Previous legume identity influence wheat rhizosphere microbial communities and grain protein content

Emmy L'Espérance[1], Vincent Poirier[2], Stéphanie Lavergne[2], Étienne Yergeau[1]*

[1]Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique, 531 boulevard des Prairies, Laval, QC, H7V 1B7, Canada.

[2] Institut de recherche en agriculture et agroalimentaire, Université du Québec en Abitibi-Témiscamingue, 79 rue Côté, Notre-Dame-du-Nord, QC, J0Z 3B0, Canada.

*Correspondance to: Etienne Yergeau, Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique, 531 boulevard des Prairies, Laval, QC, H7V 1B7, Canada. Tel.: +1 450-687-5010 ext. 8881, etienne.yergeau@inrs.ca

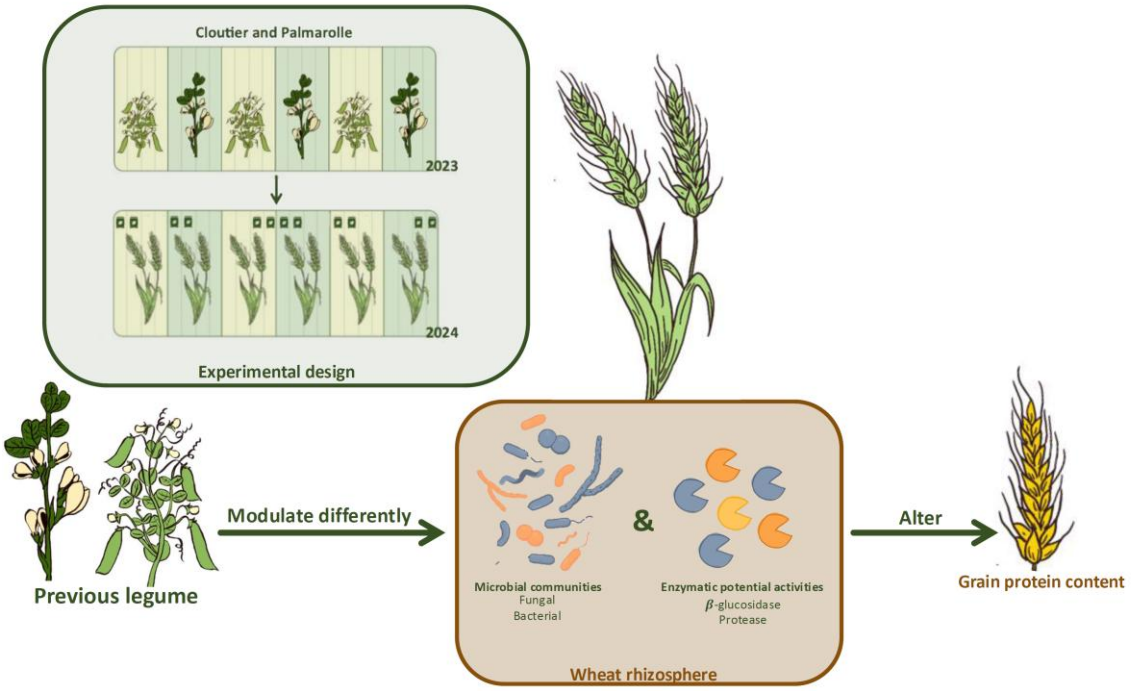
Abstract

In crop rotation systems, plant-soil feedback (PSF) effects can modulate nutrient cycling in soil, like organic carbon (C) and nitrogen (N) cycling. Organic nitrogen is present in plant and microbial necromass, and it must be depolymerized by microbes to become available for crops. Since plant identity can modulate microbial diversity and community composition, we thought that previous crop identity and its residue management would also change the microbial functional capacity, and thereby impact soil N availability, and the quality and yield of the following crop. To test this, two legumes (*Vicia faba* L., i.e. faba bean, and *Pisum sativum* L., i.e., yellow pea) were grown in two fields (Cloutier, and Palmarolle) in Abitibi-Témiscamingue, Québec, Canada (n=3 for each field). At the end of the growing season, we harvested peas and faba beans in both fields. During the following growing season, spring wheat (*Triticum aestivum* L.) was sown and received, or not granulated chicken manure as an organic fertilizer. We determined the diversity and composition of the microbial communities and their enzymatic depolymerization capacity in the soil and the rhizosphere each growing season. During wheat growth, previous legumes shaped bacterial (p-value = 0.006) and fungal (p-value = 0.001) communities without modulating the enzymatic activity of wheat-associated rhizosphere microbes. However, faba bean as a previous crop increased soil ammonium and wheat grain protein content at harvest as compared to peas at Cloutier. Altogether, our results show that faba beans can enhance wheat N nutrition, without a concomitant increase in potential protein or cellulose depolymerization, suggesting more mineralization due to increases in fungal: bacterial ratio or in the availability of substrates. Understanding plant-soil feedback in

crop rotation systems is crucial to improve our practices and sustainably meet crops' nutritional needs.

Key words

Plant-Soil feedback, Microbial communities, Rhizosphere, Depolymerization, crop rotation, Grain quality.



53 **Highlights**

54 - Previous crop identity distinctively shaped wheat rhizosphere microbial
55 communities
56 - Previous legume did not altered the enzymatic activity of wheat-associated
57 rhizosphere microbes, but increased soil ammonium
58 - Wheat grain protein content was higher following faba bean than peas
59

1. Introduction

Agricultural practices have a significant impact on belowground microbial communities, altering their functionality, community structure, and diversity (Calderon et al., 2001; Li et al., 2020; Zhao et al., 2020). Monocropping often leads to lower soil microbial diversity and decreased plant growth and yield compared to crop rotation systems (Bak et al., 2022; Adebayo et al., 2025). Crop rotation systems (i.e., growing different crop species sequentially on the same land) enhance soil nutrient content and nutrient cycling (Wang et al., 2023c), limit the establishment of plant pathogens (Zhou et al., 2023) and enhance soil health and microbial diversity (Yang et al., 2024). Crop rotation systems are therefore fundamental in organic farming to maximize yield without the use of synthetic chemical inputs (Gamage et al., 2023).

The benefits of crop rotation systems can be explained by positive plant-soil feedback (PSF). The previous crop modulates soil microbial communities and physicochemical properties, impacting the next crop (Mariotte et al., 2018; Wang et al., 2025). Indeed, plants release a myriad of compounds, shaping spatially and temporally distinct microbial communities (Zhalnina et al., 2018). Positive plant-soil feedback includes pathogen reduction and beneficial microbes' enrichment (Ma et al., 2017; Hannula et al., 2021). *Arabidopsis thaliana* changes its exudation pattern in response to the pathogenic bacterium *P. syringae* (Yuan et al., 2018). After six passages in the same soil, these changes created soil microbial communities antagonist to the pathogen, resulting in healthy plants (Yuan et al., 2018). Due to highly diversified plant traits, functions and

82 exudation patterns, PSF effects are species-specific, meaning that we cannot associate a
83 PSF effect with a plant family (Hannula et al., 2020). Plant-soil feedbacks are also not
84 limited to the original plant species and can affect other plants grown in the same soil,
85 creating multiple possibilities for crop rotation systems (Murrell et al., 2020). For instance,
86 winter wheat (*Triticum aestivum* L.) grown continuously had a lower yield compared to
87 winter wheat growing after oilseed rape, because oilseed rape changed the microbial
88 communities and the abundances of functional genes related to inorganic N-cycling
89 (Kaloterakis et al., 2025). This plant-specific rhizospheric effect can persist for many
90 growing seasons (Bay et al., 2021).
91
92 Besides changing the microbial community structure and abundance of specific genes,
93 crops also affect more generally the carbon (C) and nitrogen (N) cycles (Knops et al.,
94 2002). Plant-soil feedback effects on nutrient cycling can be attributed to both living and
95 dead crops. Plant litter origin (above vs belowground) and chemistry mediate the
96 decomposition rate (Freschet et al., 2013), and microbial decomposer abundance and
97 diversity over time (Bhatnagar et al., 2018). A classic use of PSF in agricultural settings is
98 legume-cereal rotations. Legumes are well known to enhance soil N through biological
99 fixation, and using them in crop rotation systems replenishes soil N without using
100 chemical fertilizers (Gan et al., 2015). But not all legumes are identical. For instance, faba
101 bean (*Vicia faba* L.) fixed 185 kg N ha^{-1} and forage peas (*Pisum sativum* L.) 165 kg ha^{-1},
102 resulting in different amounts of C and N in their residues (Lupwayi and Soon, 2015).
103 Living plants also influence microbial decomposer communities and their functionality

through their C-rich root exudates. Since litter and root exudates composition differ among plant species, different crops in rotation systems will not have the same effect on soil nutrient cycling and on the next crop grown (Huo et al., 2017). Therefore, the choice of rotation systems design, especially the choice of crop species, is crucial to optimize PSF effects associated with nutrient cycling. The mechanistic link between shifts in microbial communities caused by the crop's identity, soil nutrients availability, and the following crop's growth and nutrition is, however, not clear, especially in organic agroecosystems. One possible explanation could be changes in organic N depolymerization.

Organic N depolymerization is a limiting step of the enzymatic cascade leading to mineralization and nitrification, producing inorganic N available for crops (Jan et al., 2009). The input of C-rich compounds, from plant litter and/or root exudates, steers up the C: N ratio, forcing microbes to mine for nitrogen (Jones et al., 2009; Kuzyakov and Xu, 2013) by depolymerizing soil organic matter (SOM) containing organic N, such as protein, chitin and peptidoglycan (Zhu et al., 2014). When their elemental demand is met, microbes will mineralize the excess N into inorganic form (ammonium and nitrate), usable by plants. Not to mention that small organic N forms (e.g. amino acids) are also usable by plants, highlighting the importance of N depolymerization for crop nutrition (Näsholm et al., 1998). Extracellular enzymes produced by microbial decomposers mediate soil N depolymerization. Microbes will release extracellular enzymes in soil, and their enzymatic activities will be induced by the presence of their substrate (Geisseler and Horwath, 2008). Nitrogen release from depolymerization depends on the microbial decomposer

126 communities, soil physicochemical properties, and the organic substrate availability
127 (Séneca et al., 2021), all of which are influenced by the identity of the plant and the crop
128 rotation system. Increased N depolymerization may be one of the mechanisms
129 responsible for the benefits of crop rotation systems. Yet, to our knowledge, few studies
130 have tested that hypothesis under organic crop production conditions.
131
132 Here, we hypothesize that peas and faba beans, two legumes, have different lasting
133 influences on the microbial community diversity and composition, changing the microbial
134 depolymerization capacity, and thereby impacting soil inorganic N availability and quality
135 and yield of wheat grown the following season. To test this hypothesis, we grew wheat
136 with and without organic fertilizer in plots where peas or faba beans had been previously
137 grown, under production conditions at two different fields in the Abitibi-Témiscamingue
138 region (Québec Canada).
139
140

2. Materials and methods

2.1 Experimental design

We conducted a two-year field experiment in Abitibi-Témiscamingue (Québec Canada) on two farms under actual farm operating conditions, one in Palmarolle (48.665°N, 79.201°W) and in Cloutier (48.00297°N, 79.15°W). The goal of the experiment was to compare the effect of two legume crops (faba bean and peas) on the microbial communities and activities of wheat planted the following year. We also wanted to compare four fertilization strategies for wheat: granulated chicken manure (organic fertilizer), clover (*Trifolium repens*, cv. Huia) cover crop, fertilizer+cover crop, control without fertilization nor cover crop. We set up a split- plot experiment, with three blocks per field. In the first year (legume growing season), we defined randomly two plots (faba bean and peas) within each block. The following year (wheat growing season), we randomly defined two subplots (organic fertilizer and no fertilizer), and then two sub-subplots (clover cover crop and no cover crop). This resulted in 24 plots per field (48 total) (Fig. 1).

[Insert Figure 1 here]

Both sites were grassland previously cropped to perennial forages for at least three years before our experiment. Palmarolle soil texture is clay with 80, 355 and 565 g kg^{-1} of sand (2000–50 µm), silt (50-20 µm) and clay (<2 µm) particles at 0-15 cm depth, respectively. It belongs to the Gleysolic order according to the World Reference Base for Soil Resource

system (WRB, 2007) and the Canadian System of Soil Classification (Group et al., 1998). Cloutier soil texture is a silty clay with 38, 481 and 520 g kg^{-1} of sand, silt and clay particles at 0-15 cm depth, respectively, and also belongs to the Gleysolic order (Group et al., 1998) (WRB, 2007).

In fall 2022, both sites were moldboard plowed to a depth of 17 cm. In spring 2023, successive passes with a disk harrow and a field cultivator were carried out to prepare the seedbed. Faba beans and peas were sown in 2023, using the producer's equipment (Table 1). Plots at Palmarolle were 11 m wide by 100 m long, while those at Cloutier were 4 m wide by 120 m long. At Palmarolle, faba bean was buried at the end of the growing season since it did not reach full maturity, while pea was harvested. At Cloutier, both crops were harvested at the end of the season (Table 1). But, since faba bean grains did not reach full maturity at Cloutier, residues were spread and buried a few days after harvest. Therefore, faba beans were used as green manure at both fields. Thus, residue management differed between both site (Cloutier: fragmented residues were buried a few days after and Palmarolle: residues were buried at the time of harvest).

The following year (2024), the subplots were fertilized or not with the equivalent of 0.5 Mg N ha^{-1} and 1 Mg N ha^{-1} of granulated chicken manure (N:5-P:3-K:2) for Palmarolle and Cloutier, respectively. Wheat was sown on the same day as the fertilization occurred (i.e., May 30th at Palmarolle and June 04 at Cloutier). The sub-subplots were assigned or not to receive clover seeds as a cover crop (sown on the same day and not incorporated into the soil). However, at the time of sampling, the clover had not established and we disregarded

this treatment, treating the two sub-subplots from these treatments as replicates. At the end of the growing season, wheat was sampled and harvested (Table 1).

Table 1: Sowing, Harvesting and sampling dates for Cloutier and Palmarolle in 2023 and 2024

Field	Crops	Sowing rate (kg ha^{-1})	Sowing date	Harvesting date	Incorporation	Beginning of the season soil sampling date	Mid-growing season soil sampling date
Clou	Faba beans	265	2023-06-07	2023-10-17 Buried afterward	Yes	2023-06-06	2023-08-01
	Peas	265		2023-09-27	No		
	Wheat	170	2024-06-04	2024-09-12	No	2024-05-23	2024-07-31
Pal	Faba beans	265	2023-05-21	NA Buried	Yes	2023-05-21	2023-07-31
	Peas	265		2023-09-18	No		
	Wheat	170	2024-05-30	2024-09-04	No	2024-05-21	2024-07-30

2.2 Soil sampling

Every year, we sampled twice during the season, in the beginning and mid-growing season (Table 1). Wheat was approximately in the tillering phase when we sampled in 2024. We sampled five points per sub-subplot (one in the center and four in the corners, at approximately 0.5 m from the sub-subplot borders), and we combined all samples into a composite, creating one composite sample for each of the 48 sub-subplots. From the five points, we sampled the bulk soil by collecting soil (0-15cm) between rows. When present (mid-growing season), we sampled the rhizosphere of the main crops (faba bean, peas or wheat) by gently removing plants from the same five points, shaking the roots to remove the excess soil and considering the remaining soil attached to the rhizosphere (i.e., the soil influenced by plant roots).

200 Soil samples were kept in a cooler until they were brought back to the laboratory.
201 Samples were kept at 4 °C before sieving and aliquoting. We separated the rhizospheric
202 soil from the roots before sieving. We sieved (4mm) rhizospheric and bulk soil, and we
203 prepared two aliquots for each sample: one stored at 4 °C for enzymatic assays and
204 ammonium/nitrate quantification, and another stored at -20 °C for DNA extraction.
205
206 2.3 Wheat sampling
207 At the end of the growing season, we sampled wheat to determine yield and protein
208 content. Each sub-subplot was subsampled at four randomly distributed locations within
209 the plot. At each location, we sampled a quadrat of wheat by placing two 76 cm long half-
210 frames on the soil at about 45 cm width from each other, including 5 wheat rows
211 (approximately 0.342 m^2). At each subsample point, we measured the internal width
212 between half-frames to calculate precisely the sampling area, and we harvested wheat.
213 We stored wheat in paper bags and brought it back to the laboratory. Wheat was then
214 dried at 35 °C for several days and kept for further analysis.
215
216 2.4 DNA extraction and sequencing
217 We extracted soil DNA from 0.25 g of bulk and rhizosphere soil (previously sieved at 4
218 mm) samples using the DNeasy PowerLyzer power soil kit (Qiagen) following the
219 manufacturer's protocol. DNA was eluted in 50 μL of elution buffer and was stored at
220 −20°C until further analyses. We prepared libraries for 16S and ITS amplicon sequencing
221 according to Illumina's "16S Metagenomic Sequencing Library Preparation" guide

222 (Part#15044223Rev.B). We prepared libraries for the bacterial 16 S rRNA gene using
223 primers 16S: 515Y and 926R, and for the fungal ITS1 region, we used the primers ITS1F
224 and 58A2R. NextSeq (Illumina) sequencing (PE300 10M reads) was performed at the
225 *Centre d'expertise et de service Génome Québec* (CESGQ, Montréal, Canada).
226
227 2.5 Enzymatic potential activities quantification
228 We quantified the potential activity of three exoenzymes involved in SOM
229 depolymerization (deaminases, β-glucosidases and proteases) using colorimetric
230 methods. Every protocol follows the same principle: soil samples were incubated with an
231 excess of substrate under optimal conditions. Therefore, we measured the maximum
232 enzymatic capacity under optimal lab conditions, meaning that these results do not
233 reflect real-time activity in the soil.
234
235 2.5.1 Protease activity
236 To quantify the potential activity of soil protease, we used a protocol adapted from Jesmin
237 et al. (2022). Briefly, we incubated 1 g of dried sieved (4 mm) soil with 2.5 mL sodium
238 caseinate (2%) and 2.5 mL Tris buffer for two hours at 50°C and 100 rpm. After incubation,
239 we added 5 mL Trichloroacetic acid (1 g L^{-1}) to stop the enzymatic reaction, and we used
240 0.75 mL Na_2CO_3 and 0.25 mL Folin & Ciocalteu's phenol (1:3) to quantify tyrosine, the
241 product that produces a blue colour. We used 500 μL of every sample to measure the
242 absorbance at 650-700 nm using a TECAN Infinite M plate reader. The potential protease

243 activity is expressed in µmol tyrosine release g^{-1} dry soil h^{-1} incubation and was quantified
244 using a tyrosine standard curve (2-fold serial dilution from 1.0 to 0.005 mM).
245
246 2.5.2 β-glucosidases activity
247 To quantify the potential activity of soil β-glucosidase, we used a protocol adapted from
248 Eivazi and Tabatabai (1988). Briefly, we incubated 1 g of dried sieved (4 mm) soil with a
249 buffer solution containing p-nitrophenyl glycoside (25 mM) at 37°C for 1 h. After
250 incubation, we added calcium chloride (0.5 M) and Tris buffer (0.1 M, pH 12) to extract p-
251 nitrophenol, resulting from the degradation of the substrate. Extraction of the product
252 produces a yellow colour, and we measured absorbance at 415 nm using a TECAN Infinite
253 M plate reader. The potential activity is expressed in µmol p-nitrophenol released g^{-1} dry
254 soil h^{-1} incubation and was quantified using a p-nitrophenol standard curve (2-fold serial
255 dilution from 0.5 to 0.0078 g L^{-1}).
256
257 2.5.3 Deaminase activity
258 To quantify the potential activity of soil deaminases, we used a protocol adapted from
259 Killham and Rashid (1986). Briefly, we incubated 1 g of dried sieved (4 mm) soil with 1.2-
260 diamino-4-nitrobenzene (0.06 g L^{-1}) for 20 h at 25 °C, 100 rpm. The substrate is red, and
261 the colour intensity decreases with the deamination of the benzene ring. After incubation,
262 we extracted twice the remaining substrate using methanol 100 % and filtered (Whatman
263 no1) the solution to remove any impurities. We measured the absorbance at 405 nm
264 using a TECAN Infinite M plate reader. The potential activity is expressed in and was

265 quantified using a 1.2-diamino-4-itrobenzene standard curve
266 (0/0.008/0.011/0.014/0.019/0.025/0.034/0.045/0.06 g L^{-1}).
267
268 2.6 Available inorganic nitrogen quantification
269 To quantify ammonium and nitrate, we first needed to extract nitrogen from the soil,
270 using a protocol adapted from Maynard et al. (1993).
271 To do so, we incubated 1 g of dried sieved (4 mm) soil with 10 mL of KCl (2 M) for 1 h at
272 25 °C at 100 rpm. After incubation, we filtered (Whatman no. 42) the soil slurry to remove
273 any soil and debris. Soil extracts were kept at 4 °C until further analyses.
274
275 2.6.1 Ammonium quantification
276 To quantify ammonium in soil extracts, we adapted a protocol from Rhine et al. (1998).
277 Briefly, we added 100 µL of the sample (standard and/or soil extract) to a 96-well plate.
278 Using a multi-channel pipette, we added 50 µL of citrate reagent, 50 µL of phenylphenol
279 nitroprusside, 25 µL of buffered hypochlorite reagent and 50 µL of deionized water to
280 each well and incubated 45 min at room temperature. Based on the Berthelot method,
281 ammonium in soil extracts reacts with the buffered hypochlorite solution and the
282 phenylphenol nitroprusside, producing a blue product. After incubation, we measured
283 the absorbance at 660 nm using a TECAN Infinite M plate reader and the concentration
284 of ammonium is expressed in mg g^{-1} of air-dried soil and quantified using a $(NH_4)_2SO_4$
285 standard curve (2-fold serial dilution from 20 to 0.0195 mg L^{-1}).
286

287 2.6.2 Nitrate quantification

288 To quantify nitrate in soil extracts, we adapted a protocol from Hood-Nowotny et al.
289 (2010). Briefly, we added 100 µL of the sample (standard and/or soil extract) to a 96-well
290 plate. Using a multi-channel pipette, we added 100 µL of VCl_3 solution and 100 µL of the
291 Griess reagent mix (a solution containing 1:1 Griess solution 1 and Griess solution 2) to
292 each well, and we incubated the plate for 1 h at 37 °C. Based on the VCL_3/Griess method,
293 nitrate in soil extracts is converted to nitrite with VCL_3, and nitrite reacts with both Griess
294 solutions to produce a pink color. After incubation, we measured absorbance at 540 nm
295 using a TECAN Infinite M plate reader and the concentration of nitrate is expressed in mg
296 g^{-1} of soil and quantified with a standard curve of KNO_3 (2-fold serial dilution from 10 to
297 0.0195 mg L^{-1}).

298

299 2.7 Wheat grain yield and protein content

300 At the end of the 2024 growing season, wheat was hand harvested (see section 2.3) to be
301 threshed using a laboratory thresher (DL-350, Wintersteiger, Ried im Innkreis, Austria).
302 Grains were then cleaned with a sample cleaner (MNL Pfeuffer, Kitzingen, Germany).
303 Grain yield (Y_{FH}, kg ha^{-1}) was determined in both fields at the subsample level by weighing
304 clean grain harvested for the given surface area sampled at each subsampling point (see
305 section 2.3). Grain protein content (%) was determined only at Cloutier and was analyzed
306 using a Near-infrared spectrometer (Infratec 1241, FOSS, Hillerød, Denmark). Wheat yield
307 was reported on a 13.5 % relative humidity content ($Y_{13.5}$, kg ha^{-1}) as follows: $Y_{13.5} = (Y_{FH} \times$
308 $(100 - W_{FH})) / (100 - 15)$. We used $Y_{13.5}$ for our statistical analyses. We determined yield

309 and protein content (when applicable) for each subsample harvested in the field (four
310 subsamples), and we used the mean of the subsamples to perform our statistical analyses.
311
312 2.8 Bioinformatic analyses
313 We analyzed amplicon sequencing data (16S rRNA gene and ITS region) using the DADA2
314 pipeline in R to process raw reads. For 16S, we used truncLen (280,250) and maxEE (2,2)
315 to filter and trim forward and reverse reads. We used the pooled method for removing
316 chimeras, and we assigned taxonomy with the SILVA reference database. After the
317 assignation, we removed chloroplasts and mitochondria from our dataset before further
318 analysis. For ITS, we used truncLen (270,250) and maxEE (2,2) to filter and trim forward
319 and reverse reads. We used the pooled method for removing chimeras, and we assigned
320 taxonomy with the UNITE reference database.
321
322 2.9 Statistical analysis and data visualization
323 We performed Statistical analyses in R (version 4.5.1, *The R Foundation for Statistical*
324 *Computing),* and we created figures using the package ggplot2. We divided and
325 performed statistical analyses for each field (i.e., Cloutier and Palmarolle) and for each
326 year (i.e., 2023 and 2024) separately. First, we performed Shapiro-Wilk tests to check the
327 normality of the residuals and Bartlett tests to check the homogeneity of variances. When
328 the assumptions were met, we performed a full factorial analysis of variance (ANOVA).
329 For 2024, we performed ANOVAs for the effect of the previous legume crop (i.e., pea and
330 faba bean), organic fertilization (i.e., with or without granulated chicken manure), soil

331 compartment (i.e., rhizosphere and bulk soil), while including the split-split-plot design in
332 the model. For 2023, we performed ANOVAs for the effect of the previous legume crop
333 (i.e., pea and faba bean) and soil compartment (i.e., rhizosphere and bulk soil), while
334 including the split-split-plot design in the model. We did this for potential enzymatic
335 activities, soil ammonium and nitrate content. When the assumptions were not met, we
336 first tried to transform our data (log or square root transformation), and if that did not
337 work, we performed a Kruskal-Wallis non-parametric test, testing each factor
338 independently. Specifically, in the 2024 second sampling, Cloutier nitrate did not meet
339 the normality of residuals assumption, Cloutier ammonium and protease activity did not
340 meet the homogeneity of variances assumption, and deaminase data did not meet both
341 assumptions. Palmarolle deaminase data did not meet the normality of residuals
342 assumption. In the 2024 first sampling, Cloutier nitrate did not meet the homogeneity of
343 variances assumption. Palmarolle nitrate and ammonium did not meet both assumptions
344 and deaminase data did not meet the homogeneity of variances assumption. Finally, in
345 2023 second sampling, Cloutier nitrate did not meet the homogeneity of variances
346 assumption. Palmarolle nitrate did not meet both assumptions, Palmarolle ammonium
347 did not meet the normality of residuals assumption and deaminase data did not meet the
348 homogeneity of variances assumption. We performed Kruskal-Wallis nonparametric tests
349 to assess the effects of previous crop, organic fertilization, the interaction between
350 previous crop and fertilization and block on wheat yield ($Y_{13.5}$, kg ha^{-1}) and protein
351 content. We performed Kruskal-Wallis nonparametric tests to assess the effects of
352 previous crop, organic fertilization, soil compartment, and block on alpha-diversity and

353 relative abundance data, since this data did not meet the assumption of parametric
354 ANOVA. To analyze the bacterial and fungal community structure, we first transformed
355 our dataset using the Hellinger transformation. We then used Euclidean distance matrices
356 based on our transformed data to visualize the community structure using a principal
357 coordinate analysis (PCoA). We first used betadisper to test the homogeneity of
358 multivariate dispersion. We tested the effect of the previous crop, organic fertilization
359 and soil compartment on the community using PERMANOVA (999 permutations). To
360 consider the experimental design, we used the argument strata = block. To assess ASVs
361 differently abundant in wheat plots after faba bean and peas, we performed three
362 differential analysis (DEseq2, ANCOM-BC and LEfsE) and kept only ASVs found by all
363 methods.
364
365 2.10 Data availability
366 The R code used to analyze the data and generate the figures is available on GitHub
367 (https://github.com/le-labo-yergeau/). Raw sequencing reads were deposited in the NCBI
368 SRA repository under BioProject accession PRJNA1390138.
369

370 3. Results

371 We focused our microbial analysis on the mid-growing season samples (2024) to assess
372 previous legume crops' PSF effects, except when it is mentioned.

373

374 3.1 Previous crop PSF on microbial communities' composition

375 3.1.1 Cloutier 2024

376 Before performing PERMANOVA, we tested the homogeneity of multivariate dispersion
377 for both bacterial and fungal communities, and it was not significant, meaning that the
378 results of the tests were not influenced by differential dispersion across treatments. The
379 bacterial and fungal community compositions of wheat plots differed significantly
380 depending on the previous legume sown in 2023 and the addition of organic fertilization
381 (i.e. granulated chicken manure) in 2024 (Fig. 2.A and 2.C; Table S.1 and S.2). For the
382 bacterial community (Fig. 2.A), there was also a significant interaction between the
383 previous crop and the fertilization, meaning that depending on the previous crop sown,
384 fertilization's effect varied. In Figure 2.A, we can see that the addition of granulated
385 chicken manure increases the variability of the bacterial community composition,
386 especially when the previous crop was peas. Therefore, fertilization reduced the PSF
387 effect of the previous crop. For the fungal community (Fig. 2.B), there was no interaction
388 between the effect of the previous crop and the organic fertilization. The soil
389 compartment (rhizosphere and bulk soil) modulated the fungal community composition
390 (Fig. 2.B), but not the bacterial community.

391 After assessing that the previous legume crop identity shapes microbial communities in
392 wheat plots differently, we tried to find which microbes' relative abundance was different
393 between plots that received faba beans and peas. After performing DESeq2, ANCOM-BC
394 and LEfSe analysis, we did not find any bacterial ASV differentially abundant in wheat
395 plots that received faba bean and peas (Fig. 3.B). On the other hand, we found 6 fungal
396 ASVs differentially abundant in wheat plot depending on previous legume crops (Fig. 3.D;
397 Table 2). Specifically, fungal ASVs # 16, 87, 387 and 686 were significantly more abundant
398 in wheats plots that received peas as the previous legume crop and ASVs # 169 and 271
399 were significantly more abundant in wheat plots that received faba bean as the previous
400 legume crop.
401
402 3.1.2 Palmarolle 2024
403 Before performing PERMANOVA, we tested the homogeneity of multivariate dispersion,
404 and it was not significant for fungi. However, we found significant differences for bacterial
405 communities (p-value = 0.0249), which suggests that the PERMANOVA results could also
406 be due to differential dispersion of the bacterial communities among treatments. The
407 bacterial and fungal community composition of wheat plots is significantly different
408 depending on the previous crop sown in 2023 and the soil compartment (Fig. 2.C and 2.D;
409 Table S.1 and S.2). There is also a significant effect (p = 0.043) of the interaction between
410 the previous crop and the fertilization on the fungal communities' composition in
411 Palmarolle (Fig. 2.D). Like the bacterial community composition in Cloutier, fertilization
412 makes the fungal community composition more variable (Fig. 2.C).

413 After performing differential analysis (DESeq2, ANCOM-BC and LEfSe analysis), we found
414 that the bacterial ASV # 520 was significantly more abundant in wheat plots that received
415 faba bean as the previous legume crop (Fig. 4.B). We also found 8 fungal ASVs
416 differentially abundant in wheat plots depending on previous legume crops (Fig. 4.D;
417 Table 2). Specifically, fungal ASVs # 16, 162, 288, 323, 390, 580, 1155, 1159 were
418 significantly more abundant in wheat plots that received peas as the previous legume
419 crop.
420
421 [Insert Figure 2 here]
422 [Insert Figure 3 here]
423 [Insert Figure 4 here]
424
425
426
427
428
429
430
431
432
433

434 Table 2: Bacterial and fungal ASVs differentially abundant in wheat plot at Cloutier and Palmarolle found

435 with DESeq2, ANCOM-BC and LEfSe.

	#ASV	Kingdom	Phylum	Class	Genus	Previous crop	log2FCD	logFCA	lda lefse
Cloutier	16	Fungi	Ascomycota	Dothiodeomycetes	*Didymella*	peas	2.84	3.04	4.12
	87	Fungi	Ascomycota	Sordariomycetes	*Phomatospora*	peas	4.26	2.48	3.95
	169	Fungi	Ascomycota	Leotiomycetes	*Cadophora*	faba	-3.02	-1.66	3.18
	271	Fungi	Basidiomycota			faba	-3.76	-1.63	2.91
	387	Fungi	Chytridiomycota	Rhizophydiomycetes		peas	4.10	1.54	2.33
	686	Fungi	Ascomycota	Sordariomycetes	*Phomatospora*	peas	4.04	1.89	2.65
Palmarolle	16	Fungi	Ascomycota	Dothiodeomycetes	*Didymella*	peas	3.99	3.05	4.27
	162	Fungi	Ascomycota	Leotiomycetes		peas	4.56	2.29	3.59
	288	Fungi	Ascomycota	Eurotiomycetes	*Cladophialophora*	peas	3.62	1.95	3.44
	323	Fungi	Mortierellomycota	Mortierellomycetes	*Mortierella*	peas	5.56	2.57	3.03
	390	Fungi	Chytridiomycota	C_GS13		peas	3.12	1.73	3.22
	580	Fungi	Ascomycota	Eurotiomycetes	*Cladophialophora*	peas	4.10	2.12	2.95
	1155	Fungi	Ascomycota	Dothiodeomycetes	*Stemphylium*	peas	3.86	1.53	2.41
	1159	Fungi	Ascomycota	Sordariomycetes	*Metapochonia*	peas	2.73	1.25	2.31
	520	Bacteria	Actinobacteriota		*Cryobacterium*	faba	-2.51	-1.48	2.62

436

437 3.2 Potential Enzymatic activities associated with depolymerization

438 3.2.1 Cloutier 2024 and 2023

439 During wheat growth (2024 second sampling), there was significantly more β-glucosidase

440 and deaminase potential activities in the wheat rhizosphere than in the bulk soil (Fig. 5.B

441 and 5.C; Table 3). The potential β-glucosidase activity in wheat plots was higher, but not

442 significant, after faba bean than after peas. The addition of organic fertilizer at the

443 beginning of the season did not affect enzymatic activities during wheat growth. Before

444 wheat growth (2024 first sampling), the potential protease activity was higher in wheat

445 plots after peas than faba bean (Table 3). Fertilization did not affect enzymatic activities

before wheat growth. In 2023, when faba bean and peas were growing, there was significantly more protease and deaminase potential activities in both legumes' rhizosphere than in the bulk soil (Table 3).

3.2.2 Palmarolle 2024 and 2023

During wheat growth (2024 second sampling), there was significantly more protease and β-glucosidase potential activities in the wheat rhizosphere than in the bulk soil (Fig. 5.D and 5.E; Table 3). After faba bean, wheat plots had more protease and β-glucosidase activities than after peas. The addition of organic fertilizer at the beginning of the season did not affect enzymatic activities during wheat growth. Before wheat growth (2024 first sampling), fertilization and the previous crop did not influence the three exoenzymes potential activities tested. In 2023, when faba bean and peas were growing, there was significantly more protease, β-glucosidase, and deaminase potential activities in both legumes' rhizosphere than in the bulk soil (Table 3).

[Insert Figure 5 here]

Table 3: Effects of previous crop, organic fertilization and soil compartments (rhizospheric vs. bulk soil) on proteases, β -glucosidases and deaminases potential activities at 0-15 cm depth at Cloutier and Palmarolle during the wheat mid-season (2024 2 sampling), wheat beginning of the season (2024 1 sampling) and legumes mid-season (2023 2 sampling).

		Proteases				B-glucosidases				Deaminases			
		Kruskal				ANOVA (square transformed)				Kruskal			
	Stratum					B:PC	B:PC:F	B:PC:F	Within				
Clou 2024 2 sampling	Factor/term	PC	C	F	B	PC	F	PC:F	C	PC	C	F	B
	Statistic	0.41	2.93	1.58	2.09	2.40	0.04	0.42	105.61	0.82	4.42	0.79	4.35
	df	1	1	1	2	1	1	1	1	1	1	1	2
	p-value	0.523	**0.087** .	0.208	0.352	0.262	0.856	0.552	**1.16E-11** ***	0.364	**0.035** *	0.375	0.113
		ANOVA (square transformed)				ANOVA (log transformed)				ANOVA (square transformed)			
	Stratum	B:PC	B:PC:F	B:PC:F		B:PC	B:PC:F	B:PC:F		B:PC	B:PC:F	B:PC:F	
Clou 2024 1sampling	Factor/term	PC	F	PC:F		PC	F	PC:F		PC	F	PC:F	
	Statistic	32.03	0.73	0.72		0.005	0.45	0.003		0.003	0.06	5.66	
	df	1	1	1		1	1	1		1	1	1	
	p-value	**0.030** *	0.440	0.445		0.951	0.540	0.957		0.962	0.820	**0.076** .	
		ANOVA (square transformed)				ANOVA (square transformed)				ANOVA (square transfromed)			
	Stratum	B:PC	Within	Within		B:PC	Within	Within		B:PC	Within	Within	
Clou 2023 2 sampling	Factor/term	PC	C	PC:C		PC	C	PC:C		PC	C	PC:C	
	Statistic	0.53	49.67	0.30		0.37	0.04	2.19		48.71	35.89	0.07	
	df	1	1	1		1	1	1		1	1	1	
	p-value	0.542	**1.61E-08** ***	0.586		0.605	0.846	0.147		**0.020** *	**4.87E-07** ***	0.798	
		ANOVA				ANOVA (log transformed)				Kruskal			
	Stratum	B:PC	B:PC:F	B:PC:F	Within	B:PC	B:PC:F	B:PC:F	Within				
Pal 2024 2 sampling	Factor/term	PC	F	PC:F	C	PC	F	PC:F	C	PC	C	F	B
	Statistic	1.65	0.70	0.82	39.61	0.79	0.14	0.22	11.10	0.27	0.08	3.33	7.65
	df	1	1	1	1	1	1	1	1	1	1	1	2
	p-value	0.328	0.451	0.417	**4.65E-07** ***	0.467	0.731	0.667	**0.002** **	0.606	0.775	**0.068** .	**0.022** **
		ANOVA (square transformed)				ANOVA (square transformed)				Kruskal			
	Stratum	B:PC	B:PC:F	B:PC:F		B:PC	B:PC:F	B:PC:F					
Pal 2024 1 sampling	Factor/term	PC	F	PC:F		PC	F	PC:F		PC	F	B	
	Statistic	1.89	0.01	1.39		0.27	0.18	0.90		0.03	0.03	1.81	
	df	1	1	1		1	1	1		1	1	2	
	p-value	0.303	0.916	0.304		0.654	0.694	0.395		0.862	0.862	0.406	

		Kruskal				ANOVA (log transfromed)				ANOVA (no transformed)			
	Stratum					B:PC	Within		Within	B:PC	Within		Within
Pal 2023	Factor/term	PC	C		B	PC	C		PC:C	PC	C		PC:C
2 sampling	Statistic	0.02	14.35		1.82	5.924	9.024		2.58E-05	2.194	3.205		2.401
	df	1	1		2	1	1		1	1	1		1
	p-value	0.882	**0.0002**	***	0.402	0.248	**0.005**	**	0.996	0.378	**0.081**	.	0.129

.: p<0.1; *: p<0.05; **p<0.01; ***:p<0.001

B: Block; PC: Previous crop, F: Fertilization

Factor/Term, Statistic, df and p-value in bold are the significant and/or almost significant values

474 3.3 Soil inorganic N content

475 3.3.1 Cloutier 2024 and 2023

476 Figure 6 shows the amount of inorganic N in the bulk soil and the rhizosphere of wheat,
477 at mid-growing season in 2024. In the wheat rhizospheric soil at Cloutier, there is
478 significantly less inorganic N in ammonium and nitrate forms than in the bulk soil (Fig. 6.A
479 and 6.B; Table 4). There is also a trend toward an increased concentration of ammonium
480 (P=0.08) in the faba bean plots as compared to the pea plots (Fig. 6.A, Table 4). This could
481 be related to the nearly significant (P= 0.084) compartment x previous legume interaction
482 at Cloutier in 2023 (legume growing season) that stemmed from a higher concentration
483 of ammonium in the rhizosphere vs. bulk soil, but only for faba bean (Table 4). In the first
484 sampling of 2024, Cloutier nitrate concentration significantly differed between blocks.
485 When legumes were the main crop (2023 second sampling), there was less nitrate in both
486 legumes' rhizosphere than in the bulk soil.

487

488 3.3.2 Palmarolle 2024 and 2023

489 In the wheat rhizospheric soil at Palmarolle, there is significantly less nitrate, but more
490 ammonium than in the bulk soil (Fig. 6.C and 6.D; Table 6). Also, at Palmarolle, there was
491 less nitrate in wheat plots after faba beans than after peas, regardless of the
492 compartment (Fig. 6.D). In the first sampling of 2024, Palmarolle ammonium
493 concentration significantly differed between blocks. When legumes were the main crop
494 (2023 second sampling), there was less nitrate in both legumes' rhizosphere than in the
495 bulk soil and ammonium concentration differed significantly between blocks.

[Insert Figure 6 here]

Table 4: Effects of previous crop, organic fertilization and soil compartments (rhizospheric vs. bulk soil) on nitrate and ammonium content at 0-15 cm depth at Cloutier and Palmarolle during the wheat mid-season (2024 2 sampling), wheat beginning of the season (2024 1 sampling) and legumes mid-season (2023 2 sampling).

		Nitrate						Ammonium					
		Kruskal						Kruskal					
Clou 2024 2 sampling	**Stratum**												
	Factor/term	PC	C		F		B	PC	C		F		B
	Statistic	2.10	20.58		1.35		1.84	3.07	6.22		0.00		2.14
	df	1	1		1		2	1	1		1		2
	p-value	0.147	**5.73E-06**	***	0.246		0.399	**0.08**	**0.013**	*	0.984		0.343
		Kruskal						ANOVA (square transformed)					
Clou 2024 1 sampling	**Stratum**							B:PC	B:PC:F		B:PC:F		
	Factor/term	PC	F		B			PC	F		PC:F		
	Statistic	0.56	2.43		6.50			0.96	3.17		0.68		
	df	1	1		2			1	1		1		
	p-value	0.453	0.119		**0.039**	*		0.431	0.149		0.455		
		Kruskal						ANOVA					
Clou 2023 2 sampling	**Stratum**							B:PC	Within		Within		
	Factor/term	PC	C		B			PC	C		PC:C		
	Statistic	0.38	3.92		2.34			0.04	1.11		3.13		
	df	1	1		2			1	1		1		
	p-value	0.536	**0.048**	*	0.311			0.855	0.299		**0.084**	.	
		ANOVA (square transformed)						ANOVA (square transformed)					
Pal 2024 2 sampling	**Stratum**	B:PC	B:PC:F		B:PC:F		Within	B:PC	B:PC:F		B:PC:F		Within
	Factor/term	PC	F		PC:F		C	PC	F		PC:F		C
	Statistic	6.23	0.05		0.99		32.68	1.17	3.39		0.04		14.95
	df	1	1		1		1	1	1		1		1
	p-value	0.130	0.834		0.375		**2.47E-06**	*** 0.393	0.139		0.845		**5.08E-04**	***
		Kruskal						Kruskal					
Pal 2024 1 sampling	**Stratum**												
	Factor/term	PC	F		B			PC	F		B		
	Statistic	0.013	1.14		0.88			0.10	0.08		9.91		
	df	1	1		2			1	1		2		
	p-value	0.908	0.285		0.646			0.751	0.773		**0.007**	**	
		Kruskal						Kruskal					
Pal 2023 2 sampling	**Stratum**												
	Factor/term	PC	C		B			PC	C		Block		
	Statistic	0.07	5.990		0.43			0.41	0.02		13.72		
	df	1	1		2			1	1		2		
	p-value	0.798	**0.014**	*	0.807			0.523	0.898		**0.001**	**	

.: p<0.1; *: p<0.05; **p<0.01; ***:p<0.001

B: Block; PC: Previous crop, F: Fertilization

Factor/Term, Statistic, df and p-value in bold are the significant and/or almost significant values

3.4 Wheat yield and grain quality

3.4.1 Cloutier 2024

The addition of organic fertilizer significantly enhanced wheat grain protein content (Fig. 7: Table 5). Also, faba bean, when used as the previous crop, significantly increased wheat grain protein content as compared to peas (Fig. 7).

[Insert Figure 7 here]

Table 5: Kruskal-Wallis results for the effect of organic fertilization and previous crops on grain protein content and yield ($Y_{13.5}$) at Cloutier

Response variable	Factor	Statistic	p-value	p.adj	
Yield(kg ha^{-1})	Fertilization	0.093	0.7609	0.7609	
	Previous crop	1.245	0.2645	0.3174	
	Fertilization : Previous crop	7.265	0.0639	0.1024	
	Block	**10.619**	**0.0050**	**0.0148**	*
Protein content (%)	**Fertilization**	**14.087**	**0.0002**	**0.0007**	***
	Previous crop	**5.927**	**0.0149**	**0.0298**	*
	Fertilization : Previous crop	**20.106**	**0.0002**	**0.0006**	***
	Block	**10.048**	**0.0066**	**0.0158**	*

*: p<0.05; **p<0.01; ***:p<0.001

p.adj refers to the p-value with Benjamini-Hochberg correction

Factor/Term, Statistic, df, p-value and p.adj in bold are the significant values

3.4.2 Palmarolle 2024

For Palmarolle, we were only able to determine the grain yield at the end of the growing season. We found no significant effect of fertilization and the previous crop on grain yield (Table 5).

Table 5: Kruskal-Wallis results for the effect of organic fertilization and previous crops on grain yield ($Y_{13.5}$) at Palmarolle

Response variable	Factor	Statistic	p-value	p.adj
Yield (kg ha-1)	Fertilization	0.257	0.6120	0.9180
	Previous crop	0.833	0.3613	0.7225
	Fertilization : Previous crop	1.461	0.6913	0.9217
	Block	0.247	0.8838	1.0000

*: p<0.05; **p<0.01; ***:p<0.001

p.adj refers to the p-value with Benjamini-Hochberg correction

Factor/Term, Statistic, df, p-value and p.adj in bold are the significant values

4. Discussion

This study aimed to evaluate the impact of the identity of the previous legume crop on microbial community composition and functional capacity in the following growing season. We hypothesized that a part of the positive legume-cereal rotation effect on wheat yield and grain quality could be mediated by changes in the C and N depolymerization capacity of the microbial community. First, we confirmed that legume identity modifies soil microbial communities in the following growing season, and that PSF effects can be transferred to other plant species (in our case, from legumes to wheat). We also found, at Cloutier, that faba bean as previous crop increased soil ammonium content and wheat grain protein content, a key parameter of grain baking quality (Nuttall et al., 2017). We did not, however, find significant changes in potential protein and cellulose depolymerization activities, infirming our main hypothesis. Instead, the mechanism behind the effect of PSF on wheat grain quality could be an increased mineralization linked to shifts in the relative dominance of bacteria and fungi or a higher substrate availability in faba bean plots.

Under real farming conditions in fields located 100 km apart, we confirmed that the previous crops' PSF influenced the composition of microbial communities. In fact, among all factors tested, the previous crop was the only one that consistently shaped microbial communities in both fields. This highlights the strength of the previous crop effect on microbial communities. Indeed, due to logistics (e.g., the size of plots needed to match the seeder and the fertilizer applicator) and field size, we could not set up more than

553 three complete blocks. Additionally, there was quite some variation between the blocks,
554 but we still found significant evidence of a PSF effect. Previous crop PSF effects on
555 microbial community composition has been observed before (Wang et al., 2023a; Dang
556 et al., 2024). We also found ASVs significantly differently abundant depending on the
557 previous crop identity, proving the legacy effect of previous legume crops on microbial
558 communities. In both fields, fungal communities were more altered by the previous crop
559 identity and residue management than bacterial communities. Fungi are known to be key
560 players in decomposition and can form close relationships with plants, as symbionts and
561 pathogens (Zeilinger et al., 2016). Therefore, changes in fungal community composition
562 and relative abundance due to the previous crop identity are expected (Li et al., 2023).
563 Fungi are also known to be key players in plant compounds degradation, especially lignin
564 and cellulose (Schneider et al., 2012; Arcand et al., 2016). For instance, in Cloutier's wheat
565 plots after faba bean, Leotiomycetes, more specifically *Cadophora luteo-olivacea,* were
566 more abundant. *Cadophora spp.* possess a wide variety of exoenzymes associated with C
567 and N depolymerization, such as protease and carbohydrate active enzymes (Bruyant et
568 al., 2024). They could have potentially played a role in faba bean residue
569 depolymerization and wheat nutrition. In Palmarolle's wheat plots after faba bean,
570 *Cryobacterium spp.* was more abundant. *Cryobacterium spp.* is often correlated to higher
571 lignin and C content (Shamshitov et al., 2025). The incorporation of faba bean residue
572 could have enhanced *Cryobacterium spp.* abundance during the wheat growing season.
573 Fungi have a higher C:N ratio than bacteria and increases in fungal dominance in
574 depolymerization processes would result in higher mineralization rate of the

575 depolymerized organic N (Strickland and Rousk, 2010), without necessarily changing
576 potential enzymatic activities.
577
578 Faba beans and peas are two legumes often used in crop rotation systems to replenish
579 soil N pools. Both crops fix atmospheric N through a symbiosis with rhizobia (Jensen et
580 al., 2010). Their PSF effects on microbial community composition were, however, distinct,
581 supporting other findings (Yixuan et al., 2025). Faba bean is particularly efficient in
582 increasing soil N for the following crop (Herridge et al., 2008; Peoples et al., 2009). Faba
583 beans have a coarser root system (i.e., higher mean root diameter and lower specific root
584 length) and allocate a higher proportion of total plant biomass belowground than pea
585 (Guerrero-Ramírez et al., 2021), therefore adding more organic matter to the soil
586 (Nuruzzaman et al., 2006). Differences in root system sizes can also alter soil physical
587 properties, therefore changing microbial communities. With a root system four times
588 bigger than peas, faba beans promote aggregate formation, creating different
589 microenvironments for microbes in the next growing season (Oliveira et al., 2019). At
590 Cloutier, we also found higher ammonium concentration in the rhizosphere of faba beans
591 as compared to adjacent bulk soil, while there was no difference for peas. This difference
592 between faba beans and peas was maintained during the wheat growing season, which
593 could explain the higher grain protein content of wheat that followed faba beans. Indeed,
594 even with similar potential depolymerization activities, the soil with more substrate (i.e.
595 faba bean plots) will mineralize more N, as soil depolymerization activities are substrate-
596 limited (Greenfield et al., 2020).

597 Fertilization alters soil properties and microbial communities (Luo et al., 2018), and it was
598 expected that organic fertilization would affect microbial communities in our study.
599 Interestingly, microbial communities from wheat growing season did not respond the
600 same way to organic fertilization in both fields. It is important to note that the organic
601 fertilizer amount was not the same in both fields, which could in part explain the
602 difference between fields (Zhou et al., 2021). Nevertheless, organic fertilization increased
603 the variability of microbial communities coming from peas as compared to faba beans. It
604 suggests that faba beans have a stronger PSF effects due to a larger addition of root-
605 derived organic matter and, incorporation of aboveground biomass. It creates a resource-
606 rich environment, priming the microbial community to mineralize organic fertilizer and
607 making it less susceptible to changes caused by fertilizer addition (Aschi et al., 2017).
608
609 The only significant difference in potential enzymatic activity we found between the two
610 legumes was for protease activity that was higher in plots that received peas at Cloutier
611 early in the season, before wheat was sown. In contrast to our main hypothesis, this
612 increased protease activity did not seem to be beneficial for the wheat, as the wheat
613 grains harvested from the pea plots had in fact a lower protein content than the ones
614 harvested in the faba bean plots. This increase in protease potential activity, early in the
615 season and in the absence of the crops, probably led to microbial immobilization of the
616 depolymerized N. In that case, the immobilized N would have needed to be mineralized
617 or re-mobilized from the microbial biomass to reach the wheat. Other enzymes that
618 depolymerize chitin or peptidoglycan, two abundant soil organic N sources that make

619 fungal and bacterial cell walls, respectively, could have been good targets to test this idea.
620 However, since we observed less protein content in wheat grains from the pea plots, the
621 N produced from protease activity probably remained immobilized. This suggest that the
622 timing of depolymerization activities is paramount for it to be beneficial to crops. In
623 wheat, the N assimilated by the plant is mostly taken up before and during the anthesis
624 stage (Cox et al., 1985; Sharma et al., 2023). Our mid-season sampling might have missed
625 the potential increase in depolymerization activities that could have increased wheat N
626 uptake at key growth stages, resulting in higher protein content at the end of the season
627 at Cloutier. Alternatively, as mentioned above, enzymatic activities are substrate-limited,
628 and changes in potential enzymatic activities measured in the laboratory with excess
629 substrate might not represent activities in the field.
630
631 Almost every exoenzyme's potential activities were higher in the wheat rhizosphere than
632 in the bulk soil, suggesting that wheat, through its root exudates, selects for a community
633 proficient in depolymerization. Our results align with the rhizosphere priming theory, a
634 plant competitive strategy for N (Kuzyakov and Xu, 2013; L'Espérance et al., 2024). Plants
635 change the rhizosphere C: N ratio through their C-rich exudates to force microbes to
636 depolymerize soil organic matter to scavenge for N. When the C-rich exudates are all
637 consumed, microbes will mineralize the N excess, providing inorganic N to plants (Zhu et
638 al., 2014). To confirm this hypothetical rhizosphere priming effect, future studies should
639 measure rhizosphere C: N ratios and root exudates.

640 Unlike previous studies (Li et al., 2015; Lupwayi et al., 2019), the addition of organic
641 fertilizer did not have a significant effect on the enzymatic activities, except for the
642 deaminase activity at Palmarolle. This is surprising, since we added a substrate that can
643 stimulate microbes to produce and release exoenzymes. In contrast, Breza et al., (2023)
644 found that the addition of granular urea in more complex rotation systems disturbs the
645 organic N cycle, reducing the N depolymerization rate to a similar rate as monocropping.
646 In both studies, fertilization affects exoenzymes activities mediating N depolymerization
647 (Breza et al., 2023). In our case, the lack of fertilization effects can be due to the fertilizer
648 type, the application rate and/ or the sampling date. We chose to use a fertilizer used in
649 this region for organic farms, and the equivalent of only 25-50 kg N ha^{-1} was applied. The
650 amount applied – which is less than the recommended amount for spring wheat under
651 organic management (~100 kg N ha^{-1}) – and type of fertilizer was chosen to accommodate
652 farmers who must reconcile crop needs and fertilizer prices. The lack of fertilization
653 effects can also be due to the sampling date. Our mid-growing season sampling point in
654 2024 was approximately two months after the application of the organic fertilizer.
655 Organic fertilizer availability depends on the soil mineralization rate and usually takes
656 longer to be available for plants and microbes (Wang et al., 2023b). However, Lazicki et
657 al. (2020) showed that, under laboratory conditions, poultry manure-based granulated
658 fertilizer was already mineralized after a few weeks of incubation. Therefore, nutrients
659 derived from organic fertilizer were probably already immobilized in microbial or wheat
660 biomass at the time of sampling, which would explain the lack of a fertilizer effect on
661 enzymatic activities and soil ammonium and nitrate content.

662 Conducting a two-year field experiment under actual farm operating conditions is a
663 daunting task, and differences between fields are unavoidable. Pedoclimatic conditions,
664 residue management, cultivation history, plot sizes, and fertilization rates differed
665 between our sites. All these differences can alter PSF effects on microbial community
666 composition and depolymerization capacity. In our case, PSF effects were not found
667 across both fields for every parameter tested. This may be due to differences in
668 pedoclimatic conditions, which alter soil physicochemical properties across both fields
669 (A'Bear et al., 2014). Limitations created by different agricultural practices in our
670 experiment also highlighted the need to investigate PSF effects more deeply. As
671 mentioned above, different faba bean incorporation at Cloutier and Palmarolle certainly
672 influenced microbial communities and their functions. But, even with different residue
673 management, we found that the previous crops' PSF influenced microbial community
674 composition and soil ammonium content. Alternatively, any of the above-mentioned
675 differences between the two fields could have played a role. It seems, therefore, possible
676 to enhance PSF when combining crop rotations with other agricultural practices.
677

678 5. Conclusion

679 In conclusion, even with limitations encountered by doing an experiment under actual
680 farm operating conditions (field size, block number, residue management), we showed
681 that faba bean increased soil ammonium and wheat grain protein content as compared
682 to peas, at Cloutier. We had to reject our hypothesis that this effect on wheat grain
683 protein content was due to changes in potential soil enzymatic activities, as we found

little statistical support for it. We have, however, come up with two alternative explanations. First, most significant shifts in microbial communities due to previous legume identity were in fungal ASVs. This could result in a shift in the relative dominance of bacteria and fungi, which, because of differences in C:N ratio between the two groups, could result in differences in mineralization rates. Second, as soil enzymatic activities are substrate-limited, the larger root system of faba bean could have brought more substrate to these plots, increasing mineralization. Depolymerization of cellulose and proteins are widely distributed functions among soil microbial communities, and their *in-situ* activities are probably restricted by substrate availability, whereas the ratio between immobilization/mineralization probably varies with the soil microbial community composition. More field experiments with *in-situ* measurements would be needed to fully understand the role of plant-soil feedback in crop rotation systems to improve our practices and sustainably meet crops' nutritional needs.

6. Funding Sources

This work was supported by the FRQNT Programme de recherche en partenariat (grant number 322604), the Fondation de l'université du Québec en Abitibi-Témiscamingue and the Entente sectorielle biolalimentaire de l'Abitibi-Témiscamingue. ELE was supported by a Fondation Armand-Frappier doctoral scholarship.

7. Acknowledgements

We extend our gratitude to our interns: Raphaël Boisvert, Sopheaktra Honn Kuhn , Marc-Antoine Duchesne, Hubert Côté and Lisa Colleoni whose hard work during field sampling

707 greatly contributed to this project. We are thankful to Johanne Massy, Martin Jacques,
708 Werbson Lima Barroso, Mahefa Ravoavison, Sendy Augustin Salomon and Thierry
709 Nousseassi for their technical assistance, especially with harvest and assessment of wheat
710 yield and protein content. We are also thankful to the farmers who generously provided
711 access to their fields and supported this project. Their collaboration and commitment
712 were essential to the success of this study.
713
714

8.References

A'Bear, A.D., Jones, T.H., Kandeler, E., Boddy, L., 2014. Interactive effects of temperature and soil moisture on fungal-mediated wood decomposition and extracellular enzyme activity. Soil Biology and Biochemistry 70, 151-158.

Adebayo, A.A., Enagbonma, B.J., Babalola, O.O., 2025. Comparative metagenomics on community structure and diversity of rhizomicrobiome associated with monoculture and soybean precedent carrot. Scientific Reports 15, 28161.

Arcand, M.M., Helgason, B.L., Lemke, R.L., 2016. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. Applied Soil Ecology 107, 347-359.

Aschi, A., Aubert, M., Riah-Anglet, W., Nélieu, S., Dubois, C., Akpa-Vinceslas, M., Trinsoutrot-Gattin, I., 2017. Introduction of Faba bean in crop rotation: Impacts on soil chemical and biological characteristics. Applied Soil Ecology 120, 219-228.

Bak, G.-r., Lee, G.-j., Lee, J.-t., Jee, S.-n., 2022. Crop rotation affects biological properties of rhizosphere soil and productivity of Kimchi cabbage (Brassica rapa ssp. pekinensis) compared to monoculture. Horticulture, Environment, and Biotechnology 63, 613-625.

Bay, G., Lee, C., Chen, C., Mahal, N.K., Castellano, M.J., Hofmockel, K.S., Halverson, L.J., 2021. Agricultural management affects the active rhizosphere bacterial community composition and nitrification. Msystems 6, 10.1128/msystems. 00651-00621.

Bhatnagar, J.M., Peay, K.G., Treseder, K.K., 2018. Litter chemistry influences decomposition through activity of specific microbial functional guilds. Ecological Monographs 88, 429-444.

Breza, L.C., Mooshammer, M., Bowles, T.M., Jin, V.L., Schmer, M.R., Thompson, B., Grandy, A.S., 2023. Complex crop rotations improve organic nitrogen cycling. Soil Biology and Biochemistry 177, 108911.

Bruyant, P., Moënne-Loccoz, Y., Almario, J., 2024. Root-associated helotiales fungi: overlooked players in plant nutrition. Soil Biology and Biochemistry 191, 109363.

742 Calderon, F.J., Jackson, L.E., Scow, K.M., Rolston, D.E., 2001. Short-term dynamics of
743 nitrogen, microbial activity, and phospholipid fatty acids after tillage. Soil Science Society
744 of America Journal 65, 118-126.
745 Cox, M.C., Qualset, C.O., Rains, D.W., 1985. Genetic variation for nitrogen assimilation
746 and translocation in wheat. II. Nitrogen assimilation in relation to grain yield and protein
747 1. Crop Science 25, 435-440.
748 Dang, K., Ma, Y., Liang, H., Fan, Z., Guo, S., Li, Z., Li, H., Zhang, S., 2024. Distinct planting
749 patterns exert legacy effects on the networks and assembly of root-associated
750 microbiomes in subsequent crops. Science of the Total Environment 946, 174276.
751 Eivazi, F., Tabatabai, M., 1988. Glucosidases and galactosidases in soils. Soil Biology and
752 Biochemistry 20, 601-606.
753 Freschet, G.T., Cornwell, W.K., Wardle, D.A., Elumeeva, T.G., Liu, W., Jackson, B.G.,
754 Onipchenko, V.G., Soudzilovskaia, N.A., Tao, J., Cornelissen, J.H., 2013. Linking litter
755 decomposition of above-and below-ground organs to plant–soil feedbacks worldwide.
756 Journal of Ecology 101, 943-952.
757 Gamage, A., Gangahagedara, R., Gamage, J., Jayasinghe, N., Kodikara, N., Suraweera, P.,
758 Merah, O., 2023. Role of organic farming for achieving sustainability in agriculture.
759 Farming System 1, 100005.
760 Gan, Y., Hamel, C., O'Donovan, J.T., Cutforth, H., Zentner, R.P., Campbell, C.A., Niu, Y.,
761 Poppy, L., 2015. Diversifying crop rotations with pulses enhances system productivity.
762 Scientific Reports 5, 14625.
763 Geisseler, D., Horwath, W.R., 2008. Regulation of extracellular protease activity in soil in
764 response to different sources and concentrations of nitrogen and carbon. Soil Biology and
765 Biochemistry 40, 3040-3048.
766 Greenfield, L.M., Hill, P.W., Seaton, F.M., Paterson, E., Baggs, E.M., Jones, D.L., 2020. Is
767 soluble protein mineralisation and protease activity in soil regulated by supply or
768 demand? Soil Biology and Biochemistry 150, 108007.
769 Group, C.A.S.C.C.S.C.W., Canada, N.R.C., Agriculture, C., Branch, A.-F.C.R., 1998. The
770 Canadian system of soil classification. NRC Research Press.

Guerrero-Ramírez, N.R., Mommer, L., Freschet, G.T., Iversen, C.M., McCormack, M.L., Kattge, J., Poorter, H., van Der Plas, F., Bergmann, J., Kuyper, T.W., 2021. Global root traits (GRooT) database. Global Ecology and Biogeography 30, 25-37.

Hannula, S.E., Heinen, R., Huberty, M., Steinauer, K., De Long, J.R., Jongen, R., Bezemer, T.M., 2021. Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. Nature Communications 12, 5686.

Hannula, S.E., Ma, H.k., Pérez-Jaramillo, J.E., Pineda, A., Bezemer, T.M., 2020. Structure and ecological function of the soil microbiome affecting plant–soil feedbacks in the presence of a soil-borne pathogen. Environmental microbiology 22, 660-676.

Herridge, D.F., Peoples, M.B., Boddey, R.M., 2008. Global inputs of biological nitrogen fixation in agricultural systems. Plant and Soil 311, 1-18.

Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Oswald-Lachouani, P., Wanek, W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Science Society of America Journal 74, 1018-1027.

Huo, C., Luo, Y., Cheng, W., 2017. Rhizosphere priming effect: a meta-analysis. Soil Biology and Biochemistry 111, 78-84.

Jan, M.T., Roberts, P., Tonheim, S.K., Jones, D.L., 2009. Protein breakdown represents a major bottleneck in nitrogen cycling in grassland soils. Soil Biology and Biochemistry 41, 2272-2282.

Jensen, E.S., Peoples, M.B., Hauggaard-Nielsen, H., 2010. Faba bean in cropping systems. Field crops research 115, 203-216.

Jesmin, T., Margenot, A.J., Mulvaney, R.L., 2022. A comprehensive method for casein-based assay of soil protease activity. Communications in Soil Science and Plant Analysis 53, 507-520.

Jones, D.L., Nguyen, C., Finlay, R.D., 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 321, 5–33.

Kaloterakis, N., Giongo, A., Braun-Kiewnick, A., Rashtbari, M., Zamberlan, P., Razavi, B.S., Smalla, K., Reichel, R., Brüggemann, N., 2025. Rotational diversity shapes the bacterial

and archaeal communities and confers positive plant-soil feedback in winter wheat rotations. Soil Biology and Biochemistry 203, 109729.

Killham, K., Rashid, M., 1986. Assay of activity of a soil deaminase. Plant and Soil 92, 15-21.

Knops, J., Bradley, K., Wedin, D., 2002. Mechanisms of plant species impacts on ecosystem nitrogen cycling. Ecology letters 5, 454-466.

Kuzyakov, Y., Xu, X., 2013. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytologist 198, 656-669.

L'Espérance, E., Bouyoucef, L.S., Dozois, J.A., Yergeau, E., 2024. Tipping the plant-microbe competition for nitrogen in agricultural soils. Iscience 27.

Lazicki, P., Geisseler, D., Lloyd, M., 2020. Nitrogen mineralization from organic amendments is variable but predictable. Wiley Online Library.

Li, B., Liu, X., Zhu, D., Su, H., Guo, K., Sun, G., Li, X., Sun, L., 2023. Crop diversity promotes the recovery of fungal communities in saline-alkali areas of the Western Songnen Plain. Frontiers in microbiology 14, 1091117.

Li, J., Cooper, J.M., Lin, Z.a., Li, Y., Yang, X., Zhao, B., 2015. Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China Plain. Applied Soil Ecology 96, 75-87.

Li, X., Panke-Buisse, K., Yao, X., Coleman-Derr, D., Ding, C., Wang, X., Ruan, H., 2020. Peanut plant growth was altered by monocropping-associated microbial enrichment of rhizosphere microbiome. Plant and Soil 446, 655-669.

Luo, G., Rensing, C., Chen, H., Liu, M., Wang, M., Guo, S., Ling, N., Shen, Q., 2018. Deciphering the associations between soil microbial diversity and ecosystem multifunctionality driven by long-term fertilization management. Functional Ecology 32, 1103-1116.

Lupwayi, N.Z., Soon, Y.K., 2015. Carbon and nitrogen release from legume crop residues for three subsequent crops. Soil Science Society of America Journal 79, 1650-1659.

Lupwayi, N.Z., Zhang, Y., Hao, X., Thomas, B.W., Eastman, A.H., Schwinghamer, T.D., 2019. Linking soil microbial biomass and enzyme activities to long-term manure applications and their nonlinear legacy. Pedobiologia 74, 34-42.

Ma, H.-K., Pineda, A., Van der Wurff, A.W., Raaijmakers, C., Bezemer, T., 2017. Plant–soil feedback effects on growth, defense and susceptibility to a soil-borne disease in a cut flower crop: species and functional group effects. Frontiers in plant science 8, 2127.

Mariotte, P., Mehrabi, Z., Bezemer, T.M., De Deyn, G.B., Kulmatiski, A., Drigo, B., Veen, G.C., Van der Heijden, M.G., Kardol, P., 2018. Plant–soil feedback: bridging natural and agricultural sciences. Trends in ecology & evolution 33, 129-142.

Maynard, D., Kalra, Y., Crumbaugh, J., 1993. Nitrate and exchangeable ammonium nitrogen. Soil sampling and methods of analysis 1, 25-38.

Murrell, E.G., Ray, S., Lemmon, M.E., Luthe, D.S., Kaye, J.P., 2020. Cover crop species affect mycorrhizae-mediated nutrient uptake and pest resistance in maize. Renewable Agriculture and Food Systems 35, 467-474.

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M., Högberg, P., 1998. Boreal forest plants take up organic nitrogen. Nature 392, 914-916.

Nuruzzaman, M., Lambers, H., Bolland, M.D., Veneklaas, E.J., 2006. Distribution of carboxylates and acid phosphatase and depletion of different phosphorus fractions in the rhizosphere of a cereal and three grain legumes. Plant and Soil 281, 109-120.

Nuttall, J., O'leary, G., Panozzo, J., Walker, C., Barlow, K., Fitzgerald, G., 2017. Models of grain quality in wheat—A review. Field crops research 202, 136-145.

Oliveira, M., Barre, P., Trindade, H., Virto, I., 2019. Different efficiencies of grain legumes in crop rotations to improve soil aggregation and organic carbon in the short-term in a sandy Cambisol. Soil and Tillage Research 186, 23-35.

Peoples, M.á., Brockwell, J., Herridge, D., Rochester, I., Alves, B., Urquiaga, S., Boddey, R., Dakora, F., Bhattarai, S., Maskey, S., 2009. The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48, 1-17.

Rhine, E., Mulvaney, R., Pratt, E., Sims, G.K., 1998. Improving the Berthelot reaction for determining ammonium in soil extracts and water. Soil Science Society of America Journal 62, 473-480.

Schneider, T., Keiblinger, K.M., Schmid, E., Sterflinger-Gleixner, K., Ellersdorfer, G., Roschitzki, B., Richter, A., Eberl, L., Zechmeister-Boltenstern, S., Riedel, K., 2012. Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. The ISME journal 6, 1749-1762.

Séneca, J., Söllinger, A., Herbold, C.W., Pjevac, P., Prommer, J., Verbruggen, E., Sigurdsson, B.D., Peñuelas, J., Janssens, I.A., Urich, T., 2021. Increased microbial expression of organic nitrogen cycling genes in long-term warmed grassland soils. ISME communications 1, 69.

Shamshitov, A., Kadžienė, G., Pini, F., Supronienė, S., 2025. The role of tillage practices in wheat straw decomposition and shaping the associated microbial communities in Endocalcaric–Epigleyic Cambisol soil. Biology and Fertility of Soils 61, 211-231.

Sharma, S., Kumar, T., Foulkes, M.J., Orford, S., Singh, A.M., Wingen, L.U., Karnam, V., Nair, L.S., Mandal, P.K., Griffiths, S., 2023. Nitrogen uptake and remobilization from pre- and post-anthesis stages contribute towards grain yield and grain protein concentration in wheat grown in limited nitrogen conditions. CABI Agriculture and Bioscience 4, 12.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. Soil Biology and Biochemistry 42, 1385-1395.

Wang, G., van der Putten, W.H., Klironomos, J., Zhang, F., Zhang, J., 2025. Steering plant-soil feedback for sustainable agriculture. Science 389, eads2506.

Wang, P., Nie, J., Yang, L., Zhao, J., Wang, X., Zhang, Y., Zang, H., Yang, Y., Zeng, Z., 2023a. Plant growth stages covered the legacy effect of rotation systems on microbial community structure and function in wheat rhizosphere. Environmental Science and Pollution Research 30, 59632-59644.

Wang, Y., Li, Q., Li, C., 2023b. Organic fertilizer has a greater effect on soil microbial community structure and carbon and nitrogen mineralization than planting pattern in rainfed farmland of the Loess Plateau. Frontiers in Environmental Science 11, 1232527.

Wang, Y., Wu, P., Qiao, Y., Li, Y., Liu, S., Gao, C., Liu, C., Shao, J., Yu, H., Zhao, Z., 2023c. The potential for soil C sequestration and N fixation under different planting patterns depends on the carbon and nitrogen content and stability of soil aggregates. Science of the Total Environment 897, 165430.

WRB, I.W.G., 2007. World Reference Base for Soil Ressources 2006, First Update, World Soil Resources Report, Rome.

Yang, X., Xiong, J., Du, T., Ju, X., Gan, Y., Li, S., Xia, L., Shen, Y., Pacenka, S., Steenhuis, T.S., 2024. Diversifying crop rotation increases food production, reduces net greenhouse gas emissions and improves soil health. Nature Communications 15, 198.

Yixuan, C., Zhijie, D., Yu, W., Qiong, L., Ruohan, Y., Tida, G., Zhenke, Z., 2025. Faba bean enhances soil multifunctionality through shaping rhizosphere microbial communities in legume-cereal crop rotations. Frontiers of Agricultural Science & Engineering 12.

Yuan, J., Zhao, J., Wen, T., Zhao, M., Li, R., Goossens, P., Huang, Q., Bai, Y., Vivanco, J.M., Kowalchuk, G.A., 2018. Root exudates drive the soil-borne legacy of aboveground pathogen infection. Microbiome 6, 156.

Zeilinger, S., Gupta, V.K., Dahms, T.E., Silva, R.N., Singh, H.B., Upadhyay, R.S., Gomes, E.V., Tsui, C.K.-M., Nayak S, C., 2016. Friends or foes? Emerging insights from fungal interactions with plants. FEMS Microbiology Reviews 40, 182-207.

Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., Da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B.P., 2018. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiology 3, 470-480.

Zhao, Z.-B., He, J.-Z., Quan, Z., Wu, C.-F., Sheng, R., Zhang, L.-M., Geisen, S., 2020. Fertilization changes soil microbiome functioning, especially phagotrophic protists. Soil Biology and Biochemistry 148, 107863.

Zhou, J., Kong, Y., Zhao, W., Wei, G., Wang, Q., Ma, L., Wang, T., Shu, F., Sha, W., 2021. Comparison of bacterial and archaeal communities in two fertilizer doses and soil compartments under continuous cultivation system of garlic. Plos one 16, e0250571.

910 Zhou, Y., Yang, Z., Liu, J., Li, X., Wang, X., Dai, C., Zhang, T., Carrión, V.J., Wei, Z., Cao, F.,
911 2023. Crop rotation and native microbiome inoculation restore soil capacity to suppress
912 a root disease. Nature communications 14, 8126.
913 Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014.
914 Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology and
915 Biochemistry 76, 183-192.
916
917

Figure captions

Figure 1: Experimental design of Cloutier and Palmarolle field in 2024. Colors (yellow and green) represent the previous crop sown in 2023. Brown dots line represents fertilized plots in 2024, and clovers represent plots that received clover as a cover crop. The cover crop was not used in our analysis, since it was not grown when we sampled mid-growing season (2024). Our experimental design allowed us to test the effects of previous crop, fertilization and wheat compartment on exoenzymes potential activities (Protease, deaminase and B-glucosidase), on inorganic N content and on the microbial community diversity and composition.

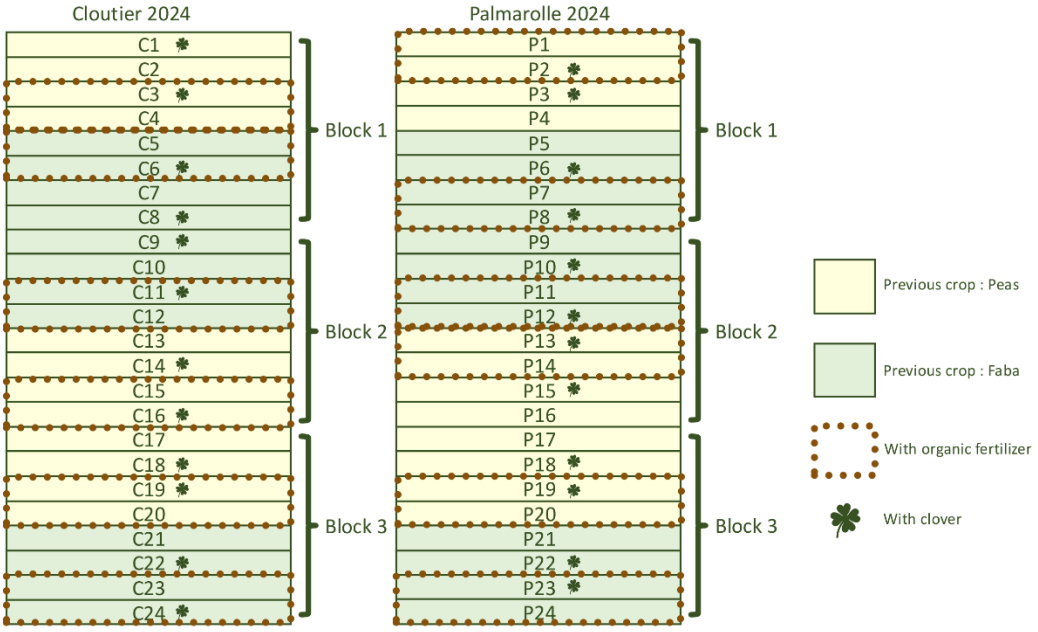
Figure 2: Beta diversity of the bacterial (16S) and fungal communities (ITS) from rhizosphere and bulk soil under wheat cropping at Cloutier (A, B) and Palmarolle (C, D) in 2024 using a Euclidean distance matrix and principal coordinate analysis. The effect of the previous crop (color) and organic fertilization (form and ellipse) is presented. For each field, all blocks are represented (n=3). The R^2 and p-value correspond to the PERMANOVA results for these variables and the compartment (i.e., rhizospheric and bulk soil) variable.

Figure 3: Compositions of the soil bacterial (A) and fungal (C) communities and Venn plot illustrating the number of differentially abundant bacterial (B) and fungal (D) ASV from rhizosphere and bulk soil under wheat cropping at Cloutier in 2024. Stacked bar charts contain the relative abundance of phyla for bacteria (A) and Classes for fungi. Venn plots

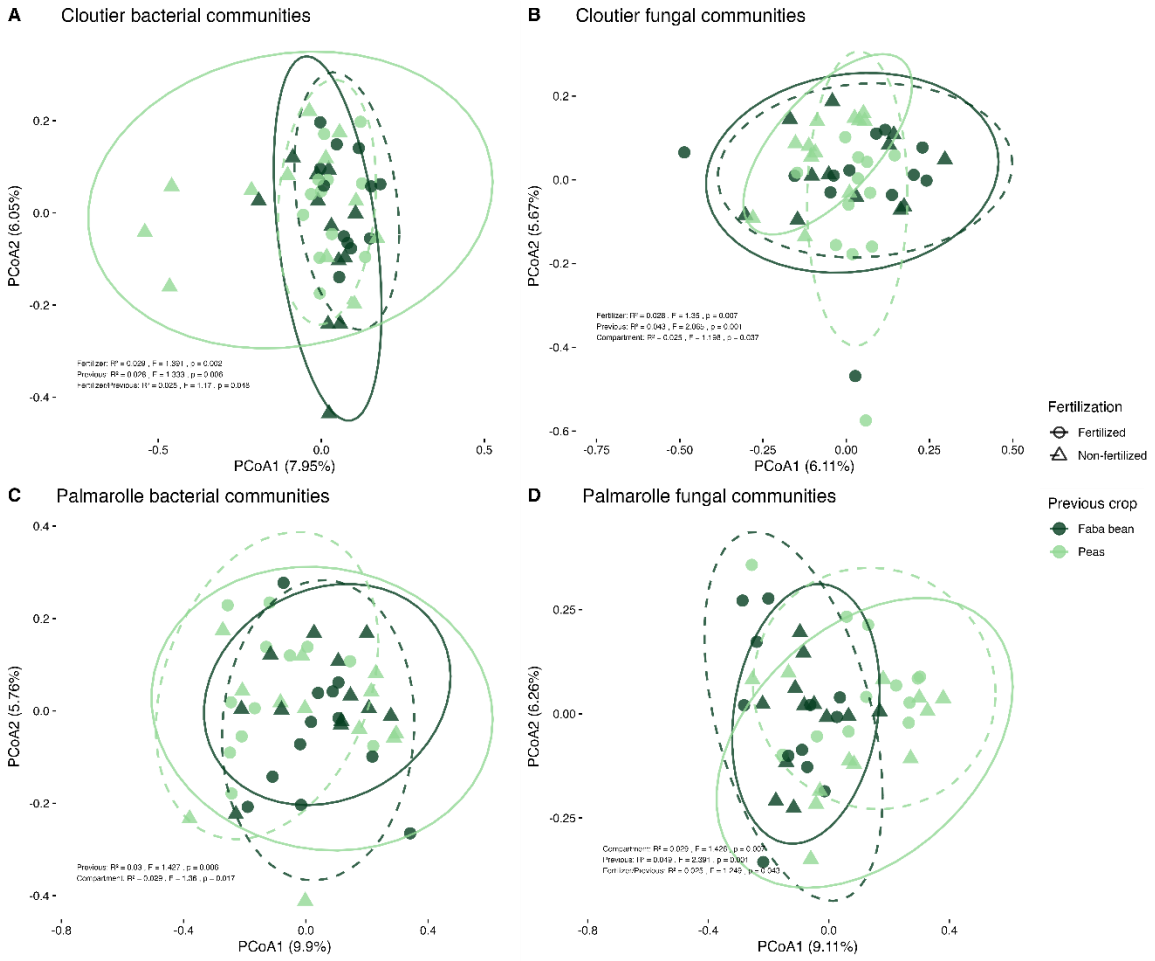
939 illustrate the number of significantly differentially abundant ASVs found by DESeq2,
940 ANCOM-BC and LEfSe analysis ($p<0.05$).
941
942 Figure 4: Compositions of the soil bacterial (A) and fungal (C) communities and Venn plot
943 illustrating the number of differentially abundant bacterial (B) and fungal (D) ASV from
944 rhizosphere and bulk soil under wheat cropping at Palmarolle in 2024. Stacked bar charts
945 contain the relative abundance of phyla for bacteria (A) and Classes for fungi. Venn plots
946 illustrate the number of significantly differentially abundant ASVs found by DESeq2,
947 ANCOM-BC and LEfSe analysis ($p<0.05$).
948
949 Figure 5: Protease, β-glucosidase and deaminases potential activities in the rhizosphere
950 and bulk soil at 0-15 cm depth under wheat cropping at Cloutier (A, B, C) and Palmarolle
951 (D, E, F) mid-growing season in 2024. The effects of the soil compartment, the previous
952 crop, and fertilization are represented. The organic fertilization is represented by points
953 (mean) and error bars (95% confidence interval) of both compartments and the previous
954 crop. For each field and potential enzymatic activities, all blocks are represented (n=3).
955 Significant results from Kruskal-Wallis or ANOVA tests for each enzyme's potential
956 activities (see Table 2) are identified with a *($p<0.05$).
957
958 Figure 6: Wheat rhizosphere and bulk soil ammonium and nitrate content mid-growing
959 season at Cloutier (A, B) and Palmarolle (C, D) at 0-15 cm depth. The effects of the
960 compartment, the previous crop, and fertilization are represented. The organic

961 fertilization is represented by points (mean) and error bars (95% confidence interval) of
962 both compartments and the previous crop. For each field and N content, all blocks are
963 represented (n=3). Significant results from Kruskal-Wallis or ANOVA tests for ammonium
964 and nitrate content (see Table 3) are identified with a *($p<0.05$).
965
966 Figure 7: Wheat grain protein content of each organic fertilization treatment and previous
967 crops after harvest at Cloutier. The effects of the previous crop and fertilization are
968 represented. Significant results from Kruskal-Wallis tests (see Table 3) are identified with
969 a **($p<0.05$).
970
971
972
973

Figure 1.


Cloutier 2024
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23
C24
Block 1
Block 2
Block 3
Palmarolle 2024
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
P13
P14
P15
P16
P17
P18
P19
P20
P21
P22
P23
P24
Block 1
Block 2
Block 3
Previous crop : Peas
Previous crop : Faba
With organic fertilizer
With clover

978 **Figure 2.**

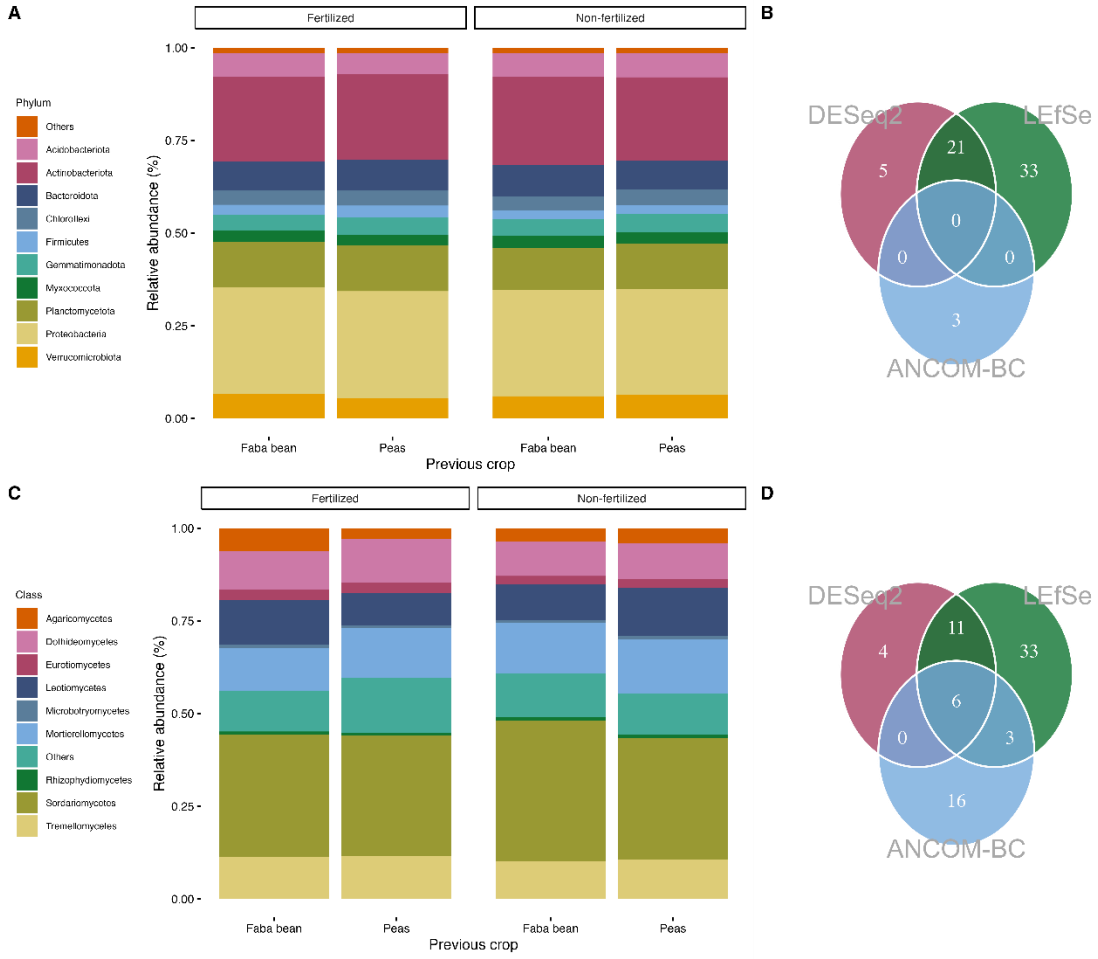


979

980

981

982 **Figure 3.**

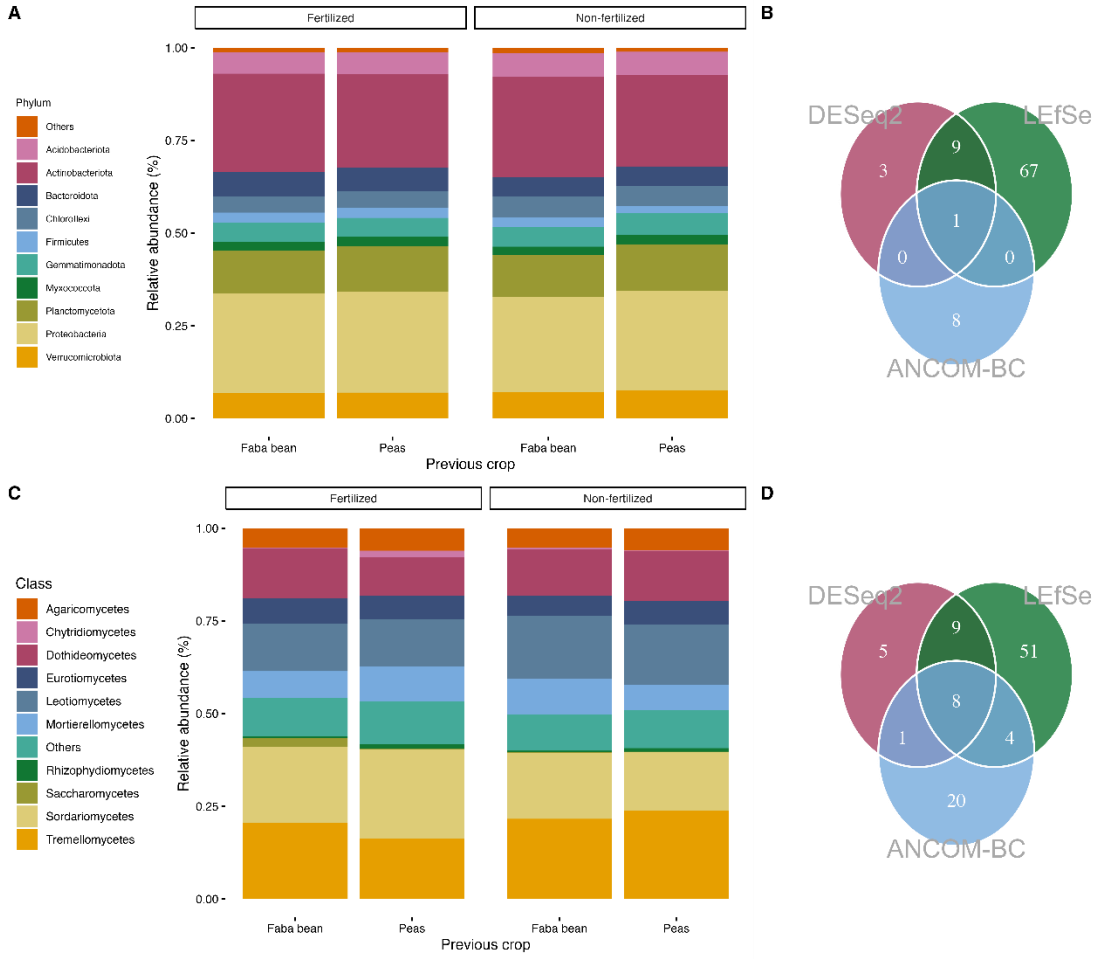


983

984

985

986 **Figure 4.**

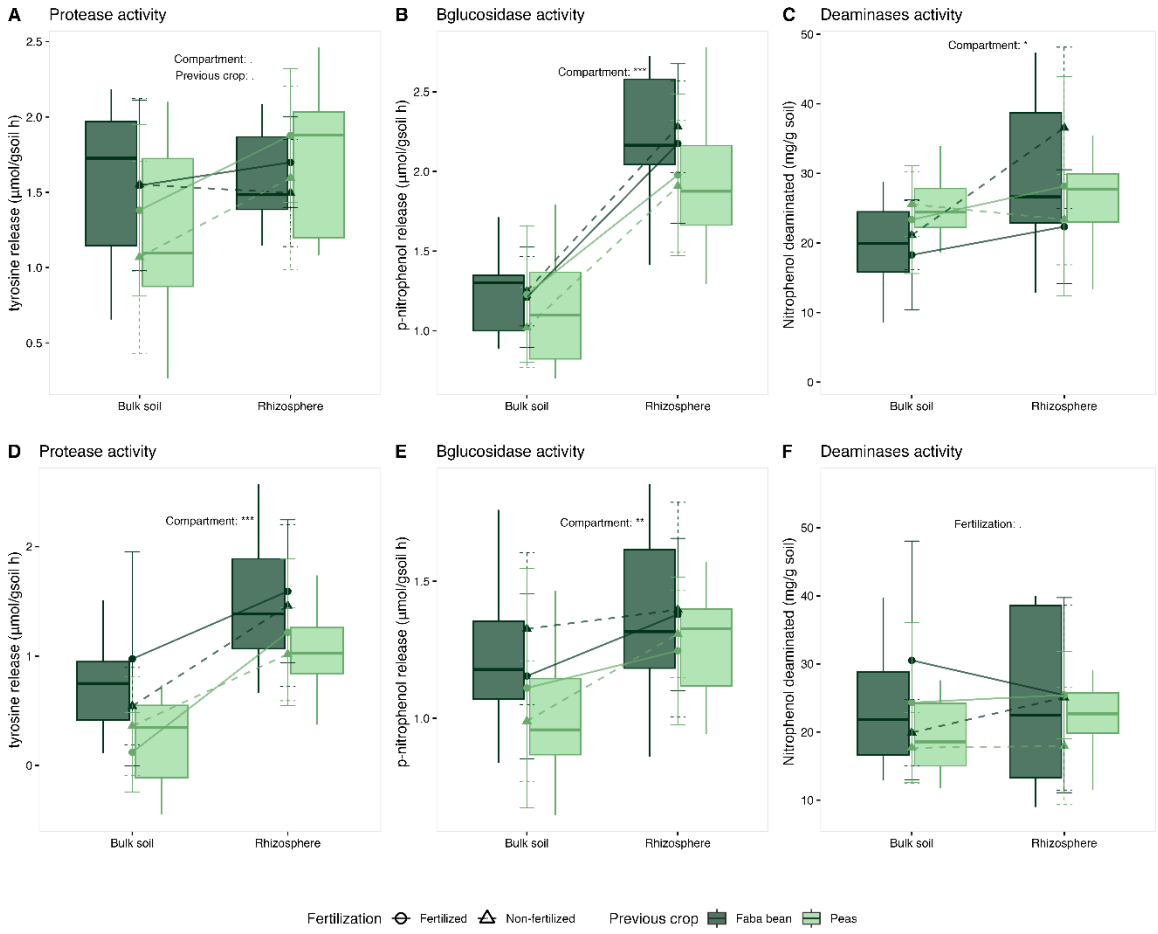


987

988

989

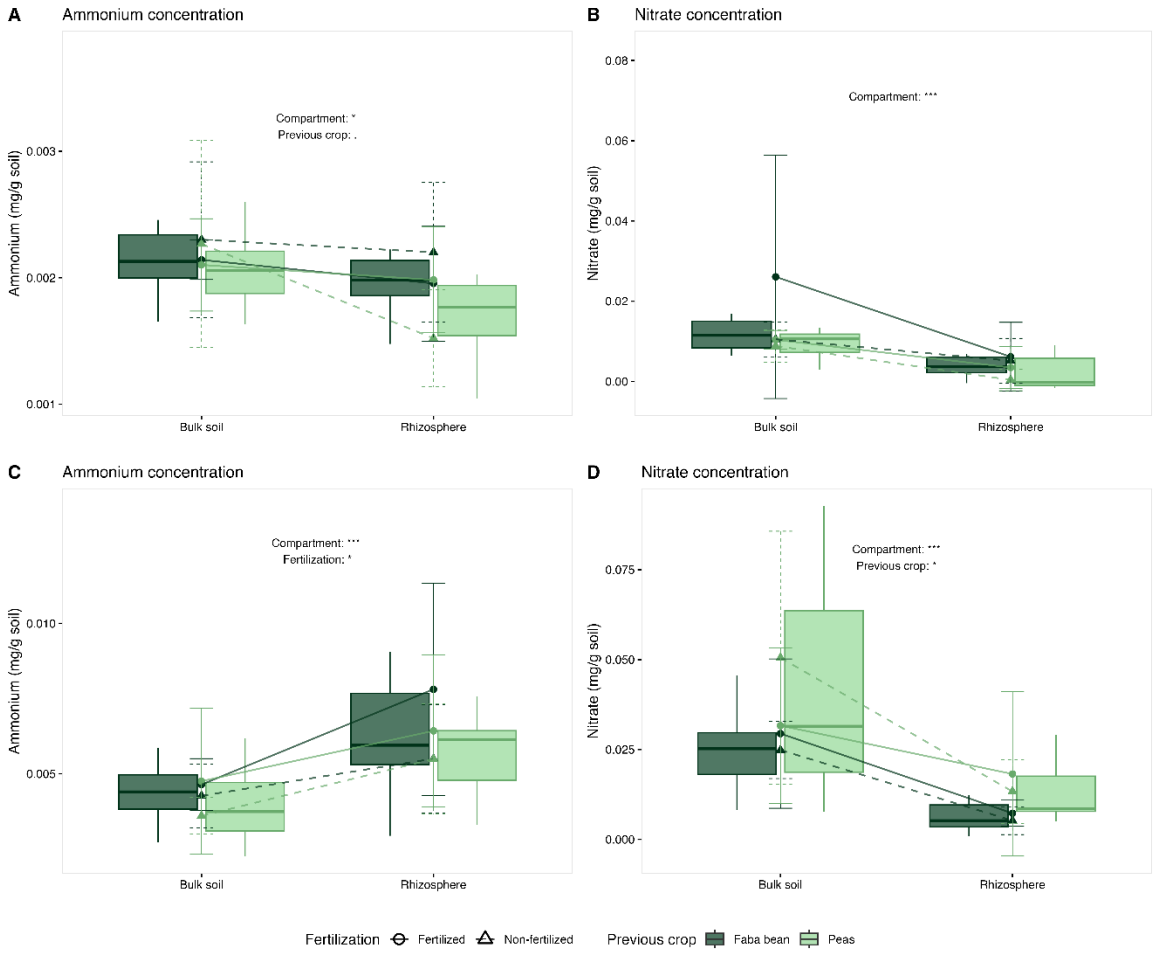
990 **Figure 5.**



991

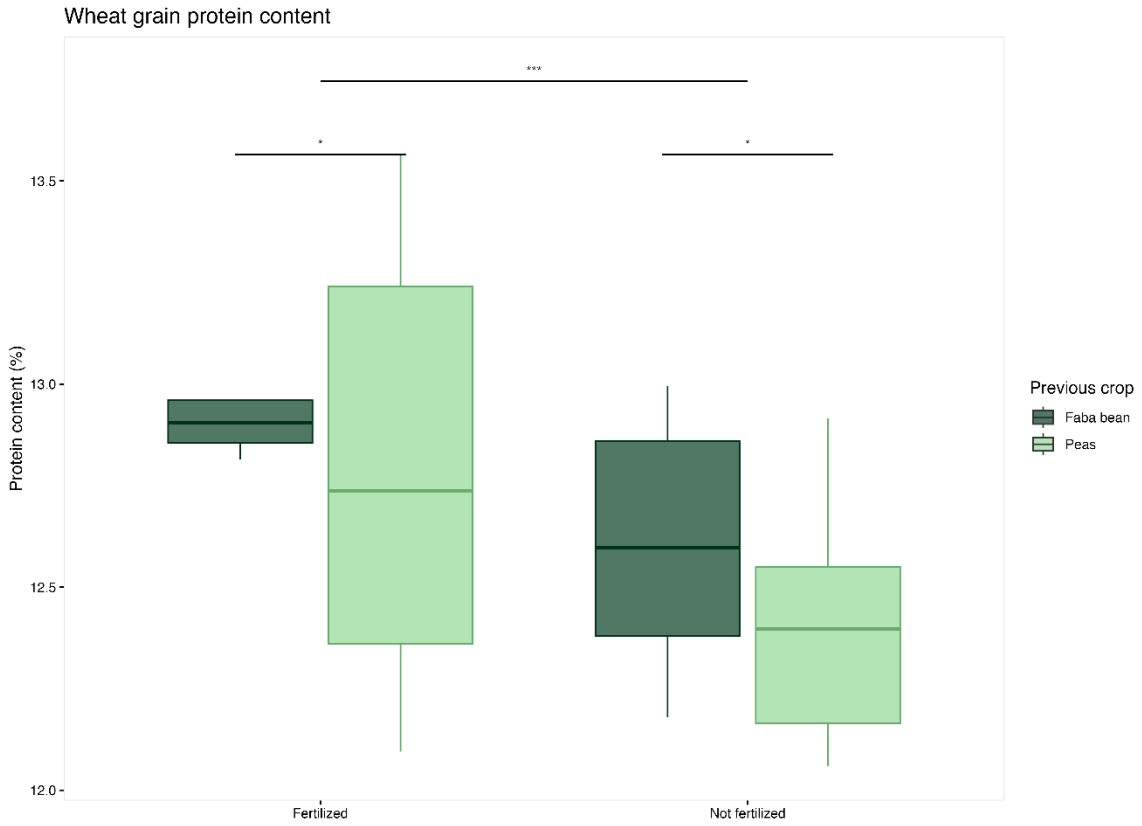
992

Figure 6.


A
Ammonium concentration
Compartment: *
Previous crop: .
Ammonium (mg/g soil)
0.003
0.002
0.001
Bulk soil
Rhizosphere
B
Nitrate concentration
Compartment: ***
Nitrate (mg/g soil)
0.08
0.06
0.04
0.02
0.00
Bulk soil
Rhizosphere
C
Ammonium concentration
Compartment: ***
Fertilization: *
Ammonium (mg/g soil)
0.010
0.005
Bulk soil
Rhizosphere
D
Nitrate concentration
Compartment: ***
Previous crop: *
Nitrate (mg/g soil)
0.075
0.050
0.025
0.000
Bulk soil
Rhizosphere
Fertilization
Fertilized
Non-fertilized
Previous crop
Faba bean
Peas

997 **Figure 7.**

998



999

1000