011). At this development stage, exponential N uptake by the crop also starts. In the last few weeks before harvest, the N uptake can reach a rate of over 60 kg ha^{-1} $week^{-1}$.

In Germany, the maximum permissible N application in vegetable crops must be derived from N demand based on the expected yield, the initial N_{min} concentration in the root zone, the soil carbon content, the previously grown cash and catch crop species, and manuring in the previous year (German Fertilizer Ordinance, 2020). According to this calculation, a maximum of 205 kg N ha^{-1} can be applied to a spinach crop. In other European countries the maximum permissible fertilizer application rate to a spinach crop is in a range between 95 and 250 kg N ha^{-1}, depending on the soil texture (D'Haene et al., 2018).

As mentioned in section 1.2, the cultivation of spinach is associated with a high risk of NO_3^- leaching. This is due to the high N application required to cover the N demand of up to three crops within a single growing season. In addition, the N uptake efficiency of spinach is low, which is caused by the shallow root system and high N_{min} concentration that must be available until harvest (Schenk, 2006; Schenk et al., 1991). Therefore, fertilization and cultivation strategies are required to reduce potential N losses without negatively affecting the marketable yield.

1.4 Organically pot-grown basil in soilless greenhouse production systems

Basil (*Ocimum basilicum* L.) is one of the most important culinary herbs worldwide. It is grown for the fresh market as well as for processing in the food, cosmetic, and pharmaceutical industries (Singletary, 2018). When grown for processing, it is typically cultivated outdoors. In contrast, fresh-cut and pot-grown culinary herbs are usually cultivated in greenhouses (Laber and Lattauschke, 2020). In this section, the market and cultivation practices of organically pot-grown basil are described. Special attention is given to the production in central Europe.

Market share and quality requirements

Potted herbs are the dominant product on the market for fresh herbs in Germany (Laber and Lattauschke, 2020; Statista, 2018). Within this segment, basil is the most important crop (CBI, 2016b). In particular, the market for organically pot-grown herbs has increased considerably in western countries (AMI, 2018; Burnett et al., 2016). Within the European Union, organically certificated products must comply with the regulations of the European Commission (2018). In addition to the European certification, many growers also produce according to specifications of private trade labels such as Bioland and Demeter.

Pot-grown basil is usually traded in 9-cm, 12-cm, and 13-cm pots (Laber and Lattauschke, 2020). An important market requirement is a homogeneous and compact habitus (Rosenbusch et al., 2021). Moreover, the leaves should be evenly colored without chlorosis and necrosis (CBI, 2016a). Basil is known to be beneficial for human health due to its composition of essential oils, phenols, and vitamins (Muráriková and Neugebauerová, 2018; Singletary, 2018). On the other hand, the NO_3^- content can be increased depending on the variety, season, and N nutrition (Muráriková and Neugebauerová, 2018).

Production of organically pot-grown basil

Basil is an herbaceous perennial that is native to tropical and subtropical climates. Thus, in temperate climates, year-round production can only be performed in heated greenhouses (Laber and Lattauschke, 2020). In addition, assimilation lighting is required to ensure a high quality of the produce during the winter season (Eghbal, 2017; Rosenbusch et al., 2021). At temperatures between 18–29 °C it takes 8 to 14 days from sowing to emergence (Treadwell et al., 2007). After the seedling stage, maximum growth can be achieved at 25 °C. However, the temperature should not drop below 16 °C to ensure proper plant growth (Chang et al., 2005; Eghbal, 2017). When growing in 9-cm pots, a cultivation time of 4–10 weeks is required, depending on the season (Eghbal, 2017).

In Europe, soilless cultivation is forbidden in the production of most organic certified crops. An exception to this is where the products are intended to be sold to the end consumer together with the growing medium, as is the case for culinary pot-grown herbs (Asp et al., 2022; European Commission, 2018). Basil is usually grown in peat-based substrates. However, private organic trade labels such as Bioland require a peat substitution of at least 20% (Bioland, 2020). In addition, many European countries have declared that they will reduce the use of peat in horticulture production by up to 100% until the year 2030 (Hirschler et al., 2022). In terms of volume, wood fibers, coir products, and composted green waste or bark are the most important organic peat substitutes used in European horticulture (Hirschler et al., 2022; Schmilewski, 2017).

Nitrogen nutrition management of organically grown basil

Basil is a fast-growing crop with a N demand of 700–900 mg (L substrate)$^{-1}$ (Laber and Lattauschke, 2020). Generally, synthetically produced N fertilizers are prohibited in organic cultivation (European Commission, 2018). Instead, organic fertilizers of natural origin can be used. However, due to the short cultivation period and small substrate volume of the pots, N supply by mineralization is usually incomplete. In order to ensure a sufficient N supply an application of 1,000 to 1,250 mg N (L substrate)$^{-1}$ is recommended when organic fertilizers are used (Eghbal, 2017; Lindner and Billmann, 2006). This N demand is usually covered by mixing granulated base fertilizers into the growing media before sowing (Burnett et al., 2016; Koller et al., 2014).

On the other hand, a high organic N supply can cause serious damage to seedlings of vegetable crops. A high salt concentration, an unfavorable substrate pH, nutrient imbalances and deficiencies, or the presence of phytotoxic allelochemicals might explain this issue (Bi et al., 2010; Burnett et al., 2016; Moncada et al., 2021; Nair et al., 2011). To prevent adverse effects on plant growth as well as to ensure a sufficient N supply, a point placement of the base fertilizer in the growing medium as well as a reduced base fertilization rate compensated by repeated drenches with liquid organic fertilizers during the later course of cultivation proved to be effective (Burnett et al., 2016; Koller et al., 2014). However, the causes of plant damage and diminished growth appear to be diverse and have not been extensively studied (Lohr and Meinken, 2018). In this context Rogers (2017) indicated the following research gaps in the soilless organic cultivation of greenhouse crops: *“(1) the mineralization rates and the microbial community structure and functionality for various soilless organic substrates and fertilizers have to be identified, (2) the timing of fertilizer applications and nutrient use efficiency has to be optimized and (3) crop productivity and quality in response to various organic amendments in greenhouses has to be measured.”*

1.5 Research questions and hypotheses

The main objective of this work was to develop strategies to reduce the risk of N losses from intensive vegetable production into the environment without negatively affecting crop yield and the quality of the produce. As described above, this objective is difficult to achieve in fast-growing crops like spinach, which are sensitive to temporary N shortages. Based on this background several fertilization trials were conducted with field-grown spinach. However, most N losses in vegetable crop rotations usually occur outside of the growing season during the winter leaching period. Therefore, further trials dealing with the crop residue management following autumn-grown spinach were conducted. In contrast to vegetable cropping in the open field, direct NO_3^- leaching losses can be neglected when crops are grown in soilless closed-loop irrigation systems in greenhouses. However, a proper N supply of organically grown crops is challenging in these cultivation systems since N supply is dependent on the mineralization of organic fertilizers in a small substrate volume. Both a high and low N mineralization rate of organic fertilizers can lead to serious plant damage and diminished crop growth. Therefore, fertilization experiments with organically pot-grown basil were performed in greenhouses. The individual research questions and corresponding hypotheses of the trials mentioned are listed below:

- Timing and split of mineral N fertilization of spinach in open-field cultivation

Research question:
How to mitigate the risk of NO_3^- leaching losses during the cultivation of field-grown spinach without adversely affecting yield and quality of the produce?

Hypotheses:

- Due to the low N uptake rates of spinach between sowing and the end of the seedling stage, there is no need for base N fertilization. Hence, the risk of NO_3^- leaching can be reduced during this period. However, the initially lower N supply must be compensated by an increased top dressing to cover N requirement and ensure normal growth of the spinach crop.
- Splitting top dressing into two applications, one at the end of the seedling stage and another just after reaching an early harvest stage, does not decrease crop performance in comparison to only a full top dressing just at the end of the seedling stage. In this way, the NO_3^- concentration peak after fertilization is flattened. In addition, the second top dressing can be omitted if the spinach crop is harvested at an early harvest, thus reducing the N_{min} residue.
- Instead of using a granulated fertilizer for the second top dressing, a frequent foliar spray between early and late harvest stages can be used to further optimize synchronization between N supply and actual spinach N demand.

- Improvement of crop residues management following autumn-grown spinach

Research question:
What post-harvest crop residues management practices of autumn-grown spinach will reduce potential N losses during the following off-season?

Hypotheses:

- A less intensive tillage practice in terms of depth and frequency decreases post-harvest mineralization of soil organic N and crop residues, NO_3^- accumulation, and subsequently N losses during the following winter leaching period.
- Postponing the tillage date from early to late autumn or early spring reduces N losses by the N uptake of resprouting spinach crop residues.
- The later the tillage date, the lower the soil temperature, which will decrease the mineralization and nitrification rate after the incorporation of the crop residues into the soil. Thus, potential NO_3^- leaching losses during the off-season are reduced.
- Nitrification following the incorporation of the spinach crop residues is delayed by the application of DMPP. Consequently, mineralized organic N will remain as NH_4^+ in the upper soil layer until the end of the winter leaching period.

- Reduction of ammonical exposures in organically fertilized pot-grown basil

Research questions:
Do ammonical exposures caused by an organic fertilization have any adverse effects on pot-grown basil? How can harmful exposures be minimized?

Hypotheses:

- Basil is adversely affected by increasing NH_4^+ and NH_3 exposures following an organic N supply.
- Moderate base N fertilization will reduce the ammonification and the associated pH increase in the growing media. As a result, NH_4^+ and NH_3 exposure levels remain low.
- The lower the substrate pH, the lower the NH_3/NH_4^+ ratio. Consequently, at a low initial substrate pH the NH_3 exposure remains on low levels.
- The amendment of mature compost into a peat-based substrate triggers the nitrification. Thus, ammonical exposures are reduced by their rapid oxidation into NO_3^- and associated decrease of the substrate pH.

Chapter 2
Scientific publications within the context of this work

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

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Citation:

Frerichs, C., Key, G., Broll, G., Daum, D. (2022a): Nitrogen fertilization strategies to reduce the risk of nitrate leaching in open field cultivation of spinach (*Spinacia oleracea* L.). J. Plant Nutr. Soil Sci. 185(2), 264–281. doi: 10.1002/jpln.202100275

Keywords:

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

Authors contributions:

Christian Frerichs: conceptualization, investigation, methodology, formal analysis, validation, visualization, and writing original draft. Georgina Key: review and editing. Gabriele Broll: review and editing. Diemo Daum: conceptualization, supervision, review, and editing.

Acknowledgements:

The authors gratefully acknowledge the Chamber of Agriculture North Rhine-Westphalia (Borken, Germany), iglo GmbH (Reken, Germany), and Rheinisch-Westfälische Wasserwerksgesellschaft mbH (RWW) (Mülheim, Germany) for their advisory support and financial participation in the European LEADER program 33.4 L1-3 by which this research was funded. For providing the fertilizers, the authors acknowledge Yara GmbH (Dülmen, Germany). The authors also like to thank all farmers (Verein zur Förderung des Feldgemüseanbaus Westmünsterland e. V., Borken, Germany) who made their fields available for the fertilization trials. For his support in the laboratory investigations, the authors like to thank Stephan Dinklage, Osnabrück University of Applied Sciences. Open Access funding enabled and organized by Projekt DEAL.

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.

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 and 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 and 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 and 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 and 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 and 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 NO_3^- 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.

Materials and methods

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 plowed 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 or cereals as a pre crop, 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.

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]	Late[b]
Spring	Green rye	1	Mar. 20, 2018	Hudson (PV)	May 09, 2018	May 22, 2018
	Green rye	2	May 09, 2020	Santa Cruz (PV)	Jun. 13, 2020	Jun. 23, 2020
	Mustard	3	Jun. 04, 2019	Ballet (Se)	Jul. 05, 2019	Jul. 15, 2019
Summer	Spinach	1	Jun. 14, 2018	SV5591 (Se)	Jul. 15, 2018	Jul. 25, 2018
	Spinach	2	Jul. 07, 2020	Rhino (RZ)	Aug. 07, 2020	Aug. 15, 2020
	Spinach	3	Jul. 30, 2019	La Paz (PV)	Sep. 01, 2019	Sep. 12, 2019
Autumn	Barley	4	Aug. 14, 2018	Solomon (Se)	Sep. 24, 2018	Oct. 09, 2018
	Triticale	5	Aug. 18, 2020	Sonoma (PV)	Sep. 19, 2020	Oct. 05, 2020
Winter	Potatoes	6	Sep. 28, 2018	Gorilla (RZ)	Apr. 07, 2019	Apr. 24, 2019
	Barley	7	Oct. 02, 2019	Sonora (RZ)	Apr. 12, 2020	Apr. 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

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 and 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% 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.

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

Season (sowing date)	Trt.	Base fertilization	Top dressings	N dressings [kg ha^{-1}]					Total crop available N [kg ha^{-1}]
				Base fertilizer (UAN/CAN)	First top dressing (CAN)	Second top dressing (CN)[a]	Foliar spray (Urea)[b]	Total	
Early spring (Mar. 20, 2018)	1	Standard	Single	70	100	0	0	170	225
	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 (May 09, 2020)	1	Standard	Single	72	83	0	0	155	235
	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 (Jun. 04, 2019)	1	Standard	Single	72	59	0	0	125	247
	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 (Jun. 14, 2018)	1	Standard	Single	36	75	0	0	110	225
	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 (Jul. 30, 2019)	1	Standard	Single	36	52	0	0	88	196
	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 (Jul. 30, 2019)	1	Standard	Single	36	51	0	0	87	219
	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 (Aug. 14, 2018)	1	Standard	Single	48	125	0	0	173	235
	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
Autumn (Aug. 18, 2020)	1	Standard	Single	54	88	0	0	143	222
	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 (Sep. 28, 2018)	3	n.a.	Single	105[c]	55	0	0	160	237
	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

(continues)

Table 2: (Continued)

Season (sowing date)	Trt.	Base fertilization	Top dressings	N dressings [kg ha^{-1}]: 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}]
	3	n.a.	Single	105[c]	55	0	0	160	199
Winter	4	n.a.	Split dose	105[c]	0	55	0	160	199
(Oct. 02, 2019)	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

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 (AlzchemAG, 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.

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 (2004b). 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.

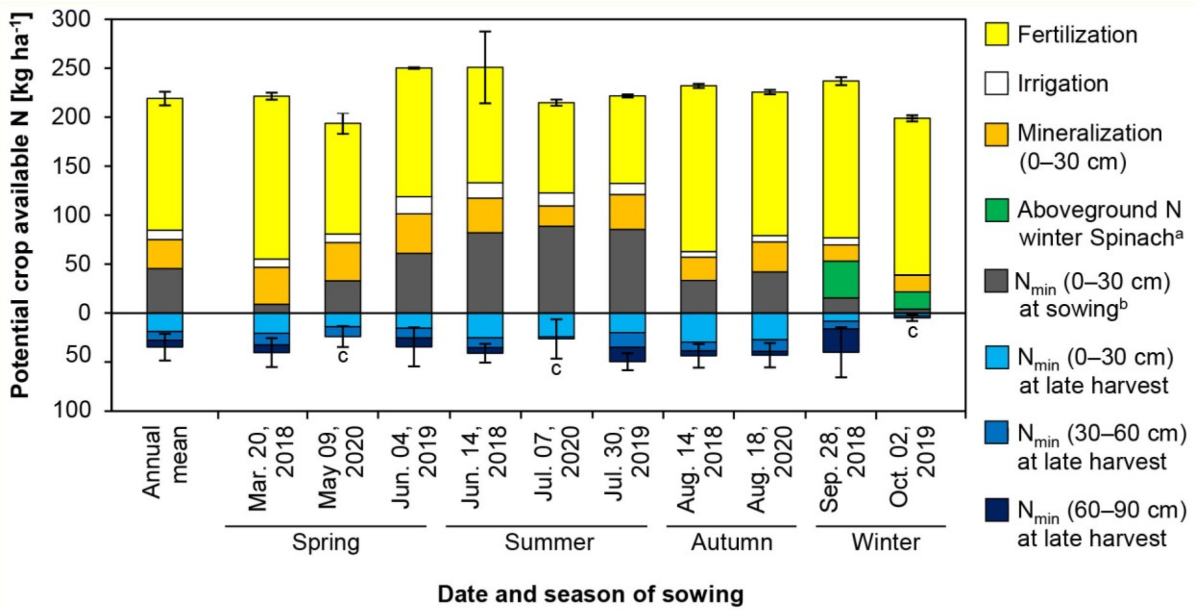


Figure 1: Sources of crop potentially available N in the top soil (0–30 cm) until late harvest as well as N_{min} residues at late harvest stage (0–90 cm) in treatment 3 along the ten field trials conducted between 2018–2020 (*n* = 3; Mean ±SD). Note: ^{a}Aboveground N of winter-grown spinach at early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

2.4 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.

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 Fmax 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).

Results

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.

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 was 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**).

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.

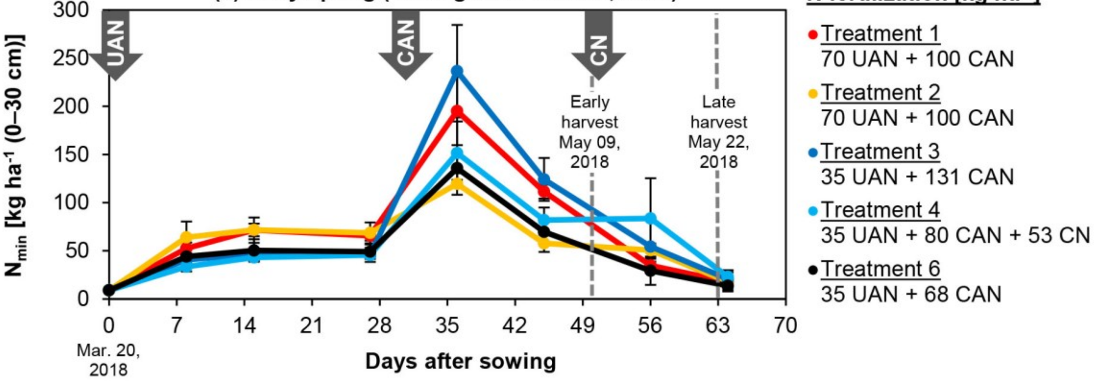
Initially, N_{min} level was low in the topsoil in early spring-grown spinach (**Figure 2A**). Therefore, a base fertilization of 70, or 35 kg N ha^{-1} was applied in treatments 1–2 and 3–6, respectively. In the following weeks N_{min} concentration ranged between 34–72 kg ha^{-1} depending on the base 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**.

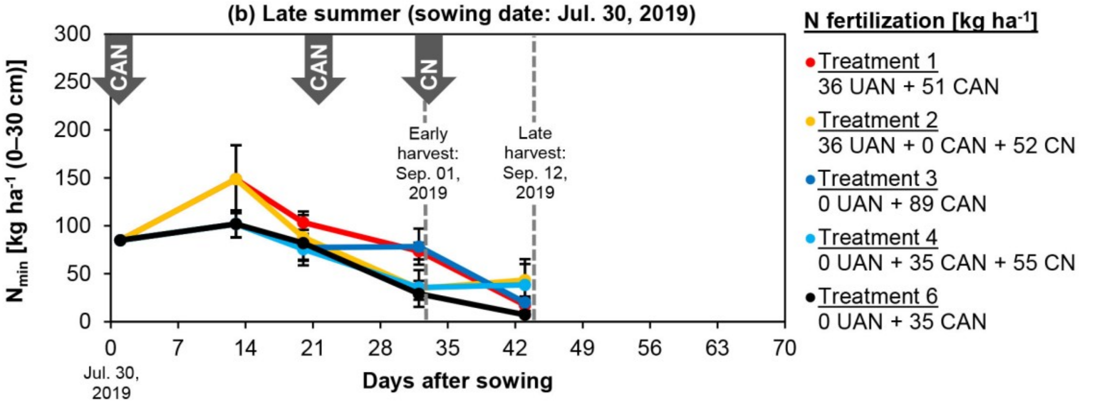
In treatments 2 and 4–6 the first top dressing rate was reduced by approximately 50–70 kg N ha^{-1}. However, this reduced the N_{min} concentration at the early harvest stage by only 20–51 kg ha^{-1} compared to treatments 1 and 3, which had already received the total N supply required from the base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

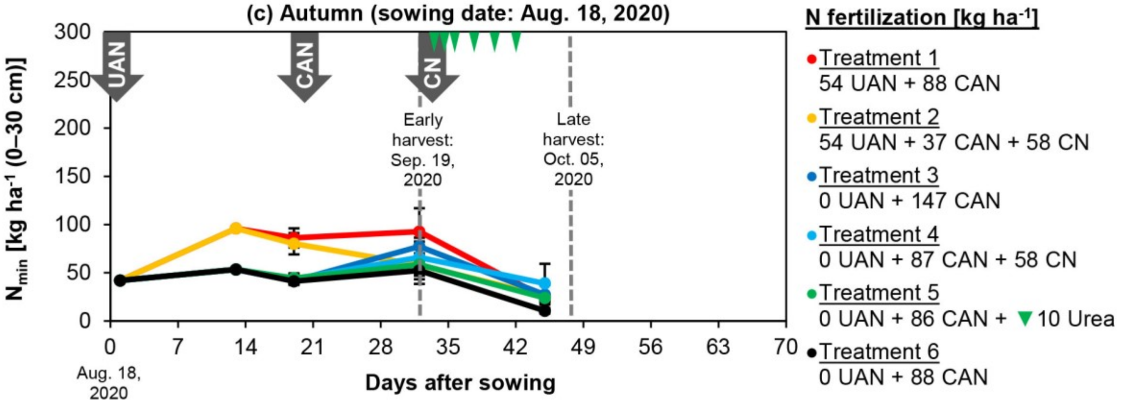
(a) Early spring (sowing date: Mar. 20, 2018)



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



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



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

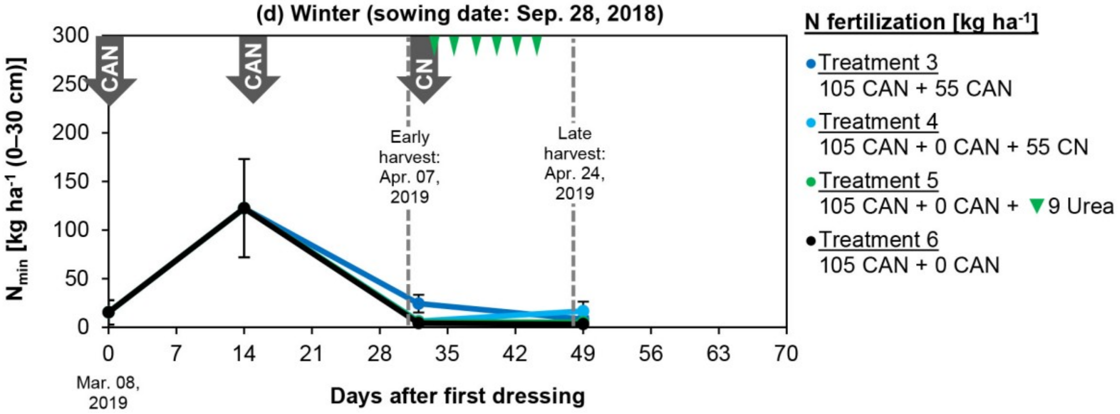


Figure 2: Soil mineral N concentration (NO_3^--N and NH_4^+-N) during the period of cultivation depending on the season (A–D) at different N base fertilization and top dressing rates (*n* = 3; Mean ±SD). Note: CAN, Calcium ammonium nitrate; CN, Calcium nitrate (Tropicote®); UAN, Urea ammonium nitrate

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**.

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 x B	0.016	n.s.
Trial x S	n.s.	0.049
B x S	n.s.	n.s.
Trial x B x S	n.s.	n.s.

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

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 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.

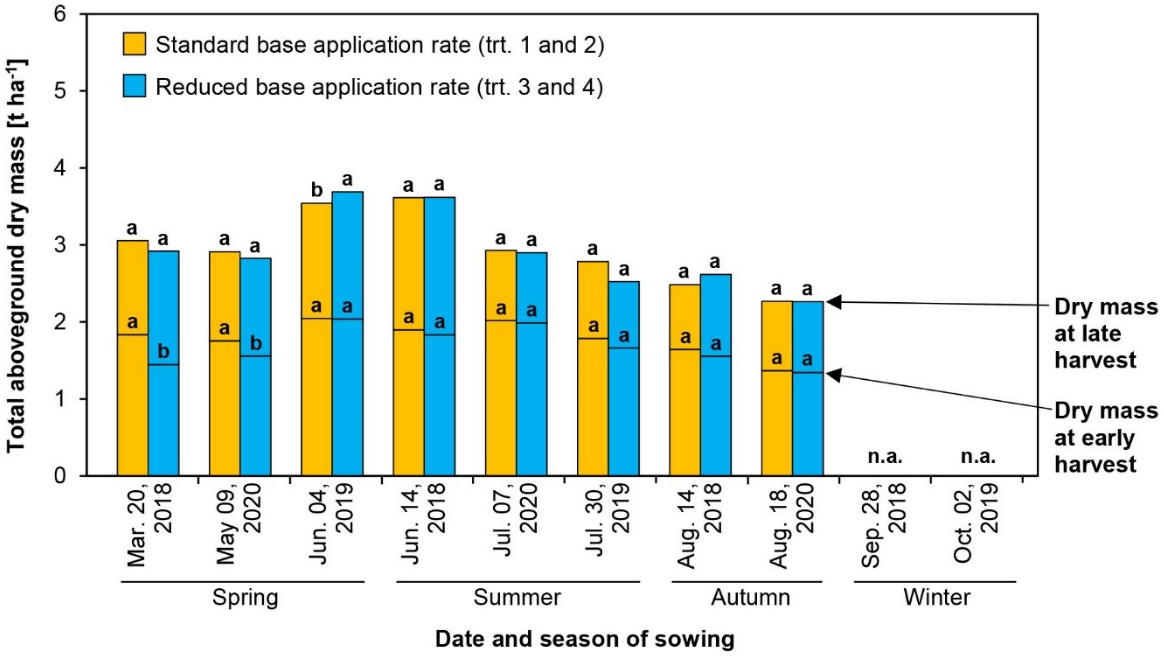


Figure 3: Mean total aboveground dry mass of spinach at different base fertilizer rates at the early and late harvest stages (within each trial and harvest stage, columns with different letters are significantly different according to Tukey's post hoc test, $p < 0.05$, $n = 6$). Note: n.a., not available;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

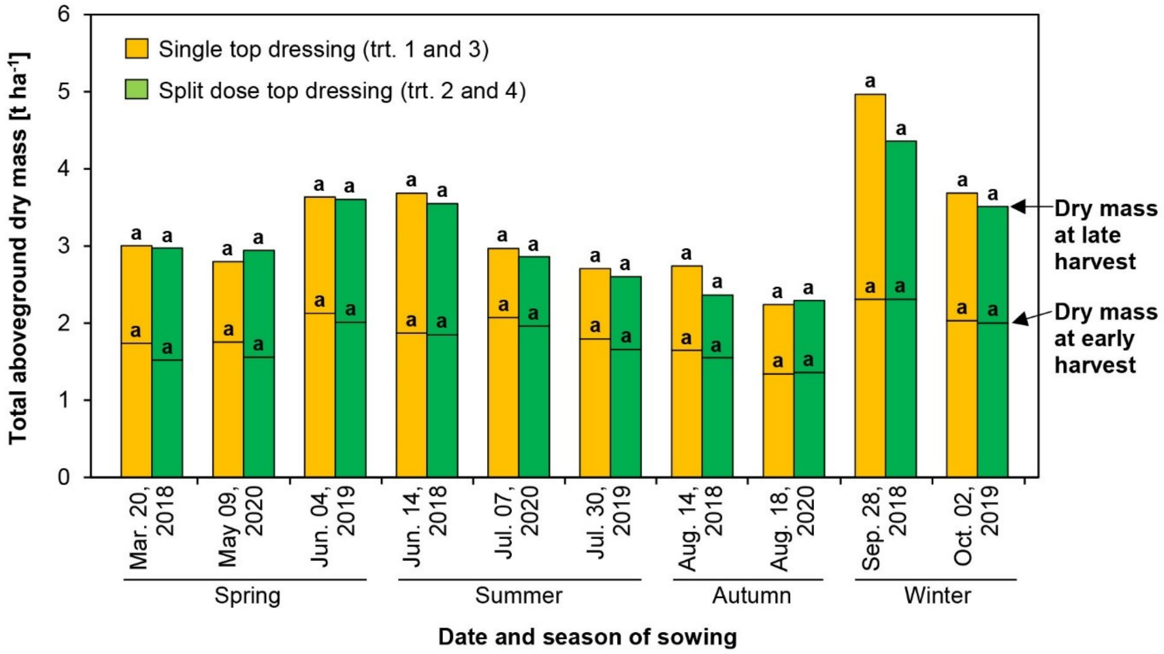


Figure 4: Mean total aboveground dry mass of spinach at a single and split dose top dressing at the early and late harvest stages (within each trial and harvest stage, columns with different letter are significantly different according to Tukey's post hoc test, $p < 0.05$, $n = 6$). Trt, Treatment; In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

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

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 ($p < 0.05$, $n = 3$)

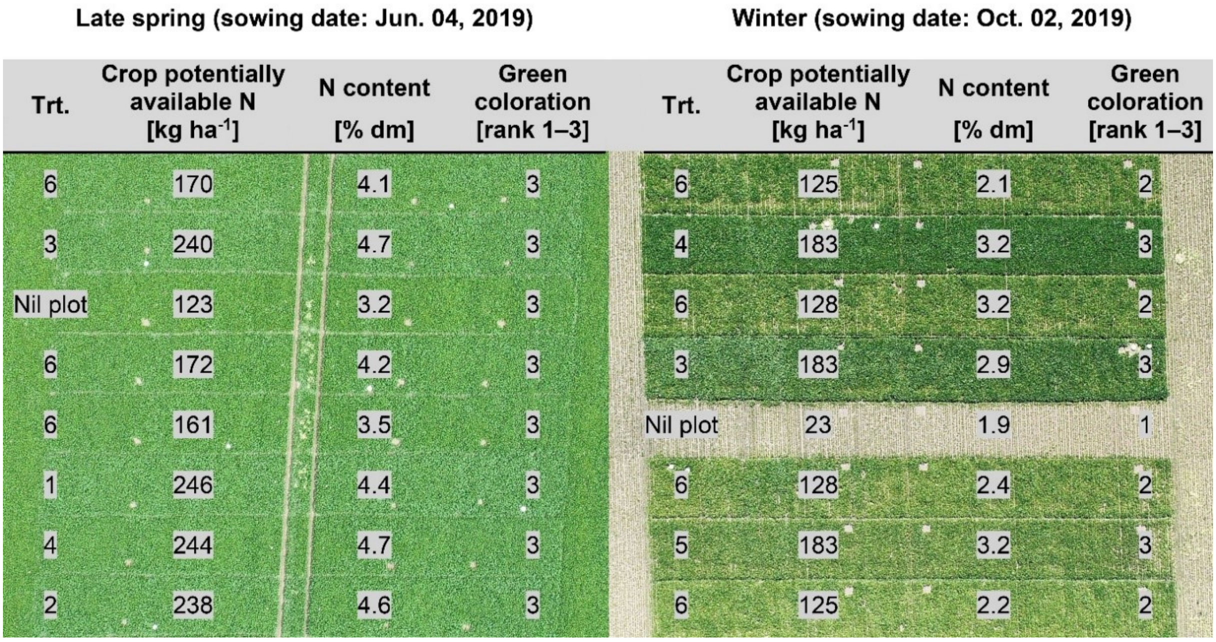
Season	**Sowing date**	**Nitrogen content [% dm]**					
		Trt. 1	**Trt. 2**	**Trt. 3**	**Trt. 4**	**Trt. 5**	**Trt. 6**
Spring	Mar. 20, 2018	3.9 a	4.0 a	4.2 a	4.0 a	n.a.	3.4 a
	May 09, 2020	4.3 a	3.8 ab	4.0 a	4.1 a	4.4 a	2.8 b
	Jun. 04, 2019	4.4 ab	4.4 ab	4.8 a	4.6 a	n.a.	3.9 b
Summer	Jun. 14, 2018	3.9 ab	4.0 a	4.1 a	4.2 a	n.a.	3.5 b
	Jul. 07, 2020	3.9 ab	4.1 a	4.2 a	4.4 a	4.5 a	3.2 b
	Jul. 30, 2019	4.9 a	4.8 a	5.1 a	4.9 a	n.a.	4.3 b
Autumn	Aug. 14, 2018	5.1 a	4.9 ab	5.1 a	4.9 b	n.a.	4.4 c
	Aug. 18, 2020	4.8 b	4.9 b	4.8 b	5.2 a	5.4 a	4.2 c
Winter	Sep. 28, 2018	n.a.	n.a.	3.6 ab	3.7 a	3.5 ab	3.1 b
	Oct. 02, 2019	n.a.	n.a.	2.7 b	2.9 a	3.1 a	2.2 c

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

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 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.


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]

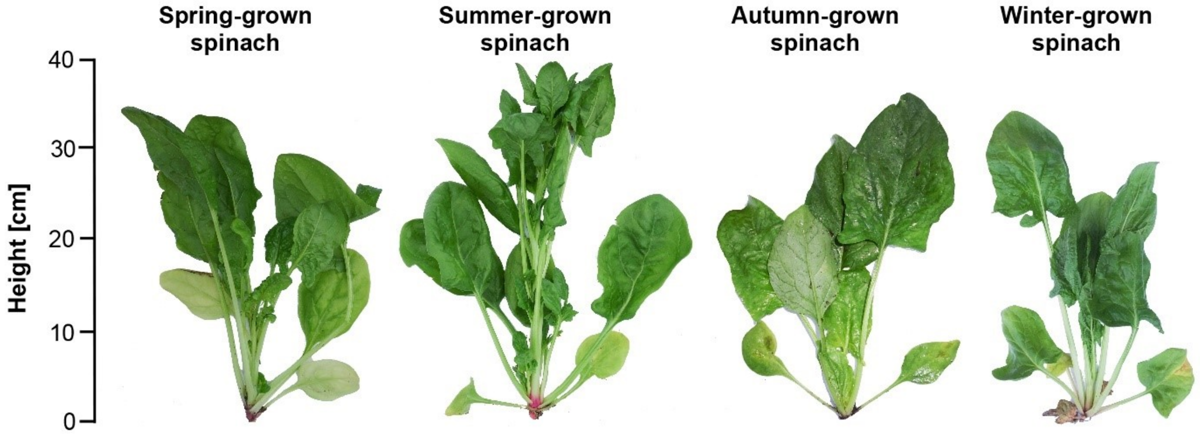


Figure 7: Spinach growth habit depending on the season at the late harvest stage

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.

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 ($p < 0.05$, $n = 3–9$)

Season	Sowing date	Shoot nitrate content [mg (kg fm)$^{-1}$]					
		Early harvest stage		Late harvest stage			
		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	2,723 a	1,613 a	1,506 a	1,531 a	773 ab	381 b
	Jun. 04, 2019	3,993 a	3,120 a	2,556 a	2,781 a	n.a.	2,764 a
Summer	Jul. 07, 2020	2,219 a	1,655 a	1,800 ab	2,400 a	1,022 ab	380 b
	Jul. 30, 2019	n.d.	n.d.	2,947 a	2,707 a	n.a.	1,542 b
Autumn	Aug. 18, 2020	2,477 a	1,504 b	1,068 ab	2,249 a	2,312 a	327 b
Winter	Sep. 28, 2018	2,587 a	1,121 a	693 a	571 ab	205 c	391 bc
	Oct. 02, 2019	758 a	179 a	100 a	132 a	44 a	47 a

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

Discussion

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 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 and 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 and 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 and 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 and 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 stage, 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 and 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.

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 seasonal 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 significant 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 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 and 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.

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 and 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 and Förster, 1989). This might also explain why the European threshold of 2,000 mg NO_3^- (kg fm)$^{-1}$ for processsed 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).

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.

Supplemental material

Table S1: Variance table for factors effects over the 10 trials

	Source of variation	**Degrees of freedom**	**Mean square (MS)**	**Computed F**
Factors effects	Trial[a] (T)	T - 1	T MS	$\frac{T\ MS}{R\ MS}$
	Replication within trial (R)	T (R - 1)	R MS	
	Base fertilization rate (B)	B - 1	B MS	$\frac{B\ MS}{E\ MS}$
	Splits of top dressing (S)	T - 1	T MS	$\frac{S\ MS}{E\ MS}$
	T x B	(T - 1) (B - 1)	T x B MS	$\frac{T\ x\ B\ MS}{E\ MS}$
	T x S	(T - 1) (S - 1)	T x S MS	$\frac{T\ x\ S\ MS}{E\ MS}$
	B x S	(B - 1) (S - 1)	B x S MS	$\frac{B\ x\ S\ MS}{E\ MS}$
	T x B x S	(T - 1) (B - 1) (S - 1)	T x B x S MS	$\frac{T\ x\ B\ x\ S\ MS}{E\ MS}$
	Pooled error (E)	T (R - 1) (B - 1) (S - 1)	E MS	

[a] Trial is considered as a fixed variable

Table S2: SPAD readings at the early and late harvest stages. Means within a line with different letters are significantly different according to Tukey's post-hoc test ($p < 0.05$, $n = 3$–9)

Season	Sowing date	Measuring date	SPAD [dimensionless]					
			Trt. 1	**Trt. 2**	**Trt. 3**	**Trt. 4**	**Trt. 5**	**Trt. 6**
Spring	Mar. 20, 2018	May 09, 2018	43.9 a	41.0 b	44.9 a	43.6 ab	n.a.	43.2 ab
		May 20, 2018	28.9 b	35.0 b	33.5 b	42.0 a	n.a.	29.5 b
	May 09, 2020	Jun. 13, 2020	34.5 a	33.8 a	34.1 a	34.2 a	33.5 a	33.2 a
		Jun. 23, 2020	27.4 bc	29.3 ab	27.0 c	30.2 a	29.6 a	25.4 c
	Jun. 04, 2019	Jul. 05, 2019	50.1 a	47.4 b	47.5 b	47.0 b	n.a.	46.6 b
		Jul. 15, 2019	43.8 a	44.6 a	43.2 a	44.6 a	n.a.	42.7 a
Summer	Jun. 14, 2018	Jul. 15, 2018	43.8 a	43.7 a	44.2 a	42.2 a	n.a.	44.8 a
		Jul. 25, 2018	41.5 abc	44.3 a	39.6 bc	43.4 ab	n.a.	38.5 c
	Jul. 07, 2020	Aug. 07, 2020	37.2 a	35.1 a	37.0 a	36.5 a	34.6 a	35.9 a
		Aug. 15, 2020	34.7 a	33.9 a	34.0 a	34.8 a	33.4 a	31.6 a
	Jul. 30, 2019	Sep. 01, 2019	36.6 a	36.3 a	35.4 a	35.4 a	n.a.	36.4 a
		Sep. 12, 2019	38.5 a	37.5 ab	37.9 ab	37.8 ab	n.a.	35.6 b
Autumn	Aug. 14, 2018	Sep. 23, 2018	42.8 a	42.0 a	39.4 a	39.4 a	n.a.	41.0 a
		Oct. 09, 2018	44.4 a	43.6 a	44.1 a	40.2 ab	n.a.	37.2 b
	Aug. 18, 2020	Sep. 19, 2020	42.0 a	41.0 a	42.2 a	40.7 a	41.6 a	41.4 a
		Oct. 05, 2020	43.1 ab	42.8 b	44.3 ab	42.5 b	46.0 a	42.8 ab
Winter	Sep. 28, 2018	Apr. 07, 2019	n.a.	n.a.	47.8 a	42.9 a	42.6 a	45.6 a
		Apr. 24, 2019	n.a.	n.a.	42.0 a	44.6 a	37.3 b	35.5 b
	Oct. 02, 2019	Apr. 12, 2020	n.a.	n.a.	50.8 a	46.8 b	47.2 b	46.9 b
		Apr. 25, 2020	n.a.	n.a.	40.5 bc	45.2 a	41.3 b	38.1 c

Trt. = treatment; n.a. = not available

Table S3: Number of bolting plants and stem length at the late harvest stage in late spring and late summer

Treatment	Late spring		Late summer	
	Bolting plants [n m^{-2}]	**SD [%]**	**Stem length [cm]**	**SD [%]**
1	119	4.2	30.0	10.4
2	154	9.1	31.0	8.7
3	131	12.1	32.1	11.1
4	136	9.7	32.0	7.2
6	137	8.2	31.0	8.5

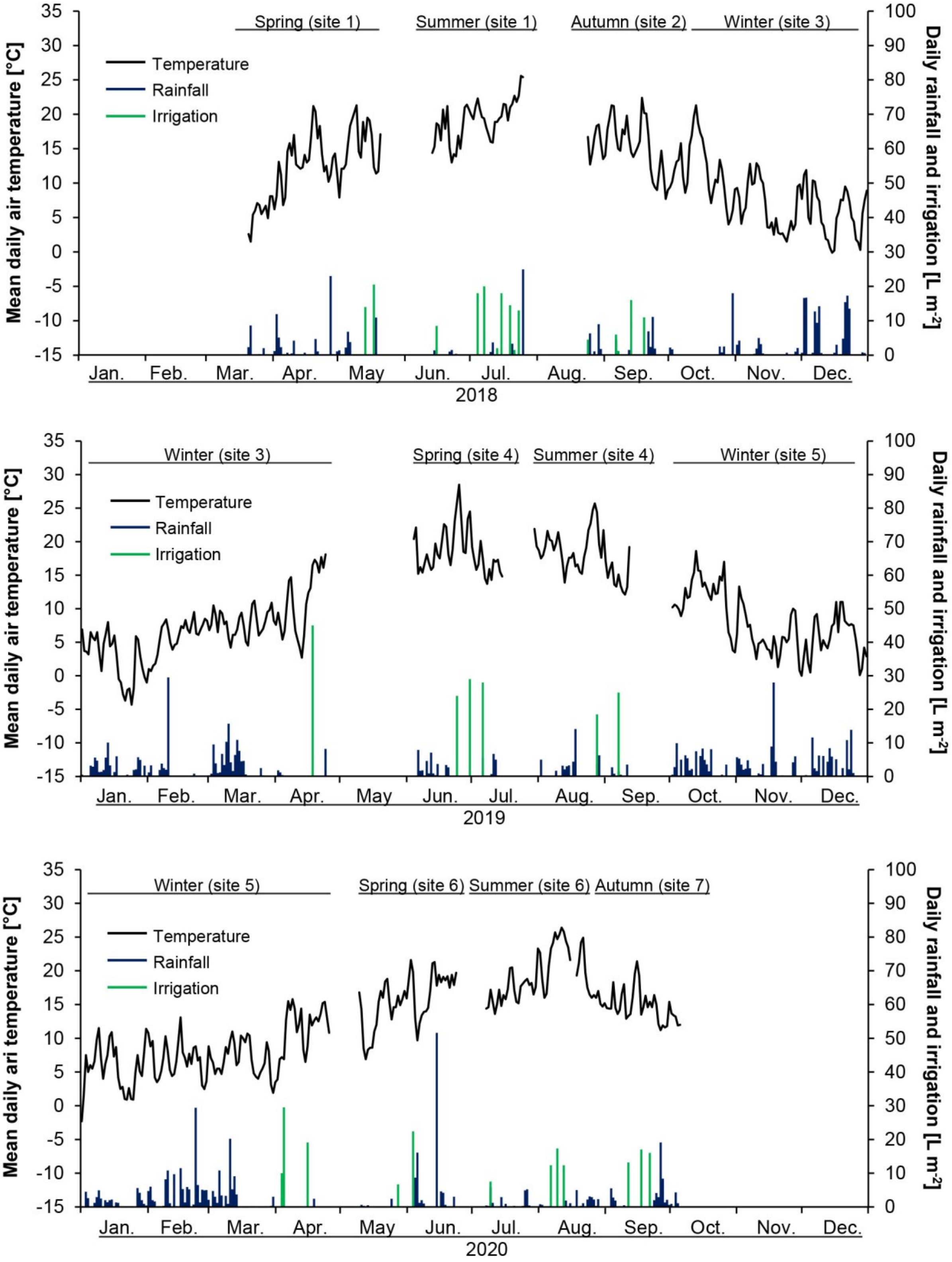


Figure S1: Mean daily air temperature (weather station Borken-Westphalia, Deutscher Wetterdienst, Germany) as well as rainfall and irrigation at the different experimental sites from 2018–2020

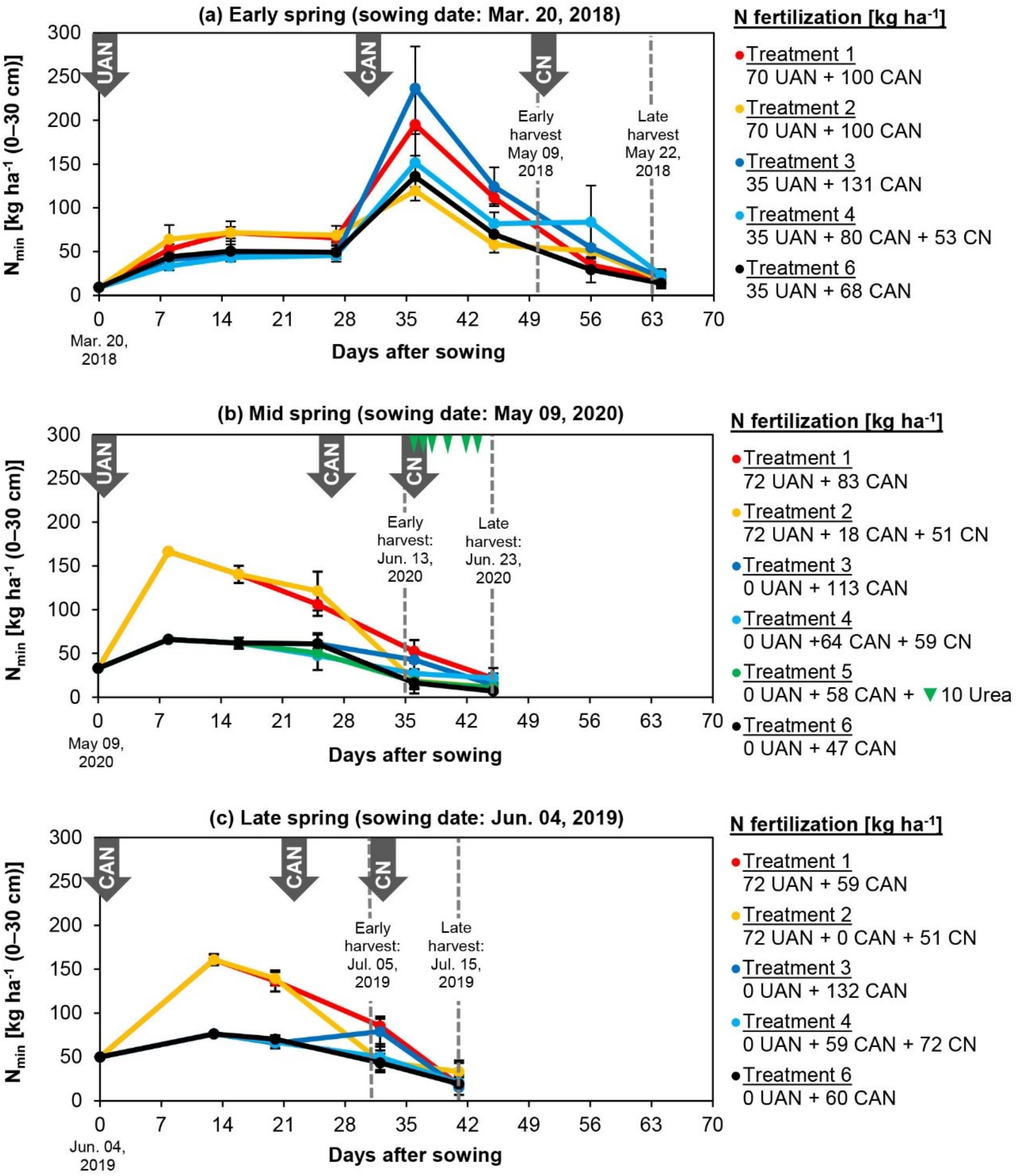


Figure S2: Soil mineral N concentration (NO_3^--N and NH_4^+-N) during the period of cultivation of early (a), mid (b), and late (c) spring-grown spinach at different N base fertilization and top dressing rates (*n* = 3; Mean ±SD). UAN = Urea ammonium nitrate; CAN = Calcium ammonium nitrate; CN = Calcium nitrate (Tropicote®)

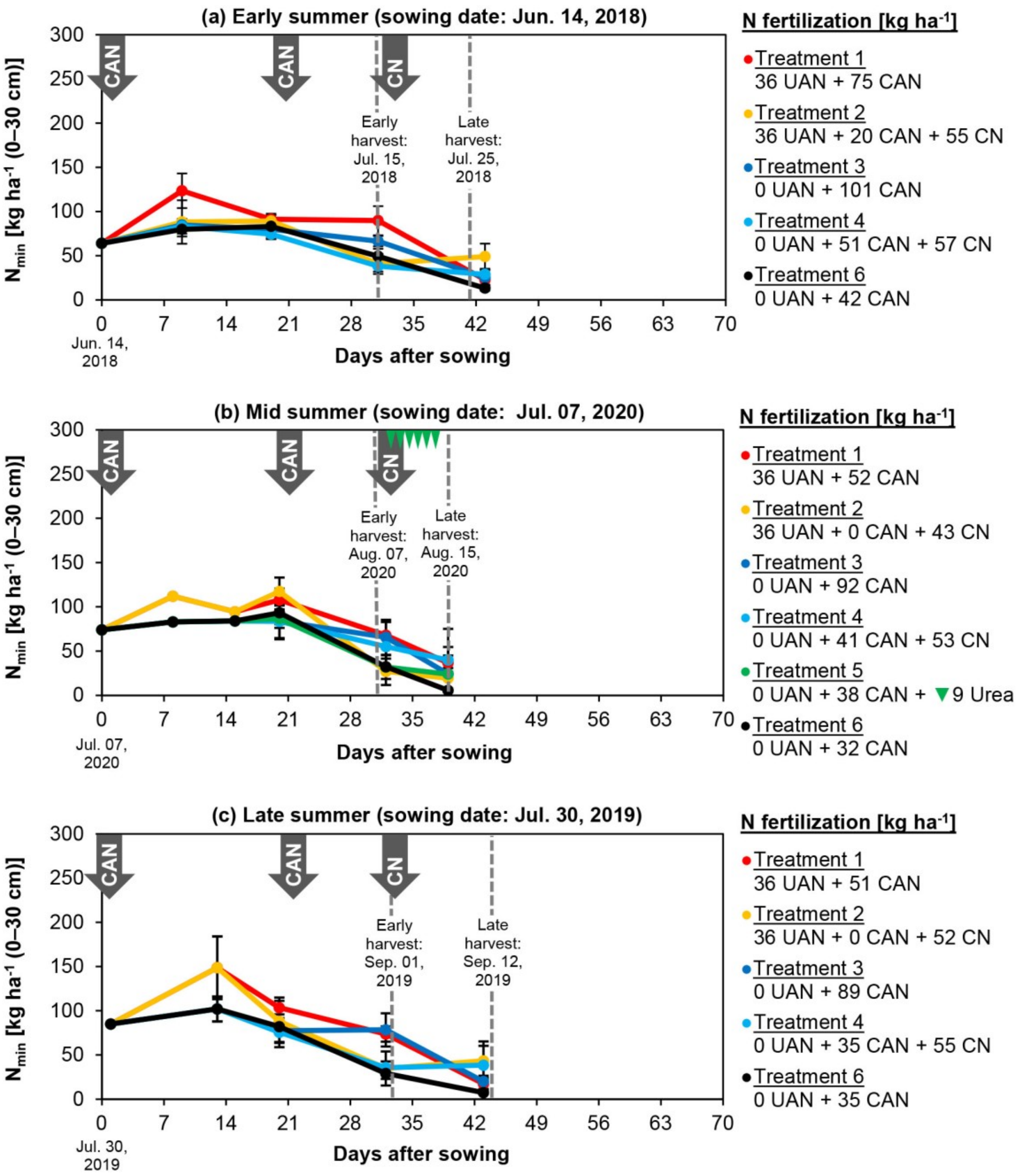
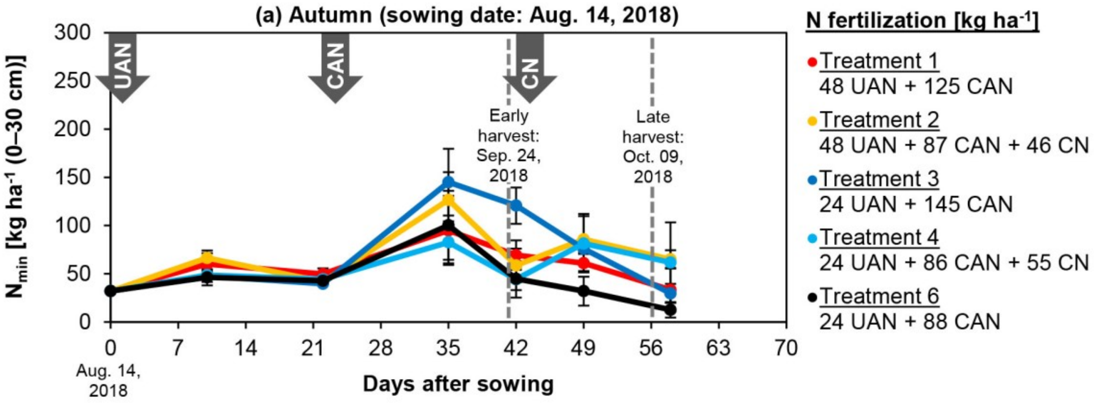
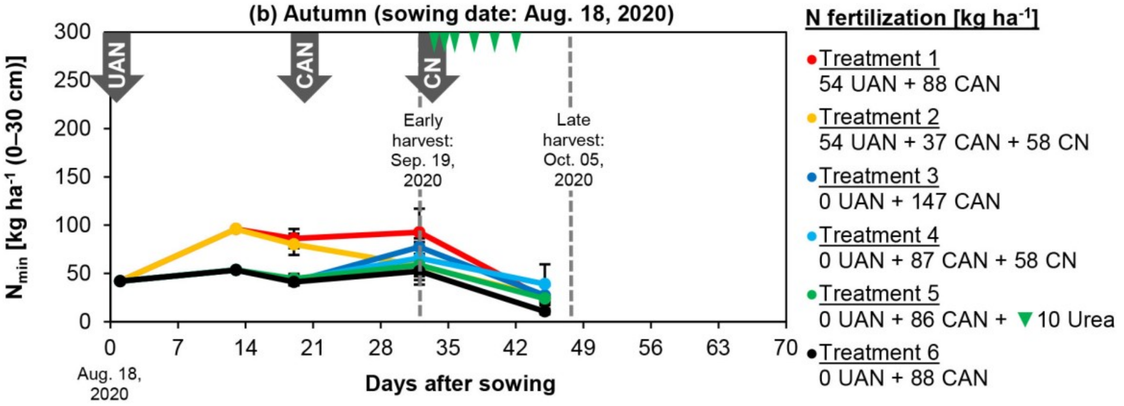
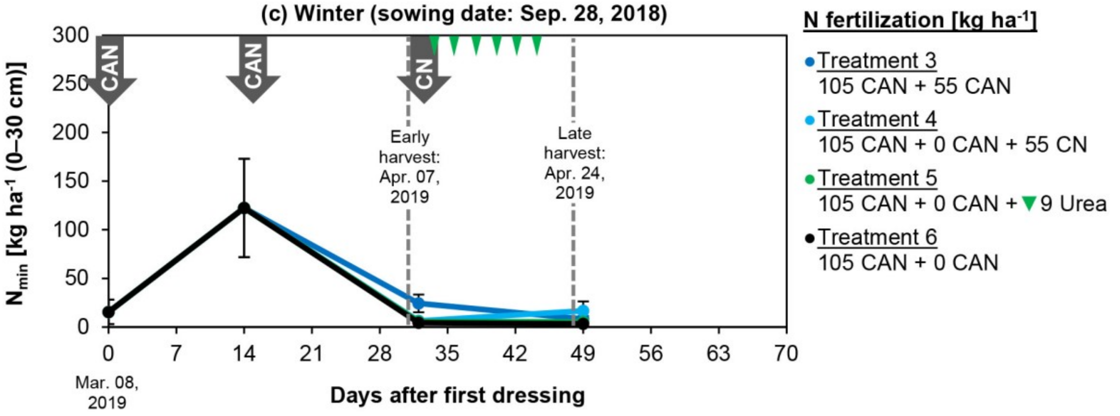


Figure S3: Soil mineral N concentration (NO_3^--N and NH_4^+-N) during the period of cultivation of early (a), mid (b), and late (c) summer-grown spinach at different N base fertilization and top dressing rates (*n* = 3; Mean ±SD). UAN = Urea ammonium nitrate; CAN = Calcium ammonium nitrate; CN = Calcium nitrate (Tropicote®)


(a) Autumn (sowing date: Aug. 14, 2018)


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


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

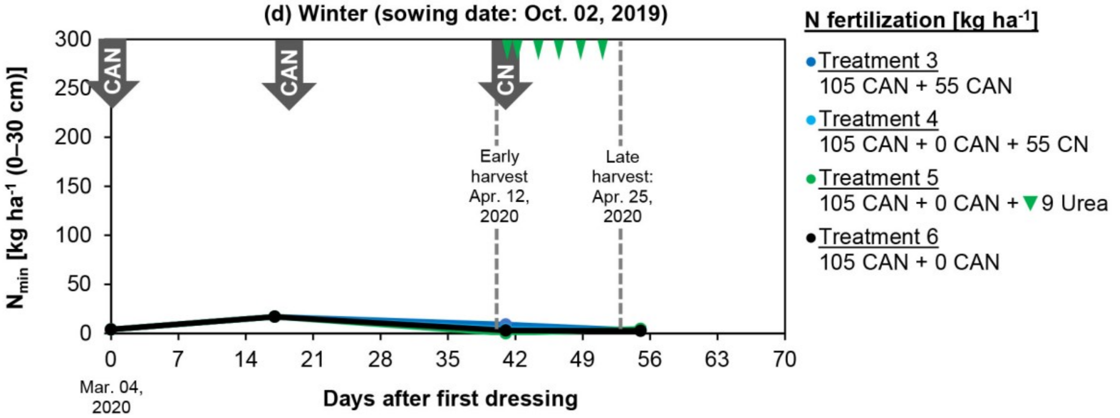

(d) Winter (sowing date: Oct. 02, 2019)

Figure S4: Soil mineral N concentration (NO_3^--N and NH_4^+-N) during the period of cultivation of autumn-grown (a, b) as well as winter-grown spinach (c, d) at different N base fertilization and top dressing rates (*n* = 3; Mean ±SD). UAN = Urea ammonium nitrate; CAN = Calcium ammonium nitrate; CN = Calcium nitrate (Tropicote®)

2.2 Crop residue management strategies to reduce nitrogen losses during the winter leaching period after autumn spinach harvest

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Citation:
Frerichs, C., Glied-Olsen, S., De Neve, S., Broll, G., Daum, D. (2022b): Crop Residue Management Strategies to Reduce Nitrogen Losses during the Winter Leaching Period after Autumn Spinach Harvest. Agronomy 12(3), 653. doi: 10.3390/agronomy12030653

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

Authors contributions:
Christian Frerichs: conceptualization, data curation, investigation, visualization, and writing original draft. Stephan Glied-Olsen: review and editing. Stefaan De Neve: review and editing. Gabriele Broll: review and editing. Diemo Daum: conceptualization, supervision, review, and editing.

Acknowledgements:
The authors gratefully acknowledge the Chamber of Agriculture North Rhine-Westphalia (Borken, Germany), iglo GmbH (Reken, Germany), and Rheinisch-Westfälische Wasserwerksgesellschaft mbH (RWW) (Mülheim, Germany) for their advisory support and financial participation in the European LEADER program 33.4 L1-3. The authors also acknowledge all farmers for providing their fields for the trials (Verein zur Förderung des Feldgemüseanbaus Westmünsterland e.V., Borken, Germany). Furthermore, the authors would also like to thank Stephan Dinklage, Osnabrück University of Applied Sciences, for his support in the laboratory investigations. We also acknowledge support by Deutsche Forschungsgemeinschaft (DFG) and Open Access Publishing Fund of Osnabrück University.

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.

Introduction

In regions with intensive vegetable production, the maximum permissible nitrate (NO_3^-) concentration of 50 mg L^{-1} groundwater is often exceeded (Council of the European Communities, 1991; European Commission, 2000; Tei et al., 2020). Nitrate leaching losses occur particularly when vegetables are grown on sandy sites, as is often the case with crops such as spinach (De Haan et al., 2009; Massa et al., 2018; Nett et al., 2015). 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 (Benincasa et al., 2017; Agostini et al., 2010). The off-season should specifically be considered in such a system approach (Congreves and von Eert, 2015).

Spinach (*Spinacia oleracea* L.), cultivated for the processing industry, is typically grown in frequent sowings and harvested from April to late October (Frerichs et al., 2022a). The crop is typically grown on sandy soils because this facilitates specific field management and reduces the impact of variable weather conditions on yield (Frerichs and Daum, 2021; Massa et al., 2018; Nett et al., 2015). 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 (Feller et al., 2011; Massa et al., 2018). 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 (Berbel and Martinez-Dalman, 2021; Vandecasteele et al., 2016). Particularly at the end of the growing season, N uptake of spinach can be reduced due to decreased solar radiation (Breimer, 1982; Gent, 2016). 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 (De Neve, 2017). 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 (Frerichs et al., 2022a; Heins, 1989).

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 (Choudhury and McRoberts, 2020; Congreves and von Eert, 2015; Lamichhane et al., 2017). 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 (De Neve et al., 1994; De Neve and Hofman, 1998; Whitmore, 1996). 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 (Agneessens et al., 2014a). 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) (Rather, 2013; Van de Sande et al., 2013; Van Dijk and Smit, 2006; VLM, 2008; VLM, 2009; VLM, 2010; Zemek et al., 2020). During the succeeding winter leaching period, the remaining NO_3^- is leached to below 120 cm in sandy soils (De Neve, 2017). Thus, even deep rooting crops may be unable to take up this NO_3^- sufficiently in the following growing season (Kage, 2000; Thorup-Kristensen, 2002).

To achieve decreased mineralization after crops are grown in the autumn, strategies such as a shallower tillage depth (Van den Bossche et al., 2009) or a postponement of the tillage date from autumn to winter or even to spring may be appropriate (Guerette et al., 2000; Mitchell et al., 2000; Rahn, 2002; Schwarz et al., 2008). 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 (Heumann and Böttcher, 2004a; De Neve et al, 1996). Furthermore, spinach is able to re-sprout after harvest and thus continue to absorb nitrogen (Suzuki et al., 2019). 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 (Agneessens et al., 2014a). 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 (Chaves et al., 2007). In contrast, if N immobilization continues after the winter season, it may have a negative impact on the yield of the following crop (Congreves and Von Eert, 2015). 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 (Viaene et al., 2017). However, spinach crop residues often only contain around 30 kg N ha^{-1} (De Neve, 2017), 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 (Agneessens et al., 2014b). 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) (Chaves et al., 2006b). This approach also proved successful in field experiments (Chaves et al., 2006a). Compared to other nitrification as well as urease inhibitors, DMPP is effective at small concentrations and less prone to leaching (Chaves et al., 2006b; Nair et al., 2020; Scheurer et al., 2016). 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.

Material and methods

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) 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" (Umweltbundesamt, 2022). 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.

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 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 (Sep. 10, 2019 – Mar. 06, 2020)	**Trial 2 (Oct. 05, 2020 – Mar. 01, 2021)**	**Trial 3 (Oct. 09, 2018 – Mar. 13, 2019)**	**Trial 4 (Oct. 10, 2019 – Mar. 06, 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	6.8
	Clay [% (w/w)]	7.5	6.3	6.6	5.8
	Organic C [% (w/w)]	1.1	1.5	1.2	3.6
	C/N ratio	15.7	12.1	9.2	25.7
	Soil pH	6.0	5.6	5.7	5.2
Crop rotation details	Crop rotation	Spinach/Spinach	Triticale/Spinach	Barley/Spinach	Carrots/Spinach
	Total N fertilization [kg ha^{-1}]				
	Liquid manure	0	170	170	170
	Mineral fertilizers	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

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: Sep. 10, 2019)	**Trial 2 (harvest: Oct. 05, 2020)**	**Trial 3 (harvest: Oct. 09, 2018)**	**Trial 4 (harvest: Oct. 10, 2019)**
1	10 (Harrow)	Early autumn	n.a.	Sep. 16, 2019	Oct. 16, 2020	Oct. 13, 2018	Oct. 19, 2019
2	10 (Harrow)	Early autumn	DMPP[1]	Sep. 16. 2019	Oct. 16, 2020	n.a.	Oct. 19, 2019
3	30 (Plow + harrow)	Early autumn	n.a.	n.a.	n.a.	Oct. 13, 2018	n.a.
4	3–4 (Direct drilling)	Early autumn	n.a.	n.a.	Oct. 16, 2020	Oct. 26, 2018	n.a.
5	10 (Harrow)	Late autumn	n.a.	Dec. 02, 2019	n.a.	Nov. 23, 2018	Nov. 16, 2019
6	10 (Harrow)	Late autumn	DMPP[1]	n.a.	n.a.	n.a.	Nov. 16, 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 (*x 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.

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. (2016). 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 (VDLUFA, 2016). Soil total N content was analyzed according to DIN EN 16168:2012.

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 (2004b). 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.

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. (2018). 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 damage 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 (1990). 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.

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 (De Vries et al, 2021). 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 (D'Haene et al., 2018; Heins, 1989; Liu et al, 2006). In order to obtain the root N of the spinach crop residues at the 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 (Evans, 1970; McLenaghen et al., 1996; Sheng and Hunt, 1991; Vos and Van der Putten, 1997). In contrast, the mixture of radish, mustard, and rye, grown in trial 1, was at an advanced development stage in early March. For this mixture, a shoot N to root N ratio of 2 was assumed (McLenaghen et al., 1996; Vos and Van der Putten, 1997; Wahlström et al., 2015).

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).

Results

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.

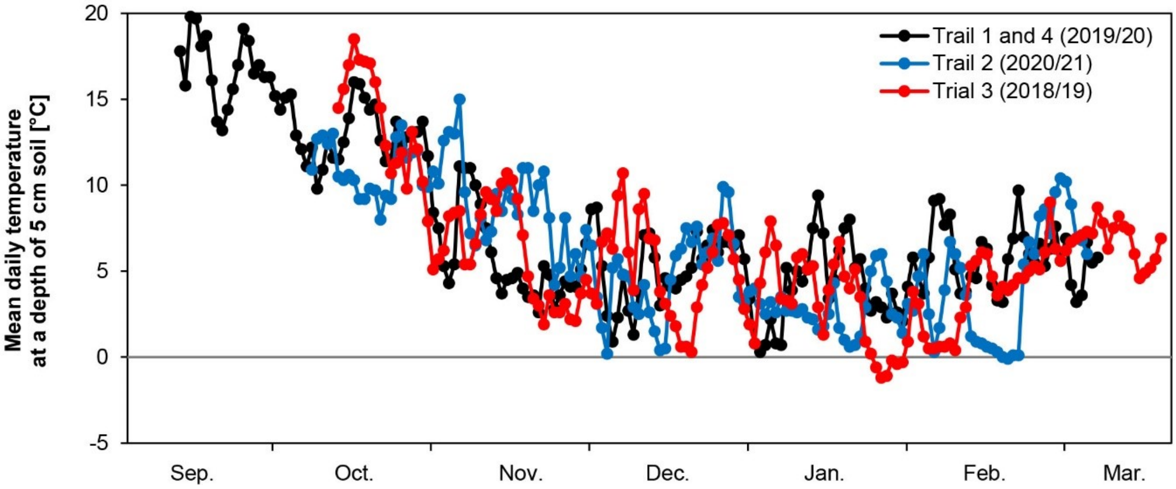


Figure 1: Mean daily temperature at a depth of 5 cm soil from spinach harvest until completion of the trials in March (weather station Borken-Westphalia, Deutscher Wetterdienst, Germany)

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.

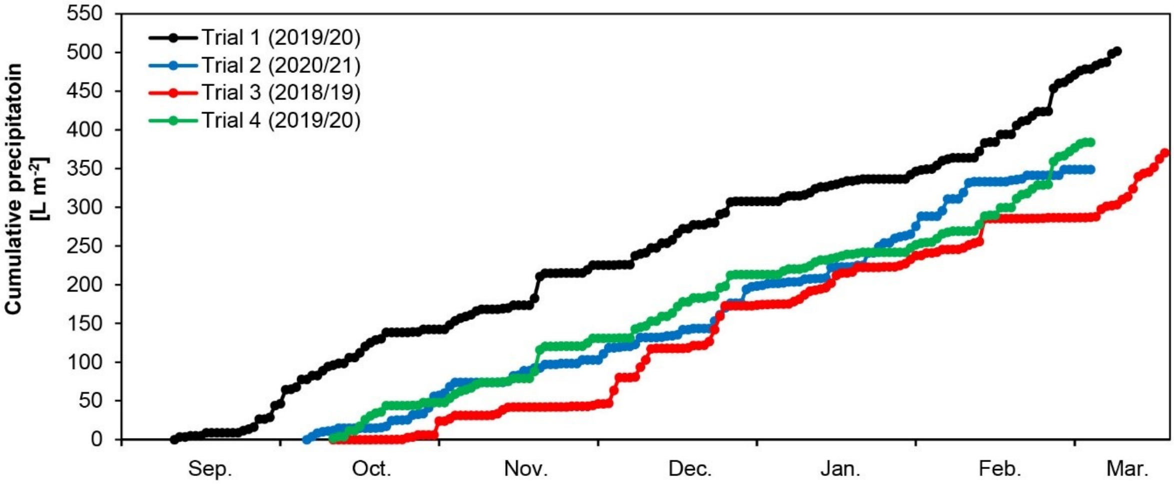


Figure 2: Cumulative precipitation from spinach harvest until completion of the trials in March (weather station Borken-Westphalia, Deutscher Wetterdienst, Germany)

Effects of the maximum tillage depth and frequency

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

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.

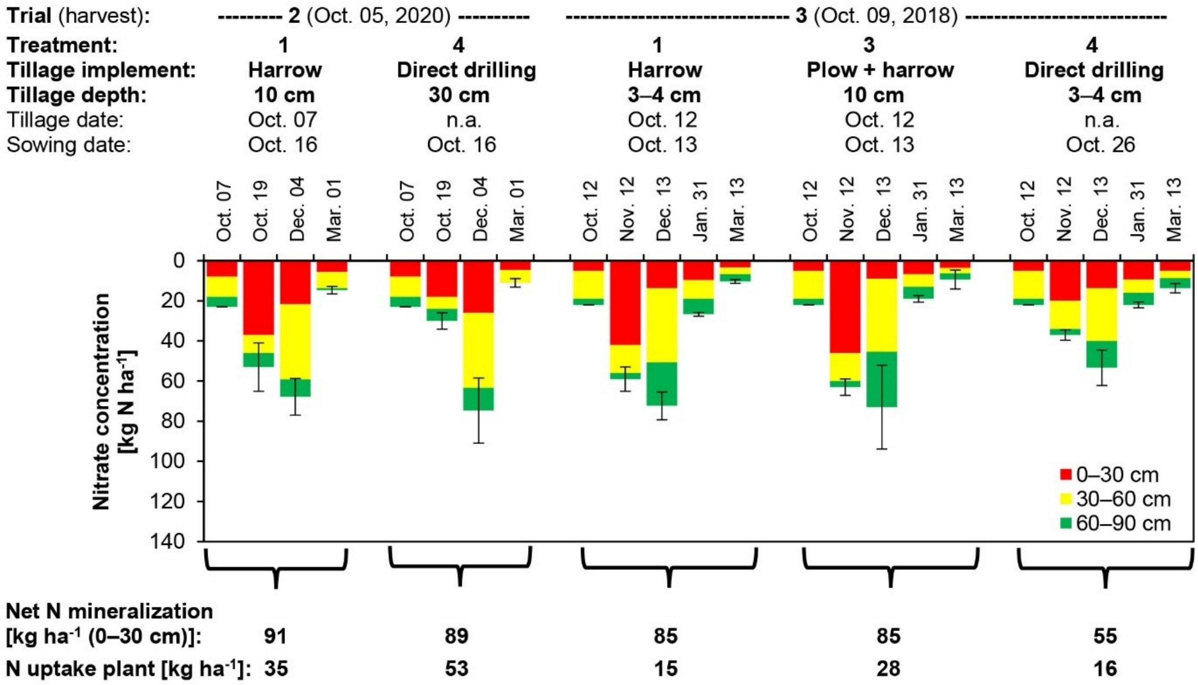


Figure 3: Soil mineral N concentration in the upper soil layers (0–30, 30–60, 60–90 cm) during autumn and winter seasons as well as net N mineralization (0–30 cm) and total N uptake by plants as a function of tillage depth after spinach harvest (*n* = 3; Mean ±SD). n.a. = not applicable

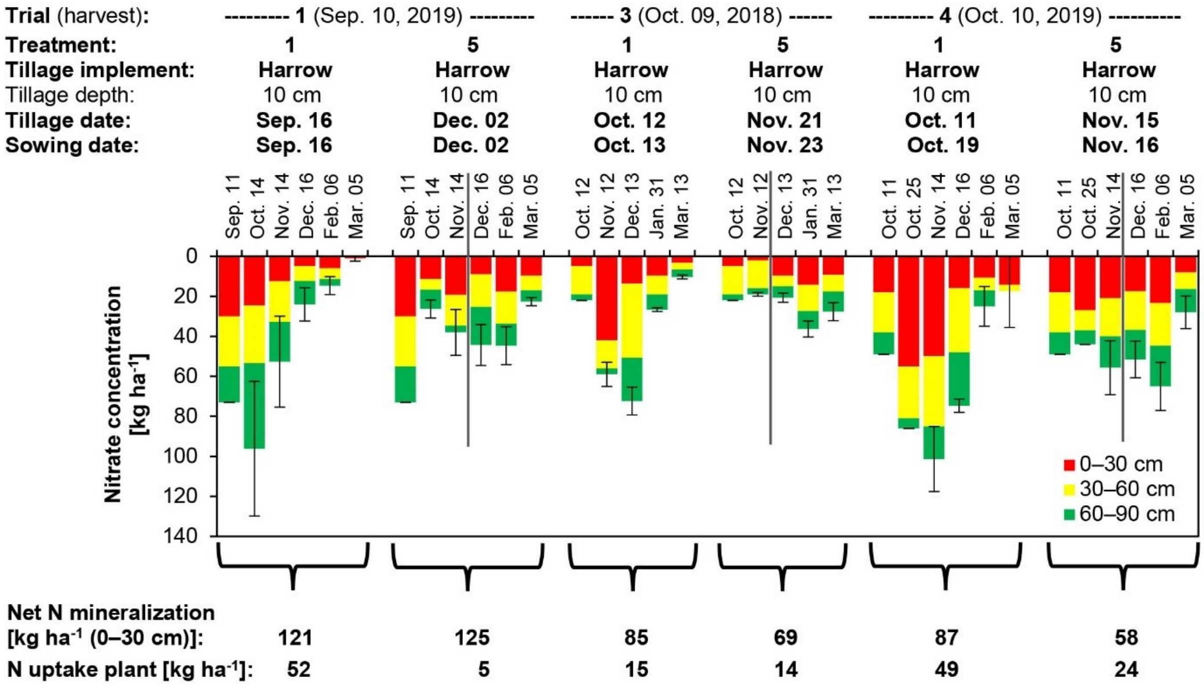


Figure 4: Soil mineral N concentration in the upper soil layers (0–30, 30–60, 60–90 cm) during autumn and winter seasons as well as net N mineralization (0–30 cm) and total N uptake by plants depending on the tillage date after spinach harvest. The gray vertical lines indicate the dates of the postponed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

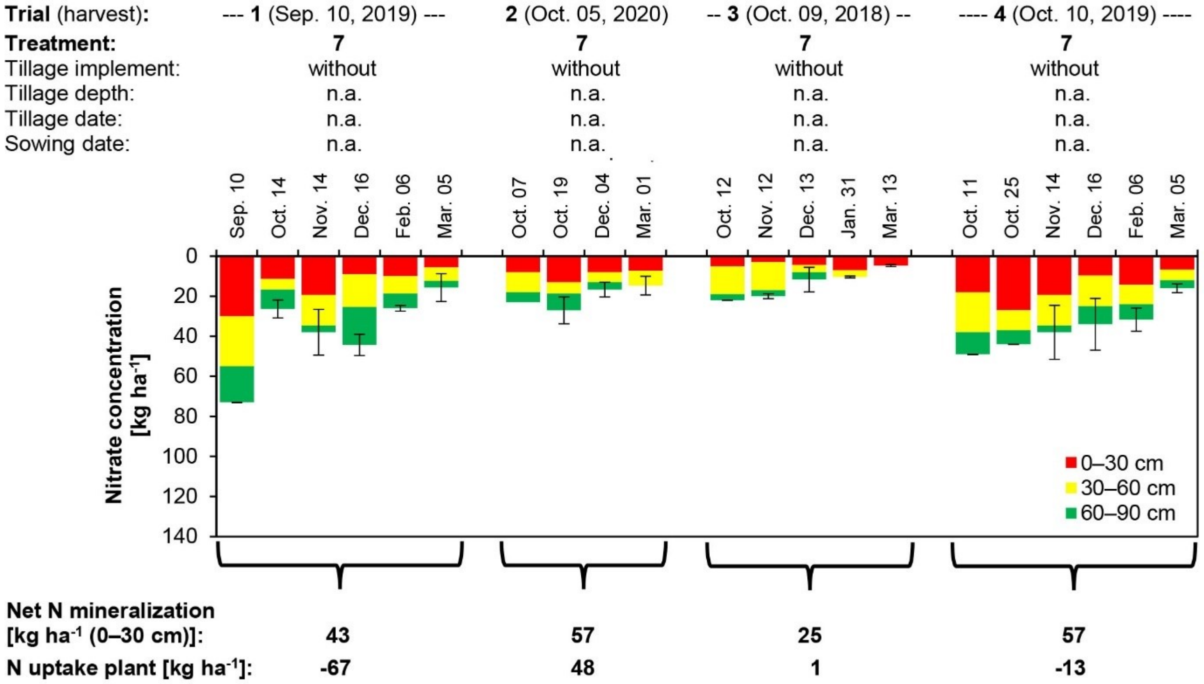


Figure 5: Soil mineral N concentration in the upper soil layers (0–30, 30–60, 60–90 cm) during autumn and winter seasons as well as net N mineralization (0–30 cm) and total N uptake by plants after spinach harvest without tillage until trials were completed in March (*n* = 3; Mean ±SD). n.a. = not applicable

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

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 December 02, 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.

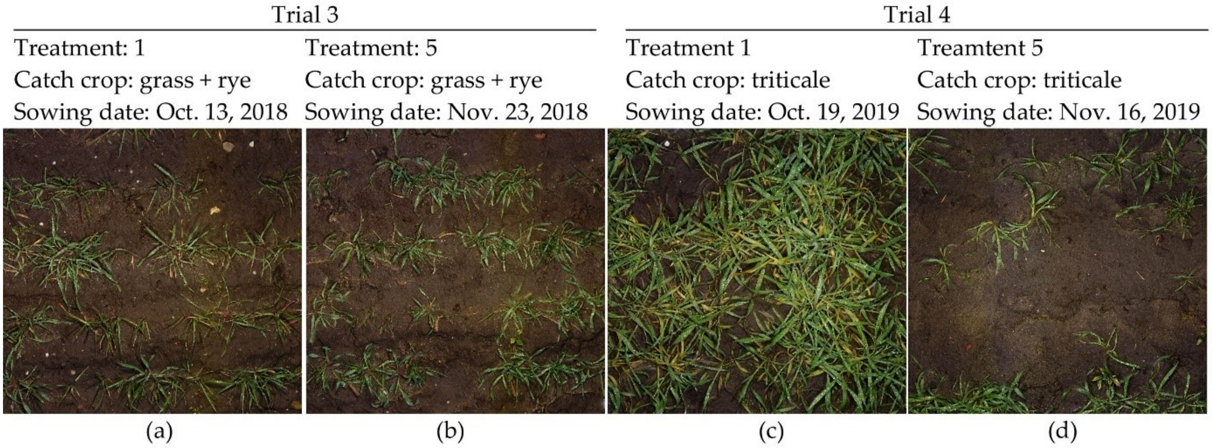


Figure 6: Aboveground biomass at the end of February depending on the catch crop species grass + rye (a, b) and triticale (c, d) as well as the sowing date in early (a, c) and late (b, d) autumn

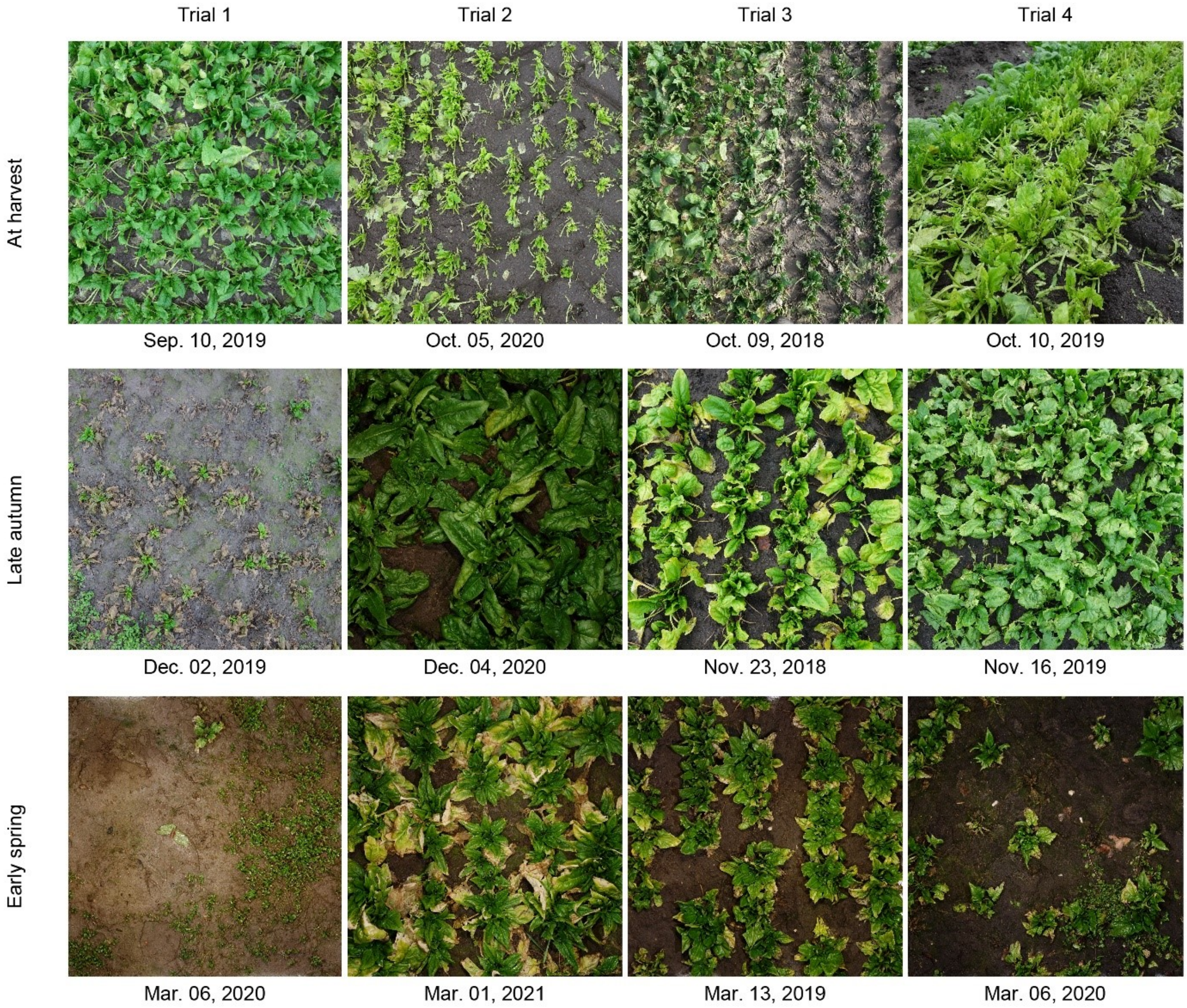


Figure 7: Spinach crop residues in treatment 7 depending on the season and trial

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 have been 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)

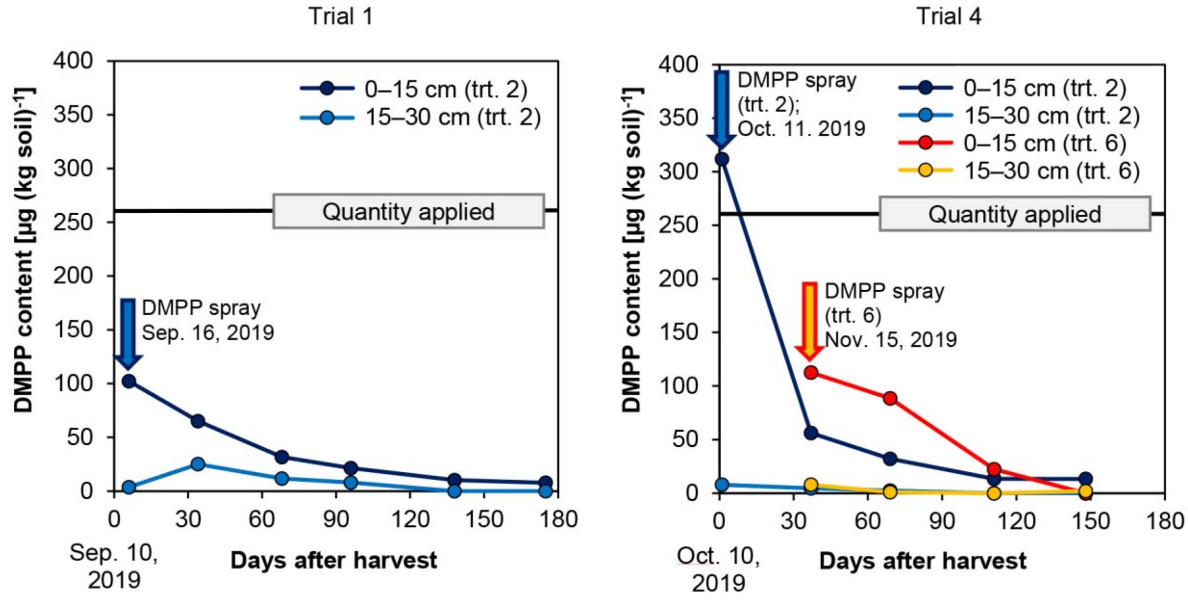
Treatment	**Tillage implement**	**Tillage depth [cm]**	**Tillage season**	**Disease severity index [%]**		
				Trial 1	**Trial 2**	**Trial 4**
1	Harrow	10	Early autumn	88 ±8	57 ±14	72 ±10
3	Plow + harrow	30	Early autumn	n.a.	n.a.	n.a.
4	Direct drilling	3–4	Early autumn	n.a.	50 ±6	n.a.
5	Harrow	10	Late 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

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 (**Figures 8** and **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.



(a) (b)

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

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. (2018) 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 (Frerichs et al., 2022a).

Aboveground crop residues contained 30–64 kg N ha^{-1} (**Table 1**), similar to levels of 25–62 kg N ha^{-1} reported earlier (Guerette et al., 2000; Neeteson and Carton, 2001; Niers, 1994; Zemek et al., 2020). 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. (2014b) and Whitmore (1996). After incorporating the aboveground autumn-grown spinach crop residues characterized by a C/N ratio of 9.6, De Neve et al. (1994) 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 (Chaves et al., 2004; Congreves et al., 2014). 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 (Rather, 2013; Van de Sande et al., 2013; Van Dijk and Smit, 2006; VLM, 2008; VLM, 2009; VLM, 2010; Zemek et al., 2020). 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 (Congreves and Von Eert, 2015). 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 (De Neve, 2017). 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 (Heumann et al., 2003; Heumann et al., 2011). 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**).

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. (2009) 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 (Curtin et al., 2014; Hansen and Djurhuus, 1997). 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 (Nett et al., 2016). This means that NO_3^- leaching after harvest seemed to be independent of post-harvest tillage intensity in sandy soils.

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 (Cookson et al., 2002; De Neve et al., 1996; Heumann and Böttcher, 2004a). 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 (Magid et al., 2001; Van Schöll et al., 1997). Furthermore, the N turnover rate can be increased, especially at fluctuating temperatures compared to constant incubation temperatures (Cookson et al., 2002). 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 (Cookson et al., 2002; Magid et al., 2001; Schwarz et al., 2008). 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 (Benincasa et al, 2017; Feaga et al., 2010; Thapa et al., 2018). 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. (2012) 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 (Thorup-Kristensen, 1994; Vos and Van der Putten, 2001; Weinert et al., 2002). Therefore, the removal of the aboveground crop residues before its decomposition begins is considered an effective measure to reduce winter N losses (Armbruster et al., 2013). 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 (Agneessens et al., 2014a).

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 (De Ruijter et al., 2010b; Velthof et al., 2002). However, Whitmore (1999) 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 (Pfab et al., 2011; Seiz et al., 2019). Most of this variation is due to the actual soil moisture content, as well as the N and C fractions in the soil (Chen et al., 2013; Zhu et al., 2013). However, differences due to tillage practice seemed to be insignificant following the incorporation of cauliflower or lettuce crop residues into sandy soils (Baggs et al., 2000; Nett et al., 2016). In contrast, the NH_3 volatilization of crop residues is often considerably reduced by their incorporation into the soil before their decomposition begins (De Ruijter et al., 2010a; Nett et al., 2015; Nett et al., 2016). However, if plant biomass decays on the soil surface, up to 5–16% of plant-N can be volatilized during winter (De Ruijter et al., 2010a). 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 (Nett et al., 2011).

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 (Nett et al., 2011; Thapa et al., 2018; Thorup-Kristensen et al., 2003; Vos and Van der Potten, 1997). 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 (Feaga et al., 2010). This is true for even the deep rooting *Brassica* species (Thorup-Kristensen et al., 2003). Despite low N uptake, catch crops mixtures including winter hard species are recommended after late harvest dates to ensure a soil cover during winter (Benincasa et al., 2017), thus reducing the risk of erosion, weed growth, and the survival of obligate diseases (Agneessens et al., 2014a; Neeteson et al., 1999). Agneessens et al. (2014b) 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 (Lamichhane et al., 2017). Therefore, spinach is usually only grown every four years on the same site in the region Borken (Pretty et al., 2008). 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 (1992), 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 damage 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.

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 (Rashti et al., 2017). 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) (Chaves et al., 2006b).

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 (Marsden et al., 2016). 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 (Vilas et al., 2019). In addition, DMPP only has an inhibitory effect on ammonium oxidizing *Bacteria* rather than on ammonium oxidizing *Archaea* or comammox *Nitrospira* (Kleineidam et al., 2011; Li et al., 2020). Especially at low soil pH, nitrification by *Archaea* can be considerable (Aigle et al., 2020; Lin et al., 2021). Thus, they can at least partially compensate for the reduced bacterial activity (Ouyang et al., 2017), 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. (2016), 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.

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 material

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)

Trial	Harvest date	DMPP application + tillage date	Ammonium/nitrate [kg N ha^{-1} (0–30 cm)]					
			Sep. 11, 2019	Oct. 14, 2019	Nov. 14, 2019	Dec. 16, 2019	Feb. 06, 2020	Mar. 05, 2020
1	Sep. 10, 2019	Sep. 16, 2019	< 2 / 30	6 / 32	< 2 / 15	< 2 / 5	< 2 / 6	< 2 / 1
4	Oct. 10, 2019	Oct. 11, 2019		* / 18	< 2 / 43	< 2 / 16	< 2 / 11	< 2 / 14
		Nov. 15, 2019			< 2 / 21	5 / 19	< 2 / 23	< 2 / 8

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)

Trial	Harvest date	DMPP application + tillage date	Ammonium/nitrate [kg N ha^{-1} (0–30 cm)]			
			Oct. 07, 2020	Oct. 19, 2020	Dec. 04, 2020	Mar. 01, 2021
2	Oct. 05, 2020	Oct. 07, 2020	< 2 / 8	2 / 34	< 2 / 24	< 2 / 6

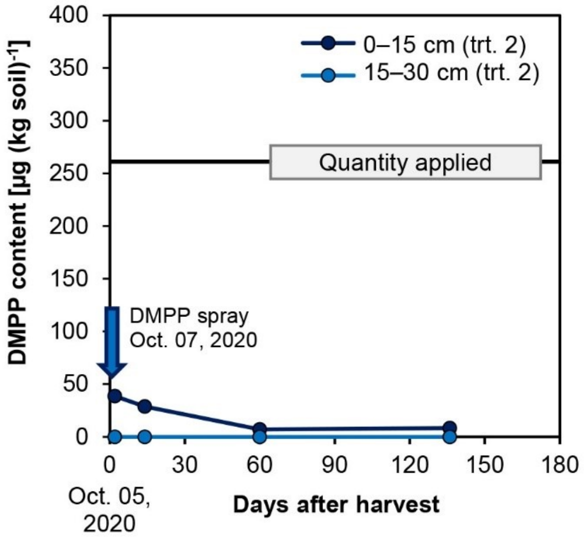


Figure S1: Content of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) within the upper soil layers (0–15 and 15–30 cm) after application to the spinach crop residues and subsequent harrowing into the soil in trial 2 (*n* = 1). Trt = Treatment

2.3 Ammonia and ammonium exposure of basil (*Ocimum basilicum* L.) growing in an organically fertilized peat substrate and strategies to mitigate related harmful impacts on plant growth

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Citation:
Frerichs, C., Daum, D., Pacholski, A. (2020): Ammonia and Ammonium Exposure of Basil (*Ocimum basilicum* L.) Growing in an Organically Fertilized Peat Substrate and Strategies to Mitigate Related Harmful Impacts on Plant Growth. Front. Plant Sci. 10, 1696. doi: 10.3389/fpls. 2019.01696

Keywords:
Pot-grown basil (*Ocimum basilicum* L.), organic cultivation, liquid amino acid fertilizer, ammonia and ammonium toxicity, substrate pH, nitrification, nitrate, mature compost

Authors contributions:
Christian Frerichs: conceptualization, investigation, methodology, formal analysis, validation, visualization, and writing original draft. Diemo Daum: conceptualization, supervision, review, and editing. Andreas Siegfried Pacholski: conceptualization, methodology, review, and editing.

Acknowledgements:
The authors gratefully acknowledge Klasmann-Deilmann GmbH (Geeste, Germany) for providing the substrate materials, fertilizers, and Dräger tubes® for the fertilization experiments. The authors would also like to thank Hans-Georg Schön, Osnabrück University of Applied Sciences, for his support with statistical analysis of the data.

Abstract

Organic pot-based production of basil (*Ocimum basilicum* L.) often has lower biomass yield than conventional cultivation. Previous investigations indicate that this growth impairment is related to high ammonium (NH_4^+) concentrations in the growing media released by the mineralization of organic nitrogen (N) fertilizers. However, as a result of this ammonification process substrate pH may also increase. Under neutral to alkaline conditions NH_4^+ is converted to ammonia (NH_3), which is known to be phytotoxic even at low concentrations. Therefore, we investigated the impact of both ammonical N species on basil grown in a peat substrate. In total three fertilization pot experiments were conducted in a greenhouse in order to compare the effect of different organic base dressings [250 and 750 mg N (L substrate)$^{-1}$ mainly supplied by a liquid amino acid fertilizer (AAF)] and two initial substrate pH levels (5.5 and 6.5). In two treatments 5% (v/v) mature compost was mixed into the peat one day and twelve days before the substrate was used for sowing, respectively. The aim of this procedure was to stimulate nitrification in this way to reduce ammonical N concentration. Ammonia concentration in the aerial plant surrounding environment was measured by using NH_3 detector tubes in combination with an open-top chamber method. The results showed that the growth of basil (number of plants, fresh matter yield, plant height) was significantly inhibited in the second and third week of cultivation by rising NH_3 and NH_4^+ exposures, as well as by a substrate pH ≥ 7.0. These adverse effects were reduced by lowering the organic base dressing rate and adjusting the initial substrate pH to 5.5. Furthermore, the addition of mature compost to peat in combination with a 12-day storage was proven to be effective for promoting nitrification in the organically fertilized substrate. As a result, plant growth was improved by both lower NH_3 and NH_4^+ exposures as well as a faster supply of nitrate (NO_3^-) as an additional N source. Using this approach, it was possible to feed organically fertilized basil right from the seedling stage with a NO_3^--N/NH_4^+-N-balanced and later on providing a predominant NO_3^--N supply.

Introduction

Along with the trend towards natural flavoring of food as well as a health-conscious nutrition, demand for potted herbs in many European countries is raising (CBI, 2016a). These plants grow in an organic substrate until use by the consumer, and thus provide optimal freshness as well as a longer shelf life than fresh-cut produce. Potted herbs make up about two-thirds of the total fresh herbs sales in Germany (Statista, 2018). Within this market segment basil (*Ocimum basilicum* L.) accounts for 50% of the turnover (CBI, 2016b). Pot-grown basil in greenhouses is produced throughout the year and about 26% of traded goods complies with the European regulations for organic farming (AMI, 2018).

Compared to conventional cultivation, organic cultivation of basil often leads to lower yield and quality. As early as one to two weeks after germination, cotyledons may become chlorotic and necrotic in organic production systems. During the winter period, these disorders are frequently accompanied by fungal diseases such as *Botrytis* (Frerichs et al., 2017). A previous investigation (Frerichs et al., 2019) showed it was possible to induce similar symptoms when basil was fed with NH_4^+ as sole N source, stabilized by a nitrification inhibitor. Even higher damage appeared following organic N fertilization. In this treatment, plants were exposed to particularly high NH_4^+ concentrations at the beginning of the cultivation period, because the added organic fertilizer was rapidly mineralized. In contrast, a NO_3^- or balanced mineral N supply with NH_4^+ and NO_3^- led to a significantly higher biomass production without any damage to plants. Similar effects of the N form on growth of basil were reported by Kiferle et al. (2013).

Overall, these results indicate that basil responds sensitively to high concentration of NH_4^+, as is well known for many plant species (Britto and Kronzucker, 2002).

Consequently, we hypothesized that growth impairments of basil in organic production are related to a temporary excessive NH_4^+ supply. Various mechanisms may contribute to this phenomenon. Beside toxicity of NH_4^+ itself (Liu and von Wirén, 2017) indirect effects such as NH_4^+-induced pH changes in the growing medium must also be taken into account. Roots release protons (H^+) when absorbing NH_4^+ to maintain a stable intracellular pH (Van Beusichem et al., 1988). Furthermore, the oxidation of one mole NH_4^+ to NO_3^- via nitrite (NO_2^-) by nitrifying bacteria generates two moles of H^+ (Sahrawat, 2008). Thus, fertilization with NH_4^+ promotes the acidification of the rhizosphere (Wiesler, 1997). However, following organic N fertilization, a transient pH increase by the ammonification process can occur before the resulting NH_4^+ is converted by nitrification (Niemiera et al., 2014; Vetanovetz and Peterson, 1990). With rising pH, the NH_4^+/NH_3 equilibrium shifts towards NH_3. Significant NH_3 formation starts from a pH of about 7.0 (Lægreid et al., 1999). Neutral to slightly alkaline conditions may arise in growing media containing substantial proportions of peat substitutes like composts and wood fibers. These constituents, common in organic production of pot plants, are relatively rich in calcium carbonate ($CaCO_3$) or have a weak buffer capacity against alkaline shifts (Neumaier and Meinken, 2015). High temperatures, typical in greenhouse cultivation, further favor the formation of NH_3 (Lægreid et al., 1999). Ammonia is known to be phytotoxic even at low concentrations, both in dissolved form in the rhizosphere and in gaseous form in the plant canopy environment (Krupa, 2003; Schenk and Wehrmann, 1979).

To separate effects of NH_3 and NH_4^+ on growth of organically fertilized basil it is necessary to monitor both compounds over the cultivation period. Diffusion and drift processes between closely adjoining plots can hamper the detection of the exact NH_3 exposure within the single plots (Gericke et al., 2011). Several approaches were developed to overcome these problems (Yang et al., 2018). A quick and easily implementable method to quantify the NH_3 volatilization even in small-scale crop units is the “Draeger-Tube-Method”, which is based on the use of NH_3 detector tubes in combination with a dynamic chamber (Pacholski et al., 2006; Pacholski, 2016). If only the NH_3 concentration in the ambient air is relevant, as in the present study, open-top chambers can also be used (Chen et al., 2012).

Given the current state of knowledge, we hypothesized that the pH of the growing medium and the dosage of the organic N base dressing are crucial factors affecting the exposure of pot-grown herbs to NH_3 and NH_4^+. An optimal initial substrate pH range is expected between 5.5–6.0 to avoid both significant NH_3 exposure under neutral to alkaline conditions (Lægreid et al., 1999) and a reduced activity of nitrifying bacteria under acid conditions (Lang and Elliott, 1991; Vetanovetz and Peterson, 1990). Concentration of ammonical N in ready-to-use growing media might be further reduced by a storage treatment for several weeks after mixing the organic fertilizer into the substrate. During this time, easily decomposable fertilizer compounds can be mineralized to a larger extent (Koller et al., 2004; Nair et al., 2011). To facilitate subsequent nitrification, the addition of mature compost might be useful as well (Delics et al., 2017). In this way, the substrate will be enriched with a well-established population of nitrifying bacteria (Chroni et al., 2009; Zeng et al., 2012).

Thus, the objective of the present study was to investigate the NH_3 and NH_4^+ exposures of pot-grown basil as affected by the amount of organic N applied with base dressing, the initial substrate pH as well as different storage treatments with and without addition of mature compost to a peat substrate. The following research questions were addressed: (a) To what extent is basil exposed to NH_3 and NH_4^+ when cultivated in organically fertilized peat-based substrates? (b) What impact does these NH_3 and NH_4^+ exposures have on the growth of basil? (c) Which practices may be suitable to minimize harmful NH_3 and NH_4^+ exposures in organic basil production?

Material and methods

In total three fertilization experiments were carried out from September 2016 to April 2017 in a greenhouse at the Osnabrueck University of Applied Sciences (**Table 1**). The first two trials focused on NH_3 and NH_4^+ concentrations in response to different levels of organic N base dressing and substrate pH conditions, as well as the development of young basil plants under these conditions. The herbs were cultivated in autumn and winter seasons for a period of 4 and 5 weeks after sowing, respectively. The third trial was conducted to evaluate the efficacy of compost amendments to decrease phytotoxic NH_3 and NH_4^+ exposures and high substrate pH under a high organic N base dressing level. Here, the plants were cultivated at the end of winter/beginning of spring for 8 weeks until they had reached a marketable size. In this way it was also examined whether plants are able to recover from high initial NH_3 and NH_4^+ exposures in the seedling stage. Basil growth and yield were determined by measuring the plant height and by harvesting the shoot biomass at weekly intervals. Simultaneously, key substrate parameters (pH, NH_4^+, NO_3^-, NO_2^-, and total water-soluble salt concentration) were analyzed and the NH_3 concentration in the canopy airspace was quantified with an open-top chamber approach as described below.

Plant material and experimental conditions

Basil (*Ocimum basilicum* L.) var. ˈEdwinaˈ was cultivated in 12-cm pots (0.6 L) filled with a peat substrate. In each pot 50 seeds were sown on the substrate surface and covered with a white fleece tissue. After 6 days seeds began to germinate and the cover was removed. In the first 15 days, plants were watered by overhead irrigation. Hereafter water and fertilizer solution were supplied by periodic flooding of the pot saucer. For irrigation and fertigation deionized water was used. Air temperature was adjusted to 16/18 °C (night/day). Between 6 a.m. and 8 p.m. the natural irradiance was supplemented by high pressure sodium lamps (44.3 $\mu mol\ m^{-2}\ s^{-1}$) when irradiance outside the greenhouse dropped below 370.4 $\mu mol\ m^{-2}\ s^{-1}$.

All experiments were performed in a randomized complete block design with three replications, each replication consisting of 25–36 pots. Every treatment and replication were positioned on a greenhouse table at a distance of 0.15–0.25 m from each other. Once a week two pots were taken from every plot according to a randomization plan. The samples were used to measure NH_3 concentration in the aerial environment of basil as well as destructively analyze plant and substrate parameters. After taking out these pots for the examinations, the remaining pots were positioned once again in a homogeneous square grid within the plot. Therefore, crop density decreased from 42 to 25 pots m^{-2} between week one and eight after sowing.

Substrate composition and treatment

The growing medium was based on a mixture of white and black peat [80% and 20% (v/v), respectively, Klasmann-Deilmann, Geeste, Germany] with a bulk density of 298 g L^{-1} and an initial N concentration of 9 mg NH_4^+-N (L substrate)$^{-1}$. It was limed according to the desired substrate pH levels. Besides the lime, the micronutrient fertilizer Radigen® (Terraflor GmbH, Iserlohn, Germany) and phosphorus (P) fertilizers were mixed into the substrate twelve days before sowing. The final substrate mix was loosely stored in a bucket at 18–22 °C with a moisture content adjusted to 49% and 47% (v/v) of the water capacity according to DIN EN 13041 (2012) in experiments 2 and 3, respectively. In experiment 1 substrates were used directly after mixing.

Adjustment of initial substrate pH

The substrate was limed with calcium carbonate ($CaCO_3$) to set a substrate pH of 5.5 (**Table 1**) at the time of sowing. A substrate pH of 6.5 was realized by the addition of calcium oxide (CaO). By this means the conditions for a low as well as a high potential NH_3 volatilization were realized.

Compost amendment

In the third experiment 5% (v/v) mature green waste compost was added to the peat substrate one day (treatment 9) or twelve days (treatment 8) before sowing (**Table 1**). The compost was characterized by a pH of 7.9 ($CaCl_2$ extraction), a total water-soluble salt concentration of 1.28 g KCl (L substrate)$^{-1}$ and a bulk density of 440 g (L substrate)$^{-1}$. Nitrate and NH_4^+ concentrations in the compost were 22 and 97 mg N (L substrate)$^{-1}$, respectively.

Nutrient demand and supply

According to the recommendations of Lindner and Billmann (2006) a total requirement of 1,125 N, 196 P, and 448 potassium (K) mg per L substrate was estimated for the whole growing period of organically cultivated basil. The contents of plant-available nutrients in peat and compost were taken into account when calculating the fertilizer demand.

Nitrogen supply

In the experiments 1 and 2 two organic N fertilization rates [250 and 750 mg N (L substrate)$^{-1}$] in combination with two initial pH levels (5.5 and 6.5) were set up to realize different NH_3 and NH_4^+ exposure levels (**Table 1**). The higher N dose corresponds to the upper fertilization range recommended for basil, which is fed solely by a base dressing. This limitation helps to prevent harmful effects on the seedling development due to high salt concentrations in the substrate, especially in the winter time (Eghbal, 2017). The lower N dose is common in organic basil production when the base dressing is complemented by repeated top dressings during the cultivation period.

Plant available N was supplied by a liquid amino acid fertilizer (AAF), manufactured from enzymatically hydrolyzed animal proteins [Fontana 9-0-0, MeMon B.V., Arnhem, Netherlands]. The batch used consisted of 8.8% (w/v) N and 54% (w/v) organic matter (OM). The C/N ratio in the fertilizer was 4. Previous incubation tests have shown that about 45% of the organic N was transformed to NH_4^+ within five days after mixing AAF into the peat substrate. Within an incubation time of 20 days no formation of NO_3^- by nitrification was observed. Hence, AAF proved to be a suitable N source for providing high exposure with ammonical N forms (NH_4^+ and NH_3) within the first weeks of plant cultivation. For base dressing a nutrient solution containing AAF was supplied at sowing. In experiment 3 additional top dressings were necessary and realized by repeated substrate drenches with the AAF solution [125 mg N (L substrate)$^{-1}$] at 45, 50, and 55 days after sowing. Corresponding to the plant fresh matter growth, which mainly determines the crop N requirement, between 250–375 mg N (L substrate)$^{-1}$ were supplied by top dressings. No top dressing was applied when herb growth was severely restrained (**Table 1**). In a control treatment, which was included in all experiments, nitrogen was supplied by calcium nitrate tetrahydrate [$Ca(NO_3)_2$ 4 H_2O pro analysi (Merck KGaA, Darmstadt, Germany) to achieve the maximum growth rate of basil under the respective trial conditions. From the literature it is well known that the biomass production of basil is optimal with predominant NO_3^--N supply (Kiferle et al., 2013; Frerichs et al., 2019). The total N fertilization rate in the control treatment was lower than in the organic treatments due to the fact that mineral N is completely phytoavailable whereas organic N fertilizers usually release not more than 60–70% of their total N content as mineral N (NH_4^+ and NO_3^-) within a period of about 8 weeks (Hartz and Johnstone, 2006; Stadler et al., 2006).

Table 1: Overview of the experimental setup in three fertilization trials. Treatments differed in initial substrate pH, N base dressing (BD), and N top dressing (TD) by using NO_3^- (control) or an amino acid fertilizer (AAF) as main component in the N supply. In addition, in experiment 3 treatments with compost addition and varying substrate storage periods were included

Experiment no.	Treatment no.	Initial substrate pH	N supply [mg L (substrate)$^{-1}$]	Compost addition and following substrate storage
1 and 2	1 (control)	6.0	200 (BD with NO_3^-)	Without compost
	2	5.5	250 (BD with AAF)	
	3	5.5	750 (BD with AAF)	
	4	6.5	250 (BD with AAF)	
	5	6.5	750 (BD with AAF)	
3	6 (control)	6.5	200 (BD with NO_3^-) 400 (TD with NO_3^-)	
	7	6.5	750 (BD with AAF) 0 (TD with AAF)	
	8	6.5	750 (BD with AAF) 375 (TD with AAF)	5% (v/v) compost was mixed into the substrate 12 days before sowing
	9	6.5	750 (BD with AAF) 250 (TD with AAF)	5% (v/v) compost was mixed into the substrate 1 day before sowing

Phosphorus (P) and potassium (K) supply

For P fertilization rock phosphate [12.7% (w/w) P], sieved with an ultra-centrifugal mill through a mesh screen of 200 µm, was used in the first two experiments. In the second experiment relatively small and dark green leaves were observed on plants of treatment 4 with an initial substrate pH of 6.5 and a base dressing of 250 mg N (L substrate)$^{-1}$. These symptoms might indicate the occurrence of P deficiency (Gibson et al., 2007). Therefore, in the third experiment the type of P fertilizer was changed to a micro-granulated bone meal [DCM ECO-FOS, 10.0% (w/w) P, 4.0% (w/w) N, Deutsche CUXIN Marketing GmbH, Otterndorf, Germany]. This P fertilizer is often used in organic basil production and was considered to maintain a sufficient P availability under these cultivation conditions. Potassium was supplied as sulfate (K_2SO_4 pro analysi, Merck KGaA, Darmstadt, Germany) via substrate drenches on the day of sowing.

Additional nutrient supply

To alleviate P deficiency, which appeared in the control treatment of experiment 3, all plants in this experiment received an additional drench with calcium dihydrogen phosphate monohydrate [$Ca(H_2PO_4)2 \times H_2O$] at a dosage of 17.4 mg P (L substrate)$^{-1}$ 33 days after sowing. In experiment 1 young leaves of basil plants in treatment 5 [initial substrate pH 6.5; 750 mg AAF-N (L substrate)$^{-1}$] showed yellowish discoloration in the last days of cultivation. These symptoms may indicate the occurrence of iron (Fe) deficiency (Bergmann, 1993; Gibson et al., 2007). To avoid related growth limitations micronutrient supply was complemented 2 and 3 weeks after sowing by overhead irrigation, each time with 50 mL per pot of 0.25 g L^{-1} Flory 10 (ProfiFlor GmbH, Pulheim, Germany) in experiments 2 and 3, respectively.

Substrate analyses and measurement of plant parameters

For the preparation of substrate samples, the entire substrate of two pots per plot and sampling date were homogenized and an aliquot portion of it was used for analysis. Substrate samples from organically fertilized treatments were taken on a weekly basis to determine NH_4^+, NO_3^-, and NO_2^- concentrations (extracted with 0.0125 M $CaCl_2$) as well as the substrate pH (0.01 M $CaCl_2$). The control treatments were sampled at the beginning and end of the trial period, in experiment 3 also at an intermediate date. Total water-soluble salt concentration was measured in experiment 1. In experiment 3, calcium chloride/DTPA (CAT) extractable P concentration was quantified. All substrate sample preparations were performed in accordance with guidelines of the Association of German Agricultural Analytic and Research Institutes e. V. (VDLUFA) (Hoffmann, 1997). The subsequent substrate analyses were conducted by using ion-selective electrodes to detect NH_4^+, substrate pH and total water-soluble salt concentration (Thermo Orion Standard Ammonia Electrode, Thermo Electron corporation, USA; Feld-pH-Meter, pH/cond 340i, Xylem Analytic Germany Sales GmbH & Co. KG, WTW, Weilheim, Germany). For NH_4^+ detection soil extract was buffered to pH > 11 by 2% ammonia ionic strength adjuster (Thermo Fisher Scientific, USA). Ion chromatography was used to detect NO_3^- (Compact IC plus 882, Deutsche Metrohm GmbH & Co. KG, Filderstadt, Germany), reflectometry to detect NO_2^- (RQflex® plus 10 and Reflectoquant® test strips, Merck KGaA, Darmstadt, Germany), as well as spectrophotometry (Specord 40-400189, Analytik Jena AG, Jena, Germany) to detect PO_4^{3-} based on the molybdenum blue reaction according to VDLUFA method A 13.1.1 (Hoffmann, 1997). Plant development was monitored by determining the number of plants, shoot fresh matter yield, and plant height (distance from substrate surface to the tip of the longest shoot) in each pot.

Aerial NH_3 measurements and related methodological evaluations

The NH_3 concentration in the aerial environment of pot-grown basil was determined by using an open-top chamber approach. The basic design and operation principles were adapted from a field measurement system described by Pacholski et al. (2006). Each measuring unit consisted of two chambers made from polyethylene (PE) pipes. Both chambers were connected by flexible PE tubes fixed by a manifold to a manual bellows pump (Accuro-Balgpumpe, Drägerwerk AG & Co. KGaA, Lübeck, Germany), which served as an air sample device (**Figure 1**). For NH_3 measurement, one basil pot was transferred in each chamber. The inlet side of the tubes was placed on the substrate surface (**Figure 2A**). Air samples were taken by using the hand pump and passed through a NH_3 detector tube (Dräger-Tube® Ammoniak 0.25/a, Drägerwerk AG & Co. KGaA, Lübeck, Germany) (**Figure 1**). By 10 pumping strokes, which require about 1 minute, 1,000 mL air was sucked through the pump and the indicator tube. The NH_3 concentration in the sampled air was immediately displayed on the scale of the detector tube by a blue reaction product. The readings were corrected afterwards to standard atmospheric pressure (1,013 hPa). Ammonia measurements took place twice a day: at sunrise and in the early afternoon to detect NH_3 at the lowest and highest daily temperature, respectively. Both measurements were averaged for further data analysis. With the described procedure, NH_3 concentrations between 0.250–3.000 ppm were detectable. By doubling the number of pumping strokes to 20, it was possible to achieve a measuring range of 0.125–1.500 ppm. To avoid cross contamination when performing NH_3 measurements, separate chamber systems for each treatment were used. Likewise, before each measurement, the sampling tubes were flushed with 500 mL air.

Preliminary investigations focusing on the reproducibility of the NH_3 measurements have shown that the coefficient of variation ranged from 8 to 12% when NH_3 concentration exceeded 0.250 ppm. At lower NH_3 concentrations, the coefficient of variation increased substantially. Under constant conditions in terms of temperature and irradiation the placing time (0–15 minutes) of the pots in the open-top chamber did not affect the detected NH_3 concentration. For further methodological evaluations, NH_3 enrichment in the canopy environment of basil was determined by two additional methods: a) acid traps used as passive NH_3 samplers and b) calculation approach based on the NH_3 and NH_4^+ equilibrium in the solution of the growing medium. The acid traps were made similar as described by Shigaki and Dell (2015) from plastic bowls (diameter: 10 cm) which were filled with 0.05 M H_2SO_4. To absorb atmospheric NH_3, the traps were positioned for 24 hours on the substrate surface (**Figure 2B**). A viscose pad placed in the plastic bowl was used to enhance the absorption capacity by a thicker surface layer. In the acid solution, NH_3 was converted immediately to NH_4^+. After dilution with demineralized water, NH_4^+ was detected by reflectometry (RQflex® plus 10 and Reflectoquant® test strips, Merck KGaA, Darmstadt, Germany). The calculation approach took into account the parameters air temperature, NH_4^+ concentration, and pH of the peat substrate, which were determined in parallel with NH_3 measurements. The theoretically formed NH_3 concentration in the solution of growing medium was estimated according to Hobiger (1996). Results obtained from both comparative methods were linearly correlated to the NH_3 concentrations determined with the open-top chamber technique as indicated by coefficients of determination (R^2, $p < 0.001$) of 0.75 (acid traps) and 0.67 (calculation approach).

NH_3 concentration in air samples of the control treatments (NO_3^- fertilization) was always below detection limit, even when NH_3 concentration in neighboring plots reached a maximum level of 1.8 ppm. In contrast, in acid traps placed on NO_3^--fed basil pots (control) a noticeable NH_4^+ accumulation was measured, which reached up to one third of the level in the organically fertilized plots. Therefore, we assume that the open-top chamber method used in our experiments was appropriate to prevent overlapping effects by NH_3 diffusion and drift processes within the arranged multi-plot set-up.

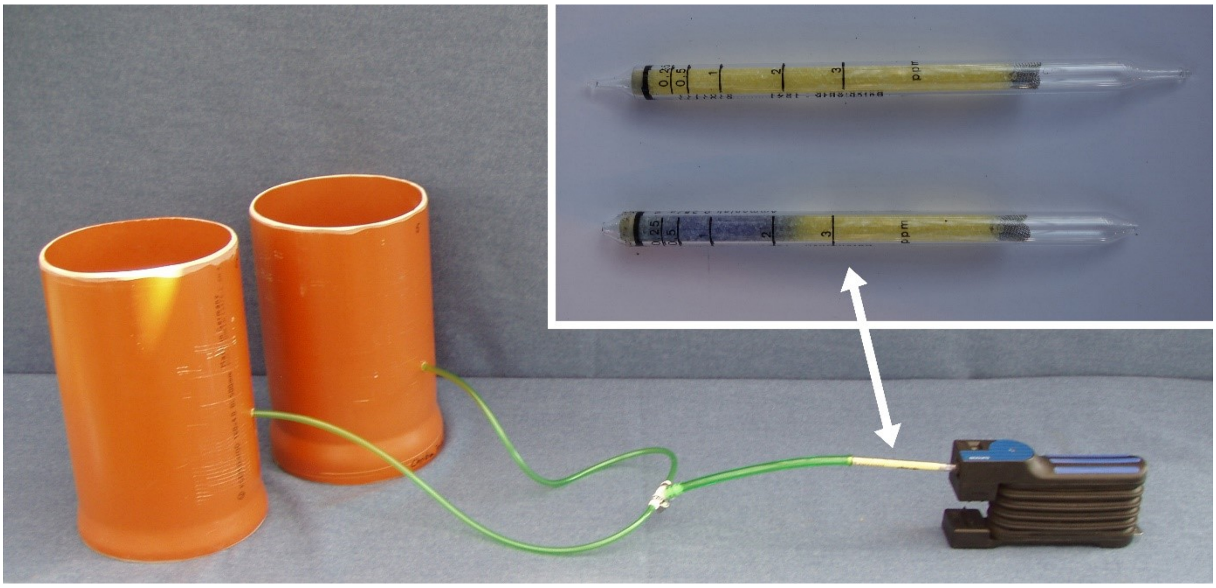

Figure 1: Ammonia measurement system consisted of open-top chambers which were connected with NH_3 sampling tubes, NH_3 detector tube, and manual bellows pump

Figure 2: Ammonia sampling tube placed on the substrate surface inside the open-top chamber (A); Acid trap used as passive NH_3 samplers (plastic bowls with viscose pad) (B)

Statistical analysis

Plant growth parameters were analyzed by ANOVA and 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 Fmax test (Köhler et al., 2012), respectively. If needed, data were logarithmically transformed to meet normal distribution and homogeneity of variance. In experiments 1 and 2 the organic treatments were analyzed by two-way ANOVA (initial substrate pH x N base dressing rate). To compare the organic treatments with the NO_3^--fed control, a one-way model was used. Likewise, experiment 3 was analyzed by one-way ANOVA. Relationships between substrate properties and plant growth performance were examined by linear regression analysis. The same approach was used to assess the effects of the aerial NH_3 concentration on basil. All analyses were performed using the statistical software SPSS, version 25 (IBM Deutschland GmbH, Ehningen, Germany).

Results

Development of substrate parameters and aerial NH_3 concentration

Substrate NH_4^+ concentration

Right from the beginning of basil cultivation, NH_4^+ concentration rose in all treatments fertilized with an amino acid fertilizer (AAF). In experiment 2, seven days after sowing approximately 56% of the supplied organic N was detected in the form of NH_4^+ when substrate pH was initially adjusted to 5.5. In contrast, at pH 6.5 only 34–43% of the supplied organic N appeared as NH_4^+ (**Figure 3A**). In experiment 3, already on the day of sowing a high concentration of NH_4^+ was available in the growing medium (**Figure 4A**). Without compost amendment, the NH_4^+ concentration remained at the same level for about 4 weeks and then decreased continuously until the end of the experiment. In the peat-compost mixtures the nitrification process was accelerated and thus NH_4^+-N concentration decreased below 100 mg (L substrate)$^{-1}$ already 3–4 weeks after sowing. In experiment 3, the top dressings slightly increased the substrate NH_4^+ concentration in the last two weeks of cultivation.

Substrate pH

In the first week after sowing, substrate pH increased by 0.5–1.0 unit in organically fertilized treatments without compost amendment (**Figures 3B** and **4B**). Afterwards, substrate pH remained on a slightly alkaline level for about four weeks when initially set to pH 6.5. Subsequently substrate pH decreased steadily. In experiment 3, which had the longest experimental period (8 weeks of cultivation), pH reached 6.3. In pots amended with compost, substrate pH dropped below 7.0 already one or two weeks after sowing when using a pre-storage treatment of 12 days or 1 day, respectively. After a top dressing of AAF, substrate pH slightly increased from 6.0 to maximum 6.5 in the last two weeks of cultivation.

NH_3 exposure in the aerial environment of basil

Under high NH_4^+ concentrations and slightly alkaline conditions in the substrate, NH_3 concentration in the canopy airspace of basil ranged initially between 0.4–1.8 ppm (**Figures 3C** and **4C**). In the following weeks NH_3 concentration declined continuously even when NH_4^+ was still the predominant N source in the growing medium and substrate pH was above 7.0 (treatments 4, 5, and 7). In the first two experiments, NH_3 remained below the detection limit (< 0.125 ppm) throughout the whole crop period when the base dressing rate was limited to 250 mg AAF-N (L substrate)$^{-1}$ and substrate pH was initially adjusted to 5.5. Likewise, with a straight NO_3^- supply (treatments 1 and 6) no NH_3 was detected. The addition of 5% (v/v) mature compost to the organically fertilized peat accelerated the decrease of the NH_3 concentration, especially when the mixed growing medium was previously stored for 12 days (treatment 8). In this case, NH_3 had almost completely disappeared two weeks after sowing. However, when the compost-amended peat was stored for just one day (treatment 9) this NH_3 depletion was delayed by one week. Repeated top dressings of AAF-N (treatments 8 and 9) applied during the last 11 days of cultivation did not stimulate NH_3 volatilization from the substrate. Aerial NH_3 concentration was always below the detection limit in this period.

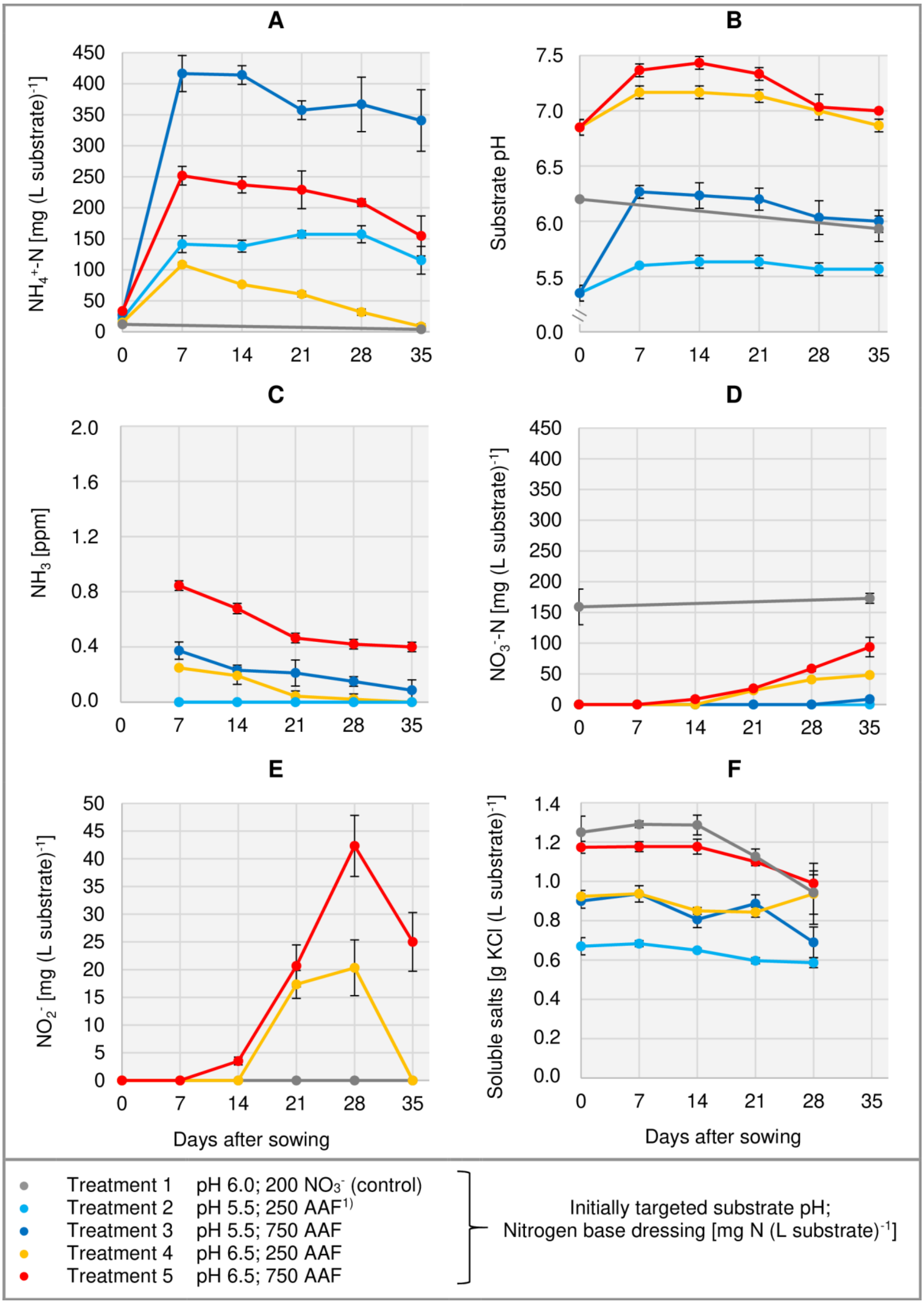

Figure 3: Development of substrate NH_4^+-N (A), NO_3^--N (D), and NO_2^- (E) concentration as well as substrate pH (B) and NH_3 concentration in the aerial environment of basil plants (C) over time in experiment 2. Total water-soluble salt concentration of the substrate in experiment 1 is shown in (F). Error bars indicate standard deviation (*n* = 3). $^{1)}$AAF, amino acid fertilizer (main component of the organic N supply)

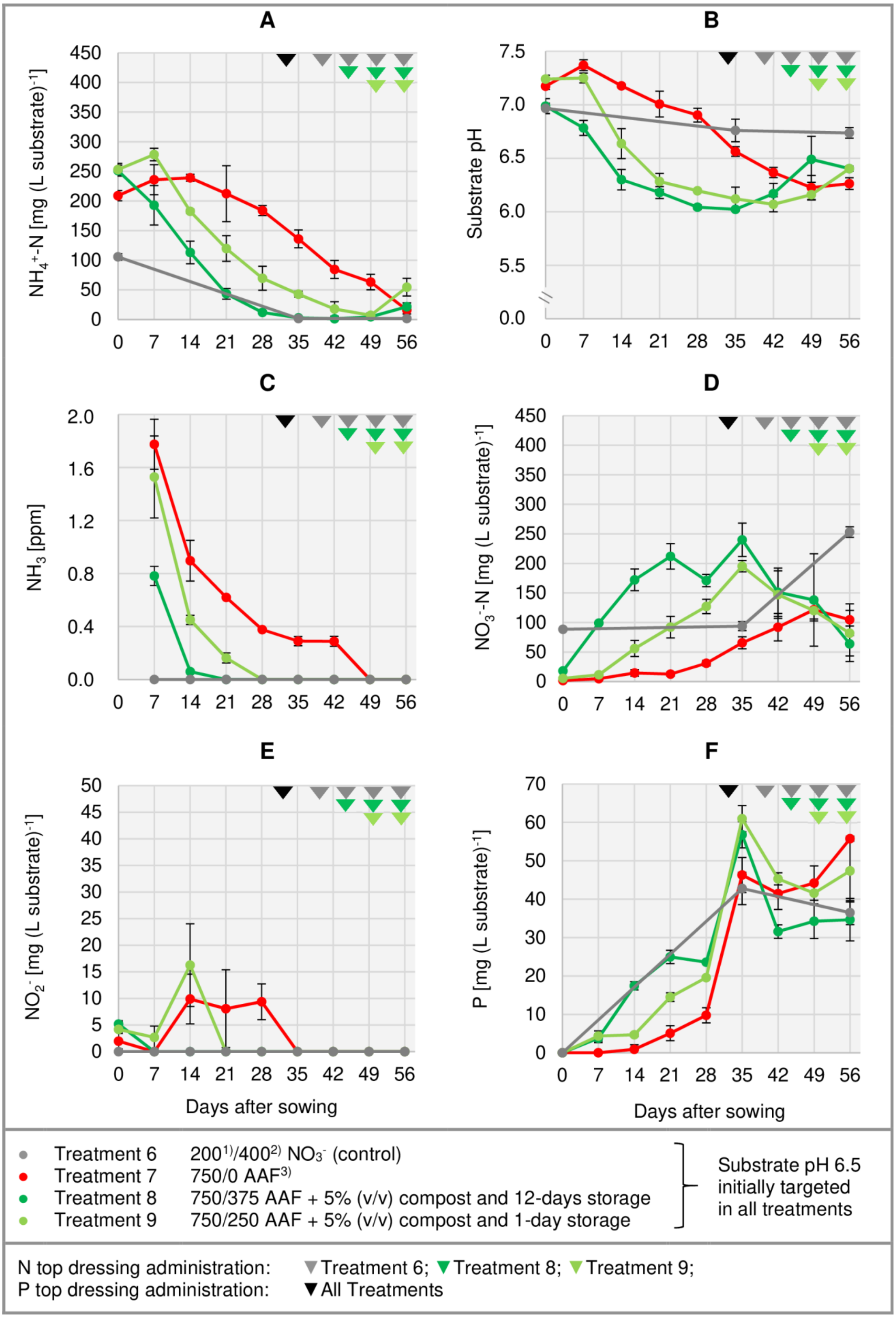

Figure 4: Development of substrate NH_4^+-N (A), NO_3^--N (D), NO_2^- (E), and P (F) concentration as well as substrate pH (B) and NH_3 concentration in the aerial environment of basil plants (C) over time in experiment 3. Error bars indicate standard deviation (*n* = 3). $^{1)}$N base dressing $^{2)}$N top dressing [mg N (L substrate)$^{-1}$]; $^{3)}$AAF, amino acid fertilizer (main component of the organic N supply)

Substrate NO_3^- concentration and NH_4^+-N/NO_3^--N ratio

The occurrence of NO_3^- in organically fertilized peat was also affected by the initial substrate pH. At pH 5.5, the NO_3^- concentration remained below 10 mg N (L substrate)$^{-1}$ for the entire trial period (**Figure 3D**). In contrast, with an initial pH of 6.5 the NO_3^- concentration rose above 50 mg N (L substrate)$^{-1}$. Nevertheless, even here 4–5 weeks had elapsed before this level was reached. Therefore, at the seedlings stage (7–21 days after sowing), basil was exposed to a high NH_4^+-N/NO_3^--N ratio. A much faster NO_3^- accumulation was observed when peat was mixed with mature compost and subsequently stored for 12 days before use (**Figure 4D; Table 4**). In this substrate the NH_4^+-N/NO_3^--N ratio was nearly balanced at the basil seedlings stage about 10 days after sowing. Another week later NO_3^- was the predominant N source in the substrate. If the storage time of the peat-compost blend was limited to one day, NO_3^- accumulation lagged two weeks behind and was less pronounced. At the end of the cultivation period, NO_3^- concentration in organically fertilized treatments ranged between 50 and 100 mg N (L substrate)$^{-1}$. A considerably higher level of NO_3^- was detected in the substrate of NO_3^--fed basil.

Substrate NO_2^- concentration

In addition to NH_4^+ and NH_3, NO_2^- is also a potentially phytotoxic N species. Its concentration in the organically fertilized substrate ranged between 0–42 mg (L substrate)$^{-1}$ and peaked 2–4 weeks after sowing (**Figures 3E** and **4E**) with the onset of an enhanced nitrification (**Figures 3D** and **4D**). In compost-amended peat NO_2^- accumulation was suppressed when the prepared growing medium was stored for 12 days. Likewise, NO_2^- concentration remained always below 2.5 mg (L substrate)$^{-1}$ (limit of quantification) in the control treatment with NO_3^- supply.

Total water-soluble salt and CAT-extractable P concentrations of the substrate

Total water-soluble salt concentration of the substrate (expressed as KCl) was measured in experiment 1 and ranged from 0.6 to 1.3 g (L substrate)$^{-1}$ (**Figure 3F**). In experiment 3 concentration of CAT-extractable P amounted to $\leq$ 25 mg (L substrate)$^{-1}$ during the first 4 weeks of cultivation. After a top dressing with calcium dihydrogen phosphate CAT-extractable P concentration rose and remained above 30 mg P (L substrate)$^{-1}$ until the end of the cultivation period (**Figure 4F**).

Effect of NH_3 and NH_4^+ exposures on the growth of basil

Germination of basil

Within the first week after sowing, radicles and germ buds emerged from seeds. In all treatments a homogeneous onset of germination was observed. However, subsequently seedling growth was stunted in treatments exposed to high NH_3 and NH_4^+ concentrations (**Figure 5**). The elongation of the radicle was inhibited and thus it did not grow into the substrate. Likewise, the development of hypocotyl and cotyledons was impaired. In addition, the primary leaves became partially chlorotic (**Figure 6E**). However, cotyledons stayed green and were free from necrotic symptoms. Three weeks after sowing, most of the stunted seedlings were dead. From the fourth week of cultivation, the number of plants remained constant. Therefore, results presented in **Tables 2–6** consider the mean NH_3 and NH_4^+ concentration in the juvenile stage of basil (7–21 days after sowing).

Compared to the NO_3^--fed control, the number of plants was reduced by up to 70% in treatments with a base dressing of 750 mg AFF-N (L substrate)$^{-1}$ at an initial substrate pH of 6.5 (**Tables 2** and **5**). The emergence of healthy plants was improved when substrate pH was initially adjusted to 5.5 and, to a much greater extent, by decreasing the base dressing rate to 250 mg AFF-N (L substrate)$^{-1}$. In this case, plant emergence rate was not negatively affected even at a higher pH level. Likewise, the addition of mature compost to peat substantially enhanced the proportion of healthy plants if the amended substrate was subjected to previous storage for 12 days. The number of plants was positively correlated to the NO_3^- concentration and negatively correlated to the NH_4^+-N/NO_3^--N ratio determined in the growing medium during the juvenile stage of plant growth (**Table 5**). When the ammonical N concentration exceeded 100 mg (L substrate)$^{-1}$ and 0.2 ppm NH_3 in the aerial plant environment, the number of plants was reduced by more than 10% compared to the NO_3^--fed control (**Table 6**). However, the adverse effect of NH_4^+ on seed germination was less pronounced as long as the NH_3 concentration remained ≤ 0.2 ppm.

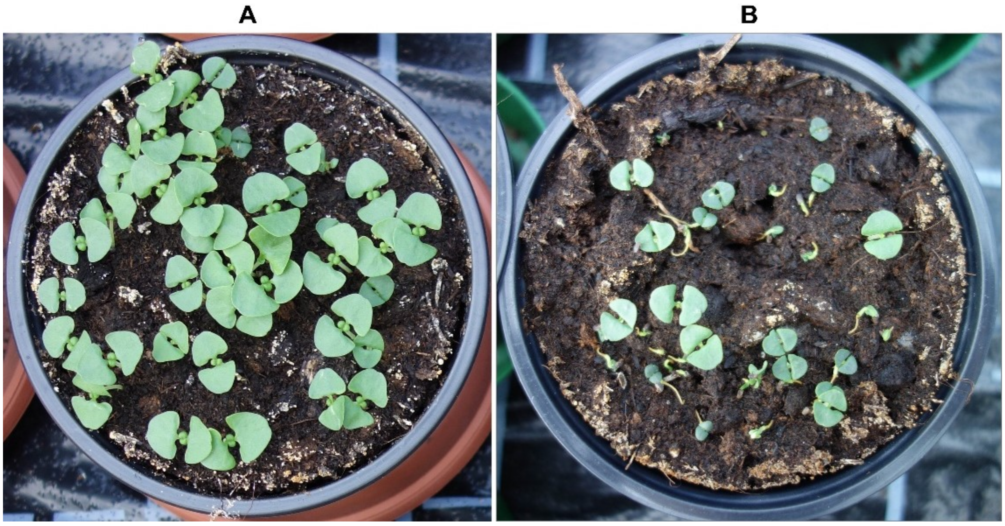

Figure 5: Typical basil seedlings 14 days after sowing in the NO_3^--fed control (A) and exposed to high NH_3 and NH_4^+ concentrations in treatment 7 (B)

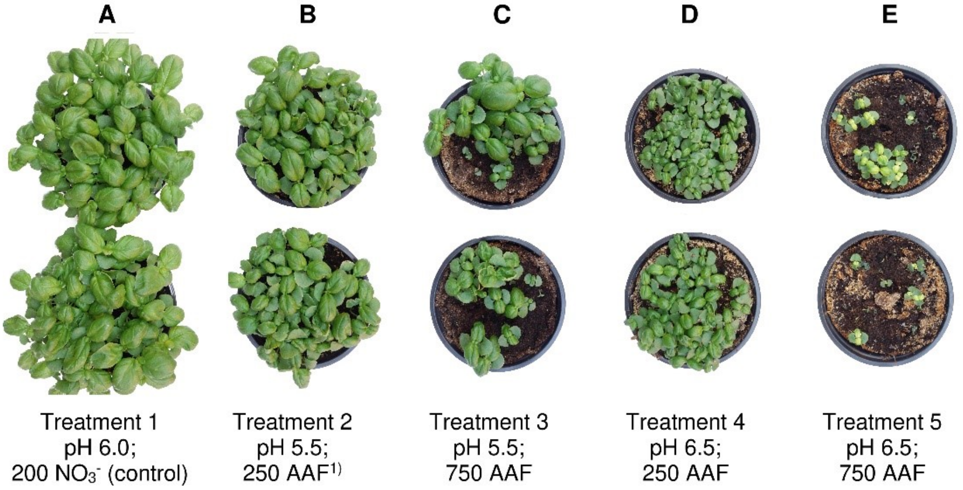

Figure 6: Basil plants 35 days after sowing in experiment 2. Nitrate-fed control (A); base dressing 250 mg AFF-N (L substrate)$^{-1}$ (B, D) and 750 mg AFF-N (L substrate)$^{-1}$ (C, E); initial substrate pH 5.5 (B, C) and 6.5 (D, E). 1)AAF, amino acid fertilizer (main component of the organic N supply)

Plant shoot biomass production and plant height

Growth impairment at the juvenile stage had a lasting negative impact on crop development. Shoot fresh matter yield was strongly reduced at the end of the experiments when basil grown in pure peat was fertilized with 750 mg AAF-N (L substrate)$^{-1}$ and/or substrate pH was adjusted to 6.5 before use (**Tables 2, 5,** and **Figures 6C–E**). ANOVA showed that crop yield was not significantly related to the number of plants ($p \geq 0.25$). Thus, biomass production of organically fertilized basil was adversely affected even beyond the impact of the reduction in plant numbers. Shoot elongation was also inhibited, as indicated by the decreased plant height. Furthermore, leaf blades remained smaller (**Figures 6D** and **6E**).

Both plant height and fresh matter yield of organically fertilized basil were negatively correlated to NH_3 and NH_4^+ exposures in the early cultivation period. Furthermore, plant growth was reduced by increasing substrate pH (**Tables 3** and **5**). Conversely, rising NO_3^- supply and declining NH_4^+-N/NO_3^--N ratio promoted crop performance (**Table 5**). Without noticeable NO_3^- formation in the first three weeks of cultivation, maximum growth of organically fertilized basil was observed with a base dressing of 250 mg AAF-N (L substrate)$^{-1}$ in combination with an initial substrate pH of 5.5. In this treatment NH_4^+ remained relatively constant at about 150 mg N (L substrate)$^{-1}$ (**Figure 3A**). With this N supply fresh matter yield and plant height were reduced by 8–34% compared to the NO_3^--fed control in the first two experiments (**Table 2**). In all other organic N fertilization treatments fresh matter yield and plant height were reduced by 44–94% and 31–66%, respectively.

Table 2: Number of plants, fresh matter yield and plant height 28 and 35 days after sowing as affected by exposure to ammonical N forms determined within 7–21 days after sowing in experiments 1 and 2, respectively (n = 3). Means within a column not sharing a letter are significantly different according to Tukey's test ($p < 0.05$)

Treatment[1]	Mean exposure (7–21 days after sowing)		Yield Parameter		
	NH_3 [ppm]	NH_4^+-N [mg L (substrate)$^{-1}$]	Number of plants [% of control]	Fresh matter yield [g pot^{-1}]	Plant height [cm]
			Experiment 1		
1 (control)[2] (pH 6.0/200 NO_3^--N)	< 0.1 c	10 e	100 a	10.8 a	5.5 a
2 (pH 5.5/250 AAF-N)	< 0.1 c	83 c	96 ab	7.2 b	3.8 b
3 (pH 5.5/750 AAF-N)	0.2 b	279 a	91 ab	6.1 bc	3.6 b
4 (pH 6.5/250 AAF-N)	0.1 bc	55 d	96 ab	3.1 cd	2.5 c
5 (pH 6.5/750 AAF-N)	0.6 a	148 b	88 b	2.5 d	2.5 c
			Experiment 2		
1 (control)[3] (pH 6.0/200 NO_3^--N)	< 0.1 c	< 5 e	100 a	10.6 a	6.1 a
2 (pH 5.5/250 AAF-N)	< 0.1 c	146 c	91 a	8.9 b	5.6 a
3 (pH 5.5/750 AAF-N)	0.3 b	396 a	66 b	3.6 c	4.2 b
4 (pH 6.5/250 AAF-N)	0.2 bc	82 d	88 a	3.9 c	3.6 b
5 (pH 6.5/750 AAF-N)	0.7 a	239 b	30 c	0.7 d	2.1 c

[1] Initially targeted substrate pH/Base dressing [mg N (L substrate)$^{-1}$]

[2] Mean concentrations detected 0 and 28 days after sowing

[3] Mean concentrations detected 0 and 35 days after sowing

Table 3: Analysis of variance results for effects of initial substrate pH and N base dressing on number of plants, fresh matter yield, and plant height in experiments 1 and 2 (28 and 35 days after sowing, respectively). Lower section of the table: Relationships between plant growth parameters and mean exposure to ammonical N forms as well as substrate pH in the organically fertilized treatments determined within 7–21 days after sowing indicated by direction (+/-) and coefficient of determination (R^2) for a linear regression model (*n* = 12)

Source of variance	Experiment 1			Experiment 2		
	Number of plants [% of control]	Fresh matter yield [g pot^{-1}]	Plant height [cm]	Number of plants [% of control]	Fresh matter yield [g pot^{-1}]	Plant height [cm]
Initial substrate pH	n.s.	**	**	**	***	***
N base dressing	*	n.s.	n.s.	***	***	***
Interaction	n.s.	n.s.	n.s.	**	**	**
	R^2 for a linear regression model					
NH_4^+ concentration	(-) 0.23 $^{\text{n.s.}}$	(-) 0.01 $^{\text{n.s.}}$	(-) 0.04 $^{\text{n.s.}}$	(-) 0.23$^{\text{n.s.}}$	(-)0.10 $^{\text{n.s.}}$	(-) 0.01 $^{\text{n.s.}}$
Substrate pH	(-) 0.07 $^{\text{n.s.}}$	(-) 0.80 ***	(-) 0.86 ***	(-) 0.33$^{\text{n.s.}}$	(-)0.70 **	(-) 0.81 ***
NH_3 concentration	(-) 0.36 *	(-) 0.35 *	(-) 0.30 $^{\text{n.s.}}$	(-) 0.93***	(-)0.92 ***	(-) 0.80 ***

Significance level: n.s. = not significant; **p* < 0.05; ***p* < 0.01; ****p* < 0.001

In experiment 3, maximum plant growth was observed when basil was grown in a compost-amendment peat that had previously been stored for 12 days (**Table 5**). In this treatment basil reached a plant height of 15 cm, which is required for marketing of the produce, 7 weeks after sowing. However, when the prepared compost-peat substrate was stored for just one day, the marketable crop size was achieved with a delay of one week (data not shown). Without compost addition plant height reached to 7.5 cm after eight weeks of cultivation, compared to 9.7 cm for the control. The relatively weak growth performance of NO_3^--fed plants in this experiment, was related to a temporary shortage in P supply during the first half of cultivation. Phosphorus deficiency symptoms became visible in the control treatment about four weeks after sowing, when cotyledons turned purple and shoot elongation lagged behind. Although a substrate drench with a water-soluble P fertilizer was conducted soon afterwards, plant growth remained distinctly lower than usually observed with N supplied as NO_3^- (e.g. in experiment 2, **Figure 6A**).

Table 4: Ammonia, NH_4^+-N, and NO_3^--N concentration as well as NH_4^+-N/NO_3^--N ratio determined within 7–21 days after sowing in experiment 3 (*n* = 3). Means within a column not sharing a letter are significantly different according to Tukey's test (*p* < 0.05)

Treatment[1)]	Mean exposure (7–21 days after sowing)			
	NH_3 [ppm]	NH_4^+-N [mg L (substrate)$^{-1}$]	NO_3^--N [mg L (substrate)$^{-1}$]	NH_4^+-N/NO_3^--N ratio
6 (control)[2)] (NO_3^--N, 200/400)	< 0.1 d	54 d	91 b	0.6 c
7 (AAF-N, 750/0)	1.1 a	229 a	10 d	68.8 a
8 (AAF-N, 750/375, 5 % compost, 12-day storage)	0.3 c	116 c	161 a	0.9 c
9 (AAF-N, 750/250, 5 % compost, 1-day storage)	0.7 b	194 b	53 c	9.9 b

[1)] Fertilized N form, base dressing/top dressing rate [mg N (L substrate)$^{-1}$], compost addition, and storage period of the prepared substrate before use; substrate pH was initially adjusted to 6.5 in all treatments of this experiment.

[2)] Mean concentrations detected 0 and 35 days after sowing

Table 5: Number of plants, fresh matter yield, and plant height 35 days and 56 days after sowing in experiment 3 ($n = 3$). Means within a column not sharing a letter are significantly different according to Tukey's test ($p < 0.05$). Lower section of the table: Relationships between plant growth parameters and exposure to mineral N forms as well as substrate pH in the organically fertilized treatments, determined within 7–21 days after sowing, indicated by direction (+/-) and coefficient of determination (R^2) for a linear regression model ($n = 9$)

Treatment[1)]	35 days after sowing			56 days after sowing	
	Number of plants [% of control]	Fresh matter yield [g pot^{-1}]	Plant height [cm]	Fresh matter yield [g pot^{-1}]	Plant height [cm]
6 (control) (NO_3^--N, 200/400)	100 a	3.4 b	2.2 bc	27.5 b	9.7 c
7 (AAF-N, 750/0)	39 c	0.7 c	1.1 c	13.0 c	7.5 c
8 (AAF-N, 750/375, 5 % compost, 12-day storage)	68 b	9.2 a	6.1 a	54.3 a	23.3 a
9 (AAF-N, 750/250, 5 % compost, 1-day storage)	35 c	2.0 b	2.8 b	31.8 b	15.3 b
	R^2 for a linear regression model[2)]				
NH_4^+ concentration	(-) 0.69**	(-) 0.91***	(-) 0.92***	(-) 0.95***	(-) 0.88***
Substrate pH	(-) 0.42$^{n.s.}$	(-) 0.90***	(-) 0.85***	(-) 0.95***	(-) 0.91***
NH_3 concentration	(-) 0.55*	(-) 0.97***	(-) 0.94***	(-) 0.98***	(-) 0.95***
NO_3^- concentration	(+) 0.63*	(+) 0.94***	(+) 0.94***	(+) 0.93***	(+) 0.86***
NH_4^+/NO_3^- ratio	(-) 0.25$^{n.s.}$	(-) 0.47*	(-) 0.49*	(-) 0.64*	(-) 0.61*

[1)] Fertilized N form, base dressing/top dressing rate [mg N (L substrate)$^{-1}$], compost addition, and storage period of the prepared substrate before use; substrate pH was initially adjusted to 6.5 in all treatments of this experiment

[2)] Significance level: n.s. = not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

Table 6: Maximum ammonical N exposure determined on average 7–21 days after sowing to generate ≥ 90% of plant yield performance, compared to the NO_3^--fed control (28 and 35 days after sowing in experiments 1 and 2, respectively)

Parameter	Ammonical exposure 7–21 days after sowing		
	NH_3 exposure [ppm]	NH_4^+-N Concentration [mg L (substrate)$^{-1}$]*	
		With NH_3 exposure ≥ 0.2 ppm	With NH_3 exposure < 0.2 ppm
Number of plants	< 0.2	< 100	< 300
Fresh matter yield	< 0.1	< 50	< 50
Plant height	< 0.1	< 50	< 50

*Substrate contained less than 15 mg NO_3^--N L^{-1}

Discussion

N dynamic in the substrate after organic N fertilization

Basil reaches its marketable size in greenhouse pot cultivation 4–10 weeks after sowing (Eghbal, 2017). To ensure sufficient N supply in organic production, the crop should be fertilized with easily decomposable organic N sources. The mineralization of the AAF investigated in this study started immediately after its incorporation into the growing medium, as indicated by the rapidly increasing concentration of NH_4^+ (**Figures 3A** and **4A**) and rising substrate pH (**Figures 3B** and **4B**) in the first week of cultivation. However, nitrification was delayed by 3–4 weeks in treatments with peat as sole substrate component. Comparable observations were made after application of different organic fertilizers such as horn shavings, blood meal, and urea to peat or wood-based substrates (Bergstrand et al., 2018; Frerichs et al., 2017; Niemiera et al., 2014). After adding the amino acid arginine to soil samples, Kemmitt et al. (2006) detected the highest NH_4^+ concentration 2–8 days later. The depletion of the amino acid pool was accelerated by liming of the natively acid soils. The correlation between the N mineralization rate and soil pH could be described by a quadratic equation that indicates a maximum NH_4^+ accumulation at pH 5.5. Similarly, in our experiments we always observed a considerably higher NH_4^+ concentration in peat substrates initially adjusted to pH 5.5 rather than to pH 6.5 (**Figure 3A**). In contrast to these findings it is well-known that most soil microorganisms prefer soil pH around 7.0 (Coyne, 1999). Thus, in several laboratory soil incubation experiments, increasing N mineralization rates were found up to this pH level (Curtin et al., 1998; Fu et al., 1987). Most probably the lower NH_4^+ accumulation in the organically fertilized peat at pH 6.5 was due to higher NH_3 emissions compared to pH 5.5 (**Figure 3C**). Based on the aerial NH_3 concentrations detected above the substrate surface, the gaseous N losses were most intensive within the first week of cultivation. Accordingly, in field experiments with urea application, NH_3 volatilization was up to one order of magnitude higher during the first days after fertilization compared to the following period. In total, more than 60% of the applied urea can be lost via NH_3 (Black et al., 1985; Pacholski et al., 2006).

The open-top chamber approach used in this study is not suitable for quantifying absolute NH_3 volatilization. Nevertheless, significant NH_3 losses from the growing medium can be assumed when taking into account the amounts of NH_4^+ detected within the first two weeks of cultivation. At this time of cultivation, uptake of mineral nutrients by plants is still negligible. On average not more than 60% and 40% of the applied AFF-N were detected as NH_4^+-N in the peat at initial substrate pHs of 5.5 and 6.5, respectively. Since amino acids are decomposed by soil microorganisms with a half-life of 1–12 h (Jones, 1999), most of the NH_4^+ release is to be expected within a few days. However, a certain part of the amino acids is retained in the microbial biomass (Barak et al., 1990). Beside this N immobilization, primarily NH_3 emissions had probably contributed to the balance gap of applied AFF-N, especially at the higher substrate pH. Gaseous N losses by denitrification are usually relatively low in peat-based substrates if waterlogging or compaction is avoided (Agner and Schenk, 2005), as ensured in the trials presented here.

The increase in substrate pH immediately after organic base dressing reflects H^+ consumption by ammonification (Ferguson et al., 1984). However, these temporary pH shifts were less pronounced with a higher initial substrate pH (**Figure 3B**). Firstly, this might be due to the logarithmic pH scale. The higher the pH, the more hydroxide ions (OH^-) are required to increase the pH for one unit. On the other hand, with rising pH, NH_3 is increasingly lost from the substrate. Each mole of emitted NH_3 will increase the concentration of H^+ by one mole (Sommer et al., 2004).

Ammonia concentration in the aerial environment of basil seedlings reached a maximum level of 1.8 ppm seven days after sowing (**Figures 3C** and **4C**). In the following weeks NH_3 exposure decreased faster than expected compared to the relatively slow decline of NH_4^+ concentration and pH in the substrate (**Figures 3A, 3B, 4A**, and **4B**). Noticeable changes in climatic conditions (e.g. air temperature, wind speed) can be excluded as possible causes. In field experiments, it was shown that the topmost millimeters of soils are most important for the volatilization of NH_3 (Pacholski et al., 2006). Therefore, we assume that the NH_4^+ concentration and pH in the upper substrate zone dropped faster compared to conditions in the whole substrate.

In pure peat, nitrification was accelerated and increased by a substrate pH close to neutrality compared to more acid conditions (**Figures 3B, 3D, 4B**, and **4D**). Similar results were reported by Lang and Elliott (1991) who identified a slightly alkaline pH as optimal for nitrification in peat-based growing media. At pH ≤ 5.4, nitrification was strongly inhibited. On the other hand, alkaline conditions can lead to an accumulation of NO_2^- due to the inhibitory effect of high NH_3 concentrations on *Nitrobacter* sp., which convert NO_2^- to NO_3^- (Bunt, 1988; Vetanovetz and Peterson, 1990). Accordingly, we observed a noticeable NO_2^- accumulation in treatments with a substrate pH ≥ 7.0 during the onset of nitrification (**Figures 3E** and **4E**). However, if peat was amended with 5% (v/v) mature green waste compost and afterwards stored for 12 days before use NO_2^- accumulation was suppressed (treatment 8). In this substrate mix pH was always between 6.0 and 7.0 and therefore in a range to prevent both inhibition of nitrification and accumulation of NO_2^- due to low and high pH levels, respectively. Already 2 weeks after sowing two thirds of the mineral N in the peat-compost blend were converted to NO_3^-. In contrast, in a peat substrate without compost amendment a similar proportion of NO_3^- was reached about 5 weeks later (**Figures 4A** and **4D**). Mature composts usually contain high numbers of nitrifying bacteria (Chroni et al., 2009; Zeng et al., 2012) and thus can serve as an inoculum to enrich peat with these microorganisms (Delcis et al., 2017). Nevertheless, apparently it took a couple of days before the nitrifying community was fully established in their new environment. This was indicated by results obtained with a peat-compost blend that was stored just one day before use (treatment 9). As a result, NO_3^- accumulation lagged about two weeks behind compared to the same substrate mix that was previously stored for 12 days.

With ongoing nitrification, substrate pH decreased slightly (**Figures 3B** and **4B**). This pattern reflects the generation of 2 H^+ ions during the microbial oxidation of NH_4^+ to NO_2^- (Sahrawat, 2008). In pure peat, the pH decline continued until the end of the trial. In the later course of cultivation, plants might also contribute to the weak acidification of the growing medium since the uptake of NH_4^+ by roots is accompanied by an equivalent H^+ efflux (Schubert and Yan, 1997). In peat-compost blends substrate pH turned once again during the last 2–3 weeks. Most probably the slight pH increase in this phase was triggered by the repeated AAF top dressings as well as the H^+-consuming NO_3^- uptake of plants.

Impact of NH_3, NH_4^+, and NO_2^- exposure on basil growth

In the experiments, basil was exposed to different concentrations of NH_3 and NH_4^+ right from the beginning of cultivation. This was done by varying AAF base dressing rates in combination with different initial substrate pH values. The emergence of radicle and germ bud from seed was not affected by any of the treatments examined. Obviously, the presence of ammonical N did not interfere with physiological processes involved with the onset of basil germination. However, shortly afterwards seedlings were suffering from increased NH_3 and NH_4^+ exposures. Under these conditions, the development of radicle, hypocotyl, and cotyledons was strongly inhibited (**Figure 5B**). Similar adverse effects of ammonical N forms on germination and seedling development were reported for several plant species (Bergstrand et al., 2018; Bremner and Krogmeier, 1989; Britto and Kronzucker, 2002; Ells et al., 1991; Qi et al., 2012).

The number of surviving seedlings and their following growth performance were negatively correlated with the intensity of NH_3 and NH_4^+ exposures occurring 7–21 days after sowing (**Tables 2, 4,** and **5**). Overall, these relationships were stronger for NH_3 and more pronounced between November to April (experiments 2 and 3) than in September/October (experiment 1). Thus, it seems that growing conditions prevailing in the winter months increased the ammonical susceptibility of basil. On the other hand, it is also conceivable that NH_3 emitted from the substrate remained for a longer time in the plant canopy due to the restricted ventilation of greenhouses in the colder season. Accordingly, it is known from commercial organic basil production that growth impairments are more severe in the winter cultivation period. Besides stunted plant growth, typical symptoms are chlorotic and necrotic cotyledons, frequently accompanied by fungal diseases such as *Botrytis* (Frerichs et al., 2017). Surprisingly, in this study cotyledons always remained green. This was contrary to previous findings even with the same basil cultivar under similar cultivation conditions (Frerichs et al., 2017). Thus, high exposure to ammonical N does not necessarily involve cotyledon discoloration.

Critical levels of ammonical N exposure in the early development stage of basil (7–21 days after sowing) were reached at concentrations of 0.1–0.2 ppm NH_3 in the aerial environment of plants and 50–100 mg NH_4^+-N L^{-1} in the growing medium. At higher concentration levels, the number of plants, fresh matter yield, and plant height were diminished by more than 10% (**Table 6**). With regard to NH_3, basil seems to be more sensitive than many other food crops, as reviewed by Krupa (2003). However, most of the published data related to this aspect were based on exposure experiments with plants in the post-emergence stage. A study on wheat has shown that the seed germination of this cereal species is unaffected at NH_3 concentration below 0.3 ppm, but completely inhibited at 0.8 ppm (Pairintra, 1973). For pot-grown basil, a poor germination and weak plant growth was observed when the NH_4^+-N concentration was between 100–130 mg (L substrate)$^{-1}$ in the first three weeks after sowing (Bergstrand et al., 2018). In contrast, Frerichs et al. (2017) reported that the germination process of basil was not adversely affected by NH_4^+-N levels at about 200 mg (L substrate)$^{-1}$. In this experiment, the substrate pH was slightly acidic throughout the entire germination period and thus, NH_3 can be assumed to be negligible. Under these circumstances, basil seemed to tolerate up to 300 mg NH_4^+-N (L substrate)$^{-1}$ (**Table 6**). However, after germination is completed and seedlings start to take up nutrients from the growing medium much lower NH_4^+-N concentrations should be present to ensure proper plant growth.

High NH_4^+ and NH_3 concentrations in the early plant development stage had a long-lasting adverse impact on the growth of basil. This became evident by the fact that plants in treatment 9 generated 41% less shoot biomass than plants in treatment 8 (**Table 5**), although both were grown under moderate to low ammonical exposure in the second half of cultivation period. However, seedlings in treatment 9 were subjected to distinctly higher concentration levels of ammonical N, especially of NH_3 (**Figure 4**).

In the organically fertilized treatments, NO_2^- concentrations reached a maximum level of 42 mg (L substrate)$^{-1}$ (**Figures 3E** and **4E**). Harmful effects to plants can be expected if NO_2^- concentration exceeds 5 mg L^{-1} in the root zone (Hoque et al., 2007; Zsoldos et al., 1993), especially in the seedling stage (Bergmann, 1993). However, NO_2^- appeared only temporarily in a few pots. Thus, NO_2^- concentration was mostly still very low in the second week of cultivation when growth impairments on seedlings became visible. Hence it seems unlikely that NO_2^- significantly contributed to these adverse effects. Nevertheless, further investigations are needed to examine the accumulation of NO_2^- in growing media after organic fertilization and to clarify the sensitivity of basil against this inorganic N species.

Impact of pH and other substrate parameters on basil growth

Plant height and fresh matter yield of basil were inversely related to the substrate pH in experiments 2 and 3 (**Tables 2, 4,** and **5**). Besides the increasing NH_3 exposure, other factors might have limited the crop development at neutral to slightly alkaline conditions. This was particularly noticeable in experiment 3. Plants predominantly fed with NO_3^- (treatment 6), were not exposed to detectable amounts of NH_3 but showed a stunted growth. Probably this was mainly due to a low availability of P in the growing medium (**Figure 4F**). According to Meinken (2008), a concentration ≥ 22 mg P (L substrate)$^{-1}$ extracted by CAT is required to supply horticultural crops having a high nutrient demand sufficiently with P. This level was not reached in the pure peat substrates within the first four weeks of cultivation. As a result, typical P deficiency symptoms such as violet discolored cotyledons emerged. Subsequently, P supply in the substrate was increased to the target range by means of top dressing with a water-soluble P fertilizer. Simultaneously, P availability was presumably improved by the declining substrate pH **(Figure 4B**), which might have increased the solubility of apatitic compounds (Alt et al., 1994).

Despite sufficient P supply in the second half of cultivation, growth of plants in the control treatment of experiment 3 remained distinctly lower than usually observed for NO_3^- fertilized basil. In both previous experiments NO_3^--fed plants generated the highest fresh matter yield (**Table 2**). On the one hand the better crop performance might be related to the generally lower substrate pH in these trials (**Figure 3B**). On the other hand, the characteristics of the different apatitic P fertilizer types could have played a role. In experiment 3 a bone meal based fertilizer was used. In principle, this product exhibits a higher P solubility at increased pH than rock phosphate (Möller, 2015), which was applied in experiments 1 and 2. However, the bone meal fertilizer consisted of coarser particles (80% < 2.0 mm) than the ultrafine-sieved rock phosphate (80% < 0.1 mm). Since the percentage of dissolution of apatitic P strongly increases with decreasing particle size (Kanabo and Gilkes, 1988), it is assumed that the bone meal fertilizer was less effective at supplying phytoavailable P.

Basil growing in compost-amended peat did not show any P deficiency symptoms, although the initial P supply was equally adjusted in all treatments. Nevertheless, in pure peat substrates lower CAT-extractable P concentrations were observed at the beginning of the cultivation period. It is most likely that the positive effect of the compost on P availability was a result of the faster pH decline in this substrate mix (**Figures 4B** and **4F**).

Iron absorption of basil was also hampered at increased substrate pH, as indicated by a yellowish discoloration of the primary leaves. These deficiency symptoms were mainly visible in the second experiment in pure peat (**Figure 6E**). At neutral to slightly alkaline conditions Fe ions react rapidly with oxygen and OH^- ions to form barely soluble compounds (Lindsay and Schwab, 1982). These precipitation processes may occur even if the fertilized Fe is chelated by ethylenediaminetetraacetic acid (EDTA) or N-(2-hydroxyethyl)ethylenediaminetriacetic acid trisodium salt hydrate (HEDTA). To alleviate arising Fe deficiency symptoms quickly, foliar sprays with Fe chelates are an effective tool (Fisher et al., 2003), as applied by an overhead irrigation in experiment 3.

A stabilization of the substrate pH against alkalization as well as acidification shifts can be attained by using peat substitutes with a high buffering capacity, such as composted bark (Neumaier and Meinken, 2015).

Approaches to mitigate harmful NH_3 and NH_4^+ effects

To improve the growth of organically fertilized basil, strategies are needed which contribute towards less intense and shorter NH_3 and NH_4^+ exposures of plants. In this respect, first of all, it seems reasonable to supply moderate organic N base dressing rates [≤ 250 mg N (L substrate)$^{-1}$] and to adjust the substrate pH to about 5.5. When basil is sown in a peat-based growing media with these characteristics, the exposure of seedlings to ammonical N will remain below or close to the above-mentioned critical concentration levels (**Figures 3A and 3C; Table 6**). To meet total N requirements of basil, base dressing can be supplemented by repeated top dressings in the later course of cultivation, without leading to harmful ammonical N concentrations. Nevertheless, even by using these measures, a retarded plant growth compared to mineral N-fertilized basil can be expected (**Table 2**). This results most probably from the high NH_4^+-N/NO_3^--N ratio in the substrate remaining for several weeks after organic base dressing. A previous study indicated that biomass production of pot-grown basil was highest at balanced to NO_3^--dominated N nutrition (Frerichs et al., 2019). In pure peat substrates it usually takes more than 4 weeks before NO_3^--N concentration reaches a similar level to NH_4^+-N. The amendment of peat with mature green waste compost has proven to be a suitable method to accelerate the NO_3^- formation in the substrate. The best results will be obtained when the compost-peat blend is stored for several days before use. In this way organically fertilized basil will be fed already from the seedlings stage onwards with adequate amounts of NO_3^-.

In the present experiments, a compost amendment of 5% (v/v) to the peat was sufficient to realize the outlined positive effects. In commercial organic basil cultivation even higher compost proportions are chosen and often required by organic farming associations as well. This may possibly boost the impact of the compost. However, Delcis et al. (2017) found a nearly similar nitrification pattern in an unstored peat-based substrate that contained 30% (v/v) green waste compost to the one that we observed by using just one sixth of this admixture with a short-term storage of the substrate blend for one day. Nevertheless, further investigations are necessary to assess the relevance of the compost/peat blending ratio for the intended purpose. Furthermore, the influence of the duration and conditions of storage (e.g. water content of the substrate mix, aeration, storage temperature) on the N dynamic in compost-peat mixtures have to be examined in more detail.

For a successful implementation of the proposed methods it is certainly essential to use fully mature compost. The maturity level of composts can be easily recognized when the temperature of the organic material approaches the ambient range and the NH_4^+-N/NO_3^--N ratio falls below 3 (Cáceres et al., 2018).

Conclusions

The exposure of seedlings to ammonical N was found to be a cause of growth impairment frequently observed in organic basil production. Critical concentrations were reached at 0.1–0.2 ppm NH_3 in the aerial environment and 50–100 mg NH_4^+-N (L substrate)$^{-1}$ in the root zone of plants. However, when NH_3 is absent and sufficient amounts of NO_3^- are available in the growing media, basil seems to tolerate higher levels of NH_4^+. Therefore, a fertilization strategy is recommended which combines a moderate organic N base dressing with repeated top dressings in the later course of cultivation to meet the total N requirement. Easily decomposable organic fertilizers such as those based on amino acids should be used to ensure a rapid N mineralization. By adjusting the initial substrate pH to 5.5–6.0 the formation of NH_3 during the ammonification of organic compounds would be prevented. Furthermore, the addition of mature green waste compost to peat-based substrates can subsequently promote the nitrification of NH_4^+, most noticeably if this substrate blend is stored for several days before use. With this approach, it seems possible to supply basil a substantial proportion of NO_3^- right from the seedling stage, and thus to improve crop growth performance. Further investigations are, however, needed to evaluate whether this procedure can be also successfully used in the organic production of other pot-grown crops.

Chapter 3
General discussion

3.1 Reducing potential nitrate leaching losses by improving the synchronization of actual N supply and crop N demand in the production of field-grown vegetables

In vegetable farming, approaches such as the “Kulturbegleitende N_{min}-Sollwerte” (KNS) method are often used to synchronize the N supply and N demand of a crop (Thompson et al., 2017). By using the KNS method, the N fertilization is usually divided into a base fertilization applied at sowing or planting and a top dressing applied at the beginning of exponential plant growth at the end of the seedling stage. The N dosages are based on the expected N demand of the crop, the soil N_{min} concentration in the root zone soon before fertilizer applications, the apparent net N mineralization and N losses as well as the minimum N_{min} concentration to allow for maximum plant N uptake (Fink and Scharpf, 2000; Tei et al., 2020). In the 1990s, KNS was implemented into the software N-Expert and has been continuously developed since then by the Leibniz Institute of Vegetable and Ornamental Crops (IGZ, 2022). However, even by means of a fertilization of vegetable crops according to approaches such as the KNS method, NO_3^- leaching losses in sandy soils are usually still too high to comply with the European guideline of maximum 50 mg NO_3^- L^{-1} groundwater (Armbruster et al., 2013; De Haan et al., 2009; Tei et al., 2020).

When assessing the risk of NO_3^- leaching, the first weeks after sowing as well as after harvest are most relevant due to the lack of N uptake by plants (Thompson et al., 2017). For instance, in sandy soils, NO_3^- leached by 6 cm following a precipitation of 13 L m^{-2} at a field moisture of initially 22% (v/v) (Niers, 1994). However, even at a high N supply in the 30–45 cm layer of the soil, N uptake of crops with a short cropping cycle like spinach and kohlrabi almost exclusively occurred in the uppermost 30 cm (Schenk et al., 1991). Thus, NO_3^- leached below 30 cm can be considered lost in such crops (D’Haene et al., 2018). Hence, reducing, delaying, or even omitting the base fertilizer application can be effective for reducing potential NO_3^- leaching losses (Gabriel and Quemada, 2017). According to Feller et al. (2011), at least a minimum of 20 kg N ha^{-1} (0–30 cm) for e.g. carrots and beans and up to 80 kg N ha^{-1} (0–30 cm) for e.g. cauliflower and broccoli should be available at sowing to ensure maximum N uptake until top dressing. For spinach, at least a concentration of 40 kg N ha^{-1} should be available in the uppermost 30 cm of the soil. However, based on N_{min} samples taken during the seedling stage of spinach, the total aboveground fresh and dry mass at an early harvest stage can be increased until a concentration of 54 and 59 kg N ha^{-1} (0–30 cm) is reached, respectively (Frerichs and Daum, 2022a). In the summer and autumn seasons, this additional N demand was often met by a high initial N_{min} concentration and mineralization during the first weeks after sowing (Frerichs and Daum, 2022b; Section 2.1, **Figure 1**, p. 20). Thus, the base fertilization rate did not affect the fresh and dry mass yield. However, in early and mid-spring the soil N supply was low. As a result, at an early harvest stage the aboveground biomass was diminished by up to 21% (annual mean: 6.5%) by reducing the base fertilization and the correspondingly increased top dressing could not fully compensate for this (Section 2.1, **Table 3** and **Figure 3**, pp. 25f). Despite a low N uptake, most vegetable crops are highly responsive to N supply at the seedling stage, especially in the spring season (Greenwood et al., 1989; Lorenz et al., 1989). Therefore, at least in the spring season a high base fertilization rate appeared to be required. To achieve both a sufficient N supply in the root zone and to reduce the NO_3^- concentration exposed to leaching, a band application of the base fertilizer close to or below the plant row instead of a broadcast application across the entire field surface can be an appropriate method. By this application technique, the risk of nutrient losses from areas in the field where crop roots are not present can be reduced. Thus, the base fertilization rate can be reduced without affecting seedlings growth (Simonne et al., 2017). However, spinach is sown at a row spacing of 12 to 25 cm (Laber and Lattauschke, 2020). Thus, the interrow space is intensively penetrated by lateral roots at harvest (Heins, 1989). Therefore, further investigations are required to examine, whether a band application can significantly increase the N uptake efficiency during the seedling stage of densely sown and fast-growing crops like spinach.

After the first true leaves become unfurled, vegetative growth and N uptake strongly increase depending on the crop and season. In cauliflower and broccoli the uptake rate reaches up to 77 and 75 kg N ha^{-1} $week^{-1}$ within the vegetative growth stage, respectively (Feller et al., 2011). To cover the N demand, top dressing is applied at the beginning of exponential plant growth. However, the actual N uptake of a crop as well as soil N supply via mineralization are difficult to predict in advance. As a result, fertilizers are often applied in excess to ensure maximum marketable yield (Tei et al., 2020; Tremblay and Bélec, 2006). To better synchronize the N supply to the actual demand of a crop, the top dressing of long-standing crops such as kale and brussels sprouts, characterized by a cultivation period of 4–6 months, is divided into two or more applications (Da Silva et al., 2020; Feller et al., 2011). In crops with a shorter cultivation period, usually only a base fertilizer or a base fertilizer in combination with a single top dressing is applied (Abras et al., 2013; Thompson et al., 2017). However, splitting the top dressing might also be appropriate for vegetables like spinach with a cropping cycle of just a few weeks. By using this approach, the NO_3^- concentration peaks after dressings were flattened and the N_{min} residue was also reduced by dispensing with the second top dressing in case of an early harvest (Section 2.1, **Figure S2–S4**, pp. 38–40). On the other hand, by splitting the top dressing the total aboveground dry mass at an early harvest stage was diminished on average by 6.0% (Section 2.1, **Table 3** and **Figure 4**, pp. 25f). This effect was the result of the reduced first top dressing. After applying the remaining N via the second top dressing spinach was able to partially recover from initial hindrances to growth. On average, the total aboveground dry mass at a late harvest stage was only reduced by 2.4%. However, at times of a high N uptake, an adequate irrigation shortly after the application of the fertilizer granules appeared to be vital to ensure a sufficient N availability in the root zone (Quemada et al., 2013). This was realized within two days after the second top dressing. In addition to the fertilizer N supply, the recovery of the spinach plants was probably due to an improved N uptake efficiency during the later course of cultivation caused by growing into deeper soil layers and a more complete penetration of lateral roots within the topsoil (Heins, 1989; Smit and Groenewold, 2005). Moreover, the N uptake rate decreases from about 60 kg ha^{-1} $week^{-1}$ at an early harvest stage to 25 kg ha^{-1} $week^{-1}$ at a late harvest stage (Feller et al., 2011; Niers, 1994). Thus, more of the crop N demand can be covered by soil mineralization and the irrigation water (Section 2.1, **Figure 1**, p. 20).

Spinach grown for the frozen food industry, is harvested as either leaf or chopped spinach at an early or late harvest stage, respectively (Frerichs and Daum, 2021). Unlike leaf spinach, chopped spinach allows for a higher proportion of leaf stalks and can therefore still be harvested after bolting has begun. However, at the time of fertilization the actual harvest stage and growth performance is unpredictable due to e.g. variable weather conditions, diseases, and the requirements of the market (Vandecasteele et al., 2016). Thus, a late harvest stage with a yield of 25 t ha^{-1} (fresh mass) is usually assumed when calculating the fertilization demand. However, with an actual fresh mass yield of about 15 t ha^{-1} (early harvest stage) the total fertilizer N supply in treatments without splitting was up to twice as much as the maximum permissible application according to the German Fertilizer Ordinance (2020) and therefore must be reduced (Frerichs and Daum, 2021). In addition, in nitrate vulnerable zones, the annual fertilizer N supply is limited to 80% of the calculated maximum permissible fertilizer N supply (German Fertilizer Ordinance, 2020). At least in some vegetable crops, fertilizer N supply can be reduced without adversely affecting marketable yield (D'Haene et al., 2018). However, in crops with a low N uptake efficiency such as spinach the mentioned legal requirements appeared to be difficult for growers to comply with (Frerichs and Daum, 2021).

Splitting the N dosage opens up the opportunity of dispensing with the final dressing in the case of a low actual N demand of the crop caused by e.g. a harvest before maximum yield was achieved (Abras et al., 2013). This strategy can help growers to comply with the above-mentioned requirements of the German Fertilizer Ordinance (2020). In addition, the N_{min} residue at an early harvest stage can be reduced in this way. However, spinach is often harvested between early and late harvest stages. Therefore, a further division of the top dressing seemed to be required to better synchronize N supply and actual N demand. However, the application of granulated fertilizers is described as being impractical when applied in small rates (Vandecasteele et al., 2016). In contrast, by using drip irrigation systems, small dosages can easily be applied by frequent fertigation close to the plant row. On the other hand, investment costs and workload can be high, limiting the practical potential of this technique. In addition, N supply via fertigation can be insufficient during rainy periods (Incrocci et al., 2017). Another way to further divide the top dressing is a frequent foliar spray with diluted liquid fertilizers. Urea appears to be an appropriate fertilizer for this purpose since it has a higher permeability on plant leaves compared to inorganic fertilizers (Fernández and Brown, 2013). Moreover, urea fertilizers have a lower salt index than NH_4^+ and NO_3^- fertilizers and are therefore less likely to cause leaf necrosis (Krishnasree et al., 2021). In experiments of Bowman and Paul (1992), no adverse effects on turf grass were observed after a concentration of about 5.0% (w/v) urea was applied. However, a frequent foliar spray with 3.0% (w/v) urea proved to be inefficient for promoting spinach growth in comparison to a treatment without a second top dressing of 50–70 kg N ha^{-1}. In addition, the foliar urea spray caused necrosis at the leaf margin (Section 2.1, **Table 4** and **Figure 6**, pp. 27f). As discussed in section 2.1 (p. 32), these observations might be due to plant stress caused by the exposure to NH_3 and the accumulation of biuret in the leaf tissues. In contrast, at an earlier developmental stage of spinach, Borowski and Michalek (2008) observed a significantly increased leaf mass yield after two foliar sprays with 1.0% (w/v) urea and NH_4^+-based fertilizers. However, at high N uptake rates of vegetable crops foliar fertilization cannot cover the nutrient demand (Singh et al., 2013). In addition, this option requires high workload and investment costs in comparison to a single application of a granulated fertilizer (Vandecasteele et al., 2016). Therefore, even by using the splitting approach, it remained difficult to achieve synchronization between N supply and the actual N demand.

By dispensing with the second top dressing of 50–70 kg N ha^{-1} the aboveground biomass at a late harvest stage was reduced on average by 7.4%. In addition, in the spring and winter seasons also the leaf coloration was adversely affected (Section 2.1, **Figure 5** and **Table S2**, pp. 28 and 36). A high sensitivity to N supply, when spinach was grown as the first crop after the winter leaching period, was also observed in previous investigations (D'Haene et al., 2018; Heins, 1989; Schmidt and Zinkernagel, 2015). Therefore, at the beginning of the growing season, even a temporary lower N supply is not recommended. In contrast to the winter and spring seasons, in summer-grown and autumn-grown spinach, reducing the total N supply by omitting the second top dressing had less effect on yield and quality of the produce (Section 2.1, **Tables 4** and **S2**, pp. 27 and 36). For autumn-grown spinach, similar observations were made in other studies (Heins, 1989; Kręzel and Kołota, 2010). The lower N demand of autumn-grown spinach might be explained by a diminished dry mass production caused by a decreasing irradiation at the end of the growing season (Breimer, 1982). In contrast, in summer, the yield was mainly limited by bolting (Section 2.1, **Table S3**, p. 36). In general, dry mass growth and N uptake of spinach is reduced after turning from vegetative into generative growth (Biemond et al., 1996; Feller et al., 2011; Smolders and Merckx, 1992). In addition, bolting reduces the quality of the produce by reducing the proportion of leaf blades (Grevsen and Kaack, 1997). Therefore, it can be hypothesized that the second top dressing should generally be dispensed with in the summer and autumn seasons. However, bolting and plant growth is dependent on the spinach variety and the actual weather conditions (Abolghasemi et al., 2021; Breimer, 1982; Chitwood et al., 2016). Thus, even in the summer and autumn seasons, omitting the second top dressing can adversely affect crop yield and the quality of the produce.

To identify situations the fertilizer N supply can be reduced without affecting the marketable yield well-fertilized reference plots can be used. Based on the aboveground N content the ratio between the field and the well-fertilized plots can be used to calculate an N nutrition index (Tremblay et al., 2011). In leafy vegetables such as spinach non-destructive optical sensors can be used to derive the N content of the crop (Liu et al., 2006; Massa et al., 2018; Muchecheti et al., 2016; Rubo and Zinkernagel, 2022). For most crops, the N nutrition index should be at least at 95% of the well-fertilized plots to ensure maximum growth (Muchecheti et al., 2016; Tremblay et al., 2011). Thus, top dressing can be dispensed with as long as the critical index does not fall below approximately 95% depending on the crop and sensor technology (Abras et al., 2013). This approach worked well in cauliflower, blanched celery, and lettuce. However, in spinach, rocket, and Welsh onion the marketable yield was adversely affected (Armbruster et al., 2013). An important disadvantage of optical sensors appeared to be the time required between the start of an N deficiency and the reflection in the readings (Garcia-Servin et al., 2022). Moreover, a rapid N availability must be guaranteed after a delayed top dressing. Therefore, spinach growth might be too fast to implement common sensor methods in fertilization planning (Armbruster et al., 2013). Furthermore, factors such as the development stage and water supply status interfere with optical measurements, which complicates the derivation of the plant N status (Liu et al., 2006; Rubo and Zinkernagel, 2022). However, in recent years, a variety of plant N status and soil monitoring technologies as well as vegetation indices derived from them have been developed and may have the potential to overcome the above-mentioned bottlenecks. In addition, a combination of these approaches as well as growth indices based on thermal time and the phenological stage of the plants seemed to be promising with regard to deriving the actual fertilizer N demand of vegetable crops (Padilla et al., 2020). This also includes a better determination of the actual soil N supply via mineralization (Frerichs and Daum, 2022b).

In contrast to adversely affecting the crop yield and leaf coloration, a reduced N supply can increase the nutritional quality of the products by decreasing their NO_3^-, NO_2^-, and oxalate contents (Kaminishi and Kita, 2006; Ranasinghe and Marapana, 2018). All three compounds can directly or indirectly pose a risk to human health. Therefore, the maximum permissible NO_3^- content is limited for spinach, salad, and rocket (European Commission, 2011). The NO_3^- content in plants is mostly dependent on their NO_3^- uptake and assimilation capacity. However, the assimilation capacity can be diminished with decreasing solar irradiation and plant stress caused by e.g. heat and drought (Breimer, 1982). Thus, even with a reduced N supply the NO_3^- content can be above the European threshold of 2,000 mg kg^{-1} (fresh matter basis) in processsed spinach (Section 2.1, **Table 6**, p. 29). The contents of NO_3^-, NO_2^-, and oxalate are only weakly correlated and affected differently by the soil N supply depending on factors such as the variety, season, weather conditions, daytime, plant age, the leaf stalk to leaf blade ratio, and post-harvest storage treatment (Cai et al., 2018; Conesa et al., 2009; Kamanishi and Kita, 2006; Koh et al., 2012). Hence, factors other than the N supply must also be considered to reduce potential risks to human health.

The results shown in section 2.1 are based on the total aboveground dry mass. However, roughly only 2/3 of the aboveground biomass is harvested in a spinach crop (Feller et al., 2011). In addition, the cutting height is dependent on the required leaf blade to leaf stalk ratio (Grevsen and Kaack, 1997). Assuming a fixed cutting height between the treatments, a reduced base fertilization and split top dressing probably affected the marketable yield by more than 10% on average. Therefore, growers need to be compensated for optimizing the fertilization strategy. On the other hand, consumers' interest in the environmental aspects of the products they consume is rising (Asioli et al., 2017; Van Rijswick, 2020). Hence, quality attributes such as the leaf coloration or the shoot to leaf blade ratio might be less crucial in the future and also consumers might be willing to pay more for sustainable produced vegetables. In addition, water suppliers may also compensate growers for a more sustainable production. Such agreements already exist with farmers in water protection areas (Gömann et al., 2020).

Overall, spinach proved to be highly sensitive to temporary N shortages in the winter and spring seasons. Therefore, the fertilizer N supply should not be reduced at the beginning of the cropping season. On the other hand, this strategy may result in high N_{min} residues. However, potential N residues deriving from a high fertilizer N supply in the first spinach crops of a calendar year can be taken up by the following crops. In this way, the risk of NO_3^- leaching losses can be effectively reduced within a few weeks. In contrast, in the summer and autumn seasons spinach was less affected by the fertilizer N supply. Therefore, the second top dressing should be omitted in order to avoid high N_{min} residues at the end of the growing season and beginning of the winter leaching period.

3.2 Crop residues management options to reduce N losses during the off-season following autumn-grown vegetables

The off-season in the context of this thesis is described as the period from the harvest of the last crop in a growing season in autumn until the first crop is sown or planted in the following growing season. Provided the N fertilization corresponds to actual N demand, the N_{min} residue at harvest ranges roughly between 0 and 60 kg ha^{-1} depending on the vegetable crop (D'Haene et al., 2018; Feller et al., 2011). For leafy vegetables, harvested within the vegetative growth stage, a minimum of 40 kg N ha^{-1} should remain in the upper 30 cm of the soil to ensure maximum N uptake of the crop until harvest. However, despite an N_{min} residue between 5 and 30 kg ha^{-1} the NO_3^- concentration exposed to leaching increased considerably within a few weeks after incorporation of the spinach crop residues (Section 2.2, **Figures 3–5**, pp. 49f). In addition, N uptake by the catch crops sown between mid-September and mid-October was low. In contrast, deep-rooting and fast-growing catch crops are effective in reducing NO_3^- leaching, when sown until early September (Agneessens et al. 2014a). Based on long-term experiments, catch crops sown in late summer appeared to be the most efficient to reduce N leaching losses in arable crop-rotations compared to optimized N fertilization and tillage practices (Constantin et al., 2010). However, the earlier the last crop in a growing season is harvested, the lower is the farmers' revenue. Therefore, this option can only be viable if growers are financially compensated for not growing vegetables that need to be harvested in autumn.

In order to develop sustainable crop residues management strategies, a long-term differentiation of all significant N loss pathways is crucial (Abalos et al., 2022a; Agnesseens et al., 2014a; Zemek et al., 2020). In the balance sheet approach, however, most of the N gains and losses were not determined directly (Section 2.2, p. 47). Based on the high NO_3^- concentration in autumn and the downwards shifting within the soil layers during the autumn and winter seasons, most of the total N losses were probably due to leaching (Section 2.2, **Figures 3–5**, pp. 49f). This conclusion was also confirmed in other studies by directly measuring NO_3^- leaching losses in vegetable crop rotations grown in sandy soils (Armbruster et al., 2013; Spiess et al., 2021). The rising NO_3^- concentration after the incorporation of the spinach crop residues was mostly explained by a net mineralization of 55–121 kg N ha^{-1} (Section 2.2, **Figures 3** and **4**, pp. 49f). In general, the mineralization rate of crop residues is negatively correlated to their C/N ratio and lignin content (De Neve and Hofman, 1996). Aboveground crop residues of most leafy vegetables are characterized by a low C/N ratio and lignin content. Thus, up to 73% of their inherent N content is mineralized within four months after their incorporation into the soil (De Neve, 2017). In contrast, net mineralization of the root biomass, which is usually characterized by a higher C/N ratio and lignin content, remained low during an incubation of 20 weeks at 21 °C (Chaves et al., 2004). For aboveground spinach crop residues approximately 50% of the organic N was mineralized within the first two weeks after incorporation. However, after this initial peak, net mineralization was insignificant within the following weeks (De Neve et al., 1994; Laber, 2015). In addition, the aboveground crop residues only contained between 30 and 64 kg N ha^{-1} (Section 2.2, **Table 1**, p. 45). Therefore, the high NO_3^- concentration and N losses during the autumn and winter seasons could only be partially explained by the N_{min} residues at harvest and mineralization of spinach crop residues. Thus, the ongoing mineralization of native soil organic N, previously incorporated crop residues and catch crops, organic fertilizers, compost, and remineralization of fertilizer N that was immobilized earlier appeared to be of importance in this context (Cameron et al., 2013; Tei et al., 2020).

At the experimental sites, the soils were intensively cropped during the past decades or even centuries and usually high amounts of mineral and organic fertilizers were applied in the fields (Section 2.2, p. 44). Such soils are highly fertile due to high quantities of easily decomposable C and N fractions (De Neve, 2017; Heumann, 2016; Luxhøi et al., 2004; Tei et al., 2020). In addition, bacteria are predominant in arable soils, which facilitate a fast mineralization after application of C or N substrates, re-wetting, and tillage (Borken and Matzner, 2009; Kuzyakov and Xu, 2013). Therefore, one of the hypotheses made in section 2.2 was that N losses during the off-season are dependent on the technique used to incorporate the crop residues into soil. It was assumed that less intensive mixing of C and N substrates into the soil leads to less activation of soil microorganisms and thus mineralization remains at a lower level. However, the tillage intensity had only little effects on the course of the NO_3^- concentration and potential N losses during the off-season (Section 2.2, **Figure 3** and **Table 3**, pp. 49 and 51). This phenomenon was often observed in coarsely textured soils. In contrast to heavier textured soils, microorganisms are less bound to aggregates in sandy soils and can therefore react faster to substrate addition (Curtin et al., 2014; Hansen and Djurhuus, 1997). In addition, in terms of the soil depth the mineralization and nitrification of crop residues and soil organic N is mostly independent from the tillage treatment in sandy soils (Kandeler and Böhm, 1996; Nett et al., 2016). This might explain, that even scratching the soil surface once by direct drilling can result in N losses similar to those observed in the harrow and plow treatments. However, a better understanding of the mineralization kinetics of crop residues and various soil organic N fractions seemed to be vital to derive appropriate incorporation techniques.

Without tillage, the NO_3^- concentration exposed to leaching as well as the potential N losses remained at low levels as long as the spinach biomass remained viable (Section 2.2, **Figures 4** and **5**, **Table 3**, pp. 50f). This was explained by a lower net mineralization rate in the soil as well as the N uptake of the resprouting spinach crop residues and conservation in the plant biomass. The lower net mineralization might be due to the reduced availability of C and N substrates in the soil as well as the shading of the soil surface by the living spinach plants (Section 2.2, **Figure 7**, p. 52). Shading by transpiring leaves has a cooling effect and can therefore slow down mineralization compared to bare soils (Kätterer and Andrén, 2009). In late autumn soil temperature dropped below 5 °C. However, net mineralization did not necessarily remain at a low level after tillage in late autumn (Section 2.2, **Figures 1** and **4**, pp. 48 and 50). Therefore, postponing the tillage from early autumn to spring appeared to be the most promising strategy to reduce potential N losses during the off-season (Section 2.2, **Table 3** and **Figure 5**, pp. 50f). As long as the root is not destroyed at harvest crops such as spinach and cabbage are able to resprout and can be used as a catch crop or can even be harvested a second time (Suzuki et al., 2019; Zemek et al., 2020). Especially at high precipitation within the first weeks after harvest, N leaching losses can effectively be reduced by this strategy by conserving N in the remaining plant biomass (Benincasa et al., 2017). However, the effectiveness of this strategy depended highly on the growth performance of the resprouting spinach crop residues. In fact, in two of the four field trials the N content in the aboveground crop residues decreased during the off-season (Section 2.2, **Figure 5**, p. 50). This indicates that spinach is no reliable substitute for common winter hard catch crop species such as cereals or oilseed radish. Based on this background, a superficial sowing of a catch crop on the soil surface into the crop residues without scratching the soil seems to be a promising strategy to ensure N uptake and a low mineralization rate. In this way nitrogen can first be conserved in the spinach biomass and after its decay N can be taken up by the winter hard catch crop. However, at dry post-harvest weather conditions catch crop germination can be severely impaired if seeds are not drilled into the soil. Therefore, further investigations are required to evaluate this option in terms of the effectiveness in reducing N losses as well as its practicability.

By postponing the tillage to late autumn or spring, the NO_3^- concentration remained low until spring. Thus, low leaching losses can be assumed. In contrast, according to the balance sheet, potential N losses in the no-till treatment were significant if the spinach biomass was decaying on the soil surface (Section 2.2, **Figures 5** and **7**, pp. 50 and 52). These losses might be due to gaseous emissions and immobilization. However, according to an incubation study with cauliflower crop residues, characterized by a C/N ratio similar to that of spinach, soil N immobilization was not increased after a mulch treatment (Rashti et al., 2017). In addition, no dead plant material was observed on the soil surface in early spring, in which nitrogen could still have been conserved (Section 2.2, **Figure 7**, p. 52). However, gaseous N losses via volatilization of NH_3 and the emission of N_2O are usually increased when immature crop residue biomass is left on the field (Janz et al., 2022). In general, the higher the N content in the decaying plant tissue, the higher the potential NH_3 volatilization (De Ruijter and Huijsmans, 2019). When decaying on the soil surface, 5–16% of the plant biomass-N was lost via NH_3. In contrast, if the plant biomass was incorporated into the soil, NH_3 losses were negligible in most cases (De Ruijter et al. 2010a; Nett et al., 2015; Nett et al., 2016). On the other hand, emissions of N_2O and N_2 after the incorporation of broccoli, sugar beet, and leek crop residues into a sandy soil ranged between 10 and 15% of the N in the residues. If the residues were decaying on the soil surface, 2 to 7% was lost via N_2O and N_2 emissions (De Ruijter et al., 2010b). Nitrous oxide is a strong climate gas. Therefore, most studies dealing with gaseous N emissions during the off-season of vegetable crop rotations only consider N_2O. However, even if only N_2O is considered, between 0.1 and 14.6% of the biomass-N was lost after mixing different vegetable and arable crop residues into sandy soils (Basalirwa et al., 2020; Nett et al., 2015; Nett et al., 2016; Rizhiya et al., 2011; Velthof et al., 2002). Besides the inherent characteristics of the plant tissues, the variation in N_2O emission was explained by soil parameters such as the NO_3^- concentration, moisture content, soil pH, and clay content. All these parameters significantly affected both denitrifying and nitrifying microorganisms in arable soils after the application of crop residues (Abalos et al., 2022a; Chen et al., 2013). Overall, gaseous N losses may partially explain N losses in both the tillage and the no-till treatment.

The removal of crop residues with a C/N ratio < 25, typically for aboveground vegetable crop residues, decreased the mean N_2O losses by 76%. In addition, NO_3^- leaching losses can be significantly reduced (Agneessens et al., 2014a; Armbruster et al., 2013; Essich et al., 2020; Spiess et al., 2021). The effectiveness of this option is dependent on the N_{min} residue at harvest and quantity of N removed with the crop residues. Hence, the removal of e.g. cabbage crop residues, with typically more than 200 kg N ha^{-1} bound in the aboveground residues, has a higher potential to reduce post-harvest N losses than the removal of spinach or lettuce crop residues with only 25–30 kg N ha^{-1} bound in the aboveground residues (Tei et al., 2020; Zemek et al., 2020). However, from an economic point of view, the removal appeared to be less attractive for growers since technical investment is required to collect vegetable crop residues (Agneessens et al., 2014b; De Noraris et al., 2022). Moreover, the biomass must be valorized via e.g. composting or ensiling and finally re-applied to the field (Abalos et al., 2022a; Agneessens et al., 2014a).

Another approach to reduce the overall N losses during the off-season is the co-incorporation of the crop residues with organic amendments rich in readily decomposable C fractions characterized by a high C/N ratio such as cereal straw, sawdust, paper waste, or immature composts. These materials can trigger N immobilization and, in this way, reduce N losses by leaching and gaseous emissions (Abalos et al. 2022a; Rahn et al., 2003a; Rahn et al., 2003b). On the other hand, the increased microbial activity after the incorporation of easily degradable C fractions can lead to a severe oxygen depletion in the soil. As a result of this depletion, denitrifying microorganisms reduce NO_3^- to N_2O (Janz et al., 2022). In addition, large amounts of these materials must be applied in order to be effective, making this option impractical and cost intensive (Agneessens et al., 2014b). A further approach to reduce overall N emissions into the environment is the co-incorporation of NO_3^- adsorbing synthetic layered double hydroxides as well as biochars made by carbonizing different types of biomasses (Borchard et al., 2019; Mohammadi et al., 2021; Torres-Dorante et al., 2009). However, the long-term effects on soil N dynamics after the incorporation of immobilizing or NO_3^- adsorbing materials are variable. Thus, crop yield in the following growing seasons can be adversely affected (Borchard et al., 2019; Chaves et al., 2007; Rahn et al., 2003b; Siedt et al., 2021). In contrast, nitrification and urease inhibitors are almost fully decomposed within a few months after their incorporation into soils (Vilas et al., 2019; Section 2.2, **Figures 8** and **S1**, pp. 54 and 59). On the other hand, their effectiveness is also diminished in this way. In section 2.2 (**Tables**

S1 and **S2**, p. 59) it was shown that nitrification was inhibited at best for a few weeks after the application of the inhibitor DMPP. This period was far too short to reduce NO_3^- leaching losses during the off-season. Generally, further long-term field trials are required for a better understanding of how the nitrification, denitrification, as well as volatilization of NH_3 are affected by the treatment of crop residues with nitrification inhibitors (Abalos et al., 2022a). In addition, the effectiveness of urease inhibitors has not yet been examined in the post-harvest management of arable crops.

Besides N losses into the environment, soil fertility and phytosanitary aspects should be addressed as well. For instance, by postponing the tillage and drilling to late autumn, catch crop growth was rather ineffective during winter (Section 2.2, **Figures 4** and **6**, pp. 50 and 52). Hence, the soil surface remained bare until spring. However, bare soils are highly prone to erosion, which can lead to reduced soil fertility in the long term (Agneessens et al., 2014a). Generally, conservation tillage can significantly reduce the overall soil losses caused by erosion (Meyer et al., 1999). Based on the growth of the crop residues, the no-till treatment appeared to be the most effective in preventing erosion (section 2.2, **Figures 6** and **7**, p. 52). On the other hand, phytosanitary aspects must be considered when allowing crop residues to re-sprout (Neeteson et al., 1999). In late summer and autumn, cool night temperatures in combination with sunny days lead to dew formation within the dense spinach canopy in the weeks before harvest. Dew formation, however, favors the infection of downy mildew (Kandel et al., 2019). Thus, resprouting of infected spinach plants allows obligate parasites such as downy mildew and soil-borne diseases to continue growing and sporulating (Lamichhane et al., 2017). As a result, adjacent spinach fields as well as following spinach crops grown in the same field can become infected by these diseases (Kandel et al., 2019). Based on the pathogenicity test, performed at the end of the off-season, the disease severity index was generally high and independent of the crop residues management (Section 2.2, **Table 4**, p. 53). To mitigate the risk of such infections, farmers usually grow spinach only every fourth year on the same site (Pretty et al., 2008). Further investigations are required to test whether this period must be extended if the incorporation of the crop residues is postponed to late autumn or spring. In addition to the mentioned risks for the soil fertility and plant health, most of the mentioned crop-residue strategies are cost intensive. Without financial compensation, this will hamper the willingness of producers to implement optimized crop residue strategies (De Notaris et al., 2022).

3.3 Nitrogen fertilization strategies to reduce harmful ammonia and ammonium exposures to organically pot-grown basil

In contrast to the open-field cultivation, direct NO_3^- leaching losses can be neglected when crops are grown in soilless closed-loop systems (Massa et al., 2020; Pignata et al., 2017). In soilless cultivation, plants grow in nutrient solution or a frequently fertigated small substrate volume. However, in the production of organically certified crops nutrient supply depends on the mineralization of organic fertilizers in the growing medium. Thus, synchronization of N demand and N supply is challenging in organic soilless cultivation (Treadwell et al., 2007). An insufficient or imbalanced nutrient supply, salt stress, an unfavorable substrate pH, and the presence of phytotoxic allelochemicals have been identified as the causes of adverse effects on plant growth following an organic fertilization of growing media (Bergstrand, 2022; Bi et al., 2010; Burnett et al., 2016; Moncada et al., 2021; Nair et al., 2011; Paillat et al., 2022). In addition, harmful NH_4^+ and NH_3 exposures were detected following a high organic N supply

(Frerichs et al., 2019; Zandvakili et al., 2019). The sensitivity to ammonical exposure is dependent on the crop species, their development stage, and the exposed plant organ (Krupa, 2003). The experiments revealed that basil seedlings are sensitive to both NH_3 and NH_4^+ as well as a high NH_4^+/NO_3^- ratio in the growing media (Section 2.3, **Tables 3**, **5**, and **6**, pp. 75f). A high NH_4^+/NO_3^- ratio can adversely affect basil growth even at low N supply levels (Frerichs et al., 2019; Kiferle et al., 2013). Moreover, a high NH_4^+ supply can lead to physiological disorders of basil and subsequently increase its susceptibility to some diseases (Dickson and Fisher, 2019; Elad et al., 2021; Yermiyahu et al., 2020).

To identify ammonical toxicity as a cause for plant damage, the growing medium can be analyzed for its NH_4^+ and NO_3^- concentrations. In addition, the NH_3 exposure can be derived by the NH_3/NH_4^+ equilibrium, which is mainly dependent on the substrate pH and temperature. However, in heated greenhouses the substrate N turnover is rapid and laboratory analysis might take too long for growers to respond to unfavorable conditions. Therefore, the development of on-site diagnostic plant and substrate tests would be beneficial (Treadwell et al., 2007). By using the open-top chambers, it was possible to determine gaseous NH_3 in the plant canopy environment within a few minutes (Section 2.3, **Figure 1** and **2**; p. 68). However, basil was adversely affected even below the detection limit of 0.125 ppm NH_3. Therefore, the sensitivity of the open-top chamber approach must be improved in the lower detection range (Frerichs et al., 2021). In other investigations indicator paper and gel pads were used to determine the NH_3 concentration (Diaz-Perez et al., 2017; Zandvakili et al., 2019). However, these approaches are also less sensitive at low exposure levels. Furthermore, other technical devices for determining NH_3 are expensive or proved to be inappropriate for detecting spatial differences between small plots (Gericke et al., 2011). Due to these bottlenecks in the measurement procedure, strategies are required by which harmful NH_3 and NH_4^+ exposures can be excluded right from the start of the cultivation.

A way to decrease the exposure of basil seedlings to NH_4^+ and its conjugate base NH_3 that can easily be implemented in practice was to reduce the N base fertilization rate (Section 2.3, **Figure 3**, p. 70). This is a strategy that is commonly used to prevent adverse effects following an organic base fertilization. To ensure a sufficient N supply in potted plants the reduced base fertilization must be supplemented by a frequent fertigation using liquid organic fertilizers such as hydrolysates (Burnett et al., 2016; Koller et al., 2014). Hydrolysates are mainly based on chemically, physically, or biologically treated biomass residues such as sugar beet molasses and feather meal from the food industry (Möller and Schultheiß, 2014). These liquid organic fertilizers are characterized by a rapid and almost complete N mineralization within 4 weeks after their application to organic growing media. Thus, a frequent fertigation of hydrolysates can help to synchronize N supply and N demand of fast-growing crops (Burnett et al., 2016; Treadwell et al., 2007). This was confirmed by using the hydrolysate AFF (Section 2.3, **Figure 4**, p. 71). In addition, no harmful accumulation of ammonical N species were observed following fertigation. This observation was probably due to the addition of only 100 mg N per drench and the fast nitrification in the growing media during the last weeks of cultivation. On the other hand, hydrolysates are more expensive than most granulated solid organic fertilizers (Möller and Schultheiß, 2014). Furthermore, liquid organic fertilizers promote the growth of microfilms, which can lead to clogging in the irrigation equipment (Burnett et al., 2016). The microbial activity in the basin of the nutrient solution generally challenges the use of these fertilizers in closed-loop fertigation systems. As a result, the recirculating solution must be discharged from time to time, leading to point emissions into surface waters (Breukers et al., 2014; Gabriel and Quemada, 2017; Laber and Lattauschke, 2020). Therefore, it might be more attractive for growers to cover plant N demand by a high base fertilization using granulated fertilizers. However, as described above, a high organic base fertilization proved to be challenging in crops such as basil, which are described as being most sensitive to ammonical N during emergence a few days after sowing (Asp et al., 2022; DeKalb et al., 2014). Strategies must therefore be developed that enable a high base fertilization without negatively affecting the crop.

Within the first 7–21 days after sowing basil seedlings were adversely affected even below a concentration of 0.1 ppm NH_3 and 50 mg NH_4^+-N (L substrate)$^{-1}$ (Section 2.3, **Table 6**, p. 76). In pure peat substrates it took at least four weeks until ammonical exposure dropped below these levels (Section 2.3, **Figures 3** and **4**, pp. 70f). In addition, maximum fresh mass yield can only be achieved by a sufficient NO_3^- supply at a low NH_4^+/NO_3^- ratio (Frerichs et al., 2017). Because the application of NO_3^- fertilizers is prohibited in certified organic cultivation (European Commission, 2018) the NO_3^- supply is dependent on mineralization and nitrification kinetics of organic fertilizers in the growing media (Cannavo et al., 2022). However, proper nitrification in pure peat substrates was delayed for several weeks after application of the base fertilizer (Section 2.3, **Figures 3D** and **4D**, pp. 70f). The issue of a high substrate ammonical N concentration and low initial nitrifying activity can be overcome by storing the growing medium until nitrification takes place (Verhagen, 2021). To eliminate general phytotoxic effects caused by organic fertilizers, amended substrates should be stored for at least 14 days before use (Diaz-Perez et al., 2017; Koller et al., 2004; Nair et al., 2011). In terms of nitrification, a temperature of 28 °C and a substrate humidity of -10 pKa was described as being the optimum after mixing organic fertilizers into growing media (Cannovo et al., 2022). However, even under favorable conditions for nitrifying microorganisms, it may take several weeks until the substrate NO_3^- concentration increases noticeably (Paillat et al., 2020; Verhagen, 2021). Such an extended and costly storage treatment seems to be impractical for the substrate industry and growers (Vandecasteele et al., 2022; Verhagen, 2021). Therefore, strategies are required to accelerate and enhance the conversion of ammonical N to NO_3^- in the growing media.

In contrast to peat and commonly used peat substitutes such as wood fibers and coir products, the microbial activity in mature composts is high and can be used as an inoculum for nitrifying cultures (Grunert et al., 2016; Pot et al., 2021). However, due to the varying chemical and biological properties of composts, care should be taken to select an appropriate batch (Atzori et al., 2021; Vandecasteele et al., 2022). Some composts may even inhibit nitrification by e.g. immobilization (Gruda, 2019; Messiga et al., 2022; Vandecasteele et al., 2017). If the intention is to promote nitrification a mature compost in which NO_3^- has already formed should be amended (DeKalb et al., 2014). This was realized in an experiment by mixing 5% (v/v) of a green waste compost containing NO_3^- into a peat substrate. However, it still tooks about two weeks before the nitrification rate increased noticeably (Section 2.3, **Figure 4D**, p. 71). Similar observations were made after mixing different growing media components with 10–30% (v/v) of composted bark or green waste composts (Cannovo et al., 2022; Delics et al., 2017; Verhagen, 2021). Overall, a certain storage period appeared to be essential to prevent harmful ammonical exposures as well as provide a sufficient NO_3^- supply right from the seedling stage.

Based on the pH-dependent NH_3/NH_4^+ equilibrium the NH_3 exposure tended to be increased at both a high NH_4^+ concentration as well as a high substrate pH (Diaz-Perez et al., 2017; Zandvakili et al., 2019). Thus, lowering the initial substrate pH decreased the NH_3 exposure after an organic base fertilization (Section 2.3, **Figure 3C**, p. 70). However, NH_4^+ oxidizing microorganisms prefer neutral to slightly alkaline conditions (Norton and Ouyang, 2019). At a substrate pH ≤ 5.4 (saturated paste extract) nitrification is strongly diminished in contrast to ammonification, which can lead to NH_4^+ accumulation (Lang and Elliott, 1991; Paillat et al., 2020). Ammonification itself is a H^+-consuming process, leading to a pH increase depending on the pH buffering capacity of the growing medium (Verhagen, 2019; Verhagen, 2021). This was confirmed by e.g. an increase of the substrate pH from 5.4 to 6.3 within the first week after an organic base fertilization of 750 mg N (L substrate)$^{-1}$ (Section 2.3, **Figure 3B**, p. 70). However, this increase was ineffective for the triggering of nitrification within the following four weeks. Therefore, a low initial substrate pH should be avoided to allow for a rapid onset of nitrification. On the other hand, at a substrate pH close to or above 7.0, the availability of P and trace elements was reduced and caused deficit symptoms (Section 2.3, **Figures 4** and **6**, pp. 71 and 73). In contrast to ammonification, the oxidation of NH_4^+ is an acidifying process, leading to a pH decrease depending on the pH buffering capacity of the substrate (Verhagen, 2019; Verhagen, 2021). In addition, if ≥ 14% of the mineral N is provided as NH_4^+, basil roots tend to decrease the rhizosphere pH via the release of H^+ (Dickson and Fisher, 2019). As a result of strong ammonification followed by a strong nitrification as well as physiological responses of the plant roots the substrate pH can vary by 1–2 units (Frerichs et al., 2019; Verhagen, 2021). Overall, the substrate pH should be buffered within a range to avoid the above-mentioned deficit symptoms as well as allow for a rapid nitrification of ammonical N species.

The pH buffering capacity of growing media is highly dependent on its cation exchange capacity (CEC) and carbonate buffer (Neumaier and Meinken, 2015; Paillat et al., 2020; Verhagen, 2019). The higher the CEC, the more H^+ ions as well as cations such as NH_4^+ can be adsorbed on negatively charged particles of the growing medium. In this way the burst of NH_4^+ ions during ammonification and the burst of H^+ ions during nitrification in the substrate solution can be reduced (Paillat et al., 2020; Verhagen, 2021). With the adsorption of NH_4^+ ions the NH_3 volatilization after an organic fertilization can also be reduced (Ells et al., 1991). The pH buffering capacity of peat and wood fiber substrates can be increased by the addition of bark or composted bark (Neumaier and Meinken, 2015; Paillat et al., 2020; Pancerz and Altland, 2020). The substrate pH of bark products is between 5.0 and 7.0. In contrast, green waste composts usually have a substrate pH above 7.0 as well as a high carbonate content which buffers H^+ ions (Neumaier and Meinken, 2015). Hence, NH_3 volatilization might be increased by the amendment of compost. However, after mixing 5% (v/v) compost into a peat substrate, the substrate pH gradually decreased from 7.0 to 6.3 within two or three weeks after sowing (Section 2.3, **Figure 4B**, p. 71). Due to the small compost volume, it can be assumed that the alkalizing effect of the compost was compensated for by the acidifying nitrification.

By using the open-top chambers, it was not possible to draw conclusions about the quantitative N losses via NH_3 volatilization. However, differences in the NH_4^+ accumulation can be used to derive these N losses. At an N supply level of 750 mg N (L substrate)$^{-1}$ the balance gap between an initial substrate pH of 5.5 and 6.5 was up to 150 mg NH_4^+-N (L substrate)$^{-1}$ (Section 2.3, **Figure 3A**, p. 70). This quantity is equivalent to 38 kg N ha^{-1}. Based on the pH-dependent NH_3/NH_4^+ equilibrium it appears that NH_3 volatilization significantly contributed to this balance gap. However, pH-dependent differences in substrate mineralization and immobilization turnover should also be considered. Besides NH_3, N can be lost via gaseous emission of N_2O and N_2. These N species are formed by the denitrification of NO_3^- and can also be emitted as a side product during the nitrification process (Deppe et al., 2017; Norton and Ouyang, 2019). However, due to the high air capacity of peat-based substrates denitrification can usually be neglected (Agner and Schenk, 2005). In addition, the NO_3^-

concentration, as a substrate for denitrifying microorganisms, was rather low in most treatments (Section 2.3, **Figures 3D** and **4D**, pp. 70f). In contrast to denitrification, no NO_3^- is required for gaseous N emissions emitted during the nitrification process. In particular, when the activity of NO_2^- oxidizing microorganisms is diminished due to e.g. a high NH_3 concentration in the substrate solution, gaseous N_2O emission can be increased (Norton and Ouyang, 2019; Venterea et al., 2015). Therefore, the high NH_3 and NH_4^+ exposures and temporary accumulation of NO_2^- could be an indication of N_2O emission (Section 2.3, **Figures 3** and **4**, pp. 70f). However, investigations dealing with N emissions after an organic fertilization of pot-grown crops are scarce and contradictory. For instance, the N_2O emission ranges between 0 and 13% of the N available in the substrate within an incubation period of 60 days after mixing composts or organic fertilizers into peat-based and coir-based substrates (Dion et al., 2020; Lévesque et al., 2018; Messiga et al., 2022).

Overall, the amendment of a peat substrate with mature green waste compost in combination with a 2-week storage period has proven to be appropriate to avoid harmful ammonical exposure levels and provide a sufficient NO_3^- supply right from the seedling stage. In this way, it was possible to realize a high base fertilization without adversely affecting basil growth. However, substrate N turnover is highly dependent on the type of organic fertilizers and growing media (Paillat et al., 2020; Paillat et al., 2022; Treadwell et al., 2007). This is challenging in view of the various organic fertilizers and substrate components used in the production of organic certified crops (Bergstrand, 2022; Burnett et al., 2016). Therefore, further investigations are required to better understand the microbial processes in soilless organic vegetable crop production systems (Rogers, 2017). This also includes the examination of different storage conditions on the biological, physical, and chemical properties of these mixtures. Besides culinary herbs consumer demand for organically certified pot-grown ornamentals and tree nursery crops is still rising (Asioli et al. 2017; Bergstrand, 2022; Burnett et al., 2016). Therefore, further investigations are required to evaluate whether the above-mentioned strategies are appropriate to prevent plant damage in crops other than basil.

Chapter 4
Conclusions

Reducing the base fertilization rate and splitting the top dressing effectively lowered the NO_3^- concentration exposed to leaching in the production of field-grown spinach. In addition, the splitting approach made it possible to dispense with the second top dressing if the crop was harvested at an early harvest stage. On the other hand, depending on the season, these measures negatively affected the marketable yield at an early harvest stage. In contrast, at a late harvest stage, spinach was less affected by the N fertilizer schedule. This shows that spinach was partially able to recover for any hindrances to growth observed in earlier development stages. A further division of the fertilizer N supply into several dressings by a frequent foliar urea spraying proved to be insufficient to promote plant growth and caused leaf necrosis in comparison to spreading of granulated fertilizers in combination with a subsequent irrigation. In the spring and winter seasons, spinach was highly responsive to the fertilizer N supply. In contrast, summer-grown and autumn-grown spinach appeared to be limited by bolting and decreasing irradiation, allowing growers to reduce the total N supply without affecting the marketable yield. To better assess whether the timing of the fertilization can be delayed or the total fertilizer N supply can be reduced, recent developments in plant and soil monitoring technologies seem to be promising and should be considered in further investigations. In view of market demands, consumers are becoming increasingly interested in the environmental aspects of the products they consume. Hence, quality attributes such as the leaf coloration might be less crucial in the future, allowing for more sustainable production.

Relative to the entire growing season, N residues resulting from a high N supply can be taken up by the following crops. However, following autumn-grown spinach winter catch crops were inefficient in taking up residual N as well as NO_3^- originating from the mineralization of crop residues and soil organic N. Thus, considerable N losses were observed during the winter leaching period. These observations were mostly independent of the tillage intensity and application of the nitrification inhibitor DMPP. In contrast, postponing the incorporation of spinach crop residues from early autumn to late autumn or spring significantly reduced potential N losses. However, the effectiveness of this strategy appeared to be mainly dependent on the growth performance of the resprouting spinach crop residues. Therefore, a superficial sowing of a winter hard catch crop without soil disturbances seems to be a promising approach to reduce N losses in the case of a low N uptake by the resprouting spinach. Based on the N_{min} concentration in the soil, most N losses were due to NO_3^- leaching. However, a direct measurement of N mineralization and immobilization kinetics, NO_3^- leaching, and gaseous emissions appeared to be vital to develop reliable post-harvest strategies. In addition, long-term phytosanitary and soil fertility aspects should also be considered since growers need to be financially compensated for implementing costly post-harvest measures.

Ammonia and NH_4^+ exposures after an organic base fertilization impaired the emergence and growth of pot-grown basil seedlings. Lowering the base fertilization rate and initial substrate pH significantly reduced ammonical exposures. However, basil is an NH_4^+-sensitive plant species and requires NO_3^- for maximum growth. To trigger the nitrification of ammonical N, mixing of mature green waste compost into a peat substrate proved to be an appropriate strategy. However, the peat-compost mixture still had to be stored for about two weeks to ensure a sufficient NO_3^- supply right from the seedling stage. Before putting this strategy into practice, also other organic N fertilizers and substrate components used in the production of organic certified crops should be considered in further trials. In addition, the effect of different storage conditions on these mixtures has to be examined.

Summary

To ensure a high yield and quality of the produce, a high fertilizer N supply is often required in vegetable crop rotations. However, the fertilizer N requirement is difficult to calculate since the N supply via mineralization and actual N uptake of the crop cannot accurately be predicted in advance. Therefore, base fertilization and top dressing are usually applied before intensive N uptake starts. However, if a crop is harvested before yield expectations are reached, this strategy can result in high N_{min} residues. In addition, high quantities of N-rich and readily mineralizable crop residues often remain in the field at harvest. In sandy soils, however, NO_3^- can be easily leached. Other significant N losses are caused by gaseous NH_3 volatilization and N_2O emission. However, the emission of these N species can directly and indirectly damage ecosystems, contribute to global warming, and contaminate sources of drinking water.

The first part of this study aimed at the risk of NO_3^- leaching losses during the cultivation of field-grown spinach. Therefore, the effect of a reduced N base fertilization as well as a split N top dressing was examined in a series of fertilization trials. The reduced base fertilization was compensated by an increased top dressing based on the soil N_{min} concentration. In a further treatment the second top dressing of 50–70 kg N ha^{-1} was applied by a frequent urea foliar spraying instead of the single application of a granulated NO_3^- fertilizer. A further part of this thesis focused on reducing post-harvest N losses following autumn-grown spinach during the winter leaching period. For this purpose, the depth, frequency, and timing of the incorporation of the spinach crop residues were varied. Furthermore, the effectiveness of spraying the crop residues with the nitrification inhibitor DMPP was examined.

The results showed that both reducing the base fertilization and splitting the top dressing significantly decreased the NO_3^- concentration exposed to leaching. In addition, the splitting approach made it possible to dispense with the second top dressing in the case of harvesting at an early harvest stage. Thus, the N_{min} residues were reduced. On the other hand, these measures diminished the crop yield at an early harvest stage by 6% on average. At a late harvest stage, spinach was less affected by the schedule of the fertilizer N supply. Due to diminished plant growth and necrosis at the leaf margin, a frequent urea foliar spray proved to be an inappropriate fertilization strategy in contrast to the application of a granulated fertilizer in combination with a subsequent irrigation. In spring-grown and winter-grown spinach, nitrogen was one of the most limiting growth factors. In contrast, in the summer and autumn seasons marketable yield appeared to be limited by bolting and decreasing irradiation, respectively. Thus, in these seasons it was possible to dispense with the second top dressing without affecting the marketable yield.

Within a few weeks after the incorporation of the spinach crop residues an intensive mineralization increased the soil NO_3^- concentration up to 100 kg N ha^{-1} (0–90 cm). Even the treatment of the crop residues with DMPP was hardly able to delay NO_3^- formation. In addition, the winter cover crops only partially absorbed the soil mineral N. As a result, high quantities of NO_3^- shifted down the soil profile during the winter leaching period. Based on an N balance sheet, potential N losses were mostly independent of the tillage intensity. In contrast, by postponing the incorporation of the crop residues from early autumn to spring the potential N losses were reduced to $\leq$ 20 kg ha^{-1}. This effect was due to the N uptake and N conservation of the resprouting spinach crop residues as well as the lower net N mineralization in the soil. However, when the spinach plants partially or completely decomposed in autumn and winter,

the potential N losses were considerable even without tillage. To ensure N uptake and a low N mineralization a superficial sowing of a winter hard catch crop into the resprouting spinach crop residues without soil disturbances seems to be a promising approach.

Unlike open-field cultivation, NO_3^- leaching losses can be effectively reduced by cultivation in soilless closed-loop irrigation systems. However, organic cultivation of fast-grown crops like basil is challenging in such systems since N supply is dependent on the mineralization of organic fertilizers in a small substrate volume. To ensure a sufficient N availability, high quantities of organic N are applied to the growing media. However, depending on the mineralization rate, this can lead to plant damage and yield depressions.

Based on this background, in the third part of this study it was examined whether pot-grown basil is affected by NH_3 and NH_4^+ exposures resulting from an organic fertilization. Therefore, basil was cultivated at different base N fertilization and substrate pH levels. The growing media consisted of pure peat or a mixture of peat and 5% (v/v) mature green waste compost.

A high base fertilization as well as high substrate pH caused considerable damage to basil seedlings and diminished crop growth. These observations were mainly explained by the ammonical exposure. Thus, both a reduced base fertilization and lower initial substrate pH mitigated these effects. However, optimum plant growth was only achieved by the amendment of compost. In addition, the peat-compost mixture had to be stored for about two weeks before sowing. In this approach an early initiation of the nitrification led to a fast and effective reduction of the NH_3 and NH_4^+ exposures right from the seedling stage. Furthermore, the resulting increased NO_3^- supply promoted basil growth. Due to the variety of organic fertilizers and substrate components used in organically grown crops, further trials are required to make general recommendations.

The optimized synchronization of N demand and N supply proved to be effective in reducing potential NO_3^- leaching losses in open fields as well as prevent harmful exposures to NH_3 and NH_4^+ in organically pot-grown basil. However, the development of specific plant and soil monitoring technologies seems to be necessary to better estimate whether the N fertilization can be delayed or even the total N supply can be reduced without affecting marketable yield in individual cases. Quantitatively, most N losses were observed in the off-season during the winter leaching period. A holistic approach to reducing N losses and emissions into the environment must therefore also include the post-harvest strategy.

Zusammenfassung

Zur Sicherstellung von Ertrags- und Qualitätszielen gemüsebaulicher Kulturen müssen zumeist hohe N-Düngergaben ausgebracht werden. Eine besondere Schwierigkeit bei der Ableitung des N-Düngebedarfes ist es, dass das N-Angebot durch Mineralisierung sowie der tatsächliche N-Bedarf einer Kultur nicht exakt vorhergesagt werden können. Grund- und Kopfdüngung werden daher bereits vor Einsetzen starker N-Aufnahme ausgebracht. Im Falle einer Ernte vor Erreichen der Ertragsziele kann diese Strategie allerdings zu hohen N_{min}-Resten führen. Zusätzlich verbleiben bei der Ernte häufig hohe Mengen an N-reichen und leicht mineralisierbaren Ernterückständen auf dem Feld zurück. In sandigen Böden unterliegt das NO_3^- allerdings einem hohen Auswaschungsrisiko. Weitere bedeutende N-Verlustpfade sind gasförmige Emissionen von NH_3 und N_2O. Der Eintrag dieser N-Spezies in die Umwelt kann jedoch direkt und indirekt Ökosysteme schädigen, zur Klimaerwärmung beitragen sowie mit der Belastung von Trinkwasserquellen einhergehen.

Der erste Teil dieser Studie zielte darauf das Risiko der NO_3^--Auswaschung im Freilandanbau von Spinat zu senken. Dazu wurde in einer Reihe von Düngungsversuchen der Effekt einer reduzierten N-Grunddüngung sowie Splittung der N-Kopfdüngung untersucht. Das N-Angebot bei reduzierter Grunddüngung wurde dabei durch eine erhöhte Kopfdüngung basierend auf der N_{min}-Konzentration ausgeglichen. In einer weiteren Variante wurde die zweite Kopfdüngung von 50–70 kg N ha^{-1} anstelle einmaligen Streuens eines granulierten NO_3^--Düngers durch regelmäßige Harnstoff-Blattspritzungen ersetzt. Ein weiteres Forschungsziel war die Minderung von N-Verlusten nach Abschluss der Anbausaison über die Auswaschungsperiode im Winter. Dazu wurden Tiefe, Frequenz und Zeitpunkt der Einarbeitung der Spinat-Ernterückstände variiert. Ergänzend hierzu wurde die Effektivität einer Behandlung der Ernterückstände mit dem Nitrifikationshemmstoff DMPP untersucht.

Die Ergebnisse zeigten, dass durch die Reduzierung der Grunddüngung sowie Splittung der Kopfdüngung die Konzentration an auswaschungsgefährdetem NO_3^- deutlich gemindert wurde. Des Weiteren ermöglichte die Splittung der Kopfdüngung es bei einer Ernte in einem frühen Erntestadium auf die zweite Kopfdüngung zu verzichten und somit die N_{min}-Reste zu reduzieren. Auf der anderen Seite gingen die erprobten Ansätze in einem frühen Erntestadium mit einem durchschnittlichen Minderertrag von 6% einher. Zu einem späteren Erntestadium war Spinat hingegen weniger empfindlich gegenüber der Staffelung der N-Gaben. im Vergleich zur Ausbringung von granulierten Düngern in Kombination mit einer anschließenden Bewässerung erwiesen sich die regelmäßigen Urea-Blattspritzungen aufgrund von Mindererträgen und Ausbildung von Blattrandnekrosen als eine ungeeignete Düngestrategie. Im Frühling und Winter war Stickstoff einer der meist limitierenden Wachstumsfaktoren. Im Sommer schienen Wachstum und Qualitätsmerkmale hingegen vordergründig durch das Einsetzen der Schosserbildung und im Herbst durch die abnehmende Sonneneinstrahlung bestimmt. So war es in diesen Jahreszeiten möglich auf die zweite N-Kopfdüngung zu verzichten ohne Ertrags- und Qualitätseinbußen hinnehmen zu müssen.

Binnen weniger Wochen nach Einarbeitung der Spinat-Ernterückstände führte eine intensive Mineralisierung zu einem Anstieg der NO_3^--Konzentration auf bis zu 100 kg N ha^{-1} (0–90 cm). Auch über die Behandlung der Ernterückstände mit DMPP konnte die NO_3^--Bildung nicht effektiv verzögert werden. Des Weiteren konnten die Winterzwischenfrüchte den mineralischen Stickstoff nur teilweise binden, sodass hohe NO_3^--Mengen über den Winter in Richtung

Grundwasser verlagert wurden. Die anhand einer Bilanz ermittelten potenziellen N-Verluste waren weitestgehend unabhängig von der Intensität der Bodenbearbeitung. Im Gegensatz dazu konnten die potenziellen N-Verluste durch die Verschiebung der Einarbeitung vom Herbst ins Frühjahr auf ≤ 20 kg ha^{-1} gesenkt werden. Ursächlich hierfür war die N-Aufnahme und N-Konservierung der wiederaustreibenden Ernterückstände sowie eine verminderte Netto-Mineralisierung im Boden. Brachen die Spinatpflanzen hingegen über den Herbst und Winter teilweise oder vollständig zusammen, waren die N-Verluste selbst ohne Bodenbearbeitung erheblich. Zur Sicherstellung der N-Aufnahme und einer geringen Mineralisierung scheint die oberflächliche Aussaat von winterharten Zwischenfrüchten in die wiederaustreibenden Ernterückstände ohne Bodenbearbeitung ein vielversprechender Ansatz.

Im Gegensatz zum Freilandanbau kann die NO_3^--Auswaschung effektiv über den erdelosen Anbau in geschlossenen Bewässerungssystemen reduziert werden. Im ökologischen Anbau von schnell wachsenden Kulturen wie Topfbasilikum stellt der erdelose Anbau allerdings besondere Anforderungen an die N-Düngung. So ist die N-Verfügbarkeit von der Mineralisierung organischer Dünger in einem nur kleinen Substratvolumen abhängig. Zur Sicherstellung einer ausreichenden N-Verfügbarkeit werden den Kultursubstraten daher hohe Mengen an organischen N-Düngern zugegeben. Allerdings kann dies in Abhängigkeit der Mineralisierungsrate mit Pflanzenschäden und Ertragsminderungen einhergehen.

Vor diesem Hintergrund wurde im dritten Teil dieser Studie geprüft, inwiefern Minderwachstum und Pflanzenschäden durch erhöhte NH_3- und NH_4^+-Expositionen nach organischer N-Düngung ausgelöst werden können. Dazu wurde Topfbasilikum bei unterschiedlich hoher N-Grunddüngung sowie unterschiedlichen Substrat-pH-Werten kultiviert. Als Kulturmedium wurde ein reines Torfsubstrat mit einer Mischung aus Torf und 5% (v/v) reifem Grüngut-Kompost verglichen.

Eine hohe N-Grunddüngung sowie ein hoher pH-Wert des Substrates gingen mit erheblichen Pflanzenschäden an den Basilikum-Sämlingen einher. Diese konnten anhand der gemessenen NH_3- und NH_4^+-Expositionen erklärt und somit durch Absenkung des pH-Wertes und Reduzierung der N-Grunddüngung vermindert werden. Optimales Pflanzenwachstum zeigte sich allerdings nur wenn dem Kultursubstrat Kompost beigemischt war und diese Mischung vor der Aussaat noch für etwa zwei Wochen gelagert wurde. In diesem Ansatz führte ein frühzeitiges Einsetzen der Nitrifikation zu einer schnellen und effektiven Reduzierung der NH_3- und NH_4^+-Expositionen. Des Weiteren wirkte sich das daraus resultierende erhöhte Angebot an NO_3^- günstig auf das Pflanzenwachstum aus. Aufgrund diverser organischer Dünger und Substratkomponenten, die im ökologischen Anbau Verwendung finden, sind weitere Untersuchungen erforderlich, um allgemeingültige Empfehlungen ableiten zu können.

Die optimierte Synchronisation von N-Bedarf und N-Angebot erwies sich als effektiv zur Minderung des NO_3^--Auswaschungsrisikos im Freiland sowie Reduzierung pflanzenschädigender NH_3- und NH_4^+-Expositionen im ökologischen Anbau von Topfbasilikum. Allerdings erscheint die Weiterentwicklung von spezifischen Boden- und Pflanzensensoren erforderlich, um im Einzelfall besser abschätzen zu können, ob die N-Düngung verzögert oder reduziert werden kann ohne den Ertrag zu mindern. Quantitativ wurden die höchsten N-Verluste allerdings erst nach Abschluss der Anbausaison über die Sickerwasserperiode im Winter beobachtet. Ein ganzheitlicher Ansatz zur Minderung von N-Verlusten und Einträgen in die Umwelt muss daher auch die Nacherntestrategie umfassen.

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Acknowledgements

Without support, help, and confidential cooperation it would not have been possible for me to conduct all the investigations, analyses, and to make the publications relating to this piece of work. Therefore, I would like to express my gratitude to the following people for contributing to the success of my research and this thesis.

Firstly, I am particularly grateful to Prof. Dr. Gabriele Broll for giving me the opportunity to carry out my PhD thesis at the University Osnabrück and for her supervision during my doctoral studies.

My deepest gratitude also goes to Prof. Dr. Diemo Daum for supervising me and the confidence that he has placed in me. He accompanied my work from the conception of the experiments to the evaluation and publication of the results. His suggestions and ideas are always a great motivation for me. After all, I have learned a lot from him about scientific work and writing, for which I am very grateful.

I also wish to express my appreciation to Dr. Andreas Pacholski for his advisory support in the development of the NH_3 measurement methods and for his contribution to the publication of the results.

Special thanks also to Mrs. Rita Schäpers from the iglo group in Reken, Germany. Her interest in my research as well as her ideas and suggestions always motivated me.

I would also like to sincerely thank Dr. Maria Vormann from the Chamber of Agriculture North Rhine-Westphalia for all her advice and reflections.

In addition, I wish to extend my special thanks to all farmers who made their land available to conduct my field studies. I am particularly grateful to Mr. Thomas Föing for sharing with me his experience in the cultivation of spinach and arable cropping in the region Borken.

Finally, deepest thanks go to my family and my girlfriend Kira for always giving me confidence in my abilities. This applies in particular to my parents Emke and Gunda, from whom I learned patience, perseverance, and conscientiousness.

Annex

Abstracts of first-authored papers

Ammonium toxicity – one cause for growth and quality impairments on organic fertilized basil?

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J. Kulturpflanzen **2017** 69(3), 101–112. doi: 10.1399/JfK.2017.03.02

Abstract

In the organic production of pot grown basil yield depressions and quality impairments are often observed. During the early development stage cotyledons become chlorotic and necrotic. Subsequently, infections with secondary parasites such as Botrytis may occur. One possible reason for this problem could be the high concentration of ammonium in the growing media released by the mineralization of organic fertilizers. Therefore, a fertilization trial was carried out including different ammonium-N/nitrate-N ratios (100/0; 50/50; 0/100) and nitrogen concentrations in the nutrient solution (8, 12 and 16 mmol N/L). Plants were cultivated in a peat substrate and fertilized by using the ebb and flow technique. The applied nutrient solution contained, beside the different nitrogen sources, equal concentrations of a base fertilizer as well as the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP). In addition, an organic fertilization treatment was realized using a solid base dressing (horn shavings and DCM ECO-MIX 4) and a liquid top dressing (Organic Plant Feed). The plants were cultivated in a peat substrate which was adjusted to an initial pH of 6.5. Basil fertilized solely with ammonium (NH_4^+) showed a diminished growth in comparison to well-developed plants receiving nitrate (NO_3^-) as nitrogen source. Germination rate, plant height and fresh matter yield of herbs were significantly reduced by NH_4^+ nutrition. Furthermore, chlorotic cotyledons and a reduction in turgidity of the shoot could be observed. Growth of plants receiving organic nitrogen initially also remained behind the NO_3^- treatment. Furthermore, with this nitrogen source cotyledons were most strongly affected by chlorosis, probably because the NH_4^+ concentration in the substrate rose up to 350 mg N/L at the beginning of the cultivation period. When nitrogen mineralization declined and NH_4^+ was increasingly converted to NO_3^-, plants exhibited improved growth. At the end of the experiment the NO_3^- content in basil shoots was highest in the organic N treatment. The most compact growth and the highest turgidity of plants were observed with balanced supply of NO_3^- and NH_4^+.

Key words:
Ocimum basilicum L., organic nitrogen fertilization, ammonium/nitrate ratio, ammonium toxicity, chlorotic cotyledons

Influence of nitrogen form and concentration on yield and quality of pot grown basil

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Acta Hortic. **2019** 1242, 209–216. doi: 10.17660/ActaHortic.2019.1242.29

Abstract

In the organic production of pot grown basil, yield depressions and quality impairments are often observed. During the early development stage, cotyledons become chlorotic and necrotic. Subsequently, fungal diseases such as botrytis occur. One possible reason for this problem could be the high concentration of ammonium in the growing media released by the mineralization of organic fertilizers. Therefore, a fertilization trial was carried out to investigate the effect of ammonium (NH_4^+) on basil in comparison to nitrate (NO_3^-). The experiment included different NH_4^+-N/NO_3^--N ratios (100/0, 50/50, and 0/100) and nitrogen (N) concentrations in the nutrient solution (8, 12, and 16 mmol N L^{-1}). Plants were cultivated in a peat substrate and fertilized with a nutrient solution which, in addition to the different N sources, contained equal concentrations of a base fertilizer as well as the nitrification inhibitor DMPP. Furthermore, an organic fertilization treatment was realized. Basil fertilized solely with NH_4^+ showed a diminished growth in comparison to well-developed plants receiving NO_3^- as N source. Germination rate, plant height and fresh matter yield were significantly reduced by NH_4^+ nutrition. Similar results occur in the organic treatment where the NH_4^+ concentration rose up to 350 mg NH_4^+-N L^{-1} substrate at the beginning of the cultivation period. Along with a reduction in biomass production, chlorotic cotyledons were observed. These effects might have been caused by NH_4^+. When N mineralization declined and NH_4^+ was largely converted to NO_3^-, plants exhibited improved growth. Within the mineral N treatments, rising NO_3^- concentration and NO_3^--N/NH_4^+-N ratio promoted plant height and reduced plant compactness due to an increased internode elongation. At the end of the experiment, the NO_3^- content in basil shoots was highest in the organic treatment and lowest with NH_4^+ as the sole N source. The best herb quality in terms of plant compactness, turgidity and healthiness of cotyledons was observed when basil was fertilized with ammonium nitrate.

Keywords:
Organic fertilization, ammonium, nitrate, nitrite, toxicity, chlorotic cotyledons

Determination of ammonia exposure of potted herbs in organic cultivation

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Acta Hortic. **2021** 1327, 167–174. doi: 10.17660/ActaHortic.2021.1327.22

Abstract

In organic production of pot-grown herbs the ammonium (NH_4^+) concentration and pH in the growing media may temporarily increase to high levels after a base dressing of organic nitrogen (N) fertilizers, thus impairing plant growth. Under these conditions, ammonia (NH_3), which is phytotoxic even at low concentrations, can also be formed. To avoid crop damage, the exposure to both ammonical N sources should be distinguished in order to develop appropriate fertilization strategies. However, the determination of NH_3 in the aerial environment of plants is challenging in small-scale in situ experiments. In order to determine the NH_3 concentration in the canopy-atmosphere of pot-grown basil, a novel small-scale open-top chamber (OTC) approach was evaluated in a series of three fertilization trials at different organic fertilization rates and initial substrate pH values. Basil pots were placed into OTCs and the air above the substrate surface was sampled and passed through an NH_3 indicator tube. The detected NH_3 concentrations were compared to both NH_3 exposure determined via passive acid traps and to calculated concentrations based on known chemical equilibria. The latter approach took into account pH and NH_4^+ concentrations in the growing media as well as air temperature at the time of NH_3 measurement. Results showed that measured NH_3 concentrations were closely correlated to values obtained from the two comparative approaches. The reproducibility of the NH_3 determination in air samples was sufficient at NH_3 concentrations above 0.2 ppm, as indicated by a coefficient of variation of ≤15%. At lower concentration levels, the variability of the readings substantially increased. However, plant growth (number of plants, fresh matter yield, and plant height) was adversely affected even at concentrations below 0.2 ppm NH_3, depending on the substrate NH_4^+ concentration. Therefore, further improvements, in particular to the sensitivity of NH_3 detection, are required to increase the reproducibility of the method in the lower concentration range.

Keywords:
Ammonia determination, open-top chamber (OTC), basil (*Ocimum basilicum* L.), organic fertilization, ammonical toxicity

Field-grown spinach production – fertilization strategies to reduce risk of nitrate leaching

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Acta Hortic. **2021** 1327, 155–160. doi: 10.17660/ActaHortic.2021.1327.20

Abstract

Spinach for the frozen food industry in Northwestern Germany is preferably cultivated in sandy soils to enable field management which is largely independent of weather conditions. However, on these sites, nitrate (NO_3^-) can easily be leached and thus contaminate the groundwater. Furthermore, short cultivation cycles (5–10 weeks) and high nitrogen (N) demand (up to 205 kg N ha^{-1} in the 0–30 cm soil layer) result in a high risk for NO_3^- leaching in spinach cultivation. In 2018–2020 ten fertilization experiments were conducted in Borken, Germany, and spread throughout the year from spring till winter. Total N dosage was calculated by the software N-Expert (IGZ Großbeeren, Germany) and split into a base and top dressing (standard procedure) or a base dressing and two top dressings. The second top dressing was applied after reaching the early harvest stage. In a third treatment the total N fertilization rate was reduced by omitting the second top dressing (50–70 kg N ha^{-1}). Overall, the fresh matter yield was reduced by splitting the N dosage into three applications and even more by reducing the total N dosage. Under reduced N supply, the foliage turned yellowish at the late harvest date. However, this phenomenon was only observed in spring. Total N dosage complied with the German Fertilizer Ordinance (2020). However, when spinach is harvested within an earlier stage, total N dosage can be above the calculated legal limit. In this case the second top dressing must be omitted to meet legislation and lower the soil mineral N (N_{min}) residue. As well as reduced N_{min} residue in an early harvest, risk of NO_3^- leaching was reduced by splitting of the total N dosage into three applications due to flatter NO_3^- peaks after fertilization. However, nutrient availability must be ensured by precipitation or irrigation immediately after the second top dressing.

Keywords:
Spinach (*Spinacia oleracea* L.), nitrogen fertilization, sandy soil, nitrate leaching, German Fertilizer Ordinance

Soil sampling depth for calculation of nitrogen base fertilization in field-grown spinach

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Acta Hortic. (in press).

Abstract

Spinach is a nitrogen (N)-demanding crop characterized by a shallow root architecture. Especially in the first weeks after sowing, significant N uptake is limited to the uppermost few centimeters of the soil. However, base fertilization is usually based on the soil mineral N (N_{min}) concentration in the upper 30 cm. Therefore, the objective of this study was to examine whether the soil sample depth for calculating the base N fertilization can be reduced to the 0–15 cm layer. In seven field trials, conducted during spring, summer and autumn seasons, either a low or high base fertilization dose was applied at sowing. Until top dressing, soil samples were frequently taken in the upper 0–15 and 15–30 cm layers to determine the average Nmin concentration in each layer. Top dressing was applied when the first true leaves had unfurled. With this fertilizer application, the total N supply was aligned between both treatments based on the Nmin concentration in the upper 30 cm of the soil. Aboveground fresh and dry masses were determined after reaching a fresh mass yield of 15–20 t ha^{-1} and related to the mean Nmin concentration in the first 3 to 4 weeks of cultivation between sowing and top dressing. It was shown that the N_{min} concentration in the upper 0–15 cm of the soil highly reflects the base fertilization rate. By contrast, the N_{min} concentration in the 15–30 cm layer remained unaffected. However, the N_{min} concentration of both top soil layers can affect fresh and dry mass yield at harvest. Therefore, the entire 0–30 cm soil layer should be considered when calculating the base N fertilization rate in field-grown spinach. Measurements revealed that spinach fresh and dry masses were increased until the N availability of between 54 and 59 kg ha^{-1} (0–30 cm) was reached at the seedlings stage, respectively.

Keywords:
Spinach (*Spinacia oleracea* L.), N_{min} concentration, base fertilization, fresh mass yield, dry mass yield

Measurement of the nitrogen supply via mineralization in field-grown spinach

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Acta Hortic. (in press).

Abstract

The mineralization of soil organic nitrogen (N) and crop residues can significantly contribute to the N supply of vegetable crops. However, short-term mineralization dynamics are difficult to predict. On the other hand, fast-growing crops like spinach are highly sensitive to N shortage. Therefore, in situ soil columns have been tested to estimate the actual N supply via mineralization in field-grown spinach. In ten fertilization trials covered soil columns (20 cm in diameter) were driven into the soil to a depth of 30 cm at the start of the cultivation. Eight columns were repeated in three blocks within a total trial area of 0.10 to 0.25 ha. Net N mineralization was derived by subtracting the soil mineral N (N_{min}) concentration in the upper 30 cm before installation from the concentration inside the columns at harvest. For comparison, a balance sheet was calculated for spinach plots receiving no N fertilization (zero plots) as well as fertilized plots and used as a proxy for net N mineralization. In this approach the initial N_{min} concentration in the upper 30 cm of the soil, the N supply via irrigation, and fertilization as well as the total aboveground N uptake by spinach and the N_{min} residue were considered. By using soil columns, N mineralization was determined with a mean coefficient of variation of 18%. A higher spatial variability of up to 43% was observed when spinach was grown as a second crop. The average net N mineralization rate ranged between 2 kg ha^{-1} $week^{-1}$ (0–30 cm) in winter-grown spinach and 3–7 kg ha^{-1} $week^{-1}$ (0–30 cm) in the other seasons. Nitrogen mineralization measured by the soil columns was qualitatively confirmed with the data obtained by the balance sheet. Soil columns enable repeated samplings during the spinach cultivation. In this way, top dressing rates can be adjusted to actual N supply.

Keywords:
Spinach (*Spinacia oleracea* L.), soil columns, balance sheet, N recovery, zero plots, crop residues, top dressing

Conference contributions and further publications

Conference contributions (oral)

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1st place Green Challange (2017) for best video, 51. Gartenbauliche Jahrestagung des Deutschen Gartenbaulichen Gesellschaft e. V. und Bundesverbandes Hochschulabsolventen/Ingenieure Gartenbau und Landschaftsarchitektur e. V.. Osnabrück, Germany. https://dgg-online.org/website_neu/tagungen/preistraeger/ (accessed Feb. 06, 2022).

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Curriculum Vitae

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Aug. 2004 – Jul. 2007	Wiesmoor Gärtnerei & Baumschule GmbH (Wiesmoor, Germany) Apprenticeship (ornamental plants)
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Erklärung an Eides statt über die Eigenständigkeit der erbrachten wissenschaftlichen Leistung

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Osnabrück, 01.03.2023 Christian Frerichs
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