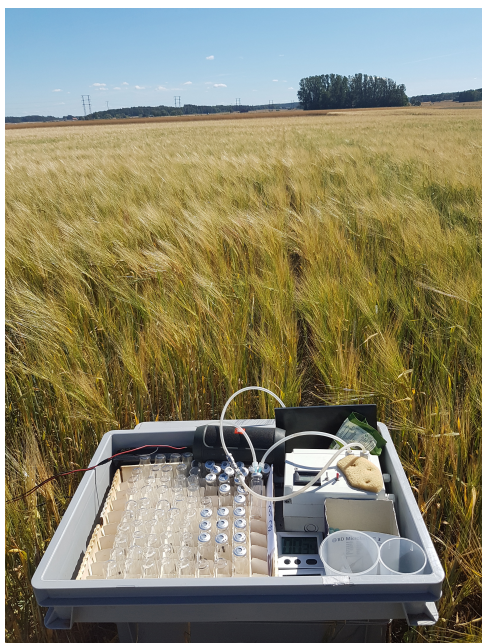




DOCTORAL THESIS No. 2023:60
FACULTY OF NATURAL RESOURCES AND AGRICULTURAL SCIENCES

Fertilizer placement for improved nitrogen use efficiency and mitigation of N_2O emissions

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SWEDISH UNIVERSITY
OF AGRICULTURAL
SCIENCES

DOCTORAL THESIS

Uppsala 2023

Acta Universitatis Agriculturae Sueciae
2023:60

Cover: Chamber greenhouse gas measurement in the field
(photo: K. Rychel)

ISSN 1652-6880

ISBN (print version) 978-91-8046-172-6

ISBN (electronic version) 978-91-8046-173-3

<https://doi.org/10.54612/a.323phkg6bv>

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Print: SLU Grafisk service, Uppsala 2023

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Abstract

Deep fertilizer placement is a promising strategy to increase crop yield and nitrogen (N) use efficiency while decreasing leached N and fertilizer-induced nitrous oxide (N₂O) emissions from soil to atmosphere. The objective was to test three fertilization depth treatments to compare greenhouse gas emissions of N₂O, methane (CH₄) and carbon dioxide (CO₂), as well as leaching and crop response. A deep fertilizer placement (Deep) at 0.20 m, a shallow placement (Shallow) at 0.07 m and a mixed-depth placement (Mixed) where the same amount of fertilizer was split between the depths of 0.07 and 0.20 m, and a non-fertilized control (Control) were evaluated. These fertilizer treatments were tested in a two-year field experiment, a five-year lysimeter experiment consisting of three years with crops, in addition to a 12-day lab incubation using labeled fertilizer. Deep placement significantly increased grain N uptake and yield and decreased N₂O and CH₄ emissions. CO₂ emissions from bare soil were not significantly affected by fertilizer placement. Further, Deep reduced N leaching and increased crop water utilization. The tracer incubation indicated that indigenous soil N was the primary contributor to total N₂O emissions in Deep, and CH₄ emissions were correlated to N₂O fluxes originating from fertilizer N. X-ray tomography of the incubated soil cores revealed that fluxes of N₂O from fertilizer were affected by compaction level. Overall N use efficiency (NUE) was evaluated in the soil-crop system via an N balance, % NUE, agronomic efficiency of N (AE_N) and the recovery efficiency of N (RE_N). Over the five years with crops, all indices indicated a higher N use efficiency with greater fertilizer placement depth.

Keywords: N use efficiency, nitrous oxide, methane, nitrate leaching, deep N fertilization, lysimeter

Dedication

To Ellison and Apollonia, with all my love, always.

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List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I. Rychel, K., Meurer, K.H.E., Börjesson, G., Strömgren, M., Getahun, G.T., Kirchmann, H., Kätterer, T. (2020). Deep N fertilizer placement mitigated N₂O emissions in a Swedish field trial with cereals. *Nutrient Cycling in Agroecosystems*, 118, pp. 133–148
- II. Rychel, K., Meurer, K.H.E., Getahun, G.T., Bergström, L., Kirchmann, H., Kätterer, T. (2023). Lysimeter Deep N Fertilizer Placement Reduced Leaching and Improved N Use Efficiency. *Nutrient Cycling in Agroecosystems*, 126, pp. 213-228
- III. Rychel, K., Koestel, J., Keck, H., Meurer, K.H.E., Kätterer, T. (2023) Greenhouse gas emissions as effected by N fertilizer placement depths and soil structure under controlled conditions (Manuscript)

Papers I and II are reproduced with the permission of the publishers.

The contribution of Katrin Rychel to the papers included in this thesis was as follows:

- I. Planned the experiment together with the co-authors. Carried out field work, data analysis, and manuscript preparation with assistance from the co-authors.
- II. Planned the experiment together with the co-authors. Carried out field work, data analysis, and manuscript preparation with assistance from the co-authors.
- III. Planned the experiment together with the co-authors. Carried out field work, data analysis, and manuscript preparation with assistance from the co-authors.

1. Introduction

Currently the world faces a great challenge to supply a greater quantity of food for a growing global population, while concurrently reducing the negative environmental repercussions that arise from crop production. Although significant progress has been made to increase the efficiency of fertilizer use, particularly in areas with greater access to technology, crop uptake of applied nitrogen (N) remains difficult to control. Globally, crop N yield is less than half of total N inputs (Lassaletta *et al.* 2014). The resulting N surplus, the difference between total N in- and outputs, is generally lost to aquatic systems or the atmosphere (Davidson *et al.* 2012). In the European Union, around 60-65% of applied fertilizer N is taken up by crops, while 85% of agricultural land is currently exceeding EU policy target thresholds for N losses to water and air (Shulte-Uebbing & Vries 2021).

The global increase of nitrate leaching into groundwater and surface waters has been linked to a wider use of fertilizer (Bijay-Singh & Craswell 2021). This leaching can pollute groundwater connected to the drinking supply that may reach unsafe levels for consumption, and if carried to surface waterbodies, may lead to eutrophication and aqueous dead zones.

Similarly, the increase in atmospheric nitrous oxide (N₂O) concentration is linked to an increasing use of fertilizer N within the agricultural sector (Nabuurs *et al.* 2022). Nitrous oxide contributes to the greenhouse gas effect, with a potency around 265 times that of carbon dioxide (CO₂) on a 100-year time scale (Myhre *et al.* 2013) and has a residence time of around 120 years. Atmospheric N₂O is either removed via microbial reduction or transported to the stratosphere and consumed in an ozone-depleting chemical reaction, making it one of the most dominant sources of ozone depletion (Ravishankara *et al.* 2009).

Thus, fertilizer management strategies aimed to increase crop yields and/or crop N uptake should also have as an objective to decrease the negative environmental impacts. Conversely, aiming to decrease the environmental damage from N fertilization should also not have an adverse effect on crop yield. Increasing the nitrogen use efficiency (NUE) in the cropping system would address both issues of increasing the uptake of applied fertilizer N in harvested biomass, while also reducing the detrimental impact of N losses to the atmosphere and via leaching. There are, however, different indices for NUE determination that vary in complexity and focus (Congreves *et al.* 2021). For example, from a grower's perspective, a simple calculation of yield per unit of applied fertilizer N or the difference between yield N and fertilizer N may be sufficient to compare different management strategies of the same type of crop, particularly for an economic analysis. However, these indices do not take into account indigenous (background) soil N, and may even overestimate NUE. A more meaningful approach to evaluate fertilizer N use would be to further incorporate background soil N as well as estimates of losses to the environment that are possible to quantify. Although fertilizer losses initially constitute an economic loss to the grower, these N losses to the environment ultimately come at a cost to society in the form of eutrophication and climate change, which is predicted to negatively impact food security globally (Challinor *et al.* 2014).

One possible strategy to increase the nitrogen use efficiency (NUE) and mitigate negative environmental impact is to increase the placement depth of N fertilizer. Previous field studies have shown that deep fertilizer placement, compared to a broadcast application, increased yields, improved NUE, and decreased N runoff (Mengel *et al.* 1982; Kelley & Sweeney 2007; Xia *et al.* 2016; Zhu *et al.* 2019). Regarding N₂O emissions, however, results are somewhat contradicting: while deep N fertilizer placement effectively lowered N₂O emissions in rice paddies (Gaihre *et al.* 2015; Wu *et al.* 2017) and in field experiments in upland regions (e.g., Chen *et al.* 2021, Pandit *et al.* 2022), as well as in combination with conservation tillage methods (Liu *et al.* 2006; Nash *et al.* 2012), other studies (e.g. Cai *et al.* 2002; Drury *et al.* 2006; Chu *et al.* 2007) found that N₂O emissions were higher from deeper N placement compared to shallow N placement.

However, the effects of this fertilization strategy on N₂O emissions have not been tested in a northern European climate, which, relative to mainland Europe, has a shorter growing season with longer daylight, cooler

temperatures, and crops are primarily reliant on precipitation. Outside of the growing season, agricultural land is subjected to freeze-thaw cycles that can result in large pulses of N₂O emissions. On a more general level, the effect of deep N fertilization on environmental impact categories such as N leaching, water use efficiency, and emissions of carbon dioxide (CO₂) and methane (CH₄) is scant in the literature.

Isotopic tracer studies have been performed to examine potential microbial reduction of the N₂O gas evolved from deep-placed fertilizer N in a packed soil column on its path of diffusion to the soil surface (e.g., Clough *et al.* 2006, Vieten *et al.* 2008). However, columns packed with sieved soil do not fully reflect GHG evolution and diffusion in the environment, and thus information linking these processes to intact soil structure is largely absent.

2. Objectives

The overall objective of this thesis was to elucidate how a deep vs. shallow or mixed-level placement of N fertilizer affected N losses. The specific objectives were to:

- Quantify GHG emissions, crop yield and N uptake, and changes in soil mineral N content in a field environment (Paper I)
- Evaluate N losses via leaching and plant-water use across multiple cropping seasons in a semi-controlled environment (Paper II)
- Examine the relationship between soil structural properties and GHG emissions on bare soil after adding N fertilizer at deep, shallow and mixed depths, and quantify the proportion of N₂O emissions from applied fertilizer in a controlled environment (Paper III)

3. Background

3.1 Environmental impact of N fertilization in upland cropping systems

3.1.1 Gaseous N losses

About 60% of global anthropogenic N₂O production comes from agriculture (Tian *et al.* 2020). Although denitrification occurs naturally in many ecosystems not subjected to fertilization, it is the input of nitrogenous fertilizer that drives higher rates of nitric oxide (NO) and N₂O emissions. The two primary microbial processes responsible for the transformation of ammonium and nitrate to nitrous oxide are nitrification and denitrification, respectively. Nitrification and denitrification are thought to occur simultaneously and in conjunction with one another, with relative contributions dependent on a variety of factors such as microbial community structure and their distribution in the soil matrix, soil physical properties, climatic conditions, and the presence or absence of oxygen (O₂) (Van Groenigen *et al.*, 2005).

Nitrification

Nitrification is a two-step aerobic process dependent on the presence of O₂ and NH₄⁺ and performed by a variety of microorganisms, but those that are known to be primarily responsible for the pathways leading to N₂O evolution along this pathway are autotrophic bacteria from the genera of *Nitrosomonas* and *Nitrospira*, referred to as ammonia oxidizing bacteria (AOB). In this process, ammonium is oxidized to nitrite, with an intermediate formation of hydroxylamine (NH₂OH), then nitrite is further

oxidized to nitrate. N_2O and NO can sometimes be formed as a byproduct during the aerobic oxidation of hydroxylamine to nitrite. (Hooper and Terry, 1979).

Denitrification

Denitrification is the anaerobic stepwise reduction of nitrate and nitrite, with some “losses” of NO and N_2O gas occurring before complete reduction to N_2 . A diverse group of heterotrophic bacteria, fungi, and archaea are capable of denitrification in soil, many of which may not have the functionality to perform each step of the entire reductive process (e.g., Graf *et al.* 2014; Zumft 1997). The primary controls on denitrification are the presence of nitrate and mineralizable carbon, along with the absence of oxygen.

There is also a distinction between denitrification performed by nitrifiers, and that of denitrifiers. Some ammonia oxidizing bacteria (AOB), produce an enzyme, nitrite reductase, which reduces nitrite to N_2O , typically under near-anaerobic conditions, when O_2 concentrations are limited in a process called nitrifier denitrification. The evolution of N_2O via this process is considered to be a strategy for these microorganisms to conserve O_2 for the energy-producing process of NH_4 oxidation, and to prevent the toxic buildup of nitrite (Poth & Focht 1985).

In contrast with nitrification, microbial denitrification can be either N_2O -producing or N_2O -consuming. A key group of microorganisms involved in N_2O reduction is a phylogenetically diverse group possessing a gene for an “atypical” *nosZ* (clade II *nosZ*) enzyme, which reduces N_2O to N_2 . Approximately 50% of these organisms are not involved in any other denitrification processes and are surprisingly abundant in some soils (Hallin *et al.*, 2018).

Factors affecting gaseous N_2O losses from the soil-crop system

Soil physical factors that affect the aeration and the balance of O_2 and CO_2 in the soil have the greatest impact on denitrification processes (Conrad *et al.*, 1983; Letey *et al.*, 1980). Pore size and distribution affect the ability for both O_2 to diffuse into the matrix and for evolved gasses from the matrix to reach the soil surface. The amount of soil water affects gas diffusivity in the soil matrix, and thus has a significant and well-known effect on the rate of denitrification, where higher levels of water filled pore space (after heavy

rainfall, for example) reduce oxygen in microsites, creating a favorable condition for anaerobic processes.

Temperature is another important factor as it affects soil respiration and thus the consumption of oxygen, driving the O₂/CO₂ balance in the soil matrix. An increase in temperature can create large anoxic areas in addition to driving higher rates of denitrification. However, while temperature affects the rate of denitrification, temperature thresholds for denitrification rates likely vary geographically due to the adaptation of microbial communities to the local climate (Powlson *et al.*, 1988).

Freeze-dry cycles have a significant effect on denitrification activity, particularly in croplands (Matzner & Borken, 2008), where thaws can be the source of more than half the yearly release of nitrous oxide (Abalos *et al.*, 2016). Wagner-Riddle *et al.* (2017) estimated that thaw cycles from frozen cropland contribute 1.07 ± 0.59 Tg of N₂O-N per year, a source that has been previously underestimated in global GHG budgets. During soil thaws, soil water content is high, thus creating anaerobic conditions. Additionally, during these cycles any trapped N₂O is liberated, and labile C and N substrates can be released from aggregates, crop residues, and dead microbial cells (Herrmann & Witter, 2002; Morley *et al.*, 1983; Skogland *et al.*, 1988).

3.1.2 Carbon dioxide and methane

The C and N cycles in soils are inherently connected. But while it is well documented that the input of N fertilizer is one of the best predictors of N₂O emissions from soils, the effect on fluxes of methane and carbon dioxide is less straightforward. Fluxes of CO₂ from the soil to the atmosphere are largely controlled by climatic factors such as temperature as well as soil moisture, which in turn affects soil O₂ concentrations and nutrient mobility.

Fluxes of CO₂ from the soil to the atmosphere are evolved primarily from two sources: the autotrophic respiration of roots, where soil O₂ is diffused into root hairs and CO₂ is released, and from heterotrophic microbial respiration, the mineralization of plant residues and soil organic carbon. The availability of mineral N in the soil may increase the microbial utilization of soil carbon, which can result in a net loss of SOC (Khan *et al.* 2007, Mulvaney *et al.* 2009). On the other hand, it has been shown that fertilization may increase microbial carbon use efficiency and lower specific respiration, leading to soil carbon accumulation (Poeplau *et al.* 2018). Meanwhile, the magnitude of photosynthetic uptake of CO₂ by the aboveground biomass of

plants is connected to crop productivity, and at least partially affected by N availability. Crop productivity in turn affects the deposition of root exudates in the rhizosphere as well as the quality and quantity of crop residues (Lee *et al.* 2007, Luo *et al.* 2010).

As a GHG, methane is 25 times more potent than CO₂ on a 100-year time scale and has a residence time in the atmosphere of around 10 years. Methane is commonly emitted from saturated (anaerobic) soils such as wetlands and flooded rice systems where it is produced by methanogenic archaea. In upland soils, net methane fluxes are generally negative, which means that these soils are sinks for atmospheric methane. This sink is mediated by methane oxidizing bacteria (Reay *et al.* 2007). Both of these processes can be affected by N fertilization, leading to higher CH₄ emissions. For example, methanogenesis can be stimulated indirectly by stimulating plant growth and rhizodeposition, which in turn increases the availability of fermentation products utilized by methanogenic bacteria. In upland soils, N fertilization has been shown to inhibit methane oxidation (Acton & Baggs 2010), although this process is not well understood (see review by Bodelier 2011).

3.1.3 N leaching

Nitrate is highly mobile in the soil, particularly when soil moisture is high. In humid climatic conditions, dissolved nitrate (NO₃⁻) and nitrite (NO₂⁻) are transported through the soil into ground- and surface water and further into streams, lakes and coastal areas, contributing to eutrophication. Around 50% of the estimated total 136.6 Tg N annual global N flows into croplands are from fertilizer input, and of that leaching accounts for 23 Tg N year⁻¹ (Liu *et al.* 2010). Of all areas globally where high groundwater concentrations of NO₃⁻ are present, 60% are in croplands (Shukla & Saxena 2018). In coastal surface waters normally limited in N, increases of N inflows by 10 to 15 times have been observed, which stimulated algal blooms and greatly increased eutrophication (Howarth 2008).

3.2 Consequences of N fertilizer placement depth

3.2.1 Increasing the path of diffusion from N₂O source to soil surface

Field studies investigating N₂O concentrations at different depths in the soil profile have shown that N₂O can be consumed as it is diffused from

deeper to shallower layers in the soil profile (e.g., Goldberg *et al.* 2008, Goldberg & Gebauer 2009, Rock *et al.* 2007). This phenomenon has also been observed using tracer studies in packed soil columns (Clough *et al.* 1998). In a review by Chapuis-Lardy *et al.* (2007), the authors note that when microbial N₂O uptake was observed, it tended to be in cases where soil moisture limited gas diffusion through the soil matrix, particularly in the absence of mineral N. When soils are amended with biochar, there can be a reduction of N₂O emissions (see for example the meta-analysis of Borchard *et al.* 2019). Harter *et al.* (2016) posited that with biochar amendment, N₂O is entrapped and then reduced in water-saturated pores. Thus, if fertilizer N were placed deeper in the soil profile, the length of diffusion from the source of N₂O formation (i.e., from N fertilizer) to the soil surface increases. Consequently, the residence time of the gas is increasing, and along with this, also the probability of its reduction to N₂.

3.2.2 Mineral N in the soil profile

Temperature and moisture are major controls on soil N turnover, availability and mobility, affecting N losses via leaching (Robinson 2002). Wet-dry cycles in soil induce pulses of N and C mineralization upon re-wetting (Schimel 2018), of which the upper topsoil is most affected via rainfall events that mobilize fertilizer N. The amplitude of temperature and moisture variability decreases with increasing soil depth, thus increasing fertilizer placement depth may be an effective method for keeping plant available N over longer periods with less rainfall due to more constant soil moisture conditions. Previous studies regarding fertilizer placement depth in non-flooded soil systems are scant and results are somewhat inconsistent. Wu *et al.* (2022a) found that deep N placement at 0.25 or 0.15 m decreased NO₃⁻ content in the 0–1 m depth compared to a shallow (0.05 m) placement in a field experiment with maize. Similarly, in a study by Grant *et al.* (2019), NO₃⁻ leaching was reduced in a laboratory experiment with intact soil monoliths when fertilizer was placed at 5 cm vs. a surface placement. Wang *et al.* (2022) reported that NO₃⁻ leaching varied with deep urea placement and depended on the amount of seasonal rainfall. Wang *et al.* (2023) similarly found that deep N fertilizer placement at 0.25 and 0.35 m under the wettest year (275 mm rainfall) led to a significant increase in NO₃⁻-N in the soil profile at 0.6 – 1.0 m depth, but in dry and normal years

3.2.3 Crop response in root distribution, N uptake, and yield

Crop roots tend to proliferate around the area of the fertilizer grain, thus deeper placement can promote root length density and enhance N uptake (Lotfollahi *et al.* 1997; Li *et al.* 2009) as well as water utilization (Singh *et al.* 1976) from deeper soil layers. Although genetics play a fundamental role in plant rooting patterns, many studies have shown that roots exhibit plasticity in response to the soil environment, particularly when nutrients are distributed heterogeneously or in patches (Hodge 2004). Because crops can obtain more than two thirds of their nutrition from deeper layers in the soil profile when nutrient availability and/or water is limited in the topsoil (Kautz *et al.* 2013), deep fertilization placement could improve plant growth, particularly during periods of little to no precipitation.

Previous field studies showed that deep fertilizer placement, compared to broadcast surface application, increased yields and improved NUE (Mengel *et al.* 1982; Kelley & Sweeney 2007; Xia *et al.* 2016; Zhu *et al.* 2019). The aforementioned study by Wang *et al.* (2022) also reported that deep urea placement promoted the proliferation of deep roots in winter wheat, which increased crop N uptake and water utilization.

4. Materials and Methods

4.1 Experimental site

The field experiment (Paper I) took place in Säby, in the southeast of Uppsala, Sweden (59°83'N, 17°71'E). Soils used in the lysimeter experiment (Paper II) were collected from an adjacent area to the experimental field plots, and columns for the incubation (Paper III) were taken directly from field plots. Rain-fed cereals have been the dominant crop cultivated in this area, which has been under agricultural use for over a century. Mean annual precipitation (1961 – 1990) is approximately 528 mm per year, around 40% of which occurs from May to August, and mean annual air temperature is 5.5°C. The soil has a silt loam texture in the topsoil and is classified by FAO as a Eutric Cambisol (FAO 2014). Soil profile characteristics can be found in Table 1.

Table 1. Soil physical properties in the soil profile from the experimental field site at Säby, Uppsala, Sweden. Soil bulk density (BD) (kg dm^{-3}), porosity (%), organic carbon (SOC) (g kg^{-1}), total nitrogen (g kg^{-1}), carbon to nitrogen ratio, calcium carbonate (CaCO_3) (g kg^{-1}), pH (H_2O), and texture represented by percentage clay, silt, and sand. Bulk density and porosity were not collected at 1.0-1.1 m depth due to groundwater infiltration. Adapted from Getahun *et al.* 2021.

Depth (m)	BD (kg dm^{-3})	Porosity (%)	SOC (g kg^{-1})	Total N (g kg^{-1})	C:N	CaCO_3 (g kg^{-1})	pH (H_2O)	Clay (%)	Silt (%)	Sand (%)
0-0.1	1.3	50.9	28.2	2.4	11.9	0.10	6.1	21.9	54.5	23.6
0.1-0.2	1.4	48.3	26.4	2.2	11.8	0.20	6.1	20.5	56.9	22.6
0.2-0.3	1.4	46.8	14.2	1.2	11.6	0.09	6.3	21.3	56.2	22.5
0.3-0.4	1.6	41.5	7.9	0.7	11.6	0.07	6.5	18.9	54.1	27.0
0.4-0.5	1.5	43.0	3.8	0.4	9.6	0.07	6.7	23.5	59.7	16.8
0.5-0.6	1.4	46.4	3.4	0.4	8.6	0.10	6.8	25.3	62.6	12.1
0.6-0.7	1.4	48.7	3.7	0.5	7.9	0.09	6.9	31.1	61.1	7.8
0.7-0.8	1.4	48.7	3.2	0.4	7.6	0.07	6.7	27.4	56.7	15.9
0.8-0.9	1.3	49.4	6.3	0.9	7.3	0.11	6.1	39.6	57.7	2.7
0.9-1.0	1.2	54.0	6.4	0.9	7.3	0.12	5.2	34.2	63.1	2.7
1.0-1.1			9.8	1.3	7.4	0.16	4.8	40.6	57.4	2.0

4.2 Environmental conditions

The field experiment ran for two cropping seasons, 2016 and 2017 (Fig 1). Lysimeters were installed in the lysimeter station at SLU and exposed to natural climatic conditions from June 2016 until August 2021. In general, the majority of months during the cropping season (May to August) between 2016 – 2021 were warmer and drier compared to long-term average (1961-1990). During the summer of 2018 there was a substantial drought characterized by high temperatures, very low precipitation and high solar radiation.

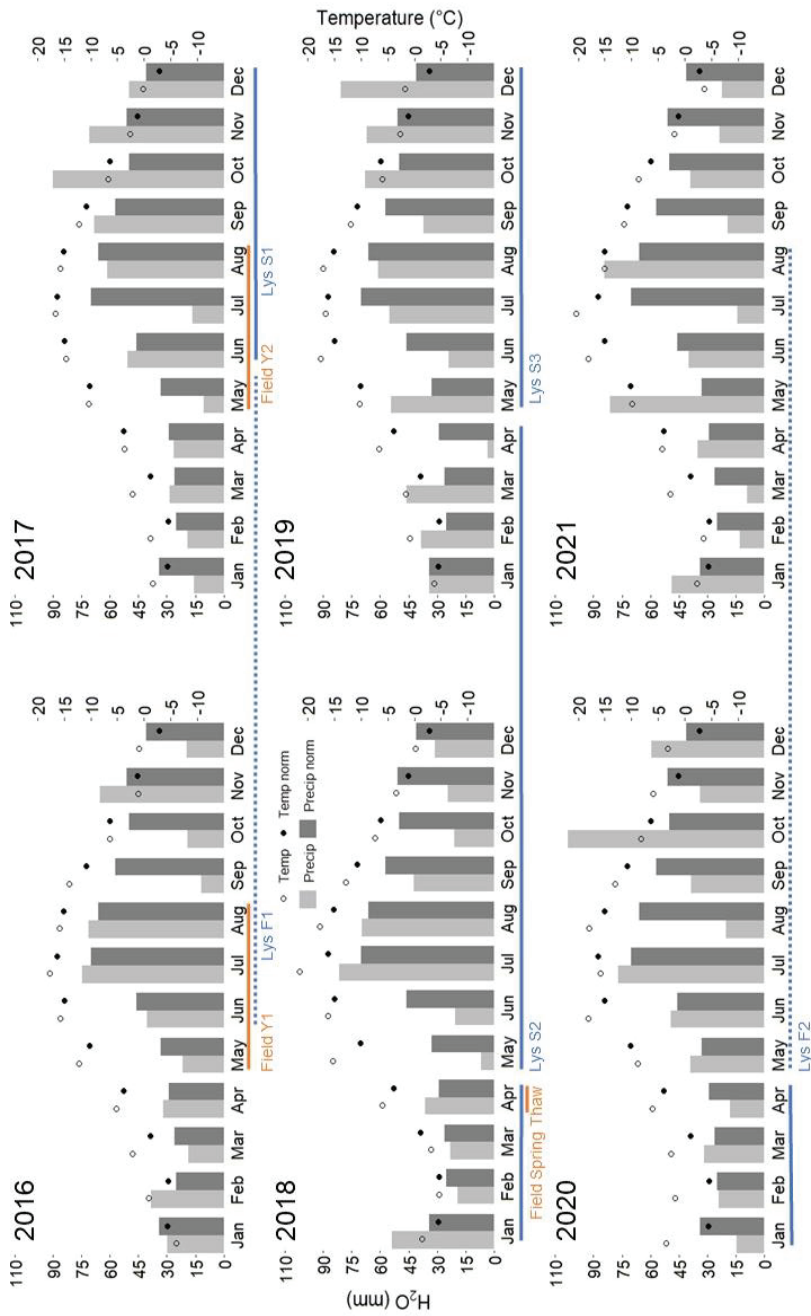


Figure 1. Sum of precipitation (mm H₂O) and average air temperature (°C) within the experimental periods compared to the long-term normal (1961-1990) for the given month. Data was collected from a Lantmet weather station on the SLU Ultuna campus in Uppsala, Sweden.

4.3 Experimental design

The local practice in SE Sweden, where cereals are the dominant crops, is to place fertilizer around 0.07 m and seeds at 0.05m depth to promote germination without the reliance on immediate rainfall. Tillage occurs in the autumn, to a depth of 0.2-0.25 m and harrowing is performed in the spring, prior to sowing, to a depth of around 0.07 m. In these experiments, this shallow fertilizer placement at 0.07 (Shallow) was used as a baseline to compare to a deeper placement (Deep) at 0.2 m, near the border of the tillage depth, as well as a mixed-level placement (Mixed) where half of the total fertilizer N was placed at 0.07 and half at 0.2 m, in the same vertical plane.

4.3.1 Field experiment

The three fertilizer placement treatments (Shallow, Mixed, and Deep) plus a non-fertilized control (Control) were tested in the field at Säby following a randomized block design. There were four blocks, each containing a 20 x 4 m plot for each of the three treatments plus control, for a total of 16 plots. The experiment encompassed two cropping seasons (2016-2017) during which spring wheat (*Triticum aestivum* L.var. ‘Quarna’) was grown in year 1 and spring barley (*Hordeum vulgare* L. var. ‘Makof’) in year 2. Ammonium nitrate (YaraBela AXAN, Yara International, Oslo, Norway) was applied at a rate of 120 kg N ha⁻¹ in the first year and 200 kg N ha⁻¹ in the second year. Fertilizer application and sowing was performed simultaneously with a Combi drill (Spirit 400C Strip Drill, Väderstad, Sweden) that allows for fertilizer and seed depth adjustment and two-level (i.e., 0.07 m and 0.2 m) fertilizer placement.

4.3.2 Lysimeter experiment

In May 2016, sixteen undisturbed soil monoliths (0.295 m diameter and 1.18 m depth) were collected from the field site in Säby with a tractor-mounted hydraulic soil auger according to the method described by Persson and Bergström (1991). The monoliths were capped on both ends and transported with minimal disturbance on a flatbed truck to the SLU-Uppsala lysimeter station. Approximately 0.08 m of soil was removed from the lower end of each monolith and replaced with pea gravel (2 – 5 mm diameter).

Stainless steel mesh was placed between the gravel layer and a perforated end cap. The topsoil (0 – 0.25 m) of all monoliths was removed and pooled, mixed together, then replaced in order to simulate tillage. The lysimeters were then carefully lowered down into the cement-walled ports where they would sit on a steel support seat, fitted on the underside with a funnel connected to piping that would transport individual lysimeter leachate into a collection flask in the basement of the lysimeter station.

The 16 lysimeters were randomized so that each of the four treatments (Shallow, Mixed, Deep, Control) contained a monolith from four different areas of the soil from which it was removed in order to represent existing heterogeneity in the field. Lysimeter installation occurred in June 2016, from which point the monoliths were exposed to weather and drained freely by gravity. To provide some time for the lysimeters to settle and to give some background information about individual lysimeter draining behavior, the fertilization experiment was not initiated until June 2017. During this initial period (F1), no crops were grown and the lysimeters were manually kept free of weeds.

The lysimeters were planted with spring-sown cereals for three consecutive growing seasons from 2017 – 2019. In 2017 (S1), the crop was spring barley (*Hodeum vulgare* L. var. ‘Makof’), followed by spring wheat (*Triticum aestivum* L. var. ‘Quarna’) in 2018 (S2), and oats (*Avena sativa* L. var. ‘Symfoni’) in the final year 2019 (S3). All sixteen lysimeters were fertilized with 40 kg K and 15 kg P ha⁻¹ yr⁻¹ at 0.07m depth. Shallow and Deep treatments additionally received 15 kg S and 100 kg N ha⁻¹ yr⁻¹ at 0.07 m depth and 0.2 m depth, respectively. Mixed treatment was fertilized with the same amount as Shallow and Deep, but the total amount of N and S was split between placement depths at 0.07 and 0.2 m. Control was not fertilized with either N or S.

The experimental time was split into five different periods consisting of two “fallow” periods F1 and F2, which preceded and followed the three growing seasons with fertilizer treatments (S1 – S3), respectively (Table 2). Similar to F1, the soil was in bare fallow during F2 and no fertilization was performed.

Table 2 Period name, duration in months, crop type, and crop sowing and harvest dates in the lysimeter experiment.

Period	Duration (mos)	Crop	Cropping period
F1	10	N/A	N/A
S1	11	Barley	12 June – 29 September 2017
S2	12	Wheat	10 May – 16 August 2018
S3	12	Oats	13 May – 2 September 2019
F2	13	N/A	N/A

4.3.3 Incubation and labelling experiment (Paper III)

In April 2018, 32 undisturbed soil cores were collected from the field site in Säby in 0.1 m-long, 0.072 m diameter steel cylinders. Half of the coil cores were collected from the 0 – 0.1 m depth and half were taken from the 0.1 – 0.2 m depth, the latter offset horizontally from where the upper core was taken, thus were not congruous. Prior to experiment initiation, all soil cores were placed in large containers in the laboratory and saturated from the bottom up over the duration of seven days by progressively adding water to the container in order to minimize trapped air. In order to simulate field conditions where the soil closer to the surface is somewhat drier, cores from the upper section (0 – 0.1 m) were placed on a sand bed at -105 cm pressure potential and cores from the lower layer (0.1 – 0.2 m) were placed on a sand bed at -100 cm pressure potential until they approximately equilibrated after seven days.

Sixteen pairs comprised of an upper and a lower column corresponding to the same plot as in the field experiment were assigned to four treatments with four replicates. Before stacking and assembling the upper and lower components, each individual core was injected using a 25 mm-long syringe with 1.25 mL of either i) 150 kg N ha⁻¹ in the form of double-N labeled 98 atom% ¹⁵N ammonium nitrate (Sigma-Aldrich) dissolved in distilled water, ii) half this N concentration (75 kg N ha⁻¹ in distilled H₂O), or iii) the equivalent amount of distilled water only. For the shallow N treatment (Shallow), the upper core was injected with the full concentration of the N fertilizer from the underside of the soil core and the lower core with distilled H₂O. For a mixed-level N treatment (Mixed), both the upper and the lower soil cores received the half N concentration injected into the underside of the

soil column. For the deep-placed N treatment (Deep), the full concentration of N fertilizer was injected into the underside of the lower soil column, and distilled H₂O was injected into the bottom of the top column. The fourth treatment received only distilled water, injected in the same manner as the other treatments. The paired cores were then stacked with the upper cores on top of the lower cores and capped at the bottom. All seams around the cap and between the two stacked cores were sealed with silicone.

The experiment was run in a temperature-regulated (22°C) control room. Soil cores were stored in the room with aluminum foil loosely covering the top soil surface to minimize moisture loss. Additionally, each column was weighed daily to monitor changes in soil moisture.

4.4 Greenhouse gas measurements

4.4.1 Field and lysimeter (Papers I and II)

Fluxes of N₂O and CH₄ were measured using static chambers to collect five chamber air concentrations in ten-minute intervals beginning immediately after chamber closure. Briefly, chambers were deployed onto fixed steel frames installed in each plot in the field (one per plot; Paper I), or fitted atop an individual lysimeter (Paper II). The chamber air was sampled at each time interval via the flow-through method where a loop was made with tygon tubing between the chamber, an air pump, and a 20 mL glass vial. Vial air concentrations of N₂O and CH₄ were determined on a gas chromatograph (Clarus 500, Perkin Elmer, USA) equipped with an automatic headspace injector (Turbo Matrix 110, Perkin Elmer, USA) and a flame ionization detector (FID) and electron capture detector (ECD).

Carbon dioxide fluxes were measured separately using a portable infrared gas analyzer (EGM-4, PP Systems, USA). Before seed emergence, an opaque chamber (SRC-2 Soil Respiration Chamber, PP Systems, USA) connected to the EGM-4 unit was used, which measured directly on the soil surface. After seed emergence, a transparent chamber was used. However, a transparent chamber was not used in the first growing season (2016) in the field experiment.

During the first year of the field experiment in 2016, GHG measurements were performed eight times between May and late July, primarily after rainfall events. In 2017, at both the field site and the lysimeter station, ten

measurements were taken in the two weeks following sowing/fertilization, then one weekly or biweekly measurement was taken for the duration of the growing season. Three additional measurements were taken in the field in April of 2018 following spring thaw. In 2018, a similar measurement scheme as the previous year was used at the lysimeter station. There were no GHG flux measurements taken during the third cropping season at the lysimeter station (S3).

4.4.2 Incubation (Paper III)

GHG fluxes from individual columns were measured once daily over the 12-day incubation period. Briefly, columns were placed one at a time into a 26 L PVC vessel with a screw-on lid for 15-20 minutes. The lid of the container was fitted with four ports with tubing connected to the inlet and outlet ports of a Picarro G2201-I ($\delta^{13}\text{CO}_2$, CH_4) and a Picarro G5131-I analyzer ($\delta^{15}\text{N}$ in N_2O). Soil $\delta^{15}\text{N}_2\text{O}$ and $\delta^{13}\text{CO}_2$ signatures from individual fluxes were determined by the y-intercept from a linear model of either $\delta^{15}\text{N}$ and $1/\text{N}_2\text{O}$ or $\delta^{13}\text{CO}_2$ and $1/\text{CO}_2$, respectively. Due to the high level of ^{15}N enrichment, the resulting $\delta^{15}\text{N}_2\text{O}$ values were converted from ‰ (per mille) to atom ‰. The relative contributions from fertilizer N fraction ($f\text{N}^{\text{fert}}$) and indigenous soil N fraction ($f\text{N}^{\text{ind}}$) to N_2O fluxes were calculated using a two-source mixing equation:

$$^{15}\text{N}_2\text{O} = ^{15}\text{N}_{\text{ind}} \times N_{\text{ind}} + ^{15}\text{N}_{\text{fert}} \times N_{\text{fert}}$$

where $^{15}\text{N}_{\text{ind}}$ and $^{15}\text{N}_{\text{fert}}$ are the respective ^{15}N enrichment of the two N sources and $^{15}\text{N}_2\text{O}$ is the ^{15}N enrichment of the N_2O flux. As the total N_2O production ($\text{N}_2\text{O}^{\text{tot}}$) constitutes the sum of the two N source fractions, i.e., $f\text{N}^{\text{fert}} + f\text{N}^{\text{ind}} = 1$, the above equation is reformulated to calculate the source fractions:

$$f\text{N}^{\text{ind}} = \frac{^{15}\text{N}_2\text{O} - ^{15}\text{N}_{\text{fert}}}{^{15}\text{N}_{\text{ind}} - ^{15}\text{N}_{\text{fert}}}$$

To obtain the N_2O fluxes originating from fertilizer ($\text{N}_2\text{O}^{\text{fert}}$), the respective fraction ($f\text{N}^{\text{fert}}$) was multiplied by $\text{N}_2\text{O}^{\text{tot}}$.

4.4.3 Flux determination (Papers I-III)

Individual GHG fluxes were determined using the R package *gasfluxes* (Fuss 2020), using the fit “robust linear” after correction for temperature and air pressure using the ideal gas law. The resulting fluxes with associated P values > 0.05 were not considered. The function `agg.fluxes` from *gasfluxes* was used to determine cumulative emissions (e.g., per period).

4.5 Crop sampling and analyses (Papers I and II)

The crop leaf chlorophyll was periodically measured throughout the growing season in both the field experiment (Paper I) and the lysimeter experiment (Paper II) using a hand-held Soil Plant Analysis Development meter (SPAD-502m, Minolta Camera Co, Osaka, Japan). Four measurements were taken and averaged from a fully expanded leaf at the top of four different plants within the same plot or lysimeter. On the same occasion, the plant height was measured from 16 plants within the same plot, or two plants from each lysimeter.

Mid-season plant biomass was collected from each field plot to determine crop N content at the growth stages of stem elongation and heading in 2016 and at booting in 2017 (Paper I). Dried biomass was ground and analyzed for N content on an organic elemental combustion instrument (LECO, USA).

Once crop maturation reached harvest, all biomass was removed manually at the base of the plant using scissors, encompassing either the entire lysimeter area, or within four randomly-placed 0.5 x 0.5 m metal frames within each field plot. Biomass was dried and threshed to separate straw from grain to determine yields of grain and crop residues. A subsample of ground straw and grain was analyzed for N content for each plot or lysimeter. Field plot grain biomass was additionally combine harvested from the center of the plots in a 34.8m² area (Paper I).

4.6 Soil collection and analysis (Paper I)

To follow the movement of fertilizer in the field over time, soil mineral N content was analyzed at three samplings occasions from the experimental plots in Säby in 2017 (Paper I), at the beginning of the experiment, mid-season (39 days after fertilization), and right after harvest. The soil was sampled to 0.4 m depth using a soil corer. The soil cores were then segmented

into five 0.05 m depth intervals between 0 – 0.3 m, and one at 0.3 – 0.4 m. A 2M potassium chloride (KCl) extraction of these soil samples was analyzed for mineral N content via colorimetric determination on a segmented flow analyzer (SEAL AutoAnalyzer 3, Seal Analytical, UK).

4.7 Leachate collection and analysis (Paper II)

Collection of lysimeter leachate began in September 2016 and the last leachate was collected in August 2021. Leaching generally occurred from autumn to early spring due to high evapotranspiration relative to precipitation in spring and the summer months. Leachate sampling did not generally occur until there was sufficient leachate present for sampling from each of the individual lysimeters. On sampling occasions, drainage water was weighed and subsampled for ammonium (NH_4^+) content and a combined concentration of nitrate (NO_3^-) plus nitrite (NO_2^-). The concentration of nitrate plus nitrite were determined via colorimetric vanadium chloride reduction and ammonium was determined colorimetrically using the salicylate method (ISO, 2013).

4.8 Nitrogen balance and NUE calculations (Papers I and II)

The N surplus was calculated for the field and lysimeter experiments. N surplus (kg ha^{-1}), the difference between measured N in- and outputs from the system, is an indicator for potential N losses to the environment. Inputs were in the form of seeds and fertilizer and outputs were harvested crop straw and grain. Additionally, leached N from the lysimeters or cumulative N_2O losses from the cropping seasons were included as losses. Inputs and outputs were calculated per plot or lysimeter and treatments were averaged.

As an indicator of resource efficiency for each treatment, the nitrogen use efficiency (NUE) was calculated according to Quemada et al., 2020:

$$NUE[\%] = \left(\frac{\sum(\text{crop N outputs}[\text{kg N ha}^{-1}])}{\sum(\text{N fertilizer inputs}[\text{kg N ha}^{-1}])} \right) * 100$$

In addition, the agronomic efficiency of N (AE_N) (Lahda *et al.* 2005), the ratio of yield to N supply, as well as the recovery efficiency of N (RE_N), the

ratio of plant N to N supply (Lahda *et al.* 2005; Dobermann 2005) were also calculated. These measures give further insight into N use efficiency by incorporating the unfertilized control treatment:

$$AE_N [kg\,kg^{-1}] = \frac{(grain\,yield_{fert} - grain\,yield_{unfert})}{N_{applied}}$$

$$RE_N[\%] = \frac{(plant\,N\,uptake_{fert} - plant\,N\,uptake_{unfert})}{N_{applied}} * 100$$

For the field experiment (Paper I), yield was scaled to N₂O emissions according to Venterea *et al.* (2011):

$$N_2O_{yield} = \frac{N_2O_{fert}[kg\,N\,ha^{-1}]}{grain\,yield\,[kg\,N\,ha^{-1}]}$$

The emission factor (EF), which evaluates N₂O emissions from anthropogenic soil inputs, i.e., fertilization, was calculated for the 2017 growing season for the field experiment:

$$EF_{N_2O-N}[\%] = \frac{(N_2O_{fert}[kg\,N\,ha^{-1}] - N_2O_{unfert}[kg\,N\,ha^{-1}])}{N_{applied}[kg\,N\,ha^{-1}]} * 100$$

For the latter two calculations, N₂O_{fert} is the cumulative N₂O emissions from a fertilized treatment. For EF, N₂O_{unfert} is the cumulative N₂O emissions from the control treatment. N_{applied} is the amount of applied fertilizer N.

4.9 X-ray imaging, processing, and analysis (Paper III)

On the final day of the incubation, all sixteen columns were scanned to obtain 3D images in a GE Phoenix v|tome|xm X-ray scanner (GE, Boston, USA) of the Department of Soil and Environment at the Swedish University of Agricultural Sciences in Uppsala. The scanner was equipped with a 240 kV X-ray tube with a tungsten target, a 0.5 mm-thick copper optical filter, and a 16'' monitor (GE DRX250RT). Scanning was performed with maximum tube voltage at 170 kV with an electron flux of 700 μA. A compound of four individual 3D scans of top, second and third quarter, and

bottom of the samples was used to produce a 3D image of each column. GE software datavox (version 2.1) was used to reconstruct the images into a 3D tomogram and exported them as 16 bit 3D TIFF images with a $100^3 \mu\text{m}^3$ voxel size.

The open source software ImageJ/FIJI (Schindelin *et al.* 2012) with the plugin SoilJ (Koestel 2018) was used for image processing. To decrease image processing time for subsequent steps, 3D image resolution was reduced by a factor of 2, resulting in an image voxel edge length of $200 \mu\text{m}$, which provided detection of imaged structures larger than $400\text{-}600 \mu\text{m}$. SoilJ was then used to automatically detect the column walls in order to straighten each column and shift the center of the canvas. The image artifacts created from X-ray scattering of the steel column walls were corrected as much as possible using an approach described in Hansson *et al.* (2017). The top, bottom, and vertical center parts in the images of the stacked columns were not included in the image analyses due to large artefacts. It was assumed that connectivity between the top and bottom column images of the stacked samples were perfect, that is, that pores on the bottom surface of the top column connected to all the pores which connect to the upper surface of the bottom column.

Image illumination was calibrated so that the same grey values were exhibited for all objects of the same density, both within individual images and across all images. The target values for grayscale standardization were obtained from the mean grey values of the steel cylinder and the 0.1 percentile of grey values for the air-filled pores inside the soil per voxel layer. The image grey values were linearly scaled to the air and aluminum target values (Koestel 2018).

To segment the air-filled pores, images were binarized using a global threshold grey value of 9000. In addition to specific macropore surface area σ (mm^{-1}) and imaged porosity ϕ ($\text{mm}^3 \text{mm}^{-3}$), measures to quantify the connectivity of the pore networks of the images were also used. These included imaged percolating pore volumes ϕ_p , pores connected to the top surface of the columns ϕ_c ($\text{mm}^3 \text{mm}^{-3}$), the Euler-Poincaré number per unit volume (mm^{-3}) (Vogel *et al.* 2010), as well as the unitless connection probability Γ (Renard & Allard 2013).

4.10 Statistical analysis

Statistical analysis was performed using R software (R Core team 2022). To determine treatment effects, Anova was used for analysis of variance and the `glht` function (*multcomp* package, Hothorn *et al.* 2008) for posthoc analysis using Tukey's all pair comparisons. For the field and lysimeter experiments, this was performed on crop yield, crop N content, SPAD and plant height. Additionally, cumulative amounts of leached H₂O and mineral N from the lysimeters, and cumulative emissions of N₂O, CH₄ and CO₂ from the field and incubation experiments were evaluated. Differences were considered significant for $P < 0.05$.

For the incubation experiment, multivariate analysis using Pearson correlation between soil structural parameters (top and bottom columns individually in addition to the total stacked column), GHG emissions and soil moisture, %C content and ¹³C (individual top and bottom columns) were performed followed by a pairwise significance test using the function `cor.mtest` (confidence level = 0.95). The resulting figure was produced using the R package `corrplot` (Wei and Simko 2021). Separate analyses were done both with and without the Control treatment (for fertilizer-derived N₂O emissions, for example, Control was excluded).

GHG emission differences in the field, lysimeter, and incubation experiments were investigated by repeated measures Anova, with a linear mixed-effects model using the `lme` function (*nlme* package, Pinheiro *et al.* 2019) with the repetitions as a random factor and the log-likelihood maximized method "ML".

To determine treatment effects over time in leachate H₂O amount (mm), N load (kg ha⁻¹), and volume-weighted concentration (mg L⁻¹), a repeated measures Anova was used, using the `lme` function to make a linear mixed model with time as a repeated factor. The `corAR1` correlation structure was used to model the error term. Treatment differences were tested at each sampling time as well as within-treatment differences at different time points using the `emmeans` function (*emmeans* package, Lenth 2022). The cropping seasons (S1-S3) were analyzed separately from the non-cropping periods (F1 and F2).

5. Results

5.1 GHG emissions

5.1.1 Nitrous oxide

Field experiment (Paper I)

Cumulative N₂O emissions were affected by N fertilization and fertilizer placement depth during both cropping seasons in the field experiment (2016 and 2017). N fertilization increased emissions between 32 - 61% in 2016 and 10 - 70% in 2017. In 2016, chamber measurements were fewer and ended earlier in the season than in 2017, thus cumulative N₂O emissions (Fig 2) were lower and treatments were more similar, making overall treatment evaluation difficult. During both cropping seasons, however, the highest emissions were from Shallow, which was the only treatment with significantly higher cumulative N₂O emissions than Control. Among fertilized treatments, Deep and Mixed reduced emissions relative to Shallow by 18% and 6% in 2016, respectively, and 35% and 21% in 2017, respectively.

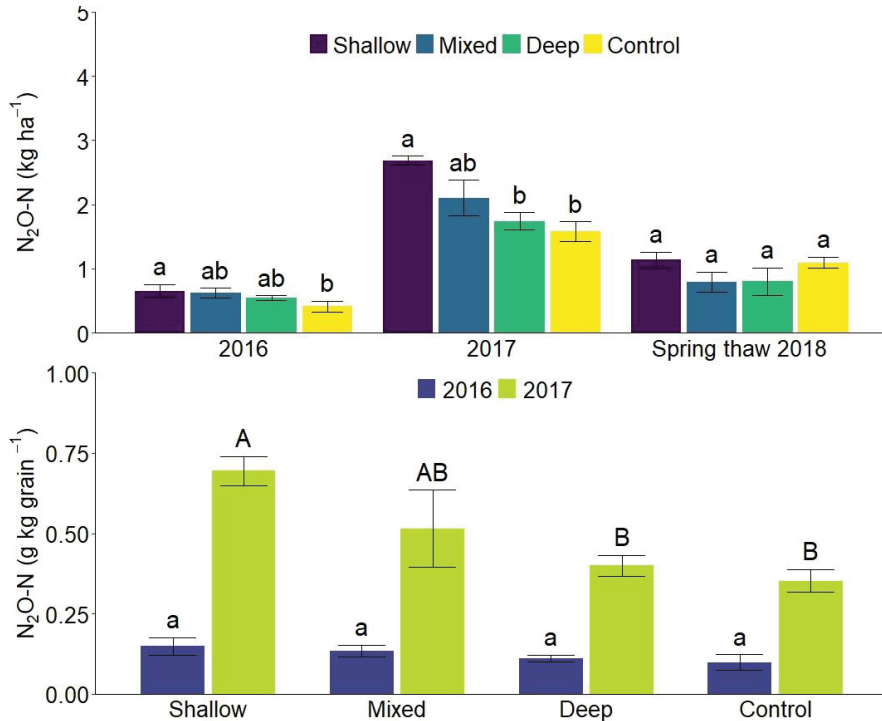


Figure 2 Cumulative N₂O emissions (kg N ha⁻¹) for the cropping seasons of 2016 and 2017 and 2-week spring thaw 2018 (top). Yield-scaled N₂O emissions (g N₂O-N kg grain⁻¹) for the 2016 and 2017 cropping seasons (bottom). Error bars indicate standard error of the mean. Lowercase/uppercase letters represent treatment differences within the same sampling time ($p < 0.05$).

Average N₂O emissions in Mixed ($69.9 \pm 49.1 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$) and Shallow ($56.9 \pm 52.9 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ ($\pm \text{SD}$)) in 2017 were not significantly different. In Deep and Control, average emissions in 2017 were similar and significantly lower than Shallow, 44.9 ± 39.2 and $43.8 \pm 37.9 \mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ ($\pm \text{SD}$), respectively.

In the first third of the 2017 cropping season, from mid-May after seed emergence to early July, fertilizer placement differences were most frequent on individual measurement occasions (Fig 3). Fluxes from Deep and Mixed were significantly lower than Shallow on eight of the 22 measurements occasions, primarily occurring during this period of intensive crop growth. On seven further occasions, fluxes from Mixed and Shallow were significantly higher than those from Deep, and on an additional three measurements, fluxes from Mixed were intermediate to those from Shallow

and Deep. Control N₂O fluxes were never significantly higher than those from fertilized treatments.

Cumulative N₂O emissions scaled to crop yield (Fig 2) decreased with increasing placement depth, a similar trend was observed in both 2016 and 2017, but only significantly in the latter. Control yield-scaled emissions were lowest in both years, 0.10 and 0.35 g N₂O-N kg grain⁻¹ in 2016 and 2017, respectively. Among fertilized treatments, yield-scaled emissions were the lowest in Deep, 0.11 in 2016 and 0.40 g N₂O-N kg grain⁻¹ in 2017, corresponding to a 26 and 43% reduction relative to Shallow. Mixed placement was intermediate to Deep and Shallow, reducing yield-scaled N₂O emissions relative to Shallow by 9% and 25% in 2016 and 2017. A similar trend was observed in the fertilizer-induced emission factor (EF), where the percentage of applied N emitted as N₂O in 2017 was 0.77 ± 0.07 , 0.58 ± 0.03 , and 0.10 ± 0.02 for Shallow, Mixed, and Deep, respectively.

The cumulative N₂O emissions, comprised of three measurements during the two-week thaw period in Spring 2018, did not indicate any treatment differences, but were very high considering the short measurement period. The emissions in this time comprised 40-70% of the total emissions from the preceding cropping season in 2017 with the highest percentage in the Control plots. Average fluxes (\pm SD) for Control, Shallow, Mixed and Deep were 412 ± 191 , 326 ± 167 , 276 ± 160 , and 319 ± 303 $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$, respectively, and five to ten times higher than previously measured fluxes.

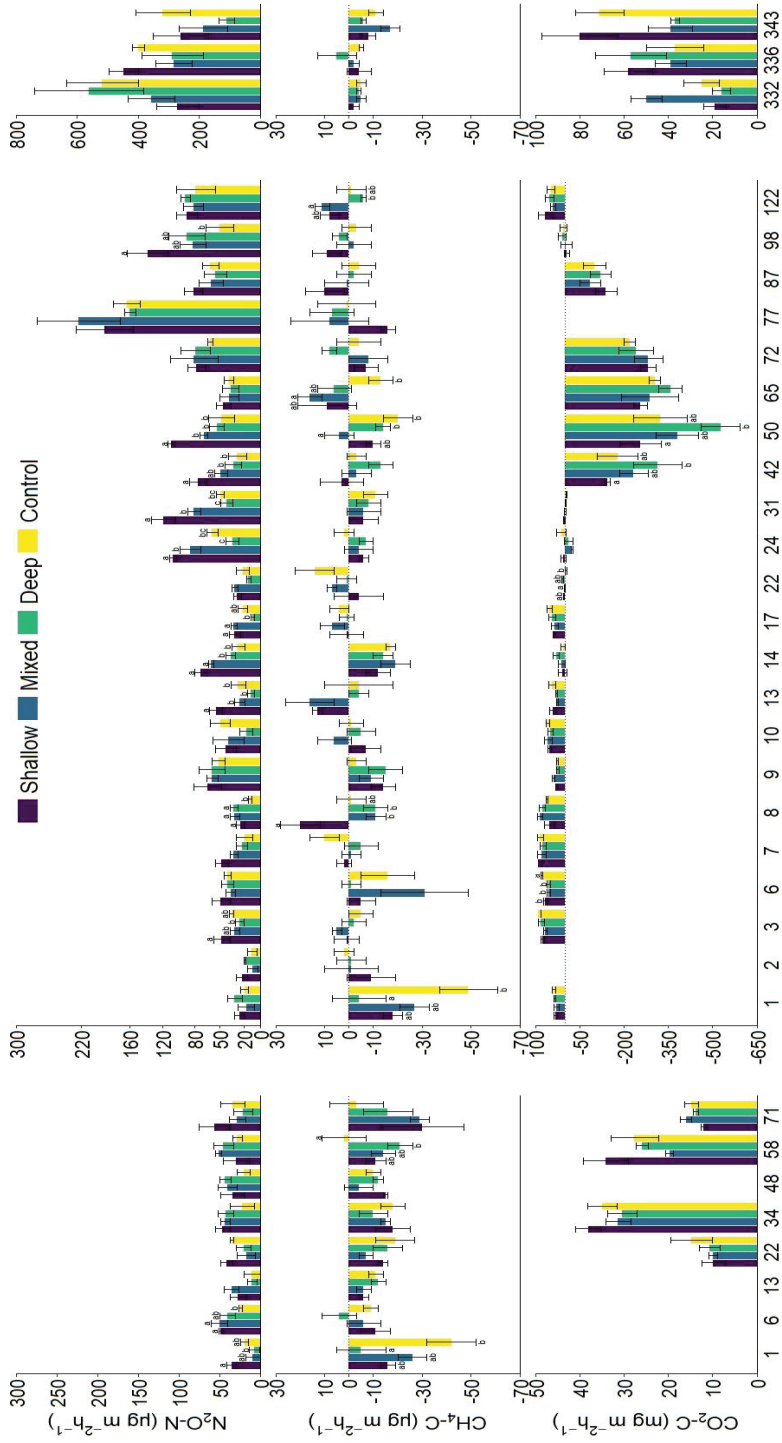


Figure 3 Mean treatment nitrous oxide fluxes ($\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$), methane fluxes ($\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) and carbon dioxide ($\text{mg CO}_2\text{-C m}^{-2} \text{h}^{-1}$). Error bars represent standard error of the mean. Lowercase letters represent treatment differences within the same sampling time ($p < 0.05$).

Incubation experiment (Paper III)

Total N_2O emissions ($\text{N}_2\text{O}^{\text{tot}}$), the sum of non-fertilizer derived and fertilizer-derived N_2O emissions, peaked in all treatments on the second day of the incubation in response to the injected water and/or fertilizer (Fig 4). Following this initial peak, $\text{N}_2\text{O}^{\text{tot}}$ fluxes gradually declined in all treatments. Deep treatment $\text{N}_2\text{O}^{\text{tot}}$ fluxes on individual days were significantly higher than Mixed on eight of the twelve days, of which fluxes from Deep were also higher than Shallow on three days. Although both Shallow and Deep cumulative $\text{N}_2\text{O}^{\text{tot}}$ emissions were elevated above the non-fertilized Control, only Deep was significantly higher (Fig 5).

Fertilizer-derived emissions ($\text{N}_2\text{O}^{\text{fert}}$) peaked on day two and three for Mixed and Shallow, respectively, and similar to $\text{N}_2\text{O}^{\text{tot}}$, declined over time. The highest daily flux of $\text{N}_2\text{O}^{\text{fert}}$ in Deep, however, was on the final day of the incubation, and had steadily increased from the pulse event on day two. The contribution of cumulative $\text{N}_2\text{O}^{\text{fert}}$ to $\text{N}_2\text{O}^{\text{tot}}$ was small, but varied by treatment, with 20, 11 and 6% of total emissions derived from fertilizer in Shallow, Mixed, and Deep, respectively (Fig 5, 6).

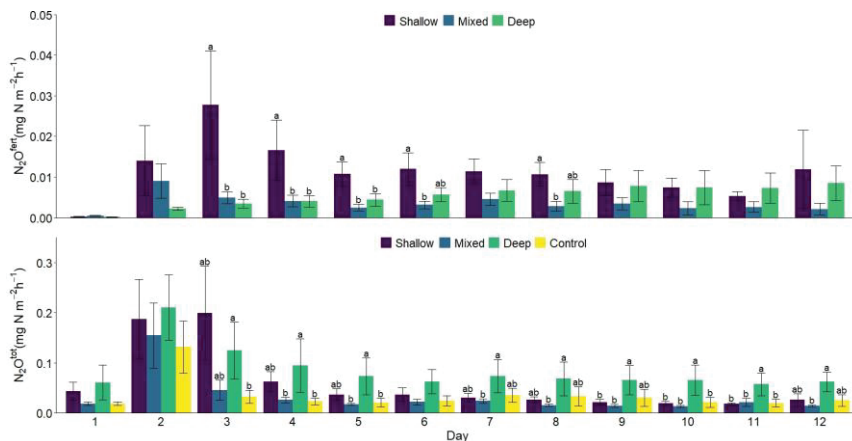


Figure 4 Mean treatment nitrous oxide fluxes from N fertilizer (N_2O^{fert}) ($mg\ N_2O-N\ m^{-2}\ h^{-1}$), mean treatment total nitrous oxide fluxes (N_2O^{tot}) ($mg\ N_2O-N\ m^{-2}\ h^{-1}$). Error bars represent standard error of the mean. Lowercase letters represent treatment differences within the same sampling time ($p < 0.05$).

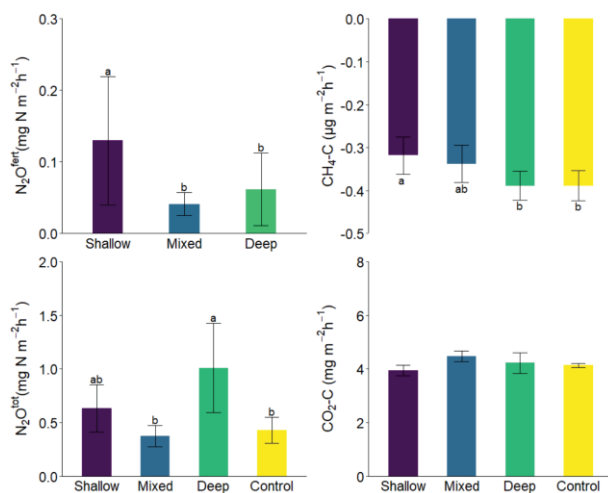


Figure 5 Cumulative emissions of nitrous oxide from fertilizer (N_2O^{fert}) and total nitrous oxide emissions (N_2O^{tot}) ($mg\ N_2O-N\ m^{-2}\ h^{-1}$), methane ($\mu g\ CH_4-C\ m^{-2}\ h^{-1}$), and carbon dioxide ($mg\ CO_2-C\ m^{-2}\ h^{-1}$). Error bars indicate standard error of the mean. Lowercase letters indicate treatment differences within the same sampling time ($p < 0.05$).

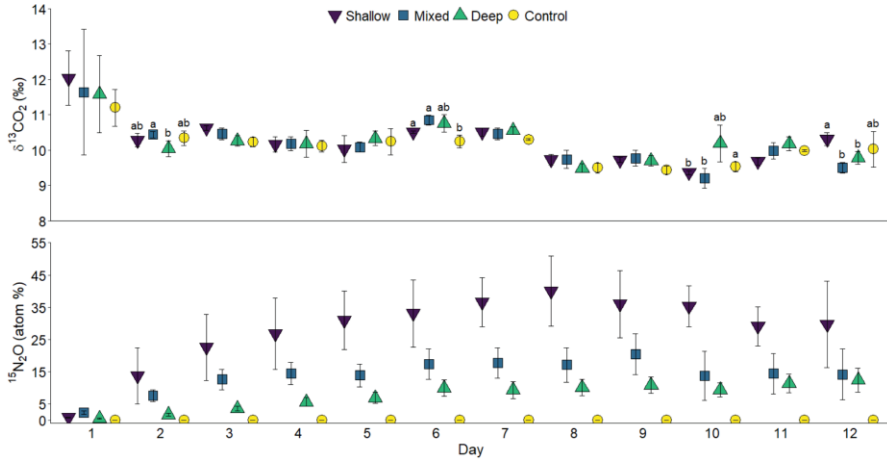


Figure 6 Mean treatment $^{13}\text{CO}_2$ enrichment (‰) and $^{15}\text{N}_2\text{O}$ enrichment (atom %). Error bars indicate standard error of the mean. Lowercase letters indicate treatment differences within the same day ($p < 0.05$).

5.1.2 Methane

Field experiment (Paper I)

Fluxes of CH_4 were primarily negative or slightly positive across treatments. There was not a significant treatment effect on cumulative fluxes in 2017, but total seasonal CH_4 uptake was greater in the Control and Deep (Fig 7). On individual days with significant treatment differences, most of which occurred in the latter part of the 2017 season, fluxes in Control and Deep were generally lower than those in Shallow and Mixed.

Shallow and Mixed had the highest average CH_4 emissions (\pm SD) in 2017, -1.7 ± 16.3 and -2.0 ± 20.0 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$, respectively. Minimum and maximum values of Mixed were -92 and 57 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$. Shallow minimum and maximum fluxes were -38 and 43 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$. Control had the greatest CH_4 uptake, with fluxes averaging (\pm SD) -5.5 ± 19.1 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$ and individual fluxes ranged from -76 to 33 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$. The average CH_4 flux in Deep was -3.9 ± 13.1 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$ with highest and lowest measured fluxes of -41.0 and 32.4 $\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$, respectively.

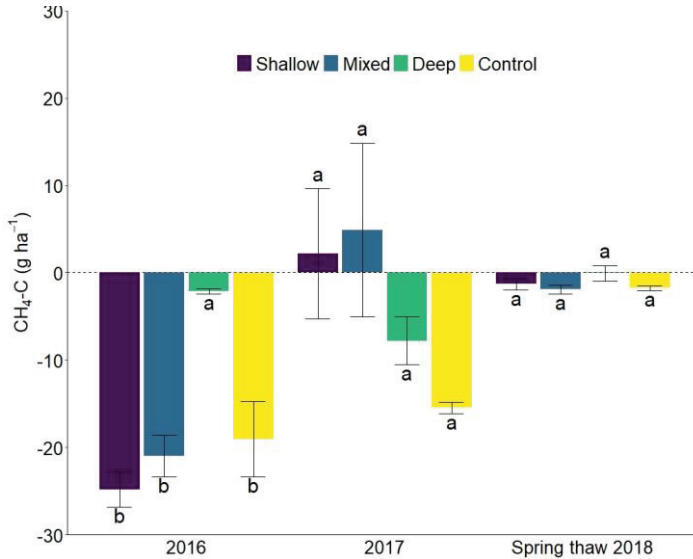


Figure 7 Cumulative fluxes of methane ($\text{g CH}_4\text{-C ha}^{-1}$) over the cropping seasons in 2016 and 2017, and the two week thaw period in April 2018.

Incubation experiment (Paper III)

Following the initial input of water and/or fertilizer at the beginning of the incubation, there was no obvious response in CH_4 fluxes (Fig 8). All fluxes during the incubation were negative, i.e., only net uptake. On the majority of days, Control had the greatest uptake, and Shallow the least, a trend that became more clear towards the end of the incubation.

Cumulatively, Deep and Control had similar methane emissions, $-0.389 \pm 0.034 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ (\pm SD), and greater uptake relative to Mixed and Shallow (Fig 5). Shallow had significantly less methane uptake, an 18% decrease, relative to Deep and Control, $-0.318 \pm 0.044 \mu\text{g CH}_4\text{-C m}^{-2} \text{ h}^{-1}$ cumulatively.

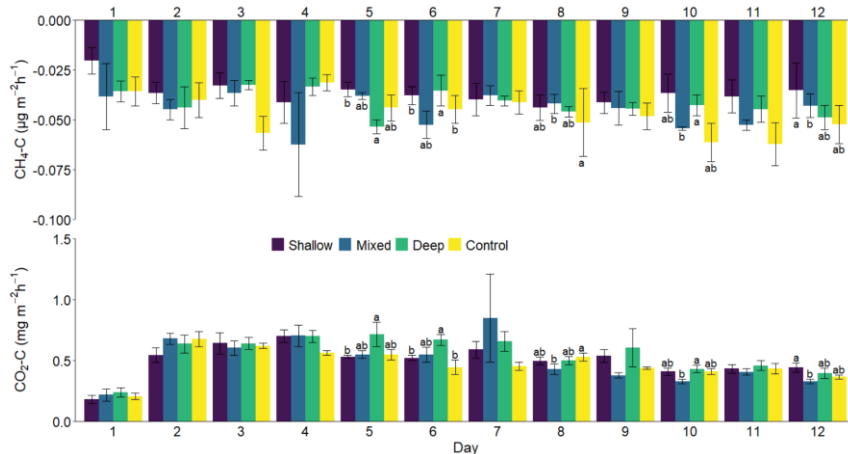


Figure 8 Mean treatment methane fluxes ($\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$) and carbon dioxide ($\text{mg CO}_2\text{-C m}^{-2} \text{h}^{-1}$) from the incubation experiment. Error bars indicate standard error of the mean. Lowercase letters indicate treatment differences within the same sampling time ($p < 0.05$).

5.1.3 Carbon dioxide (Papers I-III)

Carbon dioxide emissions, particularly from bare soil (i.e., Paper III and 2016 in the field experiment, Y1, Paper I), as a result of N fertilizer placement did not follow a distinct trend, and emissions were generally even across all treatments (Figs 3 and 5). However, with the presence of plants and the use of transparent chambers, more of a trend relating to plant growth and maturation was evident. Since the frequency of GHG measurements in the field and lysimeter experiments were clustered more heavily in the earlier part of the experiment to focus on N_2O emissions, much of the deeper understanding of the effect of fertilizer placement on crop development through the lens of photosynthetic CO_2 uptake was missed.

Though there were few data points ($n = 5$) during the period where photosynthetic CO_2 uptake was the dominant process in the second year (Y2) of the field experiment (Paper I), on the majority of those measurement occasions, Deep had the greatest uptake (Fig 3). Total uptake in Y2 (sum of negative fluxes) followed the pattern Deep > Mixed > Control > Shallow, corresponding to 1.57, 1.30, 1.13 and 1.08 $\text{g CO}_2\text{-C m}^{-2} \text{h}^{-1}$.

In the lysimeter experiment (Paper II), photosynthetic CO_2 uptake in the Control was greatest in the earlier stages of plant growth (Fig. 9) in season S1 with the Shallow treatment following a similar trend or with somewhat

less uptake than in the Control. In the drought year S2, this trend was less clear. The Mixed and Deep treatments tended to have greater CO₂ uptake later in the growing period relative to Control and Shallow. In 2017 (S1), the Mixed had the greatest CO₂ uptake but in the following year the control was highest, while in both years Shallow was the lowest. Total uptake (sum of negative fluxes) in S1 was 1.77, 1.57, 1.84, and 1.77 g CO₂-C m⁻² h⁻¹ for Control, Shallow, Mixed, and Deep respectively. In S2 the pattern of total uptake changed, and was 2.63, 2.03, 2.08, and 2.34 g CO₂-C m⁻² h⁻¹ for Control, Shallow, Mixed, and Deep respectively.

In the incubation experiment (Paper III), there was some treatment variation in ¹³CO₂ over the course of the incubation, potentially indicating a fertilization effect (Fig 6) and there was a weak negative correlation between daily ¹³CO₂ and ¹⁵N₂O when excluding Control, though ¹³CO₂ was not correlated with N₂O^{fert}.

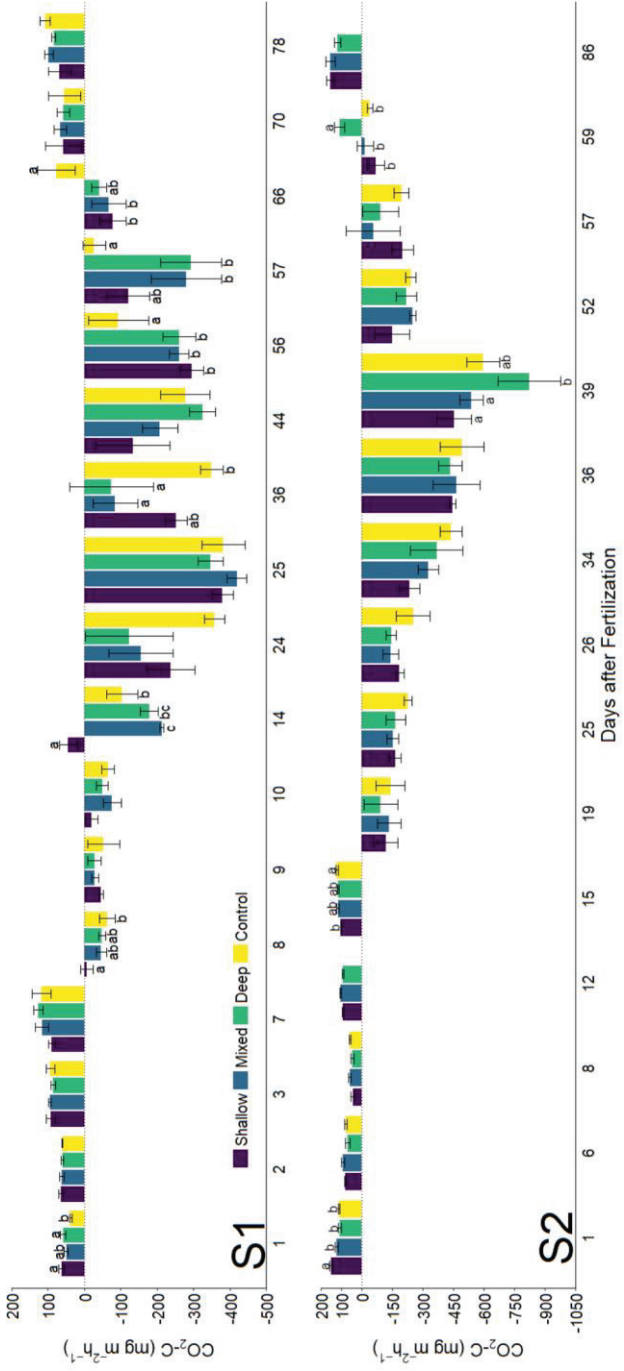


Figure 9 Mean treatment fluxes of carbon dioxide (mg CO₂-C m⁻² h⁻¹) from the lysimeter experiment. Error bars represent standard error of the mean. Lowercase letters represent treatment differences within the same sampling time (p < 0.05).

5.2 The effect of soil structure and physical properties on GHG emissions (Paper III)

Soil columns showed variability in soil structure, within and between treatment groups and upper vs. lower columns in the same stacked column (Table 3). For example, one upper column in Deep was nearly an outlier (high) among all upper columns within multiple parameters: porosity (ϕ), bottleneck diameter (d_c), porosity connected to both top and bottom surfaces (ϕ_p), and porosity connected to top surface (ϕ_c). Incidentally, the same soil core that was a near outlier in the Deep treatment also had the highest water content and cumulative N_2O^{tot} and CO_2 emissions.

Table 3 Treatment mean of soil structural and physical characteristics of the total stacked column (Tot), the upper column only (U), or the lower column (L) included in multivariate analysis. Soil H₂O content, Soil C content and Soil ¹³C were determined in individual (upper and lower) portions of the column and these two values were averaged to represent the total (Tot) column. Lowercase letters indicate statistical differences between treatments ($p < 0.05$).

Treatment	Column Component	Φ (mm ³ mm ⁻³)	Sigma (mm ⁻¹)	Gamma	Euler	Φ_p (mm ³ mm ⁻³)	Φ_c (mm ³ mm ⁻³)	MatxPerc < 1mm	Soil H ₂ O (%)	Soil C (%)	Soil ¹³ C (atom %)
Shallow	Tot	0.0377	0.187	0.2243	0.0713	0.0050	0.0157	2.5707	22.52	2.94	1.08039
	U	0.0244	0.105 ^b	0.1283	0.0457	0.0022	0.0061	2.0663	21.92	2.85	1.08041
	L	0.0510	0.268	0.4340	0.0969	0.0077	0.0181	6.0192	23.11	2.95	1.08039
Mixed	Tot	0.0393	0.218	0.2615	0.1155	0.0047	0.0155	3.3557	22.78	2.78	1.08041
	U	0.0375	0.199 ^a	0.1715	0.1082	0.0047	0.0106	3.4578	22.63	2.94	1.08047
	L	0.0411	0.237	0.2325	0.1255	0.0159	0.0170	5.5557	22.92	2.90	1.08044
Deep	Tot	0.0406	0.199	0.2490	0.0821	0.0135	0.0339	5.2650	23.43	2.95	1.08035
	U	0.0391	0.149 ^{ab}	0.2328	0.0513	0.0164	0.0217	4.1733	23.96	2.87	1.08039
	L	0.0420	0.250	0.2123	0.1130	0.0106	0.0155	6.2798	22.91	2.95	1.08030
Control	Tot	0.0354	0.152	0.1235	0.0611	0.0028	0.0193	2.8719	21.93	2.80	1.08038
	U	0.0236	0.101 ^b	0.2575	0.0479	0.0028	0.0130	2.4388	22.12	2.95	1.08042
	L	0.0472	0.203	0.2025	0.0744	0.0116	0.0203	5.1265	21.75	2.70	1.08043

Phi (ϕ) = imaged porosity (mm³ mm⁻³), *sigma* (Σ) = specific macropore surface area (mm⁻¹), *gamma* (Γ) = connection probability, *Euler* = Euler-Poincaré number per unit volume, ϕ_p = pore volume connected to both the top and bottom soil surface (mm³ mm⁻³), ϕ_c = pore volume connected to the top soil surface (mm³ mm⁻³), *matxperc* < 1mm = soil matrix within 1 mm of a percolating pore, *soil H₂O* = gravimetric water content (%), *soil C* = soil organic carbon content (%), *soil ¹³C* = ¹³C (atom %) content of soil carbon.

In terms of treatment differences in soil structure parameters, pore specific surface area (σ) in the upper column of the Mixed treatment was significantly higher than both Control and Shallow. Pore connectivity (Euler) was high in the upper columns of the mixed treatment (Anova $p = 0.04$), but not significantly according to Tukey's posthoc test. Although there was no significant correlation between these structural parameters and GHG emissions, Mixed had the lowest cumulative N_2O^{tot} emissions, as well as N_2O^{fert} , despite a higher proportion of N_2O from fertilizer relative to Deep.

Final soil moisture content in the top columns was positively correlated with cumulative emissions of N_2O^{tot} and CO_2 (Fig 10). Water content had a stronger effect on cumulative N_2O^{tot} than on CO_2 .

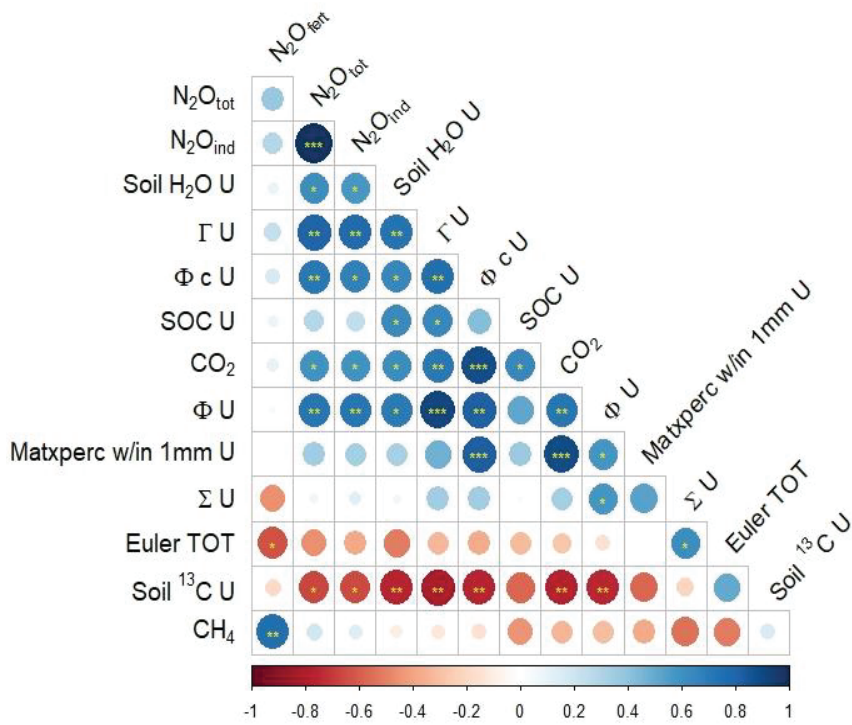


Figure 10 Pearson correlations between soil physical properties, Xray-derived structural properties, and cumulative fluxes of greenhouse gases per soil column. Pairwise comparison significance levels indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

U = upper portion of the column, TOT = total column (combined upper and lower portions). N_2O_{fert} = cumulative nitrous oxide emissions (N_2O) originating from fertilizer nitrogen (N), N_2O_{ind} = cumulative N_2O emissions originating from indigenous soil N, N_2O_{tot} = cumulative emissions of $N_2O_{ind} + N_2O_{fert}$, Soil H_2O = gravimetric water content, Gamma (Γ) = connection probability, ϕ_c = pore volume connected to the top soil surface ($mm^3 mm^{-3}$), SOC = soil organic carbon content (%), CO_2 = cumulative carbon dioxide emissions ($mg m^{-2} h^{-1}$), phi (ϕ) = imaged porosity ($mm^3 mm^{-3}$), Matxperc w/in 1mm = soil matrix within 1 mm of a percolating pore, sigma (Σ) = specific macropore surface area (mm^{-1}), Euler = Euler-Poincaré number per unit volume (mm^3), CH_4 = cumulative emissions of methane, Soil ^{13}C = ^{13}C (atom %) content of soil carbon.

Methane, however, did not correlate with any structural parameters, nor did it correlate with soil water content. $\text{N}_2\text{O}^{\text{fert}}$ was negatively correlated with Euler from the total column, and in addition was correlated with CH_4 (control excluded); both cumulative emissions and daily fluxes of $\text{N}_2\text{O}^{\text{fert}}$ and CH_4 were positively correlated ($p < 0.01$) with each other.

Cumulative CO_2 emissions were weakly correlated with cumulative $\text{N}_2\text{O}^{\text{tot}}$, but not with $\text{N}_2\text{O}^{\text{fert}}$. However, they were strongly correlated with pores connected to the surface, both in the upper portion of the column and in the total column, thus, soil properties affecting gas exchange. Additionally, average $^{13}\text{CO}_2$ signatures were positively correlated to pore diameter and water content in the upper portion of the columns.

5.3 Water and N leaching (Paper II)

5.3.1 Lysimeter water flow

Collected leachate amounts averaged 27, 10, and 14% of precipitation plus irrigation across all treatments, in seasons S1, S2 and S3, respectively. Percent total leachate quantity relative to water inputs over the three cropping years (S1 to S3), was lowest in Deep and highest in Control, following the pattern Control > Mixed > Shallow > Deep, corresponding to 20, 18, 17 and 13%.

Control had significantly higher ($p = 0.04$) mean cumulative leachate amount (\pm SE) for the three cropping years (S1-S3), 377 ± 37 mm H_2O , compared to the lowest in deep, 249 ± 12 mm. Mixed and Shallow were intermediates with mean cumulative amounts of leachate 332 ± 36 and 319 ± 25 mm, respectively. Deep had the lowest quantity of leachate for all periods except for the initial F1 period. Within the fertilized treatments, Deep leached 29% less water than Mixed and 25% less water compared to Shallow.

Water flow in the initial fallow period F1, from August 2016 to May 2017, varied slightly between individual lysimeters and treatments, but did not differ significantly between treatments. Total water input during this 12-month period was the lowest across all periods, approximately 322 mm.

The first cropping season (S1), June 2017 to April 2018, was wetter than the preceding period, particularly in the autumn and winter months (Fig 1). Mean cumulative leachate was similar across treatments in S1 and decreased

in the order Control > Shallow > Mixed > Deep corresponding to 179 ± 10 , 168 ± 6 , 160 ± 17 , and 148 ± 5 mm (\pm SE), respectively (Figs 11 and 12).

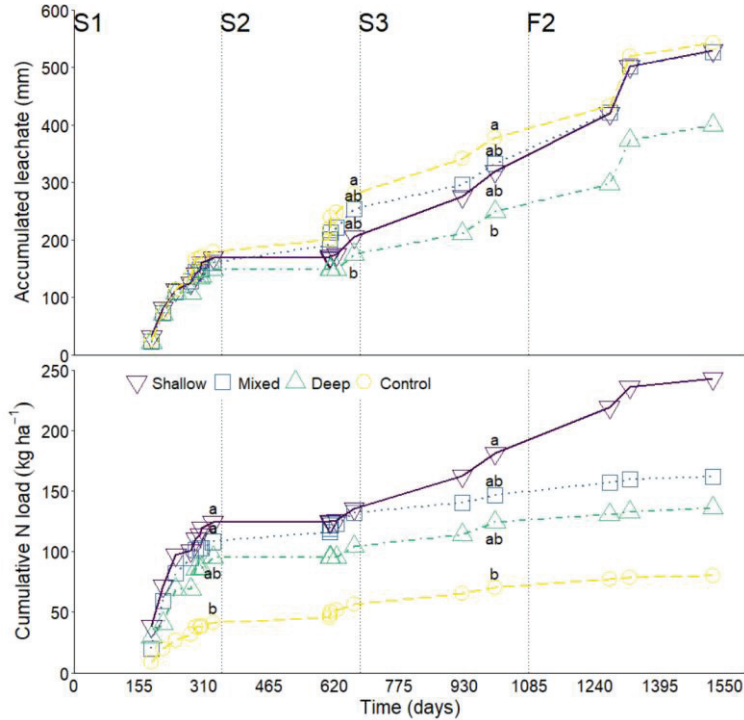


Figure 11 Cumulative water leachate curve and mineral nitrogen (N) load (kg ha^{-1}) and treatment effects ($p < 0.05$) for the experimental treatment period (S1–S3) and subsequent fallow period (F2). Lowercase letters indicate treatment differences ($p < 0.05$).

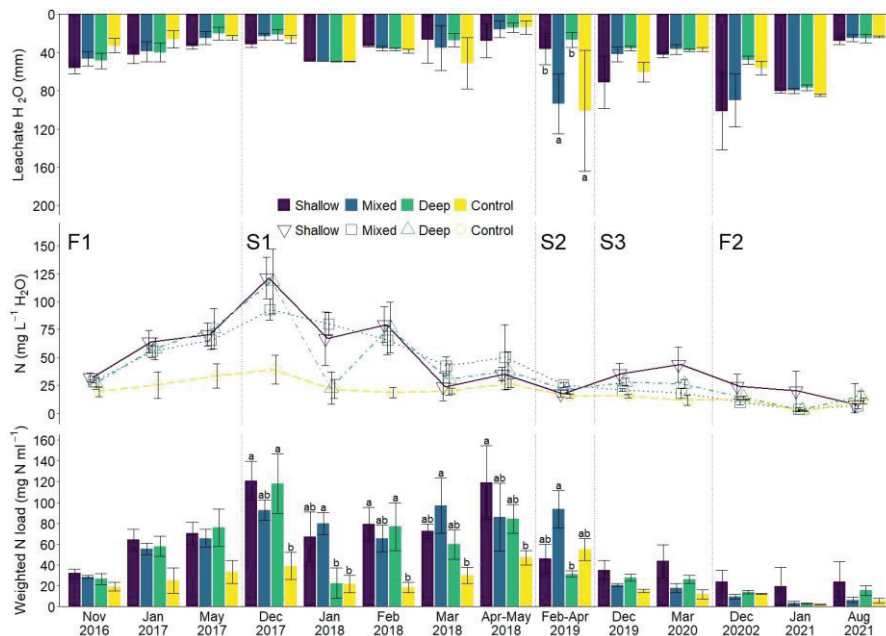


Figure 12 Mean leachate quantity (mm H₂O), leachate concentration (mg L⁻¹ H₂O), and weighted N load (mg N mL⁻¹). Lowercase letters indicate treatment differences within the same sampling time ($p < 0.05$). Each bar indicates a single sampling, except Apr-May 2018 and Feb-Apr 2019, which are comprised of the sum of multiple samplings in order to incorporate leaching from all lysimeters.

In S2, nearly every month from May 2018 to April 2019 had both higher temperatures and lower precipitation compared to the long-term normal (Fig 1), particularly during the cropping period between May and July 2018. Although precipitation for the month of July 2018 appears to have been sufficient, 96% of the rainfall occurred during a single rainfall event on a day in the latter part of the month, while the majority of the month was unusually dry. Lysimeter leaching was greatly impacted by this drought. Generally, leaching would begin in late autumn following the growing season, but in S2 leaching did not occur until February 2019, and only in two of the four treatments, Control and Mixed. Sufficient quantities of water for sampling from all treatments was not available until around April 2019 (Fig 12). Mean cumulative leachate for this period was significantly higher in Control and Mixed compared to Shallow and Deep. By leachate quantity, treatments followed the pattern of Control > Mixed > Shallow > Deep, corresponding to a percentage of leachate relative to total water inputs of 16, 15, 6 and 4%, respectively, some of the lowest of any period. Within the fertilized

treatments, leaching from Mixed was significantly higher than Shallow and Deep ($p = 0.005$ and 0.001 , respectively).

In S3, the third year with crops, climate conditions were, in contrast to S2, more similar to long-term normal, although some compensation with irrigation was still necessary, particularly in June and July 2019 (Fig 1). The pattern of mean cumulative water flow across treatments changed from the previous season where Shallow > Control > Mixed > Deep, however there were no significant differences between treatments.

During the 16-month fallow period following S3, Deep continued to leach less water than other treatments, though not significantly, despite the absence of crops (Figs 11 and 12). Mean cumulative water flow followed the order of Shallow > Mixed > Control > Deep. Most treatment effects tapered off, however, after December 2020, approximately halfway through the F2 period.

5.3.2 Nitrogen leaching

The Shallow treatment was the only fertilized treatment with significantly higher cumulative N load (S1 – S3) than the Control ($p = 0.009$), corresponding to 181 ± 21 and 70 ± 18 kg N ha⁻¹, respectively. Deep and Mixed placements were intermediates with mean cumulative N loads of 124 ± 24 and 147 ± 14 kg ha⁻¹ respectively, resulting in a reduction of leachate N losses of 37 and 21% compared to Shallow. There were no significant differences among the fertilized treatments, however.

In F1, N load steadily increased in all lysimeters after the initial disturbance of simulating tillage in the topsoil just prior to lysimeter installation (Fig 12). The flush of mineralized N peaked in S1 and declined in S2. In F1, there had not been any crops or fertilizer added, and thus N loads generally were related to quantity of leachate.

In the first cropping year S1, N loads increased in all treatments (Figs 11 and 12). Cumulative N loads for the period were significantly higher in Shallow and Mixed relative to Control ($p = 0.01$ and 0.04 , respectively). Following S1, average N load decreased in all treatments below F1 levels. However, due to the extreme drought in S2, there was only one brief period of water flow the following spring from late February to April 2019, with vastly higher flow in Mixed and Control treatments, which also had the highest N load for the period. Mixed N load was significantly higher than

Deep ($p = 0.04$) among all treatments. Among fertilized treatments, Mixed was significantly higher than both Deep ($p = 0.01$) and Shallow ($p = 0.02$).

In S3, precipitation was higher than the previous year, particularly in October to December (Fig 1), resulting in a changed pattern in leachate N load across treatments, where Shallow > Deep > Mixed > Control, but there were no significant treatment differences (Figs 11 and 12). In the second fallow period F2, subsequent to S3, the N load in the majority of lysimeters declined and became largely indistinguishable among lysimeters by August 2021.

5.4 Nitrogen in the soil-crop system (Papers I and II)

5.4.1 Crop N uptake and yield increased with placement depth (Papers I and II)

Deeper placement of N fertilizer generally led to an increase in yield relative to the shallow placement, although absolute yield varied across years in both the field and lysimeter experiments. Year 1 yield from Deep in the field was significantly higher (11%) than from Shallow, while Mixed was 5% higher than Shallow (Table 3). When taking the Control into account, the agronomic efficiency of N (AE_N) in the same year, Deep (30 %) nearly doubled that of Shallow (16 %), while Mixed was an intermediate at 25%. Similarly, in the same harvest, the recovery efficiency of N (RE_N), an expression of total harvested crop N uptake relative to Control, followed the same pattern Deep > Mixed > Shallow, corresponding to 0.37, 0.32, and 0.21. Year 2 in the field was affected by poor seed emergence followed by uneven crop development. Yield was similar across treatments, including the Control. Nonetheless, relative to the Shallow placement, indices of crop N use efficiency increased with placement depth.

A similar pattern was observed in the lysimeter experiment (Table 3), although the treatment effect became more clear over time after mineralized N from F1 had leached from the system. In S1, yield was even across all treatments, and in S2 Mixed and Deep grain yields were only marginally higher than Shallow. However, lysimeter yield in S3 was increased by 28% in Deep and 5% in Mixed relative to Shallow. AE_N in Deep increased stepwise over time between S1 and S3, nearly doubling that of Shallow and Mixed in S3. Total crop N uptake expressed as RE_N was similar in Mixed

and Deep, but higher relative to Shallow in S1 and S2, but in S3 Deep was 1.7 times higher than Mixed.

Table 4 Grain yield (tons ha⁻¹) and N content in harvest grain and straw (%) per year in the field and lysimeter experiments (Papers I and II). Lowercase letters indicate statistical difference ($\alpha = 0.05$, Tukey's HSD).

	Parameter	Shallow	Mixed	Deep	Control
2016 field	Grain yield	4.40 ± 0.12 ^{bc}	4.62 ± 0.09 ^{ab}	4.88 ± 0.1 ^a	4.18 ± 0.06 ^c
	Grain % N	2.76 ± 0.02 ^b	2.87 ± 0.02 ^a	2.83 ± 0.02 ^a	2.45 ± 0.02 ^c
	Straw % N	0.31 ± 0.01 ^a	0.33 ± 0.02 ^a	0.33 ± 0.02 ^a	0.23 ± 0.02 ^b
2017 field	Grain yield	3.87 ± 0.16	4.08 ± 0.28	4.36 ± 0.12	4.49 ± 0.12
	Grain % N	2.41 ± 0.03 ^a	2.43 ± 0.02 ^a	2.40 ± 0.02 ^a	2.11 ± 0.02 ^b
	Straw % N	0.73 ± 0.03 ^a	0.72 ± 0.03 ^a	0.68 ± 0.04 ^a	0.52 ± 0.01 ^b
2017 lysimeter	Grain yield	6.2 ± 0.4	6.7 ± 0.5	6.6 ± 0.5	6.4 ± 0.3
	Grain % N	2.26 ± 0.14 ^{ab}	2.38 ± 0.08 ^a	2.29 ± 0.07 ^{ab}	1.83 ± 0.20 ^b
	Straw % N	0.69 ± 0.08	0.72 ± 0.02	0.71 ± 0.03	0.53 ± 0.02
2018 lysimeter	Grain yield	6.1 ± 0.7 ^{ab}	7.7 ± 0.6 ^a	7.5 ± 0.2 ^{ab}	5.8 ± 0.2 ^b
	Grain % N	2.1 ± 0.09 ^a	2.0 ± 0.02 ^a	2.1 ± 0.03 ^a	1.4 ± 0.03 ^b
	Straw % N	0.43 ± 0.02 ^a	0.45 ± 0.02 ^a	0.50 ± 0.02 ^a	0.30 ± 0.02 ^b
2019 lysimeter	Grain yield	7.9 ± 1.5 ^{ab}	8.3 ± 1.1 ^{ab}	10.1 ± 0.2 ^a	6.7 ± 0.3 ^b
	Grain % N	1.97 ± 0.09 ^a	1.77 ± 0.07 ^a	1.89 ± 0.02 ^a	1.41 ± 0.02 ^b
	Straw % N	0.34 ± 0.03 ^a	0.29 ± 0.02 ^{ab}	0.27 ± 0.01 ^{ab}	0.22 ± 0.02 ^b

5.4.2 Nitrogen balance (Papers I and II)

The calculated N balance in Säby soils, in both the field experiment (Table 4) and in the lysimeters (Table 5), overwhelmingly resulted in negative values in all treatments, likely due to a high rate of N mineralization in these soils. In both experiments, the N surplus in Control remained relatively constant without leachate included. The N surplus in the fertilized treatments followed a similar pattern across most seasons (excluding leachate) where N surplus decreased with increasing fertilizer placement depth.

In the lysimeter season S1, N losses from leachate were very high (Figs 11 and 12, Table 5), even in the Control, and leachate accounted for the second-highest output from the system after harvested grain N. Although S1 yields were even among treatments (Table 3), due to the difference in grain

and straw N uptake, Mixed and Deep treatments had higher outputs in grain and straw, the latter significantly higher, compared with the Control ($p = 0.02$). When S1 – S3 N components were summed, Deep had the highest N surplus among fertilized treatments, but of that, the proportion of N output from harvested crops relative to total outputs (i.e., crop outputs plus leachate) was the highest. Shallow had the highest proportion from leachate and the lowest from crop output, and Mixed placement was an intermediate to Deep and Shallow.

Table 5 Nitrogen (N) balance components from the field experiment in 2016 (Y1) and 2017 (Y2), N use efficiency (NUE) (%), agronomic efficiency of N (AE_N) (kg kg⁻¹), and the recovery efficiency of N (RE_N) (%). N balance values are presented as treatment mean value (kg N ha⁻¹ yr⁻¹) ± standard error.

	Field Y1				Field Y2			
	Control	Shallow	Mixed	Deep	Control	Shallow	Mixed	Deep
<i>N inputs and outputs (kg N ha⁻¹)</i>								
Seeds	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Fertilization		120	120	120		105	105	105
Harvested grain	-102 ± 1	-121 ± 4	-132 ± 3	-138 ± 2	-101.1 ± 9	-97 ± 8	-104 ± 15	-111 ± 5
Harvested straw	-11 ± 1	-17 ± 1	-20 ± 1	-19 ± 1	-21 ± 3	-28 ± 3	-31 ± 3	-30 ± 3
N ₂ O loss	-0.40 ± 0.02	-0.63 ± 0.02	-0.63 ± 0.02	-0.49 ± 0.01	-1.6 ± 0.1	-2.7 ± 0.1	-2.1 ± 0.3	-1.7 ± 0.1
N surplus	-108	-13	-27	-32	-118	-17	-27	-33
NUE %	n.a.	115	126	132	n.a.	119	128	134
AE _N (kg kg ⁻¹)	n.a.	1.89	3.70	5.84	n.a.	-5.9	-3.9	-1.2
RE _N (%)	n.a.	21	32	37	n.a.	2.3	12.2	18.1

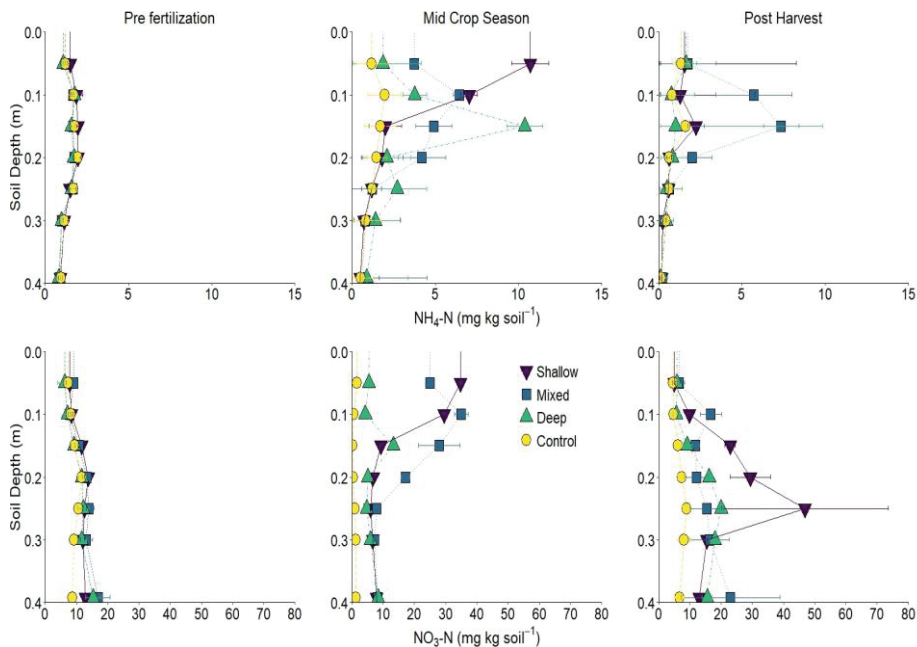
Table 6 Nitrogen (N) balance components per cropping season in the lysimeter experiment (Paper I), N use efficiency (NUE) (%), agronomic efficiency of N (AE_N) (kg kg⁻¹), and the recovery efficiency of N (RE_N) (%). N balance values are presented as treatment mean value (kg N ha⁻¹ yr⁻¹) ± standard error.

	Lysimeter S1				Lysimeter S2				Lysimeter S3						
	Control		Mixed		Shallow		Mixed		Shallow		Mixed		Deep		
	8	100	8	100	8	100	8	100	8	100	8	100	8	100	
<i>N inputs and outputs</i> (kg N ha ⁻¹)															
Seeds	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
Fertilization															
Harvested grain	-118 ± 17	-141 ± 14	-157 ± 9	-155 ± 13	-80 ± 4 ^b	-127 ± 9 ^a	-158 ± 12 ^a	-157 ± 5 ^a	-95 ± 6 ^b	-151 ± 25 ^{ab}	-145 ± 16 ^{ab}	-190 ± 5 ^a			
Harvested straw	-27 ± 2 ^b	-38 ± 6 ^{ab}	-43 ± 2 ^a	-43 ± 1 ^a	-13 ± 1 ^b	-30 ± 3 ^a	-26 ± 4 ^a	-31 ± 1 ^a	-18 ± 2 ^b	-32 ± 1 ^a	-29 ± 2 ^a	-30 ± 2 ^a			
Leachate load	-41 ± 11 ^b	-124 ± 12 ^a	-108 ± 13 ^a	-96 ± 21 ^{ab}	-15 ± 5 ^{ab}	-11 ± 2 ^{ab}	-24 ± 4 ^a	-9 ± 2 ^b	-14 ± 3	-46 ± 22	-15 ± 3	-20 ± 3			
N surplus	-179	-195	-200	-185	-100	-60	-101	-89	-119	-121	-80	-132			
NUE %	n.a.	179	200	198	n.a.	157	184	188	n.a.	183	174	220			
AE _N (kg kg ⁻¹)	n.a.	-1.8	2.5	3.1	n.a.	14.4	19.8	16.9	n.a.	11.6	15.4	33.5			
RE _N (%)	n.a.	34	55	53	n.a.	64	92	95	n.a.	70	60	107			

5.4.3 Mineral N content in the soil profile (Paper I)

The 2017 soil mineral N profile measurements (Fig 13) indicate that mineral N content decreased during the growing season in the 0.25 - 0.40 m layer in all treatments (Table 4). In Control and Deep, mineral N also disappeared from the 0 - 25 cm layer during the growing season. Shallow-placed fertilization resulted in a greater surplus of mineral N remaining in the system after harvest than in the other treatments ($38.7 \pm 13.9 \text{ kg N ha}^{-1}$), followed by the Mixed placement (6.2 ± 36.0).

The mid-crop season soil mineral N content profile essentially pinpoint where the fertilizer had been placed. Shallow, which was placed at 0.07 m depth, was primarily found between 0.05 and 0.10 m. After harvest, it appears that the bulk of Shallow soil mineral N remained in the soil, but had leached further down in the profile to 0.25 m. Just below this layer, in the 0.25–0.40 m layer, mineral N content had decreased by $1.7 \pm 2.7 \text{ kg N ha}^{-1}$. The largest mid-season N content peak in the Mixed treatment was at 0.10 m, with a gradual decline in soil N content between 0.10 and 0.25 m. Following crop harvest, the 0.10 m N content peak in Mixed had nearly halved, and another distinct N content peak was observed deeper in the soil profile at 0.40 m. Less soil mineral N was detected in Deep after harvest than in the other fertilized treatments and more N was removed from the system through harvested straw and grains resulting in a higher nutrient use efficiency of the applied fertilizer N and a higher uptake of mineral N (Table 4). Similar to the Control, mineral N in the 0 - 0.40 m layer in Deep decreased over the growing season. Only a small fraction of the negative N surplus of 118 kg in Control (Table 4) was explained by the decrease in soil mineral N ($6.2 \pm 36.0 \text{ kg N ha}^{-1}$) during the cropping season. Thus, net N mineralization during the growing season would have been at least 100 kg N, explaining the weak fertilizer response of crop yield in the fertilized treatments (Table 3).



	Shallow	Mixed	Deep	Control
<i>Soil mineral N content and changes (kg N ha⁻¹)</i>				
Soil mineral N at sowing (0–0.25 m)	42.6 ± 0.9	42.4 ± 2.7	36.8 ± 3.9	37.4 ± 2.3
Soil mineral N at sowing (0.25–0.40 m)	30.5 ± 1.8	37.4 ± 6.5	34.0 ± 4.2	22.1 ± 1.6
Soil mineral N after harvest (0–0.25 m)	82.7 ± 17.1	54.0 ± 7.8	42.2 ± 1.6	25.0 ± 3.0
Soil mineral N after harvest (0.25–0.40 m)	31.6 ± 0.7	47.6 ± 20.5	37.3 ± 2.5	16.6 ± 2.6
Δ Soil mineral N (0–0.25 m)	40.4 ± 16.5	6.9 ± 10.0	-1.9 ± 2.3	-15.7 ± 6.2
Δ Soil mineral N (0.25–0.40 m)	-1.7 ± 2.7	-0.7 ± 26.0	-4.8 ± 2.2	-8.6 ± 3.4
Total increase in mineral N (0–0.40 m)	38.7 ± 13.9	6.2 ± 36.0	-6.6 ± 4.7	-24.3 ± 9.6

Figure 13 Soil mineral N content (mg $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ kg soil⁻¹) sampled in the field experiment in 2017 in 0.05 m increments to a 0.30 m depth and at 0.30–0.40 m depth. Treatment depth averages at five days prior to fertilization (Pre fertilization) and sowing, two months after fertilization and sowing (Mid crop season), and one week after harvest (Post harvest). Horizontal bars indicate ± standard error (SE) of the mean.

6. Discussion

6.1 Placement of fertilizer N deeper in the profile reduced GHG emissions (Papers I - III)

When the total amount of fertilizer was deep-placed, the mitigation effect on N₂O emissions was evident. In the field experiment, individual N₂O fluxes from Deep were rarely elevated over Control (Fig 3) nor were cumulative emissions from the 2017 field season significantly elevated over the Control, indicating that native soil N was the primary contributor to N₂O emissions in Deep. Conversely, in the Shallow treatment, which represents the typical fertilizer placement depth for this area of Sweden, was the single fertilized treatment with consistently higher N₂O emissions than Control. The reduction in N₂O emissions from the Deep treatment compared to both Mixed and Shallow was consistent with previous studies indicating the connection between residence time of N₂O in soil and uptake or reduction in the emission of N₂O (Clough *et al.* 1998; Harter *et al.* 2016).

It was expected that Mixed, which received half the amount of fertilizer at the same depth as Shallow to be an intermediate between the highest and lowest emitters, but that was not always the case. During both cropping seasons in the field, N₂O emissions from Mixed plots were generally as high as those from Shallow, consistent with findings of Chapuis-Lardy *et al.* (2007). The higher concentration of mineral N in the upper topsoil of Mixed (Fig. 13) could explain why no significant reduction was achieved.

Similar to our findings, van Kessel *et al.* (2013) found in a meta-analysis that N₂O emissions were reduced when N fertilizers were placed at a depth ≥ 0.05 m. Moreover, the authors reported that yield-scaled emissions were significantly reduced with a deep N fertilizer placement in both no tillage and reduced tillage systems in humid climates. Similarly, yield-scaled

emissions from the 2016 and 2017 field experiment were also lower in Deep than in the Shallow placement.

Generally, treatment differences were first detectable several weeks after fertilization in the field. The strongest significant treatment effects on N₂O formation and emissions were recorded during the 3rd – 5th week after sowing and fertilization, i.e., in the first third of the 2017 growing season. It is possible that vigorous plant growth during this period, and thus soil N uptake, influenced the decreased N₂O emissions in Deep, but not in Mixed, which still had high N₂O emissions. The treatment effect on N₂O emissions largely disappeared during the latter two-thirds of the 2017 growing season (Fig. 3). Since there were fewer chamber measurements during this time, it is possible that some emission peaks, and thus treatment effects, were missed. On the other hand, in the final weeks of the 2017 growing season, N₂O fluxes from fertilized and non-fertilized plots were similar, showing that neither fertilizer depth placement nor crop utilization were important drivers for N₂O emissions at this stage when mineral N was largely utilized or had been translocated to a lower soil depth (Fig. 13). This assumption is somewhat supported by the higher soil water content observed towards the end of the experiment (41% water-filled pore space in late August and 66% in mid-September). However, soil water content was low, on average (25% water-filled pore space), throughout the 2017 growing season. This suggests that nitrification rather than denitrification was the primary process affecting N₂O production during this period.

In the incubation experiment, N₂O^{tot} emissions (sum of N₂O from both fertilizer N, if present, and indigenous soil N) were positively correlated to soil moisture and pores connected to the top surface in the upper portion of the column. These parameters were higher, on average, in Deep, relative to Shallow and Mixed (Table 3). Thus, soil N was probably more accessible (mobile) to microbes, and pore structure supported greater degassing to the soil surface in Deep. Although N₂O^{tot} was highest in Deep, the average contribution from the indigenous N fraction (fN^{ind}) was highest among the fertilized treatments, following a pattern of Deep > Mixed > Shallow, corresponding to 92.5, 87.8 and 71.8%, respectively. Additionally, in all four repetitions of the Mixed treatment, either the top and bottom of the column, or both were high in Euler but low in gamma (Table 3), indicating that the pores were largely fragmented rather than inter-connected. The same columns impacted by high Euler and low gamma additionally were low in

pores connected to the top surface and pores connecting to both the top and bottom of the soil column. Phi (porosity), which can typically be low in compacted soil, was not substantially affected in Mixed, however. This indicated that many pores were still intact, but were largely fragmented and less connected to either/both the top or bottom surface of the soil column. When columns were collected from the field, some were inadvertently collected from a tire track, but it was not clear which ones had been affected. Since N_2O^{fert} negatively correlated with Euler in the total column, this presumed compaction effect would explain why the Mixed treatment had lower emissions than would have been expected in comparison with N_2O emissions from the field experiment.

In addition to reducing N_2O emissions, deep fertilization also had a significant effect on emissions of CH_4 . Methane oxidation (i.e., uptake) was the dominant process according to the majority of fluxes in the field (Figs 3 and 7) and in the incubation experiment (Figs 5 and 8). In the 2017 field measurements and in the incubation, uptake of CH_4 from Control and Deep were highest and did not differ significantly. There were, however, more positive fluxes of methane in Mixed and Shallow in the 2017 cropping season, and similarly, Mixed and Shallow treatments had less uptake (significantly in Shallow) in the incubation experiment relative to Deep. In the latter experiment, methane was not correlated to any measured structural or physical properties, but was positively correlated to N_2O^{fert} . These results are consistent with previous studies that have linked shallow and surface N fertilization to higher CH_4 fluxes (Bodelier 2011) since CH_4 oxidation primarily occurs within 0.05 m of the soil surface (Crill *et al.* 1994, Kruger *et al.* 2001) and is inhibited by the presence of N fertilizer. Similar results were found by Wu *et al.* (2023). In their study, four deep fertilizer placements were assessed (0.05, 0.15, 0.25 and 0.3 m depth placements) and they found that the three deeper placements enhanced methane uptake relative to the shallow placement, from 42 – 169%, increasing with greater placement depth. They also found that CH_4 consumption was greatest in the upper 0.2 m of the soil profile.

Measured fluxes in the field (Fig 3), lysimeters (Fig 9), and in the incubation (Figs 5 and 8) indicated that neither fertilization nor fertilizer placement significantly influenced emissions of CO_2 on bare soil. Comparable information in the literature is largely lacking regarding a deep fertilizer N placement effect, Sosulski *et al.* (2020) found that while deep-

placed N fertilizer reduced N₂O emissions from bare soil, CO₂ emissions were higher than from a surface-applied treatment. When crop photosynthetic CO₂ uptake was greater than soil respiration, e.g., between 42 – 87 days after fertilization in the 2017 field season (Fig 3), the effect of fertilization placement was more evident. In the latter case, total measured CO₂ uptake was higher in Deep, but in the lysimeter experiment the greatest uptake was in Mixed and Control in 2017 and 2018, respectively. There is evidence that deep fertilizer N placement can delay crop senescence and increase photosynthetic capacity in maize (Guo *et al.* 2022, Wu *et al.* 2022b), which could result in a prolonged period of increased photosynthetic CO₂ uptake. As noted previously, more frequent measurements during this period of high crop CO₂ uptake in the lysimeter and field experiments would probably have improved the assessment of fertilizer depth placement.

6.2 Deep fertilization increased water use efficiency and decreased N leaching (Papers I and II)

Soil mineral N content measurements in the soil profile in the field Y2 (2017) indicated that, at two weeks after crop harvest, soil mineral N in Deep had been largely utilized (Fig 13), whereas around 39 and 6 kg N ha⁻¹ was found in the top 0.4 m in Mixed and Shallow, respectively, during that time. This remaining mineral N was primarily in the form of NO₃⁻, and would have been highly susceptible to leaching in the following autumn and winter months. A small peak of NO₃⁻ was detected in Deep between 0.2 – 0.3 m at harvest, but the concentrations were generally lower than the mineral N concentrations measured prior to fertilizing. Although these measurements indicate that most of the applied fertilizer in Deep had been utilized, it is still possible that the fertilizer N had translocated below the depth of sampling (0.4 m).

Nutrient load in the lysimeter leachate was clearly affected by fertilization placement. Shallow placement cumulative N load (S1-S3) was the only fertilized treatment significantly higher than Control, and both Mixed and Deep placements reduced N loads relative to Shallow. Among fertilized treatments, N load was lowest in every period but S3. However, it is possible that the higher N load in Shallow and Deep placements in S3 was a result of a carryover effect of previously immobilized soil N from S2 when both treatments had very little leachate flow. It is clear, however, that over time,

with diminishing background mineral N in the soil, Deep fertilizer placement led to higher crop water utilization and crop N uptake, resulting in less N losses in leachate (Table 5).

The cumulative water flow from the lysimeters, when compared to total water inputs, which could be inferred as water use efficiency, was clearly affected by fertilizer placement (Fig 14). Water use efficiency indicated a benefit of deep fertilizer placement for crop growth, particularly when plant available water was scarce.

Crop utilization of water inputs was not consistent across growing seasons in Mixed and Shallow, as the drought in S2 resulted in contrasting leaching patterns relative to S1 and S3. In terms of crop water utilization, Shallow placement was efficient during S2 compared to Mixed, likely enhanced by the supplemental irrigation that was performed during this period. Conversely, in S1 and S3, Mixed leached less than Shallow, but the differences were small. On the other hand, the Deep treatment had the lowest quantity of leachate in all periods once the treatments were initiated in S1 (Figs 11 and 12). Additionally, in contrast to all other within-treatment results, the repeat measure ANOVA analysis on water leachate revealed that within the Deep treatment, each leachate sampling was statistically similar for all periods, including the drought year. Thus, this fertilization placement depth was beneficial for crop water utilization with contrasting water availability.

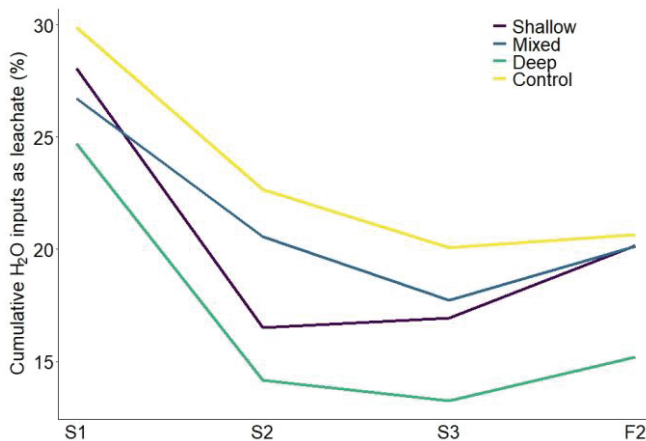


Figure 14 Recovered leachate relative to cumulative water inputs per season from the lysimeters in the three years with crops (S1 – S3) and the second fallow period F2.

Total leachate quantity in S1 – S3 was highest in Control and significantly lower in the Deep treatment than in the other treatments ($p=0.03$). Compared to Shallow, Deep placement had 25% less leachate and 29% less than Mixed. Though not significant, Deep continued to have less leachate than all other treatments during the F2 period despite the absence of crops (Figs 11 and 12). Within the fertilized treatments, however, there were no significant differences in cumulative leachate except during the drought period S2. Similar to our results, Chen *et al.* (2022) found that Deep placement of N fertilizer at 0.15 m relative to placement at 0.05, 0.25 and 0.35 m had the highest precipitation use efficiency, crop N uptake, radiation use efficiency, and also reduced soil nitrate-nitrogen residue levels in the deep layers under two years of maize followed by winter wheat. They also found that root surface area and root length density were highest at 0.15 m placement. Similarly, Wu *et al.* (2022b) found that fertilizer placement at 0.25 m significantly enhanced water use efficiency and water productivity (biomass as a function of crop evapotranspiration) in maize relative to the fertilizer placement depths at 0.05, 0.15 and 0.35 m.

In the lysimeter experiment, observed treatment differences in leachate amount, and thus crop utilization of soil water, were likely a consequence of differences in either root architecture (e.g., deep rooting), or plant allocation favoring root growth, or a combination of the two. It is known that many plants exhibit a range of root plasticity in response to heterogeneous water and nutrient distribution in the soil (Grossman & Rice 2012, Hodge 2006). Although the roots were not sampled, the belowground biomass can be inferred from aboveground plant biomass and leaching quantity. Aboveground crop biomass at harvest was negatively correlated with leachate quantity ($p < 0.0001$, $R^2 = 0.81$) when excluding the drought year S2 (Fig. 15).

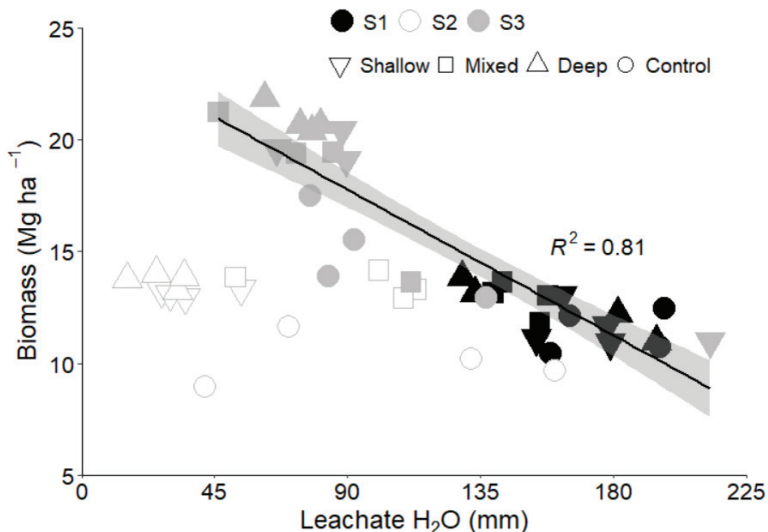


Figure 15 Total aboveground crop biomass (Dry matter in Mg ha⁻¹) and total leachate quantity of H₂O (mm) per cropping year (S1–S3) for individual lysimeters. Linear trend line and R² value for combined years S1 and S3, i.e., excluding the drought season S2.

In S1, when available soil N was likely quite high, there was only a minor effect of the N fertilizer placement on aboveground biomass and leachate quantity across treatments, and thus the correlation of biomass to leachate amount was not significant for this year individually ($p = 0.18$, $R^2 = 0.39$). However, in S3, the fertilizer treatment significantly affected crop biomass, and the correlation between aboveground biomass and leachate quantity was more clear ($p < 0.0001$, $R^2 = 0.65$). In S2, summer drought conditions likely affected the allometric relationship between roots and shoots as there were significant treatment differences in both aboveground biomass at harvest and total leachate, but they were not correlated ($p = 0.7$, $R^2 = 0.21$) (Fig. 15). Mathew *et al.* (2018) also found a weaker relationship between root to shoot ratio and shoot biomass or grain yield in wheat under drought-stressed conditions compared to non-stressed conditions. Similar to that, Meurer *et al.* (2019) found that shoot:root ratios, as well as N concentrations in living roots changed depending on irrigation and N fertilization in a field experiment with mixed grass ley in central Sweden.

A deep rather than lateral exploration by roots, earlier in the season, has been shown to be beneficial for N capture and subsoil water access, although

water in the subsoil is potentially more beneficial in the latter part of crop growth (Lynch 2013). It was expected that in the earliest stages of crop growth, the crop roots in the Shallow placement would proliferate at around 0.07 m where the P and 100% of the N fertilizers were placed, while Deep placement by contrast, would have earlier deeper root exploration and have relatively higher root biomass at and below 0.2 m. In the Mixed placement, it was expected that root proliferation would have initially occurred around the 0.07 m placement, but once N resources were exhausted, roots would explore the soil profile toward the remaining 0.2 m-placed N, but this deeper exploration would be delayed compared to the Deep treatment, and possibly mechanically impaired if subsoil moisture was low (Colombi *et al.* 2018). While this delay in the Mixed placement was not generally detrimental in terms of crop N uptake (Table 5), during drought conditions in S2 there were high N losses via leaching (Figs 11 and 12), likely due to low root biomass relative to the other fertilized treatments. Additionally, in S3, with non-drought conditions and in the absence of excess soil mineral N, the mixed placement had lower yield and lower crop N uptake compared to Deep (Tables 3 and 5).

6.3 Increase in crop yield and N uptake (Papers I and II)

Fertilizer placement effects on crop yield were obscured somewhat due to the fact that the soils on which the field experiment was performed has a high rate of mineralization and provides a high level of crop N even without fertilization. This is evident by the relatively high crop yield in the Control (Table 3), and high N balance surpluses in all measured periods. This was also evident in F1 and S1 in the lysimeter experiment, where leachate loads were very high, even in the former period, when there had been no fertilizer applied, and resulting crop yields in S1 were even across all treatments.

In addition to high indigenous soil N, different external issues affected yield in some seasons. For example, spring barley grain yield was 3.87–4.49 t ha⁻¹ in Y2 (Table 3) and in comparison, the average yield in Uppsala county for barley in that year was 5.07 t ha⁻¹ (Jordbruksverket 2018). In 2017, seed placement in the field was too shallow, and seed emergence was uneven and in some areas greatly delayed, requiring the field to be irrigated. This uneven crop development ultimately led to high variation in both yields and average nutrient uptake in all treatments. Additionally, grain yield losses in the first

two cropping seasons in the lysimeter experiment were greatly (and possibly unevenly) affected by foraging birds and to some extent by GHG chamber deployment. Thus, it was decided to discontinue GHG measurements in S3.

That the harvested grain yield in the field experiment was highest in the Control in Y2 suggests that N fertilization was not needed in this year, or that it was even counterproductive. The high values for NUE and the negative values for AE_N (Table 4), indicate that application of exogenous N did not lead to a yield increase. In contrast to Y2, values for AE_N were positive in Y1, though they were low, between 1.8 for Shallow and 5.8 kg grain kg^{-1} applied N for Deep. Similarly, grain yield in Control from the lysimeter period S1 and, to a lesser extent, from S2, was high compared with the fertilized treatments, leading to low or negative AE_N in S1. According to Dobermann (2005), common values for AE_N are 10–30 kg grain kg^{-1} applied N, with higher values in well-managed systems or at low N levels. AE_N was on the higher end of this range only in S3, and in the Deep treatment (33.5 kg grain kg^{-1} applied N). For Europe, Lahda *et al.* (2005) reported an average AE_N of 21.3 kg grain increase per applied kg N, given a similar fertilization rate as used in the field in Y2 and lysimeter experiments in S1- S3.

In contrast to AE_N , the field Y2 values for RE_N (2.3, 12.2 and 18.1% for Shallow, Mixed and Deep, respectively) indicate that, despite the low yield in the fertilized treatments relative to Control, the crops were able to acquire additional N in the grain and straw. Compared to RE_N values for cereals, as summarized by Lahda *et al.* (2005), i.e. 10 - 70%, RE_N of the Mixed and Deep treatments were at the lower range of this interval. Although Y1 was better in comparison, the highest value (in Deep, 37%) was mid-range according to Lahda *et al.* However, Dobermann (2005) reported an average range between 30 and 50%, with up to 80% achieved in well-managed systems. In the lysimeter experiment, this upper boundary was exceeded in S2 in the Mixed and Deep treatments which averaged 92 and 95%, respectively, and in S3 Deep further increased to 107%. While average N load in all lysimeters decreased (i.e., decrease in background N) over time, AE_N in Deep increased, as did treatment differences in yield. This change over time indicated that the high background levels of N introduced into the system from the combined effect of simulating tillage in F1 and the fact that the soils had been in fallow for a long period when they were collected from the field obscured the fertilizer placement effect, although the trend of improved N use efficiency in Deep was still evident. This effect could also

explain why AE_N and RE_N were somewhat low in the field Y1 even when yield from the Deep treatment was significantly higher than both Control and Shallow.

Thus, while absolute grain yield has varied across the different periods in the field and the lysimeter experiment, generally the trend was that grain yield increases with increasing fertilizer depth, with the strongest contrasts occurring with lower “background” soil N. Furthermore, even when higher background N was present, yields and N use efficiency were still improved in Deep over Shallow, the latter being the treatment that represents what is current practice in this part of Sweden.

7. Conclusions and future perspectives

Due to the dual pressures of climate change and global population growth, new management strategies need to be implemented to improve grain yields and crop N uptake and also minimize or reduce fertilizer-induced GHG emissions to the atmosphere and N losses from leaching. The experiments described in this thesis tested the environmental and agricultural benefits of deep (0.2 m) placement of N fertilizer relative to the shallow (0.07 m) subsurface placement currently in use in SE Sweden. Previously, studies were lacking on how N fertilization depth affected leaching and GHG emissions in this region of Europe.

In paper I it was concluded that placing 100% of fertilizer deeper in the soil profile at 0.2 m rather than keeping half at the shallow depth and half at the deeper depth provided the greatest potential for mitigating fertilizer-induced N₂O and CH₄ emissions relative to the shallow placement. In addition, yield and crop N uptake were improved when fertilizer was deep placed. Soil N concentrations measured in the profile over the growing season indicated that i) soil N mineralization was high in this soil and ii) deep-placed N fertilizer was largely utilized in the deep placement around crop harvest, relative to the shallow and mixed placement. Finally, the short period of GHG measurements in the spring thaw period showed that the bulk of N₂O emissions for the year were occurring during this time.

In paper II it was affirmed that deep-placed N fertilizer enhanced crop N uptake and improved yields over both shallow and mixed-level placements. Additionally, deep placement reduced N leaching and increased crop-water utilization. The results also indicated that deep placement was the most effective strategy for improving yield and NUE under drought.

In paper III, ¹⁵N tracing was utilized to identify the relative contribution of applied fertilizer N vs. indigenous soil N to total N₂O emissions in a

controlled environment. The results in paper III supported the results from paper I which indicated that N₂O emissions from deep-placed fertilizer N were primarily from indigenous soil N, even when physical and structural differences enhanced a higher degassing of total N₂O. Paper III also supported paper I results that CH₄ uptake is inhibited with increasing fertilizer N in the shallow layer (i.e., 0.07 m) and CH₄ emissions did not correlate with any physical or structural parameters, but were only correlated with N₂O emissions originating from fertilizer.

The results in this thesis indicate that a deeper subsurface fertilizer N placement than what is currently in use in SE Sweden would improve cereal grain yields as well as mitigate the negative impact associated with fertilization. Further work should be performed to assess its efficacy in other areas in Sweden and possibly in combination with different methods of precision agriculture. With weather patterns slated to change in the coming years, it is recommended to continue GHG measurements outside of the growing season, preferably year-round, as evidenced by the small but significant data from the spring thaw period in the field.

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Popular science summary

Agriculture is a prominent source of pollution to aquatic systems and contributes to the atmospheric accumulation of greenhouse gases (GHG). Fertilization associated with crop production entails the application of reactive nitrogen (N), a nutrient necessary for all life, but is in particular a vital macronutrient for crops. However, this fertilizer N, once applied to the soil, is readily transformed and mobilized by soil biota and climatic factors. N can be volatilized in the form of ammonia, and microbial transformation can lead to gaseous emissions of nitrous oxide, a potent GHG with a long residence time in the atmosphere. Due to the dual pressures to produce a greater quantity of nutritious food for a growing global population and to curb GHG emissions and water pollution, new fertilization management strategies need to be developed to both improve crop yields but also reduce the associated negative environmental impact.

In this thesis, a deep placement of N fertilizer at 0.2 m was compared to a shallow placement at 0.07 m that is currently used by farmers in central Sweden. Treatment effects on grain yield, crop uptake of N, and emissions of GHG were measured in a two-year field experiment near Uppsala, Sweden. Deep fertilizer placement improved crop yield by 11% and increased grain N uptake by 2.5% compared to the shallow placement in the first year. In both years, N₂O emissions in the deep placement treatment were lowest among the fertilized treatments and similar to the non-fertilized control.

In a 5-year lysimeter experiment that included three years with cereal crops, crop response to the fertilization placement depths as well as N leaching were investigated. The deep-placed N fertilizer had a higher nitrogen use efficiency (NUE), meaning that less N was lost through leaching, and a greater quantity of N was taken up by the crops. Deep

placement also improved crop water use efficiency and crop yield over the other treatments during a drought.

The effect of fertilizer placement depth in bare, undisturbed soil cores on GHG emissions was also investigated using labeled fertilizer and an X-ray scanner to image the soil structure. It was found that the deep-placed fertilizer, relative to the shallow placement, emitted less methane, and although N_2O emissions were highest, the majority of emissions were from indigenous soil N, not from the fertilizer. From the X-ray scans it was determined that total N_2O emissions were impacted by soil compaction.

This thesis provides information that clearly shows that the placement depth of fertilizer N in Sweden should be reassessed. Crops benefited from deeper placement, resulting in higher fertilizer N uptake and greater yields. Additionally, N_2O emissions were greatly reduced, and methane was also reduced to a lesser extent. Crop water use was improved and N leaching was reduced. Thus, deep placement is a sustainable improvement over the current shallow placement depth currently in use in central Sweden.

Populärvetenskaplig sammanfattning

Jordbruket är en framträdande källa till förorening av vattensystem och bidrar till den atmosfäriska ackumuleringen av växthusgaser. Gödsling i samband med växtodling innebär tillförsel av reaktivt kväve (N), ett näringsämne som är nödvändigt för allt liv, men är särskilt ett viktigt makronäringsämne för grödor. Detta gödselmedel N, när det applicerats på jorden, omvandlas och mobiliseras lätt av markbiota och klimatfaktorer. N kan förångas i form av ammoniak, och mikrobiell omvandling kan leda till gasformiga utsläpp av lustgas, ett potent växthusgas med lång uppehållstid i atmosfären. På grund av det dubbla trycket för att producera en större mängd näringsrik mat till en växande global befolkning och för att minska utsläppen av växthusgaser och vattenföroreningar, måste nya strategier för gödslingshantering utvecklas för att både förbättra skördarna men också minska den tillhörande negativa miljöpåverkan.

I denna avhandlingen jämfördes en djup placering av N-gödsel på 0,2 m med en grund placering på 0,07 m som idag används av lantbrukare i Mellansverige. Reningseffekter på spannmålsutbyte, grödans upptag av kväve och utsläpp av växthusgaser mättes i ett tvåårigt fältförsök nära Uppsala, Sverige. Djupt gödningsmedelsplacering förbättrade skörden med 11 % och ökade spannmålsupptaget med 2,5 % jämfört med den grunda placeringen det första året. Båda åren var lustgas utsläppen i djupplaceringsbehandlingen lägst bland de gödslade behandlingarna och liknar den icke-gödslade kontrollen.

I ett 5-årigt lysimeterförsök som inkluderade tre år med spannmålsgrödor undersöktes grödans svar på gödslingsplaceringsdjupen samt N-urlakning. Det djupt placerade kvävegödselmedlet hade en högre kväveanvändningseffektivitet (NUE), vilket innebär att mindre kväve gick förlorat genom urlakning och en större mängd kväve togs upp av grödorna.

Djupplacering förbättrade också grödans vattenanvändningseffektivitet och skördeavkastning jämfört med andra behandlingar under en torka.

Effekten av gödningsmedelsplaceringsdjup i nakna, ostörda jord på utsläpp av växthusgaser undersöktes också med hjälp av märkt gödselmedel och en röntgenscanner för att avbilda markstrukturen. Det visade sig att det djupt placerade gödselmedlet, i förhållande till den grunda placeringen, släppte ut mindre metan, och även om lustgas utsläppen var högst, var majoriteten av utsläppen från ursprunglig mark N, inte från gödselmedlet. Från röntgenundersökningarna fastställdes att de totala lustgas utsläppen påverkades av jordpackning.

Denna avhandlingen ger information som tydligt visar att placeringsdjupet för kväve gödselmedel i Sverige bör omvärderas. Grödorna gynnades av djupare placering, vilket resulterade i högre N-upptag av gödselmedel och högre skördar. Dessutom minskade lustgas-utsläppen kraftigt och metan minskade också i mindre utsträckning. Användningen av grödans vatten förbättrades och kväveläckaget minskade. Djupplacering är alltså en hållbar förbättring jämfört med det nuvarande grunda placeringsdjupet som för närvarande används i Mellansverige.

Acknowledgements

After this long (v-v-e-e-r-r-y-y long) journey, I sometimes feel like I am the PhD student with nine lives, and reflect with wonder that I managed to survive mostly intact. But who can sail without wind? Not anyone, and certainly not me. There have been so many people along the way who helped, supported, or quite literally saved my life (shout out to that temporary lab employee who rescued me from the freezer room on a Saturday in the middle of the summer!). First, I have to thank my main supervisor Thomas who was brilliant in reigning in and helping me to focus my PhD activities, was always swift to answer emails and provide assistance. I greatly appreciated your kindness and regular check-ins when I was hit with debilitating illness and facing terrifying prognoses. To Katharina, I am quite certain I would have needed yet another year of slugging through this work if I did not have your help. I deeply appreciated your enduring kindness, positivity, and understanding. To Monika, thank you for your early help in ensuring I had access to the right equipment and providing valuable input. I always felt a lift in my mood and would often come away from one of our chats with a valuable nugget of knowledge. Thanks also to Gunnar and Lars for your valuable input and contributions to my work. Holger, the last time I saw you, you gave me a hug and said there was no need to worry about my upcoming defense, something I really needed at the time. I'm sorry you did not get to be here for it like you said you wanted to.

I am deeply grateful for the support from my family. The last 11+ years have been a challenge on multiple levels, and all of you have cheered me on and held me up. To my dad, my first exposure to the concept of that mysterious other kind of doctor, whose diligent work, self-organization, and rooting for the underdog has been so important to me as a role model. Thank you for always being there for me. To my mother, thank you for always

encouraging me to do the right thing, make hard choices, and push myself to be a better person. I am not sure how I would have survived, mentally, without your daily texts throughout that long, horrible illness. Cindi, your frankness and wisdom combined with kindness is something I strive for. David, your oft-repeated phrases always made me roll my eyes, but I know them to be true. Thank you also for helping me take myself less seriously. I also feel blessed to have two brilliant older sisters who I always looked up to (sometimes with annoyance, yes, because you're both too smart!). Thank you both for your encouragement and advice around academic things and beyond.

I am also grateful for Gizachew, my long-time office mate and collaborator on PhD projects. It is not an easy thing to be working on another continent for many reasons, especially with a family, but having someone that understands and shares those difficulties and hardships (also, of course, mixed with joy) has been a true blessing. Thank you for all of your help along the way, and for the help from your family members.

Thank you, Viktoria, my co-conspirator, for (re)socializing me and showing that nature is not a place where you go to get murdered (as I grew believing). You made work life a million times more enjoyable and our adventures meant everything. I am also thankful for the friendship and solidarity at work from many, but particularly from Jenna, Mareile, Elsa, Sabina, Sabine, Jelena, Jan, Geert, John, Tino, Anna, Oksana, Hugo, Erica, Yaana, Lukas, Louis, Miyanda, Tamlyn, Maarten, Mitsuaki, Eduardo, Frederic and everyone else in the Northern lights crew.


To Ilysea (hyphae wifey!), thank you! Despite not seeing each other in over 10 years, our friendship kind of saved my life. To my friends across the world: Kyla, Jake, Yasser, Lisa, Meg, Leslie, Mavrik, India, Michelle, I <3 you.

To those at work who took the time to help me, give me advice, or just listen to me, thank you! In no particular order: Anke, Mats, Sigrun, Helena, Örjan, Lisbet, Ingrid, Abraham, Björn, Karin, and many many others.

Big thank you to Mark Waldrop for taking a chance on me as an undergrad. I am also so grateful to Gail Wilson for all the support during my time at OSU, I would not have finished without you – thank you!

Finally, following the sage advice of Dr. Tressie McMillan Cottom, I want to thank my haters and all the people who discouraged me along the way. Spite is a hell of a motivator.

Deep N fertilizer placement mitigated N₂O emissions in a Swedish field trial with cereals

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Received: 11 November 2019 / Accepted: 10 August 2020 / Published online: 25 August 2020
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Abstract Deep fertilizer placement is a proposed strategy to increase crop yield and nitrogen (N) use efficiency while decreasing nitrous oxide (N₂O) emissions from soil to atmosphere. Our objective was to test three fertilization depth orientations to compare overall N use efficiency, based on a 2-year field trial on a mineral soil cropped with cereals in Uppsala, Sweden. The field was fertilized with ammonium nitrate at a rate of 120 kg ha⁻¹ (2016) and 105 kg ha⁻¹ (2017) and a deep fertilizer placement (DP) at 0.20 m was compared to a shallow placement (SP) at 0.07 m and a mixed-depth placement (MP) where fertilizer was halved between the depths of 0.07 and 0.20 m, and a non-fertilized control (NF). In 2016, compared to SP, MP and DP increased N content in harvested grain by 3.6% and 2.5% respectively, and DP increased grain yield by 11%

($P < 0.05$). In both years, N₂O emissions were similar in DP and NF, whereas SP and MP emissions were similar but generally higher than those in DP and NF. Fertilizer-induced emission factors (EF) for the growing season of 2017 decreased with fertilizer placement depth and were 0.77 ± 0.07 , 0.58 ± 0.03 , and 0.10 ± 0.02 for SP, MP, and DP, respectively. Although deep N placement benefits are likely dependent on weather conditions and soil type, this strategy has a clear potential for mitigating N₂O emissions without adversely affecting yield.

Keywords Nitrous oxide · Deep N fertilization · Nitrogen use efficiency · Fertilizer N placement

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10705-020-10089-3>) contains supplementary material, which is available to authorized users.

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Introduction

The intensification and expansion of agriculture is on a course for rapid increase as the Earth will need to support a projected additional two billion people by 2050 (United Nations 2019). The use of mineral nitrogen (N) fertilizer directly and indirectly contributes to the microbial production of the greenhouse gas (GHG) nitrous oxide (N₂O) via soil and water systems. The residence time of N₂O in the atmosphere is about 120 years and is 265 times more potent as a GHG compared to carbon dioxide (CO₂) on a 100-year time scale (Myhre et al. 2013). Atmospheric N₂O is either removed by a sink via microbial reduction or

transported to the stratosphere and consumed in an ozone-depleting chemical reaction, making it one of the most dominant sources of ozone depletion (Ravishankara et al. 2009).

N_2O is produced by two processes, nitrification and denitrification, which occur under oxic and anoxic conditions, respectively. The former process is primarily mediated by autotrophic bacteria from the genera *Nitrosomonas* and *Nitrosospira*, and strictly a source of N_2O . The latter process, however, can be either N_2O -consuming or N_2O -producing. Though N_2O is naturally emitted, the trend of increasing emissions is due to human activities, of which around 60% comes from agriculture (Smith et al. 2014), inherently connected to the use of nitrogenous fertilizers. The near quarter-fold increase in atmospheric N_2O since the industrial revolution is attributed to a widening use of mineral N fertilizer (Park et al. 2012). Fertilization is vital for food security and cannot be excluded from crop production, necessitating a sharp focus on identifying fertilizer application strategies that can mitigate N_2O emissions. While surface-applied fertilizer can lead to N losses from both ammonia (NH_3) volatilization (Pan et al. 2016) and microbial nitrification and denitrification (Cameron et al. 2013), increasing fertilizer placement depth is a method for improving current agricultural practices, with potential to increase overall nutrient use efficiency (NUE).

Furthermore, temperature and moisture are major controls on soil N turnover, availability and mobility, affecting N losses via leaching and gaseous losses derived from nitrification and denitrification (Godde and Conrad 1999; Robinson 2002). Wet-dry cycles in soil induce pulses of N and carbon (C) mineralization upon re-wetting (Schimel 2018), of which the upper topsoil is most affected via rainfall events that mobilize fertilizer N. The amplitude of temperature and moisture variability decreases with increasing soil depth. Increasing fertilizer placement depth may be an effective method for keeping plant available N over longer periods with less rainfall due to more constant soil moisture conditions.

A deeper fertilizer placement may even improve crop growth over standard shallow or surface placements. Crop roots tend to proliferate around the area of the fertilizer grain, thus deeper placement can promote root length density and enhance N uptake (Lotfollahi et al. 1997; Li et al. 2009) as well as water utilization

(Singh et al. 1976) from deeper soil layers. Crops can obtain more than two thirds of their nutrition from deeper layers in the soil profile when nutrient availability and/or water is limited in the topsoil (Kautz et al. 2013) and deep fertilization could improve plant growth, particularly during periods of little to no precipitation. On the contrary, and particularly under high water availability, deeper placements have been shown to both increase (e.g. Ke et al. 2018) and decrease (e.g. Grant et al. 2019) N leaching and the amount of mineral N in the soil layers below the fertilizer placement.

Previous studies have indicated that augmenting the residence time of the gas in the soil matrix can decrease the $N_2O:N_2$ ratio, either by entrapment (Harter et al. 2016) or by lengthening the path of diffusion from the “source” of denitrification, i.e., location of the fertilizer grains to the soil surface (Clough et al. 1998). In studies where microbial N_2O uptake was observed, it tended to be in cases where soil moisture limited gas diffusion through the soil matrix, particularly in the absence of mineral N (Chapuis-Lardy et al. 2007). Thus, with deeper placement of fertilizer, the distance for N_2O diffusion from the fertilization layer to the soil surface would be increased, meaning a longer residence time and a potentially increased reduction of N_2O to N_2 in the upper zone of the topsoil where no fertilizer N was placed. Furthermore, deep placement concentrates fertilizer- NH_4^+ into localized areas, stimulating methane (CH_4) oxidation by soil methanotrophs and reducing CH_4 emissions (Bodelier et al. 2000a, b). Deeper root growth promoted by fertilizer placement increases the oxygen availability in the rhizosphere which is likely to enhance CH_4 consumption in deeper layers (Gilbert and Frenzel 1998; Kruger et al. 2001).

Previous field studies showed that deep fertilizer placement, compared to broadcast application, increased yields, improved NUE, and decreased N runoff (Mengel et al. 1982; Kelley and Sweeney 2007; Xia et al. 2016; Zhu et al. 2019). Regarding N_2O emissions, however, results are rather contradicting: while deep N fertilizer placement effectively lowered N_2O emissions in rice paddies (Gaihre et al. 2015; Wu et al. 2017) and field experiments comparing conservation tillage methods (Liu et al. 2006; Nash et al. 2012), other studies (e.g. Cai et al. 2002; Drury et al. 2006; Chu et al. 2007) found that N_2O emissions were higher from deeper N placement compared to shallow

N placement. In terms of CH₄ emissions, deep N placement has been found to be a promising management practice with regard to CH₄ mitigation (Linquist et al. 2012). However, the studies summarized by Linquist et al. (2012) focussing on the impact of N fertilizer placement on CH₄ emissions have been conducted in rice systems, which were either continuously flooded or rainfed. Methane measurements under different fertilizer depth management under cereals are still scarce.

The local agronomic practice in central Sweden prescribes a sub-surface placement of fertilizer around 0.07 m during seeding, which in many studies is already considered a “deep” placement. In this study, 0.07 m depth of fertilizer placement was considered as a baseline in comparison to considerably deeper placements. We tested the effect of three different mineral N fertilizer placements representing a shallow (0.07 m), deep (0.20 m), and mixed placement (half at 0.07 m, half at 0.20 m) along with a non-fertilized control on crop growth, yield, and N₂O and CH₄ emissions on a conventionally farmed mineral soil in Central Sweden. We expected that the two deeper fertilizer placements (deep and mixed) would have a positive effect on overall N use efficiency, improve crop yield, and lower N₂O and CH₄ emissions (Linquist et al. 2012; Xia et al. 2016). The mixed placement could elucidate if crops benefited from two placement depths for both early and later plant growth stages, but also if N₂O and CH₄ emissions were affected by the presence of an overlaying unfertilized zone acting as a buffer or sink.

Materials and methods

Site characteristics and experimental setup

A 2-year experiment was established in the spring of 2016 in Säby (59° 83' N, 17° 71' E), near Uppsala, Sweden on a Eutric Cambisol that has been used as cropland for at least a century. The site has a silt loam texture in the topsoil and is composed of 21.2% clay, 55.7% silt, 23.1% sand, and 6.1 p_HH₂O. The climate is cold temperate with a mean annual air temperature of 5.5 °C and precipitation of 528 mm (Table 1), of which 215 mm occur during the growing season (May–August).

In May 2016 prior to planting we sampled soil from a 20 m long · 1.5 m deep pit running parallel to the experimental plots where 24 1.5 m-deep soil columns had been removed from the field. Total soil organic carbon (SOC) and total nitrogen (TN) concentrations were analysed via dry combustion (LECO CNS Analyser, LECO Corporation, St. Joseph, MI, USA) using bulked samples taken down to 1 m depth at 0.10 m intervals at three points along the length of the pit (Table 2).

In 2016, the field was sown at a rate of 238 kg ha⁻¹ with spring wheat (*Triticum aestivum* L. var. ‘Quarna’) and fertilized with ammonium nitrate at a rate of 120 kg N ha⁻¹. The following year spring barley (*Hordeum vulgare* L. var. ‘Makof’) was sown at a rate of 200 kg ha⁻¹ and fertilized with 105 kg N ha⁻¹ ammonium nitrate. The fertilizer used in both years was YaraBela AXAN (Yara International, Oslo, Norway). The plots were sown and fertilized simultaneously using a Combi drill with the ability to adjust fertilizer and seed depth (Spirit 400C Strip Drill, Väderstad, Sweden), with two available fertilizer outlets allowing for split-level placement in the same vertical plane. Seed row spacing was 0.125 m and the fertilizer was incorporated into one or two 0.05 m-wide bands (depending on the treatment) below the seedbed. The general agronomic practice for the area is to place seeds at approximately 0.05 m and fertilizer at 0.07 m depth, so that the sub-surface soil moisture will promote seed germination without the reliance on subsequent rainfall. In 2017, because sowing depth was shallow (≤ 0.03 m) and planting occurred before a period without rainfall, seed emergence was greatly delayed in many plant rows. Irrigation is rarely used in this area, but due to poor seed emergence, plots were irrigated once after sowing with an equivalent of 17 mm rainfall on June 22nd (Fig. 1). The fields are typically cultivated in the fall, but after the 2017 growing season, a 4 m wide × 64 m long strip where the chambers had been previously established during the growing season was left uncultivated to facilitate further GHG measurements. However, field conditions after fall cultivation, particularly after rainfall and subsequent accumulation of snow and ice and then initial melt, rendered the field inaccessible and the planned GHG measurements were unobtainable for much of the autumn and winter of 2017–2018.

Table 1 Mean air temperature (°C) and sum of precipitation (mm) during the growing season in 2016 and 2017 (May–August), and climate normal in Uppsala (1961–1990); data from Ultuna meteorological station

	2016		2017		Climate normal (1961–1990)	
	May–Aug	Annual	May–Aug	Annual	May–Aug	Annual
Temperature (°C)	15.1	6.9	14.6	6.6	14.2	5.5
Precipitation (mm)	208	443	197	507	215	528

Table 2 Total C (TC) and total N (TN) (%) along the soil profile sampled in spring 2016 prior to fertilization and sowing

Soil depth (m)	TC %	TN %	n
0–0.10	2.83 ± 0.08	0.24 ± 0.005	3
0.10–0.20	2.66 ± 0.12	0.22 ± 0.010	3
0.20–0.30	1.43 ± 0.58	0.12 ± 0.050	3
0.30–0.40	0.66 ± 0.26	0.06 ± 0.030	3
0.40–0.50	0.39 ± 0.01	0.04 ± 0.002	2
0.50–0.60	0.35 ± 0.03	0.04 ± 0.004	3
0.60–0.70	0.38 ± 0.01	0.05 ± 0.001	3
0.70–0.80	0.33 ± 0.02	0.04 ± 0.003	3
0.80–0.90	0.64 ± 0.02	0.09 ± 0.004	3
0.90–1.00	0.65 ± 0.02	0.09 ± 0.003	3

Values are given as mean ± standard error. n = number of samples

The experimental setup followed a randomized block design with four repetitions of four treatments corresponding to three depths of fertilizer placement plus a non-fertilized control. Experimental plots were 4 × 20 m and consisted of an unfertilized control treatment (NF), a shallow placement of fertilizer at 0.07 m (SP), a deep placement at 0.20 m (DP) and mixed placement (MP) where half of the fertilizer was placed at 0.07 m and the other half at 0.20 m.

GHG flux measurements

Immediately after the fields were seeded and fertilized, a 0.55 × 0.35 m steel frame with a water well welded to its top and a 0.10 m lip underneath was pressed into the soil in the middle of each plot. Frames were centered encompassing the same number of crop rows, and the first gas measurement was performed within 24 h. Plant number and seed emergence within frames was monitored and found to be consistent across all plots. Before harvest, frames were removed

from the plots to avoid damage from agricultural equipment during combine harvesting and fall tillage. Static chamber measurements were performed by placing opaque polypropylene chambers (0.57 × 0.37 × 0.23 m) into the water-filled well on top of the frames. In the second year, each chamber was additionally equipped with a ventilation tube and a small battery-powered axial fan for air mixing within the chamber during sampling. When the chamber height became insufficient as crops grew taller, a riser, constructed from a similar plastic box as the chamber, but with the bottom removed, was added to the underside of the chamber to prevent crop damage and increase air movement during GHG measurements.

At each sampling occasion, chambers were closed for approximately 45 min and sampled five times at 10 min intervals beginning at time of closure. Air samples were collected using the flow-through method where air was circulated for one minute between the chamber, a 20 ml glass collection vial, and an air pump connected in a loop with tygon tubing. Air temperature inside the chamber was monitored during gas flux measurements. Thereafter, gas sample vials were stored at room temperature and analyzed within a week simultaneously for N₂O and methane (CH₄) concentration on a gas chromatograph (Clarus 500, Perkin Elmer, USA) equipped with an FID and ECD using an automatic headspace injector (Turbo Matrix 110, Perkin Elmer, USA). In the first year, eight gas flux measurements were performed during the growing season (between 18 May–27 July) timed to occur immediately following the initial fertilization and significant rainfall events. The following year, the measurement scheme was intensified so that ten measurements were performed within the first 2 weeks after sowing, two measurements per week were done during the subsequent 2 weeks, followed by weekly or biweekly measurements during the rest of the growing season. Measurements were timed to occur

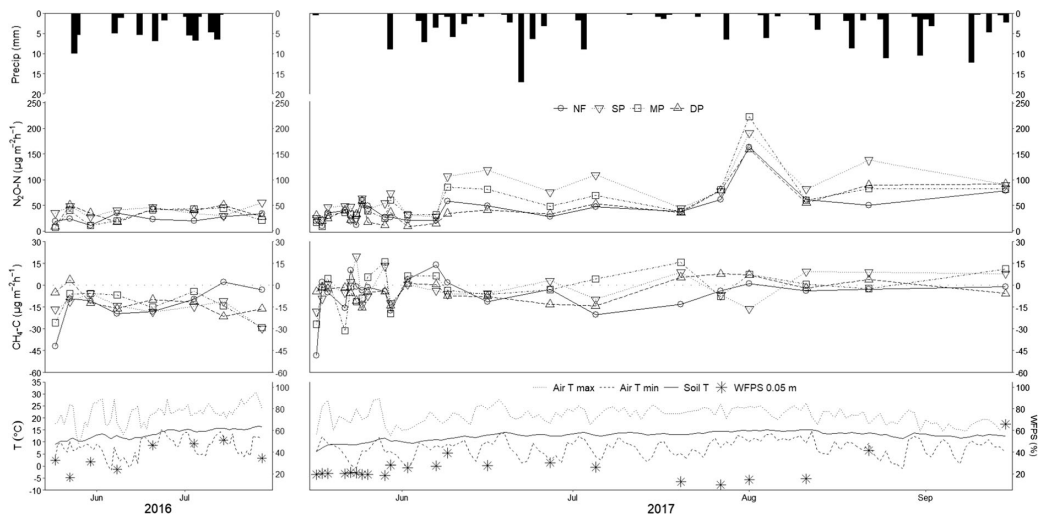


Fig. 1 2016 and 2017 cropping seasons daily precipitation and irrigation (mm), mean treatment nitrous oxide fluxes ($\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$), methane fluxes ($\mu\text{g CH}_4\text{-C m}^{-2} \text{h}^{-1}$), maximum and minimum air temperature*, soil temperature at 0.10 m soil depth* and soil water content (% WFPS) at 0–0.05 m soil depth. For clarity, error bars have been excluded from gas concentration values but can be found in supplementary material

Table S1. NF = no fertilizer, SP = shallow fertilizer placement (0.07 m), MP = mixed placement of fertilizer (half at 0.07 m, half at 0.20 m), and DP = deep fertilizer placement (0.20 m). *Accessed on 13-Feb-2019 from Uppsala Funbo-Lövsta Lantmet climate station (<http://www.ffe.slu.se/lm/LMHome.cfm?LMSUB=1>)

immediately following periods of rainfall or irrigation when possible. In addition, three measurements were done during a two-week period of spring thaw in April 2018, following the second cropping season.

Due to logistical reasons, only the measurement period in 2017 includes the whole cropping season, while measurements are limited to eight occasions in 2016 and three occasions in the spring of 2018. Trends and significant differences between the N placements will be discussed for the cropping periods in 2016 and 2017.

Biomass sampling and analysis

Above-ground biomass was sampled by hand at harvest and twice mid-season, at stem elongation and at heading, approximately Zadok’s growth stage (ZGS) 32 and 52, respectively, in 2016. In 2017 plant biomass was collected at harvest and at booting, approximately ZGS 45. The biomass was collected by removing all above-ground crop biomass within a 0.5×0.5 m metal frame randomly placed at four locations within each plot. Grain biomass was

measured both in the hand-harvested small plots and in a net plot of 34.8 m^2 in the center of each plot that was combine harvested. Collected biomass was dried, threshed at harvest, ground and analysed for N content on an organic elemental combustion instrument (LECO, USA). One to two days prior to each mid-season biomass collection, leaf chlorophyll was measured using a hand-held SPAD-502 m (Minolta Camera Co., Osaka, Japan). Four plants within four randomly chosen areas within each plot were selected, and four measurements were made on the first fully expanded leaf at the top of the selected plant. During SPAD measurements, sixteen plants within each plot were randomly chosen for measuring plant height.

Soil measurements

On gas sampling days, soil moisture was measured with a Theta probe (Delta-T Devices, Cambridge, UK) to a depth of 0.05 m at four locations both inside and outside the frames. Observed soil moisture was converted to water-filled pore space (WFPS). Soil temperature, as depicted in Fig. 1, was accessed from

a nearby climate station (Funbo-Lövsta) and was not measured at the field site. In the second year, plots were sampled to 0.40 m depth and soil cores were subdivided into 0.05 m depth increments to 0.30 m, and one at 0.30–0.40 m for analysis of mineral N content by 2 m potassium chloride (KCl) extraction followed by colorimetric determination on a segmented flow analyzer (SEAL AutoAnalyzer 3, Seal Analytical, UK). Composite soil samples were collected on three occasions from each plot, prior to fertilization, 39 days after fertilization and immediately following harvest.

Calculations and statistical analyses

The R-software R 3.4.4 (RStudio Team 2018) was used for statistical analyses. Differences between treatments, i.e. fertilizer placements, were investigated by repeated measures Anova, i.e. a linear mixed-effects model using the *lme* function (*nlme* package, Pinheiro et al. 2019) with the repetitions as random factor and the log-likelihood maximized method “ML”. Analysis of variance was done with the Anova function (*car* package, Fox and Weisberg 2019). Posthoc analysis was done by Tukey’s all pair comparisons and using the *glht* function (*multcomp* package, Hothorn et al. 2008). Differences were regarded significant for $P < 0.05$. We used a linear regression to check for climate effects (e.g., WFPS) on N_2O and CH_4 . In addition, a linear model consisting of per-plot mean WFPS, mid-season soil mineral N (0–0.20 m), and mean N_2O or CH_4 from 2017 to check for combined soil water and N effects on GHG emissions. Figures were made using *ggplot* from the *ggplot2* package (Wickham 2016) and *plot_grid* from the *cowplot* package (Wilke 2019). Nitrous oxide and CH_4 fluxes were determined from concentration increase or decrease inside the chambers and using the R package *gasfluxes* (Fuss 2019) using the “robust linear” flux calculation method. Cumulative GHG fluxes for the three measurement periods (2016 and 2017 cropping seasons and 2018 spring thawing period) were calculated by linear interpolation between the days when measurements were taken using the *aggfluxes* function from the aforementioned *gasfluxes* R package (Fuss

2019). The fertilizer-induced seasonal emission factor (EF), which evaluates the amount of N_2O emissions that result from anthropogenic N inputs into soils, was calculated over the growing season for all three fertilized treatments according to

$$EF_{N_2O-N}[\%] = \frac{(N_2O_{fert}[\text{kg N ha}^{-1}] - N_2O_{unfert}[\text{kg N ha}^{-1}])}{N_{applied}[\text{kg N ha}^{-1}]} * 100,$$

with N_2O_{fert} = cumulative N_2O fluxes from fertilized treatment, N_2O_{unfert} = cumulative N_2O flux from unfertilized treatment, and $N_{applied}$ = amount of applied fertilizer N. Yield-scaled N_2O emissions were calculated following Venterea et al. (2011):

$$N_2O_{yield} = \frac{N_2O_{fert}[\text{kg N ha}^{-1}]}{\text{grain yield}[\text{kg N ha}^{-1}]}$$

The N surplus (potential N loss to the environment) was calculated for each treatment as the difference between N inputs (N in seed and fertilization) and outputs (N in harvested grains and straw, as well as N losses in the form of N_2O). The nitrogen use efficiency (NUE) gives an indication of resource efficiency (Quemada et al. 2020) and was calculated as

$$NUE[\%] = \left(\frac{\sum(\text{crop N outputs}[\text{kg N ha}^{-1}])}{\sum(\text{N fertilizer inputs}[\text{kg N ha}^{-1}])} \right) * 100$$

However, as this measure only concerns the fertilized treatments, we calculated the agronomic efficiency of N (AE_N), which is the ratio of yield to N supply (Lahda et al. 2005) and the recovery efficiency of N (RE_N), which is the ratio of plant N–N supply (Lahda et al. 2005; Dobermann 2005). Both AE_N and RE_N take the unfertilized control into consideration:

$$AE_N[\text{kg kg}^{-1}] = \frac{(\text{grain yield}_{fert} - \text{grain yield}_{unfert})}{N_{applied}}$$

$$RE_N[\%] = \frac{\text{plant N uptake}_{fert} - \text{plant N uptake}_{unfert}}{N_{applied}} * 100$$

Results

Environmental conditions

The growing seasons in 2016 and 2017 were slightly warmer than normal and precipitation was slightly lower than normal (215 mm), particularly in the earlier part of the season of 2017 (Table 1; Fig. 1).

WFPS measured at 0.05 m ranged from 17 to 51% in 2016 with the lowest value in late May and the highest value in mid July. In 2017, observed WFPS was lower than in the previous year with the lowest value (9.6%) observed in late July and the highest value (65.7%) observed in mid September. We found no correlations between WFPS and soil mineral N content or N_2O emissions in any of the measurement periods.

Greenhouse gas emissions

Nitrous oxide

Fertilizer placement depth affected cumulative N_2O emissions during the two growing seasons in 2016 and 2017 (Fig. 2). Compared with the control NF, N fertilization resulted in an increase in cumulative N_2O emissions, between 32–61% in 2016 and 10–70% in 2017. In 2016, cumulative N_2O emissions were significantly highest in SP, and MP and DP were

intermediates between that and NF and not significantly different from the other treatments.

During the more intense measuring period in 2017, average DP emissions were similar to those in NF, but significantly lower than in SP. Emissions from MP and SP did not differ significantly. Among the fertilized treatments, N_2O emissions were significantly the lowest in DP and MP and highest in SP on 8 out of 22 occasions in 2017, primarily in the first third of the cropping season (mid-May to early July) during a period of the most vigorous crop growth and minimal precipitation (Fig. 1, Table S1). The average value (\pm SD) of measured N_2O fluxes in 2017 was highest in SP and MP, 69.9 ± 49.1 and $56.9 \pm 52.9 \mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$ respectively, and lowest in DP and NF, 44.9 ± 39.2 and $43.8 \pm 37.9 \mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$ respectively. Across all treatments, the lowest measured flux occurred early in the growing season, within either the first two days (SP and MP) or shortly after seed emergence (NF and DP), around 1.5 weeks of measurements, and the highest fluxes measured were on August 1st. Individual NF fluxes ranged from -18.9 to $210.9 \mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$ and were never statistically higher than the fertilized plots. The lowest and highest measured fluxes among all treatments during this period were in MP, -10.6 and $400.1 \mu\text{g } N_2O-N \text{ m}^{-2} \text{ h}^{-1}$ respectively. On 7 occasions, MP and SP were statistically highest but on a further 3 occasions MP was statistically lower than SP and

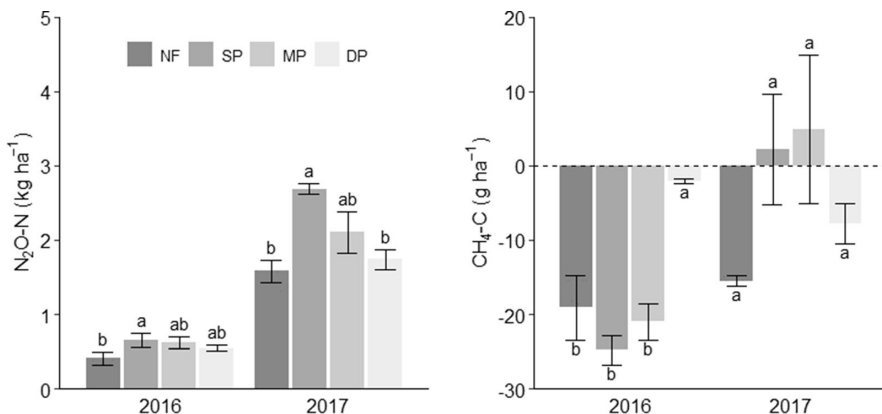


Fig. 2 Cumulative N_2O ($\text{kg } N_2O-N \text{ ha}^{-1}$) and CH_4 ($\text{g } CH_4-C \text{ ha}^{-1}$) fluxes over the cropping seasons in 2016 and 2017. Vertical error bars represent standard error (SE) of the mean. Different letters highlight significant differences between the

treatments in the respective year. NF = no fertilizer, SP = shallow fertilizer placement (0.07 m), MP = mixed placement of fertilizer (half at 0.07 m, half at 0.20 m), and DP = deep fertilizer placement (0.20 m)

either similar to DP or an intermediate between the two treatments (Table S1). Fluxes of N_2O in SP ranged from 3.7 to 291.9 $\mu\text{g } N_2O\text{-N } m^{-2} h^{-1}$. Nitrous oxide fluxes in DP ranged from 0.8 to 174.9 $\mu\text{g } N_2O\text{-N } m^{-2} h^{-1}$ (Table S1).

When crop season cumulative N_2O emissions were yield-scaled (Fig. 3), a consistent trend emerged among treatments. Fertilizer depth significantly affected yield-scaled N_2O emissions in 2017 where the GHG measurement period was longer. Yield-scaled N_2O emissions were lowest in NF both years, 0.10 and 0.35 $\text{g } N_2O\text{-N } \text{kg grain}^{-1}$ in 2016 and 2017, respectively. SP was highest in both 2016 (0.15 $\text{g } N_2O\text{-N } \text{kg grain}^{-1}$) and 2017 (0.70 $\text{g } N_2O\text{-N } \text{kg grain}^{-1}$). Among the fertilized treatments, DP yield-scaled emissions were the lowest, 0.11 and 0.40 $\text{g } N_2O\text{-N } \text{kg grain}^{-1}$ in 2016 and 2017, respectively, a reduction of 26 and 43% compared to SP. MP reduced yield-scaled emissions by 9% (0.14 $\text{g } N_2O\text{-N } \text{kg grain}^{-1}$) and 25% (0.52 $\text{g } N_2O\text{-N } \text{kg grain}^{-1}$) in 2016 and 2017 compared to SP. Fertilizer-induced emission factors (EF) calculated for the 2017 cropping season also decreased with depth of fertilizer placement. The percentage of applied N that was directly emitted as N_2O for SP, MP, and DP was 0.77 ± 0.07 , 0.58 ± 0.03 , and 0.10 ± 0.02 , respectively.

Cumulative N_2O (Figure S1) and daily emissions (Figure S2) from the two-week spring thaw

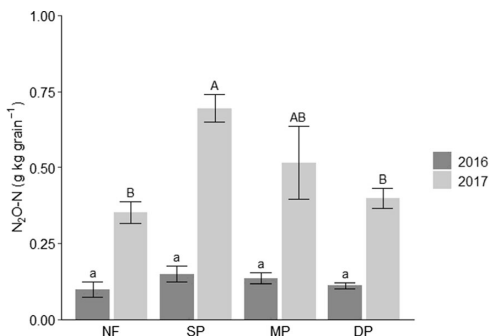


Fig. 3 Yield-scaled N_2O emissions (cumulative $N_2O\text{-N } \text{g kg grain yield}^{-1} \pm \text{SE}$) per cropping year. Different small letters are significant treatment differences in 2016 and large letters represent significant treatment differences in 2017 ($\alpha = 0.05$, Tukey's HSD). Vertical error bars represent standard error (SE) of the mean. NF = no fertilizer, SP = shallow fertilizer placement (0.07 m), MP = mixed placement of fertilizer (half at 0.07 m, half at 0.20 m), and DP = deep fertilizer placement (0.20 m)

measurement period in spring 2018 were no longer affected by fertilizer placement, but comprised between 40 and 70% of the cumulative emissions from the 2017 cropping season (see supplementary material). However, given the low number of observations, those results are less reliable. More frequent measurements over a longer period have to be made in order to make a concise statement about the impact of thaw conditions on N_2O fluxes.

Methane

Methane fluxes were generally negative or very low in all treatments (Fig. 1). There was no statistical treatment differences in cumulative emissions in 2017 (Fig. 2) and a treatment effect was detected on only four different measurement occasions (Table S1), excluding the initial disturbance effect from planting and fertilization. The non-fertilized control had the highest uptake, with fluxes averaging ($\pm \text{SD}$) $-5.5 \pm 19.1 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$ and individual fluxes ranged from -76 to $33 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$. The average CH_4 flux in DP was $-3.9 \pm 13.1 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$ with highest and lowest measured fluxes -41.0 and $32.4 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$, respectively. NF and DP were generally lower than both SP and MP on dates with significant treatment differences (Table S1). In 2017 SP and MP had the highest average CH_4 emissions ($\pm \text{SD}$), -1.7 ± 16.3 and $-2.0 \pm 20.0 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$, respectively. MP minimum and maximum values were -92 and $57 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$. Minimum and maximum fluxes in SP were -38 and $43 \mu\text{g } CH_4\text{-C } m^{-2} h^{-1}$. Methane fluxes were significantly different among treatments in the latter part of the season, up to the final measurement in mid-September at the time of harvest (Table S1).

In the 2-week spring 2018 measurement period (Figure S2), no treatment differences were detected either cumulatively or on individual measurement dates (see supplementary material). However, there are too few observations from which to draw conclusions.

Biomass and yield

Fertilization increased N concentrations mid-season in the plant biomass and in harvested straw and grain for both growing seasons, observable during mid-season

SPAD readings, and after N analysis of collected biomass and harvested grain (Table 3). In 2016, there was no detectable treatment difference in SPAD values among fertilized plots, but mid-season biomass weight increased with fertilizer placement depth in the latter part of the growing season. Early plant height, an indication of accelerated maturation, when measured around the same time, was highest in NF (0.69 m), followed by SP and DP (0.67 and 0.66 m, respectively), and was significantly lowest in MP (0.64). N content in the first mid-season biomass during elongation was highest in both MP and DP (2.42 and 2.48%, respectively), but later biomass N fertilizer placement differences during heading were not observed. Additionally, in 2016, grain yield was increased by approximately 11% in DP compared to SP, and grain N content also increased in both MP and DP. In 2017, despite higher mid-season SPAD

readings in DP and MP treatments compared to SP, no significant differences in grain yield or grain N content were observed among fertilized treatments in the second growing season.

Following the insignificant differences in 2017 grain yields and the higher yields in NF compared to the fertilized treatments, the agronomic efficiency AE_N was negative for all fertilized treatments. However, due to the higher N contents in the grains and the straw in the fertilized treatments, the N recovery efficiency RE_N was still low but above zero. They ranged from 2.3 in SP to 18.1% in DP (Table 4).

Table 3 Treatment effects on N concentration in crop biomass (dry matter) and harvested straw and grain, leaf relative chlorophyll content (SPAD-index), and harvest grain yield

(15% water content) at respective Zadok’s growth stages in the 2016 and 2017 growing seasons

	Zadok stage	NF	SP	MP	DP
2016					
Biomass N (%)	Elongation	1.74 ± 0.05 ^c	2.22 ± 0.01 ^b	2.42 ± 0.05 ^a	2.48 ± 0.07 ^a
Biomass N (%)	Heading	1.25 ± 0.05 ^b	1.63 ± 0.03 ^a	1.66 ± 0.04 ^a	1.52 ± 0.05 ^a
Plant biomass (t ha ⁻¹)	Elongation	4.06 ± 0.14 ^b	4.81 ± 0.20 ^a	4.64 ± 0.10 ^a	4.88 ± 0.13 ^a
Plant biomass (t ha ⁻¹)	Heading	7.65 ± 0.25 ^b	8.20 ± 0.33 ^{ab}	8.44 ± 0.22 ^{ab}	8.78 ± 0.26 ^a
Plant height (m)	Elongation	0.68 ± 0.005 ^a	0.66 ± 0.004 ^b	0.64 ± 0.005 ^c	0.66 ± 0.005 ^b
Plant height (m)	Heading	0.70 ± 0.005	0.71 ± 0.005	0.70 ± 0.005	0.71 ± 0.006
SPAD-index	Elongation	49.4 ± 0.4 ^b	54.2 ± 0.3 ^a	54.0 ± 0.3 ^a	53.1 ± 0.3 ^a
SPAD-index	Heading	47.9 ± 0.5 ^b	53.6 ± 0.4 ^a	53.7 ± 0.3 ^a	54.0 ± 0.3 ^a
Straw N (%)	Harvest	0.23 ± 0.02 ^b	0.31 ± 0.01 ^a	0.33 ± 0.02 ^a	0.33 ± 0.02 ^a
Grain N (%)	Harvest	2.45 ± 0.02 ^c	2.76 ± 0.02 ^b	2.87 ± 0.02 ^a	2.83 ± 0.02 ^a
Grain yield (kg ha ⁻¹)	Harvest	4.18 ± 0.06 ^c	4.40 ± 0.12 ^{bc}	4.62 ± 0.09 ^{ab}	4.88 ± 0.1 ^a
2017					
Biomass N (%)	Booting	1.49 ± 0.04	1.87 ± 0.06	1.87 ± 0.05	1.87 ± 0.03
Plant biomass (t ha ⁻¹)	Booting	5.05 ± 0.32	5.21 ± 0.31	5.74 ± 0.38	5.66 ± 0.25
Plant height (m)	Booting	0.65 ± 0.008 ^b	0.63 ± 0.007 ^b	0.66 ± 0.008 ^{ab}	0.69 ± 0.008 ^a
SPAD-index	Booting	57.6 ± 0.6 ^b	59.1 ± 0.5 ^b	60.1 ± 0.6 ^a	59.7 ± 0.5 ^a
Straw N (%)	Harvest	0.52 ± 0.01 ^b	0.73 ± 0.03 ^a	0.72 ± 0.03 ^a	0.68 ± 0.04 ^a
Grain N (%)	Harvest	2.11 ± 0.02 ^b	2.41 ± 0.03 ^a	2.43 ± 0.02 ^a	2.40 ± 0.02 ^a
Grain yield (kg ha ⁻¹)	Harvest	4.49 ± 0.12 ^a	3.87 ± 0.16 ^a	4.08 ± 0.28 ^a	4.36 ± 0.12 ^a

Values are reported as means ± standard errors. Different letters indicate statistical difference ($\alpha = 0.05$, Tukey’s HSD)

NF = no fertilizer, SP = shallow fertilizer placement (0.07 m), MP = mixed placement of fertilizer (half at 0.07 m, half at 0.20 m), and DP = deep fertilizer placement (0.20 m)

Table 4 Nitrogen balance components in the four experimental treatments for the 2017 cropping season

	NF	SP	MP	DP
<i>N inputs and outputs (kg N ha⁻¹)</i>				
Seeds	5.3	5.3	5.3	5.3
Fertilization	0	105	105	105
Harvested grains	- 101.1 ± 9.2	- 97.0 ± 8.1	- 104.4 ± 14.9	- 111.1 ± 4.8
Harvested straw	- 21.1 ± 3.3	- 27.5 ± 3.3	- 30.5 ± 2.8	- 30 ± 2.9
N ₂ O loss	- 1.6 ± 0.1	- 2.7 ± 0.1	- 2.1 ± 0.3	- 1.7 ± 0.1
<i>N surplus</i>	- 118.5 ± 5.4	- 16.9 ± 4.5	- 26.7 ± 6.8	- 32.5 ± 3.2
NUE (%)	n.a.	119	128	134
AE _N (kg ha ⁻¹)	n.a.	- 5.9	- 3.9	- 1.2
RE _N (%)	n.a.	2.3	12.2	18.1
<i>Soil mineral N content and changes (kg N ha⁻¹)</i>				
Soil mineral N at sowing (0–0.25 m)	37.4 ± 2.3	42.6 ± 0.9	42.4 ± 2.7	36.8 ± 3.9
Soil mineral N at sowing (0.25–0.40 m)	22.1 ± 1.6	30.5 ± 1.8	37.4 ± 6.5	34.0 ± 4.2
Soil mineral N after harvest (0–0.25 m)	25.0 ± 3.0	82.7 ± 17.1	54.0 ± 7.8	42.2 ± 1.6
Soil mineral N after harvest (0.25–0.40 m)	16.6 ± 2.6	31.6 ± 0.7	47.6 ± 20.5	37.3 ± 2.5
Δ Soil mineral N (0–0.25 m)	- 15.7 ± 6.2	40.4 ± 16.5	6.9 ± 10.0	- 1.9 ± 2.3
Δ Soil mineral N (0.25–0.40 m)	- 8.6 ± 3.4	- 1.7 ± 2.7	- 0.7 ± 26.0	- 4.8 ± 2.2
Total increase in mineral N (0–0.40 m)	- 24.3 ± 9.6	38.7 ± 13.9	6.2 ± 36.0	- 6.6 ± 4.7

The change in soil mineral N content was calculated from subtracting mineral N content measured one week before fertilization (at sowing) from soil mineral N content measured one week after harvest. Values are presented as mean ± standard error

NF = no fertilizer, SP = shallow fertilizer placement (0.07 m), MP = mixed placement of fertilizer (half at 0.07 m, half at 0.20 m), and DP = deep fertilizer placement (0.20 m). NUE = N use efficiency, AE_N = agronomic efficiency of N, RE_N = recovery efficiency of N

Soil mineral N profiles and N balance

Data from the 2017 soil mineral N profile measurements (Fig. 4) and resulting N balance (Table 4) indicate that mineral N content had decreased in the 0.25–0.40 m layer in all treatments (Table 4). For NF and DP, mineral N had even disappeared from the 0–25 cm layer during the growing season. SP resulted in a higher surplus of mineral N remaining in the system after harvest than in the other treatments (38.7 ± 13.9 kg N ha⁻¹). The mineral N content in the mid-crop season soil profile pinpoint more or less where the fertilizer grains had been placed; SP, which was placed at 0.07 m depth, was primarily found between 0.05 and 0.10 m. After harvest, it appears that the bulk of SP soil mineral N essentially remained in the soil, but had leached further down in the profile to 0.25 m. However, in the 0.25–0.40 m layer, mineral N content had decreased by 1.7 ± 2.7 kg N ha⁻¹. The MP treatment had its largest mid-season N content

peak at 0.10 m, with a gradual decline in soil N content from 0.10 to 0.25 m. After crop harvest, the 0.10 m N content peak of MP had nearly halved, and another distinct N content peak was observed deeper in the soil profile at 0.40 m. Only a small fraction of the negative N surplus of 118.5 kg in the unfertilized control was explained by the decrease in soil mineral N (6.2 ± 36.0 kg N ha⁻¹) during the cropping season. Thus net N mineralization during the growing season would have been at least 100 kg N explaining the weak fertilizer response of crop yield in the fertilized treatments (Table 4). Less soil mineral N was detected in DP after harvest than in the other fertilized treatments and more N was removed from the system through harvested straw and grains resulting in a higher nutrient use efficiency of the applied fertilizer N and a higher uptake of mineral N (Table 4). Similar to NF, mineral N in the 0–0.40 m layer decreased over the growing season.

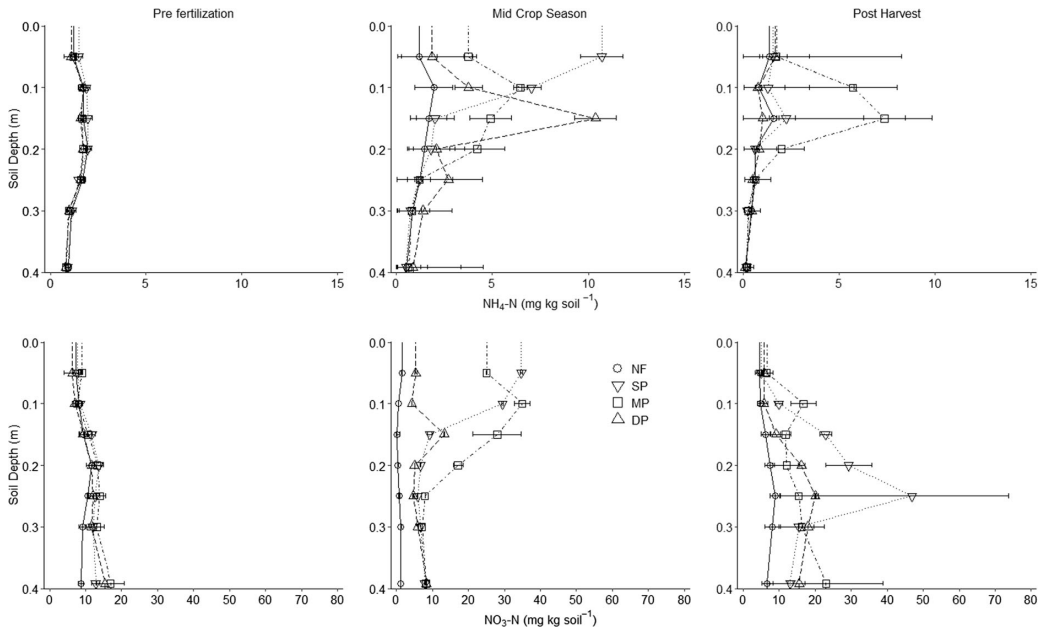


Fig. 4 Soil mineral N content (mg $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ kg soil⁻¹) sampled in 2017 in 0.05 m increments to a 0.30 m depth and at 0.30–0.40 m depth. Treatment depth averages at five days prior to fertilization and sowing, two months after fertilization and sowing, and one week after harvest. Horizontal bars

represent \pm standard error (SE) of the mean. NF = no fertilizer, SP = shallow fertilizer placement (0.07 m), MP = mixed placement of fertilizer (half at 0.07 m, half at 0.20 m), and DP = deep fertilizer placement (0.20 m)

Discussion

Effect of N placement on N_2O emissions

Different depth placement of N fertilizer had a marked effect on N_2O emissions. The reduction in N_2O emissions from the DP treatment compared to both MP and SP was consistent with previous studies pointing out the connection between residence time of N_2O in soil and uptake or reduction in the emission of N_2O (Clough et al. 1998; Harter et al. 2016). Nitrous oxide emissions from DP were generally as low as those from the unfertilized plots, a trend consistent during both the abbreviated cumulative measurement period of 2016, and for both cumulative and individual measurements in 2017. We had expected that MP, which received half the amount of fertilizer at the same depth as SP to be an intermediate between the highest and lowest emitters, but that was not always the case. During both cropping seasons, the N_2O emissions from MP plots were generally as high as

those from SP, consistent with findings of Chapuis-Lardy et al. (2007). The higher concentration of mineral N in the upper topsoil of MP (Fig. 4) could explain why no significant reduction was achieved. Compared with SP, DP reduced cumulative N_2O emissions by 18% and 35% during the GHG measurement periods during the first and second growing seasons, respectively. The fertilizer-induced N_2O emissions decreased with placement depth and the calculated emission factors for SP, MP, and DP were 0.77 ± 0.07 , 0.58 ± 0.03 , and 0.10 ± 0.02 , respectively. Similar to our findings, van Kessel et al. (2013) found in a meta-analysis that N_2O emissions were reduced when N fertilizers were placed at a depth ≥ 0.05 m. Moreover, they reported that deep fertilizer placement significantly reduced yield-scaled emissions in no tillage and reduced tillage systems in humid climates. This is similar to our findings, where yield-scaled emissions were lower from deep (DP) than from shallow (SP) placement. Gaihre et al. (2015) found that urea deep placement (0.07–0.10 m depth

placement) reduced N_2O emissions by up to 84% compared to surface broadcast application during the dry season and also increased rice grain yields by 13% in one season and gave similar yields in another season, despite a lower N application (Gaihre et al. 2018).

Generally, treatment differences were first detectable several weeks after fertilization. The strongest significant treatment effects on N_2O formation and emissions were recorded during the 3rd–5th week after sowing and fertilization, i.e., in the first third of the 2017 growing season. It can be assumed that vigorous plant growth and N uptake from soil influenced the decreased N_2O emissions in DP, but not MP, which still had high N_2O emissions.

The treatment effect on N_2O emissions largely disappeared during the latter two-thirds of the 2017 growing season (Fig. 1). This was during a time when chamber measurements were less frequent, so it is possible that some emission peaks and thus treatment effects were missed. On the other hand, in the final weeks of the 2017 growing season, N_2O fluxes from fertilized and non-fertilized plots were similar, showing that neither fertilizer depth placement nor crop utilization were important drivers for N_2O emissions at this stage when mineral N was largely utilized or had been translocated to a lower soil depth (Fig. 4). This assumption is somewhat supported by the higher WFPS observed towards the end of the experiment (41% in late August and 66% in mid-September). Apart from that, WFPS was rather low (25% on average) throughout the whole growing season in 2017. This leads to the assumption that nitrification rather than denitrification has been the major process of N_2O production. However, based on the data observed in this study, we did not find a correlation between N_2O emissions and WFPS in either of the years.

Effect of N placement on CH_4 emissions

Methane fluxes in 2017 were generally low and negative in all treatments with little differences between DP and NF. Moreover, there was lower CH_4 oxidation and consequently higher positive fluxes in the MP and SP treatment (Fig. 2). This is consistent with previous findings that have linked surface and shallow fertilizer N application to higher CH_4 fluxes (Bodelier 2011) as most CH_4 oxidation occurs in the

upper (0–0.05 m) soil layer (Crill et al. 1994; Kruger et al. 2001). For rice fields, Linquist et al. (2012) reported reduced CH_4 emissions from urea deep placement as compared to broadcast application. The studies included in their meta-analysis mostly reported lower CH_4 emissions when N fertilizer was placed below the soil surface in continuous (Schutz et al. 1989), rainfed (Rath et al. 1999) and irrigated (Setyanto et al. 2000) water management. However, when comparing irrigated and rainfed rice systems, Setyanto et al. (2000) reported higher CH_4 emissions from the deep N placement under rainfed conditions. In general, a decreasing effect of deep N placement has been related to concentrated NH_4^+ into localized areas, as well as increased O_2 availability in the rhizosphere, thus stimulating CH_4 oxidation and reducing overall emissions (Bodelier et al. 2000a, b; Gilbert and Frenzel 1998). By contrast, results from studies focussing on fertilizer placement revealed that N placement has no effect on CH_4 emissions in irrigated rice systems (Adviento-Borbe and Linquist 2016; Yao et al. 2017), upland soil under corn (Liu et al. 2006), or winter barley (Chu et al. 2007). In the study presented here, observed WFPS was comparatively low throughout the growing seasons, indicating that the soil water regime was the major driver of the low CH_4 emissions observed. Aside from the differences in the water regimes between the above-mentioned studies on rice cultivation and the results presented here, the definition of what is considered a deep placement is quite relative and varies between studies. For example, Schutz et al. (1989) and Yao et al. (2017) studied a placement depth of 0.20 and 0.10–0.15 m, respectively, which is comparable to the DP treatment presented in this study. By contrast, Rath et al. (1999) considered 0.05 m to be a deep placement, which is analogous to our SP treatment.

Biomass, N balance, and soil mineral N

Both cropping seasons had less than normal rainfall during the former part of the growing season (Table 1), which was a possible culprit for generally lower than normal yields. Spring wheat grain yield was 4.18–4.88 t ha^{-1} in 2016 and spring barley grain yield was 3.87–4.49 t ha^{-1} in 2017 (Table 3). In comparison, the average yield in Uppsala county for spring wheat in 2016 was 4.49 tons ha^{-1} , and barley in 2017 was 5.07 t ha^{-1} (Jordbruksverket 2017, 2018).

However, in 2016, nearly half of the field had been overtaken by weeds halfway through the growing season. In 2017, the uneven and delayed seed emergence from shallow seed placement resulted in differing rates of plant maturation that ultimately led to high variation in both yields and average nutrient uptake in all treatments. Despite poor growth and high variation across all plots in 2017, an increase in NUE with deeper fertilizer placement was clear (Table 4) and DP fertilization had a positive effect on yield (Table 3) compared to the other fertilized treatments, and overall improved the grain N content. In general, the high values for NUE suggest a high susceptibility to mining of N, i.e. N depletion and soil C degradation (Quemada et al. 2020). According to the EU Nitrogen Expert Panel (EUNEP 2015), the desirable NUE range is 50–90%. In our study, calculated NUEs in 2017 were 119 (SP), 128 (MP) and 134% (DP), which is above the range presented by Quemada et al. (2020) for arable farms in Denmark, Germany, and Spain. 50% of the farms included in their analysis had NUE values between 45 and 75%. For rice cultivation, deep placement of fertilizer has been found to significantly increase both NUE and grain yield, as well as agronomical N efficiency and N recovery efficiency (Das and Singh 1994; Xiang et al. 2013; Bandaogo et al. 2014; Huda et al. 2016). For flooded rice, Huda et al. (2016) reported similar floodwater $\text{NH}_4^+\text{-N}$ and ammonia (NH_3) volatilization in deep-placed treatments and unfertilized control. Using controlled-release N fertilizers in two consecutive rice growing seasons, Ke et al. (2018) found that fertilizer deep placement increased N leaching and the mineral N in the 0.40–0.60 m soil layer. However, in their study, the fertilizer was placed at a depth of 0.05 m in the deep placement, which is even more shallow than the shallow placement (SP, 0.07 m) applied in our experiment. In the study presented here, the soil-crop N balance (Table 4) was greatly influenced by remaining soil mineral N (Fig. 4), primarily in the form of NO_3^- , at the time of harvest. Interestingly, amounts of mineral N at harvest were 45 and 12 kg ha^{-1} lower in DP than in SP and MP, respectively. These differences, 6 and 17 kg ha^{-1} respectively, are partially explained by higher crop N uptake in DP, but the fate of the remaining N was unresolved (Table 4). Among the fertilized treatments in our study, SP showed the highest increase in mineral N in the whole 0–0.40 m depth ($38.7 \pm 13.9 \text{ kg N ha}^{-1}$),

which encompass an increase in 0–0.25 m depth ($40.4 \pm 16.5 \text{ kg N ha}^{-1}$), where it is susceptible to gaseous N losses, and a slight decrease in 0.25–0.40 m depth ($-1.7 \pm 2.7 \text{ kg N ha}^{-1}$). Similarly, the mineral N increased in the MP treatment, in which the fertilizer was placed at 0.07 and 0.20 m, at 0–0.25 m ($6.9 \pm 10.0 \text{ kg ha}^{-1}$) and decreased in 0.25–0.40 m depth ($-0.7 \pm 26.0 \text{ kg ha}^{-1}$), highlighting that N probably has leached further down the soil profile. Similar to NF, mineral N decreased in DP ($-6.6 \pm 4.7 \text{ kg ha}^{-1}$), which may be explained by the higher N uptake and, consequently, yield (Table 4).

Ke et al. (2018) moreover reported an increase in RE_N under the deep placement treatment compared to the broadcast application. Considering the grain yield, the positive impact of fertilizer deep placement depended on the fertilizer type and significantly higher grain yields were found for sulphur-coated urea, but not when polymer-coated urea was used. Similarly, Guo et al. (2016) found that deep placement of controlled-release fertilizer has the potential to increase N uptake and NUE in maize cultivation.

In the study presented here, the N fertilizer rate was designed for higher yields than those obtained in 2017. The fact that harvested grain yield was highest under the control treatment suggested that N fertilization was not needed in 2017 or even counterproductive as shown by the high values for NUE and the negative values for AE_N , which indicate that application of exogenous N did not lead to an increase in yield (Table 4). In contrast to 2017, values for AE_N were positive in 2016. However, they were still rather low and between 1.8 for SP and 5.8 kg grain kg^{-1} applied N for DP. According to Dobermann (2005), common values for AE_N are 10–30 kg grain kg^{-1} applied N, with higher values in well-managed systems or at low N levels. For Europe, Lahda et al. (2005) reported an average AE_N of 21.3 $\text{kg grain increase per kg N}$ applied, given a similar average fertilization rate as used in this study in 2017 ($100 \pm 13.9 \text{ kg ha}^{-1}$).

In contrast to AE_N , the positive 2017 values for RE_N (2.3, 12.2 and 18.1% for SP, MP and DP, respectively) indicate that, despite the very low yield, the plants were capable of acquiring the additional N in the grains and the straw. However, the obtained values for RE_N are much lower than common values summarized by Dobermann (2005), which range between 30 and 50%, with up to 80% achieved in

well-managed systems. Compared to RE_N values for cereals, as summarized by Lahda et al. (2005), i.e. 10 and 70%, the efficiencies of the MP and DP treatment were at the lower range of this interval.

The positive impact of deep-placed fertilizer on N uptake and N efficiencies is strongly related to the higher soil moisture in deeper layers. The occurrence of favorable nutrient and soil moisture conditions, which are expected to stimulate root proliferation, is more probable in deeper layers. Therefore, deep placement has been shown to be a successful management strategy to reach this aim (Li et al. 2009). However, the adoption of this practice might involve additional labor and costs in terms of purchasing suitable equipment for placing the fertilizer at the correct depth, as well as increased fuel consumption as compared to broadcast application.

Conclusions

Increasing the fertilizer N placement depth has the potential to both improve crop N content and yield, but also mitigate fertilizer-induced N_2O emissions, and to a smaller extent, increase methane oxidation. The GHG mitigation effect of deeper fertilizer placement was first detectable several weeks after fertilization. Deep-placed fertilizer N did not appear to have been exposed to a greater downward mobility likely because of smaller changes in soil moisture following precipitation at this depth. The benefits of increased depth placement of N are likely dependent on climate and soil type but could be a further step in precision farming and environmentally sustainable agriculture. However, further investigations are needed before deeper placement of fertilizer can be recommended as a sustainable farming practice as indicated by our study.

Acknowledgements This work was funded by FORMAS, the Swedish government research council for sustainable development, Grant # 229-2013-82. The authors would like to thank Elisabet Börjesson, Caroline Wright, Tsion Gizachew Tarekegn and Meseret Menil Abitew, Lisa Embring, Hannes Keck, Hannes Kätterer, Martin Hallinger, and Dan Johansson for their help in the field, as well as to Jan Fiedler for assistance in both the field and the GC lab, and Sabine Jordan and Abraham Joel for lending equipment. We also thank the anonymous reviewers for valuable comments to previous versions of this article.

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Funding Open access funding provided by Swedish University of Agricultural Sciences.

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Lysimeter deep N fertilizer placement reduced leaching and improved N use efficiency

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Received: 9 December 2022 / Accepted: 8 May 2023 / Published online: 14 May 2023
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Abstract Deep fertilization has been tested widely for nitrogen (N) use efficiency but there is little evidence of its impact on N leaching and the interplay between climate factors and crop N use. In this study, we tested the effect of three fertilizer N placements on leaching, crop growth, and greenhouse gas (GHG) emissions in a lysimeter experiment over three consecutive years with spring-sown cereals (S1, S2, and S3). Leaching was additionally monitored in an 11-month fallow period (F1) preceding S1 and a 15-month fallow period (F2) following S3. In addition to a control with no N fertilizer (Control), 100 kg N ha⁻¹ year⁻¹ of ammonium nitrate was placed at 0.2 m (Deep), 0.07 m (Shallow), or halved between 0.07 m and 0.2 m (Mixed). Deep reduced leachate amount in each cropping period, with significant reductions ($p < 0.05$) in the drought year (S2) and

cumulatively for S1-S3. Overall, Deep reduced leaching by 22, 25 and 34% compared to Shallow, Mixed and Control, respectively. Deep and Mixed reduced N leaching across S1-S3 compared with Shallow, but Deep further reduced N loads by 15% compared to Mixed and was significantly lowest ($p < 0.05$) among the fertilized treatments in S1 and S2. In S3, Deep increased grain yields by 28 and 22% compared to Shallow and Mixed, respectively, while nearly doubling the agronomic efficiency of N (AE_N) and the recovery efficiency of N (RE_N). Deep N placement is a promising mitigation practice that should be further investigated.

Keywords Deep N fertilization · Drought · Fertilizer placement · Lysimeter · Nitrate leaching

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10705-023-10286-w>.

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Introduction

As essential nitrogen (N) is to crop production, its use in agriculture is difficult to manage due to its mobility and rapid transformation in the soil leading to air and water pollution, partially derailing its intended path to the plant. In humid climatic conditions, dissolved nitrate (NO_3^-) and nitrite (NO_2^-) are transported through the soil into ground- and surface water and further into streams, lakes and coastal areas, contributing to eutrophication. Nitrous oxide (N_2O) emissions from microbial nitrification and denitrification of fertilizer N are major sources of greenhouse gases

(GHG) emitted within the agricultural sector (Naburs et al. 2022). Population growth, which necessitates greater crop production, and thus fertilizer, is on an upwards trajectory (FAO 2019; UN 2022), presenting a particular challenge at a time when we are seeking to reduce GHG emissions to keep the global temperature increase below the 1.5 °C target (IPCC 2018). In order to meet both United Nations sustainable development goals for increasing the food supply (goal 2), while reducing the negative environmental impacts of fertilization (e.g., goals 13 and 15) (UN 2015), we need to rapidly test and employ new methods to increase fertilizer N use efficiency (NUE).

One such method is deep fertilizer placement, which multiple studies have shown can positively influence crop production and minimize fertilizer-induced environmental damage. In the literature, what qualifies as a deep placement depth can differ widely, depending on the existing local practice for fertilization for the particular cropping system. Although placement depth, as well as climate and growing systems vary, deep N placement relative to surface fertilization has been reported to increase yields and NUE, decrease ammonification, and in some cases decrease N₂O emissions (Chen et al. 2021; Pandit et al. 2022; Rychel et al. 2020; Sosulski et al. 2020; Wu et al. 2021; Zhang et al. 2022;). However, NO₃⁻ remaining in the soil after crop uptake is susceptible to leaching and literature on the fate of NO₃⁻ following deep fertilization is scant. Ke et al. (2018) reported high NO₃⁻ losses in a flooded rice system, whereas Wu et al. (2022) found that deep placement at 0.25 or 0.15 m decreased NO₃⁻ content in the 0–1 m depth compared to a shallow (0.05 m) placement in a field experiment with maize. Wang et al. (2022) reported that deep urea placement promoted the proliferation of deep roots in winter wheat, which increased crop N uptake and water utilization, but NO₃⁻ leaching varied depending on seasonal rainfall amount. There is little to no information, however, regarding the longer-term effects of N fertilizer placement on NO₃⁻ leaching.

In a Swedish field experiment, deep N fertilization was shown to increase yield and N uptake while simultaneously decreasing N₂O emissions (Rychel et al. 2020). Although soil mineral N levels were measured multiple times during the growing season in that field experiment, the fate of the remaining N in the soil not removed by crops after the growing

season (or below our sampling depth) was unknown. Therefore, we performed an additional experiment using undisturbed soil monoliths (lysimeters) taken from the same field as the previous experiment, in which we could quantify the nutrient load in the leachate following treatments with several N fertilization strategies.

In the eastern region of central Sweden, where cereals are the dominant crops, the local agronomic practice is to place fertilizer at 0.07 m and seeds at 0.05 m simultaneously using, for example, a Combi drill. Thus, we used a baseline 0.07 m depth for a shallow fertilizer N placement and 0.2 m for deep placement, with the motivation that at 0.2 m, soil moisture and temperature are relatively more constant compared to the shallow placement depth. Thus, fertilizer N placed at 0.2 m would be less susceptible to mobilization following rainfall events and pulses in nitrification and denitrification with temperature and moisture fluctuations. Moreover, the common depth for harrowing (performed in spring) and tilling (performed in the autumn) in the region is around 0.05–0.07 m and 0.2–0.25 m, respectively, and thus we opted for N fertilizer placement on the border of these two zones in the soil profile.

In this experiment, we sought to test the effect of N fertilizer depths and depth combinations on (i) mineral N leaching and (ii) crop N uptake and yield, as well as (iii) soil emissions of carbon dioxide (CO₂), methane (CH₄) and N₂O. The latter two objectives could both corroborate field observations and provide further insight into the interplay between climate conditions, crop growth, and N losses. We hypothesized that deep-placed N fertilizer would have a beneficial affect for crop growth, and thus N uptake, resulting in less leaching of mineral N compared with a shallow N placement.

Materials and methods

Lysimeter collection and installation

Sixteen undisturbed soil columns with a diameter of 0.295 m were excavated to a depth of 1.18 m from an agricultural field in Säby (59°83'N, 17°71'E) in SE Uppsala, Sweden in May 2016 according to the method described by Persson and Bergström (1991). Briefly, the columns were extracted with a

tractor-mounted hydraulic soil auger, capped at both ends, and transported 8 km on a flatbed truck to the lysimeter station at the Uppsala campus of the Swedish University of Agricultural Sciences—SLU.

The field site where the lysimeters were excavated has been cultivated with agricultural crops, mainly cereals, for over a century. The mean annual air temperature is 5.5 °C and average precipitation is around 528 mm per year (Fig S1). In this area, as of much of Sweden, crops are primarily rain fed. The soil is characterized as a Eutric Cambisol, with a silt loam texture in the topsoil and 6.1 pH_{H2O} (Table 1). The subsoil (around 0.8 m and below) is influenced by the presence of gytja, a gel-like material originating from partially decomposed organic matter accumulated under waterbody sediment. It has an elevated organic N and carbon (C) content, high porosity, and acidic pH.

Prior to installing the lysimeters, we removed approximately 0.08 m of soil from the bottom and filled in 0.05 m of each soil column with washed pea gravel (2–5 mm diameter). Stainless steel mesh was placed between the gravel layer and the 0.3 m-thick perforated PVC lid that capped the bottom of the columns. To simulate tillage, the topsoil (0–0.25 m) was removed from all lysimeters, pooled and manually homogenized, then replaced. There was a gap of approximately 0.05 m between the top of the PVC pipe and the upper soil surface, and 0.08 m from the underside was taken up by the gravel and the cap, so

that the effective soil volume was 0.718 m³ (1.05 m length and 0.068 m² surface area). We attached a broad, nearly-flat funnel and a steel-framed supportive seat to the underside of the columns before lowering them into the concrete-walled ports. The outlet of the attached funnel fit snugly into piping that connected to outlets in the lysimeter basement, where individual lysimeter leachates could be continuously collected in 5 L glass Erlenmeyer flasks. The lysimeters were installed in June 2016 and from that point on exposed to weather and allowed to drain freely by gravity. The experiment was initiated the following year (June 2017) to provide time for the lysimeters to both settle and equilibrate as well as to collect sufficient information on individual lysimeter draining behavior and background leachate N loads. During this period, the lysimeters were not planted and were periodically weeded. Thus in the remainder of this paper we refer to this time as a fallow period.

Experimental setup

Sixteen individual lysimeters were randomly assigned to three N fertilizer depth treatments and the control, consisting of four lysimeters each. In addition to a control treatment without N fertilization (Control), was a shallow N placement (Shallow) at 0.07 m, a mixed N placement (Mixed) where half the amount of N fertilizer was placed at 0.07 m and the other half at 0.2 m, and a deep N placement (Deep) at 0.2 m.

Table 1 Soil physical properties along the soil profile sampled at lysimeter extraction from the field. Soil bulk density (BD) (kg dm⁻³), porosity (%), organic carbon (SOC) (g kg⁻¹), total nitrogen (g kg⁻¹), carbon to nitrogen ratio, calcium carbonate

(CaCO₃) (g kg⁻¹), pH (H₂O), and texture represented by percentage clay, silt, and sand. Bulk density and porosity were not collected at 1.0–1.1 m depth due to groundwater infiltration. Adapted from Getahun et al. (2021)

Depth (m)	BD (kg dm ⁻³)	Porosity (%)	SOC (g kg ⁻¹)	Total N (g kg ⁻¹)	C:N	CaCO ₃ (g kg ⁻¹)	pH (H ₂ O)	Clay (%)	Silt (%)	Sand (%)
0–0.1	1.3	50.9	28.2	2.4	11.9	0.10	6.1	21.9	54.5	23.6
0.1–0.2	1.4	48.3	26.4	2.2	11.8	0.20	6.1	20.5	56.9	22.6
0.2–0.3	1.4	46.8	14.2	1.2	11.6	0.09	6.3	21.3	56.2	22.5
0.3–0.4	1.6	41.5	7.9	0.7	11.6	0.07	6.5	18.9	54.1	27.0
0.4–0.5	1.5	43.0	3.8	0.4	9.6	0.07	6.7	23.5	59.7	16.8
0.5–0.6	1.4	46.4	3.4	0.4	8.6	0.10	6.8	25.3	62.6	12.1
0.6–0.7	1.4	48.7	3.7	0.5	7.9	0.09	6.9	31.1	61.1	7.8
0.7–0.8	1.4	48.7	3.2	0.4	7.6	0.07	6.7	27.4	56.7	15.9
0.8–0.9	1.3	49.4	6.3	0.9	7.3	0.11	6.1	39.6	57.7	2.7
0.9–1.0	1.2	54.0	6.4	0.9	7.3	0.12	5.2	34.2	63.1	2.7
1.0–1.1			9.8	1.3	7.4	0.16	4.8	40.6	57.4	2.0

The lysimeters were planted in 2017 with spring barley (*Hodeum vulgare* L. var. ‘Makof’), in the second year with spring wheat (*Triticum aestivum* L. var. ‘Quarna’), and in the third year with oats (*Avena sativa* L. var. ‘Symfoni’). All lysimeters were fertilized at 0.07 m depth with potassium phosphate at a rate of 20 kg P and 40 kg K ha⁻¹ year⁻¹. We applied 100 kg N and 15 kg S ha⁻¹ year⁻¹ in the form of ammonium sulfate and ammonium nitrate dissolved in 10 mL water to each lysimeter receiving N fertilizer. To apply the N-S fertilizer solution, we removed 0.05 m of soil from the top of each lysimeter, to simulate harrowing and seed placement depth in the field, and injected the fertilizer in 1 mL increments with a syringe at ten different sites distributed evenly over the soil surface at either 0.07 m or 0.2 m, or both. The control treatment was injected with the equivalent amount of water in place of N solution. Following fertilization, a thin layer of soil was replaced, then two rows of seeds were placed on the surface before backfilling the remainder of the ~0.05 m soil. To promote seed germination, we then irrigated each lysimeter with 1 L H₂O (14.6 mm rain equivalents) over a span of two days in 250 mL increments. In S1 (2017), spring barley was sown on June 12th and harvested September 29th. Spring wheat was sown on May 10th and harvested August 16th in S2 (2018). In 2019 (S3), oats were sown on May 13th and harvested September 2nd. In this paper, we refer to the initial period from August 2016 to May 2017, beginning from the installation of the lysimeters until the first seeding and fertilization, as Fallow 1 (F1); the first experimental growing season as S1 (June 2017–April 2018); the second growing season as S2 (May 2018–April 2019); the third growing season as S3 (May 2019–April 2020); and the final fallow period as F2 (May 2020–August 2021), ending with the final leachate collection.

Measurements

Leachate

Lysimeter leachate collection began in September 2016. Leachate water was weighed and subsampled for mineral N analysis, which consisted of ammonium (NH₄⁺) and a combined concentration of nitrate (NO₃⁻) plus nitrite (NO₂⁻). Ammonium

concentration was determined colorimetrically using the salicylate method and NO₃⁻ + NO₂⁻ concentration via colorimetric vanadium chloride-reduction (ISO, 2013). Lysimeters did not drain at the same rate, so sampling would occur when there was sufficient leachate for collection at individual lysimeters. Leaching occurred primarily in fall and winter due to climatic conditions and plant uptake of available water during summer.

Chlorophyll content, plant height and harvest

Relative plant leaf chlorophyll content was measured twice during the first growing season (S1), three times during the second season (S2), and five times in the third (S3). We used a handheld SPAD-502 m (Minolta Camera Co., Osaka, Japan) to take three averaged readings per plant leaf while four randomly chosen leaves were measured per plant. On the same day as the SPAD measurements, we measured the plant height from two plants growing in each lysimeter.

At harvest, we removed all biomass down to the base of the plant with scissors. Harvested biomass was dried and then threshed to separate grain from straw. Subsamples of ground grain and straw were analyzed for N content using an organic elemental combustion instrument (LECO CNS Analyzer, Leco Corporation, St. Joseph, MI, USA).

Greenhouse gas measurements

Measurements of N₂O, CH₄, and CO₂ were taken during the growing seasons, beginning the day after sowing and fertilizing and ending around the time of harvest. During CH₄ and N₂O gas collection, a cylindrical PVC chamber (0.022 m³ volume, with riser 0.036 m³) equipped with a small axial circulation fan and ventilation tube was fitted directly onto the lysimeter pipe. Chamber gas concentrations were collected five times per closure at 0, 10, 20, 30, and 40 min after chamber closure via the flow-through method where a loop is made with tygon tubing between a 20 ml glass vial, the chamber, and air pump. Collection vials were transported to the lab and stored for 2–14 days at room temperature before analysis for N₂O and CH₄ on a gas chromatograph (Clarus 500, Perkin Elmer, USA) equipped with an FID and ECD using an automatic headspace injector (Turbo Matrix

110, Perkin Elmer, USA). We sampled 19 times during S1, between June 13th and August 29th, with the highest frequency immediately following fertilization. In S2 we sampled 28 times, twice weekly, between May 11th and September 3rd.

Fluxes of CO₂ from each lysimeter were measured separately with a portable infrared gas analyzer (EGM-4, PP Systems, USA) on a majority of the same sampling occasions using an opaque chamber, and, once seed emergence occurred, a transparent chamber. Carbon dioxide was measured for approximately 125 s resulting in 27 respiration measurements for flux determination. The opaque chamber (SRC-2 Soil Respiration Chamber, PP Systems, USA) measured directly on the soil surface. The transparent chamber was 200 mm in diameter and 200 mm tall with an extension up to 600 mm to accommodate growing crops, and was similarly equipped with a ventilation tube and a battery-operated axial fan. Carbon dioxide was measured 17 times in S1 and 15 times during S2. Greenhouse gas measurements were not performed during S3.

Calculations and statistical analyses

Statistical analyses were performed with the R-software version 2022.07.02 “Spotted Wakerobin” (R Core Team 2022). We used R package *ggplot2* (Wickham 2016) and the *plot_grid* function from the *cowplot* package (Wilke 2020a, b) to produce data figures. To determine treatment effects on crop yield, N content, SPAD and plant height, as well as cumulative leachate amount and N load, we used the Anova function (*car* package, Fox and Weisberg 2019) to determine analysis of variance and the *glht* function (*multcomp* package, Hothorn et al. 2008) for post-hoc analysis by using Tukey’s all pair comparisons. Treatment differences were considered significant for $P < 0.05$.

To determine treatment effects over time in leachate amount (mm), N load (kg ha⁻¹), and volume-weighted concentration (mg L⁻¹), we used a repeated measures anova, using the *lme* function (*nlme* package, Pinheiro et al. 2021) to make a linear mixed model with time as a repeated factor. We used the *corAR1* correlation structure to model the error term. Using the *emmeans* function (*emmeans* package, Lenth 2022), we tested treatment differences at each sampling time as well as

within-treatment differences at different time points. The cropping seasons (S1-S3) were analyzed separately from the non-cropping periods (F1 and F2).

Lysimeter N load was calculated by multiplying the N concentration by leachate quantity at each sampling time. Mean volume-weighted concentration (mg L⁻¹) was determined by dividing the N load (mg) by leachate amount.

Greenhouse gas fluxes were calculated using the R package *gasfluxes* (Fuss 2020) using the fit “robust linear.” Fluxes with P values greater than 0.05 were not considered.

The emission factor (EF) for indirect N₂O emissions from leached N (N₂O—L) in the fertilized treatments was calculated according to

$$EF_{N_2O-L} = (N_{fert} [kgNha^{-1}] - N_{unfert} [kgNha^{-1}]) * EF5(0.011)$$

where the value for EF5 is the default value for leaching/runoff (IPCC 2019), N_{fert} = cumulative N load in leachate for fertilized treatment, and N_{unfert} = cumulative N load in leachate for the unfertilized treatment.

The N balance consisted of measured N inputs (N fertilizer and seed) and outputs (crop biomass N and leachate N) in the experimental system and their sums (kg N ha⁻¹ yr⁻¹) calculated for individual lysimeters. In addition, we calculated the nitrogen use efficiency (NUE) as an indicator for resource efficiency (Quemada et al. 2020):

$$NUE[\%] = \left(\frac{\sum (crop\ N\ outputs [kgNha^{-1}])}{\sum (N\ fertilizer\ inputs [kgNha^{-1}])} \right) * 100$$

To incorporate the control treatment that did not receive N input, we additionally calculated the agronomic efficiency of N (AE_N) according to Lahda et al. (2005) as well as the recovery efficiency of N (RE_N) (Lahda et al. 2005; Dobermann 2005):

$$AE_N [kgkg^{-1}] = \frac{(grain\ yield_{fert} - grain\ yield_{unfert})}{N_{applied}}$$

$$RE_N [\%] = \frac{(plant\ N\ uptake_{fert} - plant\ N\ uptake_{unfert})}{(N_{applied})} * 100$$

Results

Leachate quantity and climatic conditions

Across treatments, collected leachate amounts averaged 27, 10 and 14% of precipitation plus irrigation in S1, S2 and S3, respectively (Table 2). Over the three periods (S1 – S3) % total leachate quantity relative to total water inputs was lowest in Deep and highest in Control following the pattern of Control > Mixed > Shallow > Deep, corresponding to 20, 18, 17 and 13%.

The Control had significantly higher ($p=0.04$) mean cumulative leachate amount (\pm SE) for the three growing seasons (S1-S3), 377 ± 37 mm H₂O, compared to the lowest in deep, 249 ± 12 (Fig. 1). Mixed and Shallow were intermediates with mean cumulative leachate of 332 ± 36 and 319 ± 25 , respectively. The Deep placement had the lowest quantity of leachate for all periods except for the initial F1 period. Within fertilized treatments, Deep leached 25% less water compared to Shallow and 29% less than Mixed.

During the initial fallow period (August 2016 to May 2017) there were no statistical differences in water flow, although there was some variation between individual lysimeters and overall among the different treatments (Fig. 2, Table 2). Mean leachate amount was somewhat higher in Shallow (133 ± 12 mm) and lowest in the Control (83 ± 17 mm). The Mixed and Deep treatments had

intermediate water flow in F1 with 110 ± 15 and 109 ± 7 mm. Total precipitation and irrigation during the F1 period was approximately 322 mm, the lowest quantity of all periods (Fig S1, Table 2).

In the winter and spring following the first cropping season (S1), the pattern of water flow changed from the preceding F1 period. The S1 period (June 2017-April 2018) was generally wetter than the preceding period, particularly in the autumn and winter months (Fig S1). Though there were no significant differences in mean cumulative leachate, the treatments in S1 were by amount Control > Shallow > Mixed > Deep corresponding to 179 ± 10 , 168 ± 6 , 160 ± 17 , and 148 ± 5 mm, respectively (Figs. 1 and 2, Table 2).

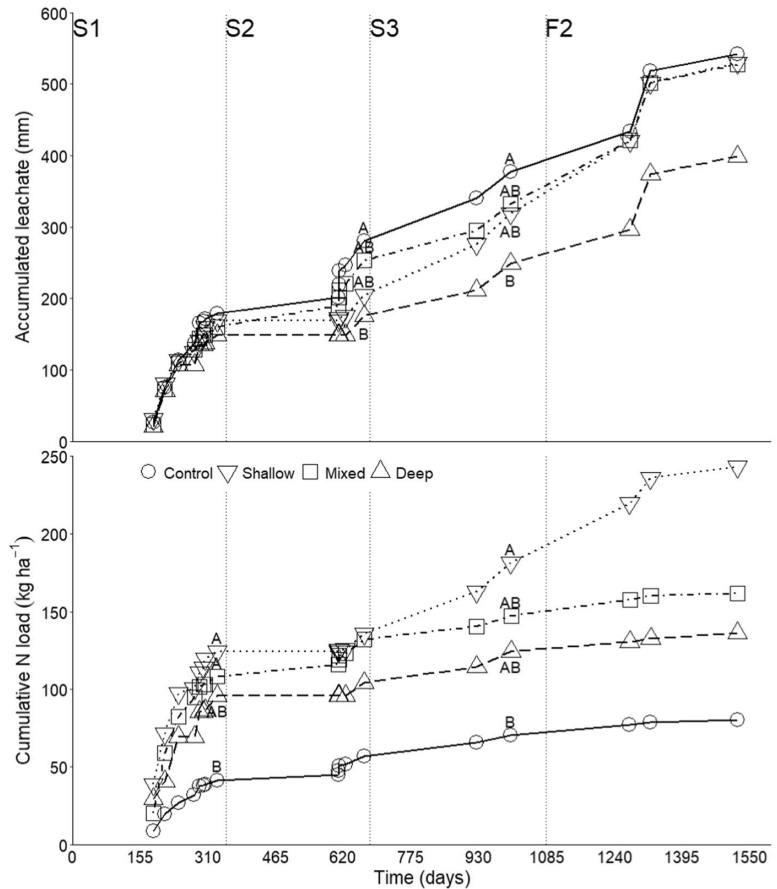
In the second cropping year (S2), nearly every month had both lower precipitation and higher average temperatures compared to the long-term normal (Fig S1), particularly during the cropping period from May to July 2018. Although it appears that July received sufficient precipitation, the majority occurred late in the month on a single day, when 79 mm out of the monthly total of 82 mm rain fell. Lysimeter leachate quantity was greatly affected by the drought and only two of the four treatments, Control and Mixed placement, were releasing water by February 2019, and in large quantities. Sufficient quantities of water for sampling did not flow from all lysimeters until around April 2019. The mean cumulative amount for S2 (\pm SE) was significantly higher

Table 2 Total precipitation and irrigation (mm), cumulative lysimeter leachate (mm) and mineral nitrogen (N) load in leachate (kg ha⁻¹) per period. Lowercase letters represent treatment differences ($p < 0.05$)

Period	Precip + Irrigation (mm)		Control	Shallow	Mixed	Deep
F1	322	Leachate (mm)	83 ± 17	133 ± 12	110 ± 15	109 ± 7
		N load (kg ha ⁻¹)	25 ± 11	69 ± 11	49 ± 4	53 ± 12
S1	599	Leachate	179 ± 10	168 ± 6	160 ± 17	148 ± 5
		N load	41 ± 11 ^b	124 ± 12 ^a	108 ± 13 ^a	96 ± 21 ^{ab}
S2	637	Leachate	101 ± 27 ^a	36 ± 6 ^{ab}	94 ± 14 ^a	27 ± 4 ^b
		N load	15 ± 5 ^{ab}	11 ± 2 ^{ab}	24 ± 4 ^a	9 ± 2 ^b
S3	642	Leachate	97 ± 14	114 ± 33	79 ± 14	74 ± 4
		N load	14 ± 3	46 ± 22	15 ± 3	20 ± 3
F2	746	Leachate	165 ± 10	211 ± 48	195 ± 38	150 ± 5
		N load	10 ± 1	62 ± 52	15 ± 9	12 ± 1

F1=Initial fallow, S1=1st year with crops, S2=2nd year with crops, S3=3rd year with crops, F2=latter fallow. Control=no N fertilizer, Shallow=shallow N fertilizer placement (0.07 m), Mixed=mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep=deep placement of N fertilizer (0.2 m)

Fig. 1 Cumulative water leachate curve and mineral nitrogen (N) load (kg ha⁻¹) and treatment effects ($p < 0.05$) for the experimental treatment period (S1–S3) and subsequent fallow period (F2). Uppercase letters indicate represent treatment differences ($p < 0.05$). S1 = 1st year with crops, S2 = 2nd year with crops, S3 = 3rd year with crops, F2 = later fallow. Control = no N fertilizer, Shallow = shallow N fertilizer placement (0.07 m), Mixed = mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep = deep placement of N fertilizer (0.2 m). Day 0 = initial fertilization



in the Control (101 ± 27 mm) and Mixed placement (94 ± 14 mm) compared with Shallow (36 ± 6 mm) and Deep (27 ± 4 mm) (Fig. 2, Table 2). Within the fertilized treatments, Mixed was also significantly higher than Shallow and Deep ($p = 0.001$). In terms of within-treatment comparisons, the Control treatment water flow in this period was significantly higher than at any other sampling time during S1–S3 with the exception of one sampling occasion immediately following, in December 2019 (Fig. 2). Similarly, leachate quantity in the Mixed placement was significantly higher than all other sampling times in S1–S3.

Rainfall was relatively closer to long-term normal during S3 in comparison with the previous year, although some compensation with irrigation

was necessary during May–July 2019 (Fig S1). Mean cumulative leachate (\pm SE) in S3 followed the order of Shallow > Control > Mixed > Deep corresponding to 114 ± 33 , 97 ± 14 , 79 ± 14 , and 74 ± 4 mm although there were no significant differences between treatments (Table 2).

Though not significant, the deep placement continued to leach less water than all other treatments into the F2 period despite the absence of crops, although most treatment effects tapered off after December 2020 (Fig. 2). Mean cumulative water flow followed the order of Shallow > Mixed > Control > Deep and corresponded to 210 ± 47 , 195 ± 37 , 165 ± 10 , and 150 ± 5 mm (\pm SE).

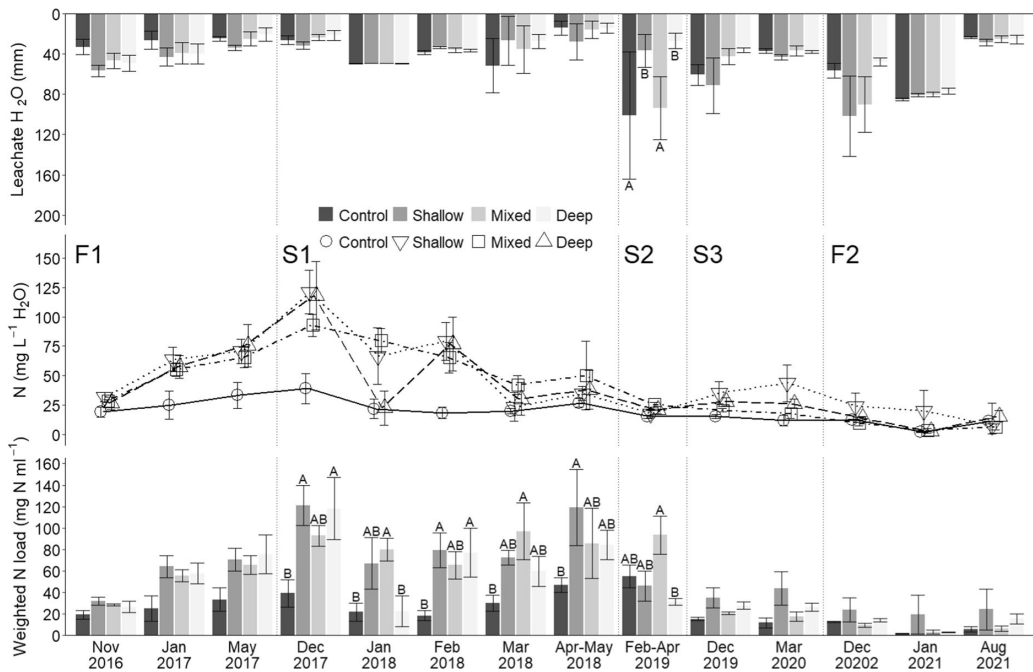


Fig. 2 Mean leachate quantity (mm H₂O), leachate N concentration (mg L⁻¹ H₂O), and weighted N load (mg N ml⁻¹). Uppercase letters indicate treatment differences within the same sampling time ($p < 0.05$). Each bar represents a single sampling, except Apr–May 2018 and Feb–Apr 2019 which are comprised of the sum of multiple samplings in order to incor-

porate leaching from all lysimeters. F1=Initial fallow, S1=1st year with crops, S2=2nd year with crops, S3=3rd year with crops, F2=latter fallow. Control=no N fertilizer. Shallow=shallow N fertilizer placement (0.07 m), Mixed=mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep=deep placement of N fertilizer (0.2 m)

N Load and N volume-weighted concentrations

Cumulative N load for all cropping seasons (S1-S3) was lowest in the Control, $70 \pm 18 \text{ kg N ha}^{-1}$, and highest in Shallow, $181 \pm 21 \text{ kg N ha}^{-1}$ with significant differences ($p=0.009$) between the two treatments. The Mixed and Deep placements were intermediates with mean cumulative N loads of 147 ± 14 and $124 \pm 24 \text{ kg ha}^{-1}$ respectively, resulting in a reduction of leachate N losses of 21 and 37% compared to Shallow. Among the fertilized treatments, however, there were no significant differences.

N losses in leachate during F1 were relatively low, but not significantly, in the Control ($25 \pm 11 \text{ kg N ha}^{-1}$) compared to the other treatments (69 ± 11 , 49 ± 4 , $53 \pm 12 \text{ kg N ha}^{-1}$ for Shallow, Mixed, and Deep respectively), even though no fertilizer had been applied to any of the treatments. Nitrogen load

steadily increased in all lysimeters after the initial disturbance at the beginning of the setup (Fig. 2) and by the end of F1, treatment differences were nearly significant ($p=0.055$). This flush of mineralized N peaked in S1 and began to decline in S2. In S1, total mean leachate N increased to 41 ± 11 , 124 ± 12 , 108 ± 21 , and $96 \pm 13 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ for Control, Shallow, Mixed, and Deep respectively, and there were significant treatment differences where Shallow, Mixed > Deep > Control, with Shallow and Mixed significantly higher than the Control (Fig. 1, Table 2). The emission factors for indirect N₂O emissions for this period due to leached N were 1.40, 1.30, and 1.18 for Shallow, Mixed, and Deep, respectively.

Subsequent to S1, average N loads decreased below F1 levels. During the growing season of S2 there was a drought during the critical part of the growing period from May to July in 2018, with low

precipitation and high temperatures compared to the long-term normal (Fig S1). Consequently, there was just one brief period of water flow the following spring (Fig. 2) from late February to April 2019, in which the Mixed placement had the highest leachate N load, significantly higher than Deep ($p=0.04$), following the pattern Mixed > Control, Shallow > Deep. S2 mean leachate N loads (\pm SE) were 15 ± 5 , 11 ± 2 , 24 ± 4 , and 9 ± 2 kg N ha⁻¹ yr⁻¹ for Control, Shallow, Mixed, and Deep respectively (Table 2). Indirect N₂O emission factors for leached N were 0.23, 0.86, and 0.13 for Shallow, Mixed, and Deep, respectively.

In the third growing season (S3), precipitation was higher, particularly in October–December, relative to the previous drought year, resulting in a different pattern in leachate N loads, where Shallow and Deep had insignificantly elevated N loads relative to Control and Mixed. Total mean N loads (\pm SE) were 14 ± 3 , 46 ± 22 , 15 ± 3 , and 20 ± 3 kg N ha⁻¹ yr⁻¹ for Control, Shallow, Mixed, and Deep respectively (Table 2). An individual lysimeter in the Shallow treatment, which also had a low yield in S3, leached N at levels 5 times greater than all other lysimeters, and continued to leach high levels of N well into F2 the following year (Fig. 1 and 2). The EF for indirect N₂O emissions due to leached N was 1.11, 0.72, and 0.66 for Shallow, Mixed, and Deep, respectively. In the last period when no fertilization or cropping had occurred, the N load in the majority of lysimeters declined. Cumulative treatment N load means for F2 (\pm SE) for the Control, Shallow, Mixed, and Deep were 10 ± 1 , 62 ± 52 , 15 ± 9 , and 12 ± 1 kg N ha⁻¹ respectively.

Greenhouse gas fluxes

About 9% of N₂O fluxes had a p -value < 0.05, which means that the individual measurements showed a significant increase or decrease. Of those, about half surpassed the variability of the detection limit of the GC (max ppm – min ppm < GC detection limit). Likewise, 25% of CH₄ fluxes had a p -value < 0.05, and of those only one was below the GC detection limit.

Photosynthetic CO₂ uptake in the Control was greatest in the earlier stages of plant growth (Fig. 3) in S1 with the Shallow treatment following a similar trend or with somewhat less uptake than the Control, but in the drought year S2, this trend was less clear. The Mixed and Deep treatments tended to have greater CO₂ uptake later in the growing period

relative to Control and Shallow. In 2017 (S1), the Mixed had the greatest CO₂ uptake but the following year the control was highest, while in both years Shallow was the lowest. Total uptake (sum of negative fluxes) in S1 was 1.77, 1.57, 1.84, and 1.77 g CO₂-C m⁻² h⁻¹ for Control, Shallow, Mixed, and Deep respectively. In S2 the pattern of total uptake changed, and was 2.63, 2.03, 2.08, and 2.34 g CO₂-C m⁻² h⁻¹ for Control, Shallow, Mixed, and Deep respectively.

Biomass yield and N balance

Nitrogen use efficiency (NUE) was high in all fertilized treatments in all seasons due to the high crop N output relative to the fertilization rate (Table 3, Fig. 4). The N surplus, calculated as the difference between N inputs to and outputs from the system, was highest in all treatments in S1 and lowest in S2, the latter of which occurred during a drought and resulted in the lowest N outputs from almost all components for all treatments.

In S1, N losses via leachate were very high (Fig. 2, Table 2), even in the control, and leachate accounted for the second-highest output from the system after harvested grain N (Table 3). Although yields were similar among treatments in S1 (Table 4), due to the difference in grain and straw N uptake, the Mixed and Deep treatments had higher outputs in grain and straw, the latter significantly higher, compared with the control ($p=0.02$). Additionally, mixed placement had significantly higher grain N content compared to the control ($p=0.045$) (Table 4).

Both AE_N, a measure of grain N uptake efficiency that accounts for N uptake in the non-fertilized control, and RE_N, where the additional N in straw is incorporated into the calculation, followed a similar trend over time, with the greatest treatment differences occurring in the final S3 season. This trend is similar to the trend in grain yield between the Mixed and Deep treatments (Table 4). During S1 and S2, both Mixed and Deep treatments had similar yields and crop N uptake, and thus similar system outputs in the form of crop N, and were greater than Shallow placement in both years. However, in S3, when there was neither drought nor an excess of mineralized N as in the previous years, Mixed placement had intermediate yields and the lowest crop N uptake among the fertilized treatments, although leachate losses were as low as the control (Table 3).

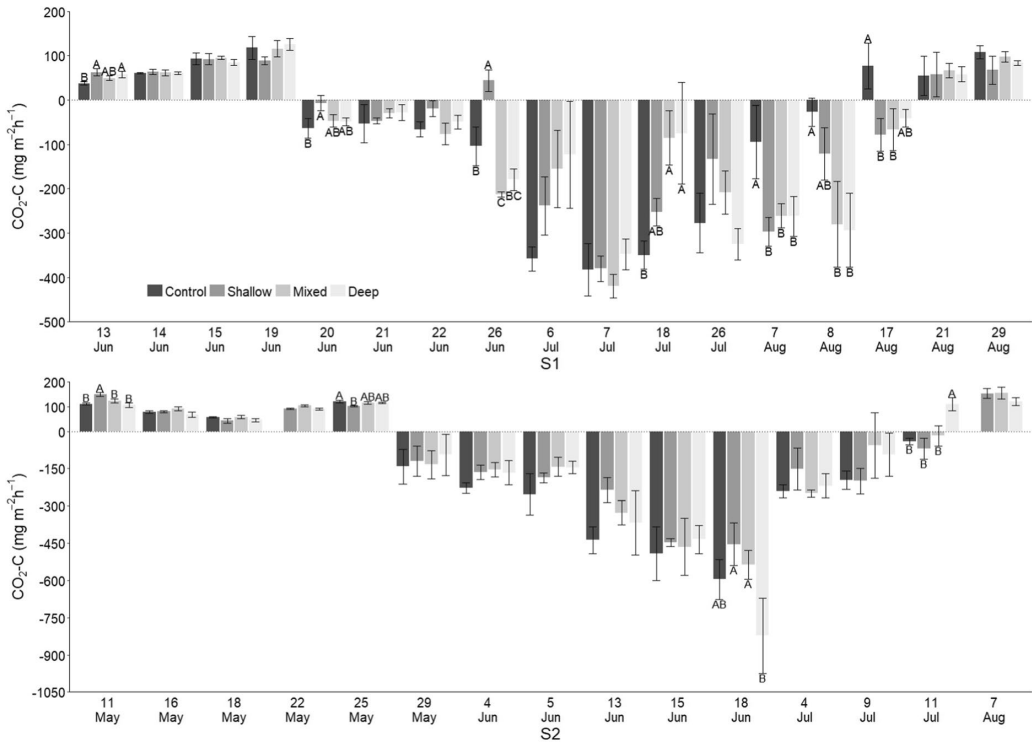


Fig. 3 Daytime CO₂ fluxes (mg m⁻² h⁻¹ CO₂-C) during the cropping periods of S1 and S2. Uppercase letters indicate represent treatment differences (*p* < 0.05). S1 = 1st year with crops, S2 = 2nd year with crops. Control = no N fertilizer, Shal-

low = shallow N fertilizer placement (0.07 m), Mixed = mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep = deep placement of N fertilizer (0.2 m)

In the cumulative N balance (sum of S1-S3) (Fig. 4), the Deep placement had the greatest N surplus but the highest amount and proportion (83%) of N output from harvested crops relative to total N outputs among the fertilized treatments. An opposite trend was observed in the Shallow placement, where outputs from crop N were lowest but those from leaching were highest, while Mixed placement was an intermediate to Deep and Shallow. The Control had the second highest N surplus, primarily due to no fertilizer input, and each N output component was the lowest among all treatments. However, the proportion of N losses in the Control were similar to the Deep placement, but approximately 1.7 times lower in each component.

Discussion

Reduced leaching through deep N placement

In general, Deep placement had the lowest quantity of leachate for all periods except for the initial fallow period (Fig. 1, Table 2), suggesting a greater water use efficiency, which in turn promoted crop N uptake, higher yields, and lower N losses via leachate (Fig. 4). Total leachate quantity in S1 – S3 was significantly lower in the Deep treatment than in the other treatments (*p* = 0.03), compared to highest in Control. Compared to Shallow, Deep placement had 25% less leachate and 29% less than Mixed. Though not significant, Deep continued to have less leachate

Table 3 Nitrogen (N) balance components per cropping season. N use efficiency (NUE) (%), agronomic efficiency of N (AE_N) (kg kg⁻¹), and the recovery efficiency of N (RE_N) (%). N balance values are presented as treatment mean value (kg N ha⁻¹ yr⁻¹) ± standard error. Lowercase letters represent significant treatment differences within the given period (*p* < 0.05). Note that actual season lengths varied

	S3											
	S1				S2				S3			
	Control	Shallow	Mixed	Deep	Control	Shallow	Mixed	Deep	Control	Shallow	Mixed	Deep
<i>N</i> inputs and outputs (kg N ha ⁻¹ yr ⁻¹)												
Seeds	8	8	8	8	8	8	8	8	8	8	8	8
Fertilization	100	100	100	100	100	100	100	100	100	100	100	100
Harvested grain	-118 ± 17	-141 ± 14	-157 ± 9	-155 ± 13	-80 ± 4 ^b	-127 ± 9 ^a	-158 ± 12 ^a	-157 ± 5 ^a	-95 ± 6 ^b	-151 ± 25 ^{ab}	-145 ± 16 ^{bc}	-190 ± 5 ^a
Harvested straw	-27 ± 2 ^b	-38 ± 6 ^{ab}	-43 ± 2 ^a	-43 ± 1 ^a	-13 ± 1 ^b	-30 ± 3 ^a	-26 ± 4 ^a	-31 ± 1 ^a	-18 ± 2 ^b	-32 ± 1 ^a	-29 ± 2 ^a	-30 ± 2 ^a
Leachate load	-41 ± 11 ^b	-124 ± 12 ^a	-108 ± 13 ^a	-96 ± 21 ^{ab}	-15 ± 5 ^{ab}	-11 ± 2 ^{ab}	-24 ± 4 ^a	-9 ± 2 ^b	-14 ± 3	-46 ± 22	-15 ± 3	-20 ± 3
N surplus	-179	-195	-200	-185	-100	-60	-101	-89	-119	-121	-80	-132
NUE %	n.a	179	200	198	n.a	157	184	188	n.a	183	174	220
AE _N (kg kg ⁻¹)	n.a	0.23	0.39	0.36	n.a	0.47	0.78	0.77	n.a	0.56	0.5	0.95
RE _N (%)	n.a	34	55	53	n.a	64	92	95	n.a	70	60	107

S1 = 1st year with crops (June 2017 – April 2018), S2 = 2nd year with crops (May 2018–April 2019), S3 = 3rd year with crops (May 2019–April 2020). Control = no N fertilizer, Shallow = shallow N fertilizer placement (0.07 m), Mixed = mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep = deep placement of N fertilizer (0.2 m)

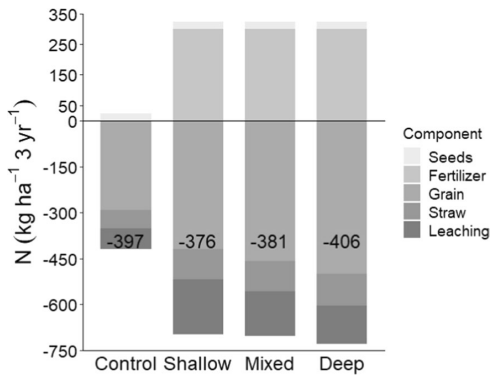


Fig. 4 Cumulative (S1-S3) N balance containing total inputs (N from seeds and fertilizer) and total outputs (N leaching, grain and straw yield N) with balance sum in kg N ha⁻¹ 3 yr⁻¹. Note that actual season lengths varied. S1 = 1st year with crops (June 2017 – April 2018), S2 = 2nd year with crops (May 2018–April 2019), S3 = 3rd year with crops (May 2019 – April 2020). Control = no N fertilizer, Shallow = shallow N fertilizer placement (0.07 m), Mixed = mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep = deep placement of N fertilizer (0.2 m)

than all other treatments during the F2 period despite the absence of crops. Within the fertilized treatments, however, there were no significant differences in cumulative leachate except during the drought period S2. Similar to our results, Chen et al. (2022) found that Deep placement of N fertilizer at 0.15 m relative to placement at 0.05, 0.25 and 0.35 m had the highest

precipitation use efficiency, crop N uptake, radiation use efficiency, and also reduced soil nitrate-nitrogen residue levels in the deep layers under two years of maize followed by winter wheat. They also found that root surface area and root length density were highest at 0.15 m placement.

In our experiment, observed treatment differences in leachate amount, and thus crop uptake of soil water, were likely a consequence of differences in either root architecture (e.g., deep rooting), root biomass, or a combination of the two. Although genetics play a fundamental role in plant rooting patterns, many studies have shown that roots exhibit plasticity in response to the soil environment, particularly when nutrients are distributed heterogeneously or in patches (Hodge 2004). Although the roots were not sampled, we can infer belowground biomass from aboveground plant biomass and leachate quantity. Aboveground crop biomass at harvest was negatively correlated with leachate quantity ($p < 0.0001$, $R^2 = 0.81$) when excluding the drought year S2 (Fig. 5).

In S1, when available soil N was likely quite high, there was only a minor effect of the N fertilizer placement on aboveground biomass and leachate quantity across treatments, and thus the correlation of biomass to leachate amount was not significant for the year individually ($p = 0.18$, $R^2 = 0.39$). Whereas in S3, the fertilizer treatment significantly affected crop biomass, and the correlation between aboveground biomass and leachate quantity was more clear ($p < 0.0001$, $R^2 = 0.65$). In S2, summer drought

Table 4 Grain yield (tons ha⁻¹), straw and grain N content (% N). Lowercase letters represent treatment differences within the given period ($p < 0.05$)

Period	Parameter	Control	Shallow	Mixed	Deep
S1	Grain yield	6.4 ± 0.3	6.2 ± 0.4	6.7 ± 0.5	6.6 ± 0.5
	Grain % N	1.83 ± 0.20 ^b	2.26 ± 0.14 ^{ab}	2.38 ± 0.08 ^a	2.29 ± 0.07 ^{ab}
	Straw % N	0.53 ± 0.02	0.69 ± 0.08	0.72 ± 0.02	0.71 ± 0.03
S2	Grain yield*	5.8 ± 0.2 ^b	6.1 ± 0.7 ^{ab}	7.7 ± 0.6 ^a	7.5 ± 0.2 ^{ab}
	Grain % N	1.4 ± 0.03 ^b	2.1 ± 0.09 ^a	2.0 ± 0.02 ^a	2.1 ± 0.03 ^a
	Straw % N	0.30 ± 0.02 ^b	0.43 ± 0.02 ^a	0.45 ± 0.02 ^a	0.50 ± 0.02 ^a
S3	Grain yield	6.7 ± 0.3 ^b	7.9 ± 1.5 ^{ab}	8.3 ± 1.1 ^{ab}	10.1 ± 0.2 ^a
	Grain % N	1.41 ± 0.02 ^b	1.97 ± 0.09 ^a	1.77 ± 0.07 ^a	1.89 ± 0.02 ^a
	Straw % N	0.22 ± 0.02 ^b	0.34 ± 0.03 ^a	0.29 ± 0.02 ^{ab}	0.27 ± 0.01 ^{ab}

*excluding outlier lysimeter 13 (Shallow treatment)

S1 = 1st year with crops, S2 = 2nd year with crops, S3 = 3rd year with crops. Control = no N fertilizer, shallow = shallow N fertilizer placement (0.07 m), mixed = mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), deep = deep placement of N fertilizer (0.2 m)

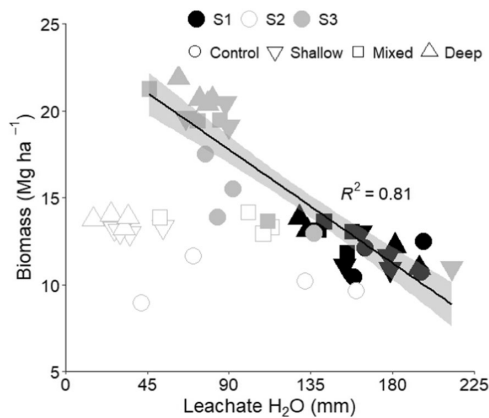


Fig. 5 Total aboveground crop biomass (Dry matter in Mg ha^{-1}) and total leachate quantity of H_2O (mm) per cropping season (S1–S3) for individual lysimeters. Linear trend line and R^2 value for combined years S1 and S3. S1=1st year with crops, S2=2nd year with crops, S3=3rd year with crops. Control=no N fertilizer, Shallow=shallow N fertilizer placement (0.07 m), Mixed=mixed placement of N fertilizer (half at 0.07 m, half at 0.2 m), Deep=deep placement of N fertilizer (0.2 m)

conditions likely affected the allometric relationship between roots and shoots as there were significant treatment differences in both aboveground biomass at harvest and total leachate, but they were not correlated ($p=0.7$, $R^2=0.21$) (Fig. 5). Mathew et al. (2018) also found a weaker relationship between root to shoot ratio and shoot biomass or grain yield in wheat under drought-stressed conditions compared to non-stressed conditions. Similar to that, Meurer et al. (2019) found that shoot:root ratios, as well as N concentrations in living roots changed depending on irrigation and N fertilization in a field experiment with mixed grass ley in central Sweden.

Deep rather than lateral exploration by roots, earlier in the season, has been shown to be beneficial for N capture and subsoil water access, although water in the subsoil is potentially more beneficial in the latter part of crop growth (Lynch 2013). We expected that in the earliest stages of crop growth, Shallow placement crop roots would proliferate around 0.07 m where the P and 100% of the N fertilizers were placed, but Deep placement by contrast, would have earlier deeper root exploration and have a relatively higher root biomass at and below 0.2 m.

In the Mixed placement, we would expect that initial root proliferation would have occurred around the 0.07 m placement initially, but once N resources were exhausted, roots would explore the soil profile toward the remaining 0.2 m-placed N, but this deeper exploration would be delayed compared to the Deep treatment, and possibly mechanically impaired if subsoil moisture was low (Colombi et al. 2018). While this delay in the mixed placement was not generally detrimental in terms of crop N uptake, during drought conditions in S2 there were high N losses via leaching, likely due to low root biomass relative to the other fertilized treatments. Additionally, in S3, with non-drought conditions and in the absence of excess soil mineral N, the mixed placement had lower yield and lower crop N uptake compared to Deep. The shallow N placement was beneficial in drought conditions (albeit with supplemental irrigation) in terms of soil water usage, since both leachate flow and N load were low, but in non-drought conditions leachate flow and N loads were high. It is possible, however, that the higher N load in Shallow and Deep placements in S3 was a result of a carryover effect of previously immobilized soil N from S2 when both treatments had very little leachate flow.

Drought obscured treatment effect on GHG emissions

Unlike our findings from the field experiment (Rychel et al. 2020), N_2O and even CH_4 fluxes to a lesser extent, were too few to allow for treatment comparisons. During the same cropping season as S1, the field N_2O fluxes averaged (\pm SD) 69.9 ± 49.1 and $56.9 \pm 52.9 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ for a shallow and mixed placement respectively, and 44.9 ± 39.2 and $43.8 \pm 37.9 \mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$ respectively for the deep and control treatments, respectively. The lack of surface N_2O fluxes in the present study is possibly due to lysimeter detachment from groundwater, which alters the soil water relative to field conditions due to the lack of capillary rise from the groundwater (Abdou and Flury 2004; Bergström 1990). Since the lysimeters are draining freely by gravity year-round, we could assume that, on average, the lysimeter soil was drier compared to natural conditions in the field, particularly in the subsoil (>0.3 m) during periods of high evapotranspiration. Supplemental irrigation was applied in small quantities in the summertime

in order to avoid creating preferential flow, usually either 3.7 or 7.3 mm per lysimeter per day, split into two watering times, and meanwhile rainfall events in May–July were normally even less. Thus, the minimum infiltration depth of supplemental irrigation (mm H₂O / soil porosity) would have been around 14.4 mm. Due to the combined effect of drier soil conditions in the subsoil along with a shallow infiltration of supplemental irrigation in the overlaying zone, we expect that any upward diffusion of N₂O produced in the (likely aerobic) zone below the infiltration depth would have been limited by the upper wetted zone where it could have undergone complete denitrification. Due to the soil moisture status during summer months, N₂O emissions in our experiment could thus be compared to studies where crops are irrigated. For example, Yang et al. (2019) and Wang et al. (2016) found that overhead sprinkler irrigation or surface drip irrigation, respectively, relative to flood irrigation, significantly reduced N₂O emissions, which primarily wetted the soil surface and did not fill soil macropores in lower soil depths.

Carbon dioxide fluxes, on the other hand, were within an expected range, although the two growing seasons differed (Fig. 3). In S1, rates and length of crop maturation as indicated by CO₂ uptake patterns were more clear between treatments and followed the pattern of Control > Shallow > Mixed, Deep. However, in S2, possibly due to climatic conditions, the rate of maturation was largely similar among treatments. Grain N content at harvest in S1 reflected the pattern of CO₂ uptake, where a longer growth period and later maturation corresponded to higher grain N accumulation, similar to the findings of Hay and Kirby (1991) and Andersson (2005). Cheng et al. (2020) and Wu et al. (2022) also found that deep N placement delayed senescence of maize so that more aboveground biomass was sustained later in the cropping season, leading to deeper and more extensive rooting, which in turn promoted both crop N uptake and higher grain yield. Plant height differences, when measured around the same time as CO₂ fluxes (Table S1), corresponded to differences in CO₂ uptake, but not relative leaf chlorophyll content readings, which instead indicated crop N uptake differences.

Conclusions

In this study, deep N fertilization was beneficial for crop N uptake and yield, but also, in contrasting climatic conditions and soil N availability, this method promoted greater crop-water use efficiency, which led to reduced mineral N losses via leaching. The effect of reduced leaching continued even into the fallow period following the three years of cropping. This study highlighted the importance of monitoring leaching behavior over a longer time period, rather than within an individual cropping season, which may be difficult to interpret.

While our results showed agronomic and environmental benefits, we recognize that the required equipment and management for implementing deep N fertilization available in Sweden may be cost-prohibitive and possibly inaccessible for some farmers. Additionally, further studies on a variety of soil textures, as well as drought studies without irrigation management, would elucidate the effectiveness of deep fertilization more generally.

Acknowledgements This work was funded by FORMAS, the Swedish research council for sustainable development, Grant # 229-2013-82. The authors would like to thank Helena Linefur and the late Göran Johansson for assistance in lysimeter collection and Maria Blomberg for technical support at the lysimeter station. Additionally, we thank Jan Fiedler for assistance in the GC lab, and Sabine Jordan, Monica Strömgren, and Örjan Berglund for lending equipment.

Author contributions Katrin Rychel ran the experiment, collected and analyzed the data, and wrote the manuscript. Katharina Meurer contributed to supervising the experiment, assisted in data analysis and manuscript revision. Gizachew Getahun contributed to collecting and analyzing the data. Lars Bergström assisted in data analysis and supervision and planning of the experiment. Holger Kirchmann led the project and contributed to planning the experiment. Thomas Kätterer was the primary supervisor of the experiment and assisted with manuscript revision. All authors reviewed the manuscript.

Funding Open access funding provided by Swedish University of Agricultural Sciences.

Declarations

Competing interests The authors declare no competing interests.

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ACTA UNIVERSITATIS AGRICULTURAE SUECIAE

DOCTORAL THESIS NO. 2023:60

This thesis investigated the impact of a deep placement of nitrogen (N) fertilizer relative to a shallow placement on greenhouse gas emissions, N leaching and crop response. The results indicated that a deeper placement reduced fertilizer-induced nitrous oxide and methane emissions, increased crop N uptake and yield as well as reduced N leaching relative to a shallow placement. These findings indicate that the current shallow placement depth currently in use in central Sweden should be reassessed.

Katrin Rychel received their graduate education at the Department of Soil and Environment at SLU in Uppsala, Sweden. They have an MS in Plant and Soil Science from the University of Oklahoma, USA and a BS in Biology from California State University, East Bay, USA.

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ISSN 1652-6880

ISBN (print version) 978-91-8046-172-6

ISBN (electronic version) 978-91-8046-173-3