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Diversifying cropping sequence reduces Nitrogen leaching risks

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Abbreviations:
SMN: Soil mineral N content

Abstract

Overuse use of chemical fertiliser in cropping systems has resulted in severe degradation of air and water quality. Diversifying cropping sequence with legumes provides a natural source of nitrogen (N), but also increases N leaching risks after their growing period. Here, we hypothesize that break crops, i.e. crops used to diversify the cropping sequence, reduce N leaching at the rotation scale due to their contribution to increasing nutrient use efficiency and crop N recovery by the following cereal crops. In two 4-year experiments conducted in northern France, we monitored agronomic performance and the changes in the soil mineral N content at field scale in six preceding crop-current crop combinations including winter wheat (*Triticum aestivum*), pea (*Pisum sativum* L) and oilseed rape (*Brassica napus* L). We quantified N leaching after each crop as a function of the preceding crop based on soil mineral N content, climate data and soil characteristics. We then simulated N leaching at the rotation scale, for 20 years of climate conditions and various cropping management systems. We show that growing pea or oilseed rape reduced both (i) soil mineral N content at harvest of the following cereals (up to mean values of -28 and -19 kg N ha^{-1} respectively), and (ii) N leaching risks during winter of the following cereals compared to the wheat-wheat cropping sequence. Although N leaching was higher during the winter after pea was cultivated, the cumulative losses over four experimental years of the pea cropping sequences were not significantly higher than the no-break cropping sequences. Over the 20 climate years, sequences including pea, oilseed rape, volunteer or catch crops reduced simulated N leaching by up to 40% compared to wheat monoculture. Our study confirms that N leaching not only depends on the current crop but is also affected by the preceding crop. A large potential reduction in nitrogen leaching could be achieved in many intensive cereal-growing regions with very limited cropping sequence diversity.

Keywords: soil mineral N content, N leaching, preceding crop, crop sequence, environmental impacts, pea, wheat, oilseed rape

Highlights

- We monitored soil mineral N during various cropping sequences
- We simulated a 20-year series of N leaching for the cropping sequences
- We found an impact of the preceding cop on N leaching after the following crop
- Catch crop and oilseed rape volunteers reduced N leaching risk
- Diversified cropping sequences can reduce N leaching by up to 40%

1. Introduction

Agroecosystems have converged in recent decades toward high intensification, with a major decrease in the number of plant species grown (*Kleijn et alk., 2009; Pretty et al., 2014),* and a marked increase in the use of synthetic nitrogen fertilisers (*Roser et Richie, 2019*). The overuse of N fertilizer has resulted in severe degradation of air and water quality, e.g., by increasing greenhouse gas emissions, particle pollution or eutrophication (*e.g., Gu et al., 2014, Lu et al., 2019).* To remain within planet boundary limits, agricultural systems must achieve both high productivity and high environmental performance (*Cambell et al., 2017).* There is increasing evidence that crop diversification contributes to higher and more stable yields (e.g., *Beillouin et al., 2021; Bowles et al., 2020; Franke et al., 2018)* and improves nutrient use efficiency (*Gardner and Drinkwater, 2009*). Yet, the impact of crop diversification on N losses toward the environment remains controversial, mainly when legumes are included (*Beaudoin et al., 2005; Plaza-Bonilla et al., 2015*). Numerous studies have reported that including pea (Pisum sativum L.) in a cropping sequence increased soil mineral N content (SMN) after harvest, leading to higher levels of N supply for the following crop, but also increasing N leaching during the following winter compared to a cereal (*Beaudoin et al., 2005; Jensen and Hauggaard-Nielsen, 2003; Maidl et al., 1996; Plaza-Bonilla et al., 2015*). Results for oilseed rape (Brassica napus L) confirmed this trend *(Ryan et al., 2006; Sieling and Kage, 2006*). Nevertheless, including break crops also has medium-term impacts on soil and crop N dynamics e.g. by improving nutrient use efficiency (e.g., for cereals: *Gardner and Drinkwater, 2009; Ghosh et al., 2007*) and the N uptake of the following crop (e.g., *Evans et al., 1991; Kirkegaard et al., 1994*; *Thomsen et al., 2001; Jensen and Hauggaard-Nielsen, 2003*). *Jabloun (2015)* and *Thomsen et al. (2001)* suggested that break crops could thus reduce the SMN after harvest and N leaching after the following crop. Analysing N leaching at the rotation scale thus seems essential (*Plazza-bonilla et al., 2015, Nemecek et al., 2008*), yet most previous studies on N leaching have not disentangled the contribution of each crop, their pre-crop and of the climate in real farming conditions (e.g., *De notaris et al., 2018; Pandey et al., 2018; Nemecek et al., 2008; Askegaard et al., 2005)*.

Accurately attributing N leaching to a preceding-crop-current crop combination is challenging. Short-term experiments or process-based model simulations do not enable the detailed investigation of the effects of the previous crop (*Yin et al., 2020)*. Nitrogen leaching displays marked spatial and temporal variability, and is strongly influenced by N fertilisation, N uptake efficiency of the current crop, and the climate. Process-based models generally do not account for the long-term and indirect effects of the crop (e.g. rotational effect on crop growth, N uptake and N leaching). Short-term experiments may lead to confounding effect between climate and the preceding crop. Experiments based on installed measurement tools (e.g., lysimeters or ceramic suction cups) generally cover small to medium areas and could lead to inaccurate measurements of N leaching. Installing these device can also alter the soil structure and create preferential flow paths (*Webster et al., 2003*). Sampling soil cores and analyzing their N content is less invasive for soil structure but requires models to interpolate N and water fluxes in the soil. These measurements can be carried out under agricultural conditions and account for large surface areas.

Our aim was thus to (i) determine the individual effect of the previous crop, the current crop, and climate in agricultural conditions (i.e., large fields, adjusted fertilisation) on N leaching; (ii) assess N leaching at the cropping sequence scale, thus addressing the overall impact of crop diversification on N leaching. To this end we monitored the soil N fluxes of different cropping sequences over four years in two sites in northern France, with precisely adjusted fertilisation. We then estimated the N leaching for 20 climate years and various cropping system management.

2. Material and methods

2.1. Experimental sites and crops

Two field experiments were carried out during four growing seasons (Table 1 – one homogenisation year in 2007 and four sampling years in the period 2008-2011) in Grignon (48.9°N 1.9°E- 40 km west of Paris) and in Holnon (48.8°N 3.2°E, in northern France). Both sites are located in the French wheat belt with an oceanic climate (Figure S1 and S2). In Grignon (Holnon resp.), the soil is a clay loam with 25.7 (18.1)% clay, 66.6 (55.2)% silt, 7.7 (26.7)% sand, 1.29 (1.09) g kg^{-1} total N, and 18 (10) g kg^{-1} organic C (measured in the 0-30 cm soil layer). Mean annual rainfall during the growing season, i.e., from October to June, reached 260, 388, 410, and 342 mm for in seasons 2007-2008 to 2010-2011 in Grignon and 482, 390, 438, and 251 mm in Holnon. Six combinations of preceding crop-current crop were tested (Wheat-wheat; Pea-wheat; Oilseed rape-Wheat; Wheat-oilseed rape, Pea-oilseed rape and Wheat-pea - Table 1). These combinations were assembled in eight cropping sequences over four years (including the homogenisation year), thus allowing all the different preceding crop-current crop combinations to be grown for several consecutive years and avoiding climate confounding effects. The experiments consisted of eight 4-year cropping sequences arranged in four randomized blocks. Each plot was 87 m² in size in Grignon and 108 m² in Holnon. Before the 2007-2008 season, homogenisation crops were cereals at both locations. The sowing densities of winter wheat were 250-255 seeds m^{-2}, 85-90 seeds m^{-2} for winter pea, 50-60 seeds m^{-2} winter oilseed rape (*Table S1*).

Nitrogen fertilisation was adjusted using the balance-sheet method for wheat (*Rémy and Hébert, 1977*) and with a specific decision tool for rapeseed, namely the "réglette N" (*Makowski et al., 2004*). As recommended in French farm conditions and due to its capacity to fix N, pea received no N fertiliser. Soil P and K contents were not limiting for crop growth, thus no P and K fertiliser was applied on the crops. Crops were fully protected against weeds and pests by chemical treatments. In the last experimental year in Grignon, however, *Septoria tritici* attacked the wheat crop in spring, slightly affecting wheat growth. The straw was removed after each crop was harvested, and the shallow stubble was ploughed under to leave the soil bare in the autumn. Moldboard ploughing was carried out before the following crop was sown.

Table 1. The cropping sequences studied in the Holnon and Grignon sites, and estimated values of N leaching, drainage of water, and N concentration in the drained water, based on the LIXIM model for the Grignon site for the years 2009 to 2011 (year 1-year 2-year 3). W: Wheat; O: Oilseed rape; P: Pea. The cropping sequences are based on six combinations of preceding crop-current crop: W-W; W-O; O-W; W-P; P-O; P-W. The crops in the first year of each cropping sequence (in brackets) were homogenisation crops

	Cropping season						
Cropping sequence	2007-2008	2008-2009	2009-2010	2010-2011	N leaching (kg N ha^{-1})	Drained water (mm)	N concentration (g N03^{-}. L^{-1})
1	(W)	W	W	O	34 – 12 - 24	147 - 73 - 121	102 - 72 - 89
2	(W)	W	O	W	10 - 14 - 28	147 - 100 - 115	92 - 62 - 108
3	(W)	O	W	W	28 - 9 - 23	146 - 69 - 97	84 - 55 - 106
4	(W)	O	W	P	28 - 16 - 28	146 - 99 - 127	84 - 70 - 96
5	(W)	W	P	O	31 – 27 - 23	90 - 102 - 106	49 - 118 - 94
6	(W)	P	O	W	52 – 14 - 29	160 - 99 - 115	143 - 63 - 110
7	(W)	W	P	W	31 - 31 - 24	147 - 113 - 103	92 - 121 - 104
8	(W)	P	W	W	44 - 16 - 14	131 - 69 - 70	149 - 99 - 89

2.2. Soil and plant sampling

Soil mineral N content (SMN - including nitrate and ammonium) was measured at both sites, each year for each crop at two dates (i) soon after harvest (~July), (ii) at the end of the winter rainy season (February/March -see Table S1 for the exact dates). In Grignon, additional measurements were taken at the beginning of the rainy season (October) to monitor soil N dynamics more precisely. Three soil cores were removed from each of the three soil layers (0-30 cm, 30-60 cm, 60-90 cm) per block and per treatment at each measurement date. The soil cores were sampled with a hydraulic coring device equipped with a 15-mm auger (Geonor, Oslo, Norway) and kept in cold storage until analysis. Soil inorganic N content was determined in a KCl (1 mol L^{-1}) extract with a Skalar auto-analyser (Skalar Analytic, Erkelenz, Germany), using copper reduction and the Griess-Ilosvay reaction for nitrate, and the indophenol method for ammonium (*Santoni et al., 2007).* Soil water content was determined by weighing the soil sample before and after oven drying for 48 h at 105 °C.

Biomass and N plant content were monitored to analyse crop N and soil N dynamics. Total shoot dry matter at harvest and grain yield were measured in four micro-plots (0.35 m² each for wheat, 0.875 m² for pea, and 1 m² for oilseed rape) per block for each crop. Vegetative aboveground parts and ears/pods were separated. The ears were threshed, the grains and straw were separately oven dried for 48 h at 80 °C and weighed. Grain yield (0% humidity) was then calculated. N content in the grains and straw was analysed using the Dumas method (*Eibeling., 1968).* This involved the combustion of dehydrated and ground plant tissue at 1,800 °C, reduction of N oxides by reduced Cu at 600 °C, and analysis of N_2 by katharometry (NA 1500 analyser, Fisons Instruments).

N-use efficiency (NUE) was calculated as the ratio between yield and soil mineral N supply (0-90cm), and N uptake efficiency (NUpE) was determined as the ratio between N in the aboveground dry matter and soil mineral N supply (*Bingham et al., 2012*).

2.3. Estimation of nitrate leaching with the LIXIM model

2.3.1. N leaching after each crop during the experiments

The amount of leached N after each crop was assessed with the LIXIM model (*Mary et al., 1999*), together with the amount of drained water and the concentration of nitrate in the drained water. LIXIM is a simple dynamic model which calculates N mineralisation and N leaching in soil by inverse modelling based on inorganic N and frequent water measurements in the soil, standard meteorological data, and soil characteristics. The model is based on an optimisation routine that estimates the N mineralisation rate and evapotranspiration that provide the best fit between observed and simulated water and nitrogen contents over the measurement period. In our case, N leaching was estimated based on measurements made at the beginning, during, and at the end of winter (i.e., before and after the rainy season, *Table S1*) of each preceding-current crop combination. It thus allows to indirectly account for all the current and preceding crop effects on soil N dynamics. The only-two model parameters, i.e., water content at field capacity (resp. permanent wilting point) were set based on measurements, at 231 (12.2), 216 (12.1), 210 (11.2) g H_2O kg^{-1} dry soil, for the soil layers 0-30 cm, 30-60 cm, and 60-90 cm, respectively. The soil apparent bulk densities were respectively, 1.3, 1.4 and 1.4 $g.cm^{-3}$, for the three layers. Climate variables (i.e., daily rainfall, potential evapotranspiration, and air temperature) were collected from *Meteo-France* weather stations close to the trials (48.9°N, 1.9°E).

Holnon and the last year of the experiment in Grignon were excluded for the LIXIM model parametrisation because the dynamics of SMN were not monitored between the beginning and the end of winter. LIXIM was evaluated based on its ability to simulate soil water content and SMN throughout the winter by comparing predicted and observed values at the end of winter for each of the three years in Grignon. The quality of the prediction was estimated for each layer through the root-mean-square error (RMSE), the bias, and the mean relative error (Figure S3).

2.3.2. N leaching in various cropping sequences for the 20 climate years

To provide conclusions that are less sensitive to specific climatic conditions, we performed N leaching simulations based on the LIXIM model on long-term climate series (20 years - 1992 to 2012 – see Figure S1). All crop combinations were simulated for each of the 20 climate years.

Based on the six combinations of preceding crop-current crop, various agronomic cropping sequences were generated, each hereafter noted by the first letter of the crops which compose them (Figure 2). The frequencies of each combinations of preceding crop-current crop varied in the cropping sequences, with for example the sequence WWW presenting only 'wheat (W)-wheat' preceding crop-current crop, and the POWW sequence presenting 'pea (P)-wheat', 'oilseed rape (O)-wheat' and 'wheat wheat' preceding crop-current crop combinations.

We also included between-crop management scenarios, i.e., bare soil in the autumn, oilseed rape volunteers in the autumn, or a sown catch-crop after pea harvest. The effect of these strategies was simulated with the LIXIM model by estimating N and water uptake by the catch crops.

For the simulations, soil data inputs were averaged from water and SMN contents measured at harvest for years where all the six preceding-current crop combinations were tested (i.e., from experimental year 2 and year 3 in Grignon). The first and last years of the experiment were not included, to avoid confounding effects with climate, as not all combinations of preceding crop and current crop were grown.

2.4. Statistical analysis

Crop agronomic performance (N uptake, N use efficiency, N uptake efficiency, shoot dry matter, and grain yield) in Grignon and Holnon was analysed for various current crops based on their preceding crops, using linear models. Multiple comparison tests were performed with the Agricolae package (*de Mendiburu, 2010*) with Tukey contrasts (alpha = 0.05).

The environmental impacts of the various preceding crops were then analysed, i.e., (i) SMN at harvest, (ii) SMN at the beginning of winter, and (iii) the proportion of SMN in the third soil layer (60-90 cm) at both dates. As all current crops were sown in autumn, the SMN in winter could be affected by their winter growth. We thus conducted our analysis considering pairs of crops: the crop that was just harvested (named the preceding crop) and the crop just sown (named the current crop). The analyses were performed independently for each site. We focused on the main effect of crops (preceding and current), and thus tested the significance of each variable using Fisher's test without considering possible interactions with the year. This allowed us to include years 1 and 4, where not all of the preceding crop-current crop combinations of crops were present, without biasing our results. The proportion of SMN variable was transformed (logit) to achieve normality.

Finally, the amount of leached N, the amount of drained water, and N concentration in drained water, estimated with the LIXIM model over 20 climatic years, were analysed with mixed models, taking into account the cropping sequence as fixed effects and the year as a random effect. Leached N and N concentrations in drained water were square-root transformed to obtain normality. Sequences were compared with Tukey's test ($\alpha = 0.05$).

For all analyses, assumptions of normal distribution and variance homogeneity were tested with Shapiro and Barttlet tests, respectively. Analyses were performed using R 2.15.3 statistical software (*R Core Team, 2013*).

3. Results

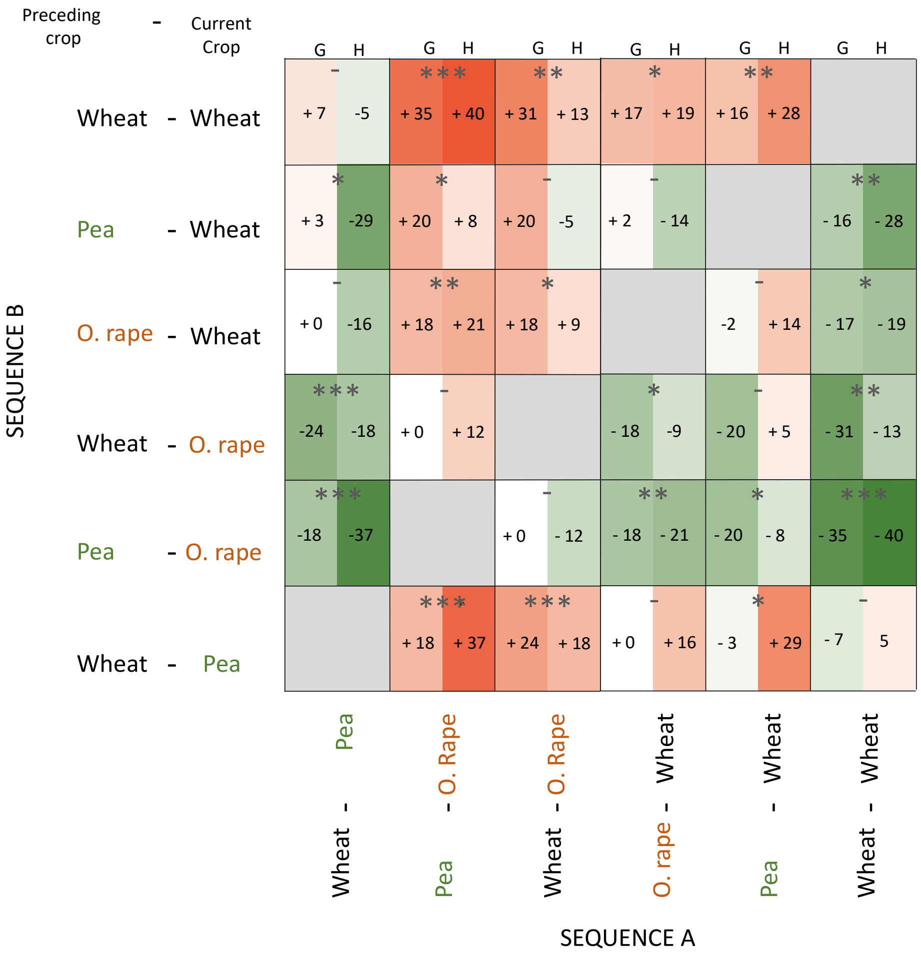
3.1. Variation in SMN according to the current crop

The average SMN after pea and wheat harvest did not differ significantly (both with wheat as the preceding crop – Figure 1). Marked variations were nonetheless observed among years (from -22 to +32 kg N ha^{-1} for pea compared to wheat, considering both sites- Table S2). Oilseed rape led to a lower post-harvest SMN than wheat, both with wheat as the preceding crop (mean of -31 kg N ha^{-1} in Grignon and -13 kg N ha^{-1} in Holnon), and with pea as the preceding crop (mean -20 kg N ha^{-1} in Grignon and -8 kg N ha^{-1} in Holnon, Figure 1). It also resulted in a lower SMN than with the pea crop (mean of -24 kg N ha^{-1} in Grignon and -18 kg N ha^{-1} in Holnon, both with wheat as the preceding crop, Figure 1).

At the beginning of winter, due to soil N mineralisation in autumn, the SMN was significantly higher after pea than after wheat (with wheat as the preceding crop - Figure S4), despite large inter-annual variability (from -33 to + 4 kg N ha^{-1}- Table S2). The SMN at this date did not differ significantly between wheat and oilseed rape (both with wheat as the preceding crop, the differences ranged from -15 to + 7 kg N ha^{-1}, Figure S4).

At harvest, the proportion of SMN in the 60-90cm soil layer was higher after pea than after wheat (33% vs. 14% for pea and wheat, respectively, with wheat as the preceding crop, Figure S5). No differences were observed between wheat and oilseed rape. At the beginning of winter, with wheat as the preceding crop, the proportion of SMN in the third layer of the soil profile was similar in the three crops (32%, 31%, and 28% of the SMN for pea, wheat and oilseed rape, respectively, Figure S6), possibly caused by N lixiviation from the 3rd layer in some situations.

Fig. 1. Mean differences in soil mineral nitrogen (SMN) at harvest between six sequences of 'preceding crop-current crop' in the Grignon (G) and Holnon (H) sites. The numbers and colours in the cells correspond to the difference between SMN when cropping sequence B (Y-axis) is grown instead of cropping sequence A (X-axis). For example, the first row of the table shows that the Wheat-Wheat succession (sequence B on the Y-axis) results in an average +7 increase (Grignon) or -5 decrease (Holnon) SMN compared with the wheat-pea sequence (sequence A in X-axis), and an increase in SMN compared to the Pea-oilseed rape, Wheat-oilseed-Rape, Oilseed rape Wheat, and Pea-wheat successions (at both the Grignon and Holnon sites). Statistical differences are mentioned for the mean value across the two sites in the upper of the cells (***: p value <0.001; **: p-value <0.01; *: p-value <0.05; -: non-significant). Values of SMN per year are listed in Table S2.



Preceding crop - Current Crop	Wheat - Pea			Pea - O. Rape			Wheat - O. Rape			O. rape - Wheat			Pea - Wheat			Wheat - Wheat		
		G	H		G	H		G	H		G	H		G	H		G	H
Wheat - Wheat	-	+ 7	-5	***	+ 35	+ 40	**	+ 31	+ 13	*	+ 17	+ 19	**	+ 16	+ 28			
Pea - Wheat	*	+ 3	-29	*	+ 20	+ 8	-	+ 20	-5	-	+ 2	- 14				**	- 16	- 28
O. rape - Wheat	-	+ 0	-16	**	+ 18	+ 21	*	+ 18	+ 9				-	-2	+ 14	*	- 17	- 19
Wheat - O. rape	***	-24	-18	-	+ 0	+ 12				*	- 18	-9	-	- 20	+ 5	**	- 31	- 13
Pea - O. rape	***	-18	-37				-	+ 0	- 12	**	- 18	- 21	*	- 20	- 8	***	- 35	- 40
Wheat - Pea				***	+ 18	+ 37	***	+ 24	+ 18	-	+ 0	+ 16	*	- 3	+ 29	-	- 7	5

3.2. Variation in SMN according to the preceding crop

The SMN after wheat harvest was significantly lower when pea was the preceding crop than in the wheat-wheat sequence (-16 and -28 kg N ha^{-1} in Grignon and Holnon, respectively, Figure 1). Similar results were observed for wheat with oilseed rape as the preceding crop compared to a wheat-wheat sequence (-17 and -19 kg N ha^{-1} in Grignon and Holnon, respectively, Figure 1).

The SMN between the wheat harvest and the beginning of winter (i.e., a few months later) increased more with pea or oilseed rape as the preceding crops than with wheat as the preceding crop (mean increase of 65, 59 and 31 kg N ha^{-1} respectively with pea, oilseed rape and wheat as preceding crops, data not shown). At the beginning of winter, there were no significant differences in SMN between the cropping sequences compared (Figure S4).

3.3. Variation in agronomic performance according to the preceding crop

Wheat produced significantly higher dry matter and grain yield when grown after pea than after wheat (Table 2 - yield ratio of 1.12 in Grignon, +0.9 t ha^{-1} and 1.16, 1.4 t ha^{-1} in Holnon). This break crop also led to a significant increase in the N use efficiency of the following wheat. The mean N uptake at harvest of the wheat crop did not differ for the pea or wheat preceding crop.

Oilseed rape as preceding crop also led to higher wheat yields (yield ratio of 1.14, + 1.0 t ha^{-1} in Grignon and 1.10 in Holnon,1.26 t ha^{-1}), significantly higher wheat N use efficiency (mean of + 5%), higher wheat N uptake in most years (20, 20 and -4 kg N ha^{-1} for year 1 to 3 respectively), but no differences in N uptake efficiency (except for year 2 – Table 2) compared to wheat as preceding crop.

Table 2. Soil N supply (0-90cm), crop N uptake in fertilised conditions (NUp), crop dry matter at flowering, N use efficiency (NUE), N uptake efficiency (NUpE) and yield of the current crop at the Grignon site. N-use efficiency (NUE) was calculated as the ratio between yield and soil mineral N supply (0-90cm); and N uptake efficiency (NUpE) was determined as the ratio between N in the aboveground dry matter and soil mineral N supply (*Bingham et al., 2012*). The soil N supply is the sum of N from mineralisation and fertiliser inputs.

Year	Preceding crop	Current crop	N supply kg N ha^{-1}	NUp kg N ha^{-1}	Dry matter t ha^{-1}	NUE (%)	NUpE (%)	Yield Mg ha^{-1}
07/08	W	W	319	237	17.7	27.8	74.0	10.4
08/09	W	W	262 b	182 b	13.9 b	25.3 b	69.9 ab	7.8 b[1]
	O	W	257 b	202 a	16.3 a	30.2 a	78.8 a	9.1 a
	P	W	283 a	193 ab	15.3 ab	26.3 a	68.8 b	8.6 ab
09/10	W	W	297 a	202 a	12.9 b	21.0 b	68.1 a	7.3 b
	O	W	279 b	198 a	15.0 a	26.0 a	76.5 a	8.1 a
	P	W	268 b	213 a	14.1 a	25.8 a	67.8 a	8.6 a
10/11	W	W	-	137 b	10.6 b	-	-	7.2 b
	O	W	-	157 a	11.8 a	-	-	8.3 a
	P	W	-	152 ab	12.4 a	-	-	7.8 ab
07/08	W	O	na	229	14.9	-	-	5.0
08/09	W	O	295 b	206 a	12.3 a	14.6 a	69.6 a	4.8 a
	P	O	314 a	230 a	13.8 a	15.7 a	73.4 a	5.4 a
09/10	W	O	201 a	128 b	7.2 b	11.8 a	64.5 a	2.6 b
	P	O	218 a	155 a	8.9 a	14.3 a	71.3 a	3.4 a

W: Winter Wheat; O: Oilseed Rape; P Winter Pea

[1] Within each year, letters denote significant differences among the cropping sequences (Tukey, 0.05)

3.4. N leaching at rotation scale

With the same cropping sequence, N leaching varied largely across the experimental years, possibly due to the effects of different climate conditions. For example, N leaching after wheat (with wheat as the preceding crop) reached 34 kg N ha^{-1} in the year 2008-2009, 12 kg N ha^{-1} in the year 2009-2010 of the sequence (W)-WOW, and 23 kg N ha^{-1} in the year 2010-2011 of the sequence (W)-OWW. It thus becomes challenging to directly extrapolate yearly results of N leaching to other climate conditions (Table 1).

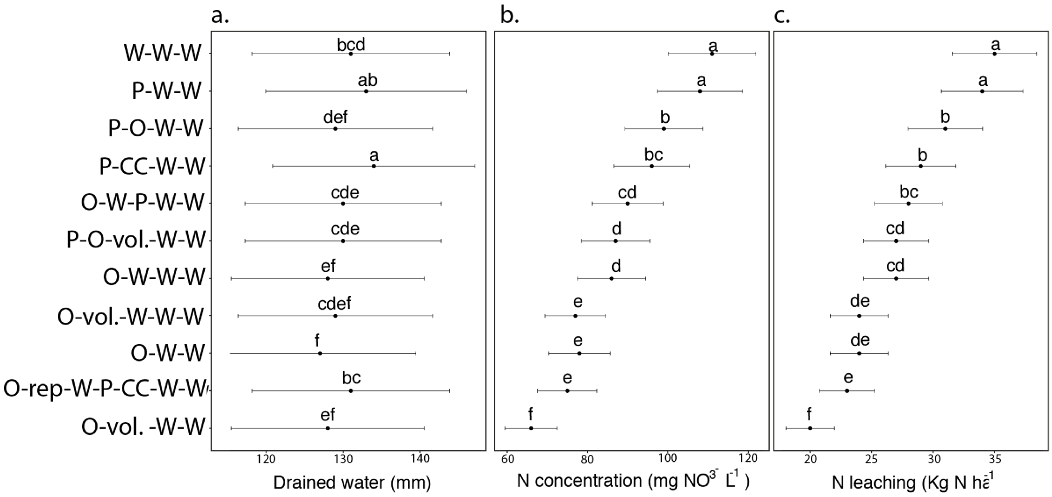
The simulation results based on the 20 climate years showed that cropping sequences with only wheat had one of the highest mean yearly values of leached N (35 kg N ha^{-1} yr^{-1} - Figure 2) and N concentration in drained water per year (111 mg NO_3^- L^{-1} yr^{-1}). Including pea in an only-wheat cropping sequence did not increase the mean N leaching (34 kg N ha^{-1} yr^{-1}), nor the N concentration in drained water (108 mg NO_3^- $L^{-1}yr^{-1}$). Adding a catch crop after pea in this sequence led to a significant decrease in mean N leaching and N concentration in drained water (28 kg N ha^{-1} yr^{-1} and 93 mg NO_3^- L^{-1} yr^{-1} respectively) than the same sequence with no cover crop.

Replacing wheat with oilseed rape in a 3-year wheat sequence led to a significant decrease in leached N (Figure 2: -9 kg N ha^{-1} $year^{-1}$) and N concentration in drained water (-43 mg NO_3^- L^{-1}). This decrease was even larger when the oilseed rape volunteers (vol) were not destroyed (-15 kg N ha^{-1} and -55 mg NO_3^- L^{-1} for O-vol-WWW compared to the WWW sequence).

Four-year sequences with pea, oilseed rape, and wheat (POWW) led to lower mean yearly leached N and N concentrations in drained water than 3-year only-wheat sequences (WWW: -4 kg N ha^{-1} yr^{-1} and –12 mg NO_3^- L^{-1}). The addition of both volunteers after oilseed rape and catch crop after pea in this 4-year sequence (O-vol-WP-CC-WW) led to even lower leached N and N concentration in drained water (-8 kg N ha^{-1} yr^{-1} and -24 mg NO_3^- L^{-1} yr^{-1} compared to the WWW sequence).

The addition of pea in a 4-year oilseed rape and wheat sequence (OWPWW *vs.* OWWW sequences) did not significantly increase mean yearly leached N and N concentration in drained water in situations with bare soils in autumn (28 kg N ha^{-1} and 90 mg NO_3^- L^{-1} vs. 27 kg N ha^{-1} and 86 mg NO_3^- L^{-1}). The two same cash cropping sequences, with the addition of oilseed rape volunteers and a catch crop after pea (O-vol-WPCCWW *vs.* O-vol-WWW sequences) resulted in similar leached N and N concentrations in drained water (23 kg N ha^{-1} and 75 mg NO_3^- L^{-1} *vs.* 24 kg N ha^{-1} and 77 mg NO_3^- L^{-1}).

Fig. 2. Mean yearly simulated drained water (mm ha^{-1} – a.), N concentration in drained water (mg NO_3^- L^{-1} – b.) and N leaching (kg N ha^{-1} – c.) over 20 climate years for cropping sequences based on wheat, oilseed-rape and pea. Bars represent the 95% confidence intervals over the 20 climate years. P= Pea, O= Oilseed rape, W= Wheat, CC= Catch crop, vol= volunteers. Letters denote yearly significant differences (Tukey, 0.05);



4. Discussion

Our study demonstrates that diversified crop rotation (e.g., including pea, oilseed rape in wheat-based crop sequence) reduced N leaching risks at the rotation scale. We identified the direct (during the winter) and indirect (during the winter after the following crop) effects of break crops on N leaching. Specifically, we showed that break crops reduce the soil mineral N content (Figure 1) and N leaching (Figure 2) during the winter after harvest of the following crop. Whereas this result was already observed by *Thomsen et al. (2001)* and *Jensen and Hauggaard-Nielsen (2003)* for legume break crops in non-fertilised situations and short term experiments, we show here for the first time, similar impacts on fertilised break crops, which is the most frequent situation in intensive cereal systems, and considering 20 climate years. This effect is quantitatively large. For instance, growing pea rather than wheat as the preceding crop amounted to a mean decrease of 16-28 kg N ha^{-1} after the following wheat harvest, depending on the study sites. This contributes directly to reducing the risk of N pollution of the environment.

This effect is possibly mediated by the positive effect of break crops on the growth and nutrient uptake of their following crops (Table 1). Such improvement of the functioning of the following crop was also shown by *Cernay et al. (2017)* in a meta-analysis of yield, and by *Luce et al. (2015)* and *Jensen and Hauggaard-Nielsen (2003),* in experimental results on N uptake. This higher N uptake or yield of the following crop could partly derive from better growth of the crop and improved functioning of the root system due to the reduced incidence of root and leaf diseases, as shown by *Stevenson and van Kessel (1996) or Perralta et al. (2018)*. It could also derive from increased P, K, and S availability, improved soil microbial activity (*McDaniel et al., 2014*), or the release of growth substances from the break crop (e.g., *Beckie et al., 1997, Lupwayi and Kennedy, 2007*). The exact contribution of each of these mechanisms on plant production remains mostly unknown (*Perralta et al., 2018*), like their indirect effect on N leaching. Understanding the precise mechanisms underlying the beneficial impacts of crop diversification on soil and plant N dynamics in the following years after harvest of the break-crop remains a key to maximizing the positive contribution of crop diversification.

Break crops also seem to favour soil N mineralisation during the autumn after the harvest of their following crops. The N release between harvest and the beginning of winter almost doubled when the preceding crop was pea or oilseed rape rather than wheat (Figure 1 – S4). The break crops have long-term effects on N mineralisation (*Grant et al., 2016*), and similar changes in N mineralisation in autumn after the crops following break crops were observed by *Engström (2010)* and *Ryan et al. (2006)*. The portion of legume residues (aboveground, roots and rhizodeposition) that is not mineralised in the first year is slowly mineralised over subsequent years, possibly at a rate as low as 5-10% per year (*Fillery, 2001; Mayer et al., 2003; Peoples et al., 2009*). Yet, the main effects of legumes on N mineralisation occur during the first month after their harvest: legume residues are characterised by a low C:N ratio (*Reeves et al., 2018*), leading to the rapid release of inorganic N (as reflected by the higher SMN in autumn measured in our experiment). In the absence of catch crops or volunteers, the high mineralisation rate increases N leaching risk in the following winter (*Beaudoin et al., 2005; Plaza-Bonilla et al., 2015*). This has led several regions in the world to make it mandatory to cover the soil in winter after these crops (e.g., in some parts of France).

We found no significant differences in SMN after pea or wheat harvest (Figure 1); but we did find a substantial variation in SMN between these two crops among years (Table S2). *Webster et al. (2003)* and *Gan et al. (2010)* also observed high inter-site and inter-year variation in SMN between these two crops, highlighting the difficulty involved in extrapolating from experiments based on a few soil-climate situations. This high variability is partly caused by differences in rainfall or evapotranspiration across years (Figure S1 and S2). Yet, a large share of the literature agrees on higher SMN after pea than after cereal harvests (from 5 to 50 kg N ha^{-1} – e.g., *Engström, 2010; Jensen et al., 2004*). SMN at pea harvest is generally more abundant in deep soil layers than at wheat harvest, as observed in our study. This can be attributed to the shallower pea root system compared to that of wheat *(Haugaard-Nielsen et al., 2001).* A denser and deeper roots system may improve the N uptake from deeper soil layers and reduce N leaching. The variability of total SMN between studies could also arise from the different fertilisation strategies (*Pandey et al., 2018*), as over-fertilization was sometimes observed. In our study, the amount of N fertiliser applied to the crop was adjusted based on the nature of the preceding crop and available management tools (e.g., the balance-sheet method) as performed in farm conditions. Nonetheless, we measured a relatively high SMN after the wheat harvest (mean value of the experimental years of 62 kg N ha^{-1} in our study) compared to other studies (e.g., *Maidl et al. (1996) and Delin et al. (2008)* measured 15-30 kg N ha^{-1} after cereal crops). Our simulations over 20 climate years allowed us to account for some of the inter-annual variability. Other experimental data or longer duration trials would make it possible to better define the effects and variability of crop functioning according to their preceding crop and their impacts on post-harvest soil mineral N.

The positive impacts of the catch crops on reducing N leaching estimated in our study (Figure 2) is consistent with the results of numerous field trials (e.g., *Rasse et al., 2000; Torstensson and Aronsson, 2000; Constantin et al., 2010; Valkama et al., 2015*) and meta-analyses (*Abdalla et al., 2019; Thapa et al., 2019*). The quantitative effects estimated by the LIXIM model are lower than the effects measured by *Constantin et al. (2010)* at three different experimental sites in France (from -36 to -62% *vs.* -15% in our study). Similarly, the reduction in the N concentration in the drained water in situations with catch crops compared to situations without catch crops was lower in our simulations (-36%) than in *Beaudoin et al. (2005),* who estimated a 50% decrease. Our results thus represent a conservative estimation of the effect of a catch crop on N leaching.

Despite the recognized ecological and agronomic benefits of crop diversification (e.g. *Beillouin et al., 2021*), cereal monocropping continues in large areas in intensive cereal producing regions (e.g., *Meynard et al., 2018* in France**,** *Becker-Rehef et al., 2018* in the US*).* Our analysis showed that a marked reduction in N losses is possible for these farming regions with the introduction of break crops, even when pea is grown during the cropping sequence. Combining break crops with catch crops or volunteers reduces the simulated N leached by up to 40% over 20 years compared to only-wheat cropping sequences. This estimation does not account for other beneficial impacts of crop diversification on the N cycle, such as fewer ecological impacts associated with the general reduction of the synthetic N fertilisers after legumes (*Nemecek et al., 2008*).

5. Conclusion

Our study, based on numerous soil mineral measurements in field conditions and optimisation-based model simulations, quantified the direct (during the winter following harvest) and indirect effects (during the winter after harvest of the following crop) of break crops, the impacts of catch crops and of climate variability in winter on N leaching. We showed that including pea in a wheat-based sequence does not increase mean N leaching at the scale of the rotation, whether associated or not with a catch crop, even if the catch crop reduces N leaching. Moreover, including oilseed rape decreased N leaching compared to only-wheat-based cropping sequences. Combining a break crop with catch crops or volunteers may reduce the N leaching up to 40% compared to wheat monoculture. Large potential reduction in N leaching could be achieved in numerous intensive cereal regions of the world where cropping systems are usually based on very few crops, mainly cereals. Understanding the mechanisms underlying the beneficial impacts of crop diversification on long-term soil and plant N dynamics is still a major scientific challenge.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Contribution

MHJ, AS, BC designed the study. EB, EC conducted the trial. DB, CC, EB, EC built the database, DB performed the statistical analyses and drafted the paper. DB, MHJ, EP discussed the results and contributed to the final version of the paper.

Supplementary information

Fig. S1. Mean temperature (a.), cumulative rainfall (b.) and cumulative evapotranspiration (c.) during the four experimental years, compared to the 1992-2012 climate years in Grignon. The years 2008-2009, 2009-2010, 2010-2011, 2011-2012 are in red, blue, green and black respectively. Values for each of the other 20 years are in gray.

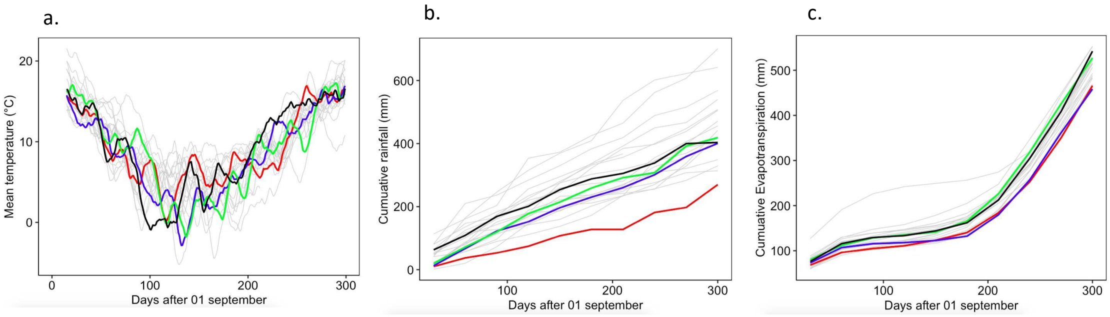


Fig. S2. Mean temperature (a.), cumulative rainfall (b.) and cumulative evapotranspiration (c.) during the four experimental years, compared to the 1992-2012 climate years in Holnon. The years 2008-2009, 2009-2010, 2010-2011, 2011-2012 are in red, blue, green and black respectively. Values for each of the other 20 years are in gray.

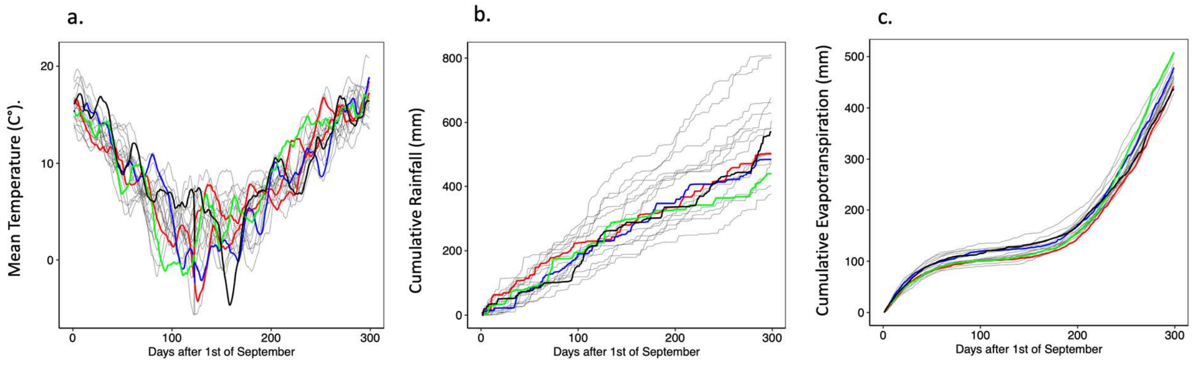


Fig. S3. Observed vs. simulated values of soil mineral N in February (end of winter) in the 0-30, 30-60, 60-90 and 0-90-cm soil layers. The symbols represent the different current crops. The solid lines correspond to the 1:1 line. (*correction needed to the figure:* 0-30 cm soil layer / 30-60 cm soil layer / Observed soil mineral N)

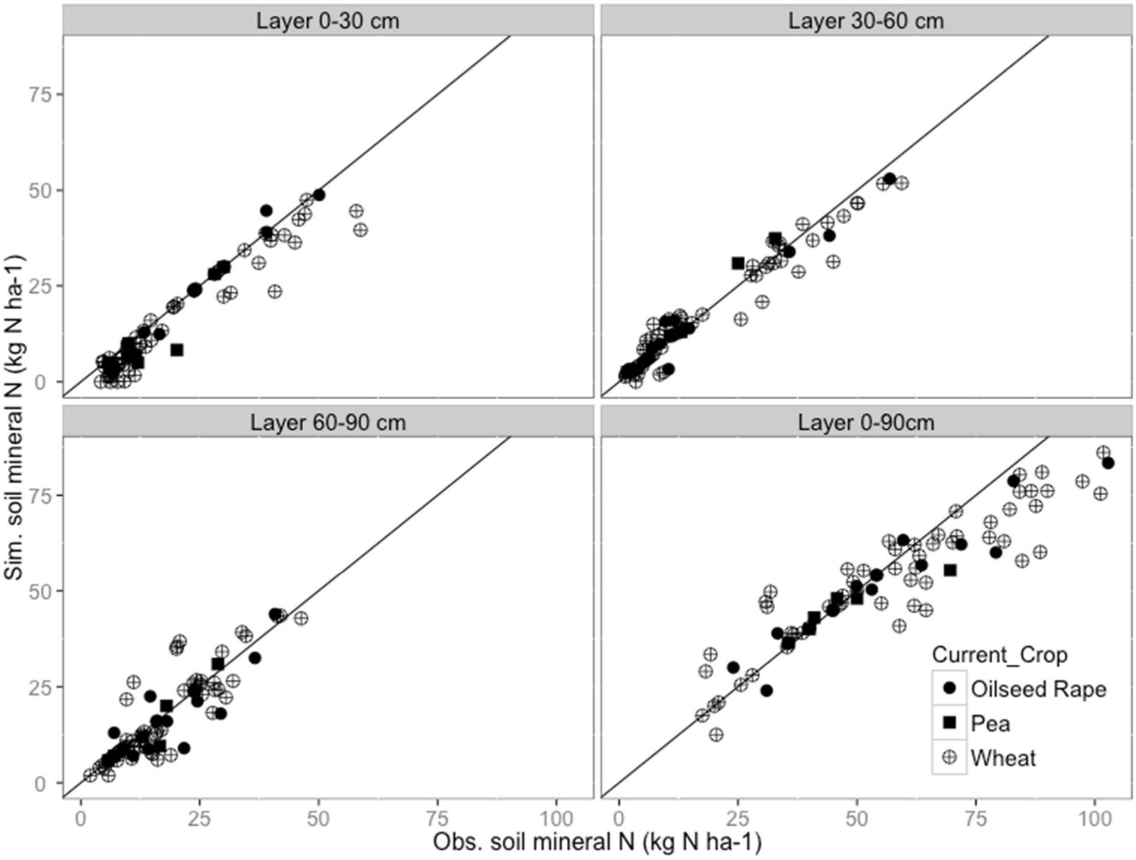
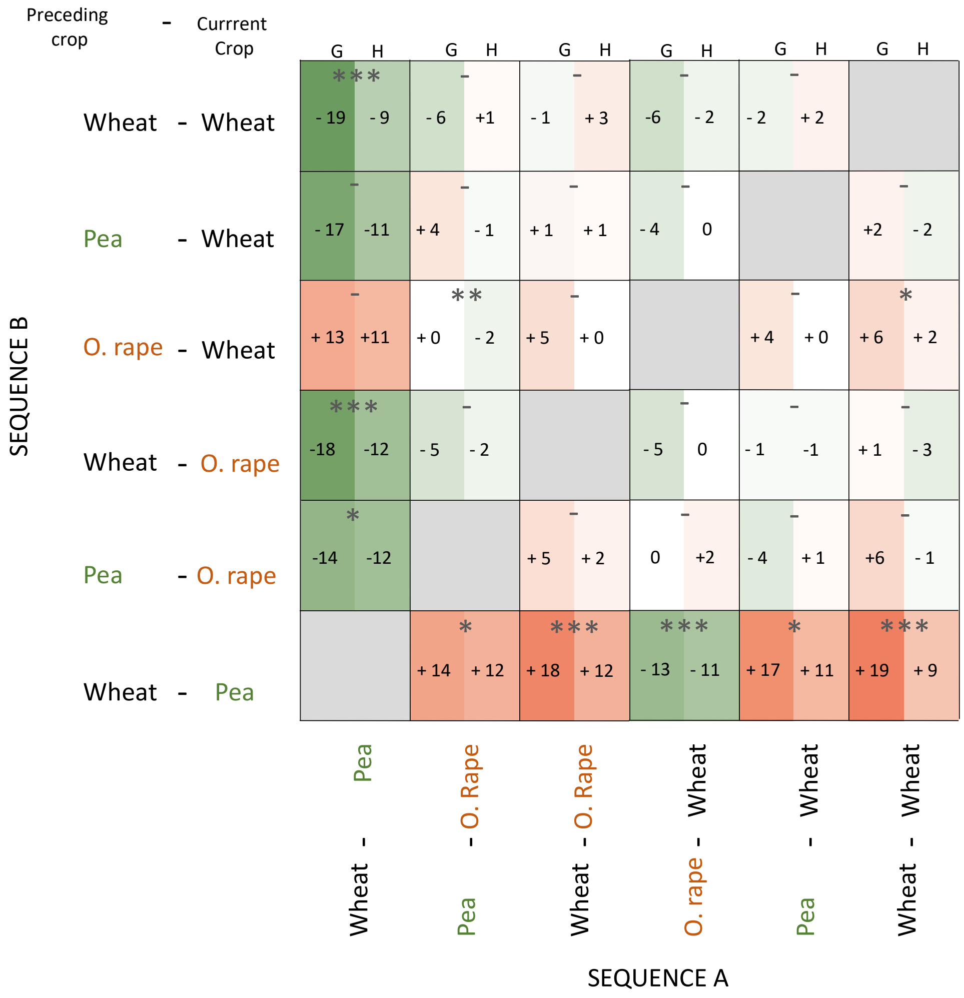


Fig. S4. Mean differences in soil mineral nitrogen (SMN) at beginning of winter between 6 sequences of ‘preceding crop-current crop’ at Grignon site. The numbers and colours in the cells correspond to the difference in SMN if cropping sequence B (Y-axis) is grown instead of cropping sequence A (X-axis). Statistical differences are given for the mean value <u>in the upper right? at the top?</u> of the cells (***: p value <0.001; **: p-value <0.01; *: p-value <0.05; -: non-significant). Values of SMN per year are listed in Table S2.

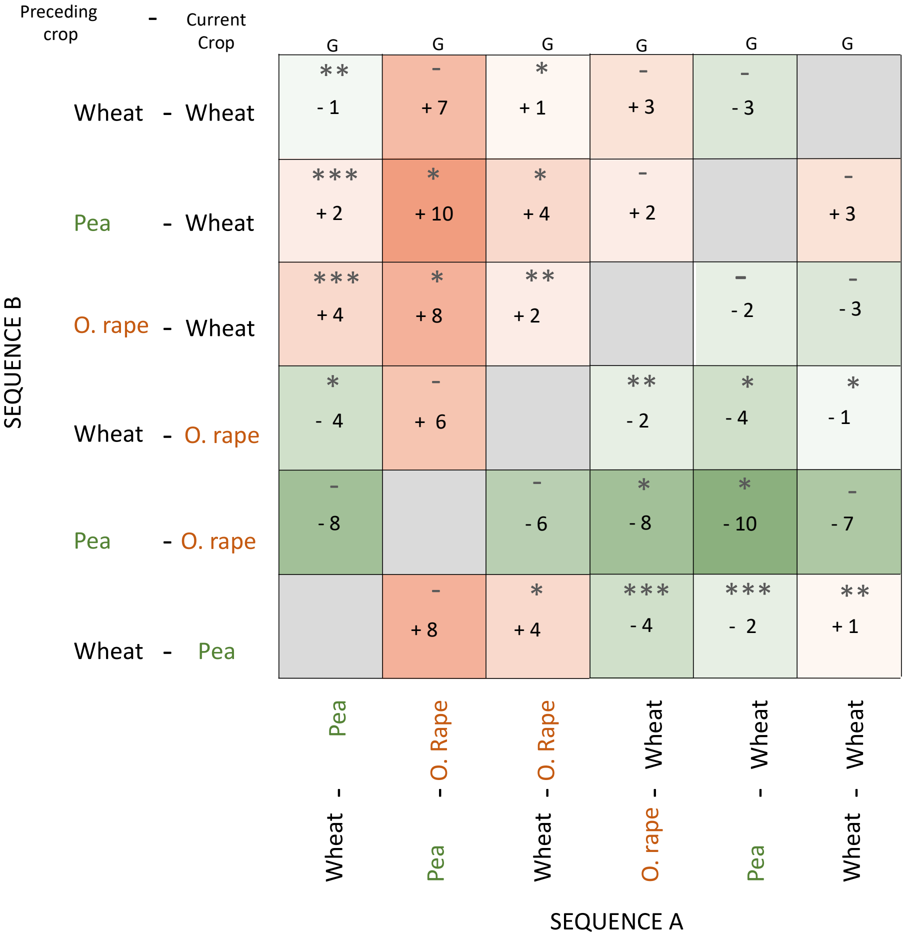
Preceding crop - Current Crop	G Wheat - Pea	G Pea - O. Rape	G Wheat - O. Rape	G O. rape - Wheat	G Pea - Wheat	G Wheat - Wheat
Wheat - Wheat	** - 18	- + 3	* - 6	- + 14	- + 18	
Pea - Wheat	*** - 28	* - 16	* - 20	- - 4		- - 18
O. rape - Wheat	*** - 24	* - 12	** - 16		- 4	- -14
Wheat - O. rape	* + 12	- + 4		** + 16	* + 20	* + 6
Pea - O. rape	- - 13		- - 4	* + 12	* + 16	- - 3
Wheat - Pea		- + 13	* - 12	*** + 24	*** 28	** + 18

Fig. S5. Mean differences in the proportion of soil mineral N (SMN) in the 60-90 cm layer (compared to the total SMN content in the 3- soil layers) at harvest between 6 sequences of ‘preceding crop-current crop’ at the Grignon (G) et Holnon (H) sites. The numbers and colours in the cells correspond to the difference of SMN if cropping sequence B (Y-axis) is grown instead of cropping sequence A (X-axis). Statistical differences are mentioned for the mean value at the top of the cells (***: p value <0.001; **: p-value <0.01; *: p-value <0.05; -: non-significant). SMN per year are listed in Table S2.



Preceding crop - Currrent Crop	Wheat - Pea		Pea - O. Rape		Wheat - O. Rape		O. rape - Wheat		Pea - Wheat		Wheat - Wheat	
	G	H	G	H	G	H	G	H	G	H	G	H
Wheat - Wheat	*** - 19	- 9	- - 6	+1	- - 1	+ 3	- -6	- 2	- - 2	+ 2		
Pea - Wheat	- - 17	-11	- + 4	- 1	- + 1	+ 1	- - 4	0			- +2	- 2
O. rape - Wheat	- + 13	+11	** + 0	- 2	- + 5	+ 0			- + 4	+ 0	* + 6	+ 2
Wheat - O. rape	*** -18	-12	- - 5	- 2			- - 5	0	- - 1	-1	- + 1	- 3
Pea - O. rape	* -14	-12			- + 5	+ 2	- 0	+2	- - 4	+ 1	- +6	- 1
Wheat - Pea			* + 14	+ 12	*** + 18	+ 12	*** - 13	- 11	* + 17	+ 11	*** + 19	+ 9

Fig. S6. Mean differences in the proportion of soil mineral N (SMN) in the 60-90 cm layer (compared to the total SMN content in the 3 soil layers) at beginning of winter when sequence B (Y-axis) is grown instead of sequence A (X-axis). Each cropping sequence is characterised by its preceding and current crop. Results are presented for the Grignon (G) and Holnon (H) sites. Statistical differences are mentioned for the mean value at the top of the cells (***: p value <0.001; **: p-value <0.01; *: p-value <0.05; -: non-significant). Raw SMN values per year are listed in Table S2.



SEQUENCE B: Preceding crop - Current Crop	G Wheat - Pea	G Pea - O. Rape	G Wheat - O. Rape	G O. rape - Wheat	G Pea - Wheat	G Wheat - Wheat
Wheat - Wheat	** - 1	- + 7	* + 1	- + 3	- - 3	
Pea - Wheat	*** + 2	* + 10	* + 4	- + 2		- + 3
O. rape - Wheat	*** + 4	* + 8	** + 2		- - 2	- - 3
Wheat - O. rape	* - 4	- + 6		** - 2	* - 4	* - 1
Pea - O. rape	- - 8		- - 6	* - 8	* - 10	- - 7
Wheat - Pea		- + 8	* + 4	*** - 4	*** - 2	** + 1

Table S1. Dates soil mineral nitrogen content were measured (dd/mm/yy) at the two experimental sites

	Grignon			Holnon	
	Harvest	Beginning of winter	End of winter	Harvest	End of winter
2007-2008	1/8/2008	17/11/2008	24/2/2009	18/9/2008	18/2/2009
2008-2009	6/8/2009	8/12/2009	16/2/2010	3/9/2009	28/1/2010
2009-2010	05/08/10	14/12/10	01/02/11	24/08/10	18/02/11
2010-2011	9/8/2011	-	-	-	-

Table S2. Details of annual soil mineral N values for the various cropping sequence at Grignon and Holnon sites.

Cropping sequence	Year	Site	Period	SMN	Number of replicates
Wheat-Wheat	1	Grignon	After harvest	49,2	(n=4)
	2	Grignon	After harvest	55,6	(n=4)
	3	Grignon	After harvest	74,1	(n=8)
	4	Grignon	After harvest	23,7	(n=20)
Wheat_oilseed Rape	1	Grignon	After harvest	26,2	(n=4)
	2	Grignon	After harvest	32,4	(n=4)
	3	Grignon	After harvest	27,4	(n=4)
	4	Grignon	After harvest		-
Wheat-Pea	1	Grignon	After harvest	62,5	(n=4)
	2	Grignon	After harvest	51,7	(n=4)
	3	Grignon	After harvest	42,4	(n=4)
	4	Grignon	After harvest		-
Pea-Wheat	1	Grignon	After harvest	-	-
	2	Grignon	After harvest	43,9	(n=4)
	3	Grignon	After harvest	56,3	(n=4)
	4	Grignon	After harvest	72,5	(n=4)
Pea-Oilseed Rape	1	Grignon	After harvest	-	-
	2	Grignon	After harvest	34,5	(n=4)
	3	Grignon	After harvest	25,1	(n=4)
	4	Grignon	After harvest	-	-
Oilseed Rape-Wheat	1	Grignon	After harvest	-	-
	2	Grignon	After harvest	42,6	(n=4)
	3	Grignon	After harvest	53,2	(n=4)
	4	Grignon	After harvest	91,4	(n=8)
Wheat-Wheat	1	Holnon	After harvest	50,8	(n=4)
	2	Holnon	After harvest	94,2	(n=16)
	3	Holnon	After harvest	79,4	(n=3)
	4	Holnon	After harvest	-	-
Wheat_oilseed Rape	1	Holnon	After harvest	69,0	(n=8)
	2	Holnon	After harvest	47,4	(n=3)
	3	Holnon	After harvest	69,7	(n=3)
	4	Holnon	After harvest	52,5	(n=3)
Wheat-Pea	1	Holnon	After harvest	72,5	(n=8)
	2	Holnon	After harvest	81,3	(n=8)
	3	Holnon	After harvest	85,4	(n=4)

	4	Holnon	After harvest		-
Pea-Wheat	1	Holnon	After harvest		-
	2	Holnon	After harvest	66,6	(n=6)
	3	Holnon	After harvest	40,6	(n=4)
	4	Holnon	After harvest		
Pea-Oilseed Rape	1	Holnon	After harvest		-
	2	Holnon	After harvest	46,8	(n=3)
	3	Holnon	After harvest	46,2	(n=3)
	4	Holnon	After harvest	61,5	(n=3)
Oilseed Rape-Wheat	1	Holnon	After harvest		-
	2	Holnon	After harvest	86,3	(n=6)
	3	Holnon	After harvest	49,0	(n=6)
	4	Holnon	After harvest		
Wheat-Wheat	1	Grignon	Beginning of winter	54,0	(n=12)
	2	Grignon	Beginning of winter	86,7	(n=4)
	3	Grignon	Beginning of winter	89,9	(n=8)
Wheat_oilseed Rape	1	Grignon	Beginning of winter	69,3	(n=4)
	2	Grignon	Beginning of winter	97,0	(n=4)
	3	Grignon	Beginning of winter	83,1	(n=4)
Wheat-Pea	1	Grignon	Beginning of winter	86,9	(n=8)
	2	Grignon	Beginning of winter	111,0	(n=4)
	3	Grignon	Beginning of winter	86,4	(n=4)
Pea-Wheat	1	Grignon	Beginning of winter	-	-
	2	Grignon	Beginning of winter	69,6	(n=4)
	3	Grignon	Beginning of winter	70,9	(n=4)
Pea-Oilseed Rape	1	Grignon	Beginning of winter	-	-
	2	Grignon	Beginning of winter	82,9	(n=4)
	3	Grignon	Beginning of	89,2	(n=4)

			winter		
Oilseed Rape-Wheat	1	Grignon	Beginning of winter	-	-
	2	Grignon	Beginning of winter	72,7	(n=8)
	3	Grignon	Beginning of winter	69,7	(n=4)

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