Science of the Total Environment 857 (2023) 159255

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Nitrate leaching losses and the fate of ^{15}N fertilizer in perennial intermediate wheatgrass and annual wheat — A field study

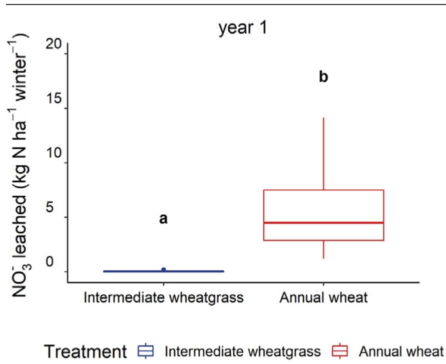
Alexandra Huddell [a,b,*], Maria Ernfors [c], Timothy Crews [d], Giulia Vico [e], Duncan N.L. Menge [a]

[a] Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York, USA
[b] Department of Environmental Science & Technology, University of Maryland, College Park, MD, USA
[c] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Alnarp, Sweden
[d] The Land Institute, Salina, KS, USA
[e] Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden

HIGHLIGHTS

- Comprehensive nitrogen budget in intermediate wheatgrass and annual wheat
- NO_3^- leaching was an order of magnitude lower in perennial compared to annual wheat.
- ^{15}N-labeled fertilizer contributed little (<3 %) to NO_3–N in leachate.
- Fertilizer recovery was high in straw, roots, and soils in intermediate wheatgrass.
- Fertilizer recovery was high in annual wheat seeds.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Wei Shi

Keywords:
Nitrate leaching
^{15}N fertilizer
Intermediate wheatgrass
Perennial grains
Agroecology

ABSTRACT

Perennial grains, such as the intermediate wheatgrass (*Thinopyrum intermedium*) (IWG), may reduce negative environmental effects compared to annual grain crops. Their permanent, and generally larger, root systems are likely to retain nitrogen (N) better, decreasing harmful losses of N and improving fertilizer N use efficiency, but there have been no comprehensive N fertilizer recovery studies in IWG to date. We measured fertilizer N recovery with stable isotope tracers in crop biomass and soil, soil N mineralization and nitrification, and nitrate leaching in IWG and annual wheat in a replicated block field experiment. Nitrate leaching was drastically reduced in IWG (0.1 and 3.1 kg N ha^{-1} yr^{-1}) in its third and fourth year since establishment, compared with 5.6 kg N ha^{-1} yr^{-1} in annual wheat and 41.0 kg N ha^{-1} yr^{-1} in fallow respectively. There were no differences in net N mineralization or nitrification between IWG and annual wheat, though there was generally more inorganic N in the soil profile of annual wheat. More ^{15}N fertilizer was recovered in the straw and all depths of the roots and soils in IWG than annual wheat. However, annual wheat recovered much more ^{15}N fertilizer in the seeds compared to IWG, which had lower grain yields. ^{15}N-labeled fertilizer contributed little (<3 %) to nitrate–N in leachate, highlighting the role of soil microbes in regulating loss of current year fertilizer N. The large reduction in nitrate leaching demonstrates that perennial grains can reduce harmful nitrogen losses and offer a more sustainable alternative to annual grains.

* Corresponding author at: Department of Ecology, Evolution, and Environmental Biology, Columbia University, New York, USA.
E-mail address: ahuddell@umd.edu (A. Huddell).

http://dx.doi.org/10.1016/j.scitotenv.2022.159255
Received 13 May 2022; Received in revised form 25 July 2022; Accepted 1 October 2022
Available online 7 October 2022

1. Introduction

Humans have more than quadrupled global reactive nitrogen (N) inputs to the terrestrial biosphere compared with natural N fixation, mostly via synthetic N fertilizer use and cultivation of N-fixing legumes (Fowler et al., 2013; Vitousek et al., 2013). N fertilizer is needed to maintain crop yields, but only about half of fertilizer N added is captured by crops each growing season (Cassman et al., 2002; Gardner and Drinkwater, 2009; Ladha et al., 2005). Much of the surplus N escapes from croplands, harming biodiversity and impairing water quality of downstream ecosystems, causing air pollution, and generating greenhouse gas emissions (Galloway et al., 2003). Most efforts to reduce reactive N losses from agriculture focus on improving the rate, type, place, and timing of fertilization, but have had only modest success (Zhang et al., 2015). Furthermore, reactive N losses from agriculture are expected to be exacerbated by climate change (Bowles et al., 2018; Martinez-Feria and Basso, 2020). Reactive N management could be improved by ecological solutions, such as the use of cover crops (Thapa et al., 2018), no-tillage (Syswerda et al., 2012), diversified crop rotations (Gardner and Drinkwater, 2009), integration of native, perennial plants on slopes (Schulte et al., 2017), or cultivation of perennial biofuel systems such as miscanthus, switchgrass, and poplar (Smith et al., 2013; Syswerda et al., 2012). Another potential management strategy, which has not received as much attention, is the cultivation of perennial grains.

Using perennial grains, such as the intermediate wheatgrass (IWG) (*Thinopyrum intermedium*), has been proposed as a solution to some of modern agriculture's greatest problems such as nitrous oxide emissions (Daly et al., 2022), soil erosion, loss of soil organic matter, and reactive N pollution (Crews et al., 2016). IWG is still in the early stages of domestication, and accordingly its grain yield is still much lower than that of annual wheat, but grain yields are rapidly improving with genomic selection breeding techniques with typical increases of 58.5 kg ha^{-1} per breeding cycle (Bajgain et al., in press; Crain et al., 2021a, 2021b). Perennial cropping systems can improve the synchrony between soil N supply and crop N demand (Crews, 2005), increase the carbon sequestration potential (Crews and Rumsey, 2017; de Oliveira et al., 2018; Zan et al., 2001), and typically lead to very low NO_3^- leaching (Smith et al., 2013). Yet, there have been only two studies on NO_3^- leaching losses in IWG compared to annual crops, on Alfisols and Mollisols soils (Culman et al., 2013; Jungers et al., 2019). Here we contrast the N cycling dynamic in a more comprehensive way, and beyond the NO_3^- leaching losses, in IWG and annual wheat, grown side-by-side at a site with a different soil order (Cambisol, i.e., Inceptisol in the USDA system) and in a drier climate than these previous two studies.

Although there has been a great focus on N fertilizer use efficiency and N uptake during the growing season of application, most of the N supply to annual crops comes from sources other than current year fertilization (Yan et al., 2020). Perennial agroecosystems may be more efficient than annual cropping systems at retaining N and recovering it in future growing seasons for several reasons. First, they translocate N to roots when aboveground biomass senesces (Li et al., 1992), which can be used in future years. Second, over time, perennial systems accumulate larger soil organic matter pools than annual systems (Culman et al., 2010), which could increase the endogenous N supply to crops and reduce fertilizer requirements. Yet, there has been no comparison between overall ecosystem recovery of fertilizer N applied to the perennial grain IWG versus annual wheat.

We aimed to characterize major differences in N cycling between IWG and annual wheat. We collected data on nitrate leaching, net nitrogen mineralization and nitrification, and the fate of ^{15}N-labeled fertilizer in plants and soils in annual and perennial grain systems in Alnarp, southern Sweden. We focused particularly on the dynamics in winter, when soil water content is high and hence N losses can be large and root and microbial biological sinks less active. We hypothesized that N losses would be lower and overall ecosystem recovery of fertilizer N applied would be greater in IWG than in annual winter wheat, because IWG has a root biomass that is larger and alive throughout the year. We also hypothesized that soil nitrogen mineralization would have increased over the three years since IWG was established, due to accumulation of soil organic matter from root inputs and reduced soil disturbance, accelerating microbially-mediated soil nitrogen cycling.

2. Methods

2.1. Site description and crop management

This study took place within the Agroecological Field Experiment (SAFE) at the Swedish Infrastructure for Ecosystem Science (SITES) Lönnstorp Field Research Station in Alnarp (55.666°N, 13.116°E), southern Sweden. The 2008–2021 mean annual precipitation is 537 mm yr^{-1} and mean annual temperature is 7.7 °C (SMHI, 2019). The study period was drier and warmer than normal (Table 1). The soil is a Eutric Cambisol within the FAO classification, which approximately translates to an Inceptisol in the USDA classification, with a pH ranging from 6.4 to 7.4 (Nilsson et al., 2010), a mix of sandy loam or loam texture across the plots, 13–24 % clay, 20–34 % silt, and 48–66 % sand, an average of 2.2 % soil organic matter in the top 20 cm. Within the SAFE experiment, we made measurements in three replicate 0.24 ha blocks in the "perennial" cropping system (Fig. S1) with IWG, *Thinopyrum intermedium*, (cycle 3 breeding population obtained from The Land Institute) and three replicate 0.12 ha blocks in reference plots. For the first year, the reference plots were of annual winter wheat (*Triticum aestivum*), variety Linus (a standard in Southern Sweden), part of an annual rotation of barley, winter oilseed rape, winter wheat, grass/legume ley cover crop and sugar beet. In the second year, our "reference" plots were left as bare fallow.

2.2. Planting and fertilization events prior to our measurement period (2016–2018)

Winter wheat was sown in row spacing of 12.5 cm at a seeding rate of 150 kg ha^{-1} on October 14, 2018, following a winter oilseed rape crop that was fertilized at a rate of 150 kg N ha^{-1} on April 8, 2018 and harvested on August 29, 2018 (Table 2). After the winter wheat harvest in 2019, the annual winter wheat plots were fallow. IWG was sown at a row spacing of 25 cm on May 2–3, 2016 (the seeding rate is unknown due to a machine malfunction). Fig. S1 depicts the experiment set up and Table 2 and Fig. S2 summarizes the timeline of events. IWG was fertilized in both May 2017 and May 2018 in the area of the field outside of our 4 × 6 m study plot at a rate of 40 kg N ha^{-1} yr^{-1} with an organic biofertilizer in the larger field plot (Table 2); 40 kg N ha^{-1} yr^{-1} is probably insufficient, but it was the maximum rate that could be applied due to regulation of cadmium levels in the biofertilizer. We wanted to align the rates and type of fertilizer used across our treatments, so we applied 160 kg N ha^{-1} of ammonium nitrate (NH_4NO_3) in 4 × 6 m plots in 3 replicate blocks to IWG on May 25, 2018 to match the rate that is typically applied in annual wheat in the region (Table 2). However, it is worth noting that 160 kg N ha^{-1} is a higher rate than would usually be applied to IWG, because of the lower amount of N harvested in those systems (roughly 50 kg N ha^{-1}), compared to roughly 100 kg N ha^{-1} in annual wheat (Table S1).

Table 1
Precipitation and temperature data over each winter (January 25–April 15) and year at this site. The last row contains means (standard deviations) from 2008 to 2021 using all available data from this weather station.

Time period	Total winter precipitation (mm winter^{-1})	Total annual precipitation (mm yr^{-1})	Mean winter temperature (°C)	Mean annual temperature (°C)
2019	128.4	582.8	4.3	9.9
2020	79.6	499.8	5.3	10.4
2008–2021	152.4 (52.7)	648.4 (183.5)	3.1 (1.5)	9.5 (0.9)

Table 2
Summary of key field operations with dates and descriptions preceding measurements (2016–2018) and during the study (2019–2020). The shaded cells correspond to the years when our experiment took place.

	2016	2017	2018	2019	2020
Planting					
Intermediate wheatgrass	Planted at a row spacing of 25 cm in May	---	---	---	---
Winter wheat	---	---	Planted at row spacing of 12.5 cm and seeding rate of 150 kg ha^{-1} in October	---	---
Fertilization					
Intermediate wheatgrass	---	40 kg N ha^{-1} with an organic biofertilizer in May	160 kg N ha^{-1} of ammonium nitrate in May	160 kg N ha^{-1} of ^{15}N- enriched ammonium nitrate split over April and May	---
Winter wheat	---	150 kg N ha^{-1} in April 2018 to prior winter oilseed rape crop	---	160 kg N ha^{-1} of ^{15}N- enriched ammonium nitrate split over April and May	---
Harvest					
Intermediate wheatgrass	---	---	---	Harvested in August	Harvested in August
Winter wheat	---	---	---	Harvested in August	(Fallow in 2020)

2.3. Fertilization events during the measurement period (2019–2020)

The observations took place in 2019 and 2020, i.e., three and four years since IWG was established in 2016. In 2019, both treatments were fertilized with 160 kg N ha^{-1} in our 4 × 6 m subfield plots (Fig. S1), and we split the application over two doses as is typical in the annual winter wheat spring fertilization for this experiment. 1.1 × 1.3 m microplots set aside within the 4 × 6 m subfield plots were fertilized on the same dates and rates with enriched ^{15}N fertilizer, which we describe in more detail below. The first 60 kg N ha^{-1}, 7 kg P ha^{-1}, and 7 kg K ha^{-1} of NH_4NO_3 NPK (27-3-3) were hand broadcast in the IWG and annual wheat plots on April 8, 2019 (Table 2). The remaining 100 kg N ha^{-1} in NH_4NO_3, 11 kg ha^{-1} of P, and 11 kg ha^{-1} of K were hand broadcast on April 19, 2019 in the IWG and annual wheat plots (Table 2). The IWG plots were not fertilized in 2020.

2.4. NO_3^- leachate measurements

Three replicate 100 cm deep soil pits were dug within our 4 × 6 m subfield plots (Fig. S1) in October and November of 2018 at least 3 m from the field edge in all of the IWG and annual winter wheat blocks. Campbell Scientific 257 soil matric potential sensors (Logan, UT, USA) were installed at 70 and 90 cm and Campbell Scientific CS655 water content reflectometers (Logan, UT, USA) were installed at 80 cm each in three replicate soil pits in two replicate blocks for both treatments (Fig. S1) by December 29, 2018. UMS SK20 suction lysimeters (UMS GmbH Munich, Germany) were installed in triplicate to a depth of 80 cm at a 45° angle between November 5–16, 2018 in all three blocks for both treatments (Fig. S1). The first lysimeter sampling in the winter wheat plots occurred January 9, 2019. Lysimeters were sampled every week when possible, and no more than two and a half weeks apart, using discontinuous vacuum sampling. To sample soil water, we added a pinch of thymol to each empty acid-washed 500 mL sample bottle, to prevent microbial activity, and then attached the bottles to the lysimeter tubing which was buried approximately 10 cm below the soil surface. A vacuum (-0.5 ± 0.025 bar) was applied to each sample bottle and the outlet tube was clamped to maintain the vacuum overnight. The sample bottles were left in insulated containers installed below the soil surface for 24–48 h and then collected in the same order in which they were attached. The vacuum did not always produce samples depending on the soil moisture content at the time of sampling. Samples were poured into acid-washed 20 mL scintillation vials, placed on ice packs in a cooler for transport, and frozen until analysis. Drainage below the rooting zone at this site generally occurs between December through May (Norberg and Aronsson, 2020). In the winter of 2019–2020, we filtered the samples to 0.2 μm using Sarstedt Filtropur S filters instead of using thymol to prevent microbial activity in the sample bottles so that the samples could be analyzed for ^{15}N-NO_3^- using the denitrifier method (Granger and Sigman, 2009) at the UC Davis Stable Isotope Facility using a ThermoFinnigan GasBench and PreCon trace gas concentration system connected to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer (Bremen, Germany), calibrated by NIST (National Institute of Standards and Technology, Gaithersburg, MD) NO_3^- standards USGS 32, USGS 34, and USGS 35. NH_4^+ and NO_3^- concentrations in soil leachate were analyzed on a 3HR Autoanalyzer from SEAL-analytical (Norderstedt, Germany). NH_4^+ concentrations were negligible in the first year, so in year 2 we only analyzed soil leachate NO_3^- concentrations on a Smartchem 170 discrete analyzer (Westco Scientific Instruments, Brookfield, CT).

2.5. Drainage and NO_3^- leaching calculations

Drainage below 80 cm was estimated based on Darcy's law, using the soil water potentials at 70 cm and 90 cm and volumetric water content at 80 cm, and van Genuchten (1980) estimation of unsaturated hydraulic conductivity, based on the equations in Huang et al. (2017) and Fan et al. (2014). The parameters of the unsaturated hydraulic conductivity (van Genuchten, 1980), θ_r, θ_s, n, and K_s, were calculated in the neural network predictions for water flow parameters based on soil texture and bulk density data (Table S2) for each plot using Rosetta v. 1.1 within HYDRUS 1-D v 4.17.0140 (Šimůnek et al., 2018). NO_3^- leaching was estimated by multiplying soil water fluxes below 80 cm with leachate NO_3^- concentrations. Concentrations were linearly interpolated between measurement dates within each season. Seasonal leachate rates were estimated by summing leaching amounts over each measurement period.

To calculate soil water fluxes below 80 cm, we applied Darcy's law to the slab of soil discretized over the slab of soil between the two soil water potential sensors 20 cm apart:

$$q_{80}(t) = \mathrm{K}(\theta) * \frac{H_{70} - H_{90} + 20}{20} \tag{1}$$

where $q_{80}(t)$ (cm d^{-1}) is the soil water flux at 80 cm soil depth at time t; H_{70} and H_{90} are the soil water potentials (cm) at 70 cm 90 cm depth; and K (cm d^{-1}) is the soil unsaturated hydraulic conductivity at 70–90 cm soil depth. K is estimated as a function of the soil volumetric water content θ (cm^3 cm^{-3}), measured at 80 cm, as

$$K(\theta) = Ks * \Delta_t * \left(\frac{\theta - \theta_r}{\theta_s - \theta_r}\right)^l * \left[1 - \left(1 - \left(\frac{\theta - \theta_r}{\theta_s - \theta_r}\right)^{\frac{1}{\left(1-\frac{1}{n}\right)}}\right)^{\left(1-\frac{1}{n}\right)}\right]^2 \tag{2}$$

where K_s (cm d^{-1}) is the soil saturated hydraulic conductivity; $\Delta_t$is the time step between measurements (d); θ (cm^3 cm^{-3}) is the soil volumetric water content measured at 80 cm; and θ_r and θ_s (cm^3 cm^{-3}) are the residual soil water content and saturated soil water content respectively, and m, n, and l are unitless parameters that depend on soil texture.

The soil water flux and the estimated leaching at time t were then obtained as:

$$NO_3^-\ (t) = \frac{mean(q_{80}(t))_i}{10} \times c(t) \tag{3}$$

where NO_3^- (t) is the NO_3^- leaching rate at 80 cm soil depth (kg N ha^{-1} d^{-1}); and $c(t)$ (mg N L^{-1}) is the NO_3^- concentration of the soil water at 80 cm depth at time t; $mean(q_{80}(t))_i$ is the mean drainage across replicates of treatment i (cm d^{-1}); and 10 converts to (kg N ha^{-1}). To thoroughly explore the variation with different combinations of drainage and the NO_3^- concentration of the soil water, we paired 25 randomly-sampled bootstrapped observations (with replacement) across the replicates for each date, treatment, and block ($n = 3$) for each of the leachate NO_3^- (mg N L^{-1}) measurements and 25 randomly-sampled bootstrapped observations (with replacement) across the replicates for each date, treatment, and block ($n = 2$) for daily drainage measurements (mm day^{-1}) to explore the variation across drainage and NO_3^- concentrations. Our drainage measurements were limited to only two out of three replicate treatment blocks due to availability of data loggers, so we bootstrapped drainage measurements for the third block from both of the first two blocks. Leachate concentrations were measured at all three blocks. Daily and cumulative drainage amounts across replicates were variable in both years for annual wheat and IWG (Fig. S3), so we used the mean daily drainage total within each treatment to calculate NO_3^- losses.

2.6. ^{15}N tracer experiment

To track the fate of N applied in fertilizer, we applied ^{15}N-enriched fertilizer to 1.1 × 1.3 m microplots within our 4 × 6 m subfield plots (Fig. S1) at the same total rate (160 kg N ha^{-1} yr^{-1}, except as explained below in two microplots where some adjustments had to be made), split over two application dates, which aligned with applications to the rest of the 4 × 6 m plot and were approximately the same time as fertilizer was applied to the whole fields. On April 8, 2019, 60 kg N ha^{-1} of 3.9 at.% $^{15}NH_4^{15}NO_3$, and 5.1 kg P ha^{-1} and 6.5 kg K ha^{-1} as KH_2PO_4 were applied in microplots where no fertilizer had previously been applied. On April 19, 2019, 100 kg N ha^{-1} of 3.9 at.% $^{15}NH_4^{15}NO_3$, and 12.6 kg P ha^{-1} and 16.0 kg K ha^{-1} as KH_2PO_4 were applied in the microplots. We divided the 1.1 m × 1.3 m microplots into 20 equal areas (28.5 cm × 25.7 cm areas) and sprayed the fertilizer by hand with small spray bottles to apply the ^{15}N-labeled fertilizer as evenly as possible. We also established one 1.1 m × 1.3 m reference microplot where no fertilizer was applied so that we could measure the natural abundance patterns of ^{15}N derived from the soil within each treatment and block (Fig. S1). We used a watering can to apply approximately 10 L of water after each microplot was fertilized to wash fertilizer off of the leaves of the plant and to the soil. Two microplots were accidentally overfertilized by a malfunction with the broadcast spreader, so we added two replacement microplots (57 cm long × 57 cm wide × 20 cm deep steel microplots) in the area that was fertilized with 160 kg N ha^{-1} of unlabeled fertilizer. We added an adjustment of 6 kg N ha^{-1} equivalent of 98 % enriched $^{15}NH_4^{15}NO_3$ on May 11, 2019 in the replacement microplots to raise the N application to 3.9 at.% $^{15}NH_4^{15}NO_3$ and added approximately 5 L of water to wash the fertilizer off of the leaves and to the soil.

The annual wheat microplots were harvested by hand on August 7, 2019. We sampled a 60 × 60 cm square from the middle of each of the original 1.1 m × 1.3 m microplots and sampled the full 57 cm × 57 cm of the two replacement microplots steel microplots where over fertilization had accidentally occurred. The IWG plots were harvested by hand on in the same way in the last two weeks of August in both 2019 and 2020. We separated heads from straw, dried all aboveground biomass at 60 °C to a constant weight, threshed the heads, and weighed straw, seeds and heads separately.

To sample roots and soil, we took three in-row and between-row pairs of soil cores from 0 to 5 cm depth using a steel cylinder (7.14 cm diameter) from each microplot. Five 0–100 cm cores were collected from each microplot with a Wintex Agro MCL3 (Vilhemsborgvej, Denmark) 1.87 cm diameter hydraulic sampler. All cores in the reference plots and two of the IWG ^{15}N-enriched plot soil cores were sampled between August 21–22, 2019. A repair needed by the hydraulic sampler delayed the rest of the samples, and the remaining ^{15}N-enriched plots were sampled September 11, 2019. In year two, soil samples were collected on August 31, 2020 from just the IWG plots. Soil samples were kept on ice and then in a refrigerator until the roots could be sampled.

Using the soil core method (Ravindranath and Ostwald, 2008) to estimate root biomass per unit area, we measured the wet masses of the soil composite samples at each depth, then took a subset of 400 g of soil from the 0–5 cm depth and 200 g of soil at each depth interval from 5 to 100 cm from each microplot (the one reference microplot and both ^{15}N microplots), and separated roots using a 2 mm sieve. Roots were carefully washed to ensure all of the soil was removed, then dried at 60 °C to a constant weight. We then extrapolated the dry root biomass data to a per area basis for each depth.

Aboveground biomass samples were coarsely milled, sub-sampled, then finely milled to 0.5 mm. Root samples above 100 mg were finely milled on a Wiley mill to 0.5 mm, root samples between 6 mg and 100 mg were ball milled, and root samples below 6 mg were broken apart by hand with a razor blade. Soil samples with no visible roots were ball milled to <0.5 mm. All homogenized plant and soil materials were then packed into tin capsules and weighed for elemental and isotopic analysis. The total carbon and nitrogen contents were measured on an elemental analyzer (Flash 2000, Thermo Scientific, Bremen, Germany) coupled in continuous flow mode to a Thermo Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Materials used for elemental analyzer mass calibration include acetanilide (Merck, Darmstadt, Germany), soil standards (Elemental Microanalysis, Okehampton, UK) or peach leaves (NIST 1547; National Institute of Standards and Technology, Gaithersburg, MD, USA).

We calculated fertilizer N uptake as follows (Guidelines on Nitrogen Management in Agricultural Systems, 2008):

$$N_{dff}(\%) = \frac{AP\ ^{15}N\ enriched\ sample - AP\ ^{15}N\ unfertilized, unlabeled\ reference\ sample}{AP\ ^{15}N\ fertilizer - AP\ ^{15}N\ unfertilized, unlabeled\ reference\ sample} \times 100 \tag{4}$$

$$Fertilizer\ N\ uptake\ (kg\ fertilizer\text{–}N\ ha^{-1}) = \frac{dry\ matter\ (kg\ \ ha^{-1}) \times N\ (\%)}{100} \times \frac{N_{dff}(\%)}{100} \tag{5}$$

$$Fertilizer\ recovery(\%) = \frac{Fertilizer\ N\ uptake(kg\ fertilizer - N\ \ ha^{-1})}{Fertilizer\ \ applied(kg\ fertilizer - N\ \ ha^{-1})} \times 100 \tag{6}$$

where N_{dff} is N derived from ^{15}N-labeled fertilizer (%), *AP* ^{15}*N enriched sample* is the atom % of ^{15}N in the enriched sample, *AP* ^{15}*N unfertilized reference sample* is the atom % of ^{15}N in the unfertilized reference sample, and *AP* ^{15}*N fertilizer* is the atom % of applied $^{15}NH_4^{15}NO_3$ fertilizer (3.9220 % ^{15}N).

2.7. N mineralization incubations

We measured net nitrogen mineralization and nitrification rates across three blocks of the IWG and annual wheat treatments. Using the "buried bag" method (Hart et al., 1994), we incubated intact soil cores which were removed from the soil, enclosed in plastic bags, and then returned to the soil to maintain the soil at field temperature and prevent changes in soil moisture content or leaching out from the bottom. 4.5 cm diameter by 10 cm deep PVC cores ($n = 4$ replicate pairs per plot), were used for the first two incubation periods, but then sturdier 7.5 cm diameter by 10 cm deep steel cores in the field ($n = 3$ replicate pairs per plot) were used in the two later incubation periods because the soils were drier. Incubations ranged from 61 to 68 days. Each measurement was taken in adjacent pairs across the plot, with an initial core sampled on day 1, and a final core left in the field. Subsamples of soil collected at the initial and final times were extracted in 2 M potassium chloride, frozen, and later analyzed for NO_3^- and NH_4^+ on a Smartchem 170 discrete analyzer (Westco Scientific Instruments, Brookfield, CT). Net mineralization was calculated by subtracting the initial from the final amount of inorganic N, and net nitrification was calculated as the change in NO_3^- over the incubation period (Hart et al., 1994). PRS cation and anion resin nutrient probes with a surface area of 10 cm^2 were vertically inserted in the top 10 cm to measure the baseline soil conditions across a wider range of nutrients (Harrison and Maynard, 2014) on December 29, 2018 and collected on January 22, 2019 (for 24 days) and analyzed. Probes were analyzed for adsorbed NO_3^--N and NH_4^+-N using colorimetric flow injection analysis and for Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, and Cd using inductively coupled plasma spectrometry.

2.8. Statistical analyses

All statistical analyses were done in R (v4.1.3) (R Core Team, 2020). We used linear mixed effects models (Bates et al., 2018) with fixed effects such as crop type (treatment), and random effects for block to test for differences between treatments in all of the statistical tests in Tables S3–12. Linear mixed effects models took the general form:

$$\text{response variable} = \beta_0 + \gamma_i + \beta_1 \times \text{treatment} + \varepsilon \tag{7}$$

where response variable is indicated in each table (such as NO_3^- concentrations (mg N L^{-1}), NO_3^- leaching through time, cumulative NO_3^- leached and more); β_0 is the fixed-effect intercept; γ_i is the random variation in the intercept for each block *i*; β_1 is the coefficient of *treatment* (a dummy variable for either annual wheat or IWG—as noted in each table); and ε is unexplained residual variation. *P*-values on linear mixed-effects models were calculated with the *lmerTest* package (Kuznetsova et al., 2017). Marginal and conditional R^2 values were calculated with the *MuMIn* package (Bartoń, 2020).

3. Results

3.1. NO_3^- leaching

NO_3^- concentrations in soil leachate were nearly two orders of magnitude lower in IWG (mean of 0.15 mg N L^{-1}) than in annual wheat (mean of 14.3 mg N L^{-1}) the first year of the study when both crops were in the field ($P < 0.01$, Fig. 1, Table S3). There was high variation in drainage, estimated based on soil moisture probes and tensiometers, across replicates (Fig. S3), but no consistent differences between treatments. Since NO_3^- concentrations were much lower in IWG but drainage was not very different between IWG and annual wheat, low NO_3^- leaching concentrations resulted in much lower total NO_3^- leaching in IWG in both years of observation (Table S4). Specifically, the mean IWG NO_3^- flux (0.1 kg N ha^{-1}) was only 1.8 % of that in the annual wheat plots (5.6 kg N ha^{-1}). In the second year, the mean NO_3^- flux in the IWG plots (3.1 kg N ha^{-1}) was 7.6 % of that in the former wheat plots, that were left fallow (41.0 kg N ha^{-1}) (Fig. 1c, d, Table S5).

3.2. Fate of ^{15}N and nitrogen use efficiency

In addition to measuring NO_3^- fluxes, we also tracked the fate of ^{15}N fertilizer in the plant, soil, and leachate to determine plant and ecosystem ^{15}N fertilizer recovery. We recovered more ^{15}N fertilizer in the soil in IWG than annual wheat at all depths ($P < 0.05$, Fig. 2a, Table S6), and total soil ^{15}N fertilizer recovery in IWG tended to be higher than in annual wheat (year one, 2019; Fig. 3c, Table S6). Total plant recovery of fertilizer (Fig. 3a) was greater in annual wheat—driven primarily by the large uptake of N in annual seeds. Total ecosystem recovery in the first year (the sum of plant and soil recovery as measured in August 2019, less the NO_3^- leaching fertilizer losses that followed in the winter of 2019–2020) was similar and remarkably high in both treatments, with an average of 92.1 % recovery in IWG and 99.7 % recovery in annual wheat (Fig. 3e, Table S1).

Straw biomass was higher in IWG (mean of 11.0 Mg ha^{-1}) than in annual wheat (mean of 7.3 Mg ha^{-1}), and root biomass at each depth was higher in IWG than in annual wheat (Fig. S4a, Table S7). The total belowground biomass to 1 m depth was about an order of magnitude greater in IWG, compared to annual wheat (Fig. S4c, Table S8). Conversely, annual wheat had much higher seed yield than IWG, with a mean of 9.8 Mg ha^{-1} compared to a mean of 0.5 Mg ha^{-1} in IWG (Fig. S4a, Table S7), and thereby greater total aboveground biomass (Fig. S4c, Table S8).

In the second year of measurements, after the ^{15}N fertilizer was applied, we also measured ^{15}N concentrations in soil leachate NO_3^-. Although there were large treatment differences in cumulative NO_3^- leaching fluxes, the proportion of ^{15}N-labeled fertilizer in leachate at 80 cm was low in both treatments. ^{15}N-labeled fertilizer accounted for only 0.2–0.7 % of N leached in the perennial treatment and 2.0–2.4 % of N leached in the fallow field that were previously annual wheat (Fig. 4, Table S9). The low ^{15}N leaching losses contributed to high rates of fertilizer recovery later that year in the 2020 harvest—much of ^{15}N-labeled fertilizer remained in the IWG plant and soil system at that time (Fig. 3f).

3.3. Soil nitrogen and carbon dynamics

In addition to tracking the fate of applied inorganic N, we also investigated N mineralization and nitrification dynamics, over year one. Using in-situ soil core incubations, we found that the net rates of N cycling in the top 10 cm did not differ between the treatments (Fig. 5a, b, and Table S10). There was more NH_4^+ and total inorganic N in the top 10 cm in IWG plots compared to annual wheat plots in January (~4 times more total inorganic N) and March (~2 times more total inorganic N) of 2019 measured at the start of each incubation period, but no differences for NO_3^- or at other measurement dates (Fig. S6a, b, c, Table S11).

We did not find evidence suggesting that the total soil carbon or total N pools increased at most depth increments from 0 to 100 cm after three years under a perennial crop by 2019 (Fig. S7). There were some differences in median soil carbon content between IWG and annual wheat between 40 and 80 cm (Fig. S7), but we believe these are due to higher carbonates in the subsoil of the IWG plots.

However, there were treatment differences in inorganic N at depth. Like the patterns observed for NO_3^- in soil water, soil inorganic N concentrations (NO_3^- + NH_4^+ μg N g soil^{-1}) were often less than half in IWG compared to annual wheat at several depth increments (Table S12). IWG maintained lower amounts of inorganic N at every depth we measured in December

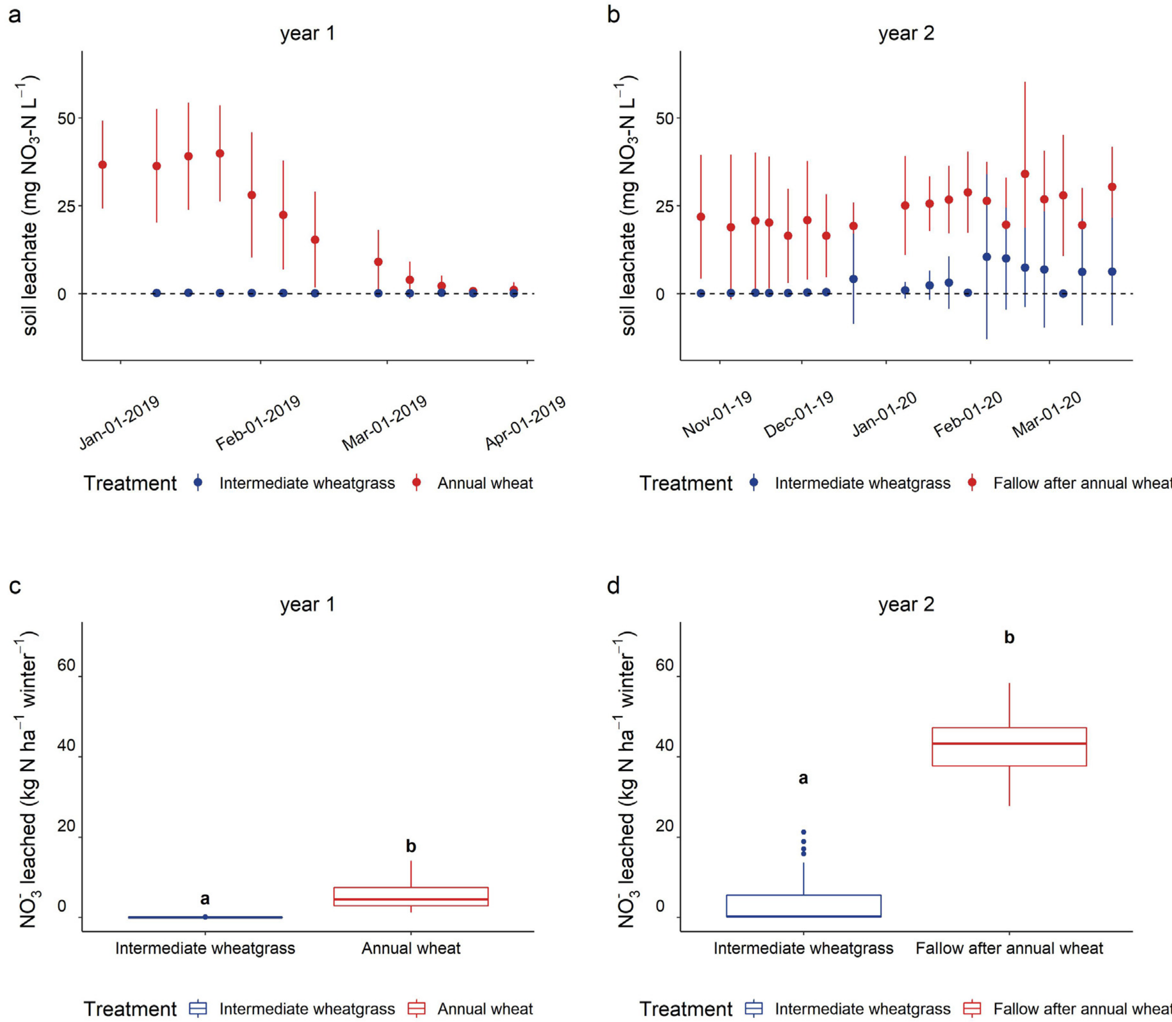


Fig. 1. Soil leachate NO_3–N concentrations at 80 cm depth vs time in year 1 (a) and year 2 (b), and total NO_3^- leached over the winter study period in year 1 (c) and year 2 (d). Each point (a, b) represents the mean across blocks and replicates, the lines are standard deviations, and the colors correspond to different treatments. The box edges (c, d) correspond to the first and third quartiles, and the middle line is the median across sums of bootstrapped estimates ($n = 25$ for each field and treatment) of total NO_3^- leached per study period (kg N ha^{-1} $winter^{-1}$). The whiskers (c, d) display 1.5 times the interquartile range, and the points are outliers. Significant differences ($P < 0.05$) between treatments are indicated with different letters (c, d). No leachate measurements were taken from mid-May 2019 to September 2019 because the soil water content was too low during the growing season; each season is summed over our $winter^{-1}$ when soil moisture was sufficient for drainage to occur (c, d) which was between December 22, 2018 and April 25, 2019 in year one, and between November 1, 2019 and May 15, 2022 year two.

of 2018, and all depths except 0–20 cm in August 2019 (Fig. S8a, b, Table S12).

4. Discussion

4.1. Nitrate leaching lower in IWG than annual wheat

IWG had over a hundred-fold lower cumulative NO_3^- leaching than annual wheat (Fig. 1). The patterns of ^{15}N fertilizer recovery in IWG and annual wheat aligned closely with biomass accumulation patterns, but overall ecosystem recovery was surprisingly high in both cropping systems in the first growing season after ^{15}N fertilizer was applied, and a large proportion of fertilizer N remained in the IWG system after two growing seasons (Figs. 2, 3).

In both years of our study, median NO_3^- leaching rates were >100 times higher in the annual wheat plots than IWG—which had been established for three years at the start of our leaching measurements in 2019. Despite anomalously lower winter precipitation in the second year—80 mm compared to 128 mm in year one and the local long term mean of 152 mm—and perhaps aided by a warmer than average winter in 2020 (Table 1), leaching losses were higher in both treatments in 2020 than in 2019 (Fig. 1d). In annual wheat, the higher leaching loss is likely due to plots left fallow in the rotation in year two. In IWG, we suspect that N limitation may have been overcome after applying a much higher fertilization rate (160 kg N ha^{-1} in 2018 and 2019) than would typically be harvested per year (roughly 50 kg N ha^{-1}, Table S1). Our cumulative NO_3^- leaching rates for annual wheat fall close to the typical range of N losses from arable land in Sweden of 15–45 kg N ha^{-1} yr^{-1} (Stenberg et al., 1999) in year one, and sometimes exceed that range in year two when the annual wheat plots were left fallow. To make a robust comparison and to investigate the different pools where fertilizer N may end up, we limited the experimental design to just two cropping systems (annual wheat, then rotated to bare fallow, and IWG) and one N fertilization rate, which is high for IWG. However, over time, perennial grain cropping systems may be less reliant on annual N fertilization, which could both reduce the energy footprint from the N fertilizer and reactive N footprints of these agroecosystems.

Our findings align well with previous conclusions that NO_3^- leaching in annual crops is much higher than in IWG (Culman et al., 2013; Jungers et al., 2019), though there is some notable variation within and across studies. In Minnesota, USA, soil solution NO_3–N concentrations were two orders of magnitude lower in IWG than maize, and average NO_3^- leaching was 0.2 kg N ha^{-1} $season^{-1}$ for IWG compared to 21.7 kg N ha^{-1} yr^{-1} for

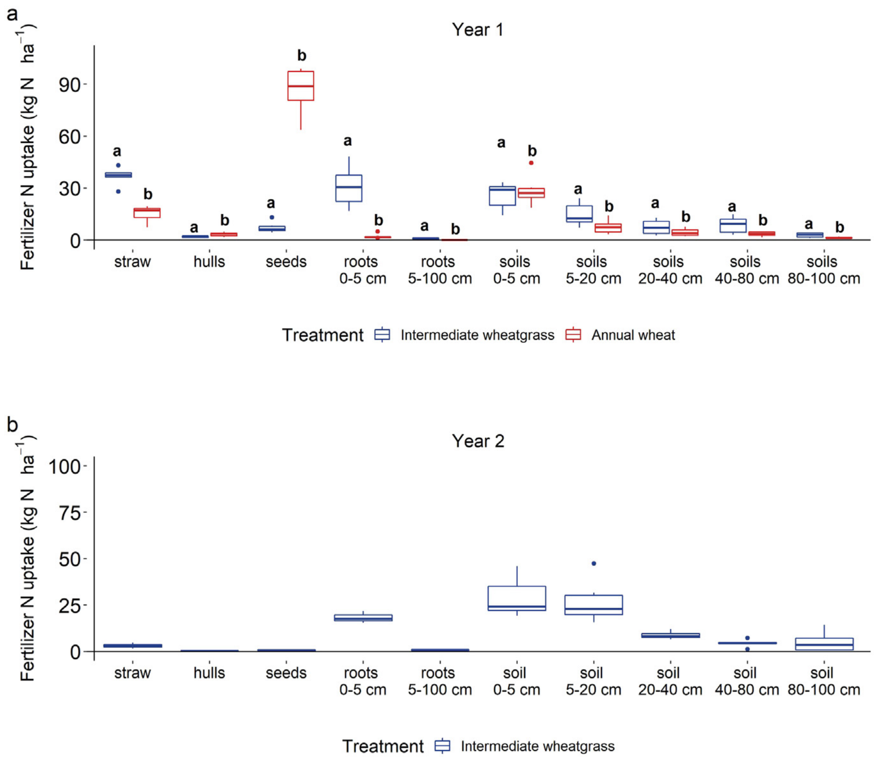


Fig. 2. Recovery of N fertilizer applied in spring of 2019 in the plant and soil across treatment and year; harvest 2019 (year one) (a) and harvest 2020 (year two) (b). Fertilizer N uptake is determined by the amount of N in each pool multiplied by the percent of N derived from fertilizer, as indicated by the proportion of ^{15}N contained in each pool. The colors indicate the treatment, the outer edges of the boxes are the first and third quartiles, and the middle line is the median. The whiskers display 1.5 times the interquartile range, and the individual points are outliers. Significant differences between treatments ($P < 0.05$) within each biomass category are indicated with different letters (a).

maize when both were fertilized at 160 kg N ha^{-1} season^{-1} (Jungers et al., 2019). In Michigan, USA, NO_3^- leaching in two-year old IWG was 9.9 kg N ha^{-1} season^{-1} in versus 69.8 kg N ha^{-1} season^{-1} in annual wheat (Culman et al., 2013). At the lower fertilization rate, 90 kg N ha^{-1} yr^{-1}, NO_3^- leaching in the second year dropped to 0.5 kg N ha^{-1} season^{-1} in IWG compared to 27.5 in kg N ha^{-1} season^{-1} in annual wheat (Culman et al., 2013). We found strong evidence at our site in Sweden, with different soils and a drier climate, that IWG dramatically reduces nitrate leaching losses. The dependence of fertilizer N use efficiency in perennial grains on climatic conditions and soils underscore the need for additional exploration. Also, we note the need to quantify N dynamics in more complex cropping systems and diverse management practices, such as perennial grains intercropped with N-fixing legumes.

4.2. Lower grain yields in IWG than annual wheat

The mean annual wheat grain yield of 8.2 Mg ha^{-1} yr^{-1} was consistent with average yields in the same region as our study site, Skåne County, where average yield was 8.3 Mg ha^{-1} yr^{-1} between 2014 and 2019 (but excluding 2018, which was anomalously low due to a severe drought) (Statistiska centralbyrån, 2019). Grain yields in the perennial IWG are still quite variable in this earlier stage of domestication and more comparable to lower yielding cereal crops such as quinoa; for example, mean global yields were 0.9 Mg ha^{-1} yr^{-1} for quinoa between 2015 and 2019 (Food and Agriculture Organization of the United Nations, 1997). IWG mean seed yield in our study was 0.51 Mg ha^{-1} yr^{-1} in 2019 and 0.29 Mg ha^{-1} yr^{-1} in 2020, which fall in the low to middle range of measurements from previous studies (0.1 to 1.7 Mg ha^{-1} yr^{-1}) and is consistent with the decline in yield observed after the second season in other studies (Culman et al., 2013; Jungers et al., 2017).

Until grain yields are raised to be more comparable with annual wheat, IWG could be used as a dual grain-forage system (Hunter et al., 2020; Pugliese et al., 2019), as a tool to reduce erosion and nutrient loss on marginal lands (Asbjornsen et al., 2014; Culman et al., 2013; Jungers et al., 2019), or to be rotated between annual crop cycles to build soil health (Ryan et al., 2018). The IWG seed in this study was from The Land Institute's breeding cycle 3 produced in 2014. The Land Institute is currently on cycle 11 and has implemented advanced breeding techniques, such as genomic selection (Crain et al., 2021a; Larson et al., 2019), which enable more rapid increases in grain yields and should accelerate the process of making perennial grains a realistic substitute for their annual counterparts.

4.3. N cycle patterns in IWG and annual wheat

We found some important differences and similarities in the N cycles between perennial and annual crops. As expected, belowground biomass in IWG far exceeded belowground biomass of annual wheat and was an important sink of fertilizer N (Figs. 2, S4). In contrast to our expectation that reduced disturbance in IWG compared to annual wheat would, over the course of several years, increase soil N supply and N cycling rates, no treatment differences in net N mineralization or nitrification were found (Fig. 5a, b). Although we did not detect differences in N cycling after three years of IWG cover, it would be important to test for these effects after longer time periods to inform IWG management.

Although current year fertilizer N is often thought to be highly susceptible to being lost, only 0.2–2.4 % of leached NO_3–N was derived from fertilizer (Table S9), demonstrating that current year's fertilizer N was not a large source of NO_3^- leaching, regardless of cropping system. A similar effect was observed in winter wheat in Portugal, where a higher but still low proportion (6.3 %) of leachate-N was lost in the second and third years after fertilization (following a dry first year after fertilization when no leaching occurred) (Carranca et al., 1999). Considering that the majority of N in crops (Yan et al., 2020) and N in NO_3^- leaching (this study) is

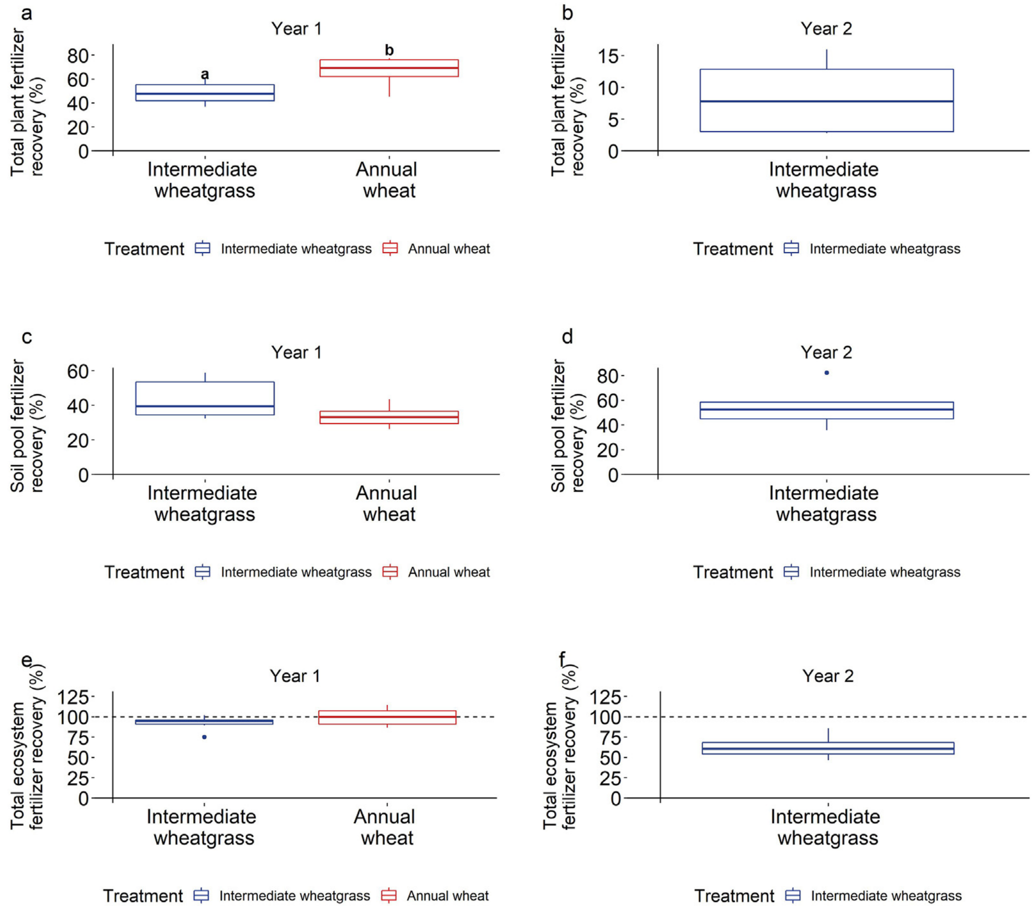


Fig. 3. Recovery of total fertilizer applied (% recovery of the total fertilizer applied in each group) in plant pools in 2019 (a) and 2020 (b), soil pools in 2019 (c) and 2020 (d), and in plant and soil pools combined (total ecosystem recovery) in 2019 (e) and 2020 (f). Significant differences between treatments ($P < 0.05$) within each biomass category are indicated by different letters, the colors indicate the treatment, the outer edges of the boxes are the first and third quartiles, and the middle line is the median. The whiskers display 1.5 times the interquartile range, and the individual points are outliers.

derived from sources other than the current year's fertilization, it seems likely that N pool substitution plays a large role in both N use efficiency in crops and N losses. N pool substitution, when much of the ^{15}N applied in a tracer study is immobilized in microbial biomass and unlabeled N is mineralized, is often observed in ^{15}N fertilizer tracer studies (Peoples et al., 2009). These low contributions of current year N fertilizer to N leaching highlight the complexity of the N cycle in agroecosystems and the need to consider the N cycle over multiple years.

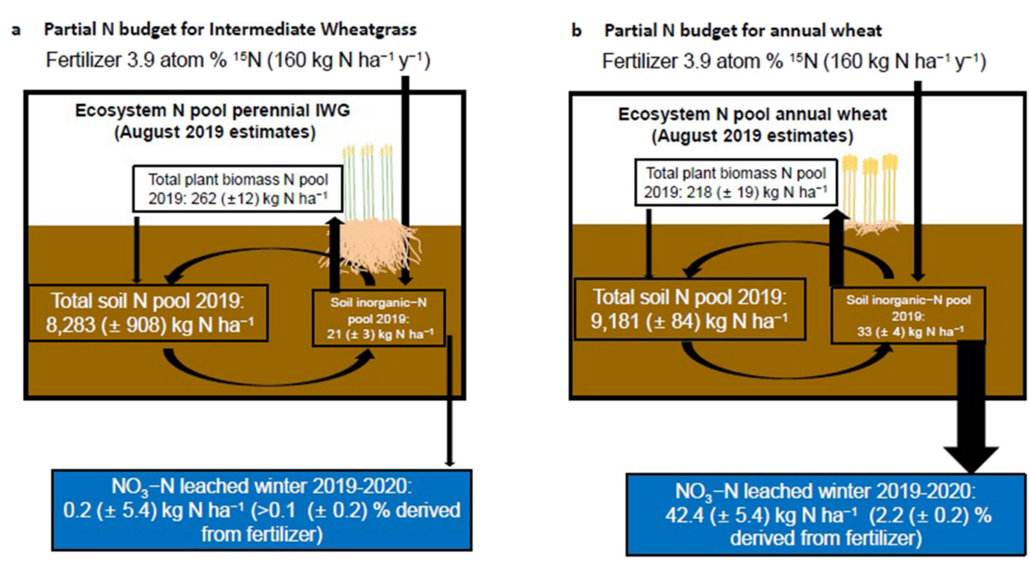


Fig. 4. Partial N budget depicting ecosystem flow of N from fertilizer to plants, soil N pools, and NO_3^- leaching in IWG (a) and annual wheat (b). Median pool sizes are reported in the boxes with standard deviations in parentheses.

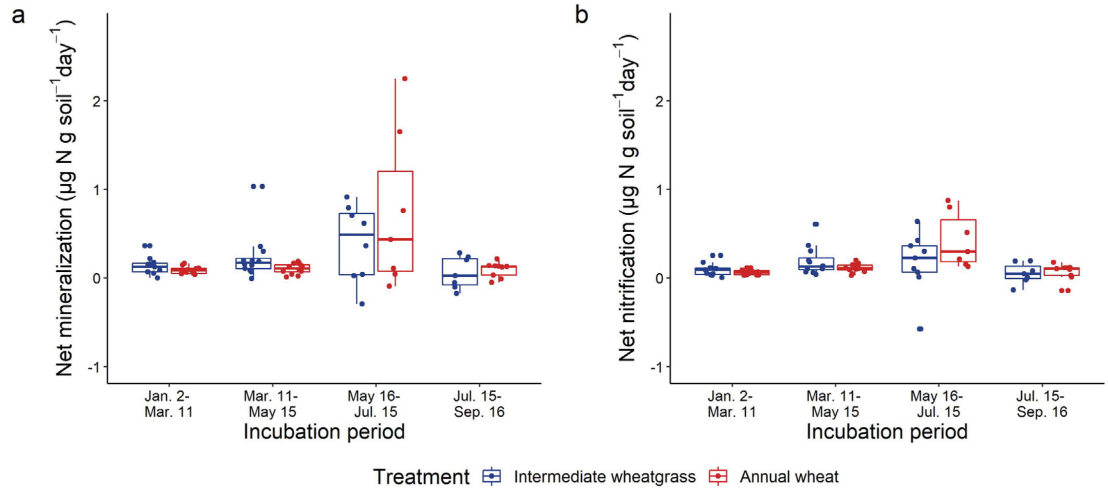


Fig. 5. Net N mineralization (a), and net nitrification (b) in the top 10 cm of soil through several incubation periods over the first year of measurements (2019), expressed as daily rates. The colors indicate the treatment, the points are individual measurements, the outer edges of the boxes are the first and third quartiles, and the middle line is the median, and the whiskers display 1.5 times the interquartile range. There were no significant differences between treatments.

We did not find evidence to support our hypothesis that ecosystem recovery of fertilizer N would be higher in perennial IWG than annual wheat in year 1 (2019), though given the high rates of NO_3^- leaching, we might predict cumulative N loss over multiple years would be higher in annual wheat. Surprisingly, both crops retained a large proportion of fertilizer N applied in the current year (mean of 96 %, Table S1). This retention rate falls at the high end of the range, well above the mean of 62 % fertilizer-N recovery in a meta-analysis of temperate grain systems (Gardner and Drinkwater, 2009), but similar to a study of a perennial grass in Sweden (Hansson and Pettersson, 1989). IWG, which we were able to track for a second year after fertilizer was applied, still had large stores of fertilizer N in its biomass and in the soil system for potential use in subsequent years (Figs. 2b, 3e). Roughly 30–60 % of the fertilizer-N applied was measured in the soil pool (Fig. 3c, d). We suspect that rapid microbial immobilization of fertilizer-N in the soil and lack of intense precipitation events held nearly all the current year's fertilizer-N in place, even as older N is exported.

Despite the high rate of fertilizer we applied, nitrate leaching under IWG remained low. Reducing nitrate leaching losses at large scales could improve drinking and ambient water quality for populations and ecosystems surrounding productive agricultural regions. Avoiding N pollution to the environment also limits subsequent indirect greenhouse gas emissions (nitrous oxide) (Turner et al., 2015), and may alleviate some of the threat nitrogen pollution poses to biodiversity (Payne et al., 2017). Our findings demonstrate that perennial grains have potential to dramatically reduce harmful N losses from grain production, thus reducing some of the negative impacts of agriculture.

Data availability

The data and code used for these analyses and visualizations are available at https://doi.org/10.5281/zenodo.6904142.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study has been made possible by the Malone Family Land Preservation Foundation, the National Science Foundation's Graduate Research Fellowship (DGE 1644869) and Graduate Research Opportunities Worldwide programs, also supported by Vetenskapsrådet and the Swedish Research Council. This work was also supported by Swedish Research Council grant number 2018-00646, and Swedish Infrastructure for Ecosystem Science (SITES) Lönnstorp. The authors would like to especially thank Ryan Davidson, Louise Rehnström, Chris Forsythe, Erik Rasmusson, Ana Barreiro and Erik Steen-Jensen from the Swedish University of Agricultural Sciences for all of their help on this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.159255.

References

Asbjornsen, H., Hernandez-Santana, V., Liebman, M., Bayala, J., Chen, J., Helmers, M., Ong, C.K., Schulte, L.A., 2014. Targeting perennial vegetation in agricultural landscapes for enhancing ecosystem services. Renew. Agric. Food Syst. 29, 101–125. https://doi.org/10.1017/S1742170512000385.

Bajgain, P., Anderson, J.A., Crain, J.L., Cattani, D.J., Larson, S.R., Altendorf, K.R., ... Turner, M.K., 2022. Breeding Intermediate Wheatgrass for Grain Production. Plant Breed. Rev. 46.

Bartoń, K., 2020. MuMIn: Multi-Model Inference. (in press).

Bates, D., Maechler, M., Bolker, B., Walker, S., 2018. Linear Mixed-Effects Models Using "Eigen" and S4.

Bowles, T.M., Atallah, S.S., Campbell, E.E., Gaudin, A.C.M., Wieder, W.R., Grandy, A.S., 2018. Addressing agricultural nitrogen losses in a changing climate. Nat. Sustain. 1, 399–408. https://doi.org/10.1038/s41893-018-0106-0.

Carranca, C., de Varennes, A., Rolston, D.E., 1999. Variation in N-recovery of winter wheat under Mediterranean conditions studied with 15N-labelled fertilizers. Eur. J. Agron. 11, 145–155. https://doi.org/10.1016/S1161-0301(99)00027-1.

Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, nitrogen-use efficiency, and nitrogen management. AMBIO J. Hum. Environ. 31, 132–140. https://doi.org/10.1579/0044-7447-31.2.132.

Crain, J., DeHaan, L., Poland, J., 2021. Genomic prediction enables rapid selection of high-performing genets in an intermediate wheatgrass breeding program. Plant Genome n/a, e20080. https://doi.org/10.1002/tpg2.20080.

Crain, J., Haghighattalab, A., DeHaan, L., Poland, J., 2021b. Development of whole-genome prediction models to increase the rate of genetic gain in intermediate wheatgrass (Thinopyrum intermedium) breeding. Plant Genome 14, e20089. https://doi.org/10.1002/tpg2.20089.

Crews, T.E., 2005. Perennial crops and endogenous nutrient supplies. Renew. Agric. Food Syst. 20, 25–37. https://doi.org/10.1079/RAF200497.

Crews, T.E., Blesh, J., Culman, S.W., Hayes, R.C., Jensen, E.S., Mack, M.C., Peoples, M.B., Schipanski, M.E., 2016. Going where no grains have gone before: from early to mid-succession. Agric. Ecosyst. Environ. 223, 223–238. https://doi.org/10.1016/j.agee.2016.03.012.

Crews, T.E., Rumsey, B.E., 2017. What agriculture can learn from native ecosystems in building soil organic matter: a review. Sustainability 9, 578. https://doi.org/10.3390/su9040578.

Culman, S.W., DuPont, S.T., Glover, J.D., Buckley, D.H., Fick, G.W., Ferris, H., Crews, T.E., 2010. Long-term impacts of high-input annual cropping and unfertilized perennial grass production on soil properties and belowground food webs in Kansas, USA. Agriculture, Ecosystems & Environment, Special Section Harvested Perennial Grasslands:

Ecological Models for Farming's Perennial Future. 137, pp. 13–24. https://doi.org/10.1016/j.agee.2009.11.008.
Culman, S.W., Snapp, S.S., Ollenburger, M., Basso, B., DeHaan, L.R., 2013. Soil and water quality rapidly responds to the perennial grain kernza wheatgrass. Agron. J. 105, 735–744. https://doi.org/10.2134/agronj2012.0273.
Daly, E., Kim, K., Hernandez-Ramirez, G., Flesch, T., 2022. Perennial grain crops reduce N2O emissions under specific site conditions. Agric. Ecosyst. Environ. 326, 107802. https://doi.org/10.1016/j.agee.2021.107802.
de Oliveira, G., Brunsell, N.A., Sutherlin, C.E., Crews, T.E., DeHaan, L.R., 2018. Energy, water and carbon exchange over a perennial Kernza wheatgrass crop. Agric. For. Meteorol. 249, 120–137. https://doi.org/10.1016/j.agrformet.2017.11.022.
Fan, Z., Lin, S., Zhang, X., Jiang, Z., Yang, K., Jian, D., Chen, Y., Li, J., Chen, Q., Wang, J., 2014. Conventional flooding irrigation causes an overuse of nitrogen fertilizer and low nitrogen use efficiency in intensively used solar greenhouse vegetable production. Agric. Water Manag. 144, 11–19. https://doi.org/10.1016/j.agwat.2014.05.010.
Food and Agriculture Organization of the United Nations, 1997. FAOSTAT Statistical Database.
Fowler, D., Coyle, M., Skiba, U., Sutton, M.A., Cape, J.N., Reis, S., Sheppard, L.J., Jenkins, A., Grizzetti, B., Galloway, J.N., Vitousek, P., Leach, A., Bouwman, A.F., Butterbach-Bahl, K., Dentener, F., Stevenson, D., Amann, M., Voss, M., 2013. The global nitrogen cycle in the twenty-first century. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 368, 20130164. https://doi.org/10.1098/rstb.2013.0164.
Galloway, J.N., Aber, J.D., Erisman, J.W., Seitzinger, S.P., Howarth, R.W., Cowling, E.B., Cosby, B.J., 2003. The nitrogen cascade. Bioscience 53, 341–356. https://doi.org/10.1641/0006-3568(2003)053[0341:TNC]2.0.CO;2.
Gardner, J.B., Drinkwater, L.E., 2009. The fate of nitrogen in grain cropping systems: a meta-analysis of 15N field experiments. Ecol. Appl. 19, 2167–2184. https://doi.org/10.1890/08-1122.1.
Granger, J., Sigman, D.M., 2009. Removal of nitrite with sulfamic acid for nitrate N and O isotope analysis with the denitrifier method. Rapid Commun. Mass Spectrom. 23, 3753–3762. https://doi.org/10.1002/rcm.4307.
Guidelines on Nitrogen Management in Agricultural Systems, 2008. Training Course Series (CD-ROM). International Atomic Energy Agency, Vienna.
Hansson, A.-C., Pettersson, R., 1989. Uptake and above- and below-ground allocation of soil mineral-N and fertilizer-15N in a perennial grass ley (Festuca pratensis). J. Appl. Ecol. 26, 259–271. https://doi.org/10.2307/2403666.
Harrison, D.J., Maynard, D.G., 2014. Nitrogen mineralization assessment using PRSTM probes (ion-exchange membranes) and soil extractions in fertilized and unfertilized pine and spruce soils. Can. J. Soil Sci. 94, 21–34. https://doi.org/10.4141/cjss2012-064.
Hart, S.C., Stark, J.M., Davison, E.A., Firestone, M.K., 1994. Nitrogen mineralization, immobilization, and nitrification. In: Weaver, R.W. (Ed.), Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties. Soil Science Society of America Book Series. Soil Science Society of America, Madison, Wis., USA, pp. 985–1018.
Huang, T., Ju, X., Yang, H., 2017. Nitrate leaching in a winter wheat-summer maize rotation on a calcareous soil as affected by nitrogen and straw management. Sci. Rep. 7. https://doi.org/10.1038/srep42247.
Hunter, M.C., Sheaffer, C.C., Culman, S.W., Lazarus, W.F., Jungers, J.M., 2020. Effects of defoliation and row spacing on intermediate wheatgrass II: forage yield and economics. Agron. J. 112, 1862–1880. https://doi.org/10.1002/agj2.20124.
Jungers, J.M., DeHaan, L.H., Mulla, D.J., Sheaffer, C.C., Wyse, D.L., 2019. Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. Agric. Ecosyst. Environ. 272, 63–73. https://doi.org/10.1016/j.agee.2018.11.007.
Jungers, J.M., DeHaan, L.R., Betts, K.J., Sheaffer, C.C., Wyse, D.L., 2017. Intermediate wheatgrass grain and forage yield responses to nitrogen fertilization. Agron. J. 109, 462–472. https://doi.org/10.2134/agronj2016.07.0438.
Kuznetsova, A., Brockhoff, P., Christensen, R., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26. https://doi.org/10.18637/jss.v082.i13.
Ladha, J.K., Pathak, H., Krupnik, J.T., Six, J., van Kessel, C., 2005. Efficiency of fertilizer nitrogen in cereal production: retrospects and prospects. Advances in Agronomy. Academic Press, pp. 85–156 https://doi.org/10.1016/S0065-2113(05)87003-8.
Larson, S., DeHaan, L., Poland, J., Zhang, X., Dorn, K., Kantarski, T., Anderson, J., Schmutz, J., Grimwood, J., Jenkins, J., Shu, S., Crain, J., Robbins, M., Jensen, K., 2019. Genome mapping of quantitative trait loci (QTL) controlling domestication traits of intermediate wheatgrass (Thinopyrum intermedium). Theor. Appl. Genet. 132, 2325–2351. https://doi.org/10.1007/s00122-019-03357-6.
Li, Y.S., Redmann, R.E., van Kessel, C., 1992. Nitrogen budget and 15N translocation in a perennial wheatgrass. Funct. Ecol. 6, 221–225. https://doi.org/10.2307/2389758.
Martinez-Feria, R.A., Basso, B., 2020. Unstable crop yields reveal opportunities for site-specific adaptations to climate variability. Sci. Rep. 10, 2885. https://doi.org/10.1038/s41598-020-59494-2.
Nilsson, Christer, Christensson, Bertil, Sveriges lantbruksuniversitet, Fakulteten för landskapsplanering, trädgårds-och jordbruksvetenskapcollab, 2010. Ett odlingssystem för integrerad produktion med låg energianvändning och hög produktionsförmåga: redogörelse för det första och andra växtföljdsomloppet i odlingssystemförsöket på Lönnstorps försöksstation, Alnarp. Fakulteten för landskapsplanering, trädgårds- och jordbruksvetenskap, Sveriges lantbruksuniversitet, Alnarp.
Norberg, L., Aronsson, H., 2020. Effects of cover crops sown in autumn on N and P leaching. Soil Use Manag. 36, 200–211. https://doi.org/10.1111/sum.12565.
Payne, R.J., Dise, N.B., Field, C.D., Dore, A.J., Caporn, S.J., Stevens, C.J., 2017. Nitrogen deposition and plant biodiversity: past, present, and future. Front. Ecol. Environ. 15, 431–436. https://doi.org/10.1002/fee.1528.
Peoples, M.B., Brockwell, J., Herridge, D.F., Rochester, I.J., Alves, B.J.R., Urquiaga, S., Boddey, R.M., Dakora, F.D., Bhattarai, S., Maskey, S.L., Sampet, C., Rerkasem, B., Khan, D.F., Hauggaard-Nielsen, H., Jensen, E.S., 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48, 1–17. https://doi.org/10.1007/BF03179980.
Pugliese, J.Y., Culman, S.W., Sprunger, C.D., 2019. Harvesting forage of the perennial grain crop kernza (Thinopyrum intermedium) increases root biomass and soil nitrogen cycling. Plant Soil 437, 241–254. https://doi.org/10.1007/s11104-019-03974-6.
R Core Team, 2020. R: A Language and Environment for Statistical Computing.
Methods for below-ground biomass. In: Ravindranath, N.H., Ostwald, M. (Eds.), Carbon Inventory Methods Handbook for Greenhouse Gas Inventory, Carbon Mitigation and Roundwood Production Projects, Advances in Global Change Research. Springer, Netherlands, Dordrecht, pp. 149–156. https://doi.org/10.1007/978-1-4020-6547-7_11.
Ryan, M.R., Crews, T.E., Culman, S.W., DeHaan, L.R., Hayes, R.C., Jungers, J.M., Bakker, M.G., 2018. Managing for multifunctionality in perennial grain crops. Bioscience 68, 294–304. https://doi.org/10.1093/biosci/biy014.
Schulte, L.A., Niemi, J., Helmers, M.J., Liebman, M., Arbuckle, J.G., James, D.E., Kolka, R.K., O'Neal, M.E., Tomer, M.D., Tyndall, J.C., Asbjornsen, H., Drobney, P., Neal, J., Ryswyk, G.V., Witte, C., 2017. Prairie strips improve biodiversity and the delivery of multiple ecosystem services from corn–soybean croplands. PNAS 114, 11247–11252. https://doi.org/10.1073/pnas.1620229114.
Šimůnek, J., Šejna, M., Saito, H., Sakai, M., van Genuchten, M.Th., 2018. HYDRUS 1-D.
SMHI, 2019. Dataserier med normalvärden för perioden 1961-1990 | SMHI [WWW Document]. URL Swedish Meteorological and Hydrological Institute Data (accessed 12.10.20) http://www.smhi.se/data/meteorologi/temperatur/dataserier-med-normalvarden-1.7354.
Smith, C.M., David, M.B., Mitchell, C.A., Masters, M.D., Anderson-Teixeira, K.J., Bernacchi, C.J., DeLucia, E.H., 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. J. Environ. Qual. 42, 219–228. https://doi.org/10.2134/jeq2012.0210.
Statistiska centralbyrån, 2019. Yield, kg per hectare by region, crop and year. URL https://www.statistikdatabasen.scb.se/pxweb/en/ssd/START__JO__JO0601/SkordarL/table/tableViewLayout1/ (accessed 1.14.21).
Stenberg, M., Aronsson, H., Lindén, B., Rydberg, T., Gustafson, A., 1999. Soil mineral nitrogen and nitrate leaching losses in soil tillage systems combined with a catch crop. Soil Tillage Res. 50, 115–125. https://doi.org/10.1016/S0167-1987(98)00197-4.
Syswerda, S.P., Basso, B., Hamilton, S.K., Tausig, J.B., Robertson, G.P., 2012. Long-term nitrate loss along an agricultural intensity gradient in the Upper Midwest USA. Agric. Ecosyst. Environ. 149, 10–19. https://doi.org/10.1016/j.agee.2011.12.007.
Thapa, R., Mirsky, S.B., Tully, K.L., 2018. Cover crops reduce nitrate leaching in agroecosystems: a global meta-analysis. J. Environ. Qual. 47, 1400–1411. https://doi.org/10.2134/jeq2018.03.0107.
Turner, P.A., Griffis, T.J., Lee, X., Baker, J.M., Venterea, R.T., Wood, J.D., 2015. Indirect nitrous oxide emissions from streams within the US Corn Belt scale with stream order. PNAS 112, 9839–9843. https://doi.org/10.1073/pnas.1503598112.
van Genuchten, M.Th., 1980. A closed-form equation for predicting the hydraulic conductivity of unsaturated soils1. Soil Sci. Soc. Am. J. 44, 892. https://doi.org/10.2136/sssaj1980.03615995004400050002x.
Vitousek, P.M., Menge, D.N.L., Reed, S.C., Cleveland, C.C., 2013. Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos. Trans. R. Soc. B Biol. Sci. 368, 20130119. https://doi.org/10.1098/rstb.2013.0119.
Yan, M., Pan, G., Lavallee, J.M., Conant, R.T., 2020. Rethinking sources of nitrogen to cereal crops. Glob. Chang. Biol. 26, 191–199. https://doi.org/10.1111/gcb.14908.
Zan, C.S., Fyles, J.W., Girouard, P., Samson, R.A., 2001. Carbon sequestration in perennial bioenergy, annual corn and uncultivated systems in southern Quebec. Agric. Ecosyst. Environ. 86, 135–144. https://doi.org/10.1016/S0167-8809(00)00273-5.
Zhang, X., Davidson, E.A., Mauzerall, D.L., Searchinger, T.D., Dumas, P., Shen, Y., 2015. Managing nitrogen for sustainable development. Nature 528, 51–59. https://doi.org/10.1038/nature15743.