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► **To cite this version:**

Maé Guinet, Bernard Nicolardot, Anne-Sophie Voisin. Nitrogen benefits of ten legume pre-crops for wheat assessed by field measurements and modelling. *European Journal of Agronomy*, 2020, 120, pp.126151. <10.1016/j.eja.2020.126151>. <hal-02963953>

HAL Id: hal-02963953

<https://institut-agro-dijon.hal.science/hal-02963953v1>

Submitted on 22 Aug 2022

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1 Nitrogen benefits of ten legume pre-crops for wheat assessed by 2 field measurements and modelling

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9

10 Highlights

- 11 • N pre-crop effects of spring and summer legumes were assessed in two biennial field trials
- 12 • The STICS model accurately predicted soil N and humidity and was used to calculate N
13 mineralisation and leaching
- 14 • Legumes had a positive effect on wheat grain and shoot N yields compared to cereals
- 15 • Part of soil N was not retrieved by the following wheat and was leached
- 16 • Half of wheat shoot N variability was explained by soil nitrogen availability

17 Abstract

18 The positive effect of grain legume pre-crops on the yield of the subsequent crop has been studied
19 widely, whereas less information is available on the nitrogen (N) processes related to this positive
20 effect, especially for a wide range of grain legume species.

21 The objective was to quantify and understand the effect of grain legume compared to cereal pre-
22 crops (sown in 2014 and 2016) on grain and shoot N yields and shoot N concentration of wheat
23 (*Triticum aestivum*) grown the following year (2015 and 2017). Spring legumes (faba bean (*Vicia*
24 *faba*), fenugreek (*Trigolia foenum-graecum*), common vetch (*Vicia sativa*), lentil (*Lens culinaris*), lupin

25 (*Lupinus albus*), and pea (*Pisium sativum*)) were compared to barley (*Hordeum vulgare*). Summer
26 legumes (chickpea (*Cicer arietinum*), common bean (*Phaseolus vulgaris*), soybean (*Glycine max*) and
27 Narbonne vetch (*Vicia narbonensis*) were compared to sorghum (*Sorghum bicolor*). Inorganic N
28 remaining in the soil at pre-crop harvest (N sparing) was measured. The STICS model which
29 accurately predicted soil humidity and soil inorganic N in the pedoclimatic conditions of the field
30 experiments was used to calculate N mineralisation from pre-crop residues and N leaching between
31 pre-crop harvest and wheat harvest.

32 Grain and shoot N yields of unfertilised N wheat were respectively 27 and 25% higher after faba bean
33 and lentil compared to barley pre-crops, and 66 and 51% higher after summer legumes compared to
34 sorghum pre-crop. In the second experiment, N fertilisation reduced the positive effect of fenugreek,
35 and lentil on wheat yield compared to barley while it did not modify the pre-crop effect of the other
36 legumes. A positive relationship between N mineralisation from pre-crop residues and wheat shoot N
37 yield was established, with higher amounts of N available for the subsequent wheat after legume
38 pre-crops. In 2014, N sparing was higher after spring pre-crops compared to summer pre-crops
39 inducing higher N leaching after spring legume pre-crops, especially in the first experiment which was
40 characterised by heavy rain in summer and autumn. Estimating N availability by taking into account N
41 sparing, N mineralisation from soil and pre-crop residues and N leaching explained 49% of wheat
42 shoot N yield variability. Unquantified N processes and non-N processes might also have contributed
43 to the positive effects of legumes on the subsequent wheat.

44 **Key words**

45 Pre-crop effect; Grain legumes; Crop residues; Mineralisation; N leaching

46 **1. Introduction**

47 Cereal crops generally have higher yields when cultivated after unrelated species (Kirkegaard et al.,
48 2008) and especially after legume crops (Angus et al., 2015; Preissel et al., 2015). Some studies also
49 highlighted higher grain N concentrations in cereals grown after legumes compared to cereals grown

50 after cereals (Biederbeck et al., 1996; Gan et al., 2003). Part of the positive effect of legumes on the
51 following cereals are due to N benefits.

52 Indeed, a substantial amount of N can be left by legume crop residues. Moreover, during crop
53 residue decomposition by soil microbes, organic N is converted into inorganic forms that can be
54 taken up by the subsequent crop. Much of the inorganic N released is rapidly immobilised by soil
55 microbial biomass. Inorganic N accumulation in the soil occurs when the amounts of N released
56 exceeds the microbial N requirements (net N mineralisation). Conversely, when inorganic N released
57 from the mineralisation of crop residues is not sufficient, micro-organisms will also assimilate soil
58 inorganic N to meet their N requirements, which results in net N immobilisation (Nicolardot et al.,
59 2001). Net N mineralisation or smaller amounts of net N immobilisation are expected for legume
60 residues compared to cereal residues, since legume residues are generally characterised by higher N
61 concentrations and lower C:N ratios.

62 Furthermore, part of the additional soil inorganic N after legume crops can be attributed to N
63 “sparing” during legume growth (Evans et al., 1987; Herridge et al., 1995). Legume superficial root
64 systems compared to those of cereals can lead to higher amounts of inorganic N remaining in the
65 soil, especially in deeper layers (Hauggaard-Nielsen et al., 2001). A positive relationship between
66 wheat yields and the amount of inorganic N left in the soil after several pre-crops was established by
67 Angus et al. (2015) for dry land field experiments, and legumes systematically presented the highest
68 post-harvest amounts of inorganic N.

69 However, the effects of legume crops on the following cereals are highly variable (Kirkegaard et al.,
70 2008; Preissel et al., 2015) and they are difficult to predict. Part of this variability can be attributed to
71 the diversity of legume species, environmental conditions and agronomic practices. Legumes species
72 may differ according to the C:N ratio of crop residues (Palm et al., 2001) or their ability to take up soil
73 inorganic N during their growth cycle (Dayoub et al., 2017; Guinet et al., 2018). The amounts of
74 inorganic N available for the following cereals, derived from N mineralisation of crop residues and/or
75 N “sparing” may therefore depend on legume pre-crop species.

76 For a given pre-crop species, the effect on the following wheat can vary considerably with
77 pedoclimatic conditions (Cernay et al., 2017). Hence, N fluxes occurring after pre-crop harvest that
78 determine the amount of inorganic N available for the following cereal are highly dependent on
79 pedoclimatic conditions. On the one hand, N mineralisation from crop residues is positively
80 stimulated by high soil moisture and temperature (Abera et al., 2012). On the other hand, soil
81 inorganic N can be lost by N leaching if heavy rainfall occurs when high amounts of inorganic N are
82 present in the soil. In European climatic conditions, N leaching mainly occurs during the autumn-
83 winter period, when the soil is bare after pre-crop harvest and/or because the following cereal crop
84 requires only very small amounts of inorganic N during its early development. Higher amounts of N
85 losses by leaching are often measured after legume crops compared to cereals because of higher N
86 sparing and the higher N mineralisation from legume residues (Plaza-Bonilla et al., 2015). Moreover,
87 N mineralisation from crop residues and N loss dynamics may also vary according to legume growth
88 cycle. Early harvested legumes (i.e. spring legumes) induce a longer fallow period before the sowing
89 of the following crop, compared to legumes harvested later in the season (i.e. summer legumes).
90 Hence, legume growth cycles could affect N leaching by modulating the synchronisation between N
91 supply by legume pre-crops and N requirements of the following crop.

92 However, N fluxes occurring between pre-crop harvest and the subsequent crop harvest are
93 particularly difficult to quantify in field conditions. Therefore, their estimation often requires
94 agronomic dynamic models that take into account the impact of both crop residues characteristics
95 (i.e. C:N ratio) and pedoclimatic conditions on N fluxes after pre-crop harvest, thus allowing the
96 calculation of N budgets over time.

97 Cereal performance after legumes are also affected by agricultural practices. Several studies showed
98 that N fertilisation on the following cereal crops reduces the yield gap between a cereal cultivated
99 after legumes and a cereal cultivated after cereals (Cernay et al., 2017; Jensen et al., 2004). Hence, in
100 situations where high levels of N fertilisation are applied, the effect of legumes on the subsequent
101 cereal performance is likely to be negligible.

102 Although it is widely acknowledged that legumes have a positive effect on subsequent crop
103 performance (Angus et al., 2015; Cernay et al., 2017; Espinoza et al., 2012; O'Donovan et al., 2014;
104 Preissel et al., 2015; Walley et al., 2007; Williams et al., 2014), it is unclear to which extent N
105 processes explain legume positive pre-crop effect on the following crop, because of the difficulty to
106 measure some N fluxes occurring after legume harvest. Moreover, little is known about the effect of
107 legume growth cycle on the dynamic of N fluxes occurring after legume harvest and on the
108 performance of the following crop.

109 Hence, the aims of our study were the following: i) to compare the effect of ten legume and two
110 cereal pre-crops on the subsequent performance of wheat; ii) to quantify N fluxes occurring between
111 the pre-crop harvest and the subsequent wheat harvest through field measurements and modelling,
112 and evaluate how they were affected by pre-crop (spring and summer) and climatic conditions; and
113 iii) to evaluate whether the subsequent wheat performance could be explained by those N fluxes. To
114 achieve these aims, a two-year field experiment was repeated twice between 2014 and 2017; i.e. the
115 first year, legume and cereal pre-crops were cultivated in the same field and followed by wheat the
116 second year, after the incorporation of pre-crop residues in the soil. Simulations with STICS model
117 were performed to estimate soil N fluxes occurring during the period spanning from pre-crop harvest
118 to wheat harvest: N mineralisation of pre-crop residues and soil organic-matter, and N leaching.

119 2. Materials and Methods

120 2.1. Field experiments

121 2.1.1. *Site and climatic conditions*

122 Two field experiments, each lasting two years were carried out at the INRA Dijon experimental site of
123 Bretenière (Eastern France; 47.241 N, 5.115 E, altitude = 206 m) in 2014-2015 (Experiment I) and
124 2016-2017 (Experiment II). The soil is classified as Cambisol (Eutric) (WRB, 2006) with a clayey surface
125 layer (depth = 0.65 ± 0.15 m) developed on an alluvial coarse layer. Before the beginning of both

126 experiments (2014 and 2016), soil samples were collected at 30 cm depth to determine soil
127 characteristics (Table 1).

128 The site is subject to a semi-continental climate but the climatic conditions were contrasted between
129 Experiments I and II. Cumulative rainfall and mean temperature between the beginning of pre-crop
130 harvest and wheat harvest were 850 mm and 11.8°C in Experiment I and 600 mm and 11.3 °C in
131 Experiment II (Fig. 1). Rainfall were unusually high between July and November 2014 (541 mm)
132 compared to 2016 (303 mm) and to the mean rainfall over the 20 past years during the same period
133 (362 mm). Rainfall between January and June were similar in 2015 (252 mm) and 2017 (293 mm), but
134 they were lower than the rainfall averaged over the 20 past years during the same period (340 mm).

135 2.1.2. Experimental design

136 Pre-crop treatments: Each experiment consisted of a two-year crop succession. Legume and cereal
137 pre-crop treatments were sown in 2014 and followed by winter wheat that was harvested in 2015
138 (Experiment I). In 2016, the same pre-crops were grown in a field nearby the first experiment, and
139 followed by winter wheat harvested in 2017 (Experiment II). This resulted in two separate data sets
140 that allowed us to evaluate the effect of pre-crops on the following wheat performance.

141 In Experiment I and II, nine legume pre-crops were included: faba bean (*Vicia faba*, cv. Espresso),
142 chickpea (*Cicer arietinum*, cv. Twist in 2014 and Vulcano in 2016), common bean (*Phaseolus vulgaris*,
143 cv. Flavert), common vetch (*Vicia sativa*, cv. Candy), lentil (*Lens culinaris*, cv. Anicia), lupin (*Lupinus*
144 *albus*, cv. Feodora), pea (*Pisium sativum*, cv. Kayanne), soybean (*Glycine max*, cv. Sultana(000)) and
145 Narbonne vetch (*Vicia narbonensis*, cv. Clara). In Experiment II, fenugreek (*Trigolia foenum-graecum*,
146 cv. Fenu-fix) was also cultivated (Table 2). Pre-crops were either sown in March or in May, depending
147 on the physiology of each species (Table 2) and were referred as spring pre-crops and summer pre-
148 crops, respectively, thereafter. Legume seeds were inoculated at sowing with species-specific strains
149 of N₂-fixing bacteria to ensure symbiotic N₂ fixation, as described in Guinet et al. (2018). No N
150 fertilisation was applied to any of the legume pre-crops in either experiment.

151 Two N fertilised cereal pre-crops were also grown in Experiments I and II: barley (*Hordeum vulgare*,
152 cv. Irina) and sorghum (*Sorghum bicolor*, cv. Quebec); they were sown in March and May,
153 respectively. Nitrogen fertilisation was applied in form of NH_4NO_3 at rates of 60 and 75 kg N ha^{-1} in
154 2014 (3rd and 28th April, respectively) for barley. In 2016, due to the extremely wet conditions in April
155 and May, only 70 kg N ha^{-1} were applied on barley on the 19th of April to reduce the risk of N
156 leaching. N fertiliser was applied twice during sorghum growth at rates of 50 kg N ha^{-1} each in 2014
157 (9th May and 13th June) and 2016 (7th June and 7th July) Pre-crops were sown on plots 1.5 m wide and
158 12 m long with four replicates per pre-crop in Experiment I and eight replicates in Experiment II.
159 Sowing densities are summarised in Table 2.

160 Pre-crop management: In order to reduce water stress, irrigation was supplied on pre-crop
161 treatments. In Experiment I, spring pre-crops were irrigated with 35, 40 and 50 mm on March 26th,
162 May 16th and June 18th 2014, respectively. Summer pre-crops were irrigated with 40, 40 and 50 mm
163 on May 16th, May 27th and June 14th 2014, respectively. In Experiment II, 50 mm was supplied to
164 summer pre-crops on July 11th 2016. Weeds were controlled by hand weeding and one insecticide
165 (Karate Zeon) was applied on April 25th 2014 and April 21th 2016 in Experiment I and II, respectively.

166 Wheat following all pre-crop treatments: Pre-crop grains were harvested with a harvesting machine.
167 No grains were harvested for chickpea in 2014 and Narbonne vetch in 2014 and 2016, due to climatic
168 conditions unsuitable for grain production. In these cases, the whole plant was considered as crop
169 residues. Two to seven weeks after pre-crop grain harvest, crop residues were chopped and
170 incorporated into the soil using a rototiller (see Table 2 for pre-crop incorporation dates). Thereafter,
171 winter wheat (*Triticum aestivum*, cv. Rubisko) was sown on the same plots on October 20th 2014 (300
172 seeds m^{-2}) in Experiment I and October 24th 2016 (350 seeds m^{-2}) in Experiment II. In order to control
173 weeds and pathogens, an herbicide (February 26th 2015, Arbalète) and a fungicide (April 30th 2015,
174 Adexar + Voxan) were applied on wheat in Experiment I. In Experiment II, irrigation was applied on
175 wheat on April 5th and 19th 2017 (27 mm and 30 mm, respectively) (Fig. 1B).

176 Wheat N treatments: In order to express pre-crop maximum effect on wheat, no N fertiliser was
177 applied in Experiments I and II, corresponding hereafter to the ON treatment.

178 In Experiment II, an additional N fertilisation treatment on wheat was studied. At the end of wheat
179 tillering (April 4th 2017), 40 kg N ha⁻¹ in the form of NH₄NO₃ was applied on half of the trial (with four
180 replicates per pre-crop treatment), corresponding to the 40N treatment.

181 *2.1.3. Sampling and measurements*

182 Pre-crop samplings: The above-ground biomass of legume and cereals pre-crops was sampled at
183 physiological maturity (Table 2) on subplots of 1 m². Straw was separated from grains and considered
184 as crop residues left on the soil at maturity (except for chickpea in 2014 and Narbonne vetch in 2014
185 and 2016 where the whole plant was considered as crop residues).

186 Wheat samplings: At physiological maturity, wheat was manually harvested on subplots of 1 m² and
187 straws were separated from grains. In order to obtain a better estimation of the wheat grain yield,
188 grains were also harvested on 6.5 m² using a harvesting machine.

189 Measurements: Pre-crop residues and wheat straw and grain samples were oven-dried for 48 h at
190 80°C before dry matter was determined. Samples were milled and N and C concentrations were
191 measured using an elemental analyser (ANCA-GLS, Sercon Ltd., Crewe, UK).

192 *2.1.4. Calculations*

193 The amount of N in pre-crop residues was calculated by multiplying dry matter of residues by N
194 concentration of residues (Table S1).

195 Total wheat shoot dry matter was calculated by multiplying wheat grain yield measured on the 6.5
196 m² surface by total wheat dry matter/wheat grain dry matter ratio measured on the 1 m² subplot.

197 The total amount of wheat shoot N (i.e. shoot N yield) was then calculated by multiplying wheat
198 shoot dry matter by N concentration of wheat shoot.

199 2.2. Estimation of N fluxes using STICS model

200 The STICS model (Simulateur multi disciplinaire pour les Cultures Standards) was developed at INRA
201 (France) to simulate crop growth, soil water, carbon and nitrogen balances as influenced by climatic
202 conditions, over either one crop cycle or several cultural successions at a daily time-step (Brisson et
203 al. (2003). The model STICS v8.50 was used to simulate N fluxes, such as N leaching, N mineralisation
204 from pre-crop residues and soil organic matter, between the pre-crop harvest and the winter wheat
205 harvest in Experiments I and II.

206 2.2.1. Model inputs

207 To run the simulations, a number of input variables are required, such as soil characteristics, initial
208 soil and pre-crop residue states, crop characteristics, crop management and climatic conditions
209 during the simulated period.

210 Soil Parameters: Permanent soil characteristics (clay, organic N, calcium, pH, etc.) were defined and
211 fixed using measurements carried out at the beginning of Experiments I and II (Table 1). Soil moisture
212 content at field capacity was estimated as the median value of water content measured on soil core
213 samples in mid-winter. Soil moisture content at wilting point and bulk density was estimated
214 according to Ugarte Nano et al. (2016), who measured these soil characteristics in a neighbouring
215 field experiment with standard reference farming practices as in the field prior to our experiment.
216 Maximal rooting depth was set at 60 cm because of the presence of an alluvial coarse layer, greatly
217 limiting root exploration below this layer. The thickness of the biological active layer for N
218 mineralisation from soil and pre-crop residues was set at ploughing depth.

219 Soil initialisation: soil inorganic N and soil moisture measurements from soil core samplings
220 performed shortly before or after pre-crop harvest were used to initiate the soil profile. In
221 Experiment I, soil core samplings were performed on August 5th 2014 for spring pre-crops and on
222 September 3rd 2014 for summer pre-crops. In Experiment II, soil core samplings were performed on
223 August 26th 2016 for spring pre-crops and on September 23rd 2016 for summer pre-crops.

224 Crop residue inputs: Pre-crop residue dry matter and its physicochemical characteristics (C:N ratio, C
225 concentrations, water content) at pre-crop harvest were used to initiate the simulations.

226 Wheat management: Winter wheat is one of several crops parametrised in STICS, using physiological
227 and morphological traits. Crop management specified dates and methods of soil tillage, wheat
228 sowing dates, sowing depth, sowing densities, row spacing, and date and amounts of water applied
229 by irrigation.

230 Climatic inputs: Climatic conditions were measured by a weather station located at the INRA
231 experimental site of Bretenière. Minimum and maximum temperatures, rainfall, global radiation and
232 Penman evapotranspiration were specified in the model at a daily time-step.

233 2.2.2. Model simulations and evaluation

234 A simulation was performed for each pre-crop treatment and for Experiments I and II focusing on the
235 0N wheat treatment, leading to a total of 23 simulations. The model was evaluated for its ability to
236 predict the daily amount of inorganic N and water content in the soil profile. Soil core samplings in
237 the 0-60 cm soil layer were performed monthly on wheat plots for all pre-crop treatments, between
238 January and July 2015 in Experiment I, and between October 2016 and July 2017 in Experiment II,
239 with a 5 cm diameter auger.

240 The amounts of soil inorganic N and soil moisture measured from the soil samples were expressed in
241 kg N ha⁻¹ and mm, respectively and compared with the simulated data on the same date and for the
242 same pre-crop treatment.

243 Three statistical criteria were used (Wallach et al., 2013) based on the comparison between model
244 predictions and observed values: RRMSE, Bias and maximal error (ME):

$$245 \text{ RRMSE} = \frac{\sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2}}{\bar{y}} \quad (1)$$

$$246 \text{ Bias} = \frac{1}{n} \sum_{i=1}^n (\bar{y}_i - \hat{y}_i) \quad (2)$$

$$247 \text{ ME} = \max |\bar{y}_i - \hat{y}_i|_{i=1}^n \quad (3)$$

248 where y_i is the observed value; \hat{y}_i the simulated value; \bar{y} the mean observed values and n the number
249 of observations (sampling dates x pre-crop treatments).

250 From the simulations, the cumulative amounts of N leached under the soil profile, and N
251 mineralisation from pre-crop residues and soil organic matter were calculated.

252 In order to understand the effect of pre-crop treatments on the N fluxes and their link with wheat
253 performance, wheat shoot N yield was plotted as a function of the cumulative amount of N
254 mineralisation from pre-crop residues, which was calculated with the STICS model.

255 The amount of inorganic N actually available for wheat during wheat growth cycle was calculated as
256 follows (eq. 4):

$$257 \quad \text{N available for wheat} = N_{\text{harv}} + N_{\text{minr}} + N_{\text{minsoil}} - N_{\text{leached}} \quad (4)$$

258 with the amount of soil inorganic N at pre-crop harvest (N_{harv}), the cumulative amounts of N
259 mineralisation from pre-crop residues (N_{minr}) of N mineralisation from soil organic matter (N_{minsoil})
260 and of N leached (N_{leached}) between pre-crop harvest and wheat harvest.

261 Wheat shoot N yield measured in the field experiments was then plotted as a function of N available
262 for wheat.

263 2.3. Data analysis

264 Statistical analyses were performed with R software version 3.4.2. (R Core Team, 2016). Two-way
265 analysis of variance (ANOVA) were performed to test for year effects (2015 and 2017), pre-crop
266 effects and year x pre-crop interaction effects on wheat grain yield, shoot N concentration and shoot
267 N yield, using data collected for unfertilized N wheat. ANOVAs were also performed to test for N rate
268 effects (0N and 40N), pre-crop effects and N rate x pre-crop interaction effects on the same three
269 response variables, using data collected in 2017 for fertilised and unfertilised N wheat. In both cases
270 spring and summer pre-crops were analysed separately. When no pre-crop by year or N rate
271 interactions were detected, pre-crop values were averaged over year or N rate. If the effects were
272 significant, contrasts among pre-crops were adjusted using the package {emmeans}.

273 The effect of year, legume growth cycle (spring and summer) and their interaction on Nleaching, was
274 also analysed. The homoscedasticity and normality of the residuals of the response variables were
275 tested using a Bartlett and Shapiro-Wilk test, respectively.

276 For all data analysis, significant differences among treatments were identified at the 0.05 probability
277 level of significance.

278 A multivariate analysis was performed to determine the relation between pre-crop characteristics, N
279 fluxes, environmental conditions and wheat performance. Pre-crop treatments were ordered
280 through a Principal Component Analysis (PCA) using the Canoco 5.10 program (ter Braak and
281 Šmilauer, 2012). The variables were standardised to obtain equal weights in the PCA.

282 3. Results

283 For both Experiments I and II, once the grains were harvested, the crop residues were incorporated
284 in the soil at two different dates for spring and summer pre-crops (Table 2). Because of the timespan
285 between the two incorporation dates, crop residues from spring and summer pre-crops were
286 subjected to different environmental conditions that may have affected their N mineralisation and
287 therefore the performance of the following wheat. Hence, in the following section, the performance
288 of wheat cultivated after spring and summer pre-crops are presented separately.

289 3.1. Effect of pre-crop treatments and year on unfertilised wheat performance

290 For spring pre-crops, species had a significant effect on grain and shoot N yields of unfertilised
291 wheat, but the interaction between year and pre-crop species was not significant (Table 3). On
292 average for both years (2015 and 2017), only faba bean and lentil increased significantly wheat grain
293 yield (+1.1 and +9 t ha⁻¹, respectively) and wheat shoot N yield (+ 18 and + 13 kg N ha⁻¹, respectively)
294 compared to barley. Conversely, wheat shoot N concentration presented no differences among
295 spring pre-crop treatments.

296 For summer pre-crops, all legume pre-crops induced higher wheat grain yields compared to wheat
297 cultivated after sorghum with an average increase of 1.6 t ha⁻¹. The interaction between year and

298 pre-crop was significant for wheat shoot N yield. Except for soybean in 2017, all legume pre-crops
299 induced higher wheat shoot N yields compared to wheat cultivated after sorghum with an average
300 increase of 24 kg N ha⁻¹ in 2015 and 26 kg N ha⁻¹ in 2017. For both experiments, Narbonne vetch pre-
301 crop treatment always induced the highest wheat grain yield and shoot N yields. Conversely, for both
302 years, wheat cultivated after sorghum, presented a slightly higher shoot N concentration compared
303 to wheat after legume summer pre-crop treatments.

304 Compared to sorghum, summer legume pre-crops induced higher wheat grain yield and shoot N yield
305 benefits than spring legume pre-crops compared to barley. This was explained by the choice of
306 reference cereal pre-crop. Indeed, wheat after sorghum always presented lower grain yields than
307 wheat after barley for both years (2015 and 2017) (Table 3).

308 3.2. Effect of N fertilisation on wheat performance

309 In 2017, N fertilisation had a significant effect on grain yield, shoot N concentration and shoot N yield
310 of wheat cultivated after all spring and summer pre-crops (Table 4). On average, for wheat cultivated
311 after legume and cereal spring pre-crops, N fertilisation increased grain yield, shoot N concentration
312 and shoot N yield by 1.2 t ha⁻¹, 0.10% and 27 kg N ha⁻¹, respectively. Considering all summer pre-
313 crops, wheat N fertilisation increased wheat grain yield by 1.6 t ha⁻¹, shoot N concentration by 0.04%
314 and shoot N yield by 29 kg N ha⁻¹. For spring pre-crops, the interaction between N rate and species
315 was significant for wheat grain and shoot N yields. While grain yield of unfertilised wheat was greater
316 after lentil and fenugreek compared to barley, it was no longer the case when wheat was N fertilised
317 (40N treatment). Likewise, wheat shoot N yield after lentil was not significantly different from wheat
318 after barley when N fertilisation was applied. For faba bean and common vetch and for all summer
319 pre-crops, grain and shoot N yields of unfertilised (0N) and fertilised (40N) wheat were always higher
320 after all legume pre-crops compared to cereal pre-crops. There were no longer significant differences
321 of wheat shoot N concentration between legumes and sorghum pre-crops when wheat was N
322 fertilised.

323 3.3. Estimation of N fluxes between pre-crop harvest and wheat harvest

324 To evaluate the model's predictive performance in our pedoclimatic conditions, simulated data were
325 compared with measured soil water content and soil inorganic N, which are known to be strong
326 drivers of NO_3^- leaching, N mineralisation from pre-crop residues and soil organic matter (Fig. 2). The
327 STICS model was efficient for predicting soil water content, leading to a small RRMSE (0.092) (Fig.
328 2A). The RRMSE (0.462) was higher for soil inorganic N (Fig. 2B).

329 There was a slight tendency to over-estimate both variables, as we obtained an overall negative bias
330 but with small absolute values: -8.9 mm for soil water (Fig. 2A) and -3.9 kg N ha⁻¹ for soil inorganic-N
331 (Fig. 2B). Soil inorganic-N was systematically under-estimated in Experiment I (bias = +12.2 kg N ha⁻¹)
332 while it was always over-estimated in Experiment II (bias = -14.2 kg N ha⁻¹). However, the
333 classification between pre-crop species according to soil inorganic N was correctly simulated in both
334 experiments. Therefore, for a given experiment, simulations can be used to compare pre-crop
335 treatments but absolute values need to be considered with caution when comparing experiments.

336 Nitrogen mineralisation from crop residues was simulated for all pre-crops and both experiments
337 (Fig. 3A: Experiment I and 3B: Experiment II). Except for Narbonne vetch, rapid N immobilisation
338 occurred just after the incorporation of crop residues in the soil, followed by net N mineralisation a
339 few weeks later. Because summer pre-crop residues were incorporated several weeks after spring
340 pre-crop residues, we observed a delay in the dynamics of N mineralisation from pre-crop residues
341 between spring (Fig. 3: dark colours) and summer (Fig. 3: light colours) pre-crop residues. At the end
342 of the simulations, the cumulative amounts of N mineralisation from pre-crop residues ranged from -
343 24 kg N ha⁻¹ (barley) to +32 kg N ha⁻¹ (Narbonne vetch) in Experiment I and from -33 kg N ha⁻¹
344 (sorghum) to +35 kg N ha⁻¹ (Narbonne vetch) in Experiment II.

345 At the beginning of the simulation, the total amount of soil inorganic N was higher after spring pre-
346 crops compared to summer pre-crops in Experiment I (Fig 3C). Consequently, soil inorganic N was
347 higher during the whole simulation period for spring pre-crop treatments compared to summer pre-

348 crops, but with smaller differences near the end of Experiment I. In Experiment II (Fig 3D) the mean
349 amount of soil inorganic N at the beginning of the simulation was on average 53 kg N ha⁻¹.

350 The simulated amounts of soil water (Fig. 3E: Experiment I and 3F: Experiment II), were also higher at
351 simulation initiation for spring pre-crop treatments compared to summer pre-crops in Experiment I
352 and for barley in Experiment II. Once rainfall had filled the soil profile up to full water capacity, the
353 amount of soil water was identical for all pre-crop treatments. However, in Experiment I, soil reached
354 field water capacity almost immediately after initiating the simulations for spring pre-crop
355 treatments while it was reached two months later for summer pre-crops (October 12th 2014). In
356 Experiment II, field soil water capacity was reached even later for all pre-crop treatments (between
357 November 4th 2016 for barley pre-crop treatment and November 21th 2016 for chickpea, common
358 bean and sorghum).

359 In both experiments, most of the N leaching occurred between wheat sowing and the end of winter
360 (Fig. 4) as the soil remained at maximum water capacity almost throughout the period (Fig 3E and F).
361 The soil profile was regularly filled by rainfall. During this period, N leaching in Experiment I was on
362 average 77 kg N ha⁻¹ for spring pre-crop treatments, with values ranging from 63 kg N ha⁻¹ (pea) to 93
363 kg N ha⁻¹ (common vetch) (Fig. 4C). For summer pre-crop treatments the average value of N leaching
364 during the same period was 52 kg N ha⁻¹ (Fig 4D). Based on all pre-crop treatments, N leaching was
365 higher in Experiment I compared to Experiment II ($F(1,19)=55.51$, $p < 0.001$), because of lower rainfall
366 in Experiment II, especially in November (Fig. 1). Considering both experiments, N leaching was
367 higher after spring pre-crops compared to summer pre-crops ($F(1,19)=14.68$ $p < 0.01$), especially in
368 Experiment I.

369 3.4. Relationships between wheat performance, pre-crop characteristics, and pedoclimatic conditions
370 The first and second axes of the PCA accounted for 42.5 and 32.7%, respectively, of the total variance
371 (Fig. 5). The cumulative amounts of N-NO₃⁻ leached between pre-crop harvest and wheat harvest
372 (19%), cumulative rainfall during the simulation period (14%), water content in the soil profile (11%)
373 and soil inorganic N content (11%) at the beginning of the simulation accounted the most for the first

374 axis. These four variables were positively correlated but all four were correlated negatively with
375 wheat shoot N concentration. C:N ratio of pre-crop residues (21%) and the cumulative amounts of N
376 mineralised from pre-crop residues (20%) contributed the most to the second axis and were
377 correlated negatively. Conversely, the cumulative amounts of N mineralised from pre-crop residues
378 was positively correlated with wheat grain yield and wheat shoot N yield.

379 A clear distinction between pre-crop treatments could be made on the first axis of the PCA. Firstly,
380 according to the year of the experiment and, secondly, between spring and summer pre-crop
381 treatments, especially in Experiment I (Fig. 5). The distinction between both experiments was mainly
382 explained by contrasting rainfall conditions leading to different amounts of leached N-NO_3^- .
383 Experiment I was characterised by higher rainfall, especially between August and November 2014,
384 compared to Experiment II.

385 The second axis of the PCA separated pre-crops species according to their effect on wheat grain yield
386 and shoot N yield. Pre-crops for which the whole plant was considered as crop residues since no
387 grains were harvested (chickpea in Experiment I and Narbonne vetch in Experiment I and II) led to
388 the highest amounts of N mineralised from pre-crop residues (Nres_{min} , Fig. 5) and to the highest
389 wheat grain yield (Wheat_{Y}) and shoot N yield (Wheat_{N}). For harvested grain pre-crops, the highest
390 amounts of N mineralised from pre-crop residues was measured for faba bean, lentil and common
391 vetch, and associated with high wheat grain yield and shoot N yield. Conversely, cereal pre-crops
392 always had low N mineralisation rates of crop residues and were associated with the lowest wheat
393 grain and shoot N yields.

394 Figure 6A shows the positive linear relationship between wheat shoot N yield and cumulative
395 amounts of N mineralised from pre-crop residues throughout the simulation period. However, for a
396 given amount of N mineralised from pre-crop residues (for example 0 kg N ha^{-1}), wheat shoot N yield
397 still varied widely (between 60 and 77 kg N ha^{-1} in our example) according to the year of the
398 experiment and between summer and spring pre-crops. Hence, climatic conditions affected the N
399 losses by leaching, and may also have influenced the mineralisation soil organic N (as it depends on

400 soil temperature and moisture). Moreover, at pre-crop harvest, the amounts of soil inorganic N left
401 in the soil (N sparing) were variable (Fig 3C and D). The amount of N actually available during the
402 wheat growth cycle was therefore calculated and was plotted versus wheat shoot N yield (Fig. 6B). A
403 unique positive linear relationship was found between both variables for Experiments I and II and for
404 spring and summer pre-crops. Generally, spring pre-crops and unharvested legume pre-crops
405 (chickpea in Experiment I and Narbonne vetch in Experiment I and II) led to the highest amounts of N
406 available for the following wheat and were associated with the highest wheat shoot N yield.
407 Conversely, the rest of summer pre-crops led to the lowest amounts of N available for the following
408 wheat and were associated with the lowest wheat shoot N yield. However, the relationship did not
409 entirely explain the variability of wheat shoot N yield.

410 4. Discussion

411 4.1. Response of wheat performance to pre-crops and N fertilisation

412 Our study confirmed the positive effect of a wide variety of grain legumes on the yield of the
413 following unfertilised cereal, with several legume species relatively poorly studied in European
414 conditions. The increases of wheat grain yields after lentil and faba bean compared to barley (on
415 average 27 %) were similar to the average yield response of cereals cultivated after legumes reported
416 by Angus et al. (2015) and Cernay et al. (2017) (on average 33% and 29 % increases, respectively).
417 Conversely, increases of wheat grain yields after summer legume pre-crops compared to sorghum
418 (on average 61 %) were close to values reported by Preissel et al. (2015) (27 – 110% improvement)
419 and Espinoza et al. (2012) (23 to 152% increases). Higher increases of wheat grain yields after
420 summer legume pre-crops vs sorghum compared to spring legume pre-crop vs barley were mainly
421 due to the choice of the reference cereal pre-crop (i.e. barley or sorghum). Indeed, wheat after
422 sorghum always presented lower grain yields than wheat after barley.
423 However, benefits to wheat grain yields showed large variations among spring and summer pre-
424 crops, respectively, reflecting differences in legume pre-crops. For spring pre-crops, wheat grain

425 yields were the highest after faba bean and lentil as highlighted by Williams et al. (2014), Wright
426 (1990), O'Donovan et al. (2014) and St Luce et al. (2016). Surprisingly, wheat grain yields after pea
427 were slightly higher but not significantly different from those of wheat after barley, while several
428 studies measured increases in cereal grain yields after pea in comparison to wheat, triticale and oat
429 (Kirkegaard and Ryan, 2014; Miller et al., 2003; O'Donovan et al., 2014, St Luce et al., 2016, Strong et
430 al., 1986). For summer legume pre crops, Narbonne vetch led to the highest increases in wheat grain
431 yields.

432 For a given year, only wheat after sorghum led to a higher shoot N concentration compared to wheat
433 after summer pre-crops. These results can certainly be explained by higher N availability per unit of
434 grain biomass, since wheat after sorghum always presented the lowest grain yields. Consequently,
435 for spring legume pre-crops the increases in wheat shoot N yield (on average 25%) were within the
436 same range as the increases in wheat grain yields (on average 27%). For summer legume pre-crops
437 wheat shoot N yield increases (on average 51%) were slightly lower than the increases in grain yield
438 (on average 66%).

439 Although wheat was supplied with small amounts of N fertilisation (40 kg N ha^{-1}), grain yield, shoot N
440 concentration and shoot N yield increased for all pre-crop treatments (legumes and cereals). It
441 reflected the limitation of N for all the pre-crop treatments when wheat was unfertilised. While
442 Preissel et al. (2015) and Cernay et al.(2017) showed that the positive effect of legume pre-crops
443 decreased when N fertilisation was applied on the following cereal, our study showed that it was only
444 the case for fenugreek and lentil compared to barley pre-crop. An average N fertilisation threshold of
445 153 kg N ha^{-1} was calculated in the meta-analysis of Cernay et al. (2017) above which legume pre-
446 crops no longer had a positive effect on the subsequent cereal grain yield compared to cereal pre-
447 crops. As the amount of N fertiliser applied in our experiment was far below this threshold, most
448 legume pre-crops still induced an increase in wheat grain yields compared to cereal pre-crops,
449 because N was still a limiting resource for wheat growth.

450 4.2. N fluxes simulated by the STICS model as affected by pre-crops and climatic conditions

451 The STICS model was used to quantify N fluxes difficult to measure in field conditions and to assess

452 the effect of pre-crops and climatic conditions on these N fluxes. Maximum differences between the

453 lowest and the highest amounts of N mineralised from pre-crop residues were 56 kg N ha⁻¹ in

454 Experiment I and 68 kg N ha⁻¹ in Experiment II. The STICS module of N mineralisation is based on the

455 C:N ratio of crop residues (Justes et al., 2009; Nicolardot et al., 2001). Hence, a single negative

456 logarithmic relationship between the cumulative amount of N mineralised from pre-crop residues at

457 the end of the simulation period and the C:N ratio of pre-crop residues was established for our two

458 experiments ($y=-32.77\ln(x)+113.4$; $R^2 = 0.94$) (Fig. S1). The STICS model allowed to distinguish pre-

459 crops species according the N mineralisation of their crop residues. Most pre-crop residues induced

460 soil inorganic N immobilisation during their decomposition, but the highest amounts of N

461 immobilised were measured for barley, sorghum soybean and lupin pre-crop residues, which had the

462 highest C:N ratios. Conversely, the highest amounts of N mineralised from pre-crop residues were

463 found for legume pre-crops for which the whole plant was considered as crop residues (chickpea in

464 Experiment I and Narbonne vetch in Experiment II) and for which C:N ratios were the lowest.

465 Part of the differences in soil inorganic-N and soil water content between spring and summer pre-

466 crops may probably have been due to the growth cycle delay between spring and summer pre-crops.

467 Once spring crops have reached physiological maturity, water and nitrogen (originating from rainfall

468 and soil mineralisation, respectively) accumulate in the soil. Meanwhile, summer pre-crops

469 continued to take up these resources to fulfil their requirements for growth. Hence, soil water and

470 inorganic N contents were not only affected by pre-crop species and climatic conditions, but also by

471 the growth period of spring and summer pre-crops and the positioning of their water and N

472 requirements for growth.

473 A part of soil inorganic-N was lost by leaching during the autumn-winter period because of a

474 desynchronisation between N uptake by the subsequent wheat and soil inorganic-N dynamics.

475 During this period, on the one hand, wheat development was very low, thus inducing low inorganic-N

476 and water uptake, whereas on the other hand, there were large amounts of soil inorganic-N and high
477 water contents remaining in the soil after the pre-crop harvest combined with rainfall during
478 autumn.

479 The amounts of N leached were certainly slightly under-estimated in Experiment I and over-
480 estimated in Experiment II since the amounts of soil inorganic N were on average under-estimated by
481 12 kg N ha⁻¹ and over-estimated by 14 kg N ha⁻¹ by the STICS model, respectively. Several studies (Beaudoin
482 et al., 2008; Constantin et al., 2012; Coucheney et al., 2015) also highlighted the difficulty of
483 accurately predicting soil inorganic N with the STICS model and Coucheney et al. (2015) found an
484 RRMSE close to our own value (0.52 vs 0.46). Soil N dynamics result from multiple and simultaneous
485 processes in interaction with the plant and water fluxes, often making prediction more difficult
486 compared to soil water dynamics (De Willigen, 1991). In the case of a maximum under-estimation of
487 12 kg N ha⁻¹ in Experiment I and a maximum over-estimation of N leaching by +14 kg N ha⁻¹ in
488 Experiment II, it supports the fact that in Experiment II N leaching was much lower than in
489 Experiment I, especially because of lower rainfall between August and November.
490

491 4.3. N fluxes induced by pre-crops partially explained wheat shoot N yield variability

492 One of our objectives was to better understand increases in wheat shoot N yield due to legume pre-
493 crops N benefits. A positive relationship was established between the amount of N available for the
494 subsequent crop and wheat shoot N yield. This positive relationship highlights a N limitation for
495 wheat growth. Hence, in all situations, wheat shoot N yield could have been further increased by
496 higher amounts of inorganic N availability if N losses had been reduced during the autumn-winter
497 period. Our study points out the necessity of synchronisation between soil inorganic N availability
498 and the N demand of the subsequent crop to maximise its growth and fulfil its N requirements.

499 Considering our relationship, the amount of N available for the subsequent wheat accounted for 49%
500 of wheat shoot N yield variability. Several N fluxes were not taken into account in our calculation and
501 could have varied among pre-crop species. Nitrogen mineralisation from pre-crop residues only took

502 into account aboveground crop residues while ignoring below-ground residues and N
503 rhizodeposition. Nor were nitrogen losses by nitrous oxide emissions taken into account.
504 Furthermore, improvements of wheat shoot N yield after legume pre-crops may not be solely
505 attributable to N benefits (Peoples et al., 2009). Introducing legumes in a cereal based rotation can
506 significantly reduce cereal soil borne diseases (Angus et al., 2015; Kirkegaard et al., 2008), help
507 control weeds at the rotation scale and enhance microbial diversity and activity (McDaniel et al.,
508 2014; Tiemann et al., 2015). These latter factors could vary between legumes due to properties
509 inherent to species (e.g. competitiveness against weed) or because of the positioning of their growth
510 cycle in a crop rotation (e.g. spring and summer species vs winter species). Yet, these factors were
511 not measured in the present study but may have contributed to improved wheat grain and shoot N
512 yields after legume pre-crops compared to cereal pre-crops. These factors might have been
513 especially important to understand wheat yield limitation after sorghum especially in Experiment I,
514 as N availability did not satisfactorily explain the very small grain yields of wheat following sorghum.

515 5. Conclusion

516 This study confirmed the relevance of reintroducing grain legumes in cereal- based cropping systems
517 in Europe, due to the positive effect of a wide range of legume pre-crops on grain and shoot N yield
518 of the subsequent wheat. Using the STIC model allowed to accurately predict N processes particularly
519 difficult to measure directly in the field conditions. Differences between legume pre-crop species
520 were highlighted according to: i) the amount of N mineralised from their residues, ii) the amount of
521 inorganic N left in the soil at their harvest, and iii) the amount of N lost after legume harvest. These
522 differences between pre-crop legumes need to be taken into account to increase the efficiency of
523 subsequent crop use of N provided by legumes while limiting N losses. Higher amounts of inorganic N
524 derived from the mineralisation of crop residues were found for species with low crop residues C:N
525 ratios and were positively correlated to the amounts of N in the subsequent wheat. Legume growth
526 cycle positioning in the year relative to soil inorganic N dynamics also modulated the amount of N
527 left in the soil at harvest and the risks of N leaching when high precipitation occurred during the

528 autumn-winter period. A longer fallow period, especially after spring legumes with earlier harvest
529 compared to summer legumes, increased the risk of N leaching due to inorganic N accumulation in
530 the soil and the more frequent rainfall during this period. Adapted management practices, such as
531 the establishment of cover-crop during the fallow period (Constantin et al., 2012; Tribouillois et al.,
532 2015), especially for long fallow period, are crucial to reduce N losses by stocking soil nitrogen before
533 the establishment of the subsequent crop. Finally, choosing a subsequent crop either with an early
534 sowing date and a high capacity to retrieve soil inorganic N during the early stages (for example,
535 oilseed rape) or with a deep rooting depth to access N leached in the deeper soil layers, might also
536 be sensible choices for reducing N losses after legume harvest (Dejoux et al., 2003).

537 **Acknowledgements**

538 M. Guinet received a grant from INRA and the Ministry of Agriculture. This experimental work was
539 supported by the ANR LEGITIMES [ANR-13-AGRO-0004], INRA Bourgogne-Franche-Comté Region
540 (PSDR project ProSys), the European Union's Horizon 2020 research and innovation programme [No
541 727247 - SolACE project] and Terres Inovia. We also thank L. Andriot, F. Bizouard, A. Coffin, M.
542 Dourneau, C. Ducourtieux, V. Durey, L. Lebeigle, M. Lefebvre, S. Girodet, F. Lombard, E. Pinet, J.
543 Ranjard, B. Serbource, J. Welter for their excellent technical assistance and L. Falchetto for the
544 agronomic management of the field experiments. Lastly, we thank the STICS team for their help in
545 showing how to use the model correctly.

546 **Author's contribution**

547 BN and A-SV designed the study. BN, and A-SV funded the experiment and provided funding for MG's
548 work. BN, A-SV and MG collected the data. MG analyzed the data. All authors were involved in the
549 interpretation of the results and contributed to writing the original version of the manuscript and
550 improving the subsequent ones.

551 **Conflict of interest**

552 The authors declare no conflict of interest.

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681

682 **Artwork**

683 **Fig. 1** Daily rainfall, irrigation and mean temperature between pre-crop harvest and wheat harvest in
684 Experiment I (**A**) and Experiment II (**B**). I: Irrigation; R: Daily rainfall; T: mean daily temperature. I, R
685 and T were measured from a weather station located in the INRA experimental site of Bretenière.
686 Horizontal dotted arrows indicate the pre-crop harvest period and vertical arrows indicate wheat
687 sowing and harvest dates.

688 **Fig. 2** Simulated vs. observed soil water content (0 - 60cm) (**A**) and soil inorganic N (0 – 60 cm) (**B**) in
689 Experiment I (blue) and Experiment II (green) between pre-crop harvest and wheat harvest. Wheat
690 was cultivated after spring pre-crops (**▲**) and summer pre-crops (**●**). The solid line corresponds to
691 the 1:1 line.

692 **Fig. 3** Cumulative amounts of N mineralised from pre-crop residues (**A, B**), soil inorganic N (**C, D**), soil
693 water content (**E, F**) and cumulative amounts of N-NO₃⁻ leached (**G, H**) simulated with STICS for
694 Experiment I (**A, C, E, G**) and Experiment II (**B, D, F, H**). Simulations were performed for the period
695 between pre-crop harvest and winter wheat harvest. Dark blue and dark green curves are the
696 simulations performed for the spring pre-crop treatments and light blue and light green curves are
697 the simulations performed for summer pre-crops treatments. Black and grey arrows indicate the
698 incorporation dates of spring and summer pre-crop residues, respectively. The red arrows indicate
699 the sowing dates of wheat. Pre-crop ID are specified in Table 2.

700 **Fig. 4** STICS model simulations obtained for the cumulative N-NO₃⁻ leached between pre-crop harvest
701 and wheat sowing (**A, B**) and between wheat sowing and the end of winter (1st March) (**C, D**) in
702 Experiment I (blue bars) and II (green bars). Simulations were performed for spring pre-crops (**A, C**)
703 and summer pre-crops (**B, D**). Legume pre-crops: solid bars; cereal pre-crops: hatch bars. Bars with a
704 heavy dark border indicate legumes for which the whole plant was considered as crop residues since
705 there was no grain production.

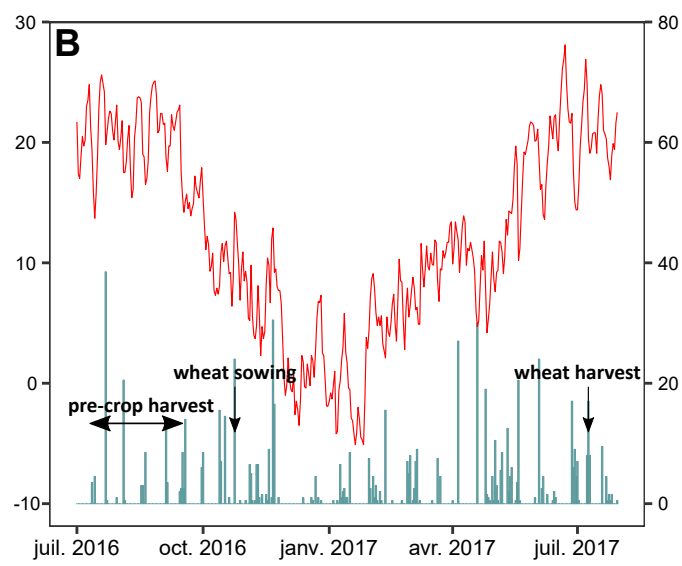
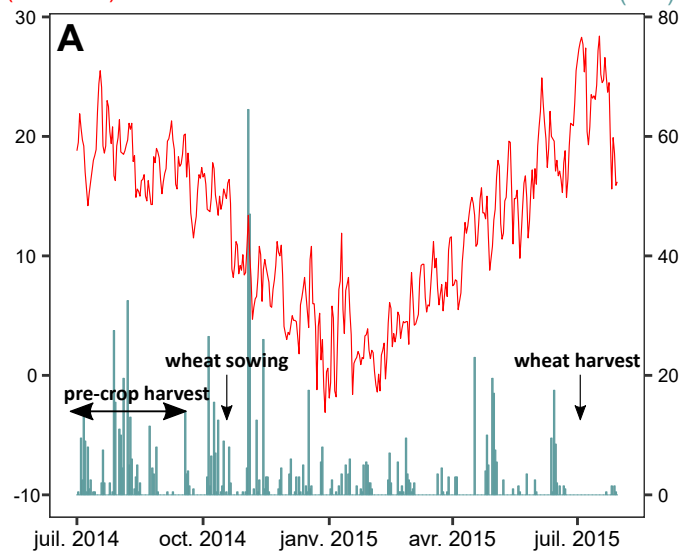
706 **Fig. 5** Biplot PCA showing the distribution of pre-crop species according to environmental conditions,
707 proprieties of crop residues and wheat performance. Amounts of N in pre-crop shoot residues
708 (Res_N); C:N ratio of pre-crop residues (Res_C:N); soil inorganic N at the start of simulation (SoilN_i);
709 water content in the soil profile at the start of simulation (SoilW_i); cumulative rainfall during the
710 simulation period (Rain); cumulative amounts of N mineralised from pre-crop residues during the
711 simulation period (Nres_min); cumulative amounts of N-NO₃⁻ leached during the simulation period

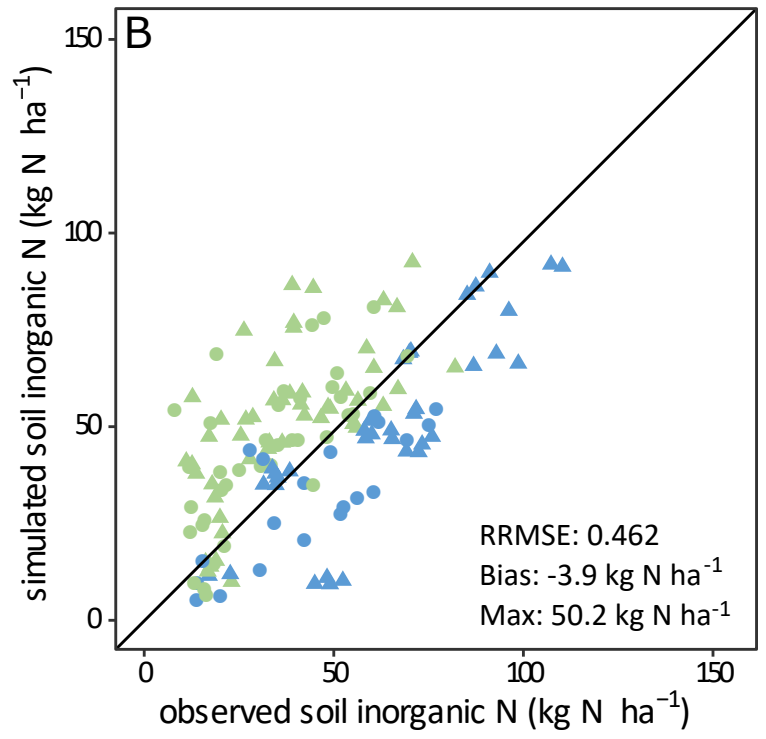
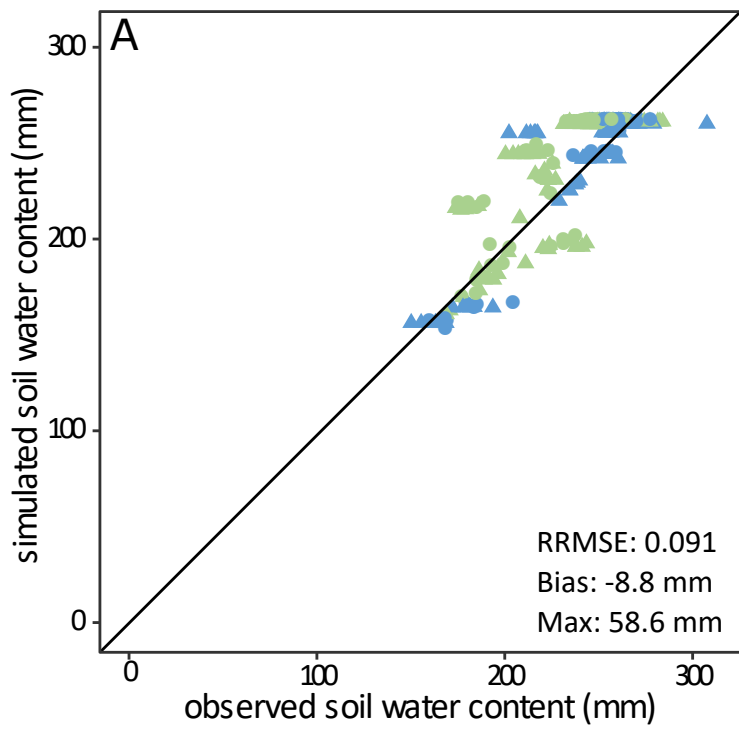
712 (Nleached); wheat grain yield (Wheat_Y); wheat shoot N yield (Wheat_N); wheat shoot N
713 concentration (Wheat_%N). Pre-crop ID are specified in Table 2.

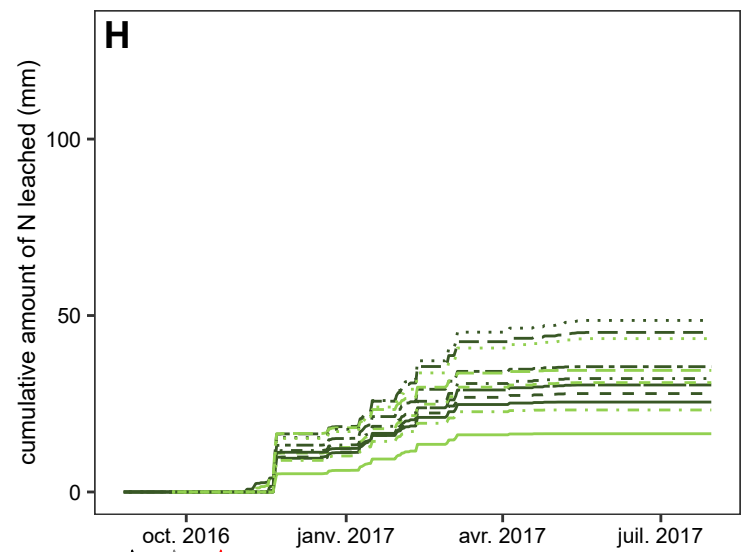
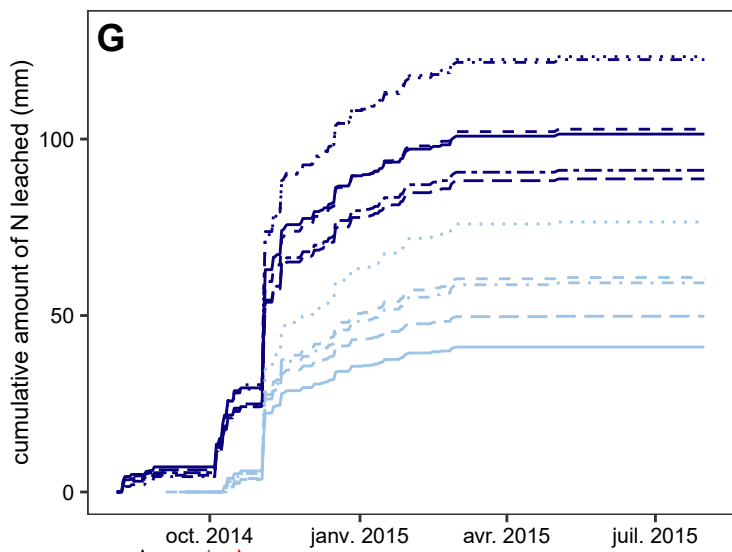
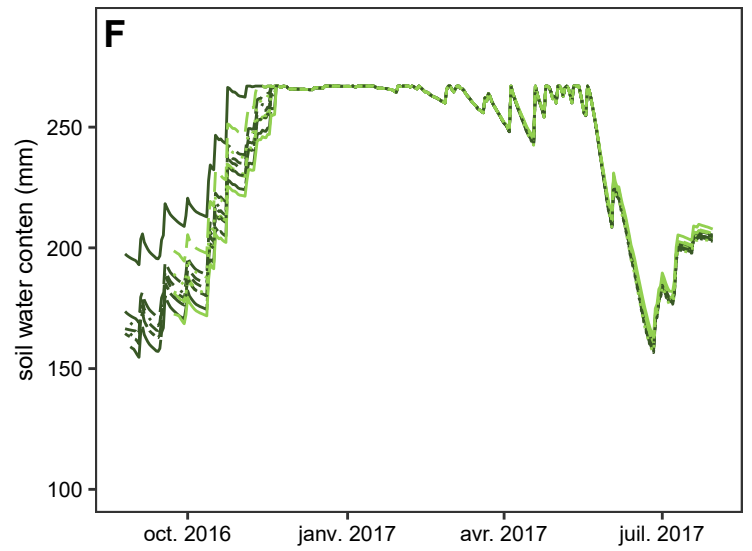
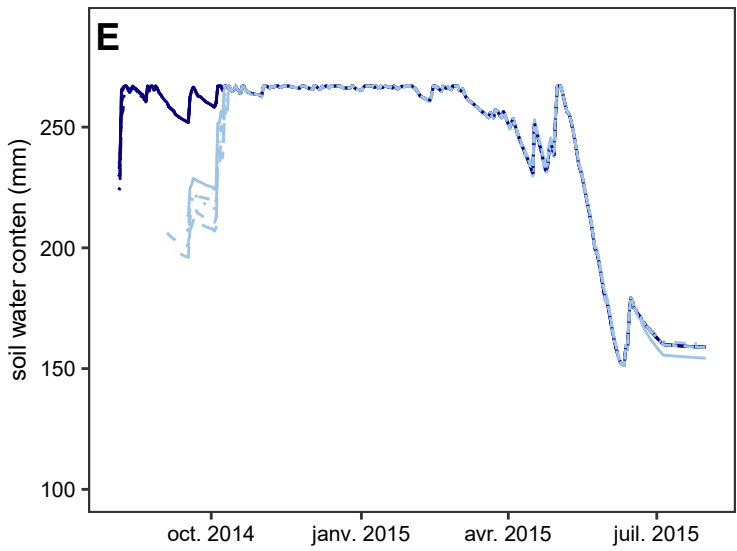
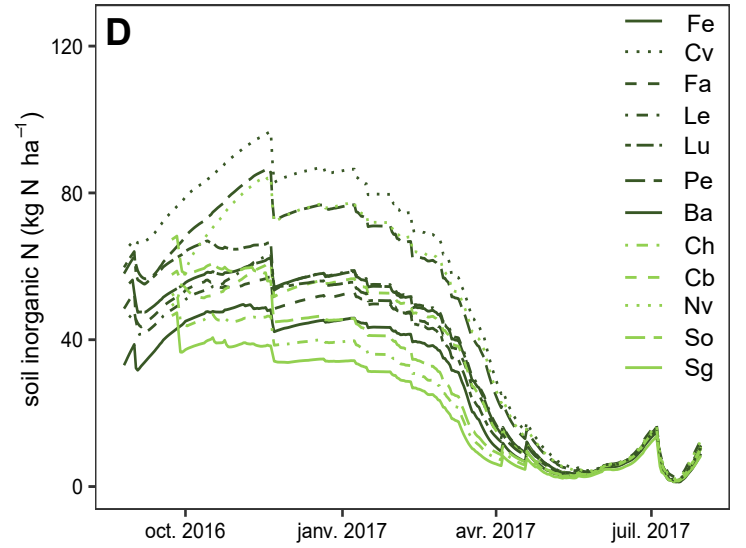
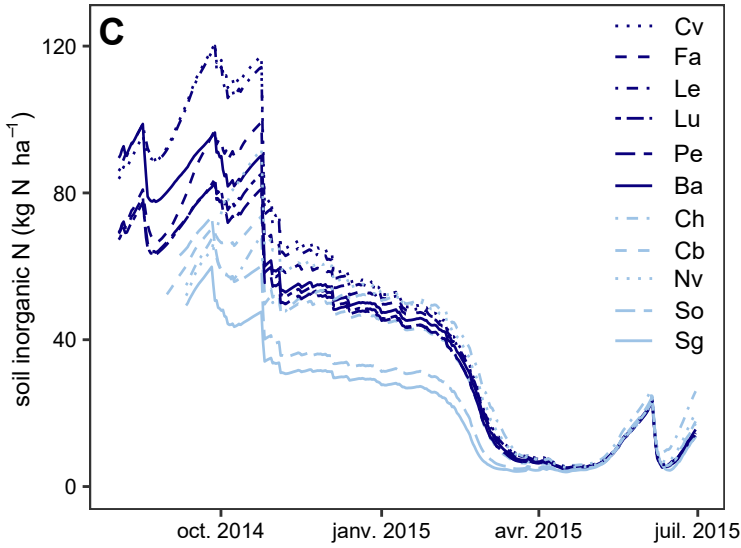
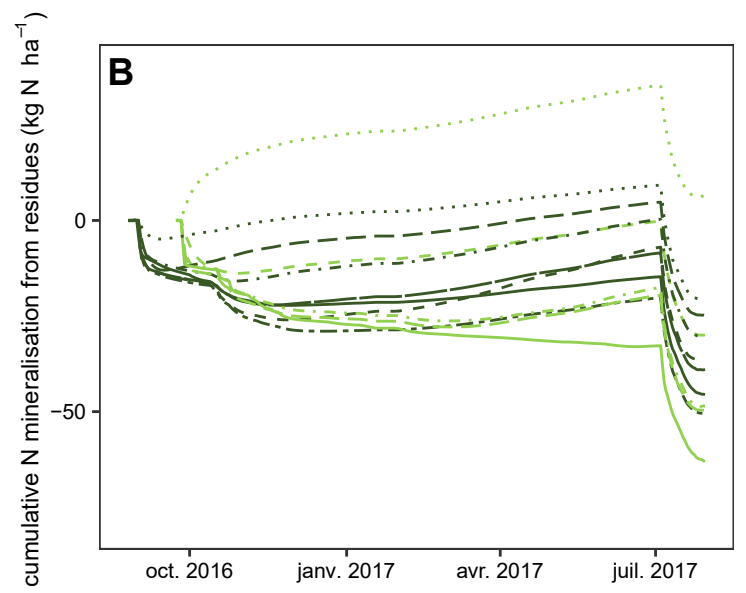
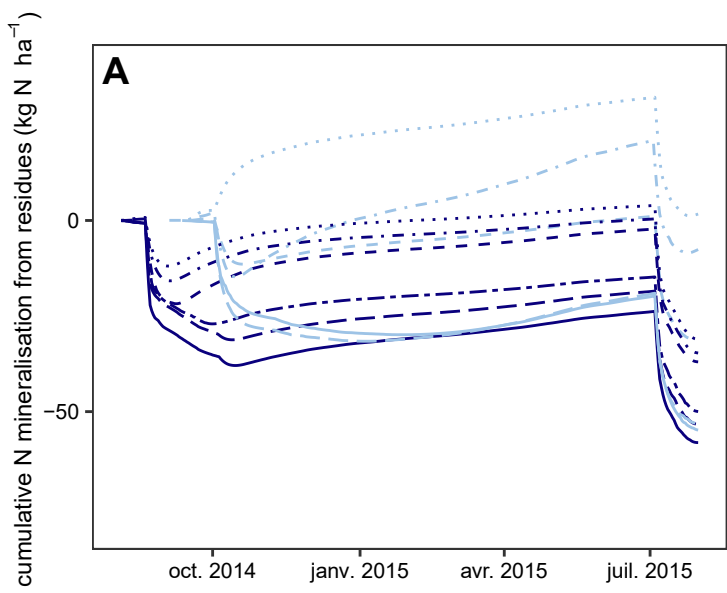
714 **Fig. 6** Relationships between wheat shoot N yield and the amount of N mineralised from pre-crop
715 residues estimated by the STICS model (**A**) and the amounts of N available for wheat (soil inorganic N
716 at pre-crop harvest + N mineralisation from pre-crop residues + N mineralisation from soil organic
717 matter - amount of N leached) (**B**). The significance of the linear regression is shown as ** $p < 0.01$
718 and *** $p < 0.001$. Pre-crop ID are specified in Table 2.

mean daily temperature
(Celsius °)

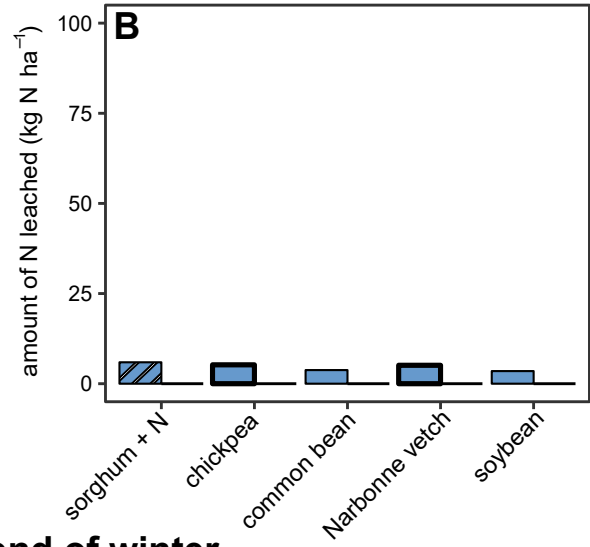
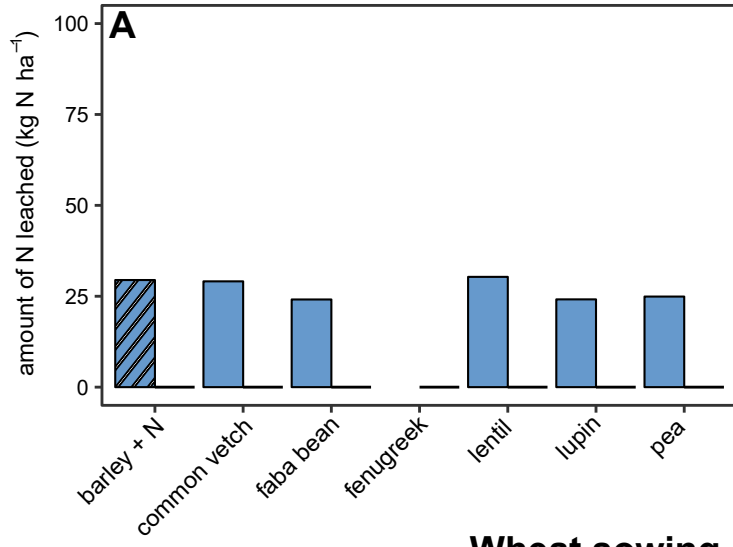
daily rainfall
(mm)



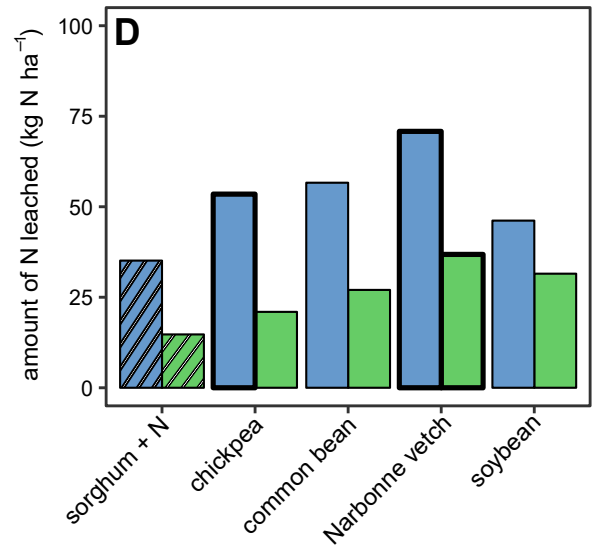
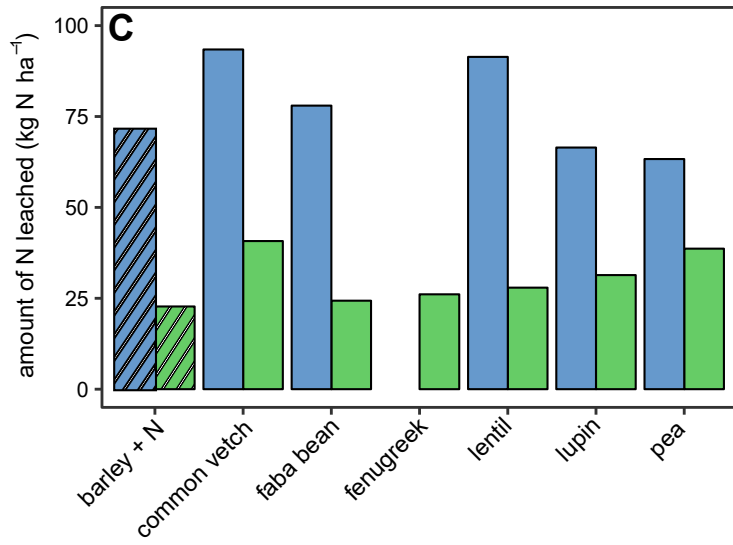


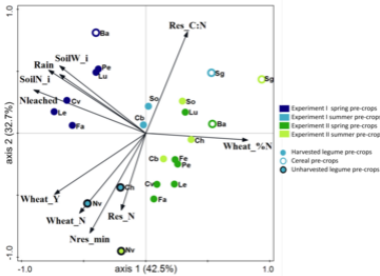


Pre-crop harvest - wheat sowing



Wheat sowing – end of winter





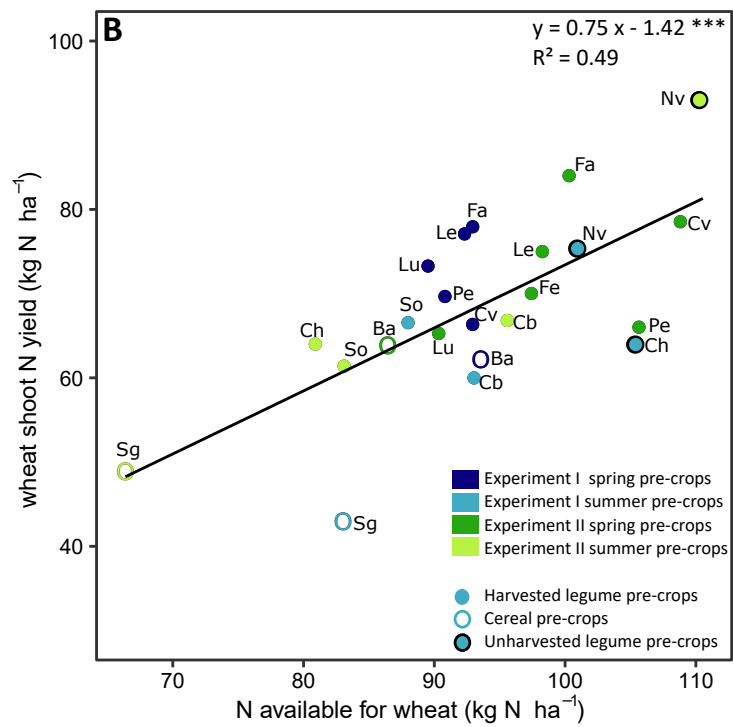
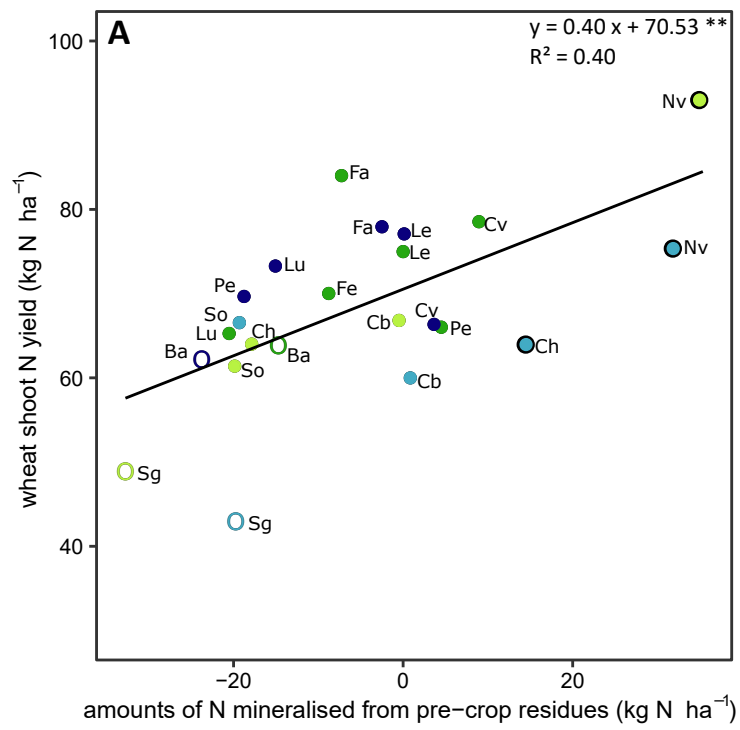


Table 1 Soil characteristics in Experiments I and II

	Experiment I (2014-2015)	Experiment II (2016-2017)
Clay (g kg⁻¹)	425	512
Silt (g kg⁻¹)	515	428
Sand (g kg⁻¹)	60	60
Organic C (g kg⁻¹)	19.3	19.6
Organic N (g kg⁻¹)	1.69	1.81
C:N ratio	11.4	10.8
pH	7.31	7.8
CaCO₃ (g kg⁻¹)	< 1	4

Table 3 Grain yields, shoot N concentration and shoot N yield of unfertilised (ON) wheat as affected by spring and summer pre-crop treatments and years (2015 and 2017). When no pre-crop by year interactions were detected, pre-crop values were averaged over year. Values are mean (n=4 or 8) \pm sd (standard deviation). Lowercase letters refer to contrasts between pre-crops. Mean value sharing the same letter ('a' and / or 'b') are not different according to Tukey's test (ns: non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	SPRING PRE-CROPS			SUMMER PRE-CROPS				
	Wheat yield 0% humidity (t ha ⁻¹)	Wheat shoot N concentration (%N)	Wheat shoot N yield (kg N ha ⁻¹)	Wheat yield 0% humidity (t ha ⁻¹)	Wheat shoot N concentration (%N)	Wheat shoot N yield (kg N ha ⁻¹)		
	2015 and 2017 pooled	2015 and 2017 pooled	2015 and 2017 pooled	2015 and 2017 pooled	2015 and 2017 pooled	2015	2017	
Pea	4.0 (0.5) bc	0.79 (0.06)	68 (6) bc	Common bean	3.7 (0.4) b	0.75 (0.02) b	60 (6) b	67 (5) b
Lupin	4.2 (0.4) abc	0.76 (0.05)	69 (6) abc	Chickpea	3.8 (0.2) b	0.77 (0.05) b	64 (4) ab	64 (2) b
Common vetch	4.2 (0.4) abc	0.79 (0.06)	73 (9) abc	Soybean	3.7 (0.8) b	0.78 (0.02) b	67 (13) ab	62 (10) cb
Lentil	4.5 (0.4) ab	0.78 (0.05)	76 (5) ab	Narbonne vetch	4.9 (0.3) a	0.76 (0.05) b	76 (2) a	93 (3) a
Faba bean	4.7 (0.9) a	0.76 (0.02)	81 (16) a	Sorghum +N	2.5 (0.3) c	0.87 (0.03) a	43 (3) c	49 (5) c
Barley +N	3.6 (0.4) c	0.79 (0.06)	63 (4) c					
year	**	***	ns	year	ns	**		*
species	***	ns	**	species	***	***		***
year*species	ns	ns	ns	year*species	ns	ns		*

Table 4 Grain yields, shoot N concentration and shoot N yield of wheat in 2017 as affected by spring and summer pre-crop treatments and N fertilisation rates (0N and 40N). When no pre-crop by N rate interactions were detected, pre-crop values were averaged over N rate. Values are mean (n=4 or 8) ± sd (standard deviation). Lowercase letters refer to contrasts between pre-crops. Mean value sharing the same letter ('a' and / or 'b') are not different according to Tukey's test (ns: non-significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

	SPRING PRE-CROPS					SUMMER PRE-CROPS				
	Wheat yield 0% humidity (t ha ⁻¹)		Wheat shoot N concentration (%N)	Wheat shoot N yield (kg N ha ⁻¹)		Wheat yield 0% humidity (t ha ⁻¹)		Wheat shoot N concentration (%N)		Wheat shoot N yield (kg N ha ⁻¹)
	0N	40N	0N and 40N pooled	0N	40N	0N and 40N pooled	0N	40N	0N and 40N pooled	
Fenugreek	4.0 (0.5) ab	4.7 (0.1) c	0.84 (0.08)	70 (8) bcd	86 (4) c	Common bean	4.9 (1.0) b	0.75 (0.03) b	0.82 (0.01)	84 (19) a
Pea	3.6 (0.4) bc	5.3 (0.3) abc	0.92 (0.11)	66 (8) cd	105 (6) ab	Chickpea	4.5 (0.8) b	0.79 (0.07) b	0.84 (0.04)	78 (16) b
Lupin	3.8 (0.1) bc	5.1 (0.3) bc	0.85 (0.08)	65 (3) d	99 (5) abc	Soybean	4.4 (1.0) b	0.79 (0.01) b	0.84 (0.05)	77 (17) b
Common vetch	4.2 (0.1) ab	5.7 (0.2) ab	0.88 (0.05)	79 (5) ab	109 (8) ab	Narbonne vetch	5.9 (0.7) a	0.80 (0.04) ab	0.90 (0.07)	108 (17) b
Lentil	4.2 (0.2) ab	4.9 (0.6) c	0.86 (0.08)	75 (3) abc	93 (15) bc	Sorghum +N	3.4 (0.9) c	0.89 (0.04) a	0.82 (0.01)	60 (13) c
Faba bean	4.7 (0.5) a	5.8 (0.3) a	0.82 (0.08)	84 (9) a	114 (9) a					
Barley +N	3.3 (0.1) c	4.9 (0.1) c	0.84 (0.04)	64 (3) d	88 (5) c					
N rate	***		***	***		N rate	***	**		***
species	***		**	**		species	***	*		***
N rate*species	**		ns	*		N rate*species	ns	*		ns