



Radish-based cover crop mixtures mitigate leaching and increase availability of nitrogen to the cash crop

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ARTICLE INFO

Keywords:

Cover crop
Species mixtures
Soil mineral N
N leaching
Biomass

ABSTRACT

Agricultural soils are at risk of nitrogen (N) leaching especially during the fallow period in autumn and winter. Cover crops are grown to capture soil mineral N that otherwise would leach to the groundwater. They can serve as green manure providing mineral N to the cash crop in spring. We investigated whether mixing species of cover crops can enhance N capture and therefore reduce N leaching more effectively than pure stands in autumn without increasing the risk of N leaching in spring. We hypothesised that mixed species with complementary traits will capture more N and accumulate more biomass. It was also expected that residues from cover crops with higher biomass and lower C:N ratio would mineralise faster and subsequently increase N leaching in spring. In a 4-year field experiment, cover crops were grown between August and February in a rotation with different cash crops. We used eight cover crop treatments, including three pure stands: radish (*Raphanus sativus*), vetch (*Vicia sativa*) and oats (*Avena strigosa*), all possible 2- and 3-species mixtures and a fallow (no cover crop). Treatment effects on leaching losses were estimated by analysing N concentrations in samples of leached pore water below the rooting zone and by modelling the volume of water leached per plot. Most N leaching occurred in autumn and winter while the amount of N leached in spring was negligible due to the lower precipitation. N leaching in autumn correlated negatively with cover crop biomass, N uptake and root length density. Radish and oats were the most productive species and dominated mixtures. Compared to the fallow, radish and mixtures that contained radish reduced N leaching by 49–73% and were characterized by quick soil cover, high N uptake and low to moderate C:N ratio. Subsequently, residues from radish and mixtures containing radish mineralized quickly, resulting in an increase in soil mineral N in spring by 70–110% as compared to fallow. This mineral N did not leach in spring and was available to the subsequent cash crop. This study demonstrates the importance of species selection in cover crop mixtures and recommends the use of radish-based mixtures if the purpose is to reduce N leaching in autumn and provide mineral N in spring.

1. Introduction

In agricultural production, high fertilisation levels in temperate regions often come with nutrient losses, especially of nitrogen (N) (Foley et al., 2011; Quemada and Baranski, 2013). A significant part of this N is often lost via leaching of nitrate (NO₃) as this form of N is highly mobile in the soil (Di and Cameron, 2002; Tilman et al., 2002). In autumn, when cash crops are usually already harvested in northwest Europe, N is most prone to leaching losses due to often significant amounts of mineral N

that remain in the soil profile after the crop is harvested (Di and Cameron, 2002). Growing cover crops during this period instead of leaving the land fallow can prevent or at least reduce these leaching losses (Thapa et al., 2018; Abdalla et al., 2019). During their growth, cover crops take up nutrients from the soil and incorporate them into their shoot and root biomass (Thorup-Kristensen et al., 2003; Kaspar and Singer, 2011). In light soils, cover crops are terminated by the end of winter or in early spring and the biomass is incorporated in the soil. The incorporated biomass serves as green manure as upon its decomposition

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nutrients are mineralised and become available to the succeeding cash crop. However, this cover crop decomposition and N mineralisation may cause leaching losses in spring if too much N becomes available too soon, before the cash crop can take it up.

Mineral N enters the soil through mineral fertilisers, SOM decomposition and through biological N fixation by free living N-fixing bacteria or in association with leguminous species. The N in the soil solution can be leached when the downward water flow drains the N below the zone that is out of reach for plant roots. Due to nutrient and water uptake by crops, the concentration of nutrients and the volume of downward water flow in the soil profile is lower in vegetated fields compared to bare fields (Kaspar and Singer, 2011; Blanco-Canqui, 2018). Cover crops have been shown to reduce nitrate (NO_3) leaching by 6–94%, depending on the type of cover crop (Kaspar and Singer, 2011). High biomass producing cover crops (such as grasses and crucifers) are generally effective in preventing N leaching. Compared to these species, leguminous cover crops that fix atmospheric N_2 and have a less extensive root system are generally less effective (Blanco-Canqui, 2018; Thapa et al., 2018). A meta-analysis by Thapa et al. (2018) showed that, compared to fallow soil, non-legume cover crops reduced leaching on average by 56% while leguminous cover crops did not reduce leaching losses. Beside biomass accumulation, rooting pattern of a cover crop can have a large influence on the amount of N leached. Plants that root deeper can intercept N further down the soil profile, while a high root length density (RLD) facilitates uptake from a larger surface area in the soil (Thorup-Kristensen, 2001).

It is known from research in semi-natural systems that increased plant species diversity can improve resource exploitation in the soil, which leads to an increased plant biomass C and N (Tilman et al., 1996; Fornara and Tilman, 2008; De Deyn et al., 2009; Cong et al., 2014). The positive effects of plant diversity are usually attributed to complementarity between plant species. Complementarity in growing strategies and resource acquisition can result in a more efficient capture of light, water and nutrients from the system as compared to pure stands (Wendling et al., 2017). For instance, complementarity could be related to root architecture (rooting depth, root length distribution) that varies widely among cover crop species (Bukovsky-Reyes et al., 2019). Therefore, mixing cover crop species with such complementary traits could lead to a higher nutrient uptake and reduce NO_3 leaching more effectively (Hauggaard-Nielsen and Jensen, 2005; Miyazawa et al., 2010; Brooker et al., 2015; Gaimaro et al., 2022). Including a leguminous species in a mixture can facilitate growth of neighbouring plants by increasing the N availability through lower mineral N uptake from the soil by the legume and by its increased N-input via biological N fixation (Blesh, 2018; Blesh et al., 2019; Bukovsky-Reyes et al., 2019). Because of this complementary resource use, mixtures of species with different functional traits are likely to have a higher biomass than expected from their monocultures (Weidlich et al., 2018). The higher average biomass of mixtures, as compared to the average of their pure stands, is known as over-yielding (Hector, 2006), which is usually attributed to limited interspecific and high intraspecific competition for resources in pure stands (Evers et al., 2019). For cover crops, it is more relevant that the biomass of mixtures is directly compared to the highest yielding pure stand. Therefore, a desirable outcome would be that mixtures produce more biomass than the most productive pure stands, which is known as transgressive overyielding (Schmid et al., 2008).

A higher N uptake by cover crop mixtures in autumn potentially results in larger N availability to the cash crop in spring (Cardinale et al., 2007). The rate at which N from cover crops that are incorporated into the soil becomes available for the cash crop depends on the cover crop biomass quality (Bichel et al., 2017). Plant biomass with a lower C:N ratio is expected to be easily decomposable and to contain more N than the soil microbes use for their growth and thus release mineral N quicker (Janssen, 1996). Quick mineralisation can potentially result in leaching losses at the start of the cash crop growing season when N availability exceeds N uptake. On the other hand, more recalcitrant biomass with

high C:N ratio could lead to N immobilisation by the soil microbes, thereby reducing N availability and suppressing crop growth (Thorup-Kristensen et al., 2003). Non-leguminous cover crops are usually less effective than leguminous cover crops at supplying plants with adequate amounts of nutrients in the spring (Thorup-Kristensen et al., 2003; Tonitto et al., 2006; Kaspar and Bakker, 2015; Marcellino and Miguez, 2017). Grasses in particular have a low N content such that their decomposition is associated with temporal N immobilisation, and thereby with reduced cash crop growth (Dabney et al., 2001; Marcellino and Miguez, 2017). Mixtures of legumes and non-legumes are expected to produce high biomass with varying C:N ratio yet with C:N ratios lower than that of the non-legume monocultures and higher than that of the legume monocultures. Therefore, mixtures of legumes and non-legumes are expected to provide a steady release of N to the soil which could potentially avoid high N losses in spring while at the same time stimulate cash crop growth. This also raises the question if there is a trade-off between high N retention in cover crop biomass in the autumn, and thus low NO_3 leaching, and a high amount of N leaching in spring after the decomposition of cover crops with a high N content.

The objective of this study was to investigate whether cover crop mixtures are more effective in reducing N leaching than pure stands during their growth in autumn, as well as after their termination and decomposition during cash crop growth. In a four-year field experiment we tested three hypotheses: 1) compared to pure stands, cover crop mixtures accumulate more biomass, take up more N and have greater RLD, 2) cover crop biomass, N uptake and RLD are negatively correlated to N leaching losses in autumn, 3) high yielding cover crops with low C:N ratio lead to the largest N losses through leaching in spring.

2. Materials and methods

2.1. Field design

The field experiment was conducted on a sandy soil (83% sand, 12% silt, 2% clay, pH 5.3, 1.09 g kg^{-1} total N, 58 mg kg^{-1} total P of which 2.2 mg kg^{-1} plant-available P, and 91 mg kg^{-1} K; Nergena, Wageningen, The Netherlands, $51^\circ 59' 42.7''\text{N}$, $5^\circ 39' 35.7''\text{E}$). Drainage pipes installed under the entire field at 100 cm deep. The drainage system discharge directly to a nearby open ditch. The field had a history of a conventional arable rotation. In 2016, before the start of the field experiment, winter wheat was grown on the entire field.

The field experiment consisted of eight cover crop treatments: three monoculture species, all possible 2- and 3- species mixtures of these monoculture cover crops and a fallow treatment. The cover crop species were radish (*Raphanus sativus*), vetch (*Vicia sativa*) and oats (*Avena strigosa*). Cover crop seeds were provided by three seed suppliers (Vandinter Semo B.V., P.H. Petersen Saat-zucht GmbH, and Joordens Zaden B.V.). The same vetch cultivar SV25 was used in all years. For oats, we used cultivar Exito (2016 and 2019), Panache (2017) and Pratex (2018). For Radish, we used cultivar Doublet (2016 and 2019), Angus (2017) and Valencia (2018). The eight treatments were replicated five times in a complete randomised block design in plots of 60 m^2 ($10 \text{ m} \times 6 \text{ m}$). Before sowing, the seedbed was prepared to a depth of 8 cm with a power harrow. The cover crops were sown on the 15th, 28th, 16th and 16th of August in 2016, 2017, 2018 and 2019, respectively. Seeds were sown with a 3 m wide no-till seed planter (Rabe Turbo drill, Germany) with a row spacing of 12.5 cm and at a depth of 2 cm. Based on the recommendation of the seed providers, seeding rates for radish, vetch and oats were 30 kg ha^{-1} , 110 kg ha^{-1} and 90 kg ha^{-1} , respectively. Species were mixed with a replacement design, using 50% and 33% of seeding rate of each species in pure stands to create 2- and 3-species mixtures, respectively. To ensure good establishment, plots with cover crops were fertilised shortly after sowing with calcium ammonium nitrate (CAN: 27% N - 6% CaO - 4% MgO; 30 kg N ha^{-1}). The fallow plots were not fertilized because, as a common and realistic practice, cover crops are fertilized, and bare soils are not.

The three growing seasons of the cover crops lasted from August until February of the next year (Fig. 1). No further growth was observed at cover crop termination. By the end of the winter, cover crops were chopped and incorporated into the top 5 cm of the soil using a cultivation machine. Each year, a different cash crop was grown after the cover crops. Cash crops were grown in all plots including the fallow plots. In 2017, maize (LG 31.211, supplied by Agrifirm) was fertilised with 300 kg K ha⁻¹ (Kali60), 59 kg P ha⁻¹ (Triple superphosphate) and 95 kg N ha⁻¹ (CAN). Herbicide was applied to maize as roundup to avoid weed growth between the rows. Due to a drought in the Netherlands, the maize was irrigated on 20–6–2017 with 30 mm. In 2018, potato (variety “eersteling”) was fertilised with 110 kg N ha⁻¹ (CAN), 46 kg P₂O₅ ha⁻¹ and 60 kg K₂O ha⁻¹. In 2019, barley (variety “IRINA”) was fertilised with 60 kg N ha⁻¹ (CAN).

2.2. Cover crop biomass

To quantify cover crop productivity of the different treatments, cover crop aboveground biomass was sampled in November of each year (at peak standing biomass, 12 weeks after sowing; Fig. 1). Plant biomass was cut just above the soil surface from two randomly allocated 1 m². The border of each plot was avoided during the sampling. Plant material was washed, dried at 70 °C for 48 h and weighed. Weeds were removed from all samples. Root biomass was sampled from the same locations as aboveground biomass. In 2016, cover crop root biomass was estimated by sampling the roots with a 12 × 30 cm² metal frame with a 30 cm depth. The metal frame was placed perpendicular on the rows in order to include multiple rows in the sample. In 2017–2019, four root cores (8 cm in diameter and 30 cm deep) were extracted from each plot, two cores within rows and two between rows. Each core was divided into three depth layers, 0–10 cm, 10–20 cm and 20–30 cm. In each plot, samples from the four cores were pooled together by depth. Samples were rinsed with water using a 0.5 mm sieve to separate roots from soil and adhering organic material. Roots were then picked with tweezers, dried at 70 °C for 48 h and weighed. A fresh root subsample from each plot was stored in alcohol and stained with “Natural Red” dye to be analysed for RLD and specific root surface area using WinRhizo; an image analysis software specialized in root morphology (WinRHIZO Pro 2013e; Bouma et al., 2000). All dried plant materials were ball-milled and the concentration of C and N in shoot and root were determined using combustion in an ElementAnalyser (Flash EA 1112, Thermo Scientific).

2.3. Pore water collection

Pore water collection and analysis was done on eighteen dates between May 2017 and January 2020 (Fig. 2). MacroRhizon tubes were used to collect pore water with minimum disturbance of soil structure

(Rhizosphere Research Products B.V., Wageningen, The Netherlands). Each MacroRhizon tube is one meter long and contains a porous suction cup from one end with a pore size of 0.15 µm. At the other end of the tube there is a female luer lock that is suitable to attach a syringe. At the west side of each plot, three MacroRhizon tubes were inserted in augured holes with an angle of 45° and at a depth of 70 cm below the soil surface. At this depth, there were no growing roots. On each sampling day, a syringe was attached to the luer lock at the end of each MacroRhizon tube and a vacuum was created by fixing a wooden retainer to the syringe. After 24 h, transparent pore water samples were collected and pooled together per plot then stored in a freezer at – 20 °C. All samples were analysed for NO₃⁻ and NH₄⁺ concentrations using an AutoAnalyzer (SEAL QuAAtro SFA system, Beun- de Ronde B.V. Abcoude, The Netherlands). The frequency of collecting leachates was around once per month, when sufficient rainfall was predicted (Fig. 2). Sufficient rainfall meant at least 5 mm of rain predicted for the next 24 h. In total, pore water was sampled 18 times over the entire period of the experiment.

2.4. Leachate estimation

We used the Soil Water Atmosphere Plant (SWAP) model to estimate leaching losses at the plot scale (Kroes et al., 2017). SWAP simulates the water movement and root water extraction in the soil, which we used to estimate the total volume of water percolation to the subsoil for the entire period between May 2017 and January 2020.

Rainfall and temperature data were obtained from a weather station of Wageningen University (the Veenkampen; 51°58'53.4"N 5°37'13.7"E) located nearby (3 km) our field experiment; reference evapotranspiration data were obtained from the KNMI (Royal Dutch Meteorological Institute). Per treatment in each year, the average of percentage soil cover by cover crops and the relative distribution of RLD at depth were used as crop input in the model. The percentage of soil covered by each cover crop was determined according to Elhakeem et al. (2019). In short, photos of soil cover were taken every week in each plot from two fixed positions using a camera that was mounted on a metal frame (1 × 0.75 m²). The fraction of soil covered by plants was analysed using DIPimage toolbox for image analysis in MATLAB (MathWorks, 2013). Subsequently, a logistic model was fit to the observed soil cover fractions then the time when 50% of maximum soil cover was reached (*T*_{50%}) was obtained from the model. Based on soil type, data of the soil hydraulic properties were obtained from the handbook of SWAP (Kroes et al., 2017). The initial soil water status was assumed at field capacity in the first simulation in 2017. The end values of each simulation were used as the initial values for the following simulation. For instance, the end values of soil water status after the simulation of maize in 2017 was used as the initial values of soil water status in the simulation of cover crops in season 2017/2018. Simulation of water infiltration, redistribution, and

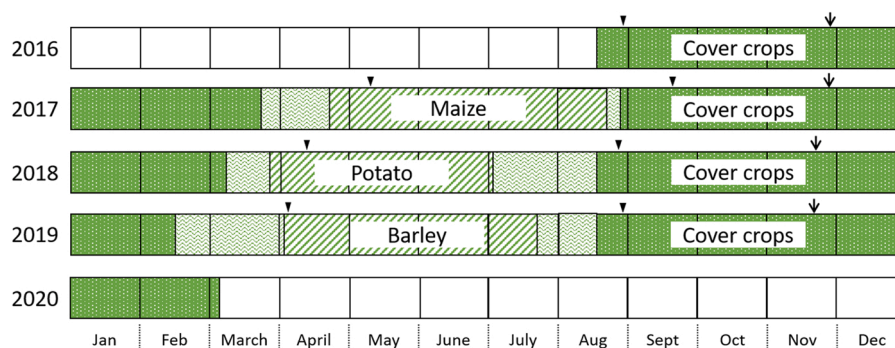


Fig. 1. Timeline of the field experiment. Dark dotted bars indicate the cover crop growing season, diagonally striped bars the cash crop growing season. Light waved bars indicate time during cash crops and cover crops, at which soil was prepared for the following crop. Arrows indicate the moment of cover crop subsampling. Triangles indicate the timing of fertilisation of the cover crop and cash crop.

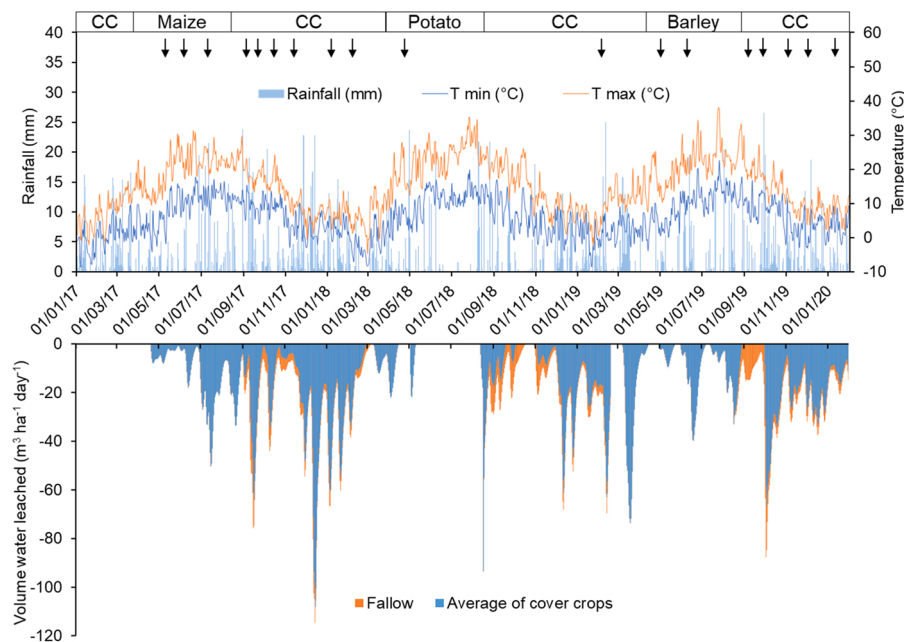


Fig. 2. Timeline of the field experiment and the observed temperature, and rainfall (top) and the modelled estimated downward flow (bottom). The top bar indicates the crop rotation with cover crops (CC) and the three different successive main crops. The black arrows indicate the time of pore water sampling.

percolation in the soil is based on the Richards equation (Kroes et al., 2017). Root water extraction is based on the potential transpiration, root density distribution and the drought reduction function (Feddes et al., 1978). Groundwater levels were estimated based on data from a groundwater well in the vicinity (51°59'54.6"N 5°39'47.7"E, ~400 m distance from field experiment). In the first SWAP simulation for maize 2017, groundwater depth was set to 180 cm below soil surface. Groundwater level during the following crops was extracted from the output file of the previous SWAP simulations. During the experiment, groundwater depth varied between 180 and 100 cm below soil surface.

The amount of N leached in each plot at each date was calculated by multiplying the mineral N concentration in the pore water and the simulated volume of water leached on that day (24 h). We summed the N leached ($\text{kg N ha}^{-1} \text{ day}^{-1}$) over the 18 measured dates to investigate the cumulative effect of cover crop treatments on N leaching. The amount of N leached was also divided into two periods: during the cover crop season (September to February) and during the cash crop season (March to August). The amount of N leached measured over 18 days does not equal the actual total amount of N leached throughout the experiment. The yearly total amount of leached N could not be measured in our design. Hence, our measurements are snap shots of the N leaching in the peak days when downward water flow was expected due to sufficient precipitation.

2.5. Bulk density and soil mineral N

Bulk density of the top-soil (0–20 cm) was measured in each plot at the start of the field experiment in August 2016. An intact soil core (from 10 cm depth) was taken by inserting a metal ring of 100 cm^3 volume. Subsequently the soil was dried at 105 °C to determine the dry weight of the soil volume sampled. To determine soil mineral N, samples were collected on the day of cover crop sowing (August), cover crop sub-sampling (November) and cover crop incorporation (February or March) from each plot. At each sampling time we pooled seven random samples, to a depth of 20 cm, of each plot together. Soil samples were dried at 40 °C for 48 h, sieved over 2 mm and subsequently extracted with a 0.01 M CaCl_2 extraction (Houba et al., 2000). Afterwards, the extracted solutions were analysed for $\text{NO}_3^- + \text{NO}_2^-$ and NH_4^+ using the Segmented Flow Analysis (SFA) system (SEAL QuAAtro SFA system, Beun- de Ronde

B.V. Abcoude, The Netherlands).

2.6. Data analysis

All statistics were carried out in R version 3.5.1 (R Core Team, 2018). Figures were made using the packages ggplot2 (Wickham, 2016) and cowplot (Wilke, 2019). We tested the effect of cover crop treatment (factor, eight levels) on biomass, N uptake, C:N ratio, RLD and N leaching using linear mixed effects models with the function lme from the package nlme (Pinheiro et al., 2019) with 'block' as a random factor. Significance was determined with analysis of variance (ANOVA). Normality and homogeneity of model residual variances were assessed by Shapiro-Wilk and Levene's tests, respectively. Data that violated the assumption of normality were transformed using power transformation then checked again for normality. To determine significant differences among cover crops, a Tukey's honestly significant difference posthoc test (HSD) was used, with the function emmeans (Lenth et al., 2019) and CLD from package multcomp (Hothorn et al., 2008). For the correlation between N leached and cover crop parameters, we fitted non-linear (nls) models $y = ae^{bx}$; where a is the intercept of the curve and b is the slope of the curve. For each curve, the hypothesis that model parameter b is different from zero was tested.

3. Results

3.1. N leaching

The estimated cumulative amount of N leached over all 18 sampling dates varied among cover crop treatments (Fig. 3, $F_{7,28} = 6.46$, $p < 0.001$). Most N was leached from fallow plots, yet that amount was not significantly different from the amount of N leached from plots with vetch, vetch-oats mixture and the three-species mixture. Compared to fallow, plots with radish, oats, vetch-radish mixture and oats-radish mixture leached significantly less mineral N (Fig. 3).

When separating estimates of N leached over the different growing seasons (Fig. 4), it became clear that the amount of N leached during the cover crop growing seasons (autumn and winter) was substantially greater than that during the cash crop growing seasons (spring and summer). The amounts of N leached during the cash crop seasons were

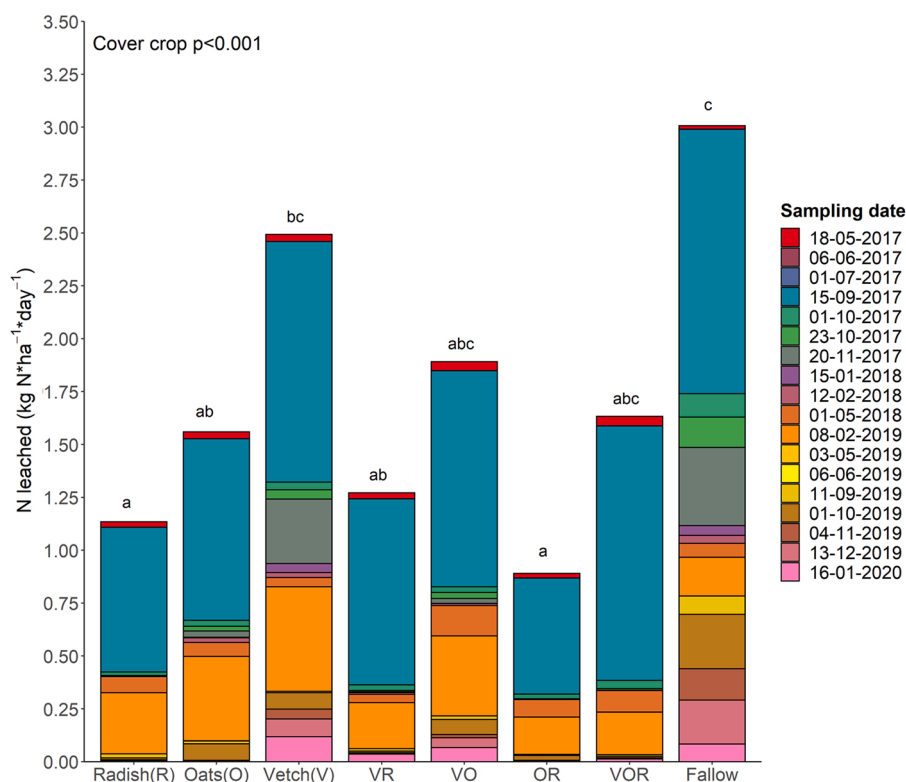


Fig. 3. Accumulated amount of N leached from 18 measuring days between 2017 and 2020. Samples were collected from plots of eight cover crop treatments. Bars with the same letter are not significantly different ($P > 0.05$; Tukey HSD test).

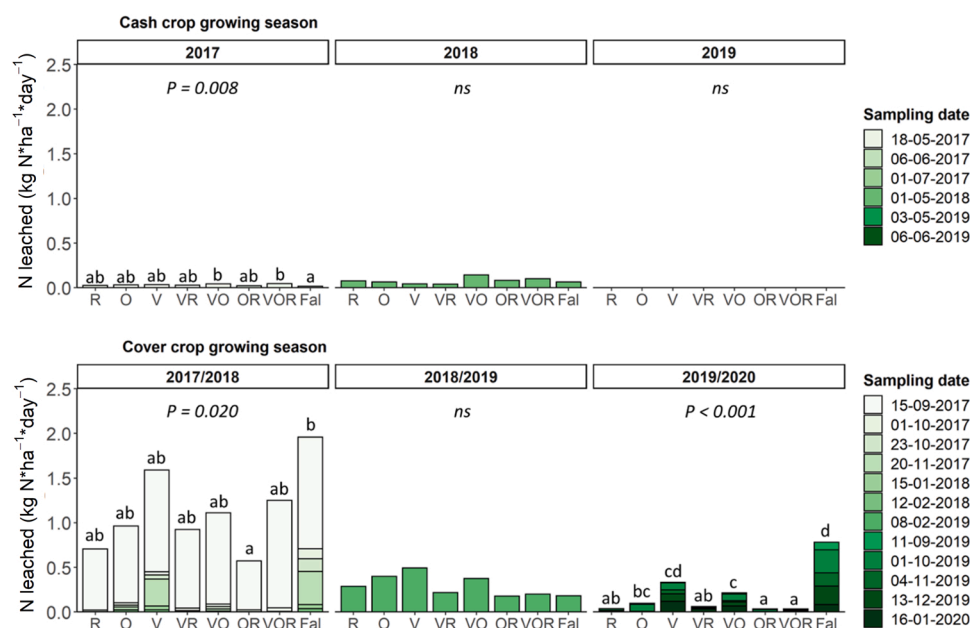


Fig. 4. Accumulated amount of N leached (kg N ha⁻¹ day⁻¹) during the cash crop growing season (upper panels; March - August) and within the cover crop growing season (lower panels; September - February). Samples were collected from eight cover crop treatments: R (radish), O (oat), V (vetch), all possible combinations and a fallow (Fal). The different shades of green represent the sampling days. Bars with the same letter are not significantly different ($P > 0.05$; Tukey HSD test); ns = no significant difference.

negligible. There was very little or no downward water flow during spring and summer, while this amount was greater in autumn and winter. Hence, the large difference between seasons in the estimated amount of leached N was mainly caused by the downward flow of water per day rather than the concentration of mineral N in the leached samples (Tables 1 and 2). This was different for the variation among treatments: N concentrations in the collected leachate varied among treatments in autumn and winter (Table 2). So, the difference among cover crop treatments in the amount of N leached in these seasons was

caused by variation in both the N concentration in the leachate and the volume of downward flow of water.

In 2017, the highest cumulative amount of leached N was under the fallow plots. Compared to fallow, only plots with oats-radish mixture had significantly lower values of cumulative N leached with 0.49 kg N ha⁻¹ day⁻¹ ($F_{7,28} = 2.93$, $p = 0.020$). In 2018, the amount of leached N was similar for all cover crop treatments. In 2019, the highest amounts were from fallow and vetch plots ($F_{7,28} = 26.42$, $p < 0.001$). Except for vetch in 2019, all cover crop treatments leached less mineral N than the

Table 1

Modelled downward water flow under eight cover crop treatments calculated with SWAP. Only data for days at which we also measured concentration of N in the downward water are shown.

Downward water flow (m ³ ha ⁻¹ day ⁻¹)										
	2017							2018		
	May 18	June 6	July 1	Sept 15	Oct 1	Oct 23	Nov 20	Jan 15	Feb 12	May 1
Radish (R)	1.33	0.00	0.00	55.79	1.06	1.22	10.00	15.18	10.64	3.48
Oat (O)	1.33	0.00	0.00	55.76	1.18	1.24	10.08	15.18	10.62	3.48
Vetch (V)	1.33	0.00	0.00	55.76	1.21	1.26	10.05	15.18	10.65	3.48
VR	1.33	0.00	0.00	55.76	1.13	1.22	10.02	15.18	10.63	3.48
VO	1.33	0.00	0.00	55.77	1.21	1.27	10.13	15.18	10.64	3.48
OR	1.33	0.00	0.00	55.76	1.09	1.20	10.01	15.18	10.63	3.48
VOR	1.33	0.00	0.00	55.76	1.16	1.23	10.03	15.18	10.63	3.48
Fallow	1.33	0.00	0.00	67.29	6.26	5.68	13.57	17.11	13.03	3.48
Downward water flow (m ³ ha ⁻¹ day ⁻¹)										
	2019							2020		
	Feb 8	May 3	June 6	Sep 11	Oct 1	Nov 4	Dec 13	Jan 16		
Radish (R)	21.16	0.02	0.00	1.51	6.96	6.60	27.56	12.93		
Oat (O)	21.16	0.02	0.00	2.03	10.23	6.54	27.56	12.93		
Vetch (V)	21.26	0.02	0.00	2.01	9.21	6.57	27.55	12.93		
VR	21.17	0.02	0.00	1.60	7.19	6.61	27.56	12.93		
VO	21.16	0.02	0.00	2.05	10.02	6.58	27.57	12.93		
OR	21.16	0.02	0.00	1.66	7.28	6.59	27.56	12.93		
VOR	21.16	0.02	0.00	1.80	7.55	6.55	27.56	12.93		
Fallow	25.47	0.02	0.00	13.54	33.89	11.09	30.91	16.46		

Table 2

Concentration of mineral N in the collected leachates measured for eight cover crop treatments. Values with the same letter within the same sample date are not significantly different ($P > 0.05$; Tukey HSD test). Asterisks indicate significant variation between cover crop treatments within the same sample date (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns = no significant difference).

Mineral N in leachate (mg L ⁻¹)										
	2017							2018		
	May 18	June 6	July 1	Sept 15	Oct 1	Oct 23	Nov 20	Jan 15	Feb 12	May 1
Radish (R)	18.8 ab	20.9 ab	13.7	11.7	15.1 a	2.9 a	0.1 a	0.0 a	0.0 a	20.8
Oat (O)	23.9 ab	25.8 ab	17.6	15.0	23.5 ab	17.0 b	3.0 b	0.2 a	2.0 abc	18.4
Vetch (V)	24.9 ab	20.0 ab	15.8	20.1	28.5 ab	34.6 d	30.0c	2.8 b	2.2 bc	12.1
VR	23.2 ab	22.8 ab	14.2	16.8	26.0 ab	4.6 a	0.2 a	0.2 ab	0.7 abc	12.1
VO	30.3 b	30.4 b	23.6	17.3	21.0 ab	21.4 bc	2.2 ab	0.1 a	0.8 abc	39.2
OR	17.4 ab	14.4 a	15.2	10.4	22.1 ab	0.6 a	0.1 ab	0.0 a	0.1 ab	25.0
VOR	32.8 b	31.5 b	20.0	20.4	32.1 b	5.8 a	0.0 a	0.0 a	0.0 a	27.8
Fallow	14.5 a	13.8 a	8.6	20.7	19.9 ab	28.0 cd	30.4c	3.0 b	3.2c	21.0
p	**†	***†	ns	ns†	*	***	***†	***†	***†	ns†
mineral N in leachate (mg L ⁻¹)										
	2019							2020		
	Feb 8	May 3	June 6	Sep 11	Oct 1	Nov 4	Dec 13	Jan 16		
Radish (R)	13.0	3.6	8.0	12.0	1.2 ab	0.2 a	0.1 a	0.3 ab		
Oat (O)	18.4	6.1	12.4	6.2	7.5 b	0.3 ab	0.1 a	0.1 a		
Vetch (V)	22.9	3.7	4.1	2.7	8.4 b	6.9 cd	3.0 bc	9.0c		
VR	10.9	7.4	10.5	7.7	1.1 a	0.2 ab	0.2 a	2.9 bc		
VO	16.9	7.1	3.3	7.9	6.7 b	2.2 bc	1.6 b	4.9c		
OR	8.9	2.8	2.7	2.8	3.5 ab	0.1 a	0.1 a	0.1 a		
VOR	9.0	3.8	6.1	4.3	1.0 ab	0.2 ab	0.1 a	0.9 ab		
Fallow	8.1	14.3	3.5	7.1	8.4 b	15.0 d	7.5c	5.7c		
p	ns	ns†	ns	ns†	***†	***†	***†	***†		

† Analysis was conducted after power transformation.

fallow plots. In the same year, the lowest cumulative amounts of leached N were from the plots with radish and all mixtures that contained radish, ranging from 0.02 to 0.06 kg N ha⁻¹ day⁻¹. Oats and the vetch-oats mixture had intermediate amounts of cumulative N leaching which were similar to that of vetch but significantly lower than that of fallow.

3.2. Downward water flow

The amount of downward water flow differed across years and that was mainly due to the variation in the amount of precipitation. Sum of precipitation in 2018 was 662 mm, which is 20% and 26% lower than that in 2017 (905 mm) and 2019 (833 mm), respectively (Supplementary Table 1). During 12 weeks of growth, the cover crops received 229, 154 and 243 mm of precipitation in 2017, 2018 and 2019, respectively. Obviously, 2018 was a dryer year with a lower downward flow of water

as compared to the other years. The major difference in the amount of downward water flow was between the fallow and the cover crop plots (the fallow having the highest flow), whereas the difference was minor among plots with cover crop treatments (Table 1).

The percentage of soil cover by cover crops over time (Table 3) was used to model downward water flow. Cover crops covered the soil fastest in 2018, a bit slower in 2019 and were much slower to cover the soil in 2017 (Table 3). On average, cover crop treatments reached 50% of the maximum soil cover ($T_{50\%}$) at 6.2, 3.0 and 3.9 weeks after sowing in 2017, 2018 and 2019, respectively. Radish was always the quickest species to establish and cover the soil, followed by oats and vetch. Mixtures had an intermediate $T_{50\%}$ as compared to their component species. Therefore, mixtures containing radish were always quicker in covering the soil and had a smaller $T_{50\%}$ than a mixture without radish (vetch-oats mixture).

Table 3

Percentage of soil cover measured between 2 and 8 weeks after sowing (WAS) and the time when 50% of maximum soil cover was reached ($T_{50\%}$). A lower $T_{50\%}$ value indicates more rapid increase in soil cover.

Year	Species	Percentage of soil covered by cover crops							$T_{50\%}$ WAS
		2WAS	3WAS	4WAS	5WAS	6WAS	7WAS	8WAS	
2017	Radish (R)	1	4	11	31	61	85	96	5.7
	Oat (O)	1	2	6	16	37	65	85	6.5
	Vetch (V)	0	1	5	14	36	66	87	6.5
	VR	1	2	8	24	53	81	94	5.9
	VO	1	2	5	13	30	54	76	6.9
	OR	1	2	8	26	58	85	97	5.8
	VOR	0	2	6	18	45	75	92	6.1
2018	Radish (R)	32	76	95	98	99	99	99	2.4
	Oat (O)	29	64	88	96	98	99	99	2.6
	Vetch (V)	6	15	32	57	78	90	94	4.7
	VR	26	67	91	97	99	99	99	2.6
	VO	12	41	77	94	98	99	99	3.2
	OR	29	75	95	98	99	99	99	2.4
	VOR	23	58	86	96	98	99	99	2.8
2019	Radish (R)	19	51	82	95	98	98	99	2.9
	Oat (O)	5	13	30	53	75	89	95	4.9
	Vetch (V)	5	16	43	75	92	97	99	4.2
	VR	14	40	73	91	97	99	99	3.3
	VO	5	14	34	62	83	94	98	4.6
	OR	12	34	65	86	95	97	98	3.5
	VOR	10	29	59	83	94	97	98	3.7

3.3. Cover crop biomass and N yield

Total cover crop biomass measured in November varied among the cover crop treatments ($F_{6108} = 57.9$; $p < 0.001$) and among the years ($F_{3108} = 83.7$; $p < 0.001$). There was a significant interaction between cover crop treatments and years ($F_{18,108} = 4.2$; $p < 0.001$). Averaged over cover crop treatments, 2017 was the least productive year with an average biomass of 2.9 Mg ha^{-1} (Fig. 5). Biomass of cover crops was significantly higher in the other years ranging from 4.9 to 6.0 Mg ha^{-1} in 2016 and 2019, respectively. The relatively lower biomass in 2017 is directly related to the delayed sowing date that was 2 weeks later than in the other three years. Subsequently, the accumulated growing degree

days (AGDD) during the 12 weeks of cover crop growth were 20% lower in 2017 (AGDD = 667) as compared to the average the other three years (ranging from 809 to 856). Among the pure stands, radish and oats were the most productive species and vetch consistently produced less. For the three pure stands, the lowest biomass was accumulated in 2017 and the highest biomass was accumulated in 2019.

All mixtures accumulated similar biomass to that of their most productive component species. For instance, in 2016, oats was the most productive species, thus all mixtures containing oats had a biomass similar to oats. In the same year, radish accumulated less biomass than oats and therefore only the mixture without oats (vetch-radish mixture) had a biomass similar to radish but also accumulated less biomass than

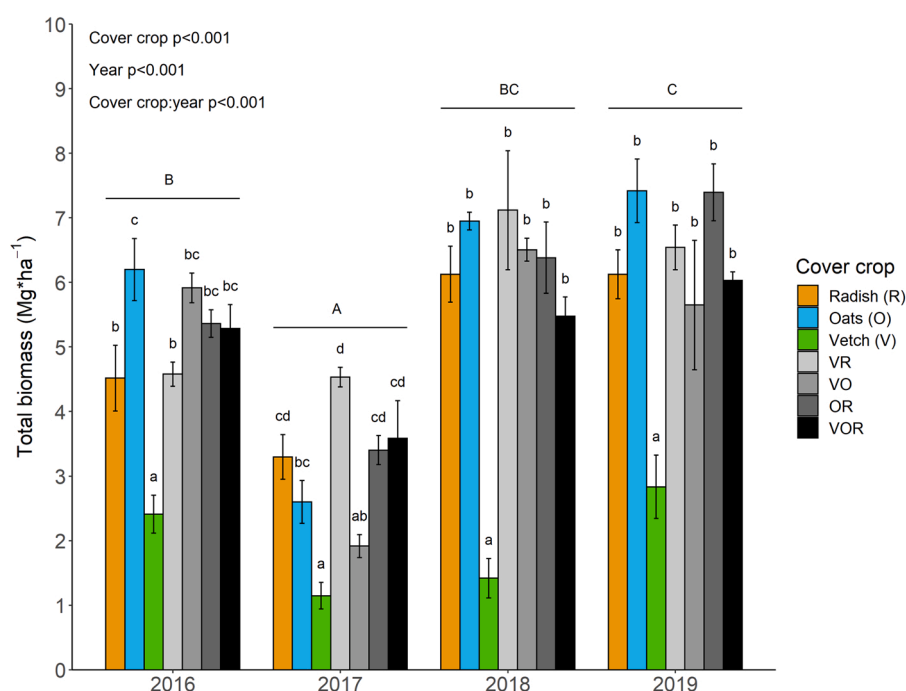


Fig. 5. Cover crop biomass (shoot + root) sampled at maximum standing biomass in November in four years 2016–2020. Error bars represent $\pm$ SE. Within each year, bars with the same lower-case letter are not significantly different. Years with the same upper-case letter are not significantly different ($P > 0.05$; Tukey HSD test).

oats ($F_{6,24} = 13.1$; $p < 0.001$). The relative contribution of vetch to mixtures' biomass was on average 11%. Radish and oats produced around 89% of the biomass when mixed with vetch. In the mixture that contain both radish and oats, radish contributed to 2/3 and oats contributed to 1/3 of the biomass.

Similar to biomass, N uptake by cover crops (shoot + root) varied among cover crop treatments ($F_{6,108} = 15.0$; $p < 0.001$) and years ($F_{3,108} = 39.8$; $p < 0.001$; Fig. 6). Years with low biomass productivity also showed low N uptake and vice versa. Averaged over the cover crop treatments, N uptake ranged from 82 kg N ha⁻¹ in 2017 to 142 kg N ha⁻¹ in 2019. N uptake in 2018 followed the same pattern but in 2016, 2017 and 2019, the N uptake by the three pure stands was similar. This was mainly because vetch, the legume species with poor biomass accumulation, had the highest N concentration (ranging from 3.2% to 3.8%) in its biomass. The N concentration was significantly lower in the most productive species, radish (N ranging from 1.6% to 2.6%) and oats (N ranging from 1.5% to 2.4%).

All mixtures took up similar amounts of N as their most productive component species. In 2016, radish had the lowest N uptake with 72 kg N ha⁻¹ ($F_{6,24} = 2.6$; $p = 0.043$), which was significantly lower than vetch-oats mixture that captured 108 kg N ha⁻¹. In the other three years, vetch-radish mixture took up the highest amount of N that ranged from 136 to 187 kg N ha⁻¹ in 2017 and 2019, respectively (2017: $F_{6,24} = 6.4$; $p < 0.001$; 2018: $F_{6,24} = 8.7$; $p < 0.001$; 2019: $F_{6,24} = 4.4$; $p = 0.004$). These amounts of captured N by vetch-radish mixture were higher than those captured by oats, vetch and their combinations in 2017, and higher than N captured by vetch in 2018 and 2019.

The combination of high N concentration and low biomass accumulation of vetch resulted in the lowest C:N ratio that ranged from 11 in 2016 to 14 in 2018 (Fig. 7; $F_{6,107} = 47.3$; $p < 0.001$). In contrast, oats combined low N concentration and high biomass accumulation; therefore, oats had the highest C:N ratio that ranged from 18 in 2017 to 29 in 2016. Radish intermediate vetch and oats with a C:N ratio that ranged from 16 in 2017 to 24 in 2016. There was a pattern that the C:N ratio of each mixture was similar to that of its most productive component species. Some exceptions were shown where the C:N ratio of a mixture

was similar to the least productive component species (i.e., vetch). In 2019, vetch-radish mixture (C:N 13) and the three-species mixture (C:N 14) had similar C:N ratios to vetch (C:N 12) that were lower than that of radish (C:N 18) and oats (C:N 22; $F_{6,24} = 25.5$; $p < 0.001$). Moreover, in 2016 and 2019, vetch-oats mixture had an intermediate C:N ratio (C:N 23 and 18, respectively) that was significantly lower than that of oats (C:N 29 and 22, respectively; 2016: $F_{6,24} = 27.6$; $p < 0.001$).

3.4. Root length density

The relative distribution of root length density (RLD) in different soil depths was used as a crop input for SWAP model. RLD over depth (in cm root cm⁻³ of soil) varied significantly among cover crop treatments (Table 4; $F_{6,80} = 15.1$; $p < 0.001$) and years ($F_{3,80} = 3.4$; $p < 0.001$). On average, RLD over depth was lowest in vetch and vetch-oats mixture and about twice as high in radish and all mixtures with radish. In 2017 and 2018, all cover crop treatments had the highest RLD in the top 10 cm of soil and RLD gradually decreased with depth. In 2019, the highest RLD was also in the top 10 cm, but the deepest soil layer (20–30 cm) had a higher RLD than the middle layer (10–20 cm). The distribution pattern of RLD across soil depths varied among species. In 2017 and 2018, radish showed a steep reduction in RLD with depth, while this reduction was less steep for vetch and oats. All mixtures had similar RLD distribution as that of their most productive component species.

3.5. Soil mineral N

Soil bulk density was measured to calculate mineral N (N_{min}) as kg N ha⁻¹; bulk density ranged from 1.45 to 1.53 g cm⁻³. In August, before sowing of the cover crops, the level of N_{min} in the soil was always higher than that in November when the biomass of cover crops reached its peak (Table 5). Soil N_{min} levels increased again in March after cover crop incorporation and before the sowing of the cash crop.

Before the sowing of the cover crops in August, soil N_{min} levels were similar in all cover crop treatments except in 2017. In this year, plots with vetch-radish (36 kg N ha⁻¹) and plots with three-species mixture

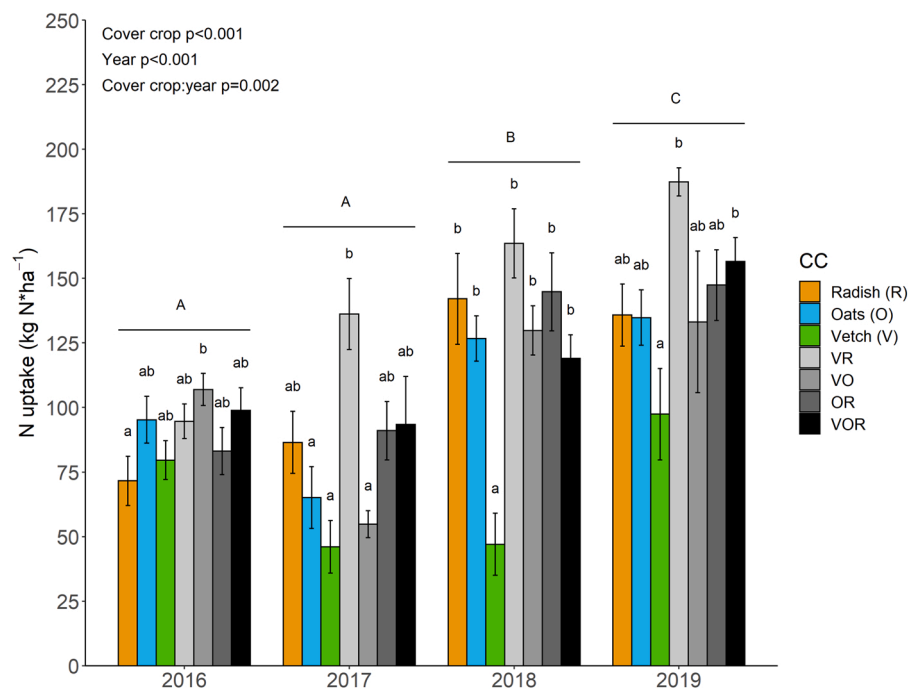


Fig. 6. Cover crop N uptake (shoot + root) measured at maximum standing biomass in November in four years 2016–2020. Error bars represent $\pm$ SE. Within each year, bars with the same lower-case letter are not significantly different. Years with the same upper-case letters are not significantly different ($P > 0.05$; Tukey HSD test).

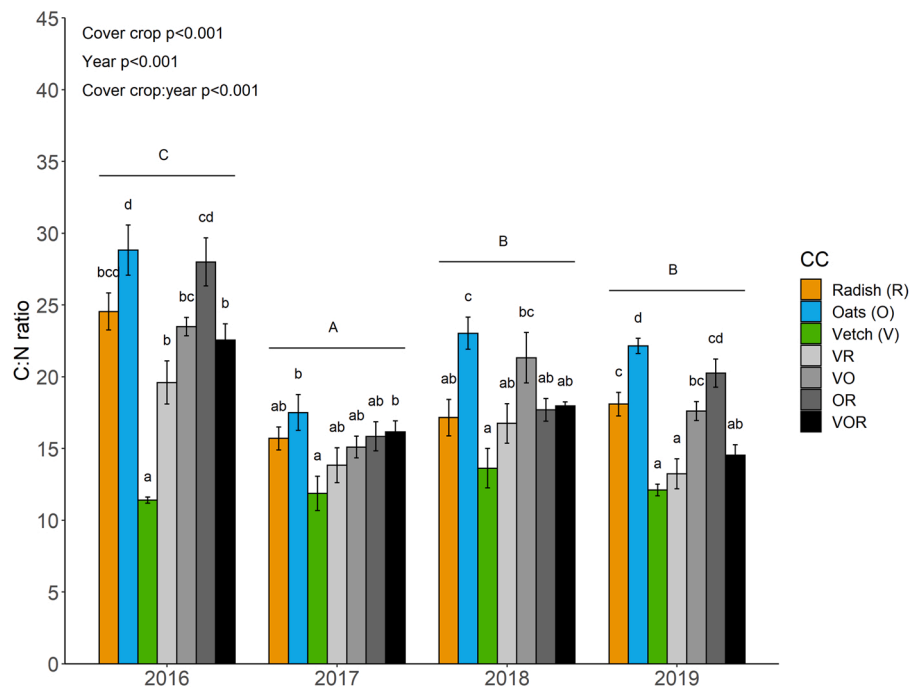


Fig. 7. C:N ratio of cover crop biomass measured in November in four years 2016–2020. Error bars represent $\pm$ SE. Within each year, bars with the same lower-case letter are not significantly different. Years with the same upper-case letters are not significantly different ($P > 0.05$; Tukey HSD test).

Table 4

Root length density (cm cm^{-3} soil) of cover crops at maximum standing biomass in November 2017–2020 from three depths. The last column in each year (0–30 cm) is the average of the three layers. Values with the same letter within each layer are not significantly different ($P > 0.05$; Tukey HSD test). Asterisks indicate significant variation between cover crop treatments within the same layer (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns = no significant difference).

	2017				2018				2019			
Cover Crop	0–10 cm	10–20 cm	20–30 cm	0–30 cm	0–10 cm	10–20 cm	20–30 cm	0–30 cm	0–10 cm	10–20 cm	20–30 cm	0–30 cm
Radish (R)	24.5 b	4.4 b	2.9 b	10.6 cd	33.6c	9.7	3.6 ab	15.6 cd	8.9	7.1	8.1	8.0
Oat (O)	8.2 a	5.0 b	3.4 b	5.5 bc	8.5 ab	7.8	4.8 ab	7.1 abc	11.2	7.2	7.1	8.5
Vetch (V)	4.7 a	1.3 a	1.1 a	2.3 a	6.0 a	4.0	1.8 a	3.9 a	10.3	3.5	4.3	6.0
VR	31.4 b	7.1 b	2.4 b	13.6 d	46.2c	9.1	5.3 b	20.2 d	9.5	6.4	6.8	7.5
VO	7.5 a	3.3 b	2.6 b	4.5 ab	8.0 ab	6.0	4.4 ab	6.1 ab	11.1	6.3	7.6	8.4
OR	21.7 b	5.1 b	3.1 b	10.0 cd	20.4 bc	8.6	5.4 b	11.5 bcd	13.2	9.1	9.0	10.4
VOR	28.7 b	4.4 b	2.3 b	11.8 cd	11.7 ab	7.9	5.6 b	8.4 abcd	8.6	6.7	10.9	8.7
<i>p</i>	*** ^t	**	*** ^t	*** ^t	*** ^t	ns ^t	*	*** ^t	ns	ns	ns	ns

^t Analysis was conducted after power transformation.

Table 5

Soil mineral N ($\text{kg N-NO}_3 + \text{N-NO}_2 + \text{N-NH}_4 \text{ ha}^{-1}$) measured for eight cover crop treatments. Values with the same letter within each sample date are not significantly different ($P > 0.05$; Tukey HSD test). Asterisks indicate significant variation between cover crop treatments at each sample date (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns = no significant difference).

	2016		2017		2018		2019		2019		2019	
Cover crop	August	November	March	August	November	March	August	November	March	August	November	August
Radish (R)	32.9	7.9 a	35.5 c	22.7	10.6 b	27.2	45.2	22.5	38.4 abc	37.1	19.1	ab
Oat (O)	30.1	6.7 a	20.9 ab	12.7 a	9.4 ab	18.6	45.8	19.7	26.8 ab	30.5	18.8	ab
Vetch (V)	33.2	13.1 b	27.2 bc	17.5 ab	11.2 b	19.1	40.1	23.0	29.7 ab	31.4	26.8	b
VR	31.8	8.5 a	38.7 c	36.3 b	11.3 b	27.7	47.8	23.1	41.8 bc	32.8	23.1	b
VO	31.1	9.7 ab	30.6 abc	16.8 ab	11.0 b	29.7	50.9	22.1	33.6 abc	33.6	19.8	b
OR	34.2	8.3 a	33.2 c	26.4 ab	10.7 ab	25.4	56.0	19.4	50.6 c	31.3	16.8	ab
VOR	31.2	7.7 a	28.8 bc	29.3 b	11.4 b	22.7	42.3	17.1	45.1 bc	29.6	18.2	ab
Fallow	33.4	8.3 a	19.8 a	15.3 ab	7.4 a	14.8	44.3	17.5	21.8 a	31.0	13.7	a
<i>p</i>	ns	*** ^t	*** ^t	*** ^t	* ^t	ns	ns ^t	ns	***	ns	***	***

^t Analysis was conducted after power transformation.

(29 kg N ha^{-1}) started with higher soil $N_{\min}$ levels than plots with oats (13 kg N ha^{-1} ; $F_{7,28} = 3.7$, $p = 0.006$).

At the peak of cover crop biomass in November, soil $N_{\min}$ level was

the lowest in the (non-fertilised) fallow plots. These amounts were not different from soil $N_{\min}$ levels in (fertilised) plots with oats. Vetch was the only treatment that consistently had a higher soil $N_{\min}$ level than

fallow. All plots with mixtures had the same soil $N_{\min}$ values as the pure stand of their most productive component species.

Incorporation of most cover crops in March, increased the $N_{\min}$ values significantly compared to the fallow plots with an amount of 20 kg N ha^{-1} in 2017 ($F_{7,28} = 8.1$, $p < 0.001$) and 22 kg N ha^{-1} in 2019 ($F_{7,28} = 5.3$, $p < 0.001$). These amounts of $N_{\min}$ in the fallow plots were not different from those in plots with oats and oats-vetch mixture. Plots with a vetch legacy had intermediate amounts of $N_{\min}$ that did not differ from that of plots with a legacy of oats. Plots with a legacy of radish and all mixtures with radish had the highest soil $N_{\min}$ in spring that ranged from 29 to 39 kg N ha^{-1} in 2017 and from 34 to 51 kg N ha^{-1} in 2019.

3.6. Relationship between cover crop biomass, N uptake, root length density and N leaching

In 2018, it was not possible to investigate the response of N leaching in autumn to changes in cover crop biomass, N uptake and RLD as there was only leaching in winter (February 2019), but no N leaching in autumn 2018. In both 2017 and 2019, the accumulated amount of N leached during sampling days in autumn was negatively correlated to cover crop biomass (Fig. 8A and D). N leaching in autumn reduced with increased RLD in 2017 (Fig. 8C) and with increased N uptake in 2019 (Fig. 8E). The same relationships were observed when the concentration of mineral N in leachate samples was used instead of the total amount of N leached (Supplementary Fig. 1). There was no relation between the same aforementioned parameters and the amount of N leached in spring as the SWAP model predicted either very little or no leaching in this period of the year.

4. Discussion

In this study we compared the effectiveness of different cover crop species and species mixtures in reducing leaching of soil mineral N. Growing a cover crop generally reduced N leaching as compared to fallow plots. Furthermore, we found that the effectiveness of a cover crop mixture to capture mineral N in autumn and winter depended on the species identity rather than on the number of species sown. In most instances, radish and mixtures containing radish were the most effective cover crop treatments to reduce N leaching. This species is characterized by quick soil cover, high biomass accumulation and N uptake. Therefore, the concentration of mineral N in downward water flow was consistently lower under plots cultivated with radish and radish-mixtures. Using the fallow as a reference, radish and radish-mixtures reduced N leaching by 49–73% and increased the availability soil $N_{\min}$ in March by 14 – 24 kg N ha^{-1} . To our knowledge, this is the first multi-year field study reporting results on N leaching under cover crop species and species mixtures in the Netherlands. Here, we highlighted the significance of including radish-based cover crops in the crop rotation to reduce N leaching in autumn and winter.

In contrast to our hypothesis, mixtures did not accumulate more biomass than their respective pure stands, i.e., transgressive overyielding did not occur. The premise that mixtures are more productive than pure stands is based on the assumption that mixed species would explore different niches and capture more resources (Vandermeer, 1992). This complementarity for resources acquisition and yield advantage was repeatedly reported in earlier studies on semi-natural systems and food crops (Tilman et al., 1996, 2006; Knops and Tilman, 1999; Fornara and Tilman, 2008; Cong et al., 2014, 2015; Yu et al., 2015; Li et al., 2020; Xu et al., 2020). However, for cover crops, it is more evident that the most productive species in a pure stand would

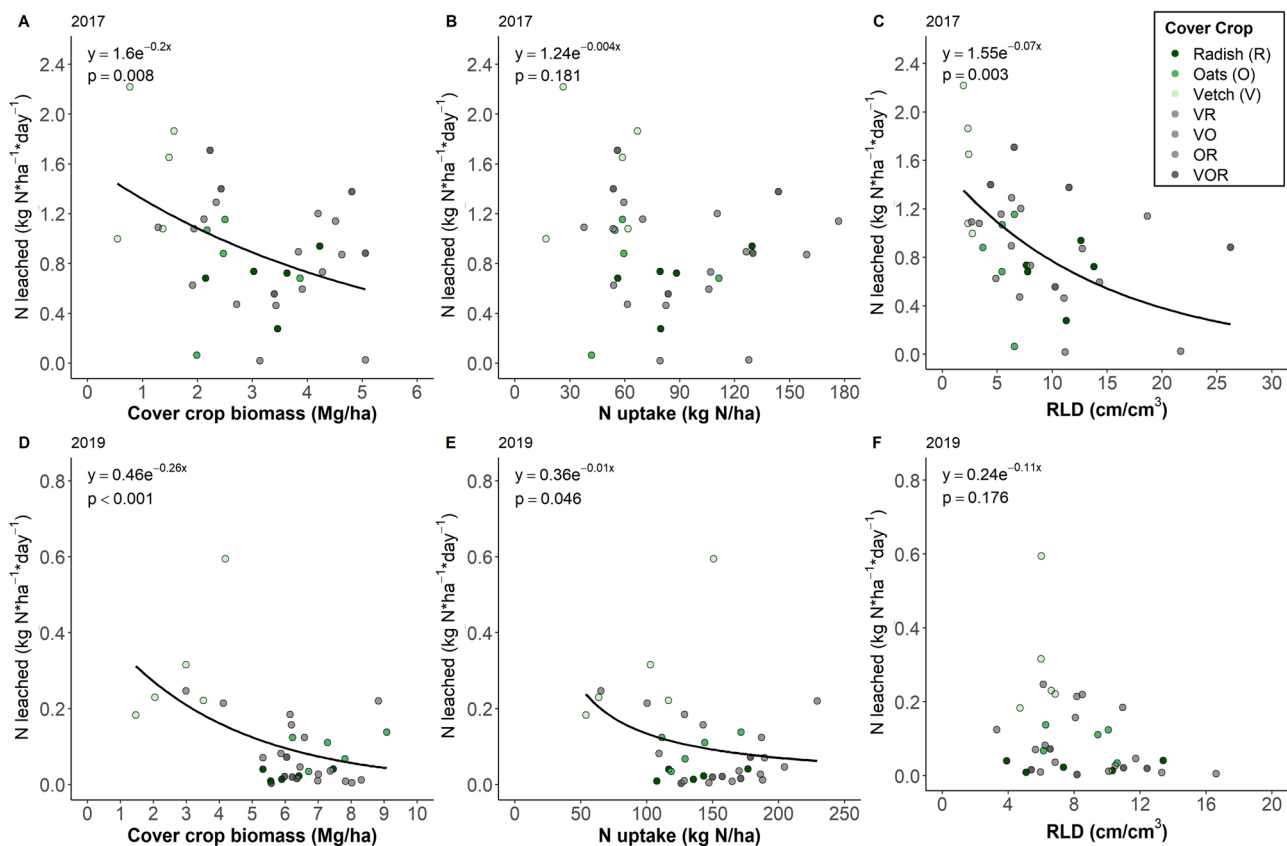


Fig. 8. Estimated amount of N leached in autumn and winter as dependant on cover crop biomass (A and D), N uptake (B and E), and root length density measured from the top 30 cm soil layer (RLD; C and F) in 2017 (A–C) and 2019 (D–F). Each point is the sum of the estimated amount of N leached during six (2017) or five (2019) sample dates during the cover crop growing period.

dominate the mixture when mixed with a less productive species, which is known as the selection effect (Loreau and Hector, 2001; Elhakeem et al., 2019). Dominance of one species or complementarity between combined species are both influenced by crop management. For instance, species of cover crops are mixed within-the-row with close proximity to maximize the benefit of niche differentiation. However, if the chosen species have considerably different rates to establish and cover the soil, then the slower species will be overgrown and out-competed (Elhakeem et al., 2019). Fertilization is another decision related to crop management that can influence complementarity and dominance within a species mixture. Wendling et al. (2017) showed that high yielding species, such as crucifers, are efficient in N uptake and accumulate biomass quickly, and therefore fertilization would enhance their growth and dominance over their companion species with a slower growth. Thus, complementarity is more explicit when resources are limited (Wendling et al., 2017). In our field, cover crops were fertilised with 30 kg N ha⁻¹ to kickstart their growth. This application may have enhanced the initial growth of radish and oats and increased their dominance over vetch. Subsequently, biomass and N uptake of mixtures were similar to that of radish and oats in pure stands, even though less seeds were sown per species in the species mixtures as we used a replacement design. This corroborates findings of earlier studies that found that crucifer-legume mixtures take up equally as much N as crucifers grown in pure stand (Elhakeem et al., 2021a, 2021b; Creissen et al., 2016; Couédel et al., 2018). Earlier studies on food crops often alluded to deeper rooting in mixtures as to explore a larger soil volume for nutrient uptake and N leached to deeper layers (Li et al., 2006; Andersen et al., 2014). Nevertheless, due to the selection effect, the RLD of cover crop mixtures was not greater than that of pure stands. Based on these results, we refute our first hypothesis that mixtures, in general, accumulate more biomass, take up more N and have a greater RLD.

We showed that biomass accumulation, N uptake and RLD are negatively correlated with the amount of leached N. Specifically, cover crop biomass had the strongest negative correlation with N leaching as compared to N uptake and RLD. The three plant parameters are related to each other and thus were expected to result in a similar correlation with N leaching. However, this was not always found due to the higher variation in data of N uptake and RLD as compared to the biomass data. Therefore, we partially accept the second hypothesis, stating that cover crop biomass accumulation, N uptake and RLD are negatively correlated with N leaching in autumn.

Contrary to our expectation, species with a high biomass and low C:N ratio did not increase N leaching in spring, after being incorporated into the soil. Therefore, we refute our third hypothesis, although we have to bear in mind that results could differ in years with more downward water flow in spring. For instance, earlier studies from the United States reported that N release after radish is sensitive to the amount of precipitation (Dean and Ray, 2009; Ruark et al., 2018; Gaimaro et al., 2022). On the one hand, soil moisture enhances decomposition and N release from plant tissues, and on the other hand, N losses increases in wet conditions. We showed that the amount of N leached in spring under all plots was negligible in three subsequent years (2017–2019) due to the low downward flow of water, which is mainly related to the lower precipitation in spring (40–60% less precipitation) than in autumn and winter (Supplementary Table 1). Although in our study mixing cover crop species did not enhance biomass quantity, biomass quality (C:N) did show significant shifts between monocultures and mixtures. Our results suggested that, in some instances, adding vetch to a mixture can improve cover crop quality (lower C:N ratio) even when vetch is overgrown by its companion species. This finding is in agreement with a recent study on cover crops showing that mixtures on average produce similar amounts of biomass but with a lower C:N ratio as compared to the most productive pure stands (Elhakeem et al., 2021b). Yet, the mechanism underlying this result is not clear. Cover crop seeds were mixed using a replacement design, therefore the number of plants from the dominant species in a mixture was either 1/2 or 1/3 of that in pure

stand. Perhaps the lower intraspecific competition in mixtures, as compared to pure stands, improved N capture by the individual plants. With a lower C:N ratio, residue from mixtures can mineralize quicker and increase mineral N in the soil. This statement is supported by our data on soil mineral N in March 2017 and 2019 (Table 5). Furthermore, it is known that cover crops containing radish can create legacies in the soil such that soil microbial communities change in composition and activity thereby enhance decomposition and N mineralisation (Barel et al., 2018, 2019). Compared to the fallow, radish-based mixtures significantly increased the availability of mineral N in spring by an average of 70% and 110% in 2017 and 2019, respectively. The higher availability of soil mineral N in spring can partially replace N input from external sources and is expected to enhance the yield of the subsequent cash crop as shown earlier (Barel et al., 2018; Abdalla et al., 2019; Chu et al., 2017).

The practical implications of our results for farming practices is that radish and radish-based mixtures are favourable when the purpose of growing a cover crop is to reduce N leaching. Radish-based species showed quick growth, high N uptake, great biomass accumulation and so reduced N leaching by 49–73%. Fertilization of cover crops should be avoided as it might increase N losses and encourage the dominance of one species in the mixture, which is not desirable. Evidence was provided that litter quality is improved in mixtures. Particularly, litter from radish-based mixtures mineralized quickly, leaving more soil mineral N available for the subsequent cash crop. To test whether growing radish-based mixtures can improve the total N budget and N use efficiency additional investigations are required to also include other N pathways, such as the yearly amount of leached N, N volatilization, N deposition and N fixation.

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.

Data Availability

Data will be made available on request.

Acknowledgements

This study is a part of the Clever Cover Cropping project which is a collaboration within Wageningen University & Research (WUR) and the Center for Soil Ecology (CSE). This study was supported by a grant from the Netherlands Organization for Scientific Research (NWO Green, grant number 870.15.071) with co-financing from seed producers (Agrifirm, Vandinter Semo, P.H. Petersen Saatzucht, and Joordens Zaden).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fcr.2022.108803.

References

- Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P., 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. *Glob. Change Biol.* 25, 2530–2543.
- Andersen, S.N., Dresbøll, D.B., Thorup-Kristensen, K., 2014. Root interactions between intercropped legumes and non-legumes—a competition study of red clover and red beet at different nitrogen levels. *Plant Soil* 378, 59–72.
- Barel, J.M., Kuyper, T.W., de Boer, W., Douma, J.C., De Deyn, G.B., 2018. Legacy effects of diversity in space and time driven by winter cover crop biomass and nitrogen concentration. *J. Appl. Ecol.* 55, 299–310.
- Barel, J.M., Kuyper, T.W., Paul, J., de Boer, W., Cornelissen, J.H., De Deyn, G.B., 2019. Winter cover crop legacy effects on litter decomposition act through litter quality and microbial community changes. *J. Appl. Ecol.* 56, 132–143.

- Bichel, A., Oelbermann, M., Echarte, L., 2017. Impact of residue addition on soil nitrogen dynamics in intercrop and sole crop agroecosystems. *Geoderma* 304, 12–18.
- Blanco-Canqui, H., 2018. Cover crops and water quality. *Agron. J.* 110, 1633–1647.
- Blesh, J., 2018. Functional traits in cover crop mixtures: biological nitrogen fixation and multifunctionality. *J. Appl. Ecol.* 55, 38–48.
- Blesh, J., VanDusen, B.M., Brainard, D.C., 2019. Managing ecosystem services with cover crop mixtures on organic farms. *Agron. J.* 111, 826–840.
- Bouma, T.J., Nielsen, K.L., Koutstaal, B., 2000. Sample preparation and scanning protocol for computerised analysis of root length and diameter. *Plant Soil* 218, 185–196.
- Brooker, R.W., Bennett, A.E., Cong, W.F., Daniell, T.J., George, T.S., Hallett, P.D., Hawes, C., Iannetta, P.P.M., Jones, H.G., Karley, A.J., Li, L., McKenzie, B.M., Pakeman, R.J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C.A., Zhang, C., Zhang, F., Zhang, J., White, P.J., 2015. Improving intercropping: A synthesis of research in agronomy, plant physiology and ecology. *N. Phytol.* 206, 107–117.
- Bukovsky-Reyes, S., Isaac, M.E., Blesh, J., 2019. Effects of intercropping and soil properties on root functional traits of cover crops. *Agric., Ecosyst. Environ.* 285.
- Cardinale, B.J., Wright, J.P., Cadotte, M.W., Carroll, I.T., Hector, A., Srivastava, D.S., Loreau, M., Weis, J.J., 2007. Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proc. Natl. Acad. Sci. USA* 104, 18123–18128.
- Chu, M., Jagadamma, S., Walker, F.R., Eash, N.S., Buschermohle, M.J., Duncan, L.A., 2017. Effect of multispecies cover crop mixture on soil properties and crop yield. *Agric. Environ. Lett.* 2 (1), 170030.
- Cong, W.F., van Ruijven, J., Mommer, L., De Deyn, G.B., Berendse, F., Hoffland, E., 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *J. Ecol.* 102, 1163–1170.
- Cong, W.F., Hoffland, E., Li, L., Six, J., Sun, J.H., Bao, X.G., Zhang, F.S., van der Werf, W., 2015. Intercropping enhances soil carbon and nitrogen. *Glob. Change Biol.* 21, 1715–1726.
- Couëdel, A., Alletto, L., Tribouillois, H., Justes, É., 2018. Cover crop crucifer-legume mixtures provide effective nitrate catch crop and nitrogen green manure ecosystem services. *Agric., Ecosyst. Environ.* 254, 50–59.
- Creissen, H.E., Jorgensen, T.H., Brown, J.K.M., 2016. Increased yield stability of field-grown winter barley (*Hordeum vulgare* L.) varietal mixtures through ecological processes. *Crop Prot.* 85, 1–8.
- Dabney, S.M., Delgado, J.A., Reeves, D.W., 2001. Using winter cover crops to improve soil and water quality. *Commun. Soil Sci. Plant Anal.* 32, 1221–1250.
- De Deyn, G.B., Quirk, H., Yi, Z., Oakley, S., Ostle, N.J., Bardgett, R.D., 2009. Vegetation composition promotes carbon and nitrogen storage in model grassland communities of contrasting soil fertility. *J. Ecol.* 97, 864–875.
- Dean, J.E., Ray, R.W., 2009. Brassica cover crops for nitrogen retention in the Mid-Atlantic Coastal Plain. *J. Environ. Qual.* 38 (2), 520–528.
- Di, H.J., Cameron, K.C., 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutr. Cycl. Agroecosystems* 64, 237–256.
- Elhakeem, A., van der Werf, W., Ajal, J., Luca, D., Claus, S., Vico, R.A., Bastiaans, L., 2019. Cover crop mixtures result in a positive net biodiversity effect irrespective of seeding configuration. *Agr. Ecosyst. Environ.* 285.
- Elhakeem, A., Bastiaans, L., Houben, S., Couwenberg, T., Makowski, D., van der Werf, W., 2021a. Do cover crop mixtures give higher and more stable yields than pure stands? *Field Crop Res.* 270.
- Elhakeem, A., van der Werf, W., Bastiaans, L., 2021b. Radiation interception and radiation use efficiency in mixtures of winter cover crops. *Field Crop Res.* 264, 108034.
- Evers, J.B., Van Der Werf, W., Stomph, T.J., Bastiaans, L., Anten, N.P.R., 2019. Understanding and optimizing species mixtures using functional-structural plant modelling. *J. Exp. Bot.* 70, 2381–2388.
- Feddes, R.A., Kowalik, P.J., Zaradny, H., 1978. Simulation of Field Water Use and Crop Yield. Pudoc, Wageningen.
- Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockström, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. *Nature* 478, 337–342.
- Fornara, D.A., Tilman, D., 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *J. Ecol.* 96, 314–322.
- Gaimaro, J., Timlin, D., Tully, K., 2022. Comparison of cover crop monocultures and mixtures for suppressing nitrogen leaching losses. *Agric. Water Manag.* 261, 107348.
- Hauggaard-Nielsen, H., Jensen, E.S., 2005. Facilitative root interactions in intercrops. *Plant Soil* 274, 237–250.
- Hector, A., 2006. Overyielding and stable species coexistence. *N. Phytol.* 172, 1–3.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric model. *Biom. J.* 50, 346–363.
- Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. *Commun. Soil Sci. Plant Anal.* 31, 1299–1396.
- Janssen, B.H., 1996. Nitrogen mineralization in relation to C:N ratio and decomposability of organic materials. *Plant Soil* 181, 39–45.
- Kaspar, T.C., Bakker, M.G., 2015. Biomass production of 12 winter cereal cover crop cultivars and their effect on subsequent no-till corn yield. *J. Soil Water Conserv.* 70, 353–364.
- Kaspar, T.C., Singer, J.W., 2011. The Use of Cover Crops to Manage Soil. Publications from USDA-ARS / UNL Faculty, p. 1382.
- Knops, J.M., Tilman, D., Haddad, D.N., Naeem, S., Mitchell, C., Haarstad, J., Ritchie, M., Howe, K., Reich, P., Siemann, E., 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. *Ecol. Lett.* 2, 286–293.
- Kroes, J.G., Dam, J.C.v., Bartholomeus, R.P., Groenendijk, P., Heinen, M., Hendriks, R.F.A., Mulder, H.M., Supit, I., Walsum, P.E.V.v., 2017. SWAP Version 4: Theory Description and User Manual. Wageningen Environmental Research, Wageningen.
- Lenth, R., Singmann, H., Love, J., Buerkner, P., Huerve, M., 2019. Emmeans: Estimated marginal means, aka least-squares means.
- Li, C., Hoffland, E., Kuypers, T.W., Yu, Y., Zhang, C., Li, H., Zhang, F., van der Werf, W., 2020. Syndromes of production in intercropping impact yield gains. *Nat. Plants* 6 (6), 653–660.
- Li, L., Sun, J., Zhang, F., Guo, T., Bao, X., Smith, F.A., Smith, S.E., 2006. Root distribution and interactions between intercropped species. *Oecologia* 147, 280–290.
- Loreau, M., Hector, A., 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412, 72–76.
- Marcillo, G.S., Miguez, F.E., 2017. Corn yield response to winter cover crops: an updated meta-analysis. *J. Soil Water Conserv.* 72, 226–239.
- MathWorks, I., 2013. MATLAB and Statistics Toolbox Release. Author Natick, MA.
- Miyazawa, K., Murakami, T., Takeda, M., Murayama, T., 2010. Intercropping green manure crops-effects on rooting patterns. *Plant Soil* 331, 231–239.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2019. nlme: linear and nonlinear mixed effects models. R. Package Version 3, 1–140.
- Quemada, M., Baranski, M., Nobel-de Lange, M.N.J., Vallejo, A., Cooper, J.M., 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. *Agric., Ecosyst. Environ.* 174, 1–10.
- R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Ruark, M.D., Chawner, M.M., Ballweg, M.J., Proost, R.T., Arriaga, F.J., Stute, J.K., 2018. Does cover crop radish supply nitrogen to corn? *Agron. J.* 110 (4), 1513–1522.
- Schmid, B., Hector, A., Saha, P., Loreau, M., 2008. Biodiversity effects and transgressive overyielding. *J. Plant Ecol.* 1 (2), 95–102.
- 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.
- Thorup-Kristensen, K., 2001. Are differences in root growth of nitrogen catch crops important for their ability to reduce soil nitrate-N content, and how can this be measured? *Plant Soil* 230, 185–195.
- Thorup-Kristensen, K., Magid, J., Jensen, L.S., 2003. Catch crops and green manures as biological tools in nitrogen management in temperate zones. *Adv. Agron.* 79, 227–302.
- Tilman, D., Wedin, D., Knops, J., 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379, 718–720.
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature* 418, 671–677.
- Tilman, D., Reich, P.B., Knops, J.M.H., 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 441, 629–632.
- Tonitto, C., David, M.B., Drinkwater, L.E., 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: a meta-analysis of crop yield and N dynamics. *Agric., Ecosyst. Environ.* 112, 58–72.
- Vandermeer, J.H., 1992. The Ecology of Intercropping. Cambridge University Press.
- Weidlich, E.W.A., Temperton, V.M., Faget, M., 2018. Neighbourhood stories: role of neighbour identity, spatial location and order of arrival in legume and non-legume initial interactions. *Plant Soil* 424, 171–182.
- Wendling, M., Buchi, L., Amosse, C., Jeangros, B., Walter, A., Charles, R., 2017. Specific interactions leading to transgressive overyielding in cover crop mixtures. *Agr. Ecosyst. Environ.* 241, 88–99.
- Wickham, H., 2016. Elegant Graphics for Data Analysis. Springer-Verlag, New York.
- Wilke, C.O., 2019. Cowplot: Streamlined plot theme and plot annotations for 'ggplot2'.
- Xu, Z., Li, C., Zhang, C., Yu, Y., van der Werf, W., Zhang, F., 2020. Intercropping maize and soybean increases efficiency of land and fertilizer nitrogen use; a meta-analysis. *Field Crop Res.* 246, 107661.
- Yu, Y., Stomph, T.J., Makowski, D., van der Werf, W., 2015. Temporal niche differentiation increases the land equivalent ratio of annual intercrops: a meta-analysis. *Field Crop Res.* 184, 133–144.