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Soil and Crop Management Options to Improve Nitrogen Cycling on Dairy Farms on Peat Soil

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Chapter 1

General introduction

In this chapter I will introduce the background to issue of soil and crop management in relation to nitrogen cycling on dairy farms on peat soil, which leads to my research objectives and major research questions. The first section of this introductory chapter focusses on the history and current characteristics of peatland use for dairy farming. The second section focusses on the nitrogen (N) cycle and loss of dairy farming systems on peat soil in the Green Heart area. In the third section, effects of too much N loss and policy developments regarding N loss from dairy farming are described. The fourth section describes measures for reducing N loss on dairy farms, and the role of the soil and crops on N cycling on dairy farms on peat soil. The objectives and research questions, and outline of the thesis are given in the final sections of this chapter.

1.1 History and current peatland use of the Green Heart area

1.1.1 Peatland formation, properties and degradation

Peat is formed in permanently waterlogged and often acidic soil conditions. In these conditions, microbial decomposition of plant material is strongly inhibited, which leads to accumulation of plant material. This allows the soil surface to rise over years, while the vegetation on top of the soil continues to grow. In the Dutch soil classification system, peat soils are defined as soils with at least 40 cm of peat within 80 cm of the soil surface. Peat is defined as material consisting of at least 15-23 mass percent organic matter at a clay percentage of 0-50% (Steur & Heijink 1991). Worldwide, peatlands have been formed over thousands of years and are estimated at present to store 5.9-25.9 Gt N (Yin *et al.* 2022). Furthermore, peatlands are known for their carbon storage as, while covering only about 3% of the planet's surface, they store more carbon than the entire forest biomass in the world (Joosten 2015; Urák *et al.* 2017).

In the Green Heart area of the Netherlands, peat was mainly formed during the Holocene, which started approximately 11,000 years ago, after the last glacial period (Mulder 1986). At present, most of the peat in the Green Heart area is eutrophic or mesotrophic; this is peat formed from species such as reed, sedges, alder and willow. In some areas, more nutrient-poor oligotrophic peat is present, which was formed mainly from peat mosses in rainwater-fed conditions (Van Kekem 2004) (Figure 1.1a). Many of the peat soils in the Green Heart area of the Netherlands have a clay-rich topsoil or contain layers of clay, which were deposited in or on top of the peat during tidal and river floods (Van Kekem 2004) (Figure 1.1b).

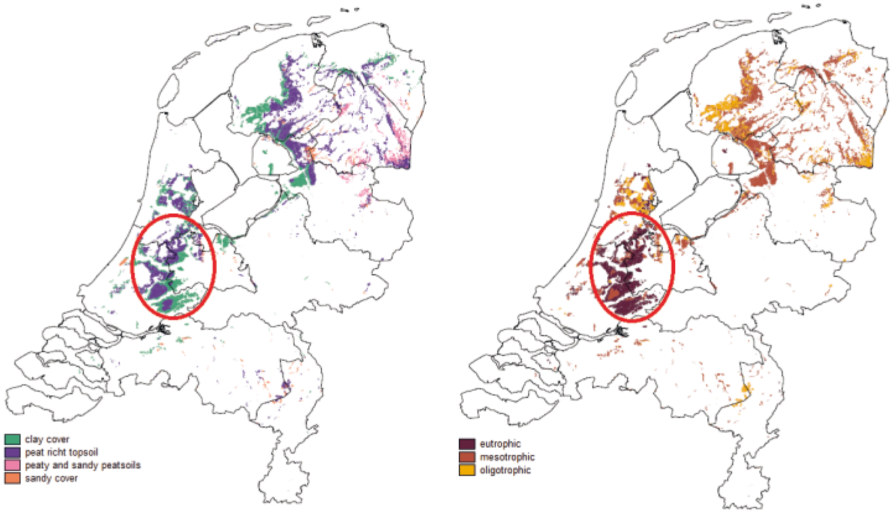


Figure 1.1a and 1.1b. The distribution of eutrophic, mesotrophic and oligotrophic peatlands (1a, left) and the distribution of clay and sand in the topsoil of peatlands (1b, right) in the Netherlands (adapted from Van Kekem 2004). The Green Heart area is in the red oval.

Worldwide, approximately 12-15% of the peat soil has been disturbed by human activity and extreme climate events, resulting in peat degradation (Joosten 2015; Urák et al. 2017). When the groundwater table of peatland is lowered under the soil surface, aerobic soil conditions lead to peat oxidation. This oxidation results in irreversible peat loss and soil subsidence, as well as in the loss to water and air of stored carbon, N components and other minerals, and to biodiversity loss (Joosten 2015). In the Netherlands, about 5% of the estimated remaining 288,340 hectares of peat soil are still peatlands where peat is actively being formed (Tanneberger *et al.* 2017), while the other remaining peat soils are mainly drained for the use as permanent grassland for dairy farming (Van Kekem 2004).

1.1.2 A short history of land use in the Green Heart area

Agricultural peatland use in the Netherlands started before the tenth century (Rijksdienst voor het Cultureel Erfgoed 2016). Around the tenth century, people started converting peat areas in a systemic way for the growth of crops such as cereals, peas, hemp and flax on the higher soils and grass on the lower soils. In addition, they started to harvest peat for fuel (Meulenkamp *et al.* 2007). Ditches and furrows were dug through the peat swamps, allowing water to drain to nearby rivers, and this resulted in soil subsidence. As a result, flooding occurred more frequently and drainage of peat areas slowly became more difficult during the centuries that followed. From around the year 1200, dykes were used to protect areas from flooding, and from around 1500, the use of windmills improved the drainage of peat areas. Mechanical water drainage was introduced in the late 19th century. During these centuries, agricultural land use of peatlands slowly changed nearly exclusively to grass production for dairy farming, as drainage became more difficult due to continuous soil subsidence, and because dairy products were becoming economically more attractive. Grass requires less deep drainage than crops and increases the bearing capacity of peat soil due the formation of a grass sod (Van de Ven 2004; Meulenkamp *et al.* 2007; Janssen *et al.* 2023).

Until the beginning of the 20th century, fertilisation was relatively low in most of the peat meadows, and dairy farming was less intensive than it is now. Fields were manured by grazing cattle or by manure collected in the barn during wintertime. In some areas, barn manure was mixed with ditch sludge and organic waste from cities and used as fertiliser, a mixture called *toemaak* in Dutch. In those days, large parts of the peat meadows consisted of managed species-rich semi-natural grasslands (Zijlstra & Vries 1935; Ellenberg 1988).

During the 20th century, milk production per hectare increased. Perennial ryegrass slowly became the dominant grassland species. Particularly in the period after the Second World War, dairy farming strongly intensified in the Netherlands (Table 1.1). This was caused by various developments, including policies aiming at higher food production levels, increased feed imports from outside of the farm, improvements in perennial ryegrass breeding and animal breeding, deeper drainage of peatland and an increased knowledge exchange between farmers, advisers and researchers, as well as technological inventions such as the milking machine, tractors and machinery, and chemical fertilisers. Dairy farming intensity and farm sizes developed similarly in the Green Heart area to dairy farms in the rest of the Netherlands (Dekker 1990), but on average farms on peat soils remained somewhat less intensive and smaller than on mineral soils. Between 2005 and 2020, the milk production in the peat areas was 3-9% lower per farm and 1-3% lower per dairy cow than the national averages (Agrimatie 2022a).

Table 1.1 Figures on the development of dairy farms in the Netherlands between 1960 and 2020 (Van Horne & Prins 2002; Wageningen Economic Research 2022).

Year	1960	1975	1985	1995	2005	2015	2020
Dairy cows per farm	9	24	41	46	67	97	107
Milk production (ton farm^{-1})	37	113	216	301	513	841	959
Milk production (ton ha^{-1})	5.5	8.9	12.5	12.0	12.7	15.4	16.4
Milk production (kg dairy cow^{-1})	4200	4625	5330	6613	7690	8240	8980
Concentrate use (kg dairy cow^{-1})	830	1890	2280	2210	2020	2230	2530

1.2 The nitrogen cycle of dairy farming systems on peat soil in the Green Heart area

The increase of farming intensity since the Second World War not only led to a higher milk production per hectare, but also led to increased losses of N to the environment.

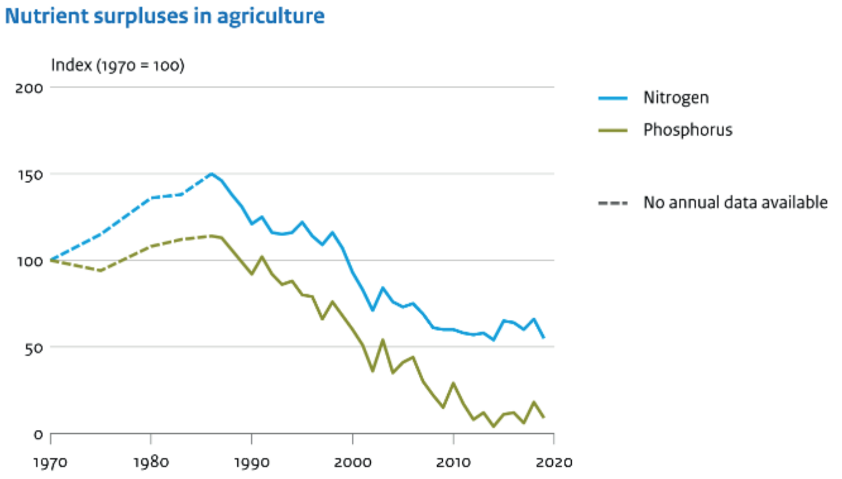
1.2.1 Nitrogen use and surplus in agriculture

Nitrogen is an essential element to all life processes, as it is a building block of amino acids, proteins, nucleic acids and DNA. On earth, most N is present as the non-reactive nitrogen gas (N_2), which makes up about 78% of the atmosphere. Plants and animals can only use N in the form of reactive N. Reactive forms of N include ammonia, ammonium, nitrite, nitrate, nitric oxide, nitrous oxide, nitrogen dioxide, urea, amines and proteins.

Before the invention of chemical fertiliser at the beginning of the 20th century, N was a limiting factor in agricultural production. At that period of time, only natural sources of reactive N were available, such as legumes living in symbiosis with nitrogen gas fixing microbes, free-living nitrogen gas fixing microbes, and piles of N-rich bird droppings from the South American coasts, called Guano (Erisman *et al.* 2015; Sanchez 2019). Industrial production of chemical N fertilisers increased strongly after the Second World War, resulting in huge increases in agricultural production, as well as strongly increased losses of N to the environment (International Nitrogen Initiative 2007; Sanchez 2019).

In agriculture, part of the N is cyclic, i.e. it remains locally in the farming system. On dairy farms, the N present in crops is fed to the cows, and then ends up in milk, meat and manure. N in manure is used as fertiliser in the soil; it is partly immobilised by soil microbes or is taken up by crops. The local N cycle of farms cannot be completely closed because different N compounds are easily lost to the environment (Oenema *et al.* 2007; Sutton *et al.* 2011). The sum of N losses in agriculture is also reffered to as N surplus, which is the difference between farm N inputs (e.g. feeds, fertilisers, atmospheric deposition) and outputs (e.g. milk, meat, crops).

N surpluses in Dutch agriculture increased between 1970 and 1986, due to the expansion and intensification of animal husbandry (Figure 1.2). Between 1986 and 2006, after the introduction of the milk quota in 1984 and environmental regulations in the nineties, the N surplus in Dutch agriculture was reduced by about 50% from 775 to 386 million kg N. In the period 2006 until 2020, the N surplus decreased further by about 20% to 307 million kg N due to stricter manure regulations. Since 2015, the N surplus has again been increasing due to various factors (Compendium voor de Leefomgeving 2022a).



Source: CBS

CBS/apr21
www.clo.nl/en009620

Figure 1.2 Development of nitrogen and phosphorus surplus in agriculture in the Netherlands since 1990 (Compendium voor de Leefomgeving 2022a). Dashed lines represent estimates for the period 1970-1985, for which no annual data were available.

1.2.2 The nitrogen cycle of dairy farms on peat soil

Oenema *et al.* (2007) conceptualised the N cycle of agricultural systems by the 'hole in the pipe' model (Figure 1.3). When applied to dairy farms on peat soils, inputs of N are fertiliser, animal manure, concentrates and other feeds, dry and wet N deposition, peat mineralisation, and occasionally sludge taken from ditches and furrows. Outputs of N are mainly milk and sold animals, and, on some farms, sold feed and manure (De Visser *et al.* 2001; De Jong *et al.* 2022). N is lost to ground and surface water via run-off and leaching of nitrate, ammonium and organic N compounds (Van Beek *et al.* 2004a). Loss of ammonia to the air occurs due to volatilisation at the barn, manure storage and field. Loss of nitrous oxide and nitrogen gas to the air occurs at the barn, manure storage and field via nitrification and incomplete denitrification. Loss of nitrogen (di)oxide mainly occurs at the barn, manure storage and silage storage (Van Bruggen *et al.* 2022).

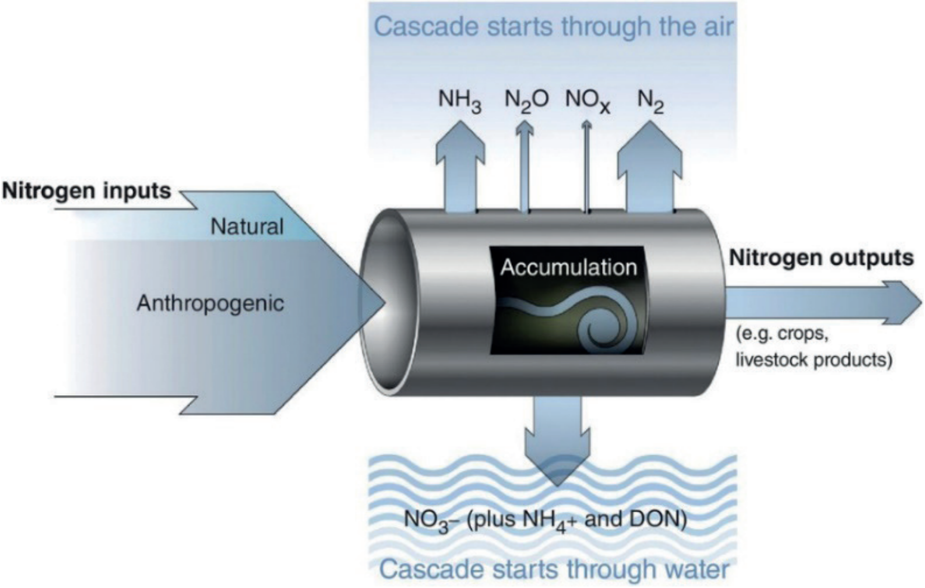


Figure 1.3 The 'hole in the pipe' model for visualisation of the N flow through and N losses of agricultural systems (International Nitrogen Initiative 2007, adapted from Oenema *et al.* 2007). In drained peat soils, peat mineralisation is an extra N input compared to mineral soils.

1.2.3 N surplus and different N losses on dairy farms on peat soil in the Netherlands

Large areas of peatlands in temperate climates in Europe and the world are used as agricultural grassland, but to a lesser extent for intensive dairy farming such as in the Netherlands (UNEP, 2022). The average intensity of dairy farming in the Netherlands, in liters milk ha^{-1}, is among the countries with highest intensities in Europe. Average N inputs, outputs and loss on dairy farms on peat soil in the Netherlans are therefore relatively high compared to other countries (Bleken et al. 2005; Uwizeye et al. 2020).

Nitrogen inputs, outputs and surpluses according to the national monitoring system

Nitrogen inputs, outputs and surpluses of dairy farms in the Netherlands have been monitored since 2002, in the national monitoring system for manure. During the period 2002-2020, dairy farms on peat soil had an average input of 110 kg N ha^{-1} via chemical fertiliser and 131 kg N ha^{-1} via concentrates and other feeds (Figure 1.4). The farms had an output of 68 kg N ha^{-1} via milk and milk products and 9 kg N ha^{-1} via sold animals and sold manure. From 2002 to 2020, the average dairy farm N surplus decreased from 172 to 133 kg N ha^{-1} in the peat regions of the Netherlands, according to the monitoring system

(Agrimatie 2022b). N inputs from deposition and peat mineralisation are not taken into account at the national monitoring system for manure policies system.

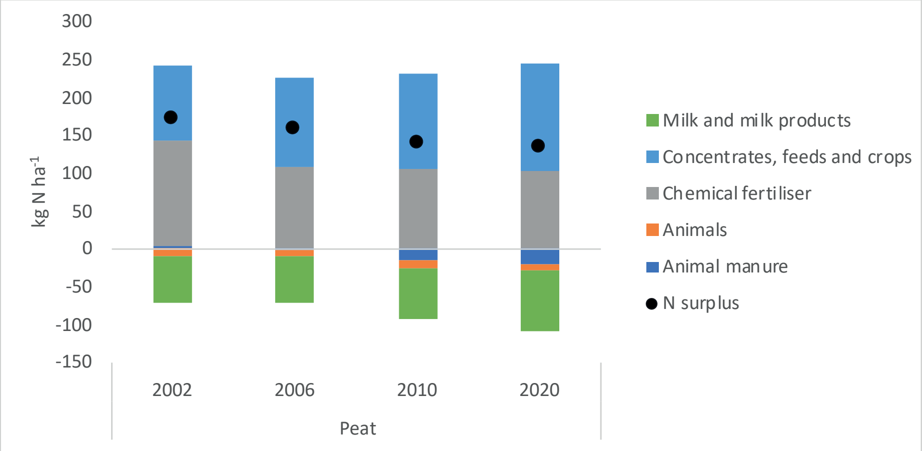


Figure 1.4. Mean annual N inputs (positive bars), outputs (negative bars) and surpluses (black dots) per hectare of dairy farms in the peat regions of the Netherlands in 2002, 2006, 2010 and 2020 according to the national monitoring system for manure policies (Agrimatie 2022b). This monitoring system does not take N inputs from deposition and peat mineralisation into account.

Estimated N cycle of dairy farms on peat soil in the Green Heart area

Figure 1.5 shows an estimated N cycle and N losses for the average dairy farm on peat soil in the Green Heart area, for the year 2020. This N cycle was defined from farm parameters described by De Jong *et al.* (2022) and was complemented with data from studies on peat mineralisation (Van Kekem 2004) and on run-off and leaching from dairy grasslands on peat soil (Schipper *et al.* 2016; Van Rotterdam *et al.* 2021). The annual nutrient cycling assessment tool (ANCA, version 2022.06) was used as a basis to define the N cycle (Aarts *et al.* 2015; Van Dijk *et al.* 2022). In addition to the parameters described by De Jong *et al.* (2022), it was assumed that manure was diluted with water (ratio 2:1) at field spreading, which is in accordance with current regulations (RVO 2021).

Peat mineralisation was considered as a farm input, and estimated at 80 kg ha^{-1} (Van Kekem 2004). In dairy grasslands on peat soil, peat mineralisation results in an extra contribution of N to herbage uptake compared to dairy grasslands on mineral soils (De Visser *et al.* 2001). Van Kekem (2004) estimated that the Soil N Supply (SNS) of dairy grassland is on average 80-85 kg ha^{-1} higher in peat soils than in mineral soils due to peat mineralisation, which is in range with earlier results (Vellinga & André 1999). SNS is defined as the herbage N uptake in the first year after cessation of N inputs, and therefore comprises N from soil organic matter that is mineralised during the growing season, mineral N that is present in the soil profile, and N from deposition and fixation of free and symbiotic living microbes in the soil (Hassink 1995). Sources of soil mineral N which contribute to the herbage and crop N uptake – other than peat mineralisation – are represented in the flows of N from fertilisation, deposition and losses from crops to the soil (Figure 1.5).

ANCA estimates ammonia and other gaseous N losses from herbage and crops, from manure in the barn and from manure storage with the National Emission Model for Agriculture (NEMA) (Van Bruggen *et al.* 2022). Soil N losses to groundwater and surface water are not differentiated from nitrogen gas losses by ANCA (Van Dijk *et al.* 2022). Therefore, N losses to groundwater and surface water were estimated based on literature data (Schipper *et al.* 2016; Van Rotterdam *et al.* 2021), and nitrogen gas losses were assumed to be equal to the difference between the soil inputs, herbage uptake and crop uptake, and ammonia, run-off, leaching and nitrous oxide losses.

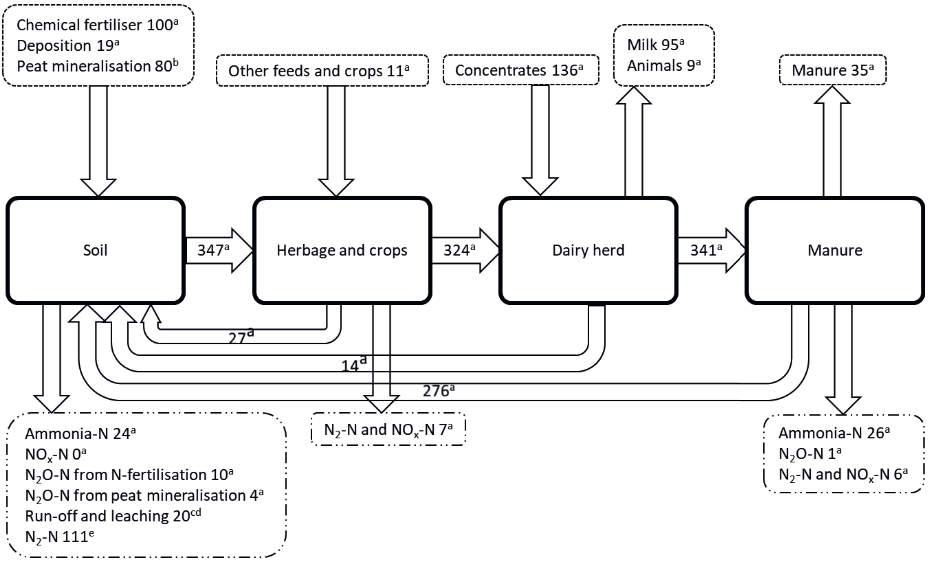


Figure 1.5. Annual estimated N flows and losses of the average dairy farm in the Green Heart area, in kg ha^{-1}, for the year 2020. Farm inputs and outputs are shown at the top of the figure in dashed rectangles. Closed rectangles represent different farm compartments. Farm losses are shown at the bottom of the figure in dashed-dotted rectangles. Arrows represent N flows. Note that the data in this figure are derived from ANCA model estimations and different experiments that were carried out at different locations, and at different spatial and temporal scales. a = ANCA model estimations (Van Dijk *et al.* 2022) from parameters described in De Jong *et al.* (2022), b = Van Kekem (2004), c = Schipper *et al.* (2016), d = Van Rotterdam *et al.* (2021), e = difference between soil inputs, herbage uptake and crop uptake, and soil ammonia, run-off, leaching and nitrous oxide losses.

Different types of N loss

Figure 1.5 shows that the N surplus of dairy farms on peat soil consists of different types of N losses which are mostly gaseous, and that most losses come from the soil. The estimated total N losses for the average dairy farm in the Green Heart area was 210 kg ha^{-1}, of which 24% are ammonia losses, 10% are run-off and leaching losses, 7% are N_2O-N losses and 60% are N_2-N and NO_x-N losses.

Ammonia

Ammonia is mainly formed when faeces comes into contact with urine. The enzyme urease, which is present in faeces, increases the formation of ammonia from the urea present in urine. Ammonia emissions mainly occur on the barn floor, during manure storage, and after manure and chemical fertiliser spreading in the field. Ammonia can be formed during grazing, but losses are smaller as faeces barely come into contact with urine during grazing (Edouard et al. 2019). Ammonia emissions were estimated at 51 kg N ha^{-1} for the average dairy farm in the Green Heart area (Figure 1.5) (De Jong et al. 2022; Van Dijk et al. 2022). This 2020 estimate for the Green Heart area is somewhat lower than the average of 58 kg N ha^{-1} reported by Plomp et al. (2018) for dairy farms in the western peat area for the years 2013-2015, which was based on 140 different estimated N cycles using ANCA. Plomp et al. (2018) found that 25% of the farms with the lowest and highest ammonia emissions had an emission of 48 and 69 kg N ha^{-1}, respectively, based on ANCA. Verloop et al. (2018) also estimated somewhat higher mean ammonia emissions of 57 kg N ha^{-1} for 881 dairy farms on peat soil for the year 2016 in the Netherlands, using ANCA. Since 2019, farmers have been obliged to dilute manure with water at field application (at least one part water to every two parts of manure) (RVO 2021). This may explain the lower estimated ammonia emissions for the dairy farm in the Green Heart area compared to the estimates of Plomp et al. (2018) and Verloop et al. (2018). At manure spreading with a trailing shoe, which is common practice on peat soils, emissions of the total ammonia N (TAN) fraction are assumed to decrease from 26.4 to 17.0% if the manure is diluted with water (ratio manure:water 2:1) (Van Bruggen *et al.* 2022).

Run-off and leaching

N can be lost to surface and groundwater via run-off and leaching, in the form of mineral N or as dissolved organic N. Leaching and run-off losses for the average dairy farm in the Green Heart area were estimated at 20 kg N ha^{-1}, following results of Schipper *et al.* (2016) and Van Rotterdam *et al.* (2021). The average N fertilisation level of 352 kg ha^{-1} at the average dairy farm on peat soil in the Green Heart area in 2020 (Figure 1.5) (De Jong *et al.* 2022) was close to the levels reported by Schipper *et al.* (2016) and Van Rotterdam *et al.* (2021). For the Krimpenerwaard area, Schipper *et al.*

(2016) estimated average leaching and run-off of N of 21 kg N ha^{-1}, with a variation between 12 and 25 kg ha^{-1}, at an N fertilisation level of around 350 kg N ha^{-1}. The Krimpenerwaard is a system of polders in the Green Heart area in which about 95% of the land use is grassland on peat soil (CBS 2023). About half of the N was estimated to originate from historic and present fertilisation, and Schipper *et al.* (2016) concluded that peat organic matter mineralisation was an important source of nutrients in the surface water. Van Rotterdam *et al.* (2021) reported total estimated annual N losses due to leaching and run-off of 19.7 kg ha^{-1} within the Gagelpolder from 2007-2016, with a range of 6-25 kg ha^{-1} for different fields. The Gagelpolder is a polder with mainly dairy grassland in the Green Heart area, where the average N fertilisation level was around 340 kg ha^{-1} before and during the study (Van Rotterdam *et al.* 2021). Van Beek *et al.* (2004a) quantified the sources of N losses to a ditch of a grassland on peat soil used for dairy farming in the Vlietpolder over two years, at a relative higher N fertilisation level of 452 kg ha^{-1}. They found a total annual N loss of 32 kg ha^{-1}, of which 24-58% originated from mineralisation of organic matter and of deeper nutrient-rich peat layers. The Vlietpolder is also located in the Green Heart area.

Nitrous oxide, nitrogen gas and nitric oxide

Drained grasslands on peat soil are prone to denitrification due to shallow groundwater levels (Koops *et al.* 1996; Velthof *et al.* 1996; Van Beek *et al.* 2004b). Total denitrification losses were estimated at 111 kg N ha^{-1} for the average dairy farm in the Green Heart area (Figure 1.5). This figure is somewhat higher than the results of Van Beek *et al.* (2004b), who measured denitrification for two years in grassland cores taken from dairy grassland on peat soil in the Vlietpolder. They found denitrification losses of 87 kg N ha^{-1} and concluded that nearly 70% of the denitrification losses originated from soil deeper than 20 cm below surface. Nitrous oxide emissions from the soil estimated for the average dairy farm in the Green Heart area by ANCA were in line with results of Velthof *et al.* (1996) and somewhat lower than the results of Koops *et al.* (1996). Velthof *et al.* (1996) found that in fertilised conditions on average 3% of the applied N was lost as N_2O on dairy grassland on peat soil. Under unfertilised conditions, they found average N_2O emissions of 5.3 kg N ha^{-1}. Koops *et al.* (1996) found that annual nitrous oxide emission was 34 kg N ha^{-1} on grassland on peat that did not receive N fertiliser, and they concluded that the subsoil (20-40 cm) contributed 25-40% of the total nitrous oxide emission in the 0-40 cm layer.

At the silage storage, barn and manure storage, nitrous oxide, nitrogen (di)oxide and nitrogen gas can be formed through different processes (Van Bruggen *et al.* 2022). These losses were estimated at in total 14 kg N ha^{-1} for the average dairy farm in the Green Heart area for the year 2020 (De Jong *et al.* 2022; van Dijk *et al.* 2022).

1.3 Too much N loss and policy developments

The different types of N loss have negative effects on the environment, climate and human health. Since the 1990s, several policy targets have been put in place to reduce N loss from agriculture.

1.3.1 Effects of too much N loss

Nowadays, the disturbance of the natural planetary biogeochemical N cycle is considered a major threat to the environment. The environmental limits within which humanity can safely operate are considered to have been exceeded (Steffen et al. 2015). Too much N loss has sequential negative impacts, not only in different environmental compartments and but also on human health. Oenema et al. (2007) summarised the following observed environmental and human health effects of reactive N losses:

- Impaired human health due to NH_3 and to NO_x-induced formation of particle matter (PM2.5) and smog;
- Plant damage due to NH_3 and to NO_x-induced ozone formation;
- Decreased species diversity of natural areas due to N enrichment through atmospheric deposition of NH_3 and NO_x;
- Soil acidification due to deposition of NH_3 and NO_x;
- Pollution of groundwater and drinking water due to nitrate leaching;
- Eutrophication of surface water due to N enrichment, leading to excess and possibly toxic algal blooms and decreased faunal and floristic species diversity;
- Global warming due to N_2O emissions;
- Stratospheric ozone destruction due to N_2O.

1.3.2 Policy developments regarding N loss from agriculture

Various national and European policies have been put in place since the 1990s, with the objective to reduce N losses to water and air from agriculture. Important policies that apply to dairy farming on peat soil are described briefly below.

Nature protection

Since 1998, different laws for the protection of nature and specific species have been put in place and have been tightened with regard to N losses in agriculture. In 2017, these laws were replaced by the Nature Conservation Act, which regulates the protection of nature and specific species in the Netherlands in the context of the EU Bird and Habitat Directives (Nederlandse Overheid 2015). In this context, Natura-2000 areas were designated, and a part of these areas were marked as nitrogen-sensitive. For these areas, a Critical Load (CL) for N deposition has been determined. If this CL is exceeded, the habitat may be significantly negatively affected by N deposition. In 2017, the CL was exceeded in more than half of the nitrogen-sensitive areas. According to Gies *et al.* (2019), if the lowest CL of specific-nitrogen sensitive areas is not to be exceeded, the N deposition in the Netherlands has to be decreased by more than 70%. N deposition rates remained stable between 2017 and 2020 (CBS 2021).

In 2021, the law on nitrogen reduction and nature improvement came into force. The objective of this law is to ensure that by 2030, 74% of the nature in Natura 2000 areas that is vulnerable to N does not receive more N deposition than the CL (https://www.aanpakstikstof.nl). Therefore, agricultural ammonia emissions must be reduced by 41% by 2030 compared to 2019 (Van der Wal 2023). Approximately 45% of the total Dutch N deposition originates from agriculture, and more than half of this N deposition originates from cattle farming (Schollaardt 2019).

Water quality

In 2000, the EU Water Framework Directive (WFD) was introduced, which aims to ensure the quality of surface and groundwater in Europe (European Parliament 2000). The objective of the WFD is that in 2027, all waters must form a healthy habitat for plants and animals living in them. Based on the concentration of dissolved N, the surface water quality was reasonable to good in the Green Heart area in 2021 (Compendium voor de Leefomgeving 2022b). Median total dissolved N concentrations in ditch waters varied between 2.1 and 2.6 mg L^{-1} in the peat areas of the Netherlands in the summer of the years 2010-2021, except in 2016 when the median concentration was 3.0 mg L^{-1} (RIVM 2022). The 2027 policy target used for dissolved N concentrations of surface waters in the Green Heart area is 2.4-2.5 mg L^{-1} (Buijs *et al.* 2020). Groenendijk *et al.* (2016) estimated that to reach the 2027 WFD target, run-off and leaching of N must be reduced by 14-38% in different parts of the Green Heart area, compared to the situation between 2010 and 2013.

Climate change

In 2018 it was agreed in the Dutch climate agreement that greenhouse gas emissions from organic soils must be reduced by 1 Mton by 2030, and that agriculture and land use must be climate neutral by 2050 (Ministerie van Economische Zaken en Klimaat 2019). Organic soils used for agriculture were estimated to emit around 0.5 Mton CO_2-equivalents of N_2O in 2003 and around 4.7 Mton CO_2-equivalents of CO_2 and methane in 2013 (Kuikman *et al.* 2005; Lof *et al.* 2017). Thus, greenhouse gasses must be reduced by about 19% in order to comply with the 2030 climate agreement target for organic soils.

Air quality

In 2008, different European directives on air quality have merged into the Ambient Air Quality Directive 2008/50/EC. According to this directive, mean annual NO_2 air concentrations in the Netherlands may not exceed 40 µg/m^3 and mean hourly concentrations may only exceed 200 µg/m^3 18 times per year. In addition, mean annual fine particle PM2.5 concentrations may not exceed 25 µg/m^3 (RIVM 2023a). Air concentrations of NO_2 and PM2.5 have been monitored since 2012. The number of cases in which NO_2 concentrations exceeded these thresholds has been reduced since 2012, and thresholds were only exceeded near busy roads (De Smet *et al.* 2022; RIVM 2023b). Mean annual fine particle PM2.5 concentrations remained well below the threshold in the period 2012-2021 (RIVM 2023a; b).

Other policy targets

There are other policy targets and ambitions related to dairy farming on peat soil, besides the targets described. These include targets and ambitions for the reduction of CO_2 and methane emissions from dairy farms, for the reduction of soil subsidence, for biodiversity preservation, for the reduction of phosphate loss to water, and for water use and safety (Van den Born *et al.* 2016). In 2022, in order to reach these targets and ambitions, the Dutch government committed to raising groundwater levels of peat soils to 20-40 cm below soil surface, so as to maintain grasslands or to change grassland cultivation to crops that grow at wet soil conditions (Ministerie van Infrastructuur en Waterstaat 2022).

1.4 Measures for reducing N loss on dairy farms and the role of the soil

To reduce the negative effects of N loss from dairy farms on the environment and human health, and to meet the different policy targets, different measures for reducing N loss have been investigated and introduced in dairy farming. A challenge that has remained specific to dairy farms on peat soil until now is controlling the flow of N from the soil to herbage, and the N concentration of the harvested herbage.

1.4.1 Measures to reduce N loss on dairy farms

It has been found that N losses from dairy farms on peat soils can be reduced substantially by combinations of measures (e.g. De Visser *et al.* 2001; Verloop *et al.* 2018). Ammonia emission reduction percentages of 25-56% have been estimated with the implementation of a combination of measures on dairy farms on peat soil, compared to benchmark situations (De Visser *et al.* 2001; Verloop *et al.* 2018). In Table 1.2, examples of measures are split into the categories end-of-pipe, reducing inputs, integral measures and a category of measures related to the cessation of dairy grassland cultivation.

Table 1.2. Examples of measures for reducing N losses on dairy farms on peat soil, arranged into different categories.

End-of-pipe	Reducing inputs	Integral measures	Cessation of dairy grassland cultivation
Low-emission barn floors Air washer at barn Low-emission manure storage Low-emission manure application	Reduction of chemical fertiliser Reduction of concentrates or other feeds from outside of the farm	Farming with increased groundwater levels Circular, regenerative and nature-inclusive agriculture, organic farming	Agriculture on nearly waterlogged to inundated soils Nature development on former dairy grasslands
	Reduction of production levels or herd size (on condition that this is a result of less farm N input)		
Development of future farm models			

Nitrogen losses per hectare and kg milk are higher on dairy farms on peat soil than on dairy farms on mineral soils (Plomp et al. 2018; Verloop et al. 2018). One reason for this is the contribution of peat mineralisation to the flow of N from soil to herbage, which makes it difficult to control herbage N uptake by adjusting the N fertilisation to the N mineralisation of the soil (Van Kekem 2004; Verloop et al. 2018). To date, limited research has been conducted on controlling and reducing the contribution of N from soil mineralisation to the N uptake of herbage and other crops at dairy farms on peat soil (Ros and Van Eekeren 2016; Deru et al. 2019).

1.4.2 Role of high N concentrations in herbage at N cycling of dairy farms on peat soil

There is a relationship between soil N mineralisation and N fertilisation, herbage and crop N uptake and N losses further in the farm N cycle of dairy farms on peat soil (Verloop *et al.* 2018). The higher the herbage N uptake, the higher the herbage crude protein (CP) concentration may be, and the more N that enters into the farm cycle (De Visser *et al.* 2001; Van Dijk *et al.* 2022). Generally, the N use efficiency of dairy cows decreases if the dietary CP contents exceed 150-160 g kg DM^{-1}, and urea N losses increase (Kebreab *et al.* 2002; Colmenero & Broderick 2006; Huhtanen & Hristov 2009), which in turn can lead to increased ammonia losses (Smits *et al.* 1995; Edouard *et al.* 2019). Decreasing dietary CP concentrations to about 150-160 g kg DM^{-1} is therefore interesting both from an environmental and from a farming N efficiency perspective. However, on dairy farms on peat soils, dietary CP contents often exceed 160 g kg DM^{-1} (Vellinga & André 1999; Verloop *et al.* 2018; Hoekstra *et al.* 2020). High grass CP contents are the most important reason for high dietary CP concentrations because diets consist of about 60-65% fresh grass and grass silage (Verloop *et al.* 2018; De Jong *et al.* 2022). Fresh grass CP concentrations may exceed 200 g kg DM^{-1} during the growing season in dairy grassland on peat soil (Eurofins

2017 in Verloop *et al.* 2018); Verloop *et al.* (2018) found that about half of the grass silages in the western peat area of the Netherlands had CP concentrations exceeding 170 g kg DM^{-1}. Grass CP concentrations are high due to a combination of relatively high SNS and N fertilisation, in dairy grasslands on peat soil (Korevaar 1986; Vellinga & André 1999). Dietary CP concentrations can be lowered by feeding low CP concentrates. Concentrates with low CP concentrations of around 110-120 g kg DM^{-1}, which mainly consist of cereals and sugar beet pulp, can for example reduce dietary CP concentrations to nearly 150 g kg^{-1} if the grass does not have a CP concentration higher than around 170 g kg DM^{-1}.

The flow of N from soil to herbage and crops is mainly determined by the SNS and fertiliser N uptake. The annual SNS of dairy grassland on peat soil with an average lowest groundwater level of 50-80 cm below field surface has been estimated at 225 kg N ha^{-1} (Van Kekem 2004) and is estimated to contribute 75% to the N uptake of herbage and crops on the average dairy farm in Green Heart area (Figure 1.5) (De Jong *et al.* 2022). Peat mineralisation, as part of the SNS, has been estimated to contribute 23% to this N uptake of herbage and crops (Figure 1.5) (Van Kekem 2004).

1.4.3 Options for controlling the N flow from the soil to herbage and crops

So far there have only been limited options to adapt fertiliser, mowing and grazing management to spatial and temporal variations in SNS and N utilisation efficiencies in dairy grassland on peat soil (Ros & Van Eekeren 2016). Insight into the factors determining spatial and temporal variation of SNS and N use efficiency of fertilisers could help improve controlling the herbage N uptake by SNS- adapted and N use efficiency adapted management strategies.

Permanent grassland has become the most suitable crop on peat soils as it results in a greater soil-bearing capacity for animals and machinery, and requires little soil tillage which minimises peat oxidation compared to the tillage of annual crops (Lohila *et al.* 2004; Taft *et al.* 2017). Breeding perennial ryegrass (*Lolium perenne* L.) or the use of alternative grass or forage species with the objective to reduce and control herbage N concentrations may be a good strategy to improve N cycling of dairy farms on peat soil, as grass crude protein contents often exceed the range of 150-160 g CP kg DM^{-1}. However, the reduction of N uptake by herbage may lead to a lower N use efficiency (NUE) at field level and increased N losses from the soil. On the other hand, there are indications that the temperate forage crop plantain (*Plantago lanceolata* L.) reduces mineralisation and nitrification in the soil (Dietz *et al.* 2013). Understanding the effects of the presence of species on the fate of N at the soil-crop level is of importance to help understand the effects on the N cycle at the soil and farming system level.

Due to social and policy pressure on reducing soil subsidence and emissions from drained peatlands, attention has increased for the concept of wet agriculture or paludiculture on rewetted peat soils (Ministerie van Infrastructuur en Waterstaat 2022). In waterlogged soil conditions, perennial ryegrass is unable to grow, but helophytes such as common reed (*Phragmites australis*) and cattail (*Typha* spp.) are able to grow (Wichtmann *et al.* 2016). In waterlogged soil conditions, the contribution of soil mineralisation to the crop N uptake may be nearly stopped as the majority of organic matter oxidation takes place in aerobic conditions (Tiemeyer *et al.* 2007; Wichtmann *et al.* 2016). Agriculture on waterlogged or inundated soils has therefore the potential to reduce N losses compared to drained conditions on dairy farms on peat soils. However, so far there has been limited research into the effects of crop cultivation on waterlogged or inundated soils, on yield potential, on nutritional value and on the N cycle of dairy farming systems on peat soil.

The sale of milk is most important source of revenue for dairy farms on peat soil in the Green Heart area (Agrimatie 2022c; De Jong et al. 2022). Therefore, measures that can reduce N losses without lowering milk yields or without increasing milk production costs may be more easily adapted by farmers than measures which may lower the milk production or increase costs (Schrijver et al. 2022).

1.5 Thesis objective and research questions

Controlling the flow of N from soil to crops so far has remained a challenge on dairy farms on peat soil. Therefore, the main objective of this thesis is to answer the question; Can N cycling on dairy farms on peat soil in the Green Heart area be improved by adapting management to the soil N supply and by using other grassland species and crops without affecting milk production? For this objective, we quantified N flows from soil to the farm N cycle, and determined the effects of changes in grassland population and species on herbage N concentrations, the farm N cycle and N losses. The following research questions are addressed:

1. What factors drive the quantity and dynamics of Soil N Supply (SNS) throughout the growing season in dairy grassland on peat soil?

2. What is the perspective of reducing herbage N concentrations of perennial ryegrass (*Lolium perenne* L.) on peat soils through breeding, and which pathway influences the N concentration?

3. Can the use of a mixture of perennial ryegrass and plantain (*Plantago lanceolata* L.), a species known to produce secondary metabolites that inhibit nitrification, improve the N cycling of dairy grassland systems on peat soils?

4. What is the potential of cattail (*Typha latifolia* L.), which can be cultivated in waterlogged conditions that may strongly slow down aerobic peat mineralisation, as an alternative forage crop for dairy farming on peat soil?

5. What is the potential effect of increased knowledge of SNS and of the changes studied in grassland populations and species on the N cycle and N loss on the farm?

1.6 Thesis outline

This thesis consists of this introductory chapter and five chapters in which the research questions are addressed.

Chapter 2 is titled *Soil nitrogen supply of peat grasslands estimated by degree days and soil organic matter content*. It explores whether the empirical understanding of SNS can be improved using soil properties and weather variables. Data from studies were used on N uptake of dairy grassland on peat soil with an average lowest groundwater level of 50-80 cm below field surface carried out between 1992 and 2017 in the western peat district of the Netherlands. Furthermore, the study explores SNS in conditions with and without calcium ammonium nitrate fertilisation.

Chapter 3 is titled *Effect of Lolium perenne population differences on shoot tissue nitrogen concentrations when grown on a peat soil*. It explores whether the use of low-N perennial ryegrass genotypes, selected on a sandy soil, could be a means to reduce grass N concentrations in dairy grassland on peat soil. Furthermore, the study explores which pathways affect grass N concentrations, as this is important for estimating potential N loss trade-offs within the agricultural system.

Chapter 4 is titled *Can the presence of plantain (Plantago lanceolata L.) improve nitrogen cycling of dairy grassland systems on peat soils?* It explores whether the presence of plantain in a perennial ryegrass plantain mixture can affect the fate of soil mineral N in dairy grassland on peat soil. This study explores whether the presence of plantain changes the potential nitrification, nitrate concentrations, nitrous oxide emissions and N use efficiencies of grasslands on peat soil.

Chapter 5 is titled *The effects of harvest date and frequency on the yield, nutritional value and mineral content of the paludiculture crop cattail (Typha latifolia L.) in the first year after planting*. It explores the potential of cattail as a forage crop for a dairy farming system on peat. In this study, the yield and its nutritional value for ruminants at different harvesting strategies is determined.

In **Chapter 6**, *General discussion*, the main findings of the research described in Chapters 2-5 are synthesised. The questions addressed are what the implication of the results of this study could be for the reduction of N losses from dairy farms on peat soil, how these reductions relate to policy targets on nitrogen, what can be learned from the results in a wider context and what future research questions may be.

Chapter 2

Soil nitrogen supply of peat grasslands estimated by degree days and soil organic matter content

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Abstract

Accurate estimates of the quantity and rate of soil nitrogen supply (SNS) are essential to increase soil and farm N use efficiencies, in particular for soils high in organic matter. The objective of this work was to enhance the empirical understanding of the SNS of dairy grasslands on peat soils, using soil properties and weather variables. Data were collected from studies on herbage N uptake carried out between 1992 and 2017 in the western peat district of the Netherlands. For the period between March to mid October, SNS was estimated from the sum of mean growing season daily temperatures, soil organic matter (SOM) and applied calcium ammonium nitrate (CAN) N with a residual standard error of 25–27 kg ha^{-1}. Each °C growing season temperature sum affected SNS by 78–90 g ha^{-1} and each g SOM per 100 g dry soil affected SNS by 3.6–3.9 kg ha^{-1}, respectively. SNS was equally estimated for conditions with and without CAN fertilisation. Validation with data from independent field trials showed similar impacts of SOM and growing season temperature sum on SNS. The error of prediction of the presented models, however, was still large for direct on-farm application and led to underestimations for a specific site. Nevertheless, the obtained models allow for an increased understanding of soil and farm N balances. The models can therefore be used for improved temporal and spatial SNS-adapted farming practice advice, for example when they are combined with estimates of previous N fertilisation rates, herbage yields and herbage N concentrations, which can potentially lead to reduced soil and farm N surpluses.

Keywords: histosols; nitrogen efficiency; mineralisation; fertilisation; pH; weather

2.1 Introduction

In temperate climates, drained peatlands are often in use as grassland for dairy farming (Joosten *et al.*, 2017; Van Kekem, 2004). Drained peatlands are associated with significant C and N losses, greenhouse gas production and soil subsidence (Urák *et al.*, 2017). Dairy farming on peat soils often results in farm N use efficiencies - defined as the amount of N output relative to the N input of the farm - that are relatively lower than that on mineral soils because of the high levels of soil N mineralisation (De Visser *et al.*, 2001; Van Beek *et al.*, 2009). Herbage N uptake and content are generally higher in dairy grasslands on peat soils than on mineral soils, largely due to higher levels of N mineralisation (De Visser *et al.*, 2001; Deru *et al.*, 2019; Van Beek *et al.*, 2009; Van Kekem, 2004). The relatively high herbage N content of dairy grassland systems on peat soils often leads to rations exceeding cows' N requirements, which consequently result in greater animal and farm N surpluses and losses to the environment, compared to dairy grassland systems on mineral soils (De Visser *et al.*, 2001).

Accurate estimates of the temporal and spatial variation of herbage N uptake from soil mineralisation, i.e. the soil N supply (SNS), are essential to improve the N use efficiencies of farming systems (Rüting *et al.*, 2018). Accurate SNS estimates can contribute to improved soil and farm N balance estimates (Schröder *et al.*, 2019) and improved SNS-adapted fertilisation, grazing and harvesting strategies (De Visser *et al.*, 2001; Hassink, 1995; Loaiza *et al.*, 2016; Lobos Ortega *et al.*, 2016; Ros *et al.*, 2011; Rütting *et al.*, 2018; Velthof *et al.*, 2009). The SNS of dairy grassland has been reported to vary between 171 and 377 kg ha^{-1} in the western peat district of the Netherlands (Deru *et al.*, 2019; Sonneveld and Lantinga, 2011). However, the estimation accuracy of SNS for dairy grasslands on peat soils has remained relatively low (Deru *et al.*, 2019; Ros and Van Eekeren, 2016; Vellinga and André, 1999). As a consequence, in the current mandatory Dutch Annual Nutrient Cycling Assessments (ANCA) and current fertilisation advice, annual SNS of dairy grasslands on peat soils is estimated at 235 and 250 kg ha^{-1}, respectively (Bemestingsadvies, 2014; Schröder *et al.*, 2019). Dutch farm management advice for peat soils is therefore currently not adapted to spatial and temporal variations of SNS (Bemestingsadvies, 2014).

Several studies showed a positive relationship between soil organic matter (SOM) content and the SNS for mineral soils (Hassink, 1995; Van Eekeren *et al.*, 2010) and peat soils (Vellinga and André, 1999). Temperature, fluctuations in groundwater level and moisture content of the soil affect the biological processes involved in mineralisation (Boxem and Leusink, 1978; Macduff and White, 1985; Verloop *et al.*, 2014). Ros *et al.* (2015) showed that progress can be made in the SNS estimation of grassland on mineral soils by integrating soil properties and weather data, and several models have been developed describing soil N mineralisation of grass- and cropland using soil properties and weather data (e.g. Van der Burgt *et al.* 2006). However, there is no known research integrating soil properties and weather variables for the estimation of annual SNS of dairy grasslands on peat soils.

Many herbage N uptake measurements have been carried out on dairy grasslands on peat soils, over the last decades. This allows for multiple data set analyses of SNS. These measurements were partly carried out in conditions without N application, which allowed for direct deduction of SNS (Hassink, 1995; Jarvis *et al.*, 1996; Warren and Whitehead, 1988; Deru *et al.*, 2019; Van Kekem, 2004; Vellinga and André, 1999), and partly in conditions with calcium ammonium nitrate (CAN)-N application. However, if the effects of CAN-N application and SNS on herbage N uptake can be unravelled, data from conditions with and without CAN-N application can both be used for site-specific SNS estimations.

Therefore, the objective of the current work is to enhance the empirical understanding of SNS of dairy grasslands on peat soils in relation to soil properties, weather variables and CAN-N applications, in order to improve the accuracy of SNS estimations. Two hypotheses were used: 1) SNS can be more accurately estimated when considering soil properties and weather variables, compared to using only soil properties; and 2) SNS is not affected by CAN-N fertilisation within a growing season, i.e. soil N mineralisation contributes equally to SNS, regardless of the presence and rate of CAN-N fertilisation.

2.2 Material and methods

2.2.1 Data collection and selection

Data were collected from studies on herbage N uptake at drained terric histosols (IUSS Working Group WRB, 2015) in the western peat district of the Netherlands carried out between 1990 and 2017. The following data selection criteria were applied:

1. Data had to have been collected from sites which were in use as production grasslands within a dairy farming system for multiple years before the year of measurements;

2. Herbage dry matter yields and total N content had to have been determined per harvest in the first year after cessation of the use of organic inputs;

3. Yields from at least two harvests had to have been determined using a 'Haldrup' small plot harvester (J. Haldrup a/s Løgstør, Denmark), mowed at a stubble height of 4–6 cm on an open plot (e.g. without potential disturbance of the plots such as top fencing);

4. If inorganic N was applied in the year of measurements, it had to have consisted only of calcium ammonium nitrate (CAN, 27% N);

5. Data had to have been collected from studies in which at least one treatment did not receive any CAN applications;

6. Data had to have been collected from sites at which the SOM content (NEN 5754) was ≥ 25 g 100 g dry soil^{-1} and soil pH (pH-KCl, NEN ISO 10390) was analysed, both at 0–10 cm below surface;

7. Data had to have been collected from sites in which ditchwater levels were maintained at maximal 60 cm below field surface, and in which the average lowest groundwater level was 50–80 cm below field surface (De Vries *et al.*, 2003). These criteria were used since ditch- and groundwater levels have been shown to affect herbage N uptake of grasslands on peat soil (Boxem and Leusink, 1978; Schothorst, 1977).

In total, data were collected of 209 treatments from 11 studies that fulfilled the selection criteria (Table 2.1). Studies were carried out at 12 experimental sites, and at the sites between 1 and 11 different fields were used (Figure 2.1).

Table 2.1. Details of studies from which data were selected

Study	Data source	Years of measurements	Experimental site(s)	Fields (n)	Collected treatment means (n)	Treatment means from conditions without N application (n)	Max. no. of harvests year^{-1}	CAN-N fertilisation ranges, kg ha^{-1} year^{-1}	Soil organic matter, g 100 g dry soil^{-1} (0–10 cm)	pH-KCl
A	Wageningen Livestock Research, unpublished	2002, 2003	G	2	33	11	5	0–260	49	4.8–4.9
B	Wageningen Livestock Research, unpublished	2002, 2003	G	2	33	11	5	0–260	41	4.9; 5.0
C	Hoving *et al.* (2008)	2004	G	4	8	4	5	0–226	46–56	4.7
D	Holshof and Van Houwelingen (2012)	2005	G	1	4	1	5	0–240	57–58	4.6–4.7
E	Deru *et al.* (2018)	2010	A, B, D, F-L	18	36	18	4	0–240	29–65	4.3–5.2
F	Deru *et al.* (2019)	2015	C, G, I	3	3	3	4	0	34–44	4.5–4.9
G	Louis Bolk Institute, unpublished	2015	G	1	2	1	4	0–120	56	4.5
J	Hofstede *et al.* (1995); Hofstede (1995a, 1995b)	1992-1994	G	3	41	14	7	0–607	33–64	4.5–5.5
K	Wageningen Livestock Research, unpublished	1995	G	7	7	7	6	0	32–56	4.6–5.2
L	Bussink *et al.* (2014)	2011, 2012	G	2	32	16	5	0–360	54–56	4.6–4.8
H	Louis Bolk Institute, unpublished	2017	E	9	9	9	5	0–250	27–52	4.5–6.6

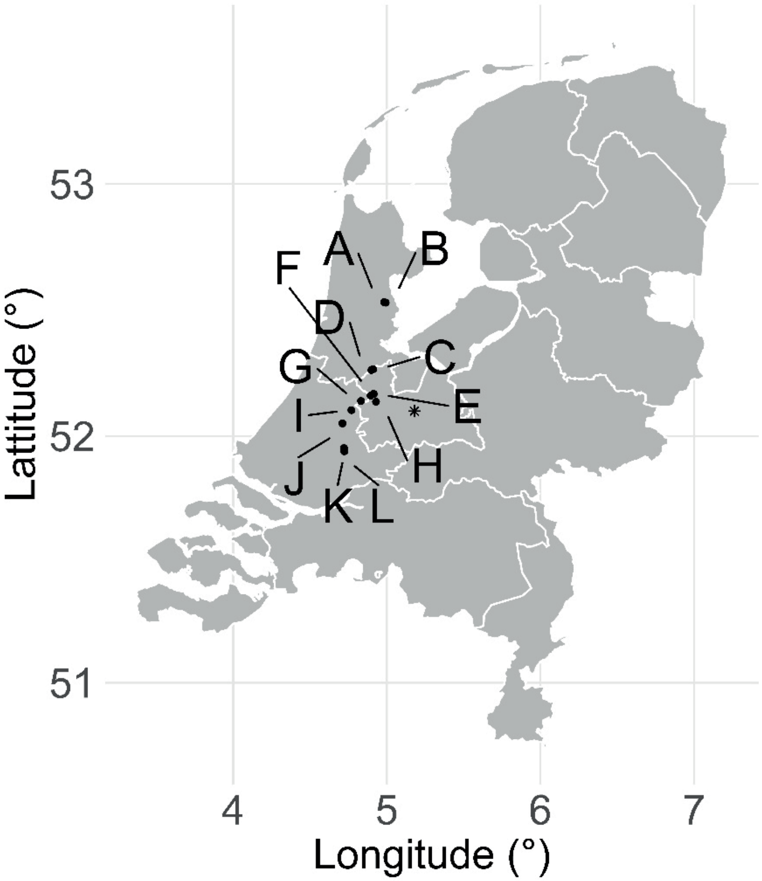

Figure 2.1. Experimental sites of studies from which data were selected (closed dots) and location of weather station de Bilt (asterisk). Experimental sites were at Zeevang (A, 2 fields; B, 2 fields), Ronde Hoep (C, 2 fields; D, 1 field), Wilnis (E, 9 fields; F, 2 fields; H, 2 fields), Zegveld (G, 11 fields), Bodegraven (I, 2 fields), Reeuwijk (J, 1 field) and Krimpenerwaard (K, 2 fields; L, 2 fields)

Soil temperature and moisture content were not recorded in most studies. Daily mean temperatures, precipitation sums and long-term weather means, for the 1970–2017 period, were retrieved from the nearest weather station (de Bilt, KNMI, the Netherlands) (Figure 2.1). Validated interpolated meteorological data were not available for each experimental site, over this period (KNMI, the Netherlands).

2.2.2 Conditions at experimental sites

Experimental sites had a humified topsoil horizon and either a mesotrophic subsoil (sites A, B, K and L) or eutrophic subsoil (sites C–J).

Mean N deposition in the study area was estimated to have decreased from 37.5 to 23.2 kg ha^{-1} between 1992 and 2017 (Wichink Kruit and Van Pul, 2018). Influence of local sources on N deposition was considered negligible, since the distance of the experimental sites to the nearest farms was at least 150 m (Sommer *et al.*, 2009).

Study sites were permanent grasslands with an almost continuous N input before the year of measurements. Therefore, it was assumed that at the sites soil organic N had reached a near-equilibrium state between the gain via organic and inorganic inputs, biomass turnover and microbially induced losses (Hassink, 1995; Sonneveld and Lantinga, 2010). SNS was determined as the herbage N uptake in the first year after cessation of organic and inorganic N inputs (Deru *et al.*, 2019; Hassink, 1995; Jarvis *et al.*, 1996; Sonneveld and Lantinga, 2010; Van Eekeren *et al.*, 2010; Van Kekem, 2004; Vellinga and André, 1999; Warren and Whitehead, 1988). In addition, data from conditions in which CAN-N was applied were selected to test the hypothesis whether CAN-N applications do affect SNS within a growing season. Data selection was limited to studies in which CAN was used as inorganic N fertiliser, as other inorganic fertiliser types than CAN were only used in a few studies and fertiliser type has been shown to affect herbage N uptake (e.g. Antille *et al.* 2015).

Before the year of measurements, organic and inorganic inputs at the experimental sites followed agricultural practices and regulations and, therefore, were rather constant. Mean N application ranged from 246 to 270 kg ha^{-1} for mineral fertiliser and from 293 to 308 kg ha^{-1} for animal manure, in the 1992–1997 period. As a result of legislations mean N application decreased to between 92 and 116 kg ha^{-1} for mineral fertiliser and between 218 and 240 kg ha^{-1} for animal manure, from 2007 onwards (LMN, 2019). During measurement years, phosphorus and potassium applications (applied as inorganic fertilisers) met recommended standards ensuring that these nutrients were not limiting herbage growth.

The mean estimated starting day for spring herbage growth was 5 March ± 17 days, of the studies from which data were selected. The mean growing period in which data were registered lasted 213 ± 15 days and during this period mean herbage dry matter yields were 11619 ± 1872 kg ha^{-1}.

2.2.3 Calculations and statistical analyses of the collected data

Herbage N uptake data from different mowing dates, fertilisation rates, experimental sites, fields or years, in each of the studies, were considered as separate treatments. Cumulative winter temperatures, a herbage N uptake starting day for the first harvest, growing season temperature sums, precipitation deficits, CAN-N application rates and herbage N yields were added to each record. Cumulative winter temperatures were calculated as the sum of mean daily temperatures (°C) between 1 December and 28 February of the winter preceding the growing season. The starting day of herbage N uptake in spring was assumed to be at a temperature sum of 240 °C, calculated as the sum of positive mean daily temperatures since 1 January (De Gooijer and Jagtenberg, 1968). The growing season temperature sum was calculated as the sum of positive mean daily temperatures (°C), cumulated from the assumed starting day of herbage N uptake onwards. Precipitation deficits were calculated as the cumulative difference between mean precipitations (1970–2017) and actual daily precipitation.

Data were split into an independent calibration and a test data set, and a subset was made of both data sets including only conditions without N application. Data from studies carried out at other experimental sites than G (Zegveld) and in which no CAN-N fertiliser was applied were evenly distributed over the calibration and test data set. Descriptive statistics of the different data sets are presented in Table 2.2.

Table 2.2. Descriptive statistics of conditions, soil properties and weather variables of the model calibration and model testing data sets (n = 49 and n = 55, respectively, for a subset of treatment means from conditions without N application) and for all treatment means (including conditions with and without N application, n = 98 and n = 119, respectively). SD = standard deviation, ND = not determined, CAN = calcium ammonium nitrate

		Calibration data set				Test data set			
Property / variable	**Unit**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Treatment means of conditions without N application (subset)									
No. of harvests		2.8	1.3	1	5	3.4	1.7	1	7
Soil N supply	kg ha^{-1}	159	73	17	353	129	72	15	306
Soil organic matter	g 100 g dry soil^{-1}	43	7	29	65	48	8	27	64
Soil pH		4.8	0.2	4.3	5.2	4.9	0.3	4.5	6.6
Precipitation deficit	mm	2	49	-94	175	ND			
Growing season mean daily temperature sum	°C	1721	847	458	3217	1510	862	229	3278
Preceding winter mean daily temperature sum	°C	253	73	17	353	335	67	194	454
All treatment means (including conditions with and without N application)									
No. of harvests		2.8	1.4	1	5	3.4	1.7	1	7
CAN-N application	kg ha^{-1}	85	84	0	260	127	138	0	607
Soil N supply	kg ha^{-1}	207	97	17	457	183	122	15	562
Soil organic matter	g 100 g dry soil^{-1}	46	6	29	65	49	9	27	64
Soil pH		4.8	0.2	4.3	5.2	4.9	0.3	4.5	6.6
Precipitation deficit	mm	14	68	-94	175	ND			
Growing season mean daily temperature sum	°C	1734	842	458	3217	1512	865	229	3278
Preceding winter mean daily temperature sum	°C	263	127	100	425	325	58	194	454

Data were tested for normality and homogeneity of variance. Subsequent statistical analyses were carried out in three steps;

1. Analyses were conducted with the objective to assess the effect of soil properties and weather variables on annual SNS per degree of growing season mean daily temperature sum, using a subset of the calibration data set with treatment means from conditions without CAN-N application. First, linear models were fitted without constant term predicting SNS by the growing season temperature sum, by which a slope coefficient per treatment mean was obtained. Then, slope coefficients were obtained from the same field, experimental site and year, by averaging treatment means differing in mowing dates. Consequently, a linear model was fitted for the prediction of these slope coefficients using stepwise weighted regression in which SOM, soil pH, precipitation deficit and winter temperature sum were candidate predictors and the number of treatment means averaged was used as weighing factor.
2. Analyses were conducted with the objective to estimate SNS for successive harvests within a growing season and to unravel effects of SNS and CAN-N application rates on herbage N uptake. Separate models were fitted on the full calibration data set and on the subset with data on conditions without N application. Mixed models for SNS estimation were fitted using stepwise linear regression, in which growing season temperature sum and CAN-N application rates were forced predictors, and SOM, soil pH,precipitation deficit, winter temperature sum and squared CAN-N application rate were candidate predictors. If there was a significant correlation between two candidate predictors, only the predictor that correlated the most with SNS was used to avoid potential multicollinearity in the models. Squared CAN-N application rate was included as candidate predictor, as herbage N use efficiencies may decrease at higher N application rates (Prins, 1983). An interaction between growing season temperature sum and SOM was allowed for, as it has been shown that air temperature correlates with soil temperature (Parton and Logan, 1981), mineralisation rate correlates with soil temperature (Macduff and White, 1985) and SOM content correlates with mineralisation rate (Vellinga and André, 1999). No constant term was taken up in the models, on the assumption that, at zero degrees growing season temperature sum, herbage N uptake had started. Means and variances of estimation residuals were analysed by ANOVA using F-probabilities to test whether additional predictors besides SOM decreased the size and variance of the SNS estimation residuals. And to test the hypothesis that SNS is estimated equally in conditions with and without N application, using the test data set.
3. Validation of the models obtained in step 2 was done by regressing SNS estimations to observations using the test data set. Therefore, the hypotheses that the estimated intercepts and slopes are equal to 0 and 1, respectively, were tested.

Statistical analyses were done using R (version 3.4.0, R core team, 2017) and the *rcorr* function of the R package *Hmisc* (version 4.0-3, Harrel *et al.*, 2017). Model selection for stepwise regression was done based on the lowest Schwarz information criterion (SIC).

2.3 Results

2.3.1 Correlations between candidate predictors of SNS

Candidate predictors of SNS per degree growing season mean daily temperature sum did not show significant mutual correlations (Table 2.3).

Table 2.3. Pearson correlations, coefficients (r) and probabilities (p-value) for different parameters of the calibration data set averaged per unique field–experimental site–year combination (n=31), for conditions without N application

		Soil N supply per degree growing season mean daily temperature sum	**Soil organic matter**	**Soil pH**	**Preceding winter mean daily temperature sum**
Soil organic matter	r	0.54			
	p-value	0.002			
Soil pH	r	-0.01	-0.05		
	p-value	0.958	0.782		
Preceding winter mean daily temperature sum	r	-0.17	0.14	-0.14	
	p-value	0.349	0.450	0.460	
Precipitation deficit	r	-0.15	-0.02	-0.01	0.22
	p-value	0.428	0.918	0.953	0.244

However, for SNS estimations for conditions without N application, the winter temperature sum correlated significantly with the precipitation deficit ($p < 0.001$, Table 2.4) and the latter was excluded as a candidate predictor. For SNS estimations of all treatment means (including conditions with and without N application), soil pH and winter temperature sum correlated significantly with SOM and precipitation deficit ($p < 0.001$, Table 2.4), and, therefore, soil pH and winter temperature sum were excluded as candidate predictors.

Table 2.4. Pearson correlation coefficients (r) and probabilities (p-value) for different parameters of the calibration data set, for conditions without N application and for the full data set (including conditions with and without N application). CAN = calcium ammonium nitrate

		Soil N supply	**Growing season mean daily temperature sum**	**CAN-N application rate**	**Soil organic matter**	**Soil pH**	**Preceding winter mean daily temperature sum**
Conditions without N application							
Growing season mean daily temperature sum	r	0.91					
	p-value	<0.001					
Soil organic matter	r	0.18	-0.03				
	p-value	0.006	0.703				
Soil pH	r	-0.05	0.03		-0.17		
	p-value	0.470	0.646		0.011		
Preceding winter mean daily temperature sum	r	0.03	0.06		0.09	0.03	
	p-value	0.707	0.376		0.160	0.710	
Precipitation deficit	r	-0.03	0.08		-0.03	-0.03	-0.42
	p-value	0.673	0.231		0.620	0.690	<0.001
All treatment means (including conditions with and without N application)							
Growing season mean daily temperature sum	r	0.81					
	p-value	<0.001					
CAN-N application rate	r	0.69	0.28				
	p-value	<0.001	<0.001				
Soil organic matter	r	0.14	-0.02	0.01			
	p-value	0.001	0.601	0.874			
Soil pH	r	-0.05	0.04	0.03	-0.32		
	p-value	0.268	0.373	0.528	<0.001		
Preceding winter mean daily temperature sum	r	-0.01	0.05	-0.06	0.11	0.05	
	p-value	0.725	0.205	0.171	0.012	0.208	
Precipitation deficit	r	0.05	0.12	0.09	-0.03	-0.02	-0.49
	p-value	0.200	0.004	0.038	0.536	0.643	<0.001

2.3.2 Estimation of SNS per degree growing season mean daily temperature sum using soil properties and weather variables, for conditions without N application

Soil organic matter correlated with SNS per degree growing season mean daily temperature sum (r = 0.54; $p = 0.001$, Figure 2.2), but not with soil pH, winter temperature sum and precipitation deficit (Table 2.3).

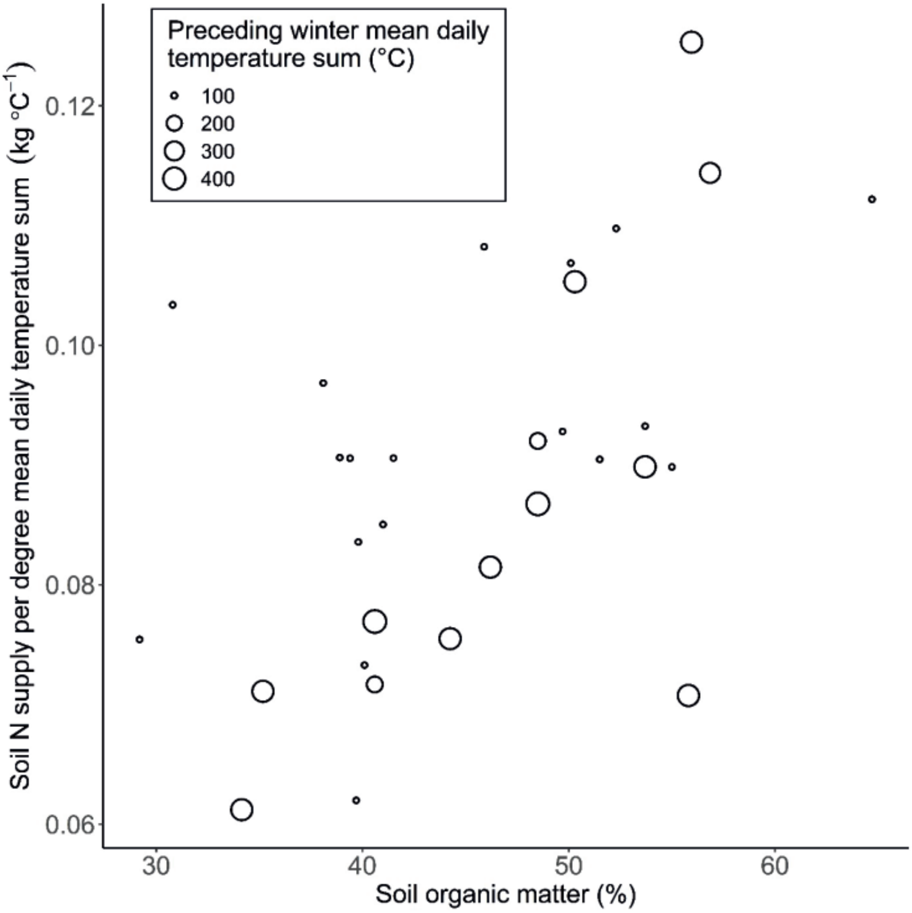

Figure 2.2. Annual soil N supply per degree growing season mean daily temperature sum versus soil organic matter content. Sizes of the data points correspond with the variation of the preceding winter (1 December – 28 February) mean daily temperature sum

Stepwise linear regression analyses revealed an effect of SOM ($p < 0.001$) and a tendency for the winter temperature sum to have an effect ($p = 0.067$) on SNS per degree growing season mean daily temperature sum (Table 2.5, model 1). Each g of SOM per 100 g^{-1} dry soil was estimated to increase SNS by 3.6 kg ha^{-1} and each

°C winter temperature was estimated to decrease SNS by 0.088 kg ha^{-1},for a growing season with a temperature sum of 3000 °C.

Table 2.5. Fitted models for the estimation of annual soil N supply per degree growing season mean daily temperature sum (kg °C^{-1} ha^{-1}, model 1) or growing season soil N supply (kg ha^{-1}, models 2–4). For each predictor, the fitted value, standard error (SE) and p-value for the hypothesis value = 0 is given. Residual standard errors (RSE) for the full models are given. CAN = calcium ammonium nitrate

Model no.	**1**	**2**	**3**	**4**
Regressor	Soil N supply per degree growing season mean daily temperature sum	Soil N supply	Soil N supply	Soil N supply
Predictor 1	<intercept>	Growing season mean daily temperature sum	Growing season mean daily temperature sum	Growing season mean daily temperature sum
Value	$3.93\cdot10^{-2}$	$8.97\cdot10^{-2}$	$2.80\cdot10^{-2}$	$2.68\cdot10^{-2}$
SE	$1.32\cdot10^{-2}$	$0.11\cdot10^{-2}$	$0.59\cdot10^{-2}$	$0.44\cdot10^{-2}$
p-value	0.006	<0.001	<0.001	<0.001
Predictor 2	Soil organic matter		Soil organic matter	Soil organic matter
Value	$1.21\cdot10^{-3}$		$4.60\cdot10^{-1}$	$4.91\cdot10^{-1}$
SE	$0.28\cdot10^{-3}$		$0.82\cdot10^{-1}$	$0.58\cdot10^{-1}$
p-value	<0.001		<0.001	<0.001
Predictor 3	Preceding winter mean daily temperature sum		Growing season mean daily temperature sum × soil organic matter	Growing season mean daily temperature sum × soil organic matter
Value	$-2.90\cdot10^{-5}$		$1.15\cdot10^{-3}$	$1.13\cdot10^{-3}$
SE	$1.52\cdot10^{-5}$		$0.14\cdot10^{-3}$	$0.09\cdot10^{-3}$
p-value	0.067		<0.001	<0.001
Predictor 4				CAN-N application
Value				$5.80\cdot10^{-1}$
SE				$0.14\cdot10^{-1}$
p-value				<0.001
RSE	$1.39\cdot10^{-2}$	31.6	24.6	26.7

2.3.3 Estimation of growing season SNS using soil properties and weather variables for conditions with and without N applications

For conditions without N application, growing season SNS coincided nearly linearly with the growing season temperature sum ($r = 0.92$, $p < 0.001$, Figure 2.3). Stepwise regression analyses resulted in the selection of growing season temperature sum, SOM and an interaction between growing season temperature sum and SOM as predictors of SNS ($p < 0.001$ for all) (Table 2.5, model 3). Soil pH and winter temperature sum were not selected as predictors of SNS.

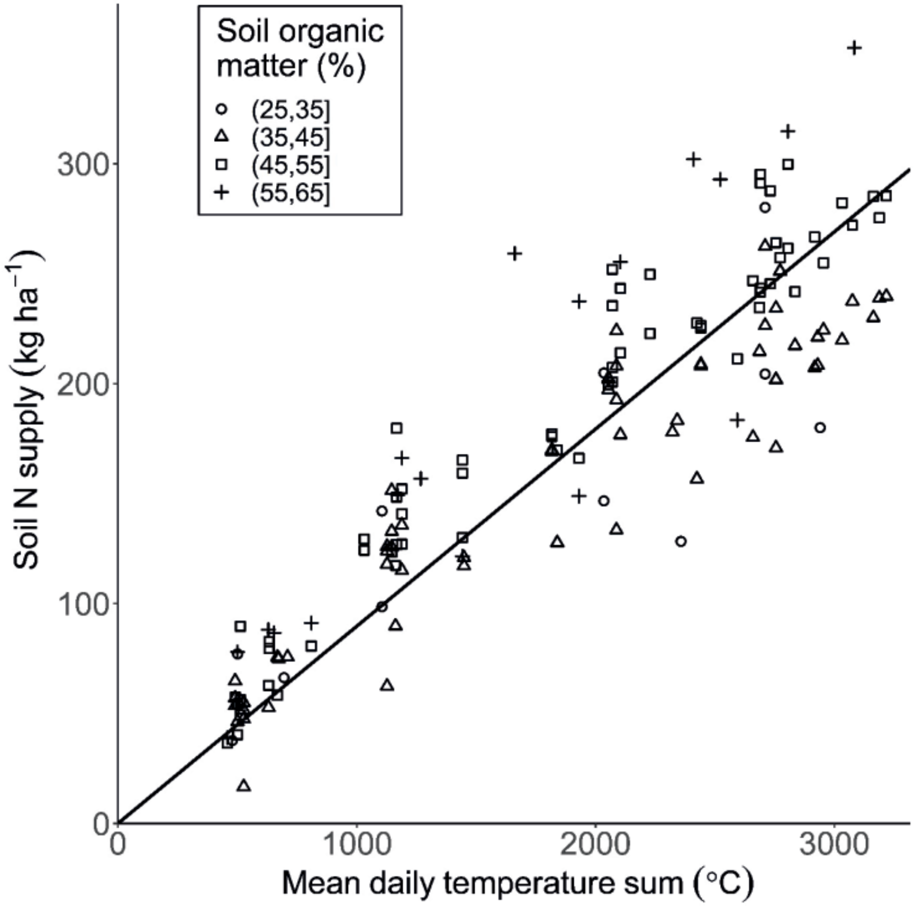

Figure 2.3. Growing season soil N supply versus growing season mean daily temperature sum, for conditions without N application. Data points represent measurements per harvest, and shapes represent different soil organic matter classes. Model details of the plotted line are shown in Table 2.5 (model 2)

For all treatment means (including conditions with and without N application), CAN-N application rate was selected as a predictor for SNS ($p < 0.001$) in addition to growing season temperature sum, SOM and an interaction between growing season temperature sum and SOM (Table 2.5, model 4). Apparent N recovery (ANR) of CAN-N applications was estimated rather constantly between the studies (58 ±14% SE). Precipitation deficit was not selected as a predictor of SNS.

Each °C growing season temperature sum and each g SOM per 100 g dry soil coincided with an SNS change of 78–90 g ha^{-1} and 3.6–3.9 kg ha^{-1}, respectively. The models estimated total SNS at 220 and 297 kg ha^{-1} $year^{-1}$ at SOM contents of 35 and 55 g 100 g dry $soil^{-1}$ for an average growing season with 3000 °C mean daily temperature sum.

The use of growing season temperature sum and the interaction between growing season temperature sum and SOM, compared to the use of only SOM as a predictor, significantly decreased SNS estimation residuals ($p < 0.001$ both for conditions without N application and conditions with and without N application).

SNS was not predicted differently for conditions without N application compared to those that included N application (Table 2.5, models 3 and 4, respectively), as means ($p = 0.520$) and variances ($p = 0.881$) of SNS estimation residuals did not differ between the models.

2.3.4 Validation of SNS estimations using the test data set

Models 3 and 4, for conditions without N application compared to those that included N application, respectively, accounted for 78% and 77% of the observed variance and predicted SNS with an RSE of 38.8 and 39.0 kg ha^{-1}. Estimations of SNS on the test data were consistent for experimental site G (Zegveld) but not for E (Wilnis) (Table 2.6). For experimental site E, measured SNS was on average 24% to 39% higher than predicted ($p < 0.001$ for conditions with and without N application), while for experimental site G, observations were in line with the estimations ($p \geq 0.146$). Compared to all other experimental sites included in the current work, observed mean SNS was markedly higher at experimental site E (339 kg ha^{-1} versus 259 kg ha^{-1} at 3000 °C growing season temperature sum). Nevertheless, SOM and growing season temperature sum explained 87% to 88% of the observed variation in SNS at this site (Table 2.6).

Table 2.6. Linear regression analyses of observed versus predicted soil N supply, for conditions without N application (estimations by model 3) and for all treatment means (including conditions with and without N application, estimations by model 4), of the test data set for experimental sites E (Wilnis) and G (Zegveld). The p-values given are for the hypotheses intercept = 0 and slope = 1. RMSD = Root mean squared deviations (RMSD) from the estimations in kg ha^{-1}, r^2 = percentage of variance accounted for by the estimation models. See Table 2.5 for details of the used estimation models

		Intercept			**Slope coefficient**			**RSMD**	**r²**
N application condition	Experimental site	Value	SE	*p*-value	Value	SE	*p*-value		
Without	E	2.42	14.2	0.865	1.35	0.08	<0.001	75.1	0.87
With and without	E	9.7	9.6	0.314	1.24	0.04	<0.001	81.4	0.88
Without	G	8.01	4.4	0.070	0.93	0.03	0.015	26.8	0.85
With and without	G	2.36	3.0	0.794	0.98	0.01	0.141	31.0	0.93

2.4 Discussion

2.4.1 Soil properties and weather variables associated with SNS

The hypothesis that SNS can be more accurately estimated by soil properties and weather variables, compared to using only soil properties, was confirmed since growing season temperature sum next to SOM as predictors of SNS significantly decreased estimation residuals.

The observed strong positive correlation between growing season temperature sum and SNS was likely the result of a positive correlation between air temperature and soil temperature (Parton and Logan, 1981), a positive correlation between soil temperature and mineralisation rate (Macduff and White, 1985) and of the phenomenon that morphological developments of grasses are closely related to the sum of mean daily temperatures (Frank *et al.*, 1985).

The observed positive correlation between SOM and SNS is in line with earlier results from studies on mineral soils (Hassink, 1995; Van Eekeren *et al.*, 2010) and peat soils (Vellinga and André, 1999). A higher soil SOM content is likely to result in a higher SNS since organic soil N is the main source for SNS, and, C:N ratios of dairy grasslands on peat soils are rather constant (Deru *et al.*, 2019). Moreover, the positive interaction between growing season temperature sum and SOM as a predictor of SNS (Table 2.5, models 3 and 4) indicates that soils with a higher SOM content are more prone to an increase in SNS at relatively higher growing season temperatures, compared to soils with a lower SOM content. Therefore, in the short term, increased growing season temperatures, e.g. as a result of climate change, may increase SNS for peat soils, especially on soils with a higher SOM content.

The found tendency for an impact of the winter temperature on the annual SNS in the following growing season might have been related to freeze-thaw events. Winters with a lower mean temperature, on average, had more or longer periods of frost than winters with a higher mean temperature (data not shown). Matzner and Borken (2008) showed that, for mineral soils, freeze-thaw events may induce gaseous and/or solute losses of N from soils, on an annual scale. They argued that freeze-thaw events induce changes in microbial biomass and populations, root turnover and soil structure. For agricultural peat soils, there are no known reports of freeze-thaw events on annual soil N dynamics, but it seems likely that such events can similarly affect the physical and biological properties of peat soils. On the other hand, the estimated effect of winter temperatures on annual SNS was rather small, and winter temperatures did not correlate with the rate of SNS per degree of the sum of the growing season temperature. Thus, the effect of winter temperature on SNS in the following growing season played only a minor role or may have been overruled by other factors, on an annual scale, in the studies used for the current work.

It is known that soil pH indirectly affects the availability of mineral N from soil mineralisation (Egan *et al.*, 2019; Jarvis *et al.*, 1996; Rashid *et al.*, 2014), although in the current work pH could not be indirectly or directly related to SNS. Soil pH variations (SD 0.2, pH was often measured per field or experimental site) and ranges (4.3 – 5.2, Table 2) in the used studies for the current work may have been too small to detect any effects of soil pH on SNS.

Although in the current work precipitation deficit was not found to affect SNS, several studies have shown that soil moisture content affects N mineralisation (Macduff and White, 1985; Paul *et al.*, 2003; Verloop *et al.*, 2014). Others suggested that water availability can be a limiting factor for herbage growth in poorly drained dairy grasslands on peat soils, due to soil desiccation at the root zone during relatively dry periods (Deru *et al.*, 2019; Schothorst, 1982; Van Kekem, 2004). Strong rainfall events might also affect herbage N uptake by inducing extra soil N losses (i.e. via denitrification or runoff) or affect soil temperatures and thereby the soil microbial community (Jarvis *et al.*, 1996). Possibly, the correlation between soil moisture content and precipitation deficit was low. Besides, since growing season SNS was modelled, short-term relationships between precipitation deficit and SNS could have been compensated for later on in the season (i.e. often short periods of precipitation deficits alternate with periods of surplus during the growing season). Therefore, using actual soil moisture concentrations as predictor and/or performing short period analyses (e.g. per season or harvest) may result in improved SNS estimations.

Estimations of SNS in the current study were in line with other studies carried out in the Netherlands. Vellinga and André (1999) estimated a mean SNS of 252 kg ha^{-1} year^{-1} for dairy grassland on peat soils, based on studies carried out over the 1934–1994 period. Van Kekem *et al.* (2004) estimated mean SNS at 225 kg ha^{-1} year^{-1}, based on studies carried out over the 1991–2003 period, and argued that mean SNS had slightly decreased since the 1990s due to slight increases in ditchwater levels, compared to levels in earlier decades.

2.4.2 CAN-N application did not affect estimated SNS

The conformation of the hypothesis that the presence and rate of CAN fertilisation does not affect SNS estimations within a growing season was in line with Egan *et al.* (2019), who found no relationship between herbage ANR of applied ammonium nitrate N (100 kg ha^{-1} year^{-1}) and soil N mineralisation of an acid sandy soil. On the other hand, studies on mineral soils have shown that inorganic N fertilisation can affect soil N transformation directly or indirectly through the alteration of soil abiotic and biotic characteristics and SOM quality (e.g. Zhang *et al.*, 2014), and therefore possibly also affect soil N mineralisation. Nitrogen fertilisation increases herbage yields and therefore also biomass turnover, which in turn could also directly increase the SOM content and SNS. However, the soil N content of grassland on peat soils is relatively high (2.01 ± 0.46 g 100 g^{-1} dry soil for 20 dairy grassland areas in the western peat area of the Netherlands, Deru *et al.*, 2019) and, in the studies used for the current work, CAN-N application amounts where relatively low (85 ± 84 kg ha^{-1} year^{-1} in the calibration data set). Furthermore, since only data of the first year after cessation of organic inputs was used, effects of N fertilisation on SNS of successive years were not included in the current analyses. This might explain the insignificant effect of CAN-N application on soil N mineralisation found in the current work.

Another important factor for the similar SNS estimations under conditions with and without CAN-N applications, was the rather constant estimated annual ANR of CAN-N application between the studies. Estimated mean annual ANR was very similar to the results from Vellinga and André (1999), who found an average ANR of nearly 60% for dairy grasslands on peat soils, based on data of studies carried out in the 1934–1994 period, and somewhat lower than Deru *et al.* (2019), who reported an average ANR of 65% for 20 dairy grasslands on peat soils for a specific year (2010). This supports the finding that annual ANR of CAN-N application was rather constant within the studied conditions.

From the current work cannot be inferred that other inorganic N fertilisers than CAN do not affect SNS within a growing season, as only studies with CAN fertiliser were used. However, effects on SNS of other inorganic fertilisers, e.g. based on ammonium or urea, applied at similar rates as in the current study, may also be limited due to moment of SNS determination (the first year after cessation of organic and inorganic inputs), the relative short period used for SNS determination (6–7 months) and due to the relative high SOM and organic N content of grassland on peat soils. The latter may overrule, at least within a growing season, effects of inorganic fertiliser types on soil N transformations and stocks.

2.4.3 SNS estimations for the test data set were inconsistent for one experimental site

The estimated SNS for experimental site E was very similar to the mean SNS (342 kg ha^{-1}) found by Sonneveld and Lantinga (2011) for a dairy grassland on a peat soil with an anthropogenic A horizon receiving relatively high historical organic inputs. According to Van Kekem (2004) and Sonneveld and Lantinga (2011), historical inputs of organic N determine at least about 60% of the annual N mineralisation of dairy grasslands on peat soils. Sonneveld and Lantinga (2011) suggested that the contribution of peat to SNS is lower when an anthropogenic A horizon is present. Soils at site E have an anthropogenic A horizon of 30–40 cm, while at site G, this horizon is not present (Stiboka, 1970). Since historical inputs were not recorded at both sites, but were limited to applicable regulations, effects of differences in historical input between sites were not expected but could not be excluded. Furthermore, soil microclimate differences could have affected SNS. Deru et al. (2019) found SNS to correlate with the Ca:Mg ratio and soil air content at pF 2. For mineral soils, Hassink et al. (1994) and Jarvis et al. (1996) suggested that physical (e.g. particles and pores) and biological properties (e.g. the composition and structure of the soil microbial community) may directly and/or indirectly affect soil N mineralisation. Therefore, in order to potentially explain differences in observed and predicted SNS for specific sites, future research on SNS of dairy grassland on peat soils should take into account actual recent historical inputs and parameters characterising the soil microclimate properties (such as temperature, moisture content, C:N ratio, pH, particle and pore size, micro mineral concentrations and ecology).

2.5 Conclusions

The current work shows that spatial variation and the temporal increase of growing season soil nitrogen supply can be accurately estimated using growing season degree days and soil organic matter content as predictors. The presented models allow for improved within growing season soil nitrogen supply adapted fertilisation, grazing and harvesting advice. Furthermore, it was found that not only measurements carried out in conditions without but also with calcium ammonium nitrate applications can be used to deduce soil nitrogen supply estimations.

However, the current work also showed that estimated soil nitrogen supply for a specific site was consistently underestimated by the presented models. To clarify inconsistencies, future research on soil nitrogen supply estimations should take into account actual growing season mean daily temperatures, soil microclimate properties and recent historical inputs.

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Conflict of interest

The authors declare that they have no conflict of interest.

Chapter 3

Effect of *Lolium perenne* population differences on shoot tissue nitrogen concentrations when grown on a peat soil

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Abstract

Grass nitrogen (N) concentrations of dairy grasslands are higher on peat soil than on mineral soils. This can lead to increased N losses to air and water from dairy farming systems on peat soils. Our hypothesis was that the use of low-N perennial ryegrass (*Lolium perenne* L.) genotypes could be a means to lower grass N concentrations, when grown on peat soils. Our objective was to determine whether perennial ryegrass populations with different shoot tissue N concentrations, recorded on a sandy soil, would show different shoot tissue N concentrations and N use efficiencies (NUE) or N uptake efficiencies (NUptE) when grown on a peat soil. First, a pot experiment lasting 62 days was carried out with nine diploid and seven tetraploid populations, followed by a field experiment with two diploid and two tetraploid populations and a control lasting 30 months. Both experiments had three N fertilisation levels. In the pot experiment, populations explained 3% of the variation in shoot tissue N concentration among tetraploids, 5% and 7% of the variation in NUE among diploids and tetraploids and 12% of the variation in NUptE among diploids. In the field experiment, populations explained 44% of the variation in NUptE. A higher NUE coincided with lower shoot tissue N concentrations among tetraploid populations in the pot experiment. We conclude that there is potential to alter the shoot tissue N concentrations of perennial ryegrass grown on peat soil, via selection for shoot tissue N concentrations and NUE.

Keywords: dairy farming; perennial ryegrass; crude protein; nitrogen use efficiency; plant breeding

3.1 Introduction

Peatlands are areas with a naturally accumulated layer of peat at the surface, and about 12% of European peatlands are drained agricultural grasslands (Byrne *et al.* 2004; Tanneberger *et al.* 2017). Peat is defined as "accumulated sedentary material of which at least 30 mass percent is dead organic matter" (Tanneberger *et al.* 2017). Grass nitrogen (N) uptake by grassland on drained peat soils is relatively high compared to mineral soils, when used in a dairy farming system. This is mainly due to the a high soil N supply (SNS), caused by a higher net organic matter mineralisation on drained peat soils compared to mineral soils (Vellinga & André 1999; De Visser *et al.* 2001). The SNS is defined as the non-fertiliser herbage shoot N uptake in the first year after cessation of N inputs, and consists therefore of organic N that is mineralised during the growing season, mineral N that is present in the soil profile in spring, N from dry and wet deposition and N fixation from free and symbiotic living microbes in the soil (Hassink 1995). Mean SNS of dairy grassland on peat with an average lowest groundwater level of 50 – 80 cm below field surface has been estimated at 25.2 g m^{-2} year^{-1}, 24 – 30 % higher than at mineral soils (Vellinga & André 1999).

Vellinga & Andre (1999) observed that at the same fertilisation rate, mean annual grass N concentrations were around 1.5 – 3.0 g kg^{-1} dry matter (DM) higher for dairy grassland on peat soils, compared to mineral soils. For dairy grassland on peat soils, grass N concentrations are often observed to exceed 24 – 26 g kg^{-1} DM, equal to about 150 – 169 g crude protein (CP) kg^{-1} DM, even under limited N fertilisation regimes (Korevaar 1986; Vellinga & André 1999; Verloop *et al.* 2018; Hoekstra *et al.* 2019). At dietary CP concentrations above this range, milk and protein yields do not generally increase, while urine urea N losses increase (Kebreab *et al.* 2002; Colmenero & Broderick 2006; Huhtanen & Hristov 2009). Increased urine urea N losses can in turn lead to increased ammonia losses (Smits *et al.* 1995; Edouard *et al.* 2019). Therefore, dietary CP concentrations above the optimal range are considered undesirable from farming and environmental perspectives.

Possible measures for optimising dietary CP concentrations for dairy farms on peat soils are; 1) adapting N fertilisation rate and timing to the seasonal SNS (Pijlman *et al.* 2020b), 2) complementing diets with low CP components such as maize or sugar beet pulp (Smits *et al.* 1995; Hristov *et al.* 2015), 3) lowering the CP content of concentrates (Hoekstra *et al.* 2020), or 4) using alternative crops such as plantain (*Plantago lanceolata* L.) (Pijlman *et al.* 2020a) and cattail (*Typha latifolia* L.) (Pijlman *et al.* 2019). However, there are various reasons why realising optimal dietary N concentrations remains difficult for dairy farms on peat soils; 1) Diets are typically grass based, as the cultivation of perennial grasses is preferred over annual low-CP crops such as maize or cereals. Annual crops require more frequent tillage, which leads to undesired increased soil organic matter decomposition, nutrient losses and soil subsidence (Lohila *et al.* 2004; Taft *et al.* 2017). Grass cultivation results in a permanent coverage of the soil by vegetation, which increases the generally low load bearing capacity for machines and cows of peat soils at high groundwater levels (Wiedow *et al.* 2016). 2) During the growing season, fertiliser inputs remain necessary to maintain or increase the nutritional value of grass and DM uptake at grazing (Peyraud & Astigarraga 1998), which generally leads to increased grass N concentrations. Furthermore, the difference between grass N concentrations on drained peat versus mineral soils, at the same fertilisation rate and timing, tends to increase during the growing season, since the SNS increases with growing degree days because of a greater mineralisation of organic matter with increasing soil temperatures (van Eekeren *et al.* 2010; Pijlman *et al.* 2020b). As a result, controlling grass N concentrations remains difficult during the growing season on these soils. 3) Farmers increasingly manage their farms with the aim of reducing mineral losses – following environmental regulations, market demand and a transition towards a circular economy – by using relatively fewer farm inputs (Fogarassy *et al.* 2016). As a result, the use of relative low CP feeds from outside the farm, such as cereals or maize, is often restricted, which limits the possibility of complementing diets with low CP feeds.

Selection of perennial ryegrass genotypes with a high DM yield per unit of N taken up (i.e. low shoot tissue N concentration in the DM) could be a potential alternative way to optimise dietary CP concentrations and to reduce environmental impacts of dairy farming on peat soils, and could be part of a more system-based plant breeding approach (Lammerts van Bueren *et al.* 2018). According to Tas (2007), decreasing the N concentration of perennial ryegrass in grass-based diets may result in a substantial improvement in animal NUE, compared to increasing the water soluble carbohydrates (WSC) concentration of perennial ryegrass. This is in line with results of other studies (Moorby *et al.* 2006; Edwards *et al.* 2007), and underlines the importance of taking N concentration into account at the breeding of grasses. Different studies pointed out that there is a selection potential for N concentration in perennial ryegrass, although heritability and variation were found to be lower than that for WSC (Humphreys 1989; Wilkins *et al.* 1997, 2000; Smith *et al.* 1998; Wilkins & Humphreys 2003; Arojju *et al.* 2020). Over the last several decades, perennial ryegrass breeding programs have mainly focussed on DM yield potential, persistence and digestibility (McDonagh *et al.* 2016). Selection for N uptake and concentration and N use efficiency (NUE) has received limited direct attention (Baert *et al.* 2007; Malmberg *et al.* 2023). It is furthermore known that trait expressions can be affected by management and environmental conditions (Conaghan & Casler 2011; Parsons *et al.* 2011; Malmberg *et al.* 2023). To our knowledge, experiments on genetic variations of perennial ryegrass N concentrations at dairy grassland on peat soils have not been reported.

There are different pathways that can lead to reduced perennial ryegrass N concentrations in the harvested DM; 1) through a more efficient use of acquired N for aboveground biomass accumulation, i.e. a higher NUE or 2) through a less efficient N uptake per unit of N supply, i.e. a lower N uptake efficiency (NUptE). Here, N supply is the sum of SNS and N fertilisation. Regarding the first pathway, different studies have shown that perennial ryegrass genotypes can differ in NUE (Humphreys 1989; Wilkins *et al.* 2000; Sandaña *et al.* 2021). Regarding the second pathway, a less efficient aboveground grass N uptake could be a result of a lower total plant N uptake per unit of N supply (Wilkins & Lovatt 1989), or a result of a shift of biomass N partitioning from aboveground to belowground biomass, at the same total plant N uptake. Brégard *et al.* (2000) observed that timothy grass populations, selected for low or high aboveground N concentrations, differed in biomass N partitioning between roots and shoots, but not in total plant N concentration.

Knowledge of pathways by which grass N concentrations are affected is of importance for the estimation of possible trade-offs within the agricultural system. For example, grasses with lower N concentrations as a result of changed N partitioning between above- and belowground biomass, may result in aboveground biomass with lower leaf to stem ratios (Brégard *et al.* 2000), which will affect the nutritional value of the grass and, consequently, farm biogeochemical cycles. A higher NUE or lower NUptE as a means to reduce grass N concentration could result in increased soil N surplus, and may lead to increased soil N losses via emission of NO_3^-, N_2O or N_2 (Wilkins *et al.* 2000; Baert *et al.* 2007).

In this study, a pot experiment and field experiment were carried out consecutively with perennial ryegrass (*Lolium perenne* L.) populations differing in previously recorded N concentrations when grown on a sandy soil. In the pot experiment, sixteen genetically differing populations were compared. The two diploid and two tetraploid populations which showed the largest differences in the pot experiment, were compared in the field experiment over a 30-month period. Both experiments were carried out on a peat soil with three different N fertilisation levels, with the objective of comparing perennial ryegrass population NUE, NUptE and N concentrations. It was hypothesised that, on a peat soil, perennial ryegrass populations selected for a different shoot tissue N concentration on a sandy soil, would have a different shoot tissue N concentration and NUE or NUptE (hypothesis 1), and that populations with a high NUE (hypothesis 2) and populations with a low NUptE (hypothesis 3) would have a lower shoot tissue N concentration.

3.2 Materials and methods

Sixteen perennial ryegrass populations, consisting of nine diploid and seven tetraploid populations with either a low or a high mean shoot tissue N concentration, were selected from a large database of perennial ryegrass populations from a commercial breeding program. This database consisted of perennial ryegrass population shoot tissue N concentrations recorded at the first three harvests in the year of seeding – 2012 or 2013 – on a sandy soil (Typic Haplohumod) (Soil Survey Staff 1999) in the Netherlands (Moerstraten, 51°32'N, 4°21'E), with a mean groundwater table greater than 120 cm below the surface. The growing season of 2012 had relatively more precipitation, while 2013 was relatively colder and had less precipitation, compared to 20-year averages (Table S3.1). At this site, N fertilisation rates were 13, 9 and 7 g m^{-2} before the first, second and third harvest, respectively, and P and K were sufficiently applied to ensure these minerals were not limiting growth (CBGV 2022). Nitrogen, P and K were applied in the form of mineral fertilisers. Shoot tissue N concentrations of the populations differed significantly (p = 0.004), and mean shoot tissue N concentrations were lower for diploids than for tetraploids (p = 0.035) (Table 3.1). This database did not contain data on dry matter yield, NNI, NUE or NUptE of the populations.

Table 3.1. Mean shoot tissue N concentration recorded for the first three harvest cuts in the year of seeding on a sandy soil for the selected diploid (n = 9) and tetraploid (n = 7) populations. SEM = standard error of the mean.

diploid		**tetraploid**	
population	**shoot tissue N concentration (g kg DM^{-1})**	**population**	**shoot tissue N concentration (g kg DM^{-1})**
1	19.2	10	20.8
2	20.3	11	21.3
3	20.4	12	21.9
4	20.6	13	24.5
5	20.8	14	25.1
6	21.0	15	25.4
7	21.3	16	25.6
8	25.6		
9	25.6		
diploid mean	21.6	tetraploid mean	23.5
diploid SEM	0.75	tetraploid SEM	0.81

3.2.1 Pot experiment

The selected nine diploid and seven tetraploid populations were tested in a pot experiment with three N fertilisation levels (0, 6 or 12 g N m^{-2}) (Table 3.2).

Table 3.2. Experimental treatments of the pot and field experiments. Crosses (x) indicate treatment combinations of populations and N fertilisation levels. Each treatment was replicated three times in the pot experiment and four times in the field experiment.

		pot experiment			**field experiment**		
		N fertilisation level (g m^{-2})			**N fertilisatwion level (g m^{-2} $year^{-1}$)**		
population	**ploidy**	**0**	**6**	**12**	**0**	**125**	**250**
1 / 2Nlow	2N	x	x	x	x	x	x
2	2N	x	x	x			
3	2N	x	x	x			
4	2N	x	x	x			
5	2N	x	x	x			
6	2N	x	x	x			
7	2N	x	x	x			
8	2N	x	x	x			
9 / 2Nhigh	2N	x	x	x			x
10 / 4Nlow	4N	x	x	x	x	x	x
11	4N	x	x	x			
12	4N	x	x	x			
13	4N	x	x	x			
14	4N	x	x	x			
15	4N	x	x	x			
16 / 4Nhigh	4N	x	x	x			x
control	2N				x	x	x

All treatments had three replicates, which was the required minimum for sufficient statistical power (see section ‘Data analyses’). The treatments were allocated to 144 pots (size 15 x 15 x 15 cm) in a randomised complete block design. The experiment lasted 62 days. At day zero (1 April 2015), the pots were filled with a standard peat-based substrate mix in which the pH was increased to 5.8 using limestone (Jiffy, Zwijndrecht, the Netherlands) (Table 3.3). The pH of the substrate was increased to improve the growing conditions and mineral availability for the grass (Egan *et al.* 2019), because peat used for substrates has typically a pH around 4.0 (Messiga *et al.* 2022). The pots were topped with 5 mm of coarse sand.

Table 3.3. Pot and field experiment. Properties of the peat substrate used in the pot experiment and of the soil in the field experiment (*n* = 2). P_{AL} = ammonium lactate-acetate soluble phosphorus. pH-KCL = pH determined at a 1:5 soil to 1.0 M KCl solution ratio (ISO 10390:2005), ND = not determined.

Soil parameter	Unit	pot experiment	field experiment
organic matter	g 100 g^{-1} dry soil	80	45.9
Clay	g 100 g^{-1} dry soil	ND	31
total C	g kg^{-1} dry soil	408	232
total N	g kg^{-1} dry soil	5.6	21.1
P_{AL}	mg P_2O_5 100 g^{-1} dry soil	ND	22
total K	mg kg^{-1} dry soil	ND	484
total S	g kg^{-1} dry soil	2.0	6.0
pH-KCl	-	5.8	4.7

The pots were placed in a greenhouse (Moerstraten, 51°32'N, 4°21'E) without artificial heating or light, and received water through sub-irrigation on a daily basis. Greenhouse temperature and soil moisture content were not recorded. Per pot, 38 germinating seeds were sown. Prior to sowing, all pots received P, K and S at a rate of 35, 5 and 10 g m^{-2}, respectively, by applying a suspension of phosphoric acid (H_3PO_4), potassium hydroxide (KOH) and sulphuric acid (H_2SO_4) on top of the soil. At day 42, N was applied at a rate of 0, 6 or 12 g m^{-2} using a 50:50 mixture of liquid urea (CH_4N_2O) and ammonium nitrate (NH_4NO_3). The N fertilisation level of 12 g m^{-2} was slightly higher compared to fertilisation levels used for a May harvest in field conditions (about 10 g m^{-2}) (Remmelink *et al.* 2018). A higher level was chosen because grass biomass growth and N uptake were expected to be higher in a greenhouse (Poorter *et al.* 2016). At days 22 and 42, aboveground biomass was harvested in order to stimulate perennial ryegrass tillering, and discarded. At day 62, grass was harvested and collected for DM (oven drying at 70° C for 48 h) and total N analyses (NEN-ISO 5983-2, Kjeldahl method, Eurofins Agro, Wageningen, the Netherlands). Grass harvests were carried out using secateurs. The stubble height after harvesting was 4 cm.

3.2.2 Field experiment

Two diploid and two tetraploid populations, with either the lowest or the highest mean shoot tissue N concentration in the pot experiment, were used in the field experiment. These were the diploid populations 1 and 9, and the tetraploid populations 10 and 16, renamed 2Nlow, 2Nhigh, 4Nlow and 4Nhigh, respectively, for the field experiment. A commercially available diploid perennial ryegrassmixture (consisting of 50% Barimero and 50% Toronto on a weight basis) was used as the control. The 2Nlow, 4Nlow and control grasses were grown at three N application levels (0, 12.5 or 25 g N m^{-2} $year^{-1}$), and the 2Nhigh and 4Nhigh grasses were grown at one N application level (25 g N m^{-2} year^{-1}) due to limited seed availability (Table 3.2). There were four replicates of each treatment. As a result, the experiment consisted of forty-four different plots (5 x 2 m), in which treatments were allocated in a randomised block design. The experiment was established on a peat soil that had been in use as a permanent dairy grassland, with mean ditch water levels 60 cm below surface (KTC Zegveld, 52°08'N, 4°50'E). Two weeks before sowing, the field was treated with 0.25 mL m^{-2} Roundup® (480 g glyphosate L^{-1}, Monsanto, Saint Louis, MO, USA), and at the day before sowing, the terminated grass sod was rotavated to a depth of 10 cm. On 2 September 2016, grasses were sown at a rate of 1327 germinating seeds m^{-2}, which was approximately equal to 3 g m^{-2} for diploids and 4.6 g m^{-2} for tetraploids. These are common sowing rates in practice for diploid and tetraploid grasses (Remmelink *et al.* 2018). The experiment was carried out until the first harvest in 2019. Each growing season, fields were fertilised with 0, 12.5 or 25 g N m^{-2} using calcium ammonium nitrate ($H_4CaN_2O_3$), 10 g K m^{-2} using potassium chloride (K_2SO_4) and 1.7 g P m^{-2} using monocalcium phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) (Table 3.4). The level and annual distribution of N fertiliser, at the treatment of 25 g N m^{-2} year^{-1}, was similar to local practice (Remmelink *et al.* 2018).

Table 3.4. Field experiment. Scheme of annually applied nitrogen (N), potassium (K) and phosphorus (P).

		applied N, K and P fertiliser per harvest cut (g m^{-2})			
	annual N fertilisation level (g m^{-2})	**end of March**	**after 1st cut**	**after 2nd cut**	**after 3rd cut**
N	0.0	0.0	0.0	0.0	0.0
	12.5	5.0	3.5	2.5	1.5
	25.0	10.0	7.0	5.0	3.0
K	0.0-12.5-25.0	4.1	3.3	2.5	-
P	0.0-12.5-25.0	1.7	-	-	-

Between May 2017 and May 2019, every five to eight weeks aboveground herbage was harvested at a stubble height of approximately 5 cm using a small plot harvester, and weighed (J. Haldrup, Løgstør, Denmark). The harvesting regime was similar to local practice (Vellinga & André 1999; Remmelink *et al.* 2018). At each cut, a representative herbage sample was taken from each plot for analyses of DM (oven drying at 70° C for 48 h) and total shoot tissue N concentration (NEN-ISO 5983-2, Kjeldahl method, Eurofins Agro, Wageningen, the Netherlands). Samples from the first cut in 2018 were analysed for water soluble carbohydrates (WSC), neutral detergent fibre (NDF), acid detergent fibre (ADF) and *in vitro* digestibility of organic matter (IVDOM) using near infrared spectroscopy (Eurofins Agro, Wageningen, the Netherlands).

Soil samples (0–10 cm depth) of all plots were taken in February 2017. These were pooled by weight into one subsample, which was used for further analyses (Table 3.3). Average daily temperatures were collected from weather station de Bilt (Royal Netherlands Meteorological Institute, 52°05'N, 5°10'E), and precipitation data were collected from weather station Zegveld (Royal Netherlands Meteorological Institute, 52°07'N, 4°50'E) (Table 3.5). These stations were located 23.6 and 2.5 km from the experimental site, respectively. Annual atmospheric N deposition (NO_x and NH_3) at the experimental site was estimated at 2.32 and 2.42 g m^{-2} in 2017 and 2018, respectively (Wichink Kruit & Van Pul 2018) (data of 2019 was not available).

Table 3.5. Field experiment. Air temperatures and precipitation at the experimental site for each growing season. SD = standard deviation.

Period	temperature (°C)		precipitation (mm)	
	Mean	SD	sum	SD
Mar.–Oct.2017	14.0	3.8	571	5
Mar.–Oct.2018	14.6	5.0	337	3
Mar.–Oct.1999–2019	13.6	4.9	587	5
Mar.–Apr. 2019	9.5	3.5	136	4
Mar.–Apr. 1999–2019	8.2	3.9	97	3

In May 2017 – the first spring after sowing – all fields received an additional calcium ammonium nitrate application of 2.5 g N m^{-2}, and all herbage was harvested at a stubble height of 7 cm and then discarded, in order to stimulate perennial ryegrass growth and tillering, and to decrease the presence of other spontaneously germinated plant species (mainly *Stellaria media*). The botanical composition of each plot was visually estimated in May 2017 and 2018.

3.2.3 Data analyses

The SNS in both experiments was determined as the shoot tissue N uptake at zero N fertilisation (Hassink 1995). The NUE was expressed by equation 1, in which ΔW is the increment of aboveground dry biomass weight between two N fertilisation levels, and ΔNupt is the increment of shoot tissue N uptake between two N fertilisation levels (Gastal *et al.* 2015).

$$equation\ 1: NUE = \frac{\Delta \mathrm{W}}{\Delta \mathrm{Nupt}}$$

The NUptE was expressed by equation 2, in which ΔN supply is the fertiliser N increment between two N fertilisation levels, assuming SNS remains constant at different N fertilisation levels (Gastal *et al.* 2015).

$$equation\ 2\text{: NUptE} = \frac{\Delta\text{Nupt}}{\Delta\text{Nsupply}}$$

Comparisons of the populations NUE and NUptE were done taking into account the nutritional N status, as suggested by Sandaña *et al.* (2021). They concluded that in order to seek for potential pathways to improve the NUE on grassland systems, both the nutritional N status and forage yield must be considered for the correct interpretation of NUE in response to genotype-nitrogen environmental conditions. We used the N nutrition index (NNI) as an assessment tool for the nutritional N status (Lemaire *et al.* 2008). The NNI is expressed by equation 3, in which $\%N_a$ is the actual shoot tissue N concentration, and $\%N_c$ is the critical N concentration on a dry matter basis (Gastal *et al.* 2015).

$$equation\ 3\text{: } NNI = \frac{\%N_a}{\%N_c}$$

The critical N concentration is considered to be the minimum plant N concentration needed for the maximum growth rate (Ulrich 1952), which can be estimated via the critical N dilution curve. We estimated the critical N dilution curve for C3-grasses by the equation of Greenwood et al. (1991) (equation 4), in which W is the aboveground biomass in Mg ha^{-1}.

$$equation\ 4\text{: } \%N_a = 4.8W^{-0.32}$$

All statistical analyses were done using R (R Core Team 2019). A *p*-value ≤ 0.05 was considered significant at all analyses. Prior to the experiments, a power analysis was performed, based on the shoot tissue N concentration variance observed on sandy soil, to determine the minimum number of observations needed for a 90% probability (ß = 0.10) that null hypotheses were not mistakenly accepted. In both experiments, analyses for differences between shoot tissue N concentration, NUE, NUptE, DM yield and fodder quality parameters were done with an ANOVA, in which population was used as factor, NNI (for NUE and NUptE) or N fertilisation (for all other variables) was used as independent variable, and replications were used as block effects. In the pot experiment, results for diploid and tetraploid populations were analysed separately, since previous recorded mean shoot tissue N concentrations on sandy soil were lower for diploids than for tetraploids. In the field experiment, results of the control, 2Nlow and 4Nlow populations at 0, 12.5 and 25 g m^{-2} N $year^{-1}$ fertilisation, and results of all populations at 25 g m^{-2} N $year^{-1}$ fertilisation, were analysed separately, since the design of the experiment was not fully balanced. In the field experiment, harvest number was used as a within-subject factor according to a repeated measures design. Pots and plots were considered experimental units in the pot and field experiment, respectively. Differences among treatments were analysed by Least Significant Differences (LSD). Correlation analyses between NUE and shoot tissue N concentration were done using Pearson correlation coefficients.

3.3 Results

3.3.1 Pot experiment

Shoot tissue N concentrations differed among tetraploid, but not among diploid populations (Table 3.6). Tetraploid population 10 had a significant lower shoot tissue N concentration compared to the other populations ($p < 0.001$). The NUE differed among diploid and tetraploid populations ($p = 0.008$ and $p = 0.005$, respectively). The NUptE differed among diploid ($p = 0.043$), but not among tetraploid populations. Dry matter yields were affected by N fertilisation ($p < 0.001$), but did not differ among populations.

Table 3.6. Pot experiment. Mean, standard error of the mean (SEM) and explained variance of shoot tissue N concentration (tissue N conc.), N use efficiency (NUE), N uptake efficiency (NUptE) and dry matter (DM) yield for diploid and tetraploid populations.

ploidy	**diploid**					**tetraploid**			
parameter	**tissue N conc.**	**NUE**	**NUptE**	**DM yield**	**parameter**	**tissue N conc.**	**NUE**	**NUptE**	**DM yield**
Unit	**g kg DM^{-1}**	**g DM g N^{-1}**	**g N$_{upt}$ g N$_{supply}$$^{-1}$**	**g m^{-2}**	**unit**	**g kg DM^{-1}**	**g DM g N^{-1}**	**g N$_{upt}$ g N$_{supply}$$^{-1}$**	**g m^{-2}**
population means					*population means*				
1	30.7	27.4^{a}	0.43^{bc}	150	10	28.4^{a}	23.9^{a}	0.48	146
2	39.3	10.9^{d}	0.41^{bc}	134	11	36.0^{b}	16.3^{bc}	0.50	138
3	41.9	15.1^{cd}	0.38^{c}	123	12	35.1^{b}	17.1^{bc}	0.48	142
4	37.5	17.4^{bcd}	0.56^{ab}	140	13	36.9^{b}	12.6^{c}	0.48	136
5	37.9	15.1^{cd}	0.42^{bc}	131	14	37.0^{b}	15.3^{bc}	0.58	138
6	35.5	22.6^{ab}	0.42^{bc}	150	15	36.8^{b}	18.9^{ab}	0.51	147
7	35.6	16.1^{bcd}	0.62^{a}	151	16	38.5^{b}	15.2^{bc}	0.46	131
8	37.4	16.6^{bcd}	0.57^{ab}	105					
9	41.2	19.1^{bc}	0.42^{bc}	131					
SEM	1.27	1.11	0.019	5.2	SEM	1.42	0.92	0.017	5.3
explained variance					*explained variance*				
population	3%ns	5%**	12%*	9%ns	population	3%***	7%**	5%ns	2%ns
N fertilisation	94%***			40%***	N fertilisation	95%***			64%***
N nutrition index		94%***	78%***		N nutrition index		84%***	74%**	
block	1%ns	0%ns	5%ns	1%ns	block	2%ns	7%*	12%ns	4%*
residual variation	5%	2%	5%	49%	residual variation	1%	2%	8%	31%

^{abc} values with an unequal superscript differed significantly ($p < 0.05$)

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant

Shoot tissue N concentrations of the tetraploid populations correlated negatively with NUE (r = -0.85 and $p = 0.014$) (Figure 3.1). On average, shoot tissue N concentrations decreased 0.8 g kg DM^{-1} per g of NUE decrease among the tetraploid populations.

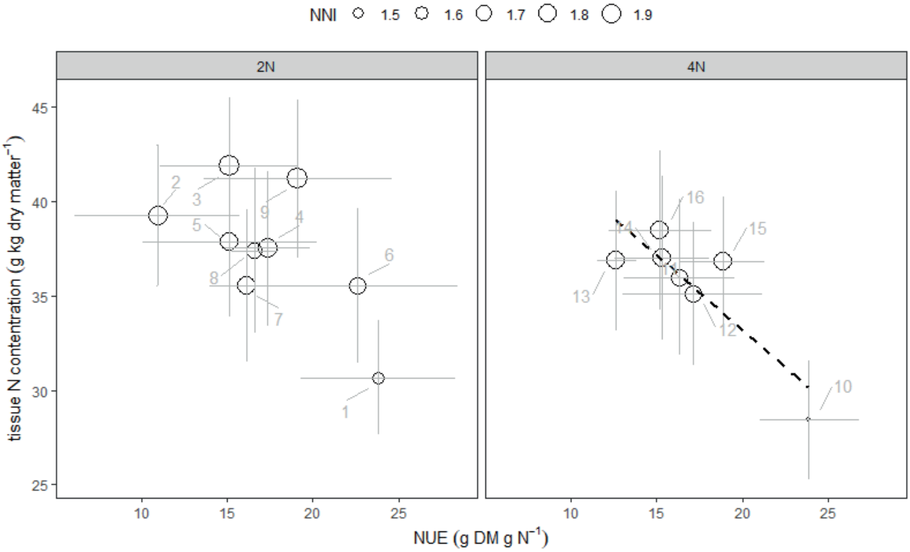

Figure 3.1. Pot experiment. Shoot tissue N concentration versus N use efficiency (NUE) of nine diploid (left; 2N) and seven tetraploid (right; 4N) perennial ryegrass populations. Symbol size reflects the N nutrition index (NNI). Horizontal and vertical bars represent standard errors of the mean. Numbers in the graphs indicate the perennial ryegrass populations. The dashed line represents the correlation between shoot tissue N concentration and NUE of the tetraploid populations (r = -0.85 and $p = 0.014$).

Within N fertilisation levels, shoot tissue N concentrations differed among the diploid populations at 12 g m^{-2} N fertilisation ($p = 0.005$), and DM yields did not differ among diploid and tetraploid populations (Table S3.2).

The mean SNS during the 20 day growing period was 2.7 ± 0.13 and 2.3 ± 0.11 g m^{-2} among diploid and tetraploid populations, respectively.

3.3.2 Field experiment. Population effects at different N-fertilisation levels

Grass shoot tissue N concentrations were affected by N fertilisation ($p < 0.001$) and blocks ($p < 0.001$), but did not differ among the populations (Table 3.7). Shoot tissue N concentration variance was largely explained by N fertilisation. The NUptE differed among populations ($p < 0.001$) and was affected by NNI ($p < 0.020$); the NUptE of population 4Nlow was lower than the control. The NUE was affected by NNI ($p = 0.001$), but did not differ among the populations. Dry matter yields of 2Nlow were higher compared to 4Nlow ($p = 0.005$), and were affected by N fertilisation ($p < 0.001$). At the first harvest of 2019, NDF differed among populations ($p = 0.038$) and was affected by N fertilisation ($p < 0.001$). Concentrations of WSC and ADF and the percentage of IVDOM did not differ among populations, at the first harvest of 2019 (Table S3.3).

Table 3.7. Field experiment. Mean, standard error of the mean (SEM) and explained variance of shoot tissue N concentration, N use efficiency (NUE), N uptake efficiency (NUptE), dry matter (DM) yield and neutral detergent fibre (NDF) of the control, 2Nlow and 4Nlow populations at all N fertilisation levels. Shoot tissue N concentration, NUE, NUptE and DM yield were determined across ten consecutive harvests, and NDF was determined at the first harvest of 2019 only.

Parameter	**shoot tissue N concentration**	**NUE**	**NUptE**	**DM yield**	**NDF**
Unit	**g kg DM^{-1}**	**g DM g N^{-1}**	**g N$_{upt}$ g N$_{supply}$$^{-1}$**	**g m^{-2}**	**g kg DM^{-1}**
population means					
control	30.7	22.7	0.41^{a}	235^{ab}	495^{ab}
2Nlow	30.3	23.1	0.33^{ab}	241^{a}	497^{a}
4Nlow	30.7	25.2	0.26^{b}	228^{b}	487^{b}
SEM	0.26	1.17	0.039	4.6	2.9
explained variance					
population	0%	3%ns	44%***	8%**	6%*
N fertilisation	90%***			74%***	64%***
N nutrition index		43%**	12%*		
block	6%***	5%ns	11%ns	1%	5%ns
residual variation	4%	49%	33%	17%	25%

^{abc} values with an unequal superscript differed significantly ($p < 0.05$)
*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant

3.3.3 Field experiment. Effects of the low and high-N populations at 25 g m^{-2} year^{-1} N fertilisation

Populations 2Nlow and 2Nhigh had a lower shoot tissue N concentration than the control ($p = 0.018$) (Table 3.8). Population 2Nlow had a higher DM yield than population 4Nhigh ($p = 0.032$). At the first harvest of 2019, population 4Nhigh had a lower NDF than the control, population 2Nlow and 4Nlow ($p = 0.015$). The WSC, ADF and percentage of IVDOM did not differ among the populations at the first harvest of 2019 (Table S3.4).

Table 3.8. Field experiment. Mean, standard error of the mean (SEM) and explained variance of shoot tissue N concentration, dry matter (DM) yield and neutral detergent fibre (NDF) of the control, 2Nlow, 2Nhigh, 4Nlow and 4Nhigh populations at a 25 g m^{-2} $year^{-1}$ N fertilisation level. Shoot tissue N concentration and DM yield were determined across ten consecutive harvests, and NDF was determined at the first harvest of 2019 only.

Parameter	**shoot tissue N concentration**	**DM yield**	**NDF**
Unit	**g kg DM^{-1}**	**g m^{-2}**	**g kg DM^{-1}**
population means			
Control	35.0^{a}	252^{ab}	511^{ab}
2Nlow	33.6^{b}	260^{a}	516^{a}
4Nlow	34.4^{ab}	243^{ab}	506^{ab}
2Nhigh	33.6^{b}	243^{ab}	494^{bc}
4Nhigh	34.7^{ab}	238^{b}	487^{c}
SEM	0.29	7.3	3.3
explained variance			
Population	36%*	56%*	56%*
Block	40%**	0%ns	4%ns
residual variation	24%	44%	41%

^{abc} values with an unequal superscript differed significantly ($p < 0.05$)
*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns = not significant

3.3.4 Field experiment. Effects within harvests

Mean shoot tissue N concentrations of the control, 2Nlow and 4Nlow populations at 0, 12.5 and 25 g m^{-2} $year^{-1}$ N fertilisation differed at the first harvests of 2017, 2018 and 2019, and at the second harvest of 2018 (Figures 3.2 and S3.1). Shoot tissue N concentrations of the control and 2Nlow were lower compared to 4Nlow at the first harvests of 2017 and 2019 ($p < 0.001$ and $p = 0.003$, respectively), and shoot tissue N concentrations of 2Nlow were lower compared to the control and 4Nlow at the first and second harvest of 2018 ($p < 0.001$ and $p = 0.015$, respectively).

Mean shoot tissue N concentrations of the control, 2Nlow, 4Nlow, 2Nhigh and 4Nhigh populations at 25 g m^{-2} $year^{-1}$ N fertilisation differed across all harvests, except at the fourth harvest in 2017, and at the third and fourth harvest in 2018 (Figures 3.2 and S3.2). Differences among population shoot tissue N concentrations were inconsistent among harvests, at 25 g m^{-2} $year^{-1}$ N fertilisation.

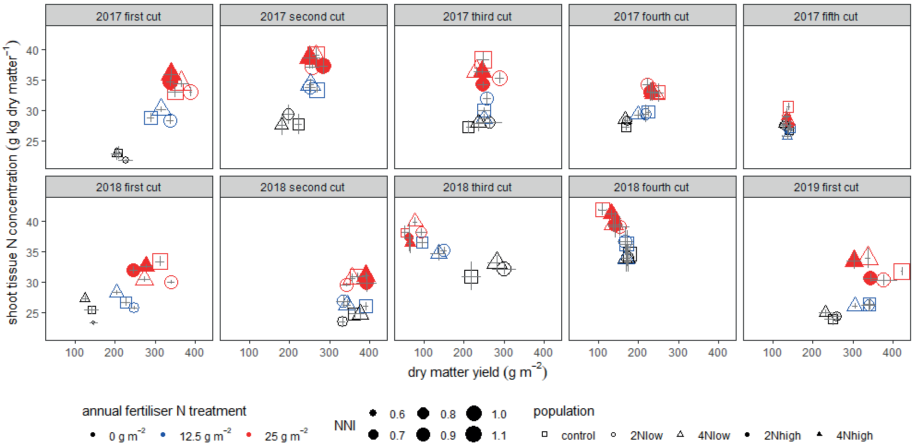

Figure 3.2. Field experiment. Relationship between shoot tissue N concentration and dry matter yield, per population, N fertilisation level and harvest cut. Symbol size reflects the N nutrition index (NNI). Horizontal and vertical bars represent standard errors of the mean.

Within harvests, NUE did not differ among the control, 2Nlow and 4Nlow populations, except at the second harvest in 2017 where 2Nlow and 4Nlow had a higher NUE than the control ($p = 0.031$) (Table S3.5). The NUptE differed among populations at the third harvest in 2017 ($p < 0.001$), at the first and second harvest in 2018 ($p = 0.003$ and $p = 0.001$, respectively) and at the first harvest in 2019 ($p = 0.001$). At these harvests, either 2Nlow, 4Nlow or both had a lower NUptE than the control. Lower shoot tissue N concentrations did not coincide with lower NUptEs at the first and second harvest in 2018 and at the first harvest in 2019.

At the second harvest in 2018, DM yields were similar among fertiliser N levels. At the third and fourth harvest in 2018, DM yields were higher in unfertilised than in fertilised conditions (Figure 3.2).

3.3.5 Field experiment. Soil nitrogen supply, estimated soil coverage and weather conditions

The mean annual SNS in 2017 and 2018 was 26.1 ± 1.2 g N m^{-2}, and the mean SNS until the first harvest of 2019 was 6.0 ± 0.7 g N m^{-2} (Table S3.6). Estimated shares of soil coverage by perennial ryegrass were 88.9 ± 3.6% and 86.8 ± 4.9% in May 2017 and 2018, respectively, and did not differ among the populations (Table S3.7). Growing season temperatures at the experimental site were higher than 20-year averages, and the 2018 growing season was relatively dry, having the fifth lowest recorded precipitation deficit since 1901 (Sluijter *et al.* 2018).

3.4 Discussion

The first hypothesis, that perennial ryegrass populations selected for different shoot tissue N concentrations on a sandy soil have a different shoot tissue N concentrations, NUE or NUptE when grown on a peat soil, was confirmed in the pot and field experiments, although results were not fully consistent between the experiments. The second hypothesis, that populations with a higher NUE will have lower shoot tissue N concentrations, was confirmed among the tetraploid populations but not among the diploid populations in the pot experiment, and not in the field experiment. The third hypothesis, that populations with a lower NUptE have lower shoot tissue N concentrations, was not confirmed in the pot nor in the field experiment.

3.4.1 Population differences in shoot tissue N concentration

Mean shoot tissue N concentrations were higher in the pot experiment (36.6 g kg^{-1} DM) and similar in the field experiment (31.3 g kg^{-1} DM), compared to mean values of sixty years of Dutch grassland experiments on peat soil (about 29 – 30 g kg^{-1} DM) (Vellinga & Andre 1999). Mean SNS, expressed on a daily basis, was 0.12 g m^{-2} in the pot experiment, 0.11 g m^{-2} in the field experiment at the growing seasons of 2017 and 2018, and 0.09 g m^{-2} until the first harvest of 2019 (29 April), respectively, and was within the range of previously observed mean estimations of SNS for dairy grasslands in the western peat area of the Netherlands (0.11 – 0.13 g m^{-2} day^{-1} at an average growing season and 0.08 – 0.12 g m^{-2} day^{-1} on average until 29April, at soil organic matter contents of 35 – 55 g 100 g^{-1} dry soil, respectively) (Vellinga & Andre 1999; Pijlman *et al.* 2020b).

Differences in shoot tissue N concentration among populations were small compared to the effect of N fertilisation in both experiments, as expected (Wilkins & Lovatt 1989; Malmberg *et al.* 2023). In the pot experiment, only the tissue N concentration of population 10 was lower compared to the other tetraploid populations, while in the field experiment the shoot tissue N concentration of population 10 (renamed to 4Nlow) was not different from tetraploid population 4Nhigh. In the pot experiment, population 10 also had a high NUE and low NNI (1.41 versus 1.79, respectively) compared to the other tetraploid populations. The low NNI indicated that population 10 had a lower luxury N consumption compared to the other populations (Lemaire *et al.* 2008). The NNI of population 10 (4Nlow) was also low compared to population 4Nhigh in the field experiment (0.82 and 0.93, respectively), in line with results of the pot experiment.

3.4.2 Population differences in N use efficiency and N uptake efficiency

The nutritional N status explained a larger part of the NUE and NUptE variance than the populations, in both experiments. This is in line with other studies, in which N fertilisation – which greatly affects the nutritional N status of plants (Lemaire *et al.* 2008) – had a greater effect than the genetic component on shoot tissue N concentration (Wilkins & Lovatt 1989; Radojevic *et al.* 1994; Sampoux *et al.* 2011; Robins & Lovatt 2016). However, these studies were carried out on mineral soils, and not all of these studies were specifically carried out to assess effects of genetic variation on shoot tissue N concentration at different N fertilisation levels.

Differences in NUptE were relatively greater than differences in shoot tissue N concentration among the diploid populations in the pot experiment and in the field experiment, similar to results of Wilkins & Lovatt (1989), who tested four perennial ryegrass varieties at three different N fertilisation levels in a field experiment on mineral soil. They reported 18% and 6% variation among varieties in N recovered to N applied and shoot tissue N concentration, respectively.

3.4.3 Relationship between shoot tissue N concentration, N use efficiency and N uptake efficiency

The negative correlation between NUE and shoot tissue N concentrations among the tetraploid populations in the pot experiment, is in line with results of earlier studies (Wilkins *et al.* 2000; Sandaña *et al.* 2021). However, the effect of NUE on shoot tissue N concentration was largely due to population 10, which had a lower tissue N concentration and a higher NUE compared to the other tetraploid populations (with exception of population 15 of which the NUE did not differ from population 10). Since NUE and shoot tissue N concentrations differed among the tetraploid populations in the pot experiment, it is likely that the selection of perennial ryegrass with a higher NUE will result in genotypes with a lower shoot tissue N concentration per unit of dry matter gain. In different studies, it was hypothesised that the negative relationship between NUE and shoot tissue N concentrations could be the result of a DM ‘dilution’ effect, caused by genetic differences in efficiency of protein synthesis and turnover (Wilkins *et al.* 2000; Baert *et al.* 2007). According to this hypothesis, reducing the shoot tissue N concentration via breeding can lead to increased DM yields at a similar shoot tissue N uptake (Wilkins & Lovatt 1989; Baert *et al.* 2007). Our results suggest that there was no correlation between NUE and DM yield among the tested populations. In the pot experiment, DM yields did not differ among populations, while there were differences in shoot tissue N concentration and NUE among the tetraploid populations. In the field experiment, across the ten harvests, DM yields differed among populations, but no differences in NUE among populations was found. Moreover, in the field experiment, at an N fertilisation rate of 25 g m^{-2} $year^{-1}$ and across the ten harvests, shoot tissue N concentrations and DM yields differed among populations, but there was no correlation between shoot tissue N concentrations and DM yields.

Our results suggest that there was no relationship between NUptE and shoot tissue N concentration in the pot and the field experiment. In the pot experiment, among the diploid populations, NUptE differed but shoot tissue N concentrations did not, suggesting that NUptE did not affect shoot tissue N concentration. In the field experiment, NUptE and shoot tissue N concentrations differed among populations at the first and second harvest of 2018 and at the first harvest of 2019, but lower shoot tissue N concentrations did not coincide with lower NUptEs at these harvests. However, we cannot exclude that genetic variation of shoot tissue N concentration and NUptE could have been masked by genotype-environmental interactions. For example, the ranking of shoot tissue N concentrations among the populations was not fully consistent between the experiments, and with concentrations recorded on the sandy soil. Genotype by environment interactions have been observed in several studies. Robins & Lovatt (2016) showed that genotype by environment interactions can affect the feeding value of perennial ryegrass, of which shoot tissue N concentration is a component, at three sites on mineral soils differing in water availability, climate and altitude. Parsons *et al.* (2011) pointed out that genotype by environment interactions affect trait expressions, and that the 'success' of traits such as high WSC or 'low respiration' largely depends on the N fertilisation rate. This means that further research is needed to confirm that NUptE is not correlated to the shoot tissue N concentration of perennial ryegrass, grown at dairy grasslands on peat soil.

3.4.4 Within harvest effects in the field experiment

Differences in shoot tissue N concentration among populations were inconsistent within harvests and years, in the field experiment. Inconsistency of shoot tissue N concentrations among populations was possibly a result of varying environmental conditions. In the field experiment, the greatest differences in shoot tissue N concentration were observed in the early harvest cuts within a season, and differences in shoot tissue N concentration among fertilisation levels were greater at the first cuts of the growing season, compared to the last cuts. This may have been the result of a combination of a higher growth rate of grass in spring compared to later in the growing season – which results in a lower soil N availability per unit of DM growth (Davies 1971; Harris *et al.* 1996; Burns *et al.* 2012) – and of a lower SNS in spring compared to summer and autumn (Vellinga & André 1999; Pijlman *et al.* 2020b), although N fertilisation rates decreased with every cut.

At the second harvest in 2018 in the field experiment, DM yields were similar among fertiliser N levels, and from the third harvest onwards, DM yields were lower in fertilised compared to unfertilised treatments. The year 2018 had a relative dry growing season. After the first harvest of 2018, soil moisture likely had a greater influence on grass regrowth and N uptake than soil mineral N availability, specifically at the N fertilised treatments which produced relatively high DM yields before dry conditions arrived (Gonzalez-Dugo *et al.* 2010). The significant effect of blocks on shoot tissue N concentration was possibly also related to the weather conditions, which may have increased the spatial and spatiotemporal variation in soil moisture and SNS (Wang *et al.* 2021). At the second harvest in 2017 and the fourth harvest in 2018, the largest differences in shoot tissue N concentration were observed among blocks (data not shown), and both of these harvests were carried out during dry periods. Variation in weather conditions and soil moisture can directly or indirectly affect grass N uptake by influencing soil moisture or SNS (Vellinga & André 1999; Gonzalez-Dugo *et al.* 2010; Sampoux *et al.* 2011; Pijlman *et al.* 2020b). The dry growing season of 2018 may also have reduced differences among populations regarding N uptake and use, because N-rich plant organs are more sensitive to water deficit than N-poor organs (Gonzalez-Dugo *et al.* 2010).

The lower NDF of population 4Nlow compared to 2Nlow across all N fertilisation levels, was likely an effect of ploidy. Tetraploids are generally leafier and have larger cells and a better digestibility than diploids, resulting in a lower NDF (Burns et al. 2012; Griffiths et al. 2017). This difference in shoot tissue NDF concentration between populations indicated that, after three years including the very dry growing season of 2018, the sown populations were still dominant in the experimental plots.

3.4.5 Limitations to the current study

There were a few limitations to the current study. By using the diploid and tetraploid populations in the field experiment, which had either the lowest or highest recorded shoot tissue N concentration in the pot experiment, it was assumed that the population variation on shoot tissue N concentration was similar in the pot and field experiment, despite differences in environment and moisture availability which could have affected genotype expressions (Robins & Lovatt 2016). Shoot tissue N uptakes were indeed higher in the pot compared to the field experiment, in line with Poorter *et al.* (2016), who concluded that inside grown plants grow faster, take up more N and may differ in morphology, compared to plants grown in field conditions.

Furthermore, there were differences between the peat-based substrate used in the pot experiment, and the peat soil at which the field experiment was carried out, although in both experiments P, K and S were considered not to be limiting grass growth (CBGV 2022). The peat-based substrate mix had a higher organic matter content and pH, compared to the soil of the field experiment, which may have resulted in a higher N availability in the pot experiment (Egan *et al.* 2019). It is also likely that the substrate and field soil differed in physical properties, due to differences in origin (Loisel *et al.* 2014) and history of use of the peat (Kechavarzi *et al.* 2010). These differences between the pot and field experiment may have influenced the expression of the genetic variation in N uptake, NUE and NUptE of the populations (Parsons *et al.* 2011; Robins & Lovatt 2016), which means that results from the pot experiment may not be completely transferable to field conditions.

The selection of populations grown on a sandy soil was only based on shoot tissue N concentration, without taking into account NNI, NUE, NUptE or morphological traits such as heading date or maturity, which may have affected the population selection for the experiments (Gastal *et al.* 2015; Sandaña *et al.* 2021). Furthermore, selection of perennial ryegrass populations for the pot experiment was based on data of first three harvests, and selection of populations for the field experiment was based on a single May harvest 62 days after sowing. This could have favoured populations differing in shoot tissue N concentration mostly at the beginning of the growing season. Possibly, selection of populations with increased summer and/or autumn growth may result in N concentrations better matching the annual SNS pattern. However, to our knowledge, there is only limited evidence of a relationship between maturity or heading date, and shoot tissue N concentration of perennial ryegrass (Wilkins & Lovatt 2011; Sampoux *et al.* 2011; Burns *et al.* 2012). Only Burns *et al.* (2012) found that later maturity was associated with higher annual herbage yields and shoot tissue N concentrations, on a clay loam soil, but this effect was not expressed in each seasonal period. Future selection experiments should therefore cover full season research across a range of weather conditions, to select for populations with consistently lower mean annual shoot tissue N concentrations.

3.5 Conclusions

For perennial ryegrass populations selected for different shoot tissue N concentrations using a sandy soil, the pot experiment using a peat substrate showed that, among tetraploid populations, lower shoottissue N concentrations coincided with a higher NUE, and that among diploid populations, NUptE but not shoot tissue N concentrations differed. The field experiment on peat soil showed that populations could explain 44% of the variation in NUptE, but no relation between NUE or NUptE and shoot tissue N concentration was found. Across ten harvests at a N fertilisation level close to local practice, selected populations had a 1.4 g kg DM^{-1} lower shoot tissue N concentrations, but not a different DM yield, compared to a commercial control. The results show potential for the selection of perennial ryegrass populations with low shoot tissue N concentration for dairy grassland on peat soil, by selecting for shoot tissue N concentration and NUE, and show potential for the selection of populations for NUptE.

Supplemental Materials

This manuscript includes seven supplementary tables and two supplementary figures; Table S3.1 on the air temperatures and precipitation near the commercial breeding site on a sandy soil; Table S3.2 on shoot tissue N concentration and DM yields within N fertilisation levels in the pot experiment; Tables S3.3 and S3.4 on the shoot tissue WSC and ADF concentrations, and percentage of *in vitro* digestible organic matter of the populations in the field experiment; Figure S3.1 on mean shoot tissue N concentrations of the control, 2Nlow and 4Nlow populations per harvest at all N fertilisation levels in the field experiment; Figure S3.2 on mean shoot tissue N concentrations of all tested populations per harvest at the 25 g N m^{-2} year^{-1} fertilisation level in the field experiment; Table S3.5 on NUE and NUptE per population and harvest in the field experiment; Table S3.6 on N uptakes per population and N fertilisation level in the field experiment, and Table S3.7 on the visually estimated soil coverage by perennial ryegrass in the field experiment.

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Author Contributions

NH, JD and NvE conceived and designed the study. JP, NH and JD conducted data gathering. JP, NH and JD performed statistical analyses. JP, NH, JD, JWE and NvE wrote the article.

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Supplementary materials

Table S3.1. Air temperatures and precipitation near the commercial breeding site on a sandy soil. SD = standard deviation. Average daily temperatures were collected from weather station Woensdrecht (Royal Netherlands Meteorological Institute, 51°27'N, 4°20'E) and precipitation data were collected from weather station Steenbergen (Royal Netherlands Meteorological Institute, 52°32'N, 3°53'E). These stations were located 10.2 and 4.6 km from the site, respectively.

Period	**temperature (°C)**		**precipitation (mm)**	
	mean	**SD**	**sum**	**SD**
Mar.–Aug. 2012	13.5	5.0	568	7.3
Mar.– Aug. 2013	12.4	6.7	295	4.0
Mar.– Aug. 1999–2019	13.8	5.3	424	5.1

Table S3.2. Pot experiment. Shoot tissue N concentration and dry matter yield means and standard errors of the mean (SEM) of diploid and tetraploid populations within N fertilisation levels. *P*-values are given for the effect of populations.

		tissue N concentration (g g DM^{-1})			**dry matter yield (g m^{-2})**		
population ploidy	**N fertilisation level (g m^{-2})**	**mean**	**SEM**	***p*-value**	**mean**	**SEM**	***p*-value**
diploid	0	26.5	2.25	0.647	94	10.9	0.700
	6	38.5	3.24	0.295	146	10.1	0.151
	12	47.5	2.00	0.408	166	14.2	0.516
tetraploid	0	24.1	1.69	0.063	93	8.7	0.942
	6	34.9	2.55	0.529	151	9.2	0.673
	12	48.1	1.96	0.005	174	9.4	0.265

Table S3.3. Field experiment. Mean shoot tissue water soluble carbohydrate (WSC) and acid detergent fibre (ADF) concentrations, and percentage of *in vitro* digestible organic matter of the control, 2Nlow and 4Nlow populations at all N fertilisation levels, at the first harvest of 2019. SEM = standard error of the mean.

parameter	**control**	**2Nlow**	**4Nlow**	***p*-value population effect**	**SEM**
WSC, g kg DM^{-1}	146	155	150	0.322	5.0
ADF, g kg DM^{-1}	255	255	250	0.195	2.7
IVDOM, %	81.5	81.3	82.0	0.125	0.17

Table S3.4. Field experiment. Mean shoot tissue water soluble carbohydrate (WSC) and acid detergent fibre (ADF) concentrations, and percentage of *in vitro* digestible organic matter of the control, 2Nlow, 2Nhigh, 4Nlow and 4Nhigh populations at a 25 g m^{-2} year^{-1} N fertilisation level, at the first harvest of 2019. SEM = standard error of the mean.

parameter	control	2Nlow	4Nlow	2Nhigh	4Nhigh	*p*-value population effect	SEM
WSC, g kg DM^{-1}	105	119	124	126	126	0.244	3.3
ADF, g kg DM^{-1}	274	271	266	259	255	0.084	2.4
IVDOM, %	81.4	82.2	82.5	82.7	83.3	0.249	0.25

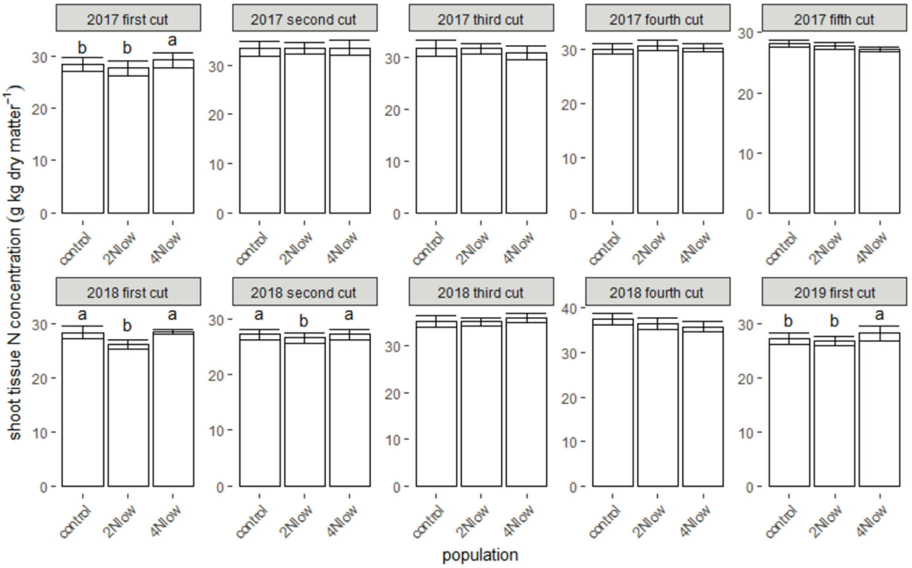

Figure S3.1. Field experiment. Mean tissue N concentration per population at ten consecutive harvests. Concentrations with an unequal superscript differed significantly ($p < 0.05$). Mean tissue N concentrations per population and N fertilisation level are presented in Figure 3.2.

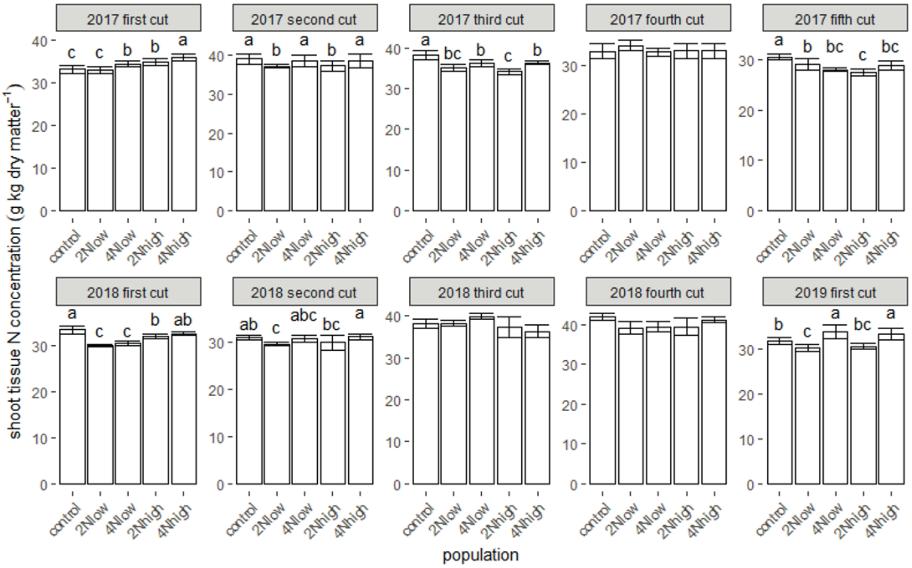

Figure S3.2. Field experiment. Mean tissue N concentration per population at ten consecutive harvests. Concentrations with an unequal superscript differed significantly ($p < 0.05$). Mean tissue N concentrations per population and N fertilisation level are presented in Figure 3.2.

Table S3.5. Field experiment. N use efficiency (NUE) and N uptake efficiency (NUptE) of ryegrass populations at ten consecutive harvests.

Year	cut	population	NUE (g DM g N^{-1})	NUptE (g N$_{upt}$ g N$_{supply}^{-1}$)
2017	1	control	21.8	0.69
		2Nlow	22.4	0.86
		4Nlow	21.5	0.88
		p-value	0.605	0.076
	2	control	13.5^{b}	0.69
		2Nlow	17.3^{a}	0.64
		4Nlow	18.1^{a}	0.89
		p-value	0.031	0.065
	3	control	14.4	0.74^{a}
		2Nlow	-2.1	0.43^{b}
		4Nlow	22.2	0.29^{b}
		p-value	0.105	<0.001
	4	control	25.3	1.29
		2Nlow	28.8	0.98
		4Nlow	27.7	0.89
		p-value	0.715	0.067
2018	1	control	30.5	0.58^{a}
		2Nlow	31.0	0.64^{b}
		4Nlow	31.9	0.48^{a}
		p-value	0.453	0.003
	2	control	11.1	0.34^{a}
		2Nlow	29.1	0.32^{a}
		4Nlow	104.4	0.06^{b}
		p-value	0.265	0.001
	3	control	39.1	-1.08
		2Nlow	34.7	-1.48
		4Nlow	32.6	-1.54
		p-value	0.505	0.085
	4	control	25.9	-0.28
		2Nlow	20.3	0.06
		4Nlow	12.0	-0.12
		p-value	0.896	0.217
2019	1	control	27.0	0.68^{a}
		2Nlow	27.1	0.51^{b}
		4Nlow	26.4	0.50^{b}
		p-value	0.965	0.001

^{ab}values with an unequal superscript differed significantly ($p < 0.05$)

Table S3.6. Field experiment. Mean shoot tissue N uptake per population and N fertilisation level. Data of 2017 and 2018 are annual figures, and data of 2019 are of the first cut. SE = standard error.

Year	N fertilisation, g m^{-2}	population	shoot tissue N uptake, g m^{-2}	
			mean	SE
2017	0	control	25.2	1.1
		2Nlow	26.6	2.0
		4Nlow	24.7	1.9
	12.5	control	35.5	1.1
		2Nlow	36.3	1.4
		4Nlow	34.7	1.7
	25	control	43.9	1.4
		2Nlow	44.2	0.2
		2Nhigh	42.3	1.2
		4Nlow	43.1	0.9
		4Nhigh	42.5	2.1
2018	0	control	25.4	0.7
		2Nlow	26.7	1.0
		4Nlow	27.9	0.5
	12.5	control	25.8	0.6
		2Nlow	26.7	0.9
		4Nlow	25.2	0.6
	25	control	28.5	0.8
		2Nhigh	27.5	0.6
		2Nlow	29.7	1.8
		4Nhigh	29.0	0.3
		4Nlow	27.7	0.2
2019	0	control	5.9	0.3
		2Nlow	6.3	0.4
		4Nlow	5.8	0.3
	5	control	9.0	0.4
		2Nlow	8.9	0.5
		4Nlow	8.0	0.2
	10	control	13.4	0.2
		2Nlow	11.4	1.1
		2Nhigh	10.5	0.7
		4Nlow	11.5	0.8
		4Nhigh	10.2	0.9

Table S3.7. Field experiment. Visually estimated soil coverage by perennial ryegrass as a percentage of the total soil surface, per population and N fertilisation level. SEM = standard error of mean.

Year	**2018**			**2019**		
N fertilisation, g m^{-2} year^{-1}	0	12.5	25	0	12.5	25
population						
Control	85.0	89.9	90.9	80.8	86.6	91.3
2Nlow	86.9	90.2	89.9	80.9	87.3	90.6
4Nlow	84.3	90.4	89.9	77.5	87.3	90.1
2Nhigh			90.9			90.9
4Nhigh			89.4			91.1
p-value	0.609	0.974	0.889	0.051	0.667	0.873
SEM	1.12	0.78	0.53	0.68	0.38	0.34


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Chapter 4

Can the presence of plantain *(Plantago lanceolata L.)* improve nitrogen cycling of dairy grassland systems on peat soils?

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Abstract

Reactive nitrogen (N) losses, and in particular nitrous oxide losses, from dairy grasslands on peat soils are generally high as a result of relative high soil organic matter contents, potential N mineralization rates and shallow groundwater levels. Effects of the inclusion of the temperate forage species plantain (*Plantago lanceolata* L.) (PL), which produces secondary compounds with biological nitrification inhibition capacity, on the fate of soil mineral N were studied in a combined mesocosm and field experiment. The experiments comprised four treatments differing in intentional herbage share of plantain versus perennial ryegrass (*Lolium perenne* L.) (100%PL, 66%PL, 33%PL and 0%PL). Potential nitrification in the mesocosm experiment was significantly lower at 100%PL versus 0%PL ($p = 0.018$), but soil nitrate concentrations were not. Nitrous oxide fluxes reduced by 39% ($p = 0.021$) in the presence of plantain in the field experiment, without an obvious link to the quantity of plantain. N use efficiency of plantain tended to increase with the quantity of plantain in the sward in the mesocosm experiment ($p = 0.098$), but not in the field experiment. Our results suggest that the presence of plantain can affect the fate of soil mineral N of dairy grasslands on peat soils.

Keywords: plantain; nitrogen; peat; grassland; nitrification; nitrous oxide

4.1 Introduction

Agriculture is the largest contributor to global reactive nitrogen (N) production (Galloway *et al.* 2008). This causes environmental pollution, disrupts N cycles of natural ecosystems and results in a significant biodiversity loss (Galloway *et al.* 2008; Erisman *et al.* 2013). It is clear that reactive N exceeds limits of interference with the global N cycle (Rockström *et al.* 2009). In commercial dairy farms N losses during conversion of N into milk and meat are often high, and include gaseous forms (N_2, N_2O, NOx, NH_3) as well as losses to groundwater through erosion, runoff or leaching of nitrate (NO_3^-) (Jarvis 1993; Kohn *et al.* 1997; Nevens *et al.* 2006; Powell *et al.* 2010). Dairy grassland systems on peat soils are particularly prone to high N_2O losses as a result of relative high soil organic matter contents, high potential N mineralization rates and shallow groundwater levels (Koops *et al.* 1996; Van den Pol-van Dasselaar *et al.* 1998).

Due to the increase of mineral N use in agriculture since the introduction of mineral fertilizers, NO_3^- nowadays accounts for > 95% of N plant uptake in modern agriculture (Subbarao *et al.* 2015), despite the fact that NO_3^- is more easily lost from the soil through leaching or denitrification than NH_4^+. Inhibition of soil nitrification has therefore been identified as a potential measure to increase herbage mineral N uptake efficiency and to reduce NO_3^- leaching and gaseous N emissions (mainly N_2O and N_2) (Di *et al.* 2009; Butterbach-Bahl *et al.* 2013; Ruser & Schulz 2015; Subbarao *et al.* 2015; Bowatte *et al.* 2016).

Synthetic nitrification inhibitors such as dicyandiamide (DCD) and 3,4-dimethypyrazole phosphate (DMPP) have shown to reduce the release of compounds such as N_2O from soil to the environment under different conditions (Ruser & Schulz 2015). But, further study on their efficacy under different conditions and long term applications is still needed, as well as on the impact of their toxicity on plant growth and human health (Ruser & Schulz 2015; Yang *et al.* 2016). Biological nitrification inhibition (BNI) might be an interesting alternative to reduce N losses from agriculture to the environment (Subbarao *et al.* 2015). BNI is defined as the release of compounds from plant roots that directly influence nitrifying organisms, i.e., via the reduction of ammonia monooxygenase (AMO) enzymatic activity by inhibiting or inactivating AMO enzymatic pathways (Arp & Stein 2003; Subbarao *et al.* 2015). For example, the tropical pasture species *Brachiaria* spp., which is adapted to low-N environments, can release significant amounts of compounds with BNI capacity depending on the presence of NH_4^+ in the soil (Subbarao *et al.* 2007). However, more recently the presence of compounds with BNI capacity has been identified in plantain (*Plantago lanceaolata* L.) (Dietz *et al.* 2013). Plantain is a pasture species which is known to grow on a wide variety of soils in temperate climates, is highly responsive to soil N, has good forage quality, has been cultivated to produce large amounts of forage (Stewart 1996; Lee *et al.* 2015) and could therefore be included in grassland mixtures in the search for improving farming system N use efficiency. The major secondary metabolites of plantain that have been identified as potentially causing BNI due to their chemical structure, are aucubin, catalpol, verbascoside and their aglyca (Dietz *et al.* 2013).

Several studies performed on sand and loamy sand soils have reported effects of plantain on soil nitrifying activity and the fate of soil mineral N. Verhagen et al. (1995) observed more than 200-fold lower numbers of nitrifying bacteria as well as lowered potential NH_4^+-oxidizing activities in the presence of plantain, after application of NH_4^+ fertilizer. Dietz et al. (2013) found that aucubin as well as soil incubated with plantain leaf material negatively affected soil mineralization and nitrification. Carlton et al. (2019) observed a lower abundance of NH_4^+-oxidizing bacteria and a significant decrease of NO_3^- eutrophication if plantain was present in the forage in a ryegrass-clover sward on which urine-N was applied in patches at a rate of 7.0 g m^{-2}. Luo et al. (2018) and Simon et al. (2019) observed lower N_2O emissions in the presence of plantain, after cattle urine applications on silt loam soils.

There are no known reports on the effects of plantain or other plants secreting compounds with potential BNI capacity on N cycling under nutrient rich conditions such as dairy grasslands on peat soils. Therefore, we studied the effects of the presence of plantain in a mixture with perennial ryegrass (*Lolium perenne* L.) on the fate of soil mineral N in a combined mesocosm experiment with peat soil and in a field experiment on a dairy grassland on peat soil. We hypothesized that the presence of plantain in a mixture with perennial ryegrass, compared to a monoculture of perennial ryegrass, (1) reduces the process of soil nitrification, (2) lowers soil NO_3^- concentrations, (3) reduces soil N_2O emissions and (4) leads to an increased efficiency of herbage N uptake.

4.2 Material and methods

4.2.1 Mesocosm experiment

The mesocosm experiment comprised four treatments differing in proportions of plantain (PL) and perennial ryegrass (RG) (100%PL, 66%PL, 33%PL and 0%PL). Thirty-two mesocosms were allocated to the treatments according to a completely randomized design, resulting in eight replicates per treatment. Mesocosms were made up of glass containers (25 cm x 25 cm x 30 cm height) which were filled with 5 cm of gravel, which in turn was topped with root fabric and 25 cm of soil. Within the gravel, a porous cup (Rhizon SMS, Eijkelkamp, Giesbeek, the Netherlands) was placed in order to collect water leaching from the system. Peat soil (terric histosol, IUSS Working Group WRB, 2015) from a depth of ± 10 - 20 cm below surface was obtained from a perennial dairy grassland with a mean groundwater table of 60 cm below surface (Knowledge Transfer Centre (KTC), Zegveld, the Netherlands, 52°08'25.7" N, 4°50'22.0" E) (see Table 4.1 for soil parameters). Before filling, the soil was thoroughly mixed by hand. Mesocosms were placed in a temperature-regulated water bath and maintained at ± 12°C soil temperature using a cryostat (ThermoFlex 1400, ThermoFisher Scientific, Waltham, MA, USA). The experiment was performed in the greenhouse facilities of Radboud University (Nijmegen, the Netherlands), and ran from 28 July till 23 December 2017 (148 days). Within the facilities, temperature was maintained between 16.6 and 17.5 °C, relative humidity at 50% and irradiance at 111 W m^{-2} or higher for 16 h per day, using grow lights if sunlight was not sufficient. Mesocosms received on average twice a week equal amounts of demineralized water.

Table 4.1. Properties of soil used (n=2 per experiment) for the mesocosm and field experiment and monthly mean air temperatures and precipitation sums for field experiment.

			Mesocosm experiment		Field experiment		
			Mean	Range	Mean	Range	SD
Soil	Organic matter	%	47.8	1.0	44.2	3.7	
	Lutum	%	36	1	27	2	
	Total C	$g\,100\,g^{-1}$	26.2	1.2	20.3	4.7	
	Total N	$mg\,100\,g^{-1}$	2244	89	2028	64	
	Total P	$mg\,100\,g^{-1}$	187	22	195	-	
	P_{AL}	$mg\,100\,g^{-1}$	7.2	0.4	12.2	3.5	
	Total K	$mg\,100\,g^{-1}$	14	-	36	1	
	pH-KCl	-	4.6	0.0	4.9	0.2	
Temperature	Mar. – Oct. 2017	°C			14.0		3.8
	Mar. – Oct. 2018	°C			14.6		5.0
	Mar. – Oct. 1999-2018	°C			13.6		4.1
Precipitation	Mar. – Oct. 2017	$mm\ month^{-1}$			72		38
	Mar. – Oct. 2018	$mm\ month^{-1}$			43		26
	Mar. – Oct. 1999-2018	$mm\ month^{-1}$			69		17

Note: SD = standard deviation.

After an acclimatization period of 17 days, plantain (*Plantoga lanceolata* L. var. "Hercules", Pelgrum Vink Materials, Lobith, the Netherlands) was planted at rates of 24, 16, 8 or 0 seedlings per mesocosm and ryegrass (*Lolium perenne* L.var. "Barimero" (50%) and var. "Toronto" (50%),BG3 Superplus, Barenbrug Holland, Nijmegen the Netherlands) was sown at rates of 0.0, 1.5, 3.0 and 4.5 g m^{-2} in the treatments 100%PL, 66%PL, 33%PL and 0%PL, respectively. The day of seeding and planting was considered as day 0 of the experiment. Plantain seeds were placed on a humid paper cloth at room temperature and seedlings were planted three days after germination.

Above ground herbage was harvested at ± 5 cm stubble height on days 41, 61, 95 and 116. At each harvest, the total herbage of each mesocosm was manually separated into plantain and ryegrass, and the dry matter (DM) content of both species was determined by oven drying at 70°C for 48 h. Ryegrass and plantain harvested at day 116 was also analysed for total N content using an elemental analyser (EA NA1500 Carlo Erba, ThermoFisher Scientific, Waltham, MA, USA), after thoroughly grinding the dried plant material using a mixer mill (MM400, Retsch, Haan, Germany).

On days 5, 74, 95 and 116 soil cores and soil leachate samples were collected. Leachate was obtained via the porous cups by connecting a vacuumed syringe to the cups until 20 mL fluid was collected. Soil cores (0-20 cm depth) were obtained by using a 17 mm diameter gouge, and the resulting hole was filled with a 20 mm diameter PVC pipe which was closed off on top to prevent interactions other than soil surface interactions with the atmosphere. Soil samples were weighed and split into two equal parts. Samples were incubated in 100 mL HDPE-bottles either with 50 mL type I purified water in order to remove water soluble ions or with 50 mL of 0.2M NaCl solution in order to remove cations. Incubated samples were shaken at 105 rpm for 2 h at room temperature and a porous cup was placed in each HDPE-bottle connected to a 100 mL vacuumed bottle to obtain fluid samples. NO_3^- and NH_4^+ concentrations in the soil fluid and leachate samples were measured colorimetrically with two continuous flow AutoAnalyzer III systems (Bran + Luebbe, Norderstedt, Germany), using hydrazine sulphate and salicylate, respectively (for details see Geurts et al. 2008). Soil core sample DM contents were determined by oven drying at 70°C for 48 h in order to express results per kg of soil DM.

At day 148, 0-10 cm depth soil cores of treatment 100%PL and 0%PL were taken for analyses of DM content and NO_3^- and NH_4^+ concentrations as described above, and for analyses of potential nitrification (ISO 15685:2012). For potential nitrification, soil was sieved over a 5 mm screen in order to remove roots. Autotrophic NH_4^+ oxidizing bacteria were exposed to ammonium sulphate in soil slurry buffered at pH 7.2. Oxidation of the nitrite, performed by nitrite-oxidizing bacteria in the slurry, was inhibited by the addition of sodium chlorate. The subsequent accumulation of nitrite was measured over a 6 h incubation period, and taken as an estimate of the potential activity of NH_4^+ oxidizing bacteria at the time of sampling. For details see Belser and Mays (1980).

4.2.2 Field experiment

The field experiment was established by sowing plantain and ryegrass at 18 May 2017 on a dairy grassland on peat with a mean groundwater table of 60 cm below surface (KTC Zegveld, 52°08'23.3"N, 4°50'12.9"E). Ten days before sowing, the field was treated with 2.5 kg ha^{-1} glyphosate (Roundup®+, Monsanto, Saint Louis, MO, USA) and at the day before sowing the present grass sod was rotavated at a depth of ± 10 cm. The treatments 100%PL, 66%PL, 33%PL and 0%PL were allocated to twenty-four plots of 2.5 x 10.0 m according to a complete randomised block design, resulting in six replicates per treatment. Plantain seed was sown at 1.00, 0.66, 0.33 and 0.00 g m^{-2} for the treatments 100%PL, 66%PL, 33%PL and 0%PL, respectively, and ryegrass seed was sown at 3 g m^{-2} in all treatments (Remmelink *et al.* 2018), following advised sowing rates for monocultures and mixtures and reported sowing rates for plantain monocultures by Minneé et al. (2013) and Lee et al. (2015). The same varieties and seedlines were used in the mesocosm and field experiment.

In June 2017, above ground herbage was mulched and left in place, and in September 2017 herbage was harvested and removed from the fields (mean stubble height ± 5 cm). In March 2018, all plots were fertilized with 5.0 g N m^{-2}, 8.3 g K m^{-2} and 1.8 g P m^{-2} and in May 2018 with 6.6 g K m^{-2}. N, K and P were appliedin the form of calcium ammonium nitrate (CAN), potassium chloride and diphosphorus pentoxide, respectively. On 12 September 2018 all fields were again fertilized with 5.0 g CAN-N m^{-2} prior to the measurements of soil N_2O fluxes.

Soil samples (0-10 cm depth) were taken before the start of the experiment and in November 2018 and were pooled per sampling moment for further analyses (Table 4.1). In the year of establishment (2017) and measurements (2018), average temperatures during the growing season (Mar. – Oct.) were 0.4 °C and 1.0 °C higher, respectively, compared to 20 year averages (KNMI, de Bilt, the Netherlands, Table 4.1). Monthly precipitations were on average 3 mm higher (2017) and 26 mm lower (2018) compared to 20 year averages. The growing season of 2018 had the 5th highest mean potential precipitation deficit since 1901 (Sluijter *et al.* 2018). Between 1 May and 31 July 2018, cumulative precipitation was 55 mm while 20 year averages for that period were 216 mm.

Herbage yields were determined at a stubble height of ± 5 cm on 16 May, 13 June, 24 July, 4 September and 29 October 2018 using a 'Haldrup' small plot harvester (J. Haldrup, Løgstør, Denmark). Two subsamples were taken per harvest date and plot. The first was used for analyses of total herbage DM content (oven drying at 70 °C for 48 h). The second subsample of at least 0.2 kg was manually separated into plantain, grasses and other herbs and forbs to obtain the sward species composition, as described in Hoekstra et al. (2018). The separated plantain, total grasses and other herbs and forbs were analysed for DM and plantain and total grasses were analysed for total N contents (NEN-ISO 5983-2, Kjeldahl method, Eurofins Agro, Wageningen, the Netherlands).

Soil N_2O fluxes were measured five times with six to eight day intervals from 20 September 2018 onwards. Within each of the 24 plots three fixed locations were randomly chosen, and each measurement day sealed off airtight for at least 30 min (exact time was recorded) using 3.1 L polyethylene caps equipped with tube fittings. Build-up of N_2O concentrations in the caps was measured using a photo-acoustic multi-gas monitor (Innova 1312, Innova AirTech Instruments, Ballerup, Denmark) by connecting Teflon tubes to the fittings. Fluxes were corrected for background concentrations, determined each fifth measurement. After correction, flux data were averaged per field. Cumulative soil N_2O fluxes were calculated assuming a linear flux between the measurement dates. For more details see Velthof et al. (2002).

4.2.3 Statistical analyses

All statistical analyses were done in R (R Core Team 2019). Soil and leachate NO_3^- and NH_4^+ concentrations measured in the mesocosm experiment were log-transformed before analyses since data did not fit a normal distribution. Analyses for statistical differences between treatments of soil, leachate, herbage and cumulative N_2O data were done by one or two-way ANOVA in which mesocosms and plots were considered as experimental unit in the mesocosm and field experiment, respectively. Block effects of the replications were included in the analyses of the field experiment data. Differences between treatments were analysed by Least Significant Differences (LSD). In all analyses a p-value ≤ 0.05 was considered as significant.

Models for the approximation of herbage N uptake of both experiments were fitted by stepwise regression. Model selection was done based on the lowest Akaike information criterion. Following the hypotheses that plantain can increase plant N use efficiency, candidate predictors for total herbage, grass and plantain herbage N uptake were plantain and grass DM yield. Linear and quadratic effects of candidate predictors were allowed.

4.3 Results

4.3.1 Mesocosm experiment

Potential soil nitrification at day 148 of the experiment was significantly lower for the 100%PL treatment compared to 0%PL (39.7 versus 65.0 µmol NO_2 g^{-1} h^{-1}, $p = 0.018$). Soil NO_3^- concentrations were negatively correlated to potential nitrification ($r = -0.83$, $p = 0.011$ for 100%PL and $r = -0.75$, $p = 0.033$ for 0%PL) (Figure 4.1).

The presence and amount of plantain did not affect leachate and soil NO_3^- and NH_4^+ concentrations, since there were no significant differences between the treatments at any of the sampling moments (Table 4.2). Soil NH_4^+ concentrations decreased during the experiment, while NO_3^- concentrations were highest at day 95 and lower at the subsequent sampling days. Soil moisture concentration was significantly higher for 100%PL versus the other treatments at day 95 ($p = 0.004$), but not significantly different between treatments at any other sampling time.

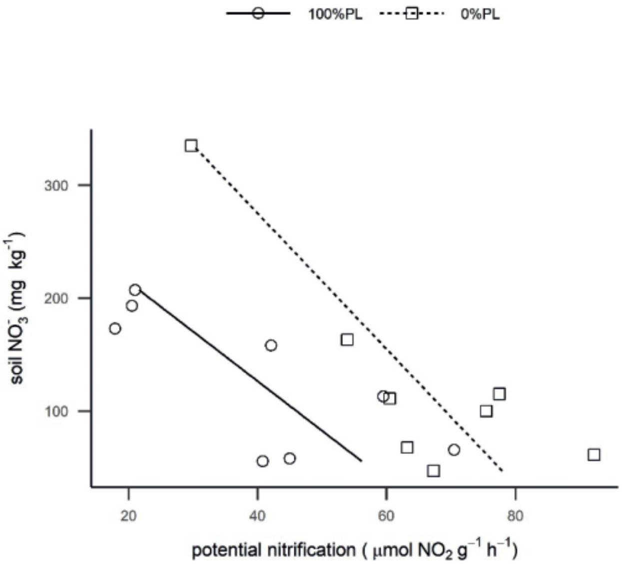

Figure 4.1. Mesocosm experiment. Correlation between soil potential nitrification and soil NO_3^- concentrations in treatments 100%PL (*open dots*, r = -0.83, *p* = 0.011) and 0%PL (*squares*, r = - 0.75, *p* = 0.033) at day 148.

Plants did not flower during the experiment. Cumulative herbage DM yield after 116 days of growth was significantly lower for 100%PL compared to the other treatments ($p < 0.001$, Table 4.2). At day 116, treatment 100%PL showed a lower N yield compared to the treatments 66%PL and 33%PL ($p = 0.045$). Ryegrass made up the greatest part of the cumulative DM yield and the N yield at day 116 in the 66%PL and 33%PL treatments. Stepwise regression revealed that plantain N yields tended to increase quadratically ($p = 0.098$) in addition of a linear effect ($p = 0.014$) to plantain DM yields, while ryegrass N yields only increased linearly per unit of ryegrass DM yield ($p < 0.001$) (Figure 4.2, Table S4.1). Total herbage N yields (plantain + ryegrass) were significantly ($p < 0.001$) positively correlated to PL and ryegrass DM yields; however the total herbage N yield increase was greater per gram of plantain DM yield ($1.94 \cdot 10^{-2}$ g g^{-1})than per gram of ryegrass DM yield ($1.60 \cdot 10^{-2}$ g g^{-1}), respectively.

No significant correlations were observed between soil NO_3^- or NH_4^+ concentrations and plantain or ryegrass N uptake at day 116, between soil NH_4^+ concentrations and potential nitrification at day 148 or between plantain or ryegrass N uptake at day 116 and potential nitrification at day 148.

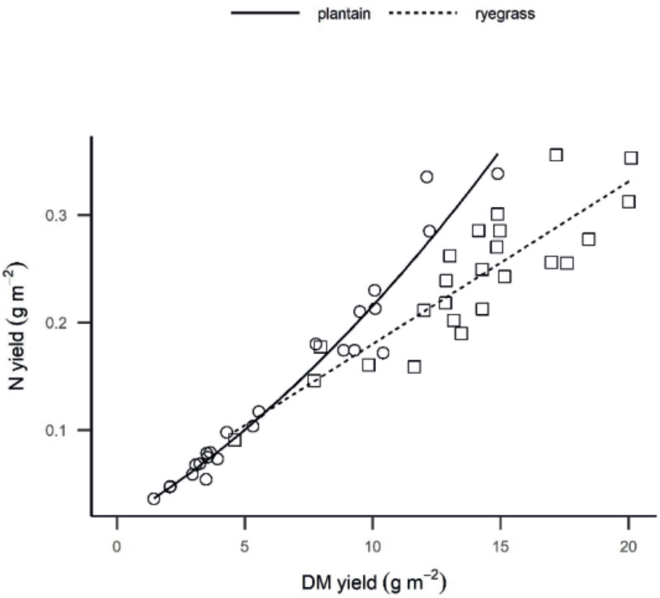

Figure 4.2. Mesocosm experiment. Nitrogen (N) yields of plantain (*open dots*) and ryegrass (*squares*) versus plantain and ryegrass dry matter (DM) yields at day 116 of the experiment, grown in a monoculture or in a plantain ryegrass mixture. For model details on the fitted lines see Table S4.1.

Table 4.2. Mesocosm experiment: Back log-transformed mean values of leachate and soil NO_3^- and NH_4^+ concentrations, and soil moisture concentrations at different sampling days.

				Treatment					
Parameter	**Depth (cm)**	**Unit**	**Day**	**100%PL**	**66%PL**	**33%PL**	**0%PL**	**p-value**	**SEM**
Leachate NO_3^-		mg L^{-1}	5	0.37	0.43	0.34	0.46	0.912	0.198
			74	0.16	0.23	0.15	0.24	0.425	0.083
			95	0.27	0.20	0.21	0.19	0.585	0.058
			116	0.45	0.44	0.55	0.53	0.977	0.587
Leachate NH_4^+		mg L^{-1}	5	3.73	2.44	3.23	3.15	0.247	0.225
			74	4.28	2.84	4.07	3.51	0.206	0.255
			95	5.28	3.76	4.57	2.39	0.216	0.343
			116	4.95	3.78	3.79	3.17	0.559	0.369
Soil NO_3^-	0-20	mg kg^{-1} DM soil	5	6.6*					
	0-20		74	103.9	90.6	86.7	77.8	0.521	5.42
	0-20		95	564.1	544.4	441.2	436.5	0.798	54.40

Table 4.2. Continued

				Treatment					
Parameter	**Depth (cm)**	**Unit**	**Day**	**100%PL**	**66%PL**	**33%PL**	**0%PL**	**p-value**	**SEM**
	0-20		116	45.4	40.8	36.0	51.8	0.540	4.52
	0-10		148	111.1	ND	ND	108.5	0.942	16.60
Soil NH_4^+	0-20	mg kg^{-1} DM soil	5	62.0*					
	0-20		74	111.8	142.3	73.2	117.8	0.113	14.02
	0-20		95	22.3	15.5	18.9	16.0	0.618	2.09
	0-20		116	2.5	2.1	1.5	2.8	0.635	0.67
	0-10		148	1.2	ND	ND	8.9	0.354	3.99
Soil moisture	0-20	g kg^{-1} soil	5	660*					
	0-20		74	727	724	690	710	0.704	7.1
	0-20		95	647^{a}	618^{b}	607^{b}	600^{b}	0.010	3.7
	0-20		116	560	560	547	574	0.925	10.1
	0-10		148	593	ND	ND	583	0.525	7.3
Total biomass DM yield		g m^{-2}	cum	294^{b}	430^{a}	483^{a}	465^{a}	<0.001	32.5
Plantain DM yield		g m^{-2}	cum	294^{a}	173^{b}	94^{c}		<0.001	14.9
Ryegrass DM yield		g m^{-2}	cum		258^{b}	390^{a}	465^{a}	<0.001	26.6
Total biomass N yield		g m^{-2}	cum	3.85^{b}	5.03^{a}	5.04^{a}	4.13^{ab}	0.045	0.19
Plantain N yield		g m^{-2}	cum	3.85^{a}	1.85^{b}	0.93^{c}		<0.001	0.26
Ryegrass N yield		g m^{-2}	cum		3.19	4.10	4.13	0.118	0.18

Note: Cumulative dry matter (DM) yields of herbage harvested at days 41, 61, 95 and 116, and N-contents and N yields of herbage harvested at day 116 of the experiment. Values not sharing the same superscript letter differ significantly ($p < 0.05$). DM = dry matter, cum = cumulative data, ND = not determined and SEM = standard error of the mean. Note the difference in soil sampling depth for the soil NO3-, NH4+ and moisture concentrations.

*pooled sample analysis

4.3.2 Field experiment

N_2O-N fluxes were significantly higher for 0%PL compared to the other treatments at 21 days after the CAN-N application (*p* = 0.009, Figure 4.3). Cumulative N_2O-N fluxes differed significantly for all combined plantain treatments compared to the control without plantain (*p* = 0.021) (Figure 4.4). Overall, the highest mean N_2O-N fluxes (0.53 ± 0.53 mg m^{-2} h^{-1}) were observed 15 days after the N application and after the first significant precipitation during the measurement period (32 mm, Figure 4.4). After day 15, observed mean fluxes were lower at each subsequent measurement day. The lowest mean N_2O-N fluxes were observed at 35 days after the CAN-N application (0.12 ± 0.06 mg m^{-2} h^{-1}).

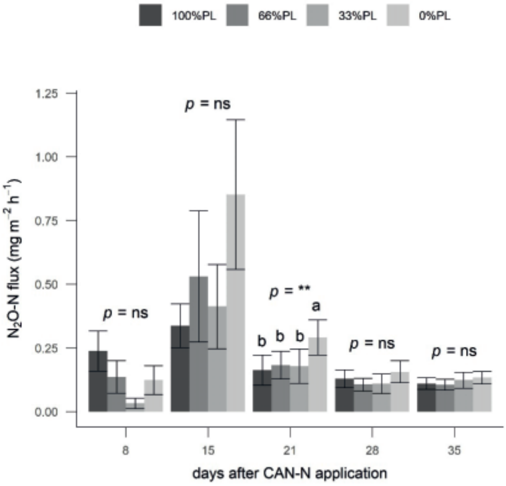

Figure 4.3. Field experiment. N_2O-N fluxes at days 8 till 35 after CAN-N application (5 g m^{-2}). Bars represent standard errors. Bars with the same letters are not significantly different ($p > 0.05$).

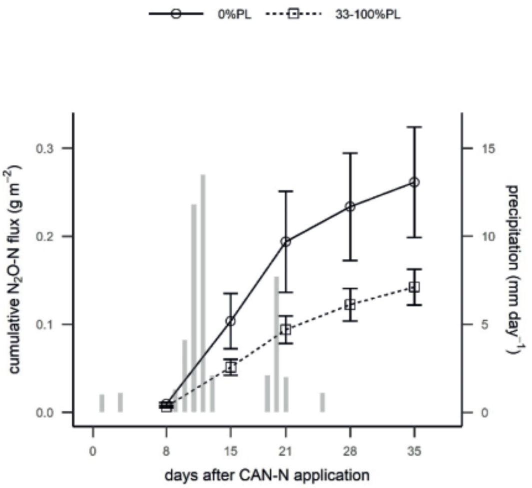

Figure 4.4. Field experiment. Precipitation (*grey bars*, KNMI, Zegveld, the Netherlands) since CAN-N application (5 g m^{-2}) and cumulative N_2O-N fluxes between 8 till 35 days after CAN-N application of treatment 0%PL (*open dots, solid line*) versus all treatments with plantain (33%PL, 66%PL and 100%PL) (*squares, dashed line*). Error bars represent N_2O-N fluxes standard errors. Cumulative N_2O-N fluxes were significantly lower in the presence of plantain ($p = 0.021$).

At each harvest, plantain had produced inflorescences while ryegrass had not. Except for the fifth harvest, treatments significantly affected the total herbage DM yield ($p < 0.001$ for all four harvests) (Figure 4.5). At the first harvest, herbage DM yields were highest for 0%PL and lowest for 100%PL, while at the second, third and fourth harvest yields were the lowest for 0%PL and at the third harvest highest for 100%PL. Total grasses N yield at the second harvest was negatively associated with the DM yield of plantain ($p = 0.049$), while total grasses N yield at the fourth harvest tended to be positively associated with the DM yield of plantain ($p = 0.066$) (Figure 4.7, Table S4.2). Total grasses N yields in the first, third and fifth harvest and plantain N yields in all harvests were only related linearly to total grasses and plantain DM yields respectively (Figures 4.6 and 4.7, Table S4.2).

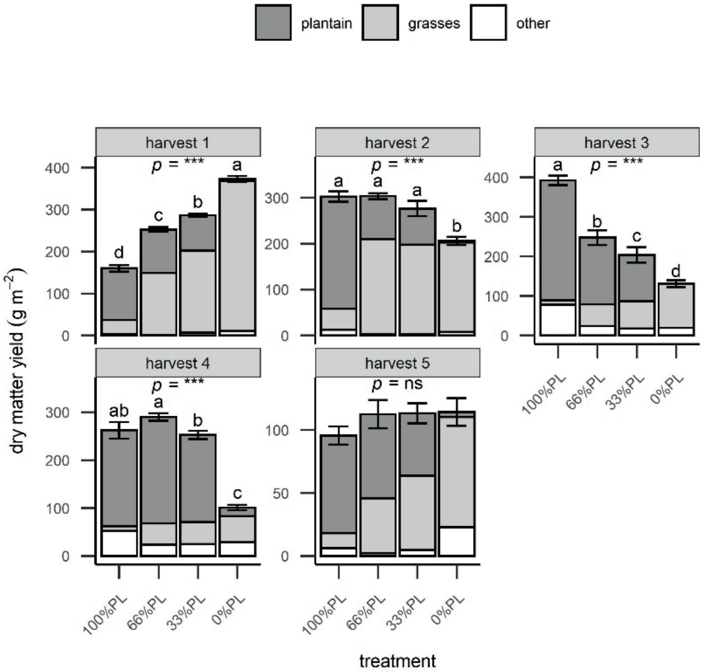

Figure 4.5. Field experiment. Total herbage dry matter yields per harvest and treatment, and contribution of plantain (*dark grey fill*), grasses (*light grey fill*) and other herbs and forbs (*white fill*) to the total herbage dry matter yield. *p*-values and errors bars represent probabilities for statistical differences and standard errors of the total herbage dry matter yield. Total herbage dry matter yields of bars with the same letters are not significantly different ($p > 0.05$).

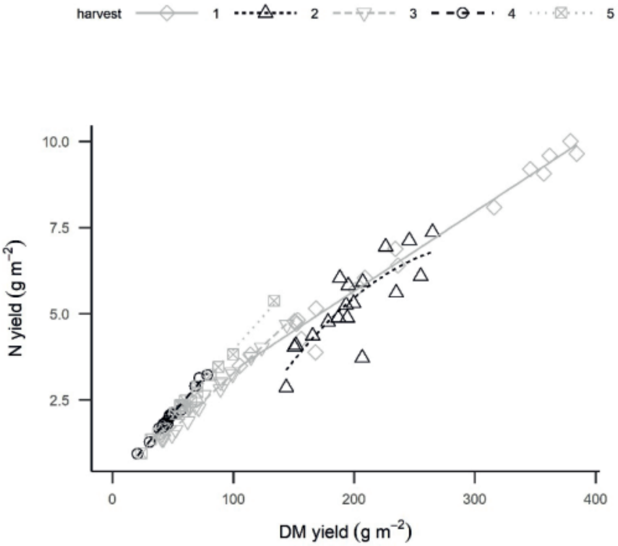

Figure 4.6. Field experiment. Plantain nitrogen (N) yields versus plantain dry matter (DM) yields per harvest, grown in a monoculture or in a plantain ryegrass mixture, respectively. For model details on the fitted lines see Table S4.2.

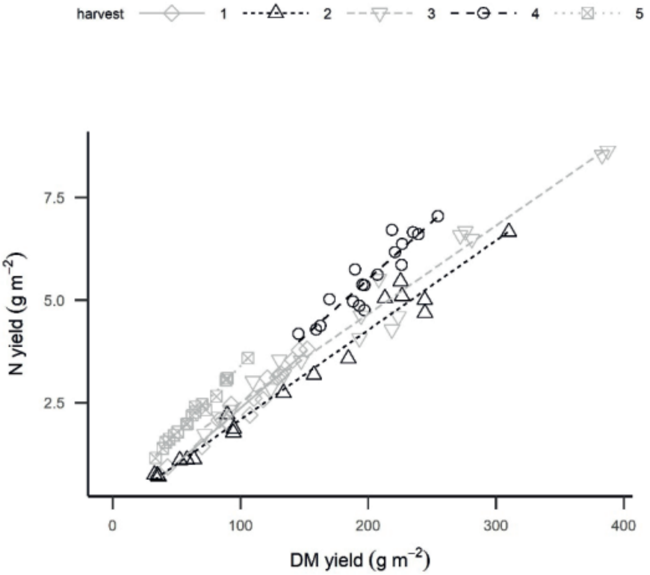

Figure 4.7. Field experiment. Grasses nitrogen (N) yields versus grasses dry matter (DM) yields per harvest, grown in a monoculture or in a plantain ryegrass mixture, respectively. For model details on the fitted lines see Table S4.2.

4.4 Discussion

4.4.1 Mesocosm experiment

The nearly 40% lower potential nitrification found for a monoculture of plantain versus a monoculture of ryegrass at day 148 of the experiment is in line with the suggested BNI capacity of plantain (Dietz *et al.* 2013; Subbarao *et al.* 2015). However, in contrast to our hypothesis that the presence of plantain would lead to lower soil NO_3^- concentrations, no differences between treatments were observed. Soil NO_3^- concentrations were rather variable, possibly as the result of spatial and temporal heterogeneity of denitrification patterns caused by the nature of the process (e.g. van den Pol-van Dasselaar et al., 1998). Furthermore, soil NO_3^- concentrations were lower when potential nitrification was higher (Figure 4.1). Low NO_3^- concentrations could be the result of a greater microbial and/or bacterivore activity coinciding with higher soil respiration and mineralisation rates (Bloem *et al.* 1994). Higher respiration rates can result locally in micro patches with lower soil oxygen concentrations favouring denitrification and decreasing soil NO_3^- concentrations (Firestone & Davidson 1989).

Plants may also influence nitrification in soils by altering the identity or activity of the microbial community as the result of effects on the physical and/or chemical soil environment (e.g. pH, moisture). Additionally, plants influence nitrification by competing with the microbial community for N and thus reducing the amount of NH_4^+ available for nitrifiers (Bowatte *et al.* 2016). Since in our experiment effects of exudated secondary metabolites with BNI capacity on nitrification were not directly assessed, we cannot exclude effects on potential nitrification caused by the soil environment or by competition for N. However, several studies showed that secondary metabolites with BNI capacity are produced by plantain and are present in above ground biomass and roots (Marak *et al.* 2000; De Deyn *et al.* 2009; Dietz *et al.* 2013; Miehe-Steier *et al.* 2015; Box & Judson 2018). Box and Judson (2018) reported for the plantain var. “Hercules”, used in our experiments, mean leaf concentrations of 39.1 mg g^{-1} DM verbascoside, 39.1 mg g^{-1} DM aucubin and < 5 mg g^{-1} DM catalpol over a year on a silt loam soil in New Zealand. Miehe-Steier et al. (2015) showed that leaf aucubin, catalpol and verbascoside concentrations are similar to root concentrations under high nutrient and high light conditions. Moreover, Fuchs and Bowers (2004) showed that aucubin and catalpol concentrations in plantain leaves increased from 0.5% dry weight at the age of 7 weeks to 6% dry weight at the age of 11 weeks. Therefore, it seems likely that in our experiment, in treatments with plantain, compounds with BNI capacity were present in the roots and rhizosphere. However, to confirm the hypotheses that the reduced potential nitrification resulted of the presence of compounds with BNI capacity, more detailed research is needed on the bacterial activity and the release and mode of action of these compounds in the rhizosphere.

According to Verhagen et al. (1995), to determine exclusive effects of secondary metabolites on inhibition of nitrification, soil mineral N conditions should not be limiting for this process. Effects of competition for N on nitrification seemed unlikely in our experiment due to the high soil organic matter, NO_3^- and NH_4^+ concentrations. In comparison, in our experiment soil NO_3^- and NH_4^+ concentrations were on average about 1.000 times higher than in experiments on sandy soils reported by Dietz et al. (2013), who observed lower soil NO_3^- and NH_4^+ concentrations after incubation with aucubin, catalpol or a plantain liquid extract compared to a water incubation. Possibly, in our experiment the level and heterogeneity of soil NO_3^- and NH_4^+ concentrations were such that the potential effects of compounds with BNI capacity on these concentrations were too limited to be determined. Moreover, genotype may have played a role, since we used a different plantain variety than Dietz et al. (2013) and concentrations of BNI compounds have been shown to be genotype specific (Box & Judson 2018).

The observed quadratic increase of plantain N yields at higher plantain DM yields partly confirmed our hypothesis that the presence of plantain can increase the N use efficiency of grassland on peat soil, since on the other hand no effects of the presence and yield of plantain on ryegrass N yields were observed (Figure 4.2). In our experiment plant N contents were low (1.94 ± 0.32 g kg^{-1} DM) compared to field conditions (typically summer and autumn N contents without fertilization are ≥ 2.40 g kg^{-1} DM) (Vellinga & André 1999; Sonneveld & Lantinga 2011; Deru *et al.* 2019). Soil mineral N, P_{AL}, K and pH conditions were not likely to have limited plant N uptake (CBGV 2022). Therefore, besides a potential effect of compounds with BNI capacity in the treatments with plantain, likely other chemical, biological and/or physical soil properties affected herbage N uptake in general in the experiment. However, since soil NO_3^- and NH_4^+ concentrations between treatments did not differ significantly (Table 4.2), apparently these conditions did not differently affect soil mineral N concentrations between treatments.

4.4.2 Field experiment

In the presence of plantain cumulative N_2O fluxes were 39% lower compared to the monoculture of perennial ryegrass (Figures 4.3 and 4.4). These substantially lower N_2O fluxes in the presence of plantain confirmed our hypothesis that plantain can reduce N_2O formation in a grassland on peat soil compared to ryegrass. Measured N_2O fluxes were in range with others studies on drained peat grasslands (Velthof et al. 1996; Koops et al. 1997; Van den Pol-van Dasselaar et al. 1998). During the N_2O flux measurement period, herbage N uptakes between treatments were similar, indicating no differences were present in available mineral N for herbage uptake between treatments.

Similar to our results, Luo et al. (2018) found lower N_2O fluxes when urine-N was applied at a rate of 62 g m^{-2} on a monoculture of plantain compared to ryegrass and lucerne on a well-drained silt loam soil, and suggested the effect of the BNI potential of plantain as the dominant mechanism reducing N_2O fluxes. Simon et al. (2019) observed a linear decreasing N_2O emission factor as the proportion of plantain increased from 0 to 100% in a ryegrass / white clover sward, after a urine-N application of 61 g m^{-2} on a silt loam soil. In our experiment, above ground biomass of plantain (plantain made up 43 to 86% of the total harvested herbage DM at 29 October in treatments 33%PL, 66%PL and 100%PL) was not correlated to cumulative N_2O fluxes. Thus, if the BNI potential of plantain was the dominant mechanism reducing N_2O fluxes, apparently the presence rather than the amount of plantain was an important factor for the reduction of the N_2O flux, at least within the studied conditions. For example differences in applied N sources, soil N concentrations and soil types resulted in different conditions between studies. Luo et al. (2018) and Simon et al. (2019) applied before N_2O measurements urine-N on silt loam soils while in the current study CAN-N was applied on a peat soil. Urine-N consists mainly of urea which first needs to be transformed to NO_3^- to be available for denitrification, while CAN-N consists of 50% NH_4^+ and 50% NO_3^-. Furthermore, urine applications temporary increase water filled pore space (WFPS) and possibly pH which both can (in)directly stimulate denitrification (Luo *et al.* 2018). Peat soils differ in many aspects from mineral soils which can (in)directly affect soil N cycling (Pihlatie *et al.* 2004); for example peat soils generally contain relative higher mineral N and organic carbon pools, have a relative higher moisture retention capacity, often have a lower pH and a different soil texture compared to mineral soils. In comparison, the grassland on silt loam soil used by Luo et al. (2018) had nearly four times lower total N and soil organic matter contents compared to the peat soil in the current study (Table 4.1). Moreover, in the current study measurements were only in the first year after glyphosate treatment and soil tillage, which likely both (in)directly affected the soil food web compared to an undisturbed grassland system (Wardle 1995), and likely thus also affected soil N cycling.

The sharp increase in the N_2O flux observed after the first significant precipitations nine to thirteen days after fertilizer application (total 32 mm, Figure 4.4) is probably the result of an increase in soil moisture content, since denitrification activity and WFPS in the top soil are positively correlated (Koops *et al.* 1996). Significant differences in soil moisture contents between the treatments seemed unlikely as we measured nitrous oxide fluxes under relative (very) dry field conditions, resulting from the high potential precipitation deficit preceding the measurement period (Sluijter *et al.* 2018). Luo et al. (2018) found WFPS to be lower in a monoculture of plantain and lucerne compared to ryegrass, and lower N_2O fluxes after urine applications on plantain versus lucerne or ryegrass pastures. However, Luo et al. (2018) concluded that effects of plantain on N_2O fluxes could not have only been induced by different soil moisture contents since they observed lower N_2O fluxes at plantain at similar WFPS contents between lucerne and plantain in their study. Therefore, it seems likely that also in our experiment root-released compounds with BNI capacity at least partially contributed to the lower N_2O flux observed in the presence of plantain.

The negative effect of plantain DM yield on grasses N yields at the second harvest and linear relations between herbage N uptake and herbage DM growth at other harvests (Figures 4.6 and 4.7, Table S4.2) suggested that our hypothesis of plantain increasing soil mineral N use efficiency in a grassland on peat soil had to be rejected, at least within the conditions of the field experiment. Lower total grasses N yields associated with higher plantain DM yields indicate increased herbage N uptake competition rather than an increase of available N for plant uptake.

Herbage N contents in the field experiment were well in range with other field measurements (Vellinga & André 1999; Sonneveld & Lantinga 2011; Deru *et al.* 2019). Possibly, the tendency towards the positive effect of plantain presence on plantain N uptake efficiency as observed in the mesocosm experiment was overruled by the relative higher herbage N uptake rate in the field experiment. Soil temperatures in the field experiment (increasing from about 11°C around 1 May to more than 20°C around 1 August) exceeded soil temperatures in the mesocosm experiment (maintained around 12°C), which positively affected soil mineralization (Bloem *et al.* 1994). Furthermore, long periods without precipitation in the second, third and fourth harvest of the field experiment may have limited denitrification and leaching of NO_3^- to below the main rooting depth, leaving relative more NO_3^- available for herbage uptake.

4.5 Conclusions

Plantain grown in mesocosms significantly reduced soil potential nitrification but did not affect soil NO_3^- concentrations. Plantain N uptake efficiency increased at a higher herbage plantain share in the mesocosm experiment, but the plantain share did not affect herbage N uptake efficiency under field conditions. Under field conditions, N_2O fluxes were nearly 40% lower in the presence of plantain, without an obvious link to the amount of plantain present. Therefore, our results strongly suggest that the presence of plantain influences the fate of soil mineral N in a dairy grassland on peat soil, but more integrated research is needed to fully confirm this.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Supplementary materials

Table S4.1. Mesocosm experiment. Models obtained by stepwise regression approximating plantain, ryegrass or total biomass N yield (g m^{-2}) of day 116 (4th harvest). PLDMY = plantain dry matter yield. RGDMY = ryegrass dry matter yield. (R)SE = (residual) standard error.

regressor	**intercept SE p-value**	**PLDMY SE p-value**	**PLDMY² SE p-value**	**RGDMY SE p-value**	**RSE**
plantain N yield	0.24	$1.41 \cdot 10^{-2}$	$3.73 \cdot 10^{-5}$		0.34
	0.26	$0.53 \cdot 10^{-2}$	$2.15 \cdot 10^{-5}$		
	0.366	0.014	0.098		
ryegrass N yield	0.46			$1.51 \cdot 10^{-2}$	0.52
	0.41			$0.18 \cdot 10^{-2}$	
	0.269			<0.001	
total biomass N yield	0.39	$1.94 \cdot 10^{-2}$		$1.60 \cdot 10^{-2}$	0.59
	0.7	$0.36 \cdot 10^{-2}$		$0.27 \cdot 10^{-2}$	
	0.586	<0.001		<0.001	

Table S4.2. Field experiment. Models obtained by stepwise regression approximating plantain and total grasses N yield per harvest (g m^{-2}). PLDMY = plantain dry matter yield, GDMY = grasses dry matter yield, (R)SE = (residual) standard error.

Harvest	**plantain N yield / grasses N yield**	**intercept SE p-value**	**PLDMY SE p-value**	**GDMY SE p-value**	**RSE**
1	plantain	-0.33	$2.69 \cdot 10^{-2}$		0.17
		0.17	$1.48 \cdot 10^{-3}$		
		0.072	<0.001		
1	grasses	1.04		$2.31 \cdot 10^{-2}$	0.35
		0.22		$8.70 \cdot 10^{-4}$	
		<0.001		<0.001	
2	plantain	-0.09	$2.18 \cdot 10^{-2}$		0.30
		0.13	$8.21 \cdot 10^{-4}$		
		0.512	<0.001		
2	grasses	0.92	$-6.50 \cdot 10^{-3}$	$2.38 \cdot 10^{-2}$	0.64
		1.08	$3.04 \cdot 10^{-3}$	$4.89 \cdot 10^{-3}$	
		0.409	0.049	<0.001	
3	plantain	0.35	$2.16 \cdot 10^{-2}$		0.37
		0.20	$9.40 \cdot 10^{-4}$		
		0.107	<0.001		

Table S4.2. Continued

Harvest	plantain N yield / grasses N yield	intercept SE p-value	PLDMY SE p-value	GDMY SE p-value	RSE
3	grasses	0.03		$3.25 \cdot 10^{-2}$	0.10
		0.07		$8.35 \cdot 10^{-4}$	
		0.710		<0.001	
4	plantain	-0.09	$2.80 \cdot 10^{-2}$		0.34
		0.56	$2.74 \cdot 10^{-3}$		
		0.873	<0.001		
4	grasses	-0.06	$3.49 \cdot 10^{-4}$	$4.22 \cdot 10^{-2}$	0.06
		0.07	$1.76 \cdot 10^{-4}$	$1.19 \cdot 10^{-3}$	
		0.459	0.066	<0.001	
5	plantain	0.12	$3.28 \cdot 10^{-2}$		0.08
		0.06	$9.29 \cdot 10^{-4}$		
		0.079	<0.001		
5	grasses	0.03		$3.92 \cdot 10^{-2}$	0.09
		0.06		$8.18 \cdot 10^{-4}$	
		0.550		<0.001	

Chapter 5

The effects of harvest date and frequency on the yield, nutritional value and mineral contents of the paludiculture crop cattail *(Typha latifolia L.)* in the first year after planting

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Abstract

The use of drained peatlands as dairy grasslands leads to long-term organic matter losses, CO_2 emissions and soil subsidence. It also yields grass with increased N and P contents compared to grass grown on mineral soils due to peat mineralisation, which often leads to greater farm surpluses of these elements. Growing *Typha latifolia* as a forage crop on rewetted peatlands (paludiculture) could reduce these issues. Therefore, the effects of harvest date and frequency on yield and nutritional value were studied in three experiments during the first growing season after establishment of two different *T. latifolia* plantations. *T. latifolia* produced 40–68 shoots m^{-2} and maximum dry matter (DM) yields of 9.81–10.89 Mg ha^{-1}. Harvesting before flowering resulted in the highest nutritional value per kg DM, of 563–575 g in vitro digestible organic matter (IVDOM), 120–128 g crude protein (CP), 287–300 g crude fibre (CF) and 1.5 g P. Surprisingly, harvesting at intervals of three or six weeks resulted in similar cumulative DM yields ($p = 0.190$). Also, average nutritional values per kg DM, especially of biomass harvested at 3-week intervals, remained similar to a May yield of 466–591 g IVDOM, 103–134 g CP and 286–303 g CF. Growing *T. latifolia* fodder for inclusion in grass-based diets could reduce the environmental impacts of dairy farming on peat.

Keywords: harvesting procedure, rewetted peatland, ruminant feed

5.1 Introduction

Peatlands cover about 3 % of the planet's surface (Urák et al. 2017) and nearly 10 % of the surface of Europe if shallow peatlands (< 30 cm peat) are also taken into account (Tanneberger et al. 2017). Oxic conditions of peat due to drainage lead to constant degradation and long-term losses of organic matter and CO2 emissions (van den Akker et al. 2008). In The Netherlands about 223,000 ha of the total 290,000 ha of peat soils are used as intensively managed dairy grassland and, therefore, have to be permanently drained (Schothorst 1977, van den Akker et al. 2008). In Dutch peatland areas used for dairy farming, the intensive management of grassland is leading to discharge of surplus N and P to surface water (van Beek 2007) and estimated soil subsidence of 1–2 cm year^{-1} (Schothorst 1977, van den Akker et al. 2007). Moreover, the constant organic matter degradation in dairy grassland on peat leads to a higher grass mineral uptake from the soil than on mineral soils, yielding more N and P dense forage (De Visser et al. 2001, van der Meer et al. 2004). When the higher nutrient density of the forage is not balanced with components low in N and P such as maize in the cows' diets, the cows will receive a dietary surplus of N and P which, in turn, will lead to a higher farm N and P surplus than for a dairy farming system of similar intensity on mineral soil (De Visser et al. 2001). Since the cultivation of maize on peat is undesirable because it requires more intensive tillage than perennial grass cultivation (and thus leads to increased peat degradation), this is not an option for most farms (De Visser et al. 2001).

Alternatively, increasing groundwater levels or completely rehydrating peat soils reduces peat degradation and reduces or completely stops soil subsidence (van den Akker et al. 2007, FAO 2014, Wilson et al. 2016). Cultivating crops in (re)hydrated conditions on peat (i.e. paludiculture) may be a viable alternative, maintaining economic activities in peatland areas (Wichtmann et al. 2016, Dragoni et al. 2017), and could be part of a more 'nature-inclusive' farming system (Erisman et al. 2016). In the Netherlands little effort has been devoted to growing flood resistant grains (e.g. *Oryza, Zizania*), submerged starch crops (e.g. *Sagitaria*) and flood tolerant grasses (*Phalaris, Glyceria*), even though large areas of Dutch agricultural land have subsided below sea level. *Typha latifolia* (broadleaf cattail) can be successfully grown and harvested in wet peatlands, is potential livestock fodder (González et al. 2000, do Nascimento et al. 2014), can produce considerable biomass, and can take up large amounts of nutrients from e.g. farm runoff and soil accumulations that have resulted from intensive farming (Maddison et al. 2005, Geurts et al. 2017).

Yields of *T. latifolia* seem to depend on time since crop establishment and harvest date, and are known to vary spatially and between years (Pfadenhauer & Wild

2001, Maddison et al. 2005, Maddison et al. 2009, Heinz 2012, Günther et al. 2015). However, to our knowledge, relatively little is known about the biomass production and nutritional value in relation to harvest date and frequency of *T. latifolia* grown as a paludiculture crop. In a dairy farming system, *T. latifolia* could possibly be grazed or harvested repeatedly during the season in order to utilise younger and nutritionally superior biomass than would be available from a single seasonal harvest. Only a few studies have been performed on the stability of *Typha* spp. regrowth after harvesting, mostly aiming to reduce the abundance of *Typha* spp. in wetlands (Hellsten et al. 1999). The objectives of the current work were to assess the production, nutritional value and mineral content of *T. latifolia* green biomass harvested at different dates and frequencies during a growing season. We hypothesised that:

1. *T. latifolia* biomass yields would peak between the middle and end of the growing season;
2. the nutritional value of *T. latifolia* would decrease with increasing crop age, with a sharp decrease before onset of flowering, as in (other) grasses; and
3. increased harvesting frequency (as in a situation with grazing) would result in a maintained nutritional value compared to harvesting before onset of flowering but would drastically reduce productivity and shoot density during a growing season.

5.2 Methods

5.2.1 Study sites

Three experiments were performed on two stands of *T. latifolia* planted on transformed dairy grassland at the Knowledge Transfer Centre Zegveld in The Netherlands (location used for Experiment I: 52° 08' 04.8" N, 4° 50' 10.4" E; location for Experiments II and III: 52° 08' 20.0" N, 4° 50' 19.6" E). The experimental sites were established by planting young *T. latifolia* plants which had been reared in a greenhouse using seeds obtained from natural stands (Aquaflora, The Netherlands). The seeds were spread in 1 × 1 m soil beds for germination, after which individual seedlings were planted in pots (4.8 × 4.5 × 11.0 cm) filled with potting soil. Before planting out, the experimental fields were prepared by mowing the grass to a stubble height of 2–3 cm then removing the topsoil layer including the grass sod (± 10 cm). The removed soil was used to make ridges around the field, in order to permanently maintain a water level of 20–30 cm above the soil surface. Water levels were maintained using a solar-powered water pump equipped with a water level sensor. At planting (June 2015 for Experiment I, July 2016 for Experiments II and III), plants were 30–60 cm high, and were planted by hand (Experiment I) or semi-mechanically (Experiments II and III). Planting densities were 15 plants m^{-2} for Experiment I and 3.5 plants m^{-2}

for Experiments II and III. Immediately after planting the experimental fields were inundated by 10–20 cm to reduce desiccation damage and repress the growth of competitive plants. In Experiment I and in the first year of Experiments II and III (2016) no additional nutrients were applied. During winter, all plants were mowed at 5–10 cm above water level using a brush cutter and cut biomass was either removed (Experiment I) or left in place (Experiments II and III). In May 2017, Experiments II and III received applications of 150 kg ha^{-1} N in the form of coated urea and 150 kg ha^{-1} K as coated potassium nitrate (Ekompany, the Netherlands). The N and K loads were based on observed N:P and N:K ratios in growing *T. latifolia* biomass (Geurts et al. 2017, Vroom et al. 2018) to simulate nutrient supply from farm runoff and to stimulate plant P uptake.

5.2.2 Experimental setup and sample collection

Experiment I consisted of a field of 60 m^2, in which ten different 0.5 × 0.5 m plots were randomly assigned to five different harvesting dates (31 May, 07 July, 03 August, 15 September and 28 October 2016) (Figure 5.1). At each harvest, two plots were harvested and a subsample of fresh biomass per plot was taken for further analyses.

Experiments II and III were performed on a field of 61.4 m^2 which was split into 24 equal plots of 1.6 × 1.6 m. For Experiment II, 22 plots in total were used to determine the effect of harvest date on biomass yield and nutritional value (Figure 5.2; plots A–I). Experiment II comprised eight different harvest dates (19 May, 02 June, 16 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017) which were randomly assigned to the 22 plots. At 19 May, eight plots (combined use with Experiment III) were used; and at the subsequent seven other harvest dates, two plots per harvest date were used.

For Experiment III, in total eight plots were used to determine the effect of a 3-week or 6-week harvest interval (Figure 5.2; plots A and B, four plots per harvest interval). Harvest dates were 19 May, 02 June (only 3-week harvest interval), 30 June, 21 July (only 3-week harvest interval), 11 August, 01 September (only 3-week harvest interval) and 22 September 2017. Treatments were randomly assigned to the plots. At each harvest, the number of shoots and flowers were counted. Thereafter, a bamboo stick was placed in the middle of each plot and plants were harvested from a circle of radius 50 cm centred on the stick, resulting in a harvested surface of 0.79 m^2. After the harvest, fresh biomass was weighed and subsamples of all plots were taken for dry matter (DM) analyses. In Experiment II all subsamples and in Experiment III two randomly selected subsamples per harvest date and scheme (n = 7 and n = 4 for the 3-week and 6-week harvest interval schemes, respectively) were taken for further nutrient and mineral analyses. In all experiments, plants were harvested at 5–10 cm above the water level using secateurs.

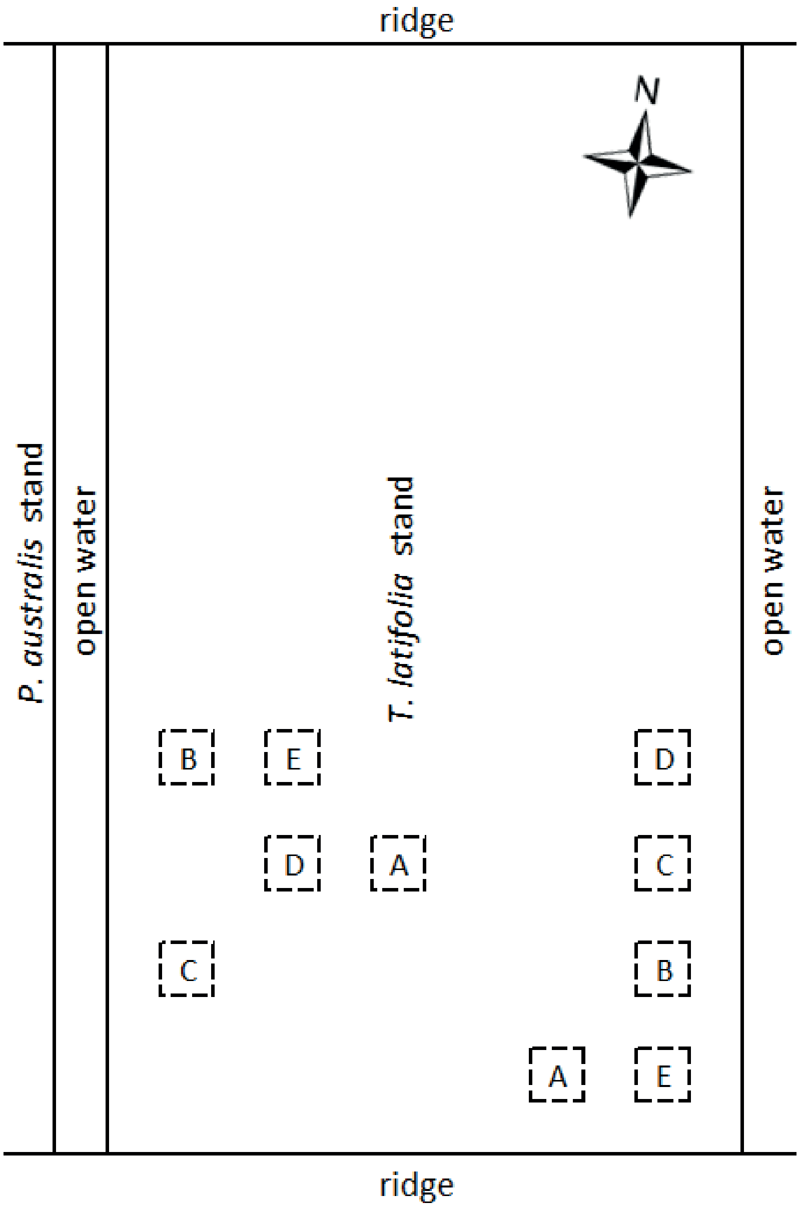

Figure 5.1. Experiment I. Schematic overview of the experimental field (6 x 10 m) used for harvesting *T. latifolia* biomass. The field was surrounded by soil ridges at north and south sides and by open water (≥ 0.75 m) at west and east sides. Harvested plot surfaces were 0.25 m^2, and harvest dates were 31 May (A), 07 July (B), 03 August (C), 15 September (D) and 28 October 2016 (E).

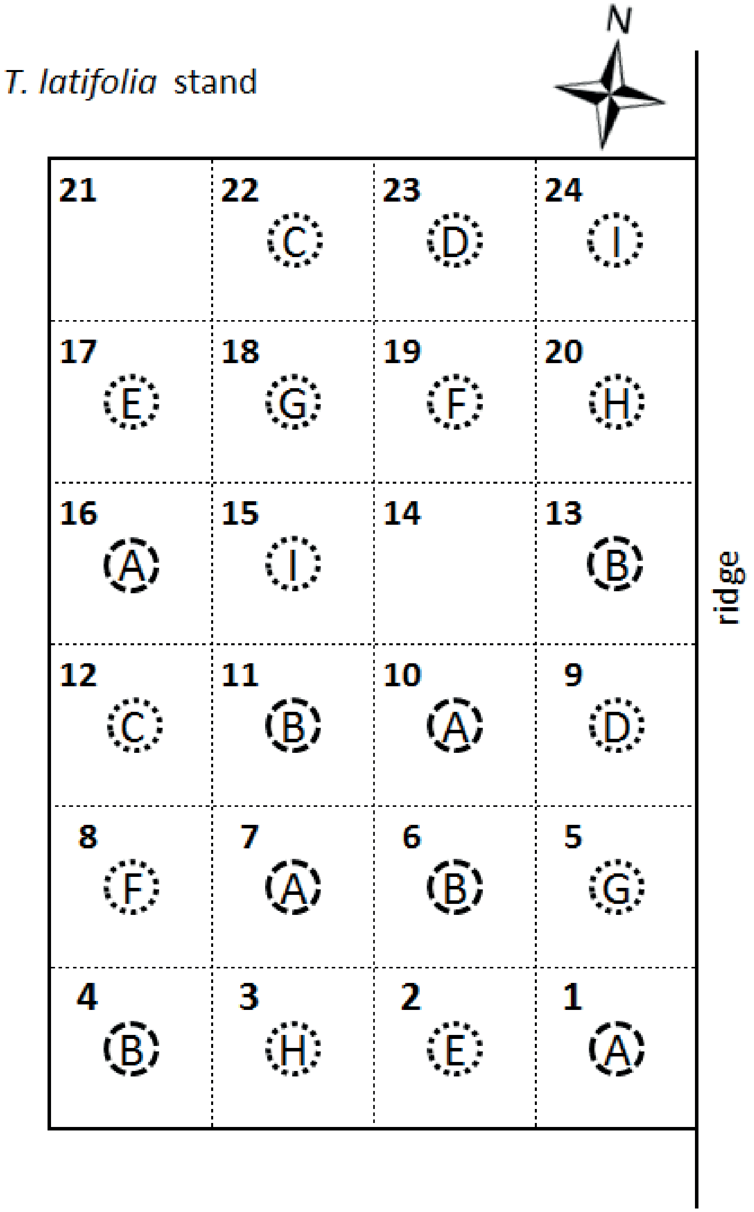

Figure 5.2. Experiment II and III. Schematic overview of experimental field (6.4 × 9.6 m) divided into 24 equal plots (1.6 × 1.6 m) used for harvesting *T. latifolia* biomass. The experimental field was a subfield (≈0.35 ha) of a planted *T. latifolia* stand. *T. latifolia* plants surrounded the experimental field on the north, west and south sides. On the east side of the field there was a soil ridge. Harvested surfaces were circles of 0.79 m^2 per plot (0.5 m radius). Plots with letters A and B (dashed circles) were used for Experiments II and III. Plots with letters C–I were only used for Experiment II (dotted circles). Plants outside the harvested surfaces (circles) were not harvested during 2017. Two subplots (nos. 14 and 21) were not used for the experiments. Harvest dates were 19 May (A, B), 02 June (C), 09 June (A), 16 June (D), 30 June (A, B, E), 21 July (A, F), 11 August (A, B, G), 01 September (A, H) and 22 (A, B, I) September 2017.

In Experiment I, soil samples were taken in May 2015, June 2017 and September 2017. Ditch water samples were taken in July and November 2015, and four times in 2016 with a monthly interval from July 2016 onwards. In Experiments II and III, soil samples were taken in July 2017 at four sub-locations distributed over the field and were analysed separately. Ditch water samples were taken four times at monthly intervals from May 2017 onwards. Average results of soil and ditch water analyses are shown in Table 5.1. During the years of sample collection, 2016 (Experiment I) and 2017 (Experiments II and III), average temperatures from March to the end of October were equal to (Experiment I) or 0.5 °C higher than (Experiments II and III) the 20-year average. Total precipitation from March to the end of October were 37 mm (Experiment I) and 11 mm (Experiments II and III) lower than the 20-year average (de Bilt, KNMI, Table 5.1).

Table 5.1. Soil, ditch water and weather averages (± standard deviations) determined at the sites used for experiments I and II + III.

	Experiment I	Experiment II and III
Soil		
Soil organic matter (%)	47 ± 0.1	75 ± 1.2
Total C (g kg^{-1})	240 ± 0.2	397 ± 0.6
Total N (g kg^{-1})	20 ± 0.4	24 ± 0.4
Total P (g kg^{-1})	1.8 ± 0.24	0.5 ± 0.15
Total K (g kg^{-1})	3.2 ± 1.94	1.8 ± 0.35
pH-NaCl	5.3 ± 0.0	4.1 ± 0.2
Ditch water		
NO_3-N (mg L^{-1})	0.20 ± 0.31	0.07 ± 0.07
NH_4-N (mg L^{-1})	0.27 ± 0.13	2.10 ± 2.00
P (mg L^{-1})	0.28 ± 0.21	0.09 ± 0.01
K (mg L^{-1})	7.6 ± 2.4	13.3 ± 1.7
Weather		
Mean temperature March-October °C, year of measurement	13.5	14.0
Precipitation March-October mm, year of measurement	545	571
Mean temperature March-October °C, 1998-2017	13.5 ± 0.5	
Precipitation March-October mm, 1998-2017	582 ± 116	

5.2.3 Analytical methods used for evaluation of nutritional values

Nutritional value was evaluated following widely used methods and component analyses for nutritional evaluation of forage and determination of ruminant dietary requirements. These were:

1. the proximate analyses which include moisture, crude ash (ash), crude protein (CP), crude fibre (CF), crude fat, starch and sugars (CVB 2016, NRC 2001, Sauvant et al. 2004);
2. the fractions neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) (Van Soest et al. 1991, CVB 2016, NRC 2001, Sauvant et al. 2004);
3. an *in vitro* organic matter digestibility (IVOMD) assay for forages (Tilley & Terry 1963); and
4. macro and micro mineral analyses of relevant minerals (CVB 2016).

Biomass samples from all experiments were analysed at Eurofins Agro (the Netherlands) for moisture content (oven drying at 70 °C for 24 h), ash (NEN-ISO 5984), CP (determined as total N × 6.25; NEN-ISO 5983-2, Kjeldahl method), CF (NEN-ENISO 6865) and IVOMD. Dry matter (DM) was calculated by subtracting the moisture content from the total sample weight, organic matter (OM) was calculated by subtracting ash from DM, and consequently the quantity of *in vitro* digested organic matter (IVDOM) was calculated using the IVOMD. Samples from Experiment I were also analysed for neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) (Van Soest et al. 1991) and samples of the first two harvest dates were analysed for crude fat (NEN-ISO 6492), starch (NEN-EN-ISO 15914) and sugars (NEN-ISO 3571).

Samples from Experiments II and III were analysed for P, Na, K, S, Ca, Mg, Mn, Cu, Zn, Fe, I, Mo (Experiment II only), Co (Experiment II only) and Se at Radboud University Nijmegen (the Netherlands). Several grams of oven-dried biomass (24 h at 70 °C) were incinerated at 550 °C for four hours in a muffle furnace (Nabertherm GmbH, Lilientahl, Germany) to obtain the crude ash fraction. This remaining fraction of the oven-dried biomass was ground in a ball mill for 4 min at 400 RPM (Fritsch Pulverisette Ball Mill, Fritsch GmbH, IdarOberstein, Germany). Total phosphorous and total potassium contents were determined by digesting 200 mg soil in 4 mL HNO_3 (65 %) and 1 mL H_2O_2 (35 %) in Teflon vessels, heated in an Ethos D microwave (Milestone, Sorisole Lombardy, Italy). Subsequently, inductively coupled plasma emission spectrometry (ICP-OES) was used to measure P, Na, K, S, Ca, Mg, Mn and Fe (IRIS Intrepid II, Thermo Electron corporation, Franklin, MA, USA). Inductively coupled plasma mass spectrometry (ICPMS) was used to measure Co, Cu, Zn, Se, Mo and I (X-series I, Thermo Electron, Bremen, Germany).

5.2.4 Analysis of data

In Experiment III cumulative (c) yields (cDM, cOM, cCP, etc.) were calculated by accumulating yields of subsequent harvests. Development of biomass yields, contents (Experiments I and II) and cumulative biomass yields (Experiment III) over time were approximated by fitting a linear, quadratic or logistic function with harvest day as dependent variable, using stepwise regression. Model selection was based on lowest Akaike Information Criterion (AIC). When a quadratic and a logistic term were both included in the model according to the lowest AIC, a maximum model with either a quadratic or a logistic term was chosen based on the highest adjusted r^2. Model response variates were biomass content or yield parameters and harvest day in which 31 May 2016 for Experiment I, and 19 May 2017 for Experiments II and III were considered as day 1. For Experiment III, effect of harvest frequency was included as a fixed factor in the model with two levels (3- or 6-weekly harvest interval), and model coefficients were analysed for statistical differences between harvest frequency using ANOVA. All analyses were performed in R (version 3.4.0, R Core Team 2017) using the functions ‘lm ’, ‘step’ and ‘anova’ (using package stats version 3.4.0).

5.3 Results

5.3.1 Experiments I and II: effect of harvest date on biomass yields and nutritional values

Average shoot densities were 68 ± 1.9 m^{-2} and 40 ± 1.9 shoots m^{-2} in Experiments I and II, respectively, and on average 16 % and 43 % of the shoots produced an inflorescence in Experiments I and II, respectively. In both experiments, lowest observed biomass DM yields were observed at the first harvest (5.20 Mg ha^{-1} for Experiment I at 31 May and 2.89 Mg ha^{-1} for Experiment II at 19 May). Peak DM yields were observed at 15 September (9.81 Mg ha^{-1}, Experiment I) and 30 June (10.89 Mg ha^{-1}, Experiment II), while later in the season observed DM yields were lower (7.39 Mg ha^{-1} for Experiment I at 28 October and 7.30 Mg ha^{-1} for Experiment II at 22 September) (Figure 5.3, Table S5.1).

In both experiments harvested biomass showed an increase in DM, OM and CF, and a decrease in IVDOM and CP, over time; with highest IVDOM and CP, and lowest CF, at the first harvest and the greatest nutritional value changes between the first and the second harvests (Figure 5.3, Table S5.1). In both experiments, the DM increase was approximated by a logistic function (r^2 adj. 0.26 and 0.99 for Experiments I and II, respectively), with the difference that in Experiment I average DM contents at 31

May and 07 July were similar while in Experiment II the DM content of wet biomass increased during that part of the season (86 g kg^{-1} increase between 02 June and 21 July). Dry biomass OM content increases (r^2 adj. 0.71 and 0.85 for Experiments I and II, respectively) and CP decreases (r^2 adj. 0.74 and 0.96 for Experiments I and II, respectively) over time were approximated by logistic functions because of the relatively strong OM increase and strong CP decrease between the first two harvest dates. In both experiments, CP decreased on average from 125 to 75 g kg^{-1} between the first two harvest dates. The IVDOM decrease and CF increase over time were approximated by linear curves for Experiment I (r^2 adj. 0.86 in both cases) and logistic curves for Experiment II (r^2 adj. 0.97 and 0.72 for IVDOM and CF, respectively).

The IVOMD decreased from 63 % to 19 % in Experiment I between 31 May and 28 October (Table 5.2) and from 69 % to 28 % in Experiment II between 19 May and 22 September. In Experiment I, at the first two harvests (31 May and 07 July), the crude fat content was on average 20 g kg^{-1}, the starch content was on average 21 g kg^{-1} and the sugar content was on average 64 g kg^{-1} (dry mass basis in all cases). Moreover, in Experiment I, the dry mass NDF, ADF and ADL contents increased between the first and last harvests. NDF, ADF and ADL averages ranged from 653 to 742, 344 to 512 and 52 to 93 g kg^{-1}, respectively (Table 5.2). In Experiment II P, K and S contents versus harvest date were approximated linearly (r^2 adj. 0.20, 0.67 and 0.27, respectively) with the highest concentrations at the first harvest date (Figure 5.4, Table S5.2). Ca, Mg, Mn, I and Se contents versus harvest date were approximated by a logistic function (r^2 adj. 0.39, 0.37, 0.09, 0.32 and 0.25, respectively), all with the highest approximated content in early June, around the time the inflorescences appeared. Fe content was lowest around the fourth harvest (30 June), and Fe content versus harvest date was approximated with a quadratic curve (r^2 adj. 0.23). For Na, Cu, Zn, Mo and Co contents versus harvest date no specific relationship seemed to be present.

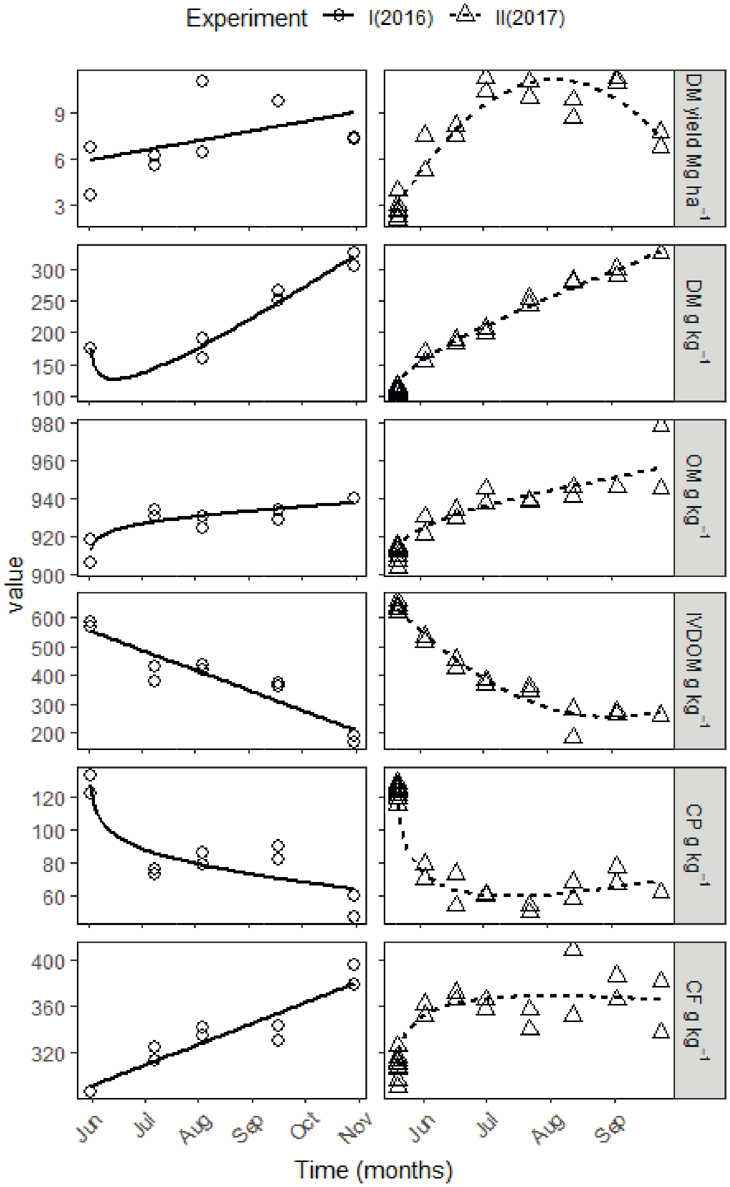

Figure 5.3. Experiment I and II: Effect of harvest day on dry matter (DM) yield and biomass DM, and on the dry matter contents of organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) in *T. latifolia* biomass one year after planting. Harvest dates were 31 May, 07 July, 03 August, 15 September and 28 October 2016 for Experiment I and 19 May, 09 June, 16 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017 for Experiment II. Each set of points per harvest date (n ≥ 2) represents the nutrient concentrations of biomass harvested for a first time in the growing season. In Experiment I DM contents of the 07 July harvest were not determined. Fit parameters of the curves are shown in Table S5.1.

Table 5.2. Experiment I: Effect of harvest day on *in vitro* organic matter digestibility (IVOMD), and the dry matter contents of crude fat (Cfat), sugars, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) in *T. latifolia* biomass one year after planting. Each figure represents measurements from two different subplots, harvested for the first time in the growing season (2016). DM = dry matter; ND = not determined.

Harvest date	IVOMD (%)	Cfat ($g\,kg^{-1}$)	Sugars ($g\,kg^{-1}$)	Starch ($g\,kg^{-1}$)	NDF ($g\,kg^{-1}$)	ADF ($g\,kg^{-1}$)	ADL ($g\,kg^{-1}$)
31 May	63.1	21	57	10	653	344	52
07 July	43.3	18	71	32	655	396	58
03 August	45.7	ND	ND	ND	663	422	69
15 September	39.3	ND	ND	ND	676	438	67
28 October	19.0	ND	ND	ND	742	512	93

5.3.2 Experiment III: effect of harvest frequency on *T. latifolia* stand development, biomass yields and nutritional values

Frequent cutting apparently prevented the plants from producing inflorescences in Experiment III. On average, the observed number of shoots decreased during the experiment from 29 to 15 shoots m^{-2} at the 3-week harvest interval and 35 to 26 shoots m^{-2} at the 6-week harvest interval. However, the developments of the number of shoots (Figure 5.5 and Table S5.3) appeared difficult to approximate by linear functions (r^2 adj. 0.29). According to the linear models, the number of shoots differed significantly at the first harvest date (19 May) ($p = 0.003$) but the approximated linear decrease did not differ significantly between the 3-week and 6-week harvest intervals ($p = 0.711$). Average cumulative DM yields did not differ significantly ($p = 0.190$, $n = 4$ per harvest interval) and were 5.16 ± 0.57 Mg ha^{-1} (3-week harvest interval) and 6.51 ± 0.71 Mg ha^{-1} (6-week harvest interval). DM yields per cut and harvest interval decreased between 19 May and 22 September from 2.97 Mg ha^{-1} to 0.09 Mg ha^{-1} at the 3-week harvest interval and from 2.80 Mg ha^{-1} to 0.43 Mg ha^{-1} at the 6-week harvest interval.

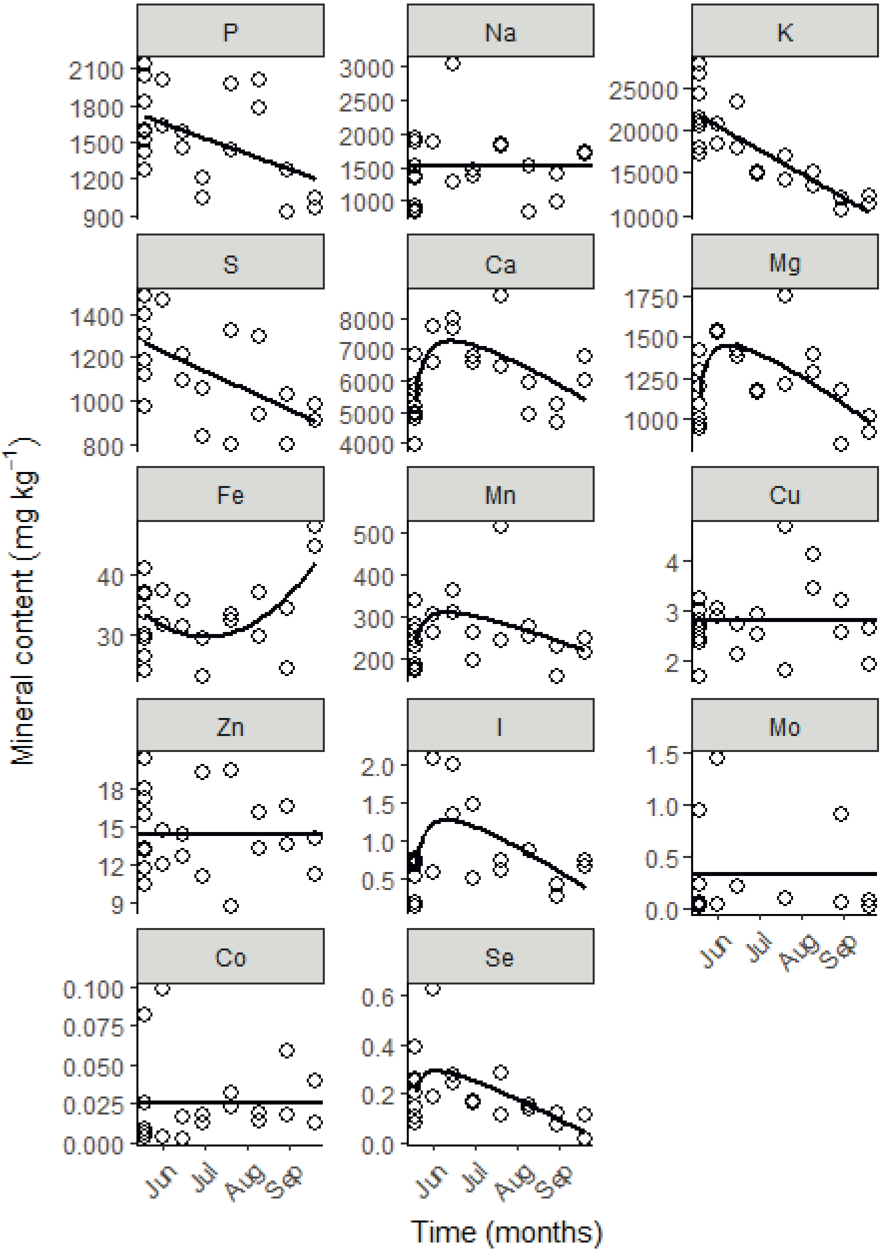

Figure 5.4. Experiment II: Effect of harvest day on the dry matter contents of minerals in *T. latifolia* biomass one year after planting. Harvest dates were 19 May, 02 June, 16 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017. Each set of points per harvest date (n ≥ 2) represents biomass mineral concentrations from two different subplots, harvested for the first time in the growing season. Fit parameters of the curves are shown in Table S5.2.

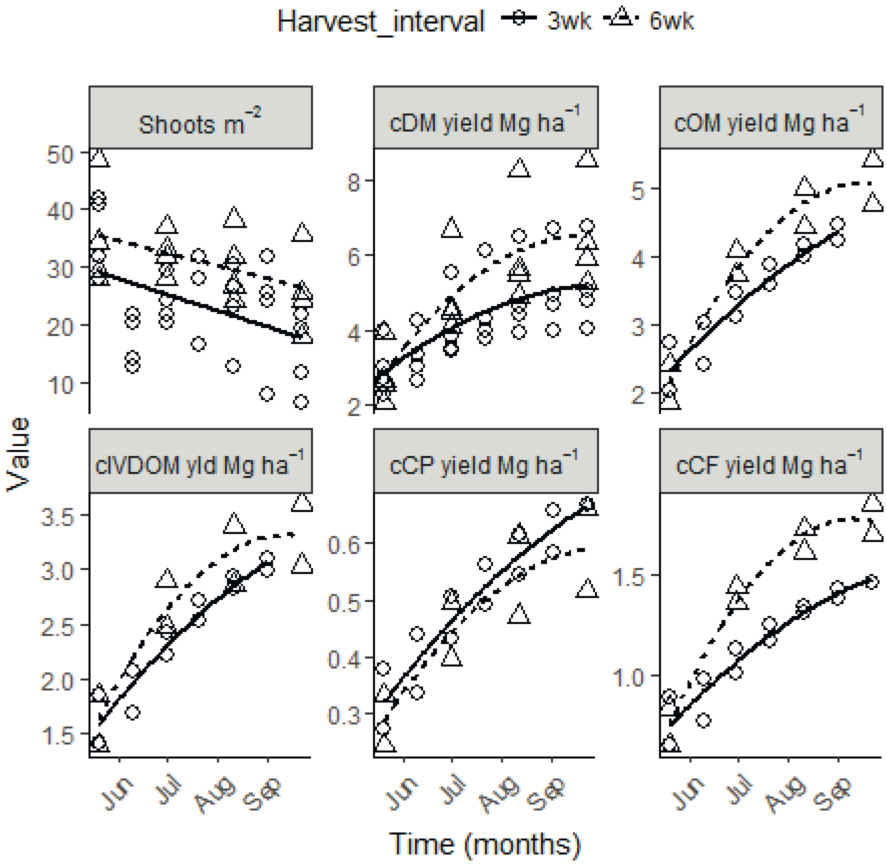

Figure 5.5. Experiment III: Effect of harvesting frequency (3-week or 6-week intervals) on shoot density and cumulative dry matter (DM), organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) yields of *T. latifolia* biomass contents one year after planting. Harvest dates were 19 May, 02 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017. Fit parameters of the curves are shown in Table S5.3.

The 6-weekly harvesting frequency resulted in a faster increase of cCF yields (p = 0.015 and p = 0.032 for the linear and quadratic effects, respectively) and a tendency towards a faster increase of cOM yield (p = 0.072 for the linear slope coefficient of cOM) compared to the 3-weekly harvesting frequency. Harvesting frequency did not significantly affect the development of cDM, cOM, cIVDOM and cCP yields, since there were no significant differences between the slope coefficients of the different harvesting frequencies. When comparing the average IVDOM, CP and CF contents of biomass harvested after May to biomass harvested in May, the nutritional value of biomass harvested at 3-week intervals remained similar to the nutritional quality of spring biomass harvested in May while biomass harvested at 6-week intervals had lower IVDOM ($p < 0.001$) contents (Table 5.3). DM contents of biomass harvested after May was higher at both harvest frequencies compared to the harvest at 19 May ($p < 0.001$).

Table 5.3. Experiment III. Dry matter (DM) yield, and dry matter contents of organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) for the May harvest (Experiment II) and average contents of harvested biomass from the 3-weekly (6 cuts) and 6-weekly (3 cuts) subsequent harvests. SEM = standard error of the mean.

	19 May harvest (first cut)	**3-week harvest intervals, 08 June to 22 September**	**6-week harvest intervals, 30 June to 22 September**	**SEM**	***p*-value**
DM (g kg^{-1})	112^{c}	153^{b}	195^{a}	7.9	<0.001
OM (g $kgDM^{-1}$)	911^{ab}	901^{a}	917^{b}	2.6	0.020
IVDOM (g $kgDM^{-1}$)	624^{a}	591^{a}	466^{b}	16.3	<0.001
CP (g $kgDM^{-1}$)	123^{ab}	134^{a}	103^{b}	5.1	0.023
CF (g $kgDM^{-1}$)	304	286	303	5.7	0.328

^{abc} Values with an unequal superscript differ significantly ($P \leq 0.05$)

Harvesting frequency did not significantly affect the development of cumulative mineral yields over time. However, for cSe a tendency towards a faster yield increase for the 6-weekly compared to the 3-weekly harvesting frequency was observed (Figure 5.6 and Table S5.4). Harvesting frequency had a significant effect on the intercept of the approximated yield developments for cNa ($p = 0.002$), cMg ($p = 0.039$), cCu ($p = 0.015$), cI ($p = 0.037$) and cSe ($p = 0.001$).

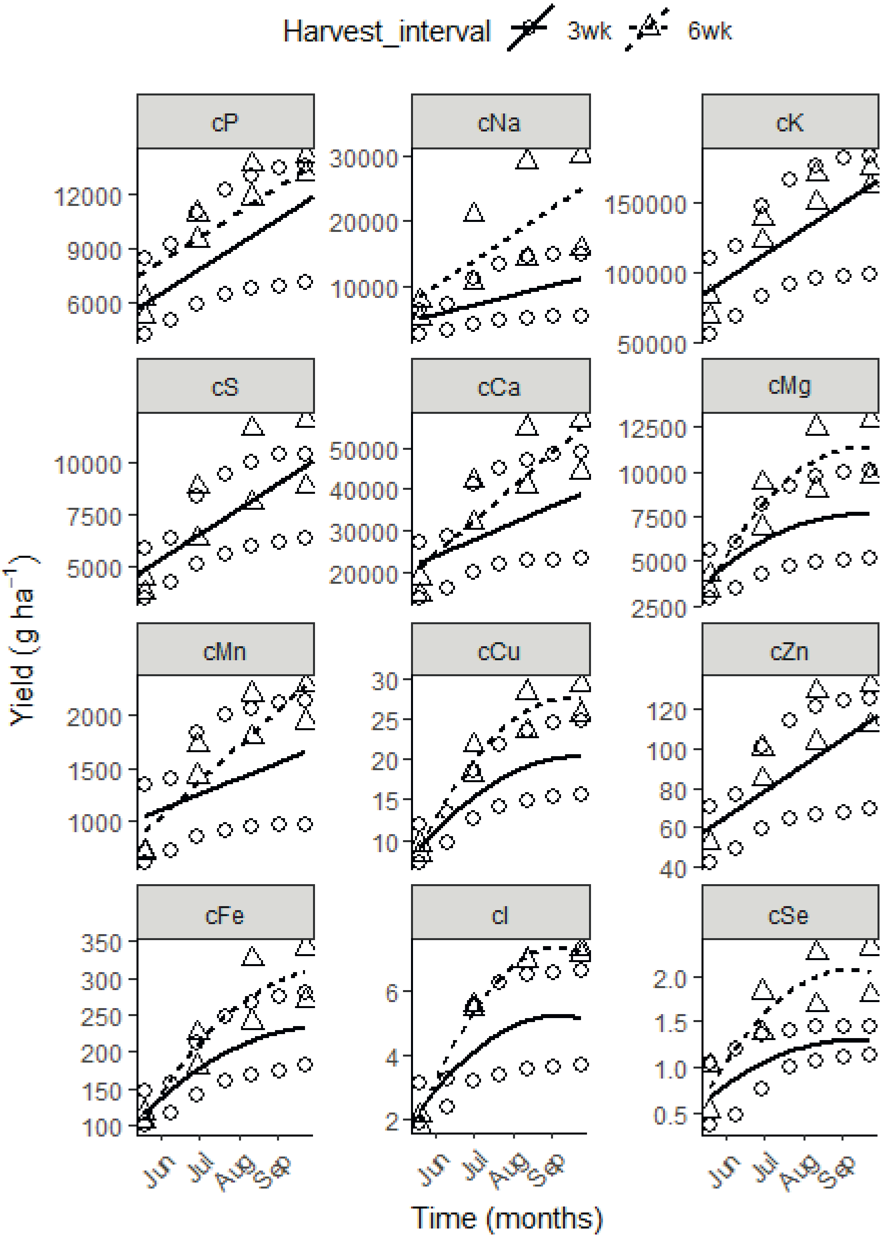

Figure 5.6. Experiment III: Effect of harvest interval (3-week or 6-week intervals) on cumulative (c) mineral yields of *T. latifolia* biomass one year after planting. Harvest dates were 19 May, 02 June, 30 June, 21 July, 11 August, 01 September and 22 September 2017. Each set of points per harvest date (n = 2) represents biomass nutrient concentrations from two different subplots. Fit parameters of the curves are shown in Table S5.4. Cumulative yields of K, S and Zn were approximated by (nearly) the same curve for both harvest intervals, therefore only the fitted curve for the 3-week interval is visible for these minerals.

5.4 Discussion

5.4.1 Effect of harvest date on yields and nutritional values

We hypothesised that biomass yields would peak between the middle and end of the growing season and that, as in (other) grasses, the nutritional value of *T. latifolia* would decrease with increasing crop age, with a sharp decrease before onset of flowering. These hypotheses were confirmed in Experiments I and II. The IVOMD of *T. latifolia* was, especially from June onwards, low (on average < 50 %). Thus, for optimal forage quality *T. latifolia* biomass should be harvested in May, before the appearance of inflorescences and the onset of nutrient translocation to belowground biomass as known for other perennial grasses such as *Lolium perenne* (Parsons 1988). From June onwards we observed a decrease of 42 and 50 % of CP content in Experiments I and II, respectively. Similar results were reported by Grosshans (2014) who observed that due to translocation the content of nutrients in the shoot tissue can decrease by more than 50 % in *Typha glauca*, and by Maddison et al. (2009) who found that N and P were translocated from shoots to reserve organs (mainly rhizomes but also inflorescences) after the flowering stage. Maddison et al. (2009) measured, at a water N load of 138 kg ha^{-1} $year^{-1}$, dry matter CP contents between 79 and 151 g kg^{-1} during the growing season, which corresponds well to our results from Experiments I and II (CP content of 75 to 125 g kg^{-1}). In our Experiments I and II we observed similar CP content decreases despite a fertiliser N amendment and higher soil and ditch water N contents at the site of Experiment II compared to Experiment I. It is possible that plant N uptake or growth was limited by other factors since we observed very similar CP levels in both experiments.

Apparently, 0.5 °C lower average temperatures during the growing season, lower N concentrations in soil and ditch water (Table 5.1), no N and K amendments and biomass removal in the year of planting in Experiment I compared to Experiment II had limited effects on the biomass yield and contents in the consequent year, since we observed similar peak DM yields (9.81–10.89 Mg ha^{-1}) and developments of OM, IVDOM, CP and CF in Experiments I and II, although peak yields were in September for Experiment I and in June for Experiment II. Biomass nutrient removal from the site could have significantly affected nutrient cycling (Jordan et al. 1990). Possibly, the higher average plant density (and thus lower yield per shoot) in Experiment I (68 ± 1.9 shoots m^{-2}) compared to Experiment II (40 ± 1.9 shoots m^{-2}) compensated the yield per ha.

DM yields in our study could have been limited because the stand was not fully developed, despite the observed great increases in shoot densities (increase from

15 to 68 in Experiment I and from 3.5 to 40 shoots m^{-2} between the first year of planting and the second year of measurements). Possibly, plants put most of their resources into offspring when competition is limited. Fiala (1978) observed that each *T. latifolia* plant can produce up to 46 new shoots in the first year after planting when there was no competition. Heinz (2012) reported summer dry matter (DM) yields of up to 12 Mg ha^{-1} in the second year after establishment and Pfadenhauer & Wild (2001), who planted *T. latifolia* at a density of 0.5–2 plants m^{-2} in 1998, observed average increases to 75 and 102 shoots m^{-2} in the growing seasons of 1999 and 2000, respectively, with corresponding DM yield increases to about 8.9 Mg ha^{-1} in 1999 and 20.1 Mg ha^{-1} in 2000. Furthermore, observed yields might have been affected by (unknown) inter-annual variations. Maddison et al. (2009), Heinz (2012) and Günther et al. (2015) reported inter-annual variations of 2–8 Mg ha^{-1} DM yield in different *T. latifolia* stands, which only in some of the cases could be related to events such as late winter or spring harvests before the growing season, storms and spatial variations in stand densities. Thus, to determine the effects of increased stand ages and inter-annual variations on the sustainability of biomass production potential and biomass contents of *T. latifolia* in any particular situation, the current work should be considered as tentative and long-term effects need further investigation.

5.4.2 Effect of harvest frequency on yields and nutritional values

We hypothesised that an increased harvesting frequency (as in a situation with grazing) may maintain nutritional value but reduce productivity and shoot density during a growing season. The reduction of productivity is not confirmed by the results of Experiment III because the results showed that *T. latifolia* can be harvested repeatedly and intensively (at intervals of either 3 or 6 weeks) during a growing season and still produce biomass after each harvest. However, frequent harvesting seemed to have adverse effects on shoot density, which decreased over time, and this may influence productivity in the years to come. Also, DM yields decreased during the season and cumulative DM yields in Experiment III were lower than the yield from a single harvest per growing season when harvested in mid-June or later in the season (Experiments I and II), which may have been related to the decreasing shoot density. Therefore, the effects of frequent harvesting and grazing on biomass production in subsequent years need further investigation as our observations are limited to one growing season. Possibly, harvesting only two or three times instead of four or seven times during a growing season could result in an improved balance between yields, fodder quality, plant regrowth and stand development. Our observation that *T. latifolia* regrowth persisted during the most intensive harvesting scheme is in line with the findings of Hellsten et al. (1999) and Lishawa et al. (2017), who studied effects of mowing *Typha* spp. underwater with the objective of plant removal and concluded that above-water biomass removal is not an efficient strategy to completely remove natural stands.

The average fodder quality of cumulatively harvested biomass, when defined as highest IVDOM and CP and lowest CF contents, was very similar to that of a single harvest in May, especially for biomass harvested at 3-week intervals. This confirmed our hypothesis that intensive harvesting may lead to a rather stable nutritional value. Three-week harvest intervals led to a slightly better nutritional value compared to 6-weekly harvest intervals, which is similar to grasses such as *L. perenne* where more frequent harvesting also leads to improved fodder value (Parsons 1988).

5.5 General findings

We concluded that a harvest in July and August was optimal for yielding the greatest quantity of *T. latifolia* fodder. For the highest nutritional value, the optimal harvest was around May, before the appearance of inflorescences and at relatively low standing crop. The nutritional value of *T. latifolia* was inferior to fresh biomass from grassland dominated by *L. perenne* (Eurofins Agro 2017) when using dairy cow requirements (CVB 2016) as a quality standard, even when harvested before the appearance of inflorescences.

Furthermore, we found that the regrown biomass harvested at either 3-week or 6-week intervals after a first harvest in May can be nutritionally similar to biomass harvested in May. Thus, *T. latifolia* could potentially be grazed by adapted cattle (e.g. *Bubalus arnee*). However, cumulative DM yields were lower than a single yield per growing season when harvested in mid-June or later in the season. For the interpretation of our results, it is important to point out that the experiments described here were done at a relatively low nutrient availability and at a young stand age, which probably resulted in lower seasonal biomass DM yields than could be obtained from older or further developed *T. latifolia* stands with higher nutrient availabilities (Maddison et al. 2009, Pfadenhauer & Wild 2001, Heinz 2012).

Based on our results, we suggest that *T. latifolia* biomass harvested later in the season could be used as fibrous roughage at low dietary inclusion rates, whereas the biomass harvested before the appearance of inflorescences could be used at higher inclusion rates in grass based dairy rations. This introduces a promising prospect for lowering dietary N and P contents, which would allow the reduction of N and P losses to the environment from dairy farms on peat soils. Also , *T. latifolia* biomass appeared to have higher contents of Se, which is interesting because biomass from grassland dominated by *L. perenne* is typically deficient in Se in terms of dairy cow requirements (Table 5.4; CVB 2016, Eurofins Agro 2017).

Table 5.4. Average dry matter mineral contents in *T. latifolia* biomass (Experiment II) and fresh grass from *L. perenne* dominated dairy grasslands in the Netherlands (Eurofins Agro 2017).

	***T. latifolia* biomass**	***L. perenne* dominated fresh grass biomass**
P (g kg DM^{-1})	1.7	4.2
Na (g kg DM^{-1})	1.3	2.3
K (g kg DM^{-1})	22.1	34.1
S (g kg DM^{-1})	1.2	3.5
Ca (g kg DM^{-1})	5.3	5.3
Mg (g kg DM^{-1})	1.1	2.4
Fe (mg kg DM^{-1})	32.2	169.0
Mn (mg kg DM^{-1})	237.8	73.0
Cu (mg kg DM^{-1})	2.6	8.4
Zn (mg kg DM^{-1})	15.0	40.0
I (mg kg DM^{-1})	0.6	0.2
Mo (µg kg DM^{-1})	263	1900
Co (µg kg DM^{-1})	25	67
Se µg kg DM^{-1}	199	56

To further unlock the potential of *T. latifolia* as a (complementary) forage in combination with (intensive) dairy farming on peat, further research is needed on aspects such as breeding, establishing and maintaining crops, managing optimal nutrient supplies, and optimising harvesting and conservation techniques. Furthermore, effects of dietary inclusion on animal performance and possible nutritional constraints should be investigated, as well as the impact on the economy and ecology of farms and surrounding regions.

Acknowledgements

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Supplementary materials

Table S5.1. Experiments I and II: Effect of harvest day on dry matter (DM) yield and DM, and on the dry matter contents of organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) in *T. latifolia* biomass one year after planting. Fitted parameters to the (maximum) function $y = intercept + harvest\ date + harvest\ date^2 + \log(harvest\ date) + error$, in which *y* represents DM yield or biomass content variables and *harvest date* the number of days after the first harvest date (31 May for Experiment I, 19 May for Experiment II). SE = standard error.

	Experiment	**Intercept**		**Harvest date**		**Harvest date^2**		**log(Harvest date)**		**r^2 adj.**
		Value	**SE**	**Value**	**SE**	**Value**	**SE**	**Value**	**SE**	
DM yield (Mg ha^{-1})	I	5.90	1.11	0.021	0.012					0.26
	II	2.87	0.38	0.219	0.019	$-1.44 \cdot 10^{-3}$	$1.61 \cdot 10^{-4}$			0.90
DM (g kg^{-1})	I	172.9	11.2	1.88	0.24			-27.35	6.60	0.96
	II	108.4	2.4	1.21	0.07			13.43	1.57	0.99
OM (g kgDM^{-1})	I	912.4	3.9	0.04	0.06			3.72	1.82	0.71
	II	910.6	2.5	0.18	0.07			4.68	1.60	0.85
IVDOM (g kgDM^{-1})	I	554.6	27.3	-2.28	0.30					0.86
	II	634.9	9.7	-7.35	0.52	$3.54 \cdot 10^{-2}$	$4.61 \cdot 10^{-3}$			0.97
CP (g kgDM^{-1})	I	126.3	9.2	-0.06	0.15			-10.59	4.28	0.74
	II	122.4	2.2	0.38	0.07			-20.94	1.49	0.96
CF (g kgDM^{-1})	I	290.8	6.9	0.59	0.08					0.86
	II	309.1	5.9	-0.24	0.17			18.00	3.78	0.72

Table S5.2. Experiment II: Effect of harvest day on dry matter mineral contents of *T. latifolia* biomass one year after planting. Fitted parameters to the (maximum) models *y = intercept + harvest day + harvest day 2+ error* or *y = intercept + harvest day + log(harvest day)+ error*, in which *y* represents biomass concentration and *harvest day* the harvest date where the 19 May 2017 was set as day 1. SE = standard error.

	Intercept		**Harvest day**		**Harvest day^2**		**log(Harvest day)**		**r^2 adj.**
	Value	**SE**	**Value**	**SE**	**Value**	**SE**	**Value**	**SE**	
P (mg kg^{-1})	1705	99	-4.00	1.61					0.20
Na (mg kg^{-1})	1517	106							
K (mg kg^{-1})	21693	832	-89.70	13.53					0.67
S (mg kg^{-1})	1262	59	-2.84	0.96					0.27
Ca (mg kg^{-1})	5330	335	-31.78	9.78			842.23	215.09	0.39
Mg (mg kg^{-1})	1132	65	-7.05	1.91			152.34	42.05	0.37
Fe (mg kg^{-1})	33.3	1.9	-0.171	0.097	$1.89\cdot10^{-3}$	$8.18\cdot10^{-4}$			0.23
Mn (mg kg^{-1})	237.8	25.7	-1.419	0.752			33.36	16.53	0.09
Cu (mg kg^{-1})	2.8	0.1							
Zn (mg kg^{-1})	14.4	0.7							
I (mg kg^{-1})	0.6	0.1	-0.014	0.004			0.318	0.094	0.32
Mo (mg kg^{-1})	$3.22\cdot10^{-1}$	$1.29\cdot10^{-1}$							
Co (mg kg^{-1})	$2.60\cdot10^{-2}$	$6.06\cdot10^{-3}$							
Se (mg kg^{-1})	$2.09\cdot10^{-1}$	$3.80\cdot10^{-2}$	$-3.12\cdot10^{-3}$	$1.11\cdot10^{-3}$			$4.78\cdot10^{-2}$	$2.44\cdot10^{-2}$	0.25

Table S5.3. Experiment III: Effect of harvesting frequency (3-weekly or 6-weekly) on shoot density and cumulative (c) dry matter (DM), organic matter (OM), *in vitro* digestible organic matter (IVDOM), crude protein (CP) and crude fibre (CF) yields of *T. latifolia* biomass contents one year after planting. Fitted parameters to the (maximum) model *y = intercept × harvest interval + harvest day × harvest interval + harvest day2 × harvest interval + error*, in which *y* represents the biomass yield, *harvest interval* is 3 weeks or 6 weeks and *harvest day* is number of days after 19 May 2017 (set as day 1). SE = standard error.

	Intercept					Harvest day	
Harvest interval	**3 weeks**		**6 weeks**			**3 weeks**	
	Value	**SE**	**Value**	**SE**	***p*-value**	**Value**	**SE**
Shoot density (m^{-2})	29.1	2.6	8.9	4.1	0.003	-0.091	0.034
cDM (Mg ha^{-1})	2.702	0.414	0.613	0.556	0.016	4.06·10-2	1.30·10-2
cOM (Mg ha^{-1})	2.315	0.195	2.117	0.285	0.001	2.49·10-2	8.58·10-3
cIVDOM (Mg ha^{-1})	1.492	0.133	1.666	0.183	0.014	2.42·10-2	4.35·10-3
cCP (Mg ha^{-1})	0.310	0.033	0.292	0.044	0.223	4.18·10-3	1.03·10-3
cCF (Mg ha^{-1})	0.739	0.055	0.733	0.081	<0.001	8.63·10-3	2.09·10-3

Table S5.4. Experiment III: Effect of harvest interval (3 weeks or 6 weeks) on cumulative (c) mineral yields of *T. latifolia* biomass one year after planting. Fitted parameters to the (maximum) model *y = intercept × harvest interval + harvest day × harvest interval + harvest day2 × harvest interval + error* or *y = intercept × harvest interval + harvest day × harvest interval + log(harvest day) × harvest interval + error*, in which *y* represents biomass yield variables, *harvest interval* the harvesting interval (3-week or 6-week interval) and *harvest day* the number of days after the first harvest on 19 May 2017. SE = standard error.

	Intercept					Harvest day	
Harvest interval	**3 weeks**		**6 weeks**			**3 weeks**	
	Value	**SE**	**Value**	**SE**	***p*-value**	**Value**	**SE**
cP (g ha^{-1})	5918	1069	7698	1156	0.140	45.4	12.7
cNa (g ha^{-1})	4689	2659	8113	4209	0.002	50.5	34.7
cK g (ha^{-1})	86694	12596				592.4	162.3
cS g (ha^{-1})	4855	724				39.3	9.3
cCa (g ha^{-1})	17847	6046	17506	8116	0.127	363.8	189.3
cMg (g ha^{-1})	3725	1230	3959	1652	0.039	75.2	38.5
cMn (g ha^{-1})	1040.4	231.1	894.5	365.7	0.257	4.79	3.02
cCu (g ha^{-1})	8.2	2.1	9.3	2.8	0.015	0.22	0.06
cZn (g ha^{-1})	59.6	8.6	60.0	0.1	0.195	0.43	0.11
cFe (g ha^{-1})	110.2	24.9	109.8	33.4	0.061	1.91	0.78
cI (g ha^{-1})	2.0	0.7	2.1	0.9	0.037	0.07	0.02
cSe (g ha^{-1})	0.603	0.173	0.814	0.232	0.001	$1.42\cdot10^{-2}$	$5.41\cdot10^{-3}$

Harvest day			Harvest day²					
6 weeks			3 weeks		6 weeks			
Value	SE	*p*-value	Value	SE	Value	SE	*p*-value	r² adj.
0.054	0.053	0.711						0.29
2.36·10-2	7.09·10-3	0.141	-1.73·10-4	9.48·10-5	-1.73·10-4	9.48·10-5		0.54
4.86·10-2	1.16·10-2	0.072	-5.11·10-5	7.71·10-5	-2.00·10-4	9.70·10-5	0.147	0.91
2.52·10-2	2.66·10-3	0.696	-9.46·10-5	3.68·10-5				0.87
3.86·10-3	5.84·10-4	0.587	-1.16·10-5	7.90·10-6	-1.16·10-5	7.90·10-6		0.80
1.81·10-2	3.09·10-3	0.015	-2.21·10-5	1.66·10-5	-7.84·10-5	2.38·10-5	0.032	0.94

Harvest day			Harvest day²		
6 weeks			3 and 6 weeks		
Value	SE	*p*-value	Value	SE	r² adj.
					0.39
133.0	53.8	0.142			0.52
					0.37
					0.44
498.3	103.5	0.211			0.43
107.1	21.1	0.148	-0.37	0.28	0.50
10.92	4.68	0.207			0.33
0.27	0.04	0.124	$-1.00\cdot10^{-3}$	$4.74\cdot10^{-4}$	0.71
					0.42
1.90	0.01	0.140	0.659	0.426	0.64
0.08	0.01	0.122	$-3.42\cdot10^{-4}$	$1.52\cdot10^{-4}$	0.64
$1.94\cdot10^{-3}$	$2.96\cdot10^{-3}$	0.097	$-7.27\cdot10^{-5}$	$3.96\cdot10^{-3}$	0.66

Chapter 6

General discussion

6.1 Introduction

Dairy farming on peat soil is a source of reactive nitrogen (N) losses to water (mineral and organic N), and of emissions to the air (ammonia (NH_3), nitrous oxide (N_2O) and nitrogen (di)oxide (NO_x)). The severity of these losses depends on the N intensity of the dairy farming system, farm management, the circumstances in which N losses can take place, and the sensitivity of the surrounding environmental system (**Chapter 1**). There is increasing pressure to reduce these N losses and to mitigate their negative effects on the environment, climate and human health. Ambitious targets for N deposition, chemical water quality and greenhouse gas emissions have been put in place by the Dutch government. Peat soil drainage, required for dairy farming on peat soil, also leads to soil subsidence, CO_2 emissions, pressure on biodiversity, the loss of other nutrients to water, and issues regarding water use and safety (Van Beek *et al.* 2004a; Hoogland *et al.* 2012; Van den Born *et al.* 2016; Deru *et al.* 2018). The combination of these issues has resulted in increased pressure on the current way of land use and farming on peat soils.

The main objective of this thesis was to answer the question; Can N cycling on dairy farms on peat soil in the Green Heart area be improved by adapting management to the soil N supply and by using other grassland species and crops without affecting milk production? The following research questions were addressed:

1. What factors drive the quantity and dynamics of Soil N Supply (SNS) throughout the growing season in dairy grassland on peat soil?

2. What is the perspective of reducing herbage N concentrations of perennial ryegrass (*Lolium perenne* L.) on peat soils through breeding, and which pathway influences the N concentration?

3. Can the use of a mixture of perennial ryegrass and plantain (*Plantago lanceolata* L.), a species known to produce secondary metabolites that inhibit nitrification, improve the N cycling of dairy grassland systems on peat soils?

4. What is the potential of cattail (*Typha latifolia* L.), which can be cultivated in waterlogged conditions that may strongly slow down aerobic peat mineralisation, as an alternative forage crop for dairy farming on peat soil?

5. What is the potential effect of increased knowledge of SNS and of the changes studied in grassland populations and species on the N cycle and N loss on the farm?

Section 6.2 synthesises the main findings of the research and gives, based on the results, potential options for dairy farms on peat soil which may reduce farm N losses. Section 6.3 discusses the potential impacts of the options on N cycling and loss. Section 6.4 discusses important uncertainties of the options. In section 6.5, the estimated reduction of N losses of the options explored is compared to policy targets. Section 6.6 provides a reflection on the current research and suggestions for future research. General conclusions of this thesis are given at the end of this chapter.

6.2 Main findings of this thesis

6.2.1 Synthesis of results

A study with the objective to determine factors controlling the quantity and dynamics of SNS was described in **Chapter 2**. It was found that the SNS of dairy grasslands on peat soil can be estimated by soil organic matter (SOM) content and degree days. At an average growing season, the total SNS was estimated at 220 and 297 kg N ha^{-1} at SOM contents of 35 and 55 g 100 g dry soil^{-1}, respectively. The dynamics and the quantity of SNS were estimated similarly in conditions with and without calcium ammonium nitrate applications. The estimation models had residual standard errors of 25-27 kg ha^{-1}.

The use of low-N perennial ryegrass populations was investigated in a pot experiment using a peat substrate and a field experiment on peat soil, described in **Chapter 3**. The objective of the study was to determine whether perennial ryegrass populations with different shoot tissue N concentrations recorded on a sandy soil would also show different shoot tissue N concentrations and N use efficiencies (NUE) or N uptake efficiencies (NUptE) when grown on peat soil. Results of the pot experiment showed that, among tetraploid populations, higher N use efficiencies – but no lower N uptake efficiencies – coincided with lower tissue N concentrations, and that among diploid populations, N uptake efficiencies but not shoot tissue N concentrations differed. The field experiment showed that populations could explain 44% of the variation in N uptake efficiency, but no relation with shoot tissue N concentration was found. Two selected diploid populations had a relatively low and a relatively high recorded shoot tissue N concentration in the pot experiment. Compared to a commercial control, both had a 1.4 g kg^{-1} (4%) lower dry matter (DM) shoot tissue N concentration and a similar DM yield, at a N fertilisation level of 250 kg ha^{-1} in the field experiment (Table 6.1).

Table 6.1. Mean, standard error of the mean (SEM) and explained variance of shoot tissue N concentrations and dry matter (DM) yield of the control, 2Nlow, 2Nhigh, 4Nlow and 4Nhigh populations, at a N fertilisation level of 250 kg ha^{-1} year^{-1}. Shoot tissue N concentrations and DM yields were determined across ten consecutive harvests in the field experiment (**Chapter 3**).

Parameter	**shoot tissue N concentration**	**DM yield**
Unit	g kg DM^{-1}	g m^{-2}
population means		
Control	35.0^{a}	252^{ab}
2Nlow	33.6^{b}	260^{a}
4Nlow	34.4^{ab}	243^{ab}
2Nhigh	33.6^{b}	243^{ab}
4Nhigh	34.7^{ab}	238^{b}
SEM	0.29	7.3
p-value population effect	0.018	0.032

^{abc} values with an unequal superscript differed significantly ($p < 0.05$).

Chapter 4 described a study with the objective to determine the effect of the presence of plantain on the fate of soil mineral N in dairy grassland on peat soil. The study consisted of a mesocosm and a field experiment. In the mesocosm experiment, it was found that plantain significantly reduced potential nitrification, and that the N uptake efficiency of plantain increased at higher plantain shares in the herbage. Soil nitrate concentrations were not affected by the presence of plantain. In the field experiment, N_2O fluxes after mineral fertiliser N application were reduced by 39% in the presence of plantain, compared to grassland without plantain (Figure 6.1). No relation was found between plantain share in the herbage and herbage N uptake efficiency. At a sowing rate of 33% plantain, compared to perennial ryegrass without plantain, no significant differences in annual herbage N concentration, N uptake and DM yields were observed. At a sowing rate of 66% plantain compared to perennial ryegrass without plantain, N uptake and DM yields were 5 and 7% lower, respectively.

Chapter 5 describes a study on the cultivation of cattail on rewetted former dairy grassland on peat soil. At two different sites, three field experiments were carried out in the first year after planting; the objective was to determine the effects of harvest date and frequency on the yield and nutritional value of cattail. Cattail produced a maximum of 9.8-10.9 ton DM ha^{-1} of harvestable biomass, when harvested once a year. The nutritional value was the highest before flowering in May. However, cattail had high crude fibre (CF) concentrations (287-300 g kg DM^{-1}) and low crude protein (CP) (125 g kg DM^{-1}) and phosphorus (1.5 g kg DM^{-1}) concentrations, and a low *in vitro* digestibility of organic matter (IVDOM) (63-69%) compared to perennial ryegrass (means for fresh grass on peat soil; 205 g CF kg DM^{-1}, 204 g CP kg DM^{-1}, 4.1 g P kg DM^{-1}, 83% IVDOM) (CVB 2022). Harvesting in intervals of three or six weeks in the period from May to September resulted in similar cumulative DM yields (5.2 and 6.5 ton DM ha^{-1}, respectively) and a nutritional value similar to the May harvest.

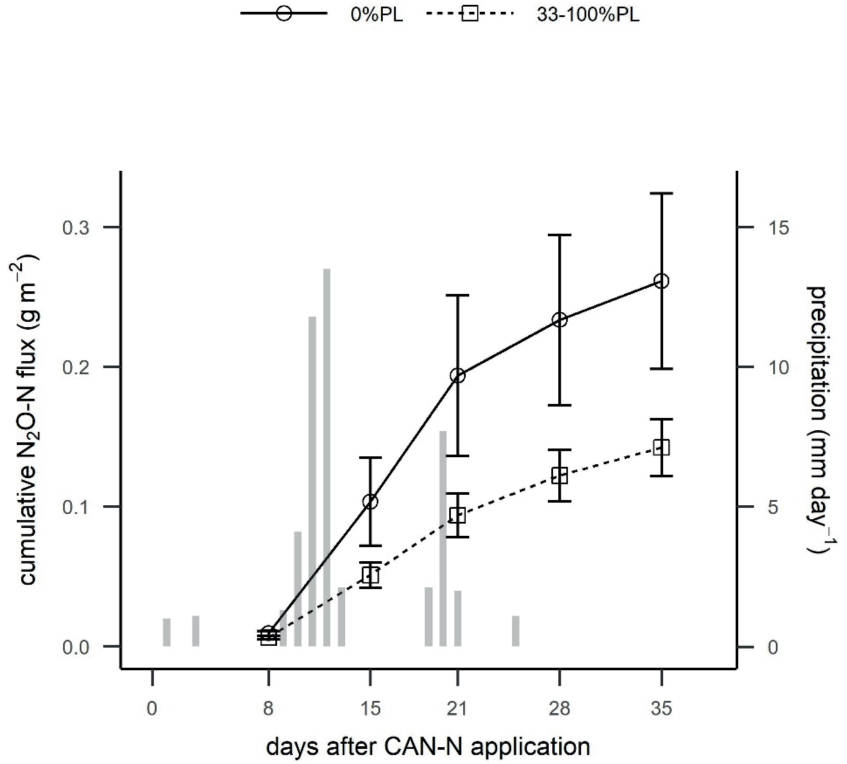

Figure 6.1. Cumulative N_2O-N fluxes were 39% lower in the presence of plantain ($p = 0.021$) after a chemical fertiliser N application of 50 kg ha^{-1} in the field experiment described in **Chapter 4**. The figure shows precipitation (*grey bars*, KNMI, Zegveld, the Netherlands) after CAN-N application, and cumulative N_2O-N fluxes from 8 to 35 days after CAN-N application of the treatment without plantain (*open dots, solid line*) versus all treatments with plantain (33%, 66% and 100% plantain) (*open squares, dashed line*). Error bars represent N_2O-N fluxes standard errors.

6.2.2 Potential implications

The results of the different studies have potential implications for the N cycle of dairy farms on peat soil.

The SNS estimation can be used for more accurate estimations of farm N cycling and losses, and for management adjustments. For example, the Dutch Annual Nutrient Cycling Assessment (ANCA) tool and the Dutch fertiliser advice for dairy grassland on peat soil considers SNS to be constant, regardless of weather and soil variations (CBGV 2022; Van Dijk *et al.* 2022). Moreover, the SNS dynamics estimated by the models can be valuable for the fertilisation practices of farmers at the level of fields or groups of fields when they are combined with different estimates. These are estimates of previous N fertilisation rates, herbage yields and herbage N concentrations, the actual N fertilisation rates and the timing of grazing or mowing can be adapted to yield herbage with a targeted CP concentration. With this approach, field differences in SNS and NUE can be taken into account, and the control on herbage CP concentrations and farm N losses may be improved.

The N flow from soil to herbage may be reduced by about 4% with the use of selected low-N perennial ryegrass populations. This reduction was recorded in the field experiment at a N fertilisation level of 250 kg N ha^{-1} (Table 6.1). Of the range of N fertilisation levels in that experiment, 250 kg N ha^{-1} was the closest to the current practice in the Green Heart area. The mean N fertilisation level on dairy farms on peat soil was 238 kg available N ha^{-1} in the year 2020, assuming that 45% of the N in animal manure is equally available for herbage uptake as chemical fertiliser N (Agrimatie 2022d; RVO 2022), and that mineral fertiliser is not used on extensively managed grassland or for the cultivation of maize (De Jong *et al.* 2022).

At a 33% sowing rate of plantain, N_2O emissions from fertilisation in dairy grasslands on peat can be reduced by 39% (Figure 6.1). At this sowing rate, plantain may have limited effects on milk production because herbage DM and N yields were not different from perennial ryegrass without plantain.

Feeding cattail to dairy cows can reduce the levels of dietary CP and may consequently reduce NH_3 and other N losses on the farm. Furthermore, since cattail can grow in rewetted soil conditions, the use of the crop might result in roughage production while strongly reducing peat oxidation. Mitigating or ceasing peat oxidation can reduce the flow of N from the soil to the crop, compared to drained soil conditions, and may also reduce other N losses as a result of a reduced soil N mineralisation (Williams 1974; Kieckbusch & Schrautzer 2007; Cabezas *et al.* 2012; Hoving *et al.* 2018).

6.3 Potential impact of options on the N cycle of dairy farms on peat soil

6.3.1 Impact of the options

The investigated aspects and options may lead, either individually or combined, to management adjustments that can improve N cycling and reduce N losses on dairy farms on peat soil. To estimate the potential effects of the options on dairy farms on peat soil in the Green Heart area, five options (B-F) were compared to the average situation of 2020 (A), all at current milk production levels. The options were reducing the use of chemical N fertiliser by adapting N fertilisation and grassland management to SNS (B); using low-N perennial ryegrass populations (C); using plantain perennial ryegrass mixtures (D); changing grassland cultivation to the cultivation of cattail (E) or a combination of all options (F).

The ANCA tool (version 2022.06) (Aarts *et al.* 2015; Van Dijk *et al.* 2022) was used to estimate the effects on total farm losses of NH_3 and N_2O, and on losses of NO_x and nitrogen gas (N_2) in and around farm buildings. The potential effects on N losses to water were estimated based on the results of Schipper *et al.* (2016) and Van Rotterdam *et al.* (2021). What was not estimated were the effects of soil tillage at the establishment, and the effects of an establishment period of low-N ryegrass populations, plantain and cattail on N cycling and N losses of the farm. Further assumptions are described in more detail below.

A. *Assumptions for the current situation* Average dairy farm on peat soil in the Green Heart area

An average dairy farm on peat soil in the Green Heart area was defined from parameters described by De Jong *et al.* (2022) for the year 2020. Details of the estimated N cycle of this average farm are given in **Chapter 1**,section 1.5. The farm cultivates 43.3 hectares of productive grassland, 6.7 hectares of extensive grassland and 4 hectares of maize on 75% peat soil and 25% clay soil. The farm herd consists of 105 dairy cows and 52.5 calves and heifers, and the average milk production is 17,403 kg ha^{-1}. Annually 125 kg N ha^{-1} of chemical fertiliser is applied to production grassland, assuming that mineral fertiliser is not used on extensively managed grassland or for the cultivation of maize (De Jong *et al.* 2022).

B. *Assumptions for the option* Adapting chemical fertiliser N use to SNS

- By adapting the fertiliser N use to SNS, the use of chemical fertiliser on production grassland is reduced by 20% up to a maximum of 100 kg N ha^{-1},without an effect on grassland DM yields. This assumption is based on experiences of farmers and measurements in dairy grasslands on peat soil in the polder Groot-Wilnis Vinkeveen (Pijlman *et al.* 2020c; Van Rotterdam *et al.* 2021);

- The herbage N uptake from soil mineralisation and the uptake efficiencies of N from chemical fertiliser and animal manure remain equal. This assumption is based on results of Vellinga & André (1999), who modelled a nearly constant apparent N recovery of chemical fertiliser N between fertilisation rates of approximately 180-250 kg N ha^{-1}. This option leads to a decrease in the mean available N fertilisation level of the production grassland from 238 to 213 kg ha^{-1}, with the assumption that 45% of the N in animal manure is equally available for herbage uptake as chemical fertiliser N (RVO 2022).

C. *Assumptions for the option* Using low-N perennial ryegrass populations

- With the use of low-N perennial ryegrass populations, the annual herbage N concentration decreases by 4% (1.1 g N kg DM^{-1}), but the annual herbage DM yields do not change compared to the average situation, following the results of the field experiment described in **Chapter 3** at 250 kg N ha^{-1} fertilisation.
- Except for CP, the feeding value of the grass remains equal, which is in line with the results of the field experiment (**Chapter 3**).
- There is no shift in partitioning of N uptake between aboveground and belowground biomass of the grass, compared to the current situation.

D. *Assumptions for the option* Sowing 33:67% plantain perennial ryegrass mixtures

- Plantain perennial ryegrassswards consisting for about one third of plantain are established in all grassland of the farm.
- Based on the results of the field experiment described in **Chapter 4**,the presence of plantain does not affect herbage DM and N yields, or N concentrations.
- Dairy cows do not reduce their voluntary feed intake and milk production at a one third plantain inclusion in the herbage, compared to perennial ryegrass-based herbage without plantain. In a feeding experiment with dairy cows who were given a grass-silage-based diet with inclusion of 28% plantain, feed intake and milk production were not reduced compared to a diet without plantain (Pijlman *et al.* 2021).
- The N fertilisation level in all grassland remains equal compared to the average situation, because in the field experiment described in **Chapter 4**, at a sowing rate of 33% plantain, no effect was found of the presence of plantain on herbage N uptake.

E. *Assumptions for the option* The conversion of production grassland to cattail cultivation

- Cattail is planted on former dairy grassland on peat soil, after the removal of the nutrient rich topsoil (approximately 10 cm).
- Cattail is harvested every six weeks during the growing season, and yields 5,543 kg DM ha^{-1} of silage per year. This yield is based on a gross cattail yield of 5,835 kg DM ha^{-1} (experiment III, **Chapter 5)** and estimated average DM losses of 5% at silage production, similar to DM losses observed for grass silage production (Van Dijk *et al.* 2022).
- Cattail receives 150 kg N ha^{-1} chemical fertiliser and no animal manure, and CP concentrations of cattail silage are 120 g kg DM^{-1} (**Chapter 5**). Chemical fertiliser N inputs are therefore slightly higher at farm level compared to the average situation. Animal manure which was applied to the grassland that is converted to cattail in this option leaves the farm.
- 5% of the dietary grass silage inclusion of dairy cows can be substituted by cattail silage, without affecting feed uptake and milk production (Bestman *et al.* 2019). Together, these assumptions result on farm level in a conversion of 3.8 ha production grassland to cattail cultivation.
- The decrease in silage production, due to the lower DM production of cattail compared to grassland cultivation, is assumed to be compensated for by attracting extra grass silage from outside the farm.

F. *Assumption for the combination of options B-E*

- All options (B-E), based on the assumptions described, are combined for an average farm, which means 20% less chemical fertiliser use, the use of low-N perennial ryegrass populations mixed with one third plantain and the cultivation of 3.8 ha cattail.

Table 6.2 presents the estimated potential effects of the options on N cycling and N losses for an average dairy farm on peat soil in the Green Heart area.

Table 6.2. Estimated effect of four potential options (B-E) and a combination of all options (F) at current production levels on annual farm N cycling and losses, for an average dairy farm on peat soil in the Green Heart area (A). Milk production was assumed to remain equal for all options. The potential effects of the options were estimated for a situation after the establishment of new populations or species. Changes in losses were estimated compared to the average situation. Arrows indicate if losses are estimated to increase (↑) or decrease (↓). Estimates of changes in losses are indicated with percentages, or by points of different sizes(· •) for relatively smaller or larger expected effects when a quantitative estimate could not be made.

	A	B
Parameter	**average farm in 2020**	**20% less chemical N fertiliser**
farm parameters		
production grassland, ha [a, b]	43.3	43.3
extensive grassland, ha [a, b]	6.7	6.7
maize cultivation, ha [a, b]	4	4
cattail cultivation, ha [a, b]		
dietary CP level for dairy cows, g kg DM^{-1}[a, c]	173	168
estimated N cycle, kg N ha^{-1}		
inputs		
chemical fertiliser [a]	100	80
deposition [c]	19	19
peat mineralisation [b]	80	80
concentrates [a]	136	136
other feeds and crops [a]	11	11
outputs		
milk and animals [a]	103	103
manuresold [a, b]	35	35
losses		
ammonia in barn and manure storage [c]	26	↓4%
ammonia in soil [c]	24	↓6%
nitrous oxide, nitrogen gas and nitrogen (di)oxide from silage storage, barn and manure [c]	14	↓4%
nitrous oxide in soil from fertilisation	10[c]	↓6%
nitrous oxide in soil from peat mineralisation	4[c]	no effect
leaching and run-off in soil	20[e]	↓4%
nitrogen gas in soil	111[f]	↓14%

a = De Jong *et al.* (2022), b = described assumptions, c = ANCA (Aarts *et al.* 2015; Van Dijk *et al.* 2022), d = **chapter 4** , e = Schipper *et al.* (2016); Van Rotterdam *et al.* (2021), f = difference between soil inputs, herbage and crop uptake on the one hand, and soil ammonia, run-off, leaching and nitrous oxide losses on the other.

Option			
C	D	E	F
low-N perennial ryegrass populations	plantain perennial ryegrass mixture	cattail cultivation	B-E combined
43.3	43.3	39.5	39.5
6.7	6.7	6.7	6.7
4	4	4	4
		3.8	3.8
169	173	172	167
100	100	102	84
19	19	19	19
80	80	unclear	unclear
136	136	136	136
11	11	17	17
103	103	103	103
35	35	43	43
↓4%	negligible effect	no effect	↓4%
↓4%	negligible effect	↓1%	↓7%
↓3%	negligible effect	↓1%	↓7%
negligible effect	↓37%[d]	↓7%	↓•
negligible effect	unclear	↓7%	↓·
negligible effect	unclear	unclear	unclear
negligible effect	unclear	unclear	unclear

6.3.2 N losses from the soil

A 20% reduction (25 kg N ha^{-1}) of chemical fertiliser N use on productive grassland, as a result of adapting N fertilisation to SNS, was estimated to decrease N_2O losses from fertilisation by 6%, leaching and run-off losses by 4%, and N_2 emissions by 13% (Table 6.2) (Van Rotterdam *et al.* 2021; Van Dijk *et al.* 2022). Van Rotterdam *et al.* (2021)reported that N losses through leaching and run-off could be reduced by about 4% if the chemical fertiliser N use is reduced from 123 to 98 kg ha^{-1} in dairy grassland on peat soil.

The use of low-N perennial ryegrass populations was estimated to lead to 4% lower NH_3 losses at manure application, and to similar N_2O losses from N fertilisation, N_2, and leaching and run-off losses. This is because the total soil N surplus was estimated at 170 kg ha^{-1} in this option, nearly equal to the 169 kg ha^{-1} in the average situation. Lower shoot tissue N concentrations resulted in an estimated decrease in the flow of N from the soil to herbage of 12 kg ha^{-1}. However, the flow of N from the soil to yielded herbage, to cows, and back to the soil via manure was also estimated to decrease by 11 kg ha^{-1}, due to the lower N uptake of the perennial ryegrass.

The use of plantain was estimated to decrease N_2O emissions from N fertilisation by 37% at farm level, following results of the field experiment described in **Chapter 4**. It was not determined whether the presence of plantain had an effect on N_2O and N_2 emissions from denitrified N of peat mineralisation in the field experiment (**Chapter 4**). It is unclear if plantain has an effect on N_2O and N_2 emissions from mineralisation which takes place in deeper soil layers. Van Beek *et al.* (2004) found that almost 70% of the N loss from denitrification came from soil layers deeper than 20 cm on dairy grassland on peat soil, while the majority of the roots of plantain grow in the upper 20-30 cm soil layer (Cranston *et al.* 2016). What also remains unclear is the effect of the presence of plantain on N leaching and run-off in dairy grassland on peat soil. The inhibition of nitrification could theoretically lead to increased soil ammonium (NH_4^+) concentrations, which in turn may lead to decreased soil nitrate (NO_3^-) leaching, from NO_3^- formed at nitrification of NH_4^+. A reduction in NO_3^- leaching in the presence of plantain has been observed in some studies in dairy grassland on mineral soils (e.g. Woods *et al.* 2018; Carlton *et al.* 2019). In the mesocosm experiment on peat soil described in **Chapter 4**, no effect of the presence of plantain was found on soil and leachate NH_4^+ and NO_3^- concentrations.

At the cultivation of cattail, a 7% decrease in N_2O emissions from fertilisation and soil mineralisation was estimated, equal to the decrease in grassland surface at the farm. Hardly any N_2O formation is expected in permanently waterlogged or inundated soil conditions (Velthof & Rietra 2018; Vroom *et al.* 2018). Compared to grassland, cultivation of cattail may lead to decreased N leaching, run-off and N_2 losses, because organic matter mineralisation rates are expected to be lower in waterlogged conditions than in drained soil conditions, resulting in decreased mineral N availabilities (Williams 1974; Tiemeyer *et al.* 2007). On the other hand, the use of chemical fertiliser N may result in increased leaching, run-off and N_2 losses. Consequently, the net effect of the conversion of grassland to cattail cultivation on leaching, run-off and N_2 losses is unclear.

6.3.3 N losses at farm level

The 20% reduction in chemical fertiliser N use, the use of low-N perennial ryegrass populations, the conversion of 3.8 ha grassland to cattail cultivation for roughage production and a combination of these options were all assumed to reduce herbage N concentrations, and consequently dietary CP levels (Table 6.2). In all options, dietary CP levels remained well above the threshold of 150-160 g kg DM^{-1},below which negative effects on milk production may be expected (Kebreab *et al.* 2002; Colmenero & Broderick 2006). The combination of all options was estimated to reduce NH_3 losses by 7% at farm level. All the options were also estimated to result in a 1-7% decrease in N_2O and NO_x emissions from silage storage, barn and manure, except for the plantain ryegrass swards option, where no effect on these emissions was expected. The use of plantain did not change the flow of N from the soil to herbage, from herbage to animals and from animals to manure, because it was assumed that feed intake and feed N concentrations did not change in this option.

6.4 Variations in the effects of the options on farm N losses

Several aspects may influence the variation and persistence of the options on farm N cycling and losses, as well as milk production. Some main aspects include soil tillage, the period of establishment of a productive vegetation, the perennial persistence of species, the consistency of effects of species on farm N cycling and losses and effects of species on the nutrtitonal value of grazed or harvested herbage. These aspects are discussed in this section.

6.4.1 Species sowing or planting and persistence

The method of soil tillage at sowing or planting, the establishment period of a productive vegetation and the perennial persistence of species are expected to affect farm N cycling and losses, because N compounds are more easily lost during the establishment period of perennial ryegrass, plantain and cattail (Schils *et al.* 2007; Seidel *et al.* 2009; Quadra *et al.* 2023; Offermanns *et al.* 2023).

Soil tillage on peat soil and previous grass sod termination lead to increased organic matter mineralisation and to N and other mineral losses (Lohila *et al.* 2004; Seidel *et al.* 2009; Taft *et al.* 2017; Offermanns *et al.* 2023). The amount of nutrients that is lost at the establishment of new species depends on various factors, such as the moment of soil tillage, the depth of soil tillage, the used soil tillage and seeding methods, and soil chemical and biological properties (Schils *et al.* 2007; Janssen & Verhoeff 2021; Quadra *et al.* 2023). Topsoil removal before peatland rewetting has been shown to mitigate net carbon emissions, and could lead to reduced soil NH_4^+ concentrations at the cultivation of cattail; however, the removed topsoil may continue to degrade at the location where the topsoil was transported, resulting in losses at this location (Quadra *et al.* 2023). Concentrations of mineral N in the soil and the degree of peat soil degradation may also affect the amount of N losses at rewetting (Tiemeyer *et al.* 2007; Cabezas *et al.* 2012). In the field experiments with low-N ryegrass populations, plantain and cattail, the previous grass sod was either killed and rotavated, or, in the case of cattail cultivation, completely removed (**Chapters 3-5**). A less invasive option leading to less mineral losses could be over-sowing or planting in the previous sod (Schils *et al.* 2007; Janssen & Verhoeff 2021), but the success of the establishment of new species at over-sowing is often lower than at complete sward renewals (Schils *et al.* 2007).

Monocultures of perennial ryegrass can hardly be maintained in dairy grasslands on peat soil (Wilman *et al.* 2001). Thus, low-N perennial ryegrass populations are likely to become mixed with other species after time, such as *Poa pratensis* (Deru *et al.* 2019). Plantain is less competitive under high fertility conditions than perennial ryegrass, and plantain has been observed to disappear after four years or more (Foster 1988; Stewart 1996; Sanderson *et al.* 2003). Moreover, it is not clear whether the increase of groundwater levels to 20-40 cm below surface and the management adaptations required at these groundwater levels, such as increased grazing for a sufficient soil load-bearing capacity, have an effect on the persistence of perennial ryegrass and plantain, compared to the current situation where groundwater levels are 60 cm or more below surface during the growing season (Hoving *et al.* 2018; Living Lab Boeren op Hoog Water 2022). When cattail was harvested three and six times per week, shoot densities decreased during the year of measurement (**Chapter 5**) and continued to decrease in the year after the measurements (Bestman *et al.* 2019). In the second year after planting, cumulative DM yields were 5-8 ton ha^{-1} lower in the plots harvested three or six times per week, compared to plots which were harvested once or twice per year (Bestman *et al.* 2019). Thus, cattail might be less suitable for frequent harvesting for perennial forage production. Cattail may also be succeeded by other species such as common reed (Williams & Pendleton 1982; Diggelen *et al.*

1996; Bestman *et al.* 2022). Long-term results from cultivated sites with cattail are still scarce (Geurts *et al.* 2020).

6.4.2 Variations between fields and in time

The effects on farm N cycling and losses, as well as on milk production, of adapting fertiliser N rates to SNS, or of using low-N perennial ryegrass populations, plantain or cattail, are likely to vary between locations and in time.

The SNS models have a random estimation error, and for specific locations the models were found to underestimate the SNS (**Chapter 2**). This estimation error is probably due to the fact that other chemical and biological factors can affect N mineralisation, besides SOM and degree days (Hassink 1995; Jarvis *et al.* 1996; Sonneveld & Lantinga 2011; Deru *et al.* 2019). Furthermore, suitable moments for fertilisation, grazing and mowing are determined by previous management decisions, weather variations and policy regulations, which affect soil conditions such as the moisture content and the load-bearing capacity (Janssen *et al.* 2023), or may limit the time of year at which activities are allowed (such as regulations on the application moment of fertilisers and regulations on the protection of meadow birds).

Differences in shoot tissue N concentrations of perennial ryegrass populations may vary in time and location. Shoot tissue N concentration differences among populations were inconsistent at the different N fertilisation levels in the field experiment, and between the pot and field experiments (**Chapter 3**). Lower N concentrations of low-N populations, compared to the other tested populations, were furthermore more often observed at the first three cuts of the growing season while in dairy grassland on peat soils, herbage N concentrations are typically undesirably high in the autumn (Eurofins Agro in Verloop *et al.* 2018).

Effects of the presence of plantain on N_2O emission reductions and soil N cycling have been observed to vary at a temporal scale. For example, a 26% reduction of N_2O emissions in the presence of plantain was observed at the same experimental site in the year after the study described in **Chapter 4** (Pijlman *et al.* 2020d). Others also reported varying effects of ryegrass-plantain swards on N_2O emissions in mineral soils (Luo *et al.* 2018; Simon *et al.* 2019; Bracken *et al.* 2021). The reduction in N_2O emissions in the presence of plantain is related to different factors and processes. Concentrations of BNI compounds in plantain have been reported to have a seasonal variation, and to vary between different plantain populations (Box & Judson 2018; Box *et al.* 2019), which may affect the inhibition of nitrification. Bracken *et al.* (2021) found indications that under drier soil moisture conditions (31-75% WFPS), but not under wetter soil conditions (43-77% WFPS), the inclusion of plantain may biologically inhibit nitrification. Nyameasem *et al.* (2021) suggested that belowground resource allocation dynamics is a possible means by which the sward composition affects N_2O emissions from the soil.

6.4.3 Variations in nutritional value

Changes in the nutritional value of grazed or harvested herbage can affect milk production and farm N cycling, and the expected effect on N losses (Van Dijk *et al.* 2022). Low-N perennial ryegrass populations could be the result of populations with lower leaf-to-stem ratios (Brégard *et al.* 2000). Morphological characteristics, such as the leaf-to-stem ratio, interact with the nutritional value of the grass (Beecher *et al.* 2015). However, no effects were observed on the content of water soluble carbohydrates and the percentage of *in vitro* digestible organic matter at the first cut of the growing season.

Feeding plantain may also influence milk production. Stewart (1996) reported that compared to perennial ryegrass, plantain had on average less CP and similar physical breakdown characteristics with less water soluble carbohydrates, cellulose, neutral and acid detergent fibre, but more lignin. In the year after the field experiment with plantain, described in **Chapter 4**, DM yields of the perennial ryegrass without plantain and the plantain perennial ryegrass mixtures remained equal, but plantain shoot biomass had on average 15% lower N concentrations than perennial ryegrass (Pijlman *et al.* 2020e). Plantain silage, with a plantain share of 81-96%, made in another field at the same experimental farm, had a 9% lower estimated net energy value for dairy cows, and 0-17% lower total N concentrations at the same cut, compared to perennial ryegrass without plantain (Pijlman *et al.* 2021). These lower CP and NE values are in line with findings by Stewart (1996).

The relatively low feeding value of cattail, as a result of the high fibre content and low organic matter digestibility, is likely to limit the use of cattail as feed for dairy cattle if milk production levels are not affected. Too high dietary fibre inclusions have negative effects on feed intake and consequently on energy intake, which may negatively affect the milk production of dairy cows (Dado & Allen 1995). Heifers and dry cows have lower daily energy requirements (CVB 2022), making dietary cattail inclusion a more interesting option for these animals.

6.5 Reduction in N losses for the explored options compared to policy targets

At current production levels, all the options have – on their own or in combination – the potential to reduce N losses on dairy farms on peat soil (Table 6.2). However, these reductions are not sufficient to meet policy targets. Below, the estimated reductions of the options are compared with policy targets and suggestions are made about what is needed to meet these targets in the future.

6.5.1 Policy targets until 2030

There are three important policy targets that require a reduction in N losses from dairy farming on peat soils in the Green Heart area before 2030. Ammonia losses from agriculture must have been reduced by 41% by 2030 compared to 2019, dissolved N concentrations in surface waters must be reduced by about 14-38% to meet the water framework directive (WFD) targets for 2027, and greenhouse gas emissions from peat soils must have been reduced by about 19% by 2030 compared to 2018 (**Chapter 1**, section 1.6). Here, I assumed these targets must be met at farm level.

Adapting lower chemical N fertilisation levels, the use of low-N perennial ryegrass genotypes and the cultivation of cattail can help to achieve policy objectives for NH_3 losses (reduction of 1-5% at farm level), but it will not lead to a sufficient reduction in losses to meet policy targets. The reduction by 20% (25 kg ha^{-1})in chemical fertiliser N use also helps to reduce N leaching and run-off losses (-4%), but this reduction is not sufficient to meet targets of surface water N concentrations. The 37% reduction in N_2O emissions from fertilisation, which is assumed to be achieved with the use of plantain-ryegrass swards, resulted in a 3% overall reduction in greenhouse gas emissions from dairy grassland on peat soil, assuming that N_2O emissions from fertilisation account for 7% of the total greenhouse gas emissions from dairy grasslands on peat soils (Velthof *et al.* 1996; Kuikman *et al.* 2005; Lof *et al.* 2017; Van Dijk *et al.* 2022). The use of plantain thus helps to reduce greenhouse gas emissions, but the emission reduction is not sufficient to meet the 2030 climate agreement target for organic soils. The cultivation of cattail is also expected to help to meet the climate agreement target, since it is expected to reduce not only N_2O emissions but also CO_2 emissions from organic matter oxidation (Joosten 2015). In fact, greenhouse gas emissions per hectare can potentially be reduced by more than 50% by the cultivation of cattail, depending on conditions such as the mean water table, factors influencing methane (CH_4) formation, and the quantity of greenhouse gas emissions before rewetting the soil (Vroom *et al.* 2018; Tiemeyer *et al.* 2020; Bianchi *et al.* 2021; Quadra *et al.* 2023). This reduction would still not be sufficient to meet the greenhouse gas reduction target of 19% on farm level, because only 11% (3.8 ha) of the grasslands of an average farm could be converted to cattail cultivation, to maintain current milk production levels (Table 6.2).

The explored options should thus be combined with other options, to meet NH_3 emission, water quality and greenhouse gas policy targets. Some examples of other N loss reduction options include the following:

- Raising the groundwater table with the use of submerged drains to maintain higher groundwater levels in grasslands during the growing season. This is expected to decrease soil subsidence and the SNS of grasslands on peat soil (Pijlman et al. 2018; Verloop et al. 2018; Van Rotterdam et al. 2021);
- Increasing the grazing time and fresh grass uptake because NH_3 losses are considerably lower at grazing compared to feeding in the barn, where faeces and urine are mixed (Verloop et al. 2018; Van Bruggen et al. 2022; Van Dijk et al. 2022);
- Implementing unfertilised strips of land along ditches and furrows, which can lead to lower N losses from run-off (Van Rotterdam et al. 2021);
- Further reducing fertiliser N use, to reduce the N flow and consequently losses on the farm (Verloop et al. 2018; Van Bruggen et al. 2022; Van Dijk et al. 2022);
- Further optimising dietary CP and energy to CP concentrations, to reduce both NH_3 losses and N concentrations in manure (Smits et al. 1995; Verloop et al. 2018; Edouard et al. 2019; Van Dijk et al. 2022);
- Optimising productivity, reproductive efficiency and longevity of cattle, so as to increase the share of milk-producing animals at the farm (Lahart et al. 2021);
- Optimising grassland fertilisation, grazing and mowing management, which is aimed at increasing N use efficiency and reducing losses (Verloop et al. 2018; Egan et al. 2019; Van Dijk et al. 2022);
- Technical adaptations in barns and of manure application, mainly to reduce NH_3 losses (Verloop et al. 2018; Van Bruggen et al. 2022; Van Dijk et al. 2022);
- Developing dairy farming models with lower production levels per hectare at higher groundwater levels. This has been proposed and evaluated in response to the various policy targets that apply to dairy farming on peat soil, including policy targets on N emissions (De Jong *et al.* 2022; Schrijver *et al.* 2022).
- The development of biomass production and harvesting in waterlogged or inundated soil conditions, ideally without N fertilisation, as an alternative business model for dairy farming (paludiculture). For example, this could be the cultivation of cattail or reed for the production of biobased building materials or the cultivation of peat moss for the production of potting material (Wichtmann *et al.* 2016; Liu *et al.* 2023).

Different combinations of options may be adapted on dairy farms, to reach greater reductions in farm N losses (De Visser *et al.* 2001; Verloop *et al.* 2018). For the options of this thesis, no interactions were assumed between the amount of N used for milk production and the amount of N lost, as production levels were not assumed to be affected by the options (Table 6.2). However, combining several options may result in an interaction between the amount of N used for production and the amount of N losses (Powell & Rotz 2015), resulting in a lower milk production. For example on organic dairy farms in the Netherlands, where no chemical fertiliser N is used and the application of animal manure is restricted to a maximum of 170 kg ha^{-1}, not only the estimated farm N surpluses per hectare are more than 50% lower than on conventional farms, but the milk production per hectare is on average also more than 40% lower (Thomassen *et al.* 2008; Agrimatie 2022e). More extensive farms generally have higher costs per unit of milk, and therefore need a higher milk price per unit of milk to compensate revenues for the lower milk production per hectare (De Jong *et al.* 2022; Schrijver *et al.* 2022).

There have been different studies on combining various options for lower N losses on dairy farms on peat soil. De Visser *et al.* (2001) estimated that NH_3 emission reductions of 56% were possible without an effect on production on an average dairy farm on peat soil in the Green Heart area with NH_3 emissions of 69 kg ha^{-1}. They arrived at this estimate by combining options such as decreased N fertilisation levels, optimisation of grass and silage quality, reduction of concentrate use, lower replacement rate of cows, improved slurry management and improved N recovery of fertilisers. Verloop *et al.* (2018) estimated that annual farm NH_3 emission reductions of at least 25% were possible for dairy farms on peat soil in the Netherlands, without an effect on milk production, compared to the situation before 2018 when average NH_3 losses were 57 kg ha^{-1}. They arrived at this estimate by combining options such as diluting manure with water (ratio 2:1) at manure spreading, lowering dietary CP contents for dairy cows and heifers, optimising the dietary energy-to-protein ratios of dairy cows, and increasing groundwater levels of grassland on peat soils by using submerged drainage systems. The dilution of manure with water at field spreading (ratio 2:1) with the use of a trailing shoe manure applicator, which resulted in a 20% reduction of NH_3 losses from dairy farms on peat soil according to Verloop *et al.* (2018), was made mandatory by the Dutch government as of 2019 (RVO 2021).

In a comparative study on different dairy farming intensities on peat soil for the Green Heart area, De Jong *et al.* (2022) made estimations for a farm which combines water infiltration systems with a 50% lower milk production per hectare, compared to the current situation. Water infiltration systems consist of a combination of submerged drains and a pumping system, which allow for pressurised water infiltration in the soil (Hoving *et al.* 2018). De Jong *et al.* (2022) estimated that NH_3 and field N surpluses per hectare could be reduced by 51% and 29%, respectively, but that milk N production per hectare would also decrease by 46-57% in these scenarios, compared to the situation in 2020. Moreover, this estimated reduction in N surpluses was on the assumption that N_2O losses from denitrification remained equal at increased groundwater levels. Maintaining shallower groundwater levels may lead to more favourable conditions for N_2O formation (Tiemeyer *et al.* 2016), but studies on the effect of water infiltration systems on N_2O emissions from dairy grasslands on peat soils are still scarce.

6.5.2 Policy ambitions after 2030

Policy ambitions for NH_3 and greenhouse gas emissions after 2030 are much stricter but less accurately defined than before 2030. If, to comply with European laws, the Critical Load (CL) of N deposition on 'nitrogen-sensitive' nature areas may not be exceeded after 2030, total N deposition rates must decrease by more than 70% (Gies *et al.* 2019). Here, I assumed that NH_3 losses per hectare from dairy farming on peat soil must be reduced at farm level by 70% after 2030, compared to the current situation. In the Dutch climate agreement, it was agreed that agriculture and land use should be climate neutral by 2050 (Ministerie van Economische Zaken en Klimaat 2019). However, what was not described is how this climate neutral state should be reached. For example, are greenhouse gas emissions still acceptable in parts of the peatlands if they are compensated elsewhere, or should the peatlands be completely climate neutral themselves? Here, I assumed that all peatlands in the Green Heart area must themselves be climate neutral by 2050.

As mentioned above, a reduction in NH_3 losses of up to 51% may be reached through a combination of management and technical options, and through extensification of the milk production per hectare (De Visser *et al.* 2001; Verloop *et al.* 2018; De Jong *et al.* 2022). In fact, it is likely that even a reduction in NH_3 losses of more than 70% is possible via these options, by combining for example a further extensification with management and technical options (De Visser *et al.* 2001; Verloop *et al.* 2018; Van Rotterdam *et al.* 2021; De Jong *et al.* 2022; Living Lab Boeren op Hoog Water 2022). The combination of options and the level of extensification are still to be determined, and different combinations may be possible to reach the NH_3 emission policy target (De Jong *et al.* 2022; Living Lab Boeren op Hoog Water 2022).

For bringing N_2O emissions on peat soils to zero, it is likely that groundwater levels need to be maintained at or above the soil surface, and that high NO_3^- concentrations in soil and water should be avoided. Peat soils which emit hardly any N_2O are typically waterlogged and low in NO_3^- concentrations (Regina *et al.* 1996; Tauchnitz *et al.* 2015; Tiemeyer *et al.* 2020). In waterlogged conditions the soil is anaerobic, and consequently, aerobic organic matter mineralisation and nitrification are strongly inhibited, resulting in low NO_3^- concentrations. With low soil oxygen concentrations, denitrification rates are typically low and denitrification is mostly complete, which results in very low N_2O emissions (Tauchnitz *et al.* 2015; Vroom *et al.* 2018; Kandel *et al.* 2019). Nitrous oxide emissions may be low or close to zero for the cultivation of crops such as cattail and reed if they can grow without NH_4^+ and NO_3^- fertilisation, in waterlogged soil conditions (Vroom *et al.* 2018; Kandel *et al.* 2019) and in unfertilised peat grasslands and semi-natural peatlands (Schrier-Uijl *et al.* 2014).

Meeting the stricter greenhouse gas targets of 2050, in combination with other policies and ambitions such as reducing or stopping soil subsidence, preserving biodiversity and reducing phosphate losses to water, as well as targets related to water use and safety-related targets, probably means that other land use options need to be explored and evaluated (Strootman *et al.* 2020). Future land use of peatlands in the Green Heart area could therefore consist of a mix of relatively extensive dairy or cattle farming at relatively high groundwater levels, with adapted management and technologies for low N losses, and the cultivation of crops that grow in waterlogged (or near-waterlogged) and relatively poor soil mineral N conditions, in unfertilised semi-natural peatlands and in restored natural peat ecosystems (Freeman *et al.* 2022). Several factors may influence the mix of this land use, such as the soil chemical heterogeneity of the area, hydrology, current farming intensity and sizes, current land use and socio-economic aspects (Hoving *et al.* 2020; Freeman *et al.* 2022; Liu *et al.* 2023). It will also be important how policymakers will deal with the more short-lived greenhouse gas methane compared to CO_2 to strive for climate-neutral agriculture and land use (Lynch & Garnett 2021). Rumen fermentation and cattle manure are currently the main methane sources in dairy farming in the Green Heart area (De Jong *et al.* 2022; Van Dijk *et al.* 2022). Ditches of peatlands have also been identified as “significant” sources of methane (Tiemeyer *et al.* 2020), and at peatland rewetting, there could be a partial trade-off between the reduction of CO_2 emissions from organic matter mineralisation at drained soil conditions and the formation of methane that occurs mainly at rewetted soil conditions (Tiemeyer *et al.* 2020; Bianchi *et al.* 2021). Furthermore, future land use options will depend on the level at which policy targets must be met: whether this is at the level of the country, region or field, or at another level. When future land use options are investigated, environmental and technical performances of farms should be evaluated in an integrated way, as each combination of options may lead to trade-offs between emissions and productivity, with effects on the economic feasibility for land users, as well as on the societal perception and acceptance (Erisman *et al.* 2020).

6.6 Reflection on the current research and future research suggestions

The estimated effects of the options on farm N losses are relatively small, compared to for example earlier studies in which several options were combined or which investigated the extensification of milk production per hectare as an option (De Visser *et al.* 2001; Verloop *et al.* 2018; De Jong *et al.* 2021; Agrimatie 2022e). However, the research themes of this thesis may be considered innovative because so far research has been scarce on the quantity and dynamics of SNS, on the effects of perennial ryegrass populations on shoot tissue N concentrations, on the effects of plantain on soil N cycling, and on the yield and nutritional value of cattail in (former) dairy grassland on peat soils (Davis 2021). The options, or variants of the options such as sod-seeding of low-N perennial ryegrass populations or plantain, are also options that farmers are more easily willing to experiment with or work with, because they can be implemented relatively easily, are relatively inexpensive, and effects on milk production may be small (Bestman *et al.* 2019; Pijlman *et al.* 2021; Janssen & Verhoeff 2021). Cattail cultivation is an exception because a rewetted peat soil cannot easily be taken back as productive grassland after the removal of the nutrient rich topsoil (Quadra *et al.* 2023). Moreover, the options may all positively influence other policy targets than nitrogen-related targets. For example, reducing the use of chemical fertiliser N reduces energy use and the CO_2 emissions released during the production of fertilisers (Walling & Vaneeckhaute 2020). Insights into the possibilities of lowering N levels in perennial ryegrass on peat soil may also be useful for dairy grasslands on mineral soils (Wilkinson *et al.* 2020). The use of plantain can increase the drought resilience of grassland (Stewart 1996), also of grassland on mineral soils, and it may also contribute to lower methane emissions from dairy cows (Della Rosa *et al.* 2022). Cattail cultivation can contribute to the reduction of soil subsidence and CO_2 emissions from peat oxidation, and it may also increase the biodiversity of areas with dairy grassland on peat soil (Tanneberger *et al.* 2020; Bianchi *et al.* 2021).

The studies in this thesis were all based on a combination of a pot and field experiment, or on field experiments. Pot experiments and more fundamental research are generally useful to study mechanisms and the ‘proof of principle’ of options, but at the same time, research under practical conditions is needed for the translation of results into practice. It is known that plants grown inside grow faster, take up more N and may differ in morphology, compared to plants grown in field conditions (Poorter *et al.* 2016). At the same time, many questions remain unanswered after only one or a few field experiments, because there are so many variables in field conditions, such as variations in soil chemistry, biology, hydrology, management and weather conditions. Therefore, it is important that farmers can experiment with potential options, in their own situation and context, so they can experience and evaluate options for themselves, and share experiences with others.

Several aspects of the options, which were not investigated but are relevant for estimating the effects on farm N losses and productivity, are discussed in greater detail in section 6.4, and these may well be future research topics. Future research could therefore focus on soil tillage, on seeding or planting methods and species persistency, and on the feeding value and the effect of feeding low-N perennial ryegrass populations, plantain and cattail on dairy cow and farm performances, in pot or lab and field experiments. Furthermore, the effect of using low-N perennial ryegrass populations, plantain and cattail on N cycling and losses could also be investigated on mineral soils. The effects of the investigated options on other policy targets could be also examined, such as the effects of reduced use of fertiliser N as well as of the cultivation of low-N perennial ryegrass populations, plantain and cattail on soil and dairy cow greenhouse gas emissions.

In the years up to 2050, dairy farming in the Green Heart area will be facing stricter policy targets, on the assumption that national policy targets must be met at regional or farm level. Given the strictness of the policy targets, it may be questioned whether research into and experimentation with options that can be applied relatively easily in current farming practices and in which milk production levels can be maintained, will provide options that are sufficiently effective to meet the 2050 policy targets. Another approach could be to investigate such options that have been hypothesised to help meet multiple policy targets for 2050, but that imply greater changes in farming practices. For example, raising groundwater levels close from the soil surface at grassland with water infiltration systems, more extensive land use with livestock or the cultivation of cattail, reed and other crops grown in nearly waterlogged to inundated peat soils without N fertilisation (paludiculture) may be options that allow a form of agriculture on peat soils that could strongly reduce nitrogen and greenhouse gas emissions compared to the current situation (Wichtmann *et al.* 2016; Liu *et al.* 2023). Furthermore, the biomass of paludiculture crops may have a greater value as for example food or biobased building material, compared to the use as feed (Wichtmann *et al.* 2016), and paludiculture has the potential to strongly reduce the societal costs associated with the current peatland use for dairy farming (Liu *et al.* 2023). However, large-scale implementation of these options is currently not feasible from an economic perspective (De Jong *et al.* 2021; Freeman *et al.* 2022; Liu *et al.* 2023). Therefore, there is only limited support for research into extensification and farming at raised groundwater levels or inundated soils among current land users and stakeholders of the current land use, and few farmers are currently willing to invest in these options (Ziegler *et al.* 2021; Freeman *et al.* 2022). Consequently, I suggest that future research on the socio-economic aspects of options of future land use is highly relevant for the Green Heart area to meet the various 2050 policy targets.

6.7 General conclusions

In this thesis, it was found that the soil organic matter content and the air temperature are correlated to the dynamics and quantity of the soil N supply of dairy grasslands on peat soil, and that these correlations were similar in conditions with and without calcium ammonium nitrate applications. It was also found that selected low-N perennial ryegrass populations, plantain and cattail can change soil and crop N dynamics in dairy grasslands on peat soils. The results of this thesis may have practical implications for farm management decisions regarding grazing, mowing and fertilising dairy grasslands, as well as implications for the choice of grown species populations and herbage species, which may lead to lower N losses from dairy farms on peat soils.

The options of lowering chemical fertiliser N use, as a result of adapting fertilisation to SNS, and the cultivation of plantain, cattail and low-N perennial ryegrass populations were estimated to reduce farm NH_3, N_2O from N fertilisation and N losses from run-off and leaching by 7%, 37% and 4%, respectively, for an average dairy farm on peat soil in the Green Heart area.

None of the options, either individually or together, were estimated to have the potential to sufficiently reduce N losses from dairy farming on peat soil to comply with the NH_3, water and greenhouse gas policy targets for 2030. Therefore, it is suggested that the options may be combined with other reduction options to reach these policy targets, especially since policy targets will be stricter after 2030.

This thesis comprised research themes that could lead to relatively easily implementable options at low costs for decreasing N losses on dairy farms on peat soil, with the exception of cattail cultivation. Several aspects may affect the variation and persistence of the reduction of farm N losses of these options, and these are potential future research topics. Combining more options to meet 2050 policy targets may lead to a decrease in milk production and revenues. This is why I suggest that future research should also take the socio-economic effects of options into account. I would like to conclude by stating that the research themes of this thesis may also be relevant for mineral soils, and for cattail this would mean mineral soils that are permanently waterlogged or inundated.

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Appendices

Summary
Samenvatting
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Summary

There is increasing pressure to reduce nitrogen (N) losses from dairy farming on peat soil. Ambitious targets for N deposition, chemical water quality and greenhouse gas emissions have been put in place by the Dutch government.

Peat consists of organic matter from plant remains which are not completely broken down but have been left behind and accumulated in water-saturated and often acidic conditions. Peat soils worldwide store large amounts of N and carbon. Drainage of peat soils for agricultural use has a history of thousands of years in the Green Heart area of The Netherlands. At drainage, oxygen enters the peat soil, which allows microbes to oxidize the peat at which stored N becomes available for crop uptake or can be lost. The current agricultural land use of peat soils in the Green Heart area is mainly grassland for dairy farming. Especially after the Second World War, dairy farming in the Green Heart area has strongly intensified, at which N losses per hectare have also increased.

Completely closing the N cycle of an agricultural system is impossible because several N compounds are easily lost to the environment. In general, the intensity of N supply, farm management, and the conditions under which N losses can occur determine the amount of N losses in agricultural systems. Peat mineralization is an extra N supply at dairy grasslands on peat soil, compared to dairy grasslands on mineral soils. In 2020, the estimated N losses of an average dairy farm in the Green Heart area were 210 kg N per hectare, of which 24% were ammonia (NH_3) losses, 10% were run-off and leaching losses such as nitrate (NO_3^-) and organically bound N, 7% were nitrous oxide (N_2O) losses and 60% were nitrogen (di)oxides (NO_x) and nitrogen gas (N_2) losses.

NH_3, NO_3^-, N_2O and NO_x are forms of reactive N. Excessive losses of reactive N have negative effects on, for example, biodiversity, water quality, climate and human health. Since the 1990s, environmental policy has focused on reducing reactive N losses from agriculture. Currently, relevant policy targets regarding nitrogen for dairy farming in the Green Heart area are; the reduction of NH_3 emissions by 41% in 2030 compared to 2019; the reduction of N emissions to water by 14-38% compared to the period 2010-2013; and the reduction of greenhouse gases from peat soils by 19% by 2030. In addition, there are also policy targets and ambitions for CO_2 and methane emissions, soil subsidence and soil rewetting, biodiversity, chemical and ecological water quality, and water use and safety.

There are various options to reduce N losses from dairy farms, but, to date, it has remained a challenge to reduce the contribution of N from soil mineralisation to the N uptake of herbage and farm N losses. The main objective of this thesis was to

answer the question; Can N cycling on dairy farms on peat soil in the Green Heart area be improved by adapting management to the soil N supply and by using other grassland species and crops without affecting milk production? This thesis comprised four studies, which focused on the following;

1. Estimating the soil N supply of dairy grassland on peat soil;
2. Lowering N levels of perennial ryegrass grown on peat soil through population selection;
3. Investigating the effect of plantain on the soil N cycle of dairy grassland on peat soil;
4. Investigating the yield and nutritional value of cattail as an alternative roughage, which can be grown on waterlogged or inundated soils.

Ad 1. The objective of this study was to determine factors controlling the quantity and dynamics of the soil N supply of dairy grassland on peat soil. It was found that the soil N supply can be estimated by soil organic matter content and degree days. The dynamics and the quantity of soil N supply were estimated similarly in conditions with and without calcium ammonium nitrate applications.

Ad 2. The objective of this study was to determine whether perennial ryegrass populations with different shoot tissue N concentrations recorded on sandy soil, would also show different shoot tissue N concentrations and N use efficiencies or N uptake efficiencies when grown on peat soil. In a pot experiment using a peat substrate, among tetraploid populations, lower shoot tissue N concentrations coincided with a higher N use efficiency, and among diploid populations, N uptake efficiencies but not shoot tissue N concentrations differed. In a field experiment on peat soil, populations could explain 44% of the variation in N uptake efficiency, but no relation with shoot tissue N concentration was found. Selected populations had, on average, 1.4 g per kg dry matter (4%) lower shoot tissue N concentrations, but not a different dry matter yield, compared to a commercial control at a fertilisation level close to practice.

Ad 3. The objective of this study was to determine the effect of the presence of plantain on the fate of soil mineral N in dairy grassland on peat soil. In the mesocosm experiment, it was found that plantain significantly reduced potential nitrification, and that the N uptake efficiency of plantain increased at higher plantain shares in the herbage. In the field experiment, N_2O fluxes after mineral fertiliser N application were reduced by 39% in the presence of plantain, compared to grassland without plantain. At a sowing rate of 33% plantain, compared to perennial ryegrass without plantain, no significant differences in annual herbage N concentration, N uptake and DM yields were found.

Ad 4. The objective of this study was to determine the effects of harvest date and frequency on the yield and nutritional value of cattail, in the first year after planting on a rewetted dairy grassland on peat soil. Cattail produced a maximum of 9.8-10.9 tons of dry matter per hectare of harvestable biomass, when harvested once a year. The nutritional value was the highest before flowering in May. Cattail had high crude fibre concentrations (287-300 g per kg dry matter) and low crude protein (125 g per kg dry matter) and phosphorus (1.5 g per kg dry matter) concentrations, and a low *in vitro* digestibility of organic matter (63-69%) compared to perennial ryegrass. Harvesting in intervals of three or six weeks in the period from May to September resulted in similar cumulative dry matter yields (5.2 and 6.5 tons of dry matter per hectare, respectively) and a nutritional value similar to the May harvest.

The results of the various studies may lead to options for dairy farms on peat soil that can limit N losses while maintaining milk production levels, which were estimated as follows for this thesis;

1. When soil N supply estimates are combined with estimates of previous N fertilisation rates, herbage yields and herbage N concentrations, the actual N fertilisation rates and the timing of grazing or mowing can be adapted to yield herbage with a targeted CP concentration. Practical experiences, which were not part of this thesis, showed that the fertiliser N use on dairy grassland on peat soil could be reduced by an average of 25 kg per hectare by adapting N fertilisation to the soil N supply. It was estimated for an average dairy farm in the Green Heart area, based on results from this thesis and other literature, that a reduction of 25 kg N fertiliser per hectare could reduce soil N_2O losses from fertilisation by 6%, soil N losses from leaching and run-off by 4%, and farm NH_3 losses by 5%.
2. The use of low-N perennial ryegrass populations may reduce the protein content of grass by 4%. This could lead to 4% lower farm NH_3 losses and comparable soil N_2O losses from N fertilisation and soil N losses from leaching and run-off for an average dairy farm in the Green Heart area.
3. Sowing plantain at a sowing percentage of 33% at all grasslands may reduce N_2O emissions from N fertilisation by 37% at the farm level for an average dairy farm in the Green Heart area. This may have no effect on milk production, because grass yields and N contents at this sowing percentage did not differ from perennial ryegrass without plantain.
4. Feeding cattail can reduce the protein content in the dairy cattle ration. Moreover, since cattails can grow in fully waterlogged or inundated peat soils, cattails may provide forage production while greatly reducing peat oxidation, which may also limit the N supply from peat mineralisation. It was estimated that the cultivation

of cattail could lead to a reduction of soil N_2O emissions by 7% and farm NH_3 losses by 1% for an average dairy farm in the Green Heart area.

However, several aspects of the options may affect these estimated N losses, as well as milk production. Examples include (the method of) grassland renewal, the persistence of species, the consistency of species effects, such as the effect of the presence of plantain on N_2O emissions, and the effects of lower N fertilisation or other species on the nutritional value of feed. These aspects were not investigated.

The estimated N loss reductions of the investigated options were relatively small. Even when the options were combined, they were not sufficient to achieve policy targets regarding nitrogen up to 2030. The estimated N loss reductions were also lower compared to previous studies, in which, for example the extensification of milk production per hectare was investigated. However, the themes of this thesis were innovative and research on these themes on peat soils has been scarce until now. Also, the options studied are relatively easy to implement on a dairy farm, and are low in cost. The cultivation of cattail is an exception to this because a rewetted peat soil cannot easily be returned to productive grassland after the nutrient-rich topsoil has been removed. Moreover, the options can all positively influence policy targets other than policy targets related to nitrogen.

The policy ambitions for NH_3 and greenhouse gas emissions after 2030 are stricter, which may mean that the possible land use of peat soils will change further. Given the strictness of long-term policy targets, it may be questioned whether options that can be applied relatively easily in current farming practices and in which milk production levels can be maintained, will be sufficient to meet these targets.

Other options should be investigated to integrally reach multiple policy targets for 2050 in the Green Heart area. However, these options will imply greater changes in farming practices, such as the extensification of livestock farming or the cultivation of crops that can grow in nearly waterlogged or inundated peat soils. Therefore, research into socio-economic aspects of future land use options is also recommended for the Green Heart area. In addition, the research themes of this thesis may also be relevant to mineral soils, and in the case of cattail for mineral soils that are waterlogged or flooded.

Samenvatting

Er is een toenemende druk om stikstof(N)verliezen van de melkveehouderij in de veenweiden terug te dringen. Ambitieuze doelen voor N-depositie, chemische water kwaliteit en broeikasgasemissies zijn door de Nederlandse overheid opgesteld.

Veen bestaat uit organische stof van plantenresten welke in waterverzadigde en vaak zure omstandigheden niet volledig zijn afgebroken maar zijn achtergebleven en geaccumuleerd. Veengronden wereldwijd bevatten grote hoeveelheden N en koolstof. De ontwatering van veengronden voor agrarisch gebruik kent een geschiedenis duizenden jaren in het Groene Hart van Nederland. Ontwatering zorgt voor zuurstof in het veen, waardoor micro-organismen het veen kunnen oxideren en opgeslagen N kan worden opgenomen door gewassen of verloren gaan. Het huidig agrarisch landgebruik van veengronden in het Groene Hart is voornamelijk veenweiden voor de melkveehouderij. Met name na de Tweede Wereldoorlog is de melkveehouderij in het Groene Hart sterk geïntensiveerd, waarbij ook de N-verliezen per hectare zijn toegenomen.

Het volledig sluiten van de N-kringloop in een landbouwsysteem is onmogelijk, omdat verschillende N-verbindingen gemakkelijk verloren gaan naar het omliggende milieu. In algemene zin geldt dat de intensiteit van N-aanvoer, bedrijfsmanagement en de omstandigheden waaronder de N-verliezen kunnen plaatsvinden, de hoeveelheid N-verlies bepalen van landbouwsystemen. Veenmineralisatie is een extra N-aanvoer in veenweiden in vergelijking tot melkveegraslanden op minerale gronden. In 2020 waren de geschatte N-verliezen van een gemiddeld melkveebedrijf in het Groene Hart 210 kg N per hectare, waarvan 24% ammoniak (NH_3), 10% nitraat (NO_3^-) en organisch gebonden N richting water, 7% lachgas (N_2O) en 60% stikstofoxiden (NO_x) en stikstofgas (N_2).

NH_3, NO_3^-, N_2O en NO_x zijn vormen van reactief N. Te grote verliezen van reactief N hebben negatieve effecten op bijvoorbeeld biodiversiteit, waterkwaliteit, klimaat en humane gezondheid. Sinds de jaren ’90 is milieubeleid erop gericht om reactieve N-verliezen uit de landbouw terug te dringen. Op dit moment zijn relevante beleidsdoelen rondom stikstof voor de melkveehouderij in het Groene Hart; het verminderen van NH_3-emissies met 41% in 2030 ten opzichte van 2019; het verminderen van N-emissies richting water met 14-38% ten opzichte van de periode 2010-2013; en het terugdringen van broeikasgassen uit veengronden met 19% in 2030. Daarnaast zijn er ook beleidsdoelen en –ambities op gebied van CO_2- en methaanemissies, bodemdaling en vernatting, biodiversiteit, chemische en ecologische waterkwaliteit, en watergebruik en veiligheid.

Er zijn diverse opties om N-verliezen van melkveebedrijven te beperken, maar tot nu toe is het een uitdaging gebleven om de bijdrage van N uit veenmineralisatie aan de gewasopname en bedrijfs-N-verliezen te beperken. Het hoofddoel van dit proefschrift was het beantwoorden van de vraag; Kan de N-kringloop van melkveebedrijven op veengrond in het Groene Hart verder worden gesloten door management aanpassingen aan de N-levering van de bodem en via het gebruik van andere graslandsoorten en gewassen, zonder een effect op de melkproductie? Het onderzoek omvatte vier studies, welke waren gericht op het volgende:

1. Het schatten van de N-levering uit de bodem van veenweiden;
2. De mogelijkheid om N-gehalten van Engels raaigras op veengrond te verlagen via veredeling;
3. Het onderzoeken van het effect van smalle weegbree op de N-cyclus van de bodem;
4. Het onderzoeken van de productie en voederwaarde van lisdodde als alternatief ruwvoer, wat geteeld kan worden in waterverzadigde of ondergelopen bodems.

Ad 1. Deze studie had als doel factoren te bepalen die de bodem-N-levering hoeveelheid en dynamiek van veenweidenbodems bepalen. Het bleek dat de N-levering kon worden geschat op basis van het gehalte aan organische stof in de bodem en graaddagen. De hoeveelheid en dynamiek van de N-levering werden vergelijkbaar geschat in omstandigheden met en zonder N-kunstmest.

Ad 2. Deze studie had als doel te bepalen of Engels raaigras populaties met verschillende bovengrondse N-concentraties, gemeten op een zandgrond, verschillende N-concentraties, N-gebruiksefficiënties of N-opname-efficiënties hadden op veengrond. In een potexperiment met potgrond vielen bij tetraploïde populaties lagere bovengrondse N-concentraties samen met een hogere N-gebruiksefficiëntie, en bij diploïde populaties verschilden de N-opname-efficiënties maar niet de bovengrondse N-concentraties. In een veldexperiment op veengrond konden populaties 44% van de variatie in N-opname-efficiëntie verklaren, maar er werd geen relatie met bovengrondse N-concentratie gevonden. Geselecteerde populaties hadden gemiddeld 1,4 g per kg droge stof (4%) lagere bovengrondse N-concentraties, maar geen andere drogestofopbrengst, vergeleken met een commerciële controle bij een bemestingsniveau dat dicht bij de praktijk ligt.

Ad 3. Deze studie had als doel om het effect van de aanwezigheid van weegbree op mineraal N in de bodem van een veenweide te bepalen. In een mesocosm-experiment verminderde weegbree de potentiële nitrificatie, en de N-opname-efficiëntie van weegbree nam toe bij hogere weegbree-aandelen in een Engels raaigras-weegbree mengsel. In een veldexperiment waren de N_2O-verliezen na toediening van N-kunstmest 39% lager in de aanwezigheid van weegbree, in vergelijking tot grasland zonder weegbree. Bij een zaaipercentage van 33% weegbree waren er geen verschillen in jaarlijkse N-concentraties, N-opnames en drogestofopbrengsten, in vergelijking tot Engels raaigras zonder weegbree.

Ad 4. Deze studie had als doel om de effecten van oogstdatum en -frequentie op de opbrengst en voederwaarde van lisdodde te bepalen, in het eerste jaar na aanplanten op een vernatte afgeplagde veenweide. Lisdodde produceerde maximaal 9,8-10,9 ton droge stof per hectare aan oogstbare biomassa, als het één keer per jaar werd geoogst. De voederwaarde was het hoogst vóór de bloei in mei. Lisdodde had hogere concentraties ruwe celstof (287-300 g per kg drogestof) en lagere ruw eiwit (125 g per kg drogestof) en fosfor (1,5 g per kg drogestof) concentraties, en een lagere *in vitro* verteerbaarheid van organische stof (63-69%) in vergelijking tot Engels raaigras. Elke drie of zes weken oogsten in de periode mei tot september resulteerde in vergelijkbare cumulatieve droge stofopbrengsten (5,2-6,5 ton droge stof per hectare), en een voederwaarde vergelijkbaar met de oogst in mei.

De resultaten van de verschillende onderzoeken kunnen leiden tot opties om N-verliezen te beperken op melkveebedrijven op veengronden, welke als volgt waren geschat voor deze thesis.

1. Wanneer N-leveringschattingen worden gecombineerd met schattingen van eerdere N-mestgiften, grasopbrengsten en gras-N-concentraties, kunnen huidige N-mestgiften of het moment van beweiden of maaien worden aangepast voor een beoogde ruw eiwitconcentratie in het gras. Uit praktijkpilots, welke geen onderdeel van deze thesis waren, kwam naar voren dat het N-kunstmestgebruik in veenweiden gemiddeld met 25 kg per hectare kon worden verlaagd door N-bemesting aan het NLV aan te passen. Op basis van de resultaten van dit proefschrift en andere literatuur, werd voor een gemiddeld melkveebedrijf in het Groene Hart geschat dat door een reductie van 25 kg N kunstmest per hectare, bodem-N_2O-verliezen uit bemesting met 6% afnamen, bodem-N-verliezen door uit- en afspoeling met 4% afnamen en NH_3-verliezen op bedrijfsniveau met 5% afnamen.
2. Het gebruik van laag-N Engels raaigras populaties kan het eiwitgehalte van gras met ongeveer 4% verlagen. Dit zou kunnen leiden tot 4% lagere NH_3-verliezen op bedrijfsniveau en tot vergelijkbare bodem-N_2O-verliezen uit bemesting en

bodem-N-verliezen door uit- en afspoeling, voor een gemiddeld melkveebedrijf in het Groene Hart.

3. Door weegbree in te zaaien met een zaaipercentage van 33% op alle graslanden kan de N_2O-emissie uit N-bemesting op bedrijfsniveau met 37% worden verminderd, voor een gemiddeld melkveebedrijf in het Groene Hart. Mogelijk heeft dit geen effect op de melkproductie, omdat opbrengsten en N-gehalten bij dit zaaipercentage niet verschilden van Engels raaigras zonder weegbree.
4. Het voeren van lisdodde kan het eiwitgehalte in het melkveerantsoen verlagen. Bovendien, aangezien lisdodde kan groeien in volledig vernatte of ondergelopen bodems, zou lisdodde ruwvoerproductie kunnen opleveren terwijl de veenoxidatie sterk wordt verminderd, wat ook de N-aanvoer vanuit veenmineralisatie kan beperken. Geschat werd dat de teelt van lisdodde zou kunnen leiden tot een reductie van de N_2O-emissie uit de bodem met 7% en een reductie van de NH_3-verliezen op de boerderij met 1%, voor een gemiddeld melkveebedrijf in het Groene Hart.

Verschillende aspecten kunnen echter van invloed zijn op deze geschatte N-verliezen, evenals op de melkproductie. Voorbeelden hiervan zijn (de methode van) graslandvernieuwing, de persistentie van soorten, de consistentie van effecten van soorten, zoals het effect van de aanwezigheid van weegbree op N_2O-emissies, en effecten van een lagere N-bemesting of van andere soorten op de voederwaarde van voer. Deze aspecten zijn niet onderzocht.

De geschatte effecten op N-verliezen van de onderzochte opties waren relatief klein. Ook wanneer de opties werden gecombineerd, waren ze niet voldoende om beleidsdoelen tot 2030 te halen op het gebied van stikstof. Ook waren de geschatte effecten op N-verliezen lager in vergelijking met eerdere studies, waarin bijvoorbeeld extensivering van de melkproductie per hectare is onderzocht. De thema's van dit proefschrift waren echter vernieuwend en onderzoek naar deze thema's op veengrond was tot nu toe schaars. Ook zijn de onderzochte opties relatief eenvoudig te implementeren op een melkveebedrijf, en hebben ze lage kosten. De teelt van lisdodde vormt hierop een uitzondering, omdat een vernatte veengrond na het verwijderen van de voedselrijke bovengrond niet eenvoudig weer als productief grasland kan worden teruggenomen. Bovendien kunnen de opties allemaal een positief effect hebben op andere beleidsdoelen dan beleidsdoelen gerelateerd aan stikstof.

De beleidsambities voor NH_3 en broeikasgasemissies na 2030 zijn strikter, wat kan betekenen dat het mogelijke landgebruik van veengronden verder verandert. Gezien de striktheid van lange termijn beleidsdoelen, kan men zich afvragen of met opties welke relatief eenvoudig toepasbaar zijn in de huidige landbouwpraktijk, en waarbij melkproductie niveaus kunnen worden behouden, voldoende zijn om deze doelen te halen.

Andere opties zouden moeten worden onderzocht om integraal de verschillende beleidsdoelen voor 2050 in het Groene Hart te halen. Deze opties zullen een grotere verandering betekenen voor de huidige landbouwpraktijk, zoals extensivering van de veehouderij of de teelt van gewassen in waterverzadigde bodems. Daarom is onderzoek naar sociaaleconomische aspecten van toekomstige landgebruikopties ook aan te bevelen voor het Groene Hart. Daarnaast kunnen de onderzoeksthema's van dit proefschrift ook relevant zijn voor minerale bodems, en in het geval van lisdodde betekent dit minerale bodems die drassig of overstroomd zijn.

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Toen ik in 2007 startte met de studie Dierwetenschappen in Wageningen kwam ik door mijn interesse in melkgeiten in contact met het Louis Bolk Instituut (LBI), waar ik een afstudeeronderzoek deed. Dit is een basis geweest voor mijn huidige werk, en daar ben ik Nick en Anneke dankbaar voor. Ik was erg onder de indruk van de verbinding tussen onderzoek en praktijk die het LBI maakt. Tijdens mijn studie ging ik een aantal weken helpen op het landbouwbedrijf van de familie Diard in Frankrijk. Stéphane was degene die mij een spiegel voorhield met de vraag: "Waarom ga je niet promoveren?" Stéphane, merci de m'avoir posé cette question.

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Curriculum vitae

Jeroen Pijlman was born in 1984 in Heerenveen, the Netherlands. He grew up on a dairy farm bordering the Weerribben-Wieden National Park. During his secondary education period at the Emelwerda College in Emmeloord (1996-2001), he became inspired by his family and friends to study animal husbandry at Aeres University of Applied Sciences in Dronten, where he obtained a Bachelor's degree in 2005. In Dronten he had not only learned about animal husbandry but also gained valuable work experience at various arable farms.

After a two-year period in which he worked as a salesman and on various farms in Australia and the Netherlands, Jeroen decided to study animal sciences with a specialisation on animal nutrition and grassland sciences at Wageningen University & Research. He did two master's theses; one at the Institute of Biological, Environmental and Rural Sciences of Aberystwyth University on rumen nitrogen dynamics and one at the Louis Bolk Institute on grazing of organic dairy goats. He received his Master's degree in 2009.

From 2009 to 2015, Jeroen worked for a year at the Grassland Sciences Foundation as researcher in the field of animal nutrition, grassland and milk quality, in combination with farm work in Brittany, France, and for five years at Schothorst Feed Research as researcher on feedstuff evaluation, feed formulation and animal nutrition.

In 2016 he started working as a researcher in sustainable livestock production at the Louis Bolk Institute. Since then, Jeroen has initiated, led and executed several projects on topics related to soil, crops, animal nutrition, fertilisation and their effects in relation to crop and animal production, nutrient cycling and losses, with a focus on peatlands and nitrogen cycling. He has moreover worked on economic aspects of farming in relation to reducing environmental pressure. The first two projects he worked on, *Proeftuin Veenweiden* and *Veen, Voer en Verder* provided the majority of the data for this PhD thesis. In 2021, he was admitted as an external PhD candidate at the Institute of Environmental Sciences, at Leiden University, in parallel with his work at the Louis Bolk Institute.

List of publications

Scientific articles included in this thesis

Pijlman, J., Berger, S.J., Lexmond, F., Bloem, J., van Groenigen, J.W., Visser, E.J.W., Erisman, J.W. & van Eekeren, N. (2020). Can the presence of plantain (*Plantago lanceolata* L.) improve nitrogen cycling of dairy grassland systems on peat soils? New Zealand Journal of Agricultural Research, 63, 1–17.

Pijlman, J., Geurts, J., Vroom, R., Bestman, M., Fritz, C. & van Eekeren, N. (2019). The effects of harvest date and frequency on the yield, nutritional value and mineral content of the paludiculture crop cattail (*Typha latifolia* L.) in the first year after planting. Mires and Peat, 25, 1–19.

Pijlman, J., Hoekstra, N., Deru, J.G.C., Erisman, J.W. & van Eekeren, N. (2023). Effect of *Lolium perenne* population differences on shoot tissue nitrogen concentrations when grown on a peat soil. The Journal of Agricultural Science, First View, 1-13.

Pijlman, J., Holshof, G., van den Berg, W., Ros, G.H., Erisman, J.W. & van Eekeren, N. (2020). Soil nitrogen supply of peat grasslands estimated by degree days and soil organic matter content. Nutrient Cycling in Agroecosystems, 117, 351–365.

Scientific articles relevant to the subject of this thesis

De Jong, M., van Hal, O., **Pijlman, J.**, van Eekeren, N. & Junginger, M. (2021). Paludiculture as paludifuture on Dutch peatlands: An environmental and economic analysis of *Typha* cultivation and insulation production. Science of The Total Environment, 792, 148161.

de Klein, C.A.M., Bowatte, S., Simon, P.L., Arango, J., Cardenas, L.M., Chadwick, D.R., **Pijlman, J.**, Rees, R.M., Richards, K.G., Subbarao, G.V. & Whitehead, D. (2022). Accelerating the development of biological nitrification inhibition as a viable nitrous oxide mitigation strategy in grazed livestock systems. Biology and Fertility of Soils, 58, 235–240.

Jeroen Pijlman is also (co-)author of professional magazine articles, reports, brochures and conference papers. For an up-to-date list, see https://www.louisbolk.nl/over-ons/medewerkers/jeroen-pijlman