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Article

Do Conservative Agricultural Practices Improve the Functional Biological State of Legume-Based Cropping Systems?

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Abstract: This study evaluated the response of soil microbial functions to the presence and placement of faba beans in crop rotations (rotation without legumes versus rotations with faba beans as the crop previous to wheat, with the faba beans sown three years before the wheat crops) combined with two tillage systems (conventional and reduced tillage). The study included 36 wheat-producing plots, and two agricultural practice types were defined: high-frequency–low-intensity (HF–LI) and low-frequency–high-intensity (LF–HI). The results demonstrated a significant increase in the total carbon and nitrogen (N) content under reduced tillage. Furthermore, the general path analysis suggested that arylamidase and β -glucosidase activities significantly affect N fluxes. The enzyme activities were modified by changing the soil's physicochemical properties. These findings highlighted the significance of introducing legumes as the crop preceding wheat, especially when applying conventional tillage. Moreover, it was revealed that farmers' management of these conservative practices is a leading factor in regulating soil functions. Pesticides and inorganic fertilization inputs were classified as HF–LI practices, while organic matter (OM) inputs and liming treatments were qualified as LF–HI practices. For instance, LF–HI practices (OM inputs and liming) directly and indirectly influenced the soil functions related to the N cycle, while HF–LI practices (pesticide, inorganic N fertilization, and previous crops) resulted in fewer soil function changes.

Keywords: conservative practices; enzyme activities; faba bean placement; mineralization–immobilization turnover; tillage systems



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1. Introduction

The development of agriculture in recent decades has caused several ecological problems, such as soil compaction, wind and water erosion, salinization, and biodiversity and organic matter (OM) loss [1,2]. Various agricultural approaches have emerged to address these threats, including sustainable, ecologically intensive, agroecological, multifunctional, and conservation agriculture [3]. Most of these approaches are characterized by three linked factors, i.e., minimal soil disturbance, permanent soil cover, and crop rotation diversification [4–6]. As a result, reducing or suppressing soil tillage, recycling crop residues, and including leguminous plants in crop rotation have been recognized as conservative agricultural practices that potentially increase soil functioning, OM content, and biodiversity [7–11]. Moreover, these conservative practices can alter the structure and composition of the microbial communities that support soil functions. In addition, intensive agricultural practices indirectly impact soil microorganisms and their trophic resources by changing their habitats through alterations in the soil's physicochemical properties [12–14] and modifying the OM amounts or carbon (C) and nitrogen (N) dynamics in the soil [15–17].

Therefore, shifts in tillage systems alter crop residue distribution and the vertical stratification of OM, modifying soil microbial communities [14,18,19]. Furthermore, introducing leguminous crops, such as faba beans (*Vicia faba* L.), into the crop rotation enables agrosystem diversification [20–22] and improves the N and phosphorus (P) quantities available in the soil for the subsequent crops [23,24].

The effects of faba beans on soil microbial communities have been widely investigated. It was reported that soil-cultivable microorganisms are more abundant after introducing faba beans into the soil [25]. Biolog data analysis and PhosphoLipid Fatty Acid (PLFA) profiling indicated that rotations that include faba beans modify microbial populations [26], induce variations in the catabolic capacity of soil microbial communities, and enhance mycorrhizal colonization [27,28]. Yang et al. [29] also suggested that wheat (*Triticum aestivum* L.) and faba bean intercropping could significantly improve the efficiency of microbial C use and alter the microbial community structure in the rhizosphere of the faba beans. Other research also indicated that wheat–faba bean intercropping could increase the abundance of bacteria, fungi, and actinomycetes in the rhizosphere [30]. Although the impacts of faba beans on microbial communities are well-documented, their effects on biological soil functions have been poorly investigated. The available studies were conducted during the crop’s growth phases or on the crop immediately following the leguminous crop cultivation year [21], providing no information regarding leguminous interest for other crops in the rotation.

Numerous studies have been performed on experimental sites, ensuring a homogeneous pedoclimatic context. This is essential to better understanding the underlying mechanisms of the soil’s biological and functional responses to agricultural practices. Nevertheless, the strict control of field conditions impedes achieving a clear consensus on the impacts of agricultural practices across broader climatic and edaphic contexts. Additionally, conservative methods are frequently evaluated independently, i.e., crop rotations are contrasted with other crop rotations [31,32], and tillage methods are compared with other tillage practices [33]. Consequently, the combined effects of these conservative practices under actual field conditions are poorly documented.

Thus, the primary objective of this study was to evaluate the effects of two conservation agriculture practices—implementing leguminous plants in crop rotations and tillage reduction—on soil biological functioning in field conditions with Normandy farm’s wheat crops. Specifically, this study aimed to assess the effects of (i) the presence of a legume (faba bean) in a crop rotation, (ii) the position of this legume crop within the rotation, and (iii) the interaction of these modifications with the tillage practices (conventional tillage or reduced tillage). The enzyme activities involved in soil C and N dynamics and gross N fluxes were measured to assess the soil’s functional state. It was hypothesized that combining faba bean introduction and reduced tillage would increase the soil’s enzyme activities and gross N fluxes. In addition, the long-term impact of these changes on the subsequent crops was examined by varying the placement of the faba bean within the rotation. Finally, this study’s experimental approach, “under real farming conditions”, encompassed the variability in the farmers’ implementation of these two conservative practices contributed. The second step evaluated how variations in farming practices and soil physiochemical properties drive soil enzyme activity and gross N fluxes.

2. Materials and Methods

2.1. Field Experimental Design and Soil Sampling

This study was conducted in the Normandy region (northwestern France, 48°52′47.532″N, 0°10′16.511″E). This region has an oceanic climate characterized by annual rainfall ranging from 900 to 1000 mm and a mean annual temperature of approximately 10.5 °C.

A total of 36 agricultural plots located in 20 farms in Normandy were selected for the experimental design, accounting for the presence and placement of the faba beans in the crop rotations and the soil tillage type. Winter wheat was sown at the sites during

the sampling year. Only one crop type (wheat) was targeted to homogenize rhizospheric properties and crop management. Thus, the objective was to compare how the tillage and inclusion of faba beans in the rotation affected soil enzyme activity and N cycling using the same crop in various conditions. Six experimental treatments were established by combining two tillage systems and three rotation types. The tillage systems consisted of conventional tillage (CT) with moldboard plows and reduced tillage (RT) using chisel plows. CT involved soil inversion to a depth of 25–30 cm, while RT involved mechanical disturbance at a depth of less than 8 cm without soil inversion. The three crop rotations were (1) faba beans grown as the crop before wheat (N-1), (2) faba beans grown three years before wheat (N-3), and (3) the absence of faba beans and any other leguminous crops in the rotation during the preceding five years (Control (CTL)). The six experimental treatments were CTL_CT, CTL_RT, N-1_CT, N-1_RT, N-3_CT, and N-3_RT. Six plots were selected as independent replications for each treatment, leading to 36 wheat plots (six plots multiplied by six treatments), as shown in Figure 1.

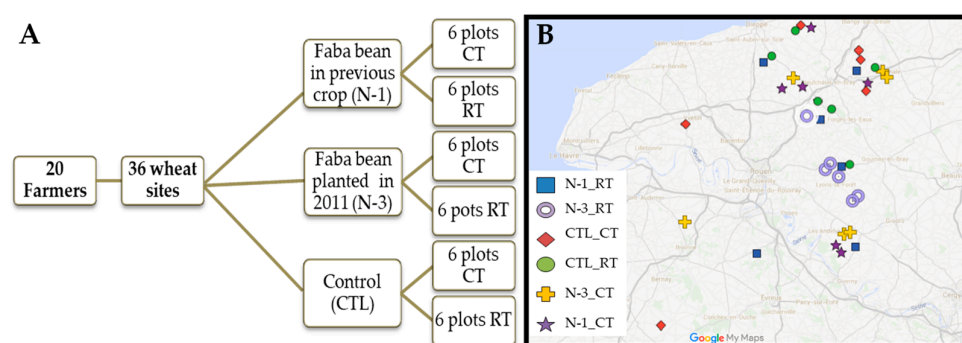


Figure 1. In situ experimental design. (A) The sampling design used to identify the impacts of the tillage systems and faba bean placement on soil enzyme activity and gross N fluxes. (B) The geographical location of the sampled plots. CT: conventional tillage; RT: reduced tillage; N-1: faba beans as the crop before wheat; N-3: faba beans sown three years before wheat; CTL: rotation without faba beans and any leguminous crops during the preceding five years.

The sampling procedure was uniform across all plots. Three composite soil samples were collected along the seed line (spacing of 30 m) during the spring of 2014 (in April). Each composite soil sample comprised five soil cores collected from the 0–10 cm soil depth using a hand auger. Each of the 108 composite samples (six plots by six treatments by three samples) was filtered through a 2 mm sieve and subsequently homogenized. Fresh soil was used for biological analyses, and air-dried soil was used for physical and chemical analyses.

2.2. Farmer Surveys of the Studied Plots

The present study was conducted in actual agricultural settings required to consider the variability caused by the current management methods associated with implementing the two studied practices: faba bean introduction and reduced tillage. The variability was assessed using a survey distributed to the farmers of the examined farms. This survey addressed the implemented management practices, the study year, and the previous five years in the wheat plots selected for soil sampling, i.e., information on inorganic fertilization, pesticides, tillage, liming, OM inputs, and previous crops.

Three modalities in global agricultural practices were identified based on the temporality of the treatment relating to OM inputs and liming treatments. The first modality involved plots receiving a recent OM input (during the sampling year or one year before). This modality was called “Recent”. The second modality, “Intermediate”, considered the plots that received OM between two and three years before the sampling date. Finally, the third modality, “Old”, included plots that received OM at least four years before the sampling date. The Treatment Frequency Index (TFI) was calculated according to the study conducted by Lechenet [34] based on the number of registered doses applied for each

pesticide per hectare on the wheat plots before sampling. The pesticide treatments were then classified according to three intensity modalities related to the amount typically used for wheat, i.e., low if $TFI < 1.5$, middle if $1.5 \leq TFI \leq 3.8$, and high if $TFI > 3.8$. Additionally, based on the nitrogen unit (NU) applied during the sampling year in the wheat crops, three modalities of inorganic N fertilization were identified, i.e., low if less than 60 NU was applied, middle when 60 NU–140 NU was applied and high if more than 140 NU was applied in the plots. Finally, the crops sown in the studied plots during the last five years were examined. These practices were classified as high-frequency (HF) practices due to inorganic fertilization, pesticide treatments, and preceding crops representing practices that occurred once or several times during the year before sampling. These HF practices were also characterized by their applied doses.

Due to the environmental risks, farmers typically adhere to the recommendations of agricultural advisors and apply low doses of pesticides and inorganic fertilizers. Thus, these practices were also qualified as low-intensity (LI) and referred to as high-frequency–low-intensity (HF–LI) practices. In contrast, OM inputs and liming treatments that reach 20–30 tons per application were qualified as high-intensity practices (HI). These treatments were identified as low-frequency–high-intensity practices (LF–HI) because they are typically applied only once in a rotation. The farmer surveys generated categorical data encoded in a matrix for statistical analysis, as detailed below.

2.3. Chemical and Physical Characterization

Soil texture was determined using 5 g of air-dried soil which was initially treated with a hydrochloric acid (HCl) solution to remove soil carbonate; then, 33% H_2O_2 was added to eliminate soil OM; finally, a hexametaphosphate solution was used to break up the soil particles. Reference concentrations were obtained according to NFX-31-107. Particle size distribution percentages were measured using laser diffraction (Malvern Mastersizer 2000, Malvern Panalytical, Malvern, UK) according to the manufacturer's protocol. The moisture content was recorded after drying the soil at $105^\circ C$ for 48 h. The INRA Laboratory of Soil Analysis performed cation exchange capacity (CEC) at Arras according to the extraction method detailed in NFX-31-130 using a cobalt hexamine trichloride solution. The pH of the soil's water was measured in a 1:2.5 soil-in-water suspension using a glass electrode (NF ISO 10390, 2005). Total organic carbon (TOC) and total nitrogen (TN) were analyzed following a dry combustion method using an elemental analyzer CHN Flash 2000 from Thermo Scientific (Milan, Italy). TOC was measured with a TOC Analyzer (Schimadzu-TOC-5050A, Shimadzu, Kyoto, Japan) using 0.20 mm milled dry soil sub-samples. Permanganate oxidizable carbon (i.e., active carbon, POXC) was extracted from 2.5 g of air-dried soil by quantifying the carbon oxidized by MnO_4 using a spectrophotometer (Varian Cary 50 Scan, Apeldoorn, The Netherlands) at 550 nm, as described by Culman et al. [35].

2.4. Measurement of Enzyme Activities

The activities of four enzymes were measured in this study. Three of the enzymes are involved in the C cycle (N-acetyl-glucosaminidase (NAG, EC: 3.2.1.30), β -glucosidase (GLU, EC: 3.2.1.21), and cellulase (CEL, EC: 3.2.1.4)), and the final enzyme is involved in the N cycle (Arylamidase (ARYL, EC: 3.5.1.5)). ARYL activity was measured according to Acosta-Martinez and Tabatabai [36], and CEL, GLU, and NAG activities were assessed using the detailed protocols presented in Trap et al. [37] (Table S1). NAG, GLU, CEL, and ARYL activities were examined using <2 mm field-moist samples at their optimal pH values. A unit of enzyme activity (U) was expressed as a nanomole of hydrolyzed substrate per minute and per gram of dry soil weight.

2.5. Measurement of Gross N Fluxes

Gross N fluxes were estimated using the ^{15}N pool dilution technique described by Barraclough [38]. A 70 g aliquot of the 108 fresh soil samples was thinly spread and sprayed

with a $^{15}\text{(NH}_4\text{)}_2\text{SO}_4$ solution ($15 \mu\text{g NH}_4^+\text{-N g soil}^{-1}$ enriched at 10 atoms % ^{15}N). Then, the soil was mixed, separated into two sub-samples, placed in a hermetic receptacle, and incubated at $20 \text{ }^\circ\text{C}$ for 2 h and 72 h. After each incubation period, the soil sub-samples were removed from the incubator and shaken with 100 mL of 1 M KCl for 30 min at 30 rpm. The supernatants were filtered using glass microfiber filters (Whatman GF/D, 47 mm, Whatman, Maidstone, UK) after centrifugation (3500 rpm, 15 min). Then, the KCl extracts were frozen ($-20 \text{ }^\circ\text{C}$) until ready for analysis. Furthermore, a micro-diffusion technique [39] was used to estimate the NH_4^+ and NO_3^- content, and the ^{15}N abundance in the extracts was determined using an elemental analyzer (Euro EA, EUROVECTOR, IT) coupled with mass spectrometry (DELTA V Advantage, Thermo-Electron, DE, Thermo Fisher Scientific, Waltham, MA, USA). Gross mineralization, nitrification, and soil N organization were calculated using dilution equations described by Barraclough [38].

2.6. Statistical Analysis

The 36 wheat plots were selected to analyze the effect of faba bean placement in crop rotations under different tillage systems in real farm settings. However, implementing these practices under real farm conditions implies altering several other practices, risking confounding effects during results interpretation. Therefore, a two-step statistical procedure was adopted. The first step involved comparing the means of the variables measured across the six treatments tested. The second step included implementing a path analysis to identify and rank direct and indirect links across all the agricultural practices (including those reported by the survey) and the soil microbial functions assessed in the 36 wheat plots.

Given the lack of normality and homogeneity in microbial and physicochemical variables (assessed according to the Wilk–Shapiro test and the Bartlett test, respectively), the variations in the six treatments were assessed using the nonparametric Tukey’s test and a permutation-based multiple analysis of variance (PERMANOVA) [40]. The data were analyzed using the tillage system and the faba bean rotation placement as factors, and the PERMANOVA analyses were performed using 999 permutations. Post hoc Tukey’s test results were displayed when the interaction was significant. In contrast, a post hoc Tukey’s test for the main effects was displayed when the interaction was insignificant, but a single effect of the faba bean placement or the tillage systems was recorded. The Adonis function in the “vegan” package was used in the statistical platform R (R Core Team, Vienna, Austria) to run this statistical test. Additionally, Spearman correlation coefficients were calculated to detect potential correlations between the soil’s physicochemical properties and biological variables. Then, the significant relationships between the variables were used to build the path analysis model.

In the second step, a generalized multilevel path analysis was performed to test the multivariate causal hypothesis on the mechanisms through which agricultural practices and soil physicochemical properties drive N fluxes and enzyme activities in soils. This method is valuable for establishing causal relationships from experimental data and detecting complex associations through indirect effects among system components (Shipley 2000). Finally, a conceptual model of the hypothesized relationships was developed using ecological theory and prior knowledge of the system (based on the first step analysis, i.e., PERMANOVA, as described above) to define the paths of interest (Figure S1).

The second step required continuous data accounting for all the agricultural practices to run the path analysis. Nevertheless, the farmer surveys generated categorical data, and the factor dimensionality related to the agricultural practice’s matrix was reduced using a preliminary multiple correspondence analysis (MCA) (Axis 1 = 29%, Axis 2 = 19%) (Figure S2) followed by the hierarchical clustering (method = Ward) (Figure S3). Thus, an MCA was applied to the data matrix built with the information provided by the farmer surveys. The MCA enables the transformation of categorical data to continuous data. Specifically, the coordinates of each field plot on Axes 1 and 2 of the MCA were extracted and used to represent a gradient of the intensity of agricultural practices for the generalized multilevel path analysis. The MCA results are displayed in Figure 2. Finally, the soil’s physicochemical properties were analyzed using a principal

component analysis (PCA) (Figure S4). The PCA coordinates of each field plot were extracted from Axes 1 and 2 and applied in the generalized path analysis as a gradient of changes in soil physicochemical properties.

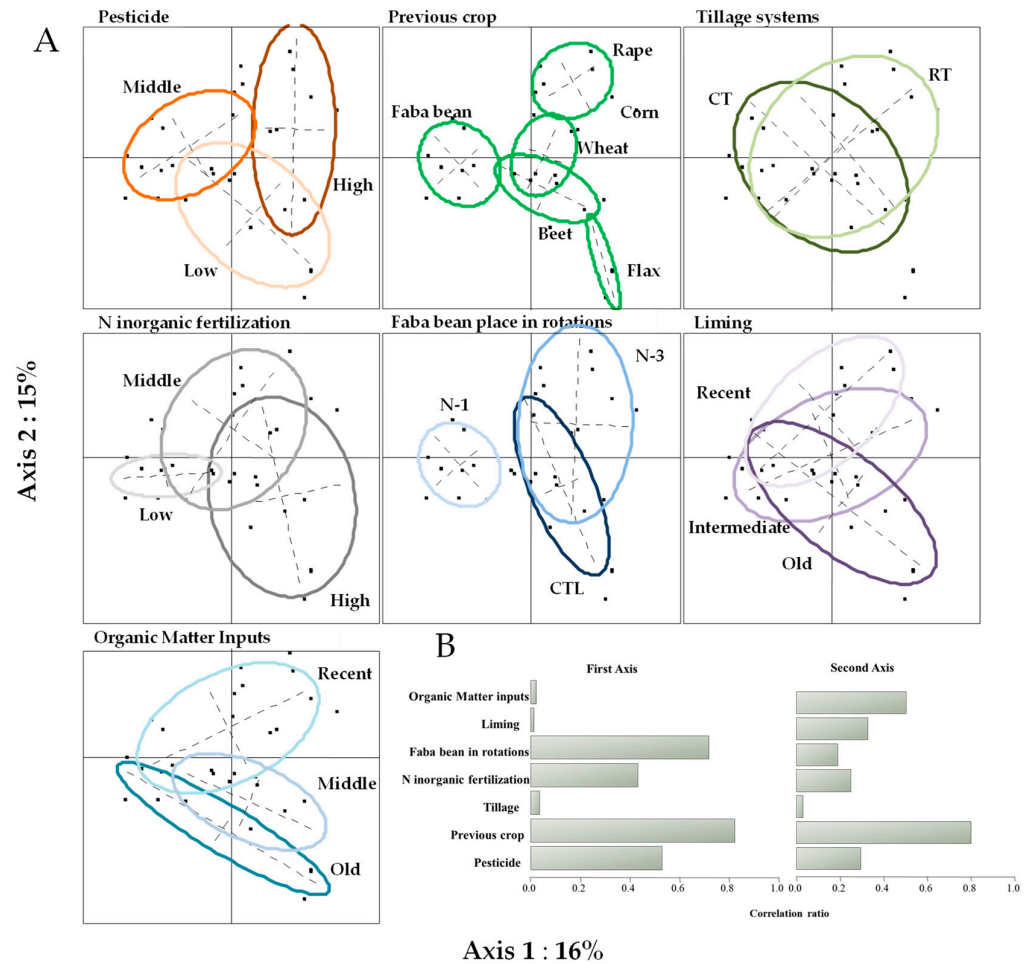


Figure 2. (A) Multiple correspondence analyses were performed on the data matrix of agricultural practices collected from farmers. The relative inertias supported by axes 1 and 2 were 16% and 15%, respectively. (B) The correlation ratio of the agricultural practices with the first two axes of a multiple correspondence analysis performed on the data matrix of categorical variables related to agricultural practices. Conventional tillage (CT); Reduced tillage (RT); Faba bean as the preceding crop (N-1); Faba bean sown three years before wheat (N-3); Rotation without faba bean or any leguminous crops during the preceding five years (CTL).

Furthermore, two main gradients of physical and chemical properties were identified: a gradient of increasing soil TOC, TN, and POXC values and a gradient of increasing pH and CEC values. The data were transformed using $f(x) = \log_{10}(x)$ where necessary [41]. In addition, GLU and ARYL values were integrated into these path analyses to understand the impact of soil enzyme activities on N gross fluxes.

Finally, a saturated global model was built, and the weakest correlations were sequentially removed until the criteria for the Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI), and Standardized Root Mean Square Residual (SRMR) were met (RMSEA < 0.05; Df \neq 0; Pchi2 > 0.05; CFI > 0.90, SRMR < 0.10). Then, a final model was selected according to the lowest value of Akaike's information criterion (AIC).

3. Results

3.1. Soil Physicochemical Properties

The inclusion and the placement of faba beans in the crop rotation combined with the tillage systems slightly impacted certain soil physicochemical properties (Table 1). Across the six treatments, the ranges of TOC and TN content were 9.9–15.5 g kg⁻¹ dry soil and 1.1–1.6 g kg⁻¹ dry soil, respectively. TOC and TN significantly responded to the tillage systems. However, they did not respond to the presence or the placement of the faba beans or the interaction between these factors (Table 1). Reduced tillage exhibited the highest values with 13.4 ± 4.3 g kg⁻¹ dry soil for TOC and 1.4 ± 0.4 g kg⁻¹ dry soil for TN. In addition, significant correlations were observed between TOC and the other soil physicochemical properties. For example, TOC was positively correlated to POXC ($p < 0.001$), TN content ($p < 0.001$), and soil CEC ($p < 0.05$) (Table 2).

Regarding POXC content, the mean value observed was approximately 512 ± 55 mg kg⁻¹ dry soil (Table 1). The tillage systems and their interactions with faba bean position significantly impacted the POXC content of the soil. The N-3_RT treatment exhibited the highest mean value (609 ± 56 mg kg⁻¹ dry soil), and the CTL_CT treatment displayed the lowest mean value (450 ± 48 mg kg⁻¹ dry soil). The other four treatments showed intermediate POXC values.

The tillage systems had a slight effect on the soil pH–water ($p < 0.1$), and there were no significant differences in CEC between the various treatments (Table 1). Moreover, soil pH–water and CEC were positively correlated (Table 2).

Table 1. Response of physicochemical properties, enzyme activities, and N gross fluxes to faba bean placement in crop rotations, tillage systems, and their interactions assessed using a PERMANOVA.

Faba Bean in Rotations	Tillage	pHwater		CEC (cmol ⁺ kg ⁻¹ DW _{soil})		Moisture (%)		Total N (g kg ⁻¹ DW _{soil})		TOC (g kg ⁻¹ DW _{soil})		POXC (mg kg ⁻¹ DW _{soil})		Gross N Mineralization (mg kg ⁻¹ Dry Soil Day)		Potential N Immobilization (mg kg ⁻¹ Dry Soil Day)		Potential N Nitrification (mg kg ⁻¹ dry Soil Day)		ARYL (U)		GLU (U)		CEL (U)		NAG (U)	
		f	p	f	p	f	p	f	p	f	p	f	p	f	p	f	p	f	p	f	p	f	p	f	p	f	p
Permanova																											
Tillage		4.82	§	0.15	ns	0.15	ns	3.61	*	7.57	**	4.56	*	1.44	ns	0.40	ns	2.14	ns	0.34	ns	0.13	ns	0.68	ns	0.70	ns
Faba bean in rotations		0.66	ns	1.10	ns	0.39	ns	0.60	ns	0.52	ns	1.72	ns	1.74	ns	0.38	ns	0.10	ns	3.20	§	0.20	ns	0.80	ns	0.66	ns
Tillage x faba bean in rotations		1.68	ns	1.51	ns	0.67	ns	1.99	ns	1.81	ns	3.87	*	0.80	ns	1.50	ns	2.20	ns	2.02	ns	2.39	ns	0.70	ns	0.01	ns
Tukey test interaction effect																											
CTL	RT	6.99 ± 0.62		10.06 ± 1.50		20.99 ± 2.08		1.54 ± 0.58		13.27 ± 4.31		525.87 ± 84.11^{ab}		2.35 ± 1.97		1.73 ± 0.91		2.85 ± 1.92		0.44 ± 0.26		5.48 ± 2.57		0.46 ± 0.28		1.53 ± 0.49	
	CT	7.05 ± 0.46		9.44 ± 1.91		19.38 ± 2.62		1.19 ± 0.14		10.48 ± 1.43		450.06 ± 47.86^b		2.06 ± 0.83		2.15 ± 1.08		3.48 ± 2.48		0.34 ± 0.13		4.13 ± 0.93		0.38 ± 0.07		1.44 ± 0.21	
N-1	RT	6.89 ± 0.66		9.94 ± 2.07		19.28 ± 2.95		1.25 ± 0.54		11.64 ± 5.37		483.90 ± 73.81^{ab}		1.52 ± 0.58		2.09 ± 1.04		2.23 ± 0.65		0.46 ± 0.12		4.59 ± 1.45		0.65 ± 0.45		1.70 ± 0.95	
	CT	7.47 ± 0.43		11.87 ± 2.78		19.34 ± 1.76		1.31 ± 0.39		11.09 ± 2.08		525.08 ± 116.12^{ab}		2.66 ± 1.80		1.29 ± 0.48		5.45 ± 2.76		0.73 ± 0.44		4.32 ± 0.73		0.44 ± 0.17		1.51 ± 0.28	
N-3	RT	6.47 ± 0.94		10.77 ± 1.58		19.55 ± 3.44		1.62 ± 0.27		15.52 ± 2.98		609.00 ± 55.96^a		2.66 ± 1.29		2.06 ± 0.51		3.01 ± 0.94		0.63 ± 0.21		4.15 ± 0.41		0.41 ± 0.07		1.41 ± 0.24	
	CT	7.31 ± 0.44		10.39 ± 1.31		20.16 ± 1.00		1.15 ± 0.08		9.92 ± 0.92		480.03 ± 61.49^{ab}		3.64 ± 2.02		1.88 ± 0.95		5.77 ± 3.18		0.60 ± 0.13		5.26 ± 1.09		0.46 ± 0.23		1.28 ± 0.69	
Tukey test Main effect																											
Faba bean in rotations	CTL	7.02 ± 0.52		9.75 ± 1.67		20.19 ± 2.41		1.37 ± 0.44		11.87 ± 3.39		487.97 ± 76.32		2.20 ± 1.45		1.94 ± 0.98		4.23 ± 4.16		0.39 ± 0.20^B		4.80 ± 1.97		0.42 ± 0.20		1.46 ± 0.37	
	N-1	7.18 ± 0.61		10.91 ± 2.55		19.32 ± 2.32		1.28 ± 0.45		11.37 ± 3.89		504.49 ± 95.23		2.09 ± 1.40		1.69 ± 0.87		3.84 ± 2.54		0.60 ± 0.34^{AB}		4.46 ± 1.10		0.53 ± 0.28		1.61 ± 0.68	
	N-3	6.89 ± 0.83		10.58 ± 1.40		19.86 ± 2.44		1.38 ± 0.31		12.72 ± 3.60		544.51 ± 87.62		3.14 ± 1.69		1.96 ± 0.73		4.39 ± 2.66		0.61 ± 0.17^A		4.71 ± 0.97		0.43 ± 0.16		1.35 ± 0.50	
Tillage	CT	7.26 ± 0.52^A		10.57 ± 2.22		19.63 ± 1.84		1.22 ± 0.24^B		10.49 ± 1.54^B		485.06 ± 82.21		2.79 ± 1.67		1.77 ± 0.90		4.90 ± 2.85		0.56 ± 0.31		4.57 ± 1.01		0.43 ± 0.16		1.40 ± 0.43	
	RT	6.81 ± 0.72^B		10.26 ± 1.67		19.95 ± 2.81		1.47 ± 0.48^A		13.48 ± 4.38^A		539.59 ± 86.40		2.18 ± 1.41		1.96 ± 0.81		3.41 ± 3.28		0.51 ± 0.21		4.74 ± 1.71		0.49 ± 0.27		1.55 ± 0.61	

Data presented: means ± SD. Lowercase letters (a,b) refer to significant interaction effects of faba bean placement in rotations and tillage systems between all modalities following the PERMANOVA and post hoc Tukey's test. Capital letters (A,B) refer to the significant main effect of isolated faba bean placement in rotations or tillage systems in isolation, i.e., significant differences between tillage treatments or faba bean placement in rotations when the main effect was detected. Bold characters indicates a significant effect of studied factors; *f* refers to degrees of freedom; *p* refers to the significance of the tested effect (*p*-value) accepted until 0.1 (§: *p* < 0.1; *: *p* < 0.05; **: *p* < 0.01; ns: *p* > 0.05). Conventional tillage (CT); Reduced tillage (RT); Faba bean as the previous crop (N-1); Faba bean sown three years before wheat (N-3); Rotation without faba bean or any leguminous crops during the preceding five years (CTL). Nanomole of hydrolyzed substrate per min and per gram of dry soil weight (U).

Table 2. Spearman’s correlations between physicochemical properties and enzymes activity.

	TOC	POXC	TN	pH _{water}	CEC	GLU	CEL	NAG	ARYL	Gross N Mineralization	Potential N Immobilization	Potential N Nitrification
TOC												
POXC	0.70	***										
TN	0.96	***	0.74	***								
pH Water	−0.09	ns	0.19	ns	−0.06	ns						
CEC	0.38	*	0.66	***	0.47	**	0.67	***				
GLU	0.24	ns	0.35	*	0.33	*	0.54	***	0.54	***		
CEL	0.17	ns	0.35	*	0.20	ns	0.18	ns	0.36	*	0.38	*
NAG	0.52	***	0.32	*	0.48	**	0.27	ns	0.39	*	0.46	**
ARYL	0.18	ns	0.58	***	0.27	ns	0.39	*	0.56	***	0.18	ns
Gross N Mineralization	0.29	ns	0.47	**	0.40	*	0.42	**	0.50	**	0.33	*
Potential N Immobilization	0.23	ns	0.09	ns	0.25	ns	−0.19	ns	−0.09	ns	−0.25	ns
Potential N Nitrification	0.01	ns	0.36	*	0.14	ns	0.63	***	0.61	***	0.30	ns

Significant correlations are in bold ($r > 0.31$, $n = 36$), and significantly adjusted p -values are indicated as *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ns: $p > 0.05$.

3.2. Soil Enzyme Activities

This study's results showed that NAG, GLU, and CEL activities did not differ across the six treatments. Only ARYL activity was slightly modified ($p < 0.1$) by the faba bean crop's presence and placement; the highest value was observed for N-3, and the lowest value was observed for CTL. GLU and CEL activities were positively correlated and related to POXC and CEC, respectively (Table 2). NAG was positively correlated with GLU, TOC and TN content, and CEC. ARYL activity was positively correlated with POXC content, soil pH–water, and CEC.

3.3. Nitrogen Fluxes

Gross N mineralization, potential nitrification, and gross immobilization were not significantly affected by the presence or placement of the faba bean crops, tillage systems, or the interaction of these two factors (Table 1). Gross N mineralization was positively correlated with POXC, TN, soil pH–water, GLU, and ARYL activities. Potential nitrification was significantly correlated with ARYL activity, gross N mineralization, soil pH–water, and POXC content (Table 2).

3.4. Direct and Indirect Effects of Agricultural Practices on N Fluxes

3.4.1. Farmer Survey Data Analysis

The agricultural surveys generated categorical variables that were encoded and studied using MCA. This statistical method was selected to provide continuous variables (coordinates of each sampled plot on axes 1 and 2 of the MCA), which were used in the generalized multilevel path analysis (detailed in the statistical analysis section). The first axis (16% of relative inertia) was oriented using four studied practices: the placement of faba bean in the crop rotations, pesticide treatment, inorganic N fertilization, and wheat as the preceding crop (Figure 2A). The results differentiated between the N-1, N-3, and CTL treatments. This first axis also distinguished plots with low or medium pesticide inputs associated with faba beans as the preceding crop and high pesticide inputs associated with other previous crops. Moreover, inorganic N fertilization showed a similar gradient application dose of pesticides along the first axis. Regarding the effect of being the preceding crop to wheat, faba bean was distinct from other crops. The MCA second axis (15% relative inertia) was significantly correlated with the elapsed time since the last OM modification and liming treatment. Axis 2 distinguished modalities of LF practices performed to maintain soil fertility for an intermediate term, as liming treatments were applied every six to seven years, and OM was applied every three to four years. In contrast, the modalities of HF practices performed to ensure the short-term soil fertility and the productivity of the actual wheat crop (e.g., pesticide and inorganic fertilization) mainly correlated with Axis 1 (Figure 2B).

3.4.2. The Effects of Farming Practices

LF–HI practices, i.e., OM inputs and liming treatments (MCA–Axis 2, Figure 2), directly and positively impacted gross N mineralization in the soil (Figure 3A). The recent lime application and OM inputs increased the gross N mineralization in the soil. These practices also increased the soil pH–water and CEC and raised GLU and ARYL activities, mediating gross N mineralization. Conversely, HF–LI practices, i.e., pesticide usage, high inorganic N fertilization, and the absence of legume in the crop rotation (MCA–Axis 1, Figure 2) decreased soil pH and CEC, the activity of the two enzymes and, consequently, the gross N mineralization (Figure 3A). This highlights the antagonistic effect of annual versus occasional management practices, i.e., HF–LI versus LF–HI practices. Regardless of the agricultural practice effects, TOC, POXC, and TN content directly and positively impacted GLU and ARYL activities, increasing gross N mineralization.

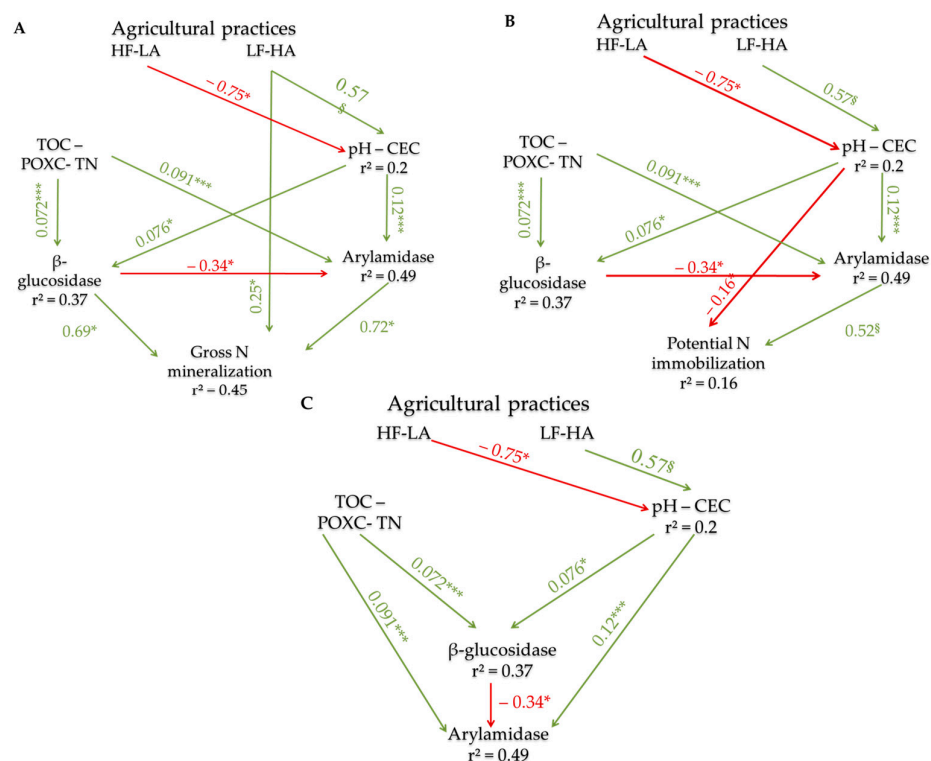


Figure 3. Generalized multilevel path models assessing the response of gross N mineralization (A), potential N immobilization (B), and potential nitrification with arylamidase activity as a proxy (C) were mediated by agricultural practices, soil physicochemical properties, and soil enzyme activities according to the general conceptual model presented in Figure S1. All the models were well supported by the data. The selected models did not imply any independence claims that were statistically significant at $\alpha = 0.05$. The alternative candidate models have a significantly worse fit for the data ($\Delta AIC > 2$). These selected models provided the best fit for the data and were well supported (model A: $\chi^2 = 47.59$, $df = 8$, $p = 0.967$; model B: $\chi^2 = 49.68$, $df = 9$, $p = 0.787$; model C: $\chi^2 = 44.94$, $df = 5$, $p = 0.868$). The path coefficients are range-standardized (partial) regression coefficients. The arrow widths are proportional to the standardized path coefficients. Each endogenous variable's marginal coefficients of determination (r^2) are reported. Only significant correlated errors are reported for clarity. Each component model of the global path model was examined for normal distribution and homogeneity of variances. *, **, ***, and § indicate that the F-value differs significantly from 0 at $p = 0.05, 0.01, 0.001$, and 0.10 , respectively. Cation exchange capacity (CEC), pH measured in a 1:2.5 soil in water suspension using a glass electrode, total organic carbon (TOC), active carbon (POXC), and total nitrogen (TN). Based on the ordination scores of the two first axes of the MCA analysis conducted on data pertaining to agricultural practices, High-Frequency–Low-Intensity (HF-LI) and Low-Frequency–High-Intensity (LF-HI) practices were identified. The data were obtained from farmers (Figure 2; MCA axes 1 and 2 explained 31% of the total dataset variation).

The conservative practices did not directly affect the gross N immobilization in soil. However, indirect regulating factors were identified (Figure 3B). HF-LI practices (high-pesticide treatments and N fertilization) and the absence of leguminous plants in the crop rotation negatively affected pH–water and CEC and significantly increased the gross N immobilization. This was also connected to OM inputs and liming through a marginal relationship with ARYL activity, which significantly amplified following an increase in soil pH–water and CEC (Figure 3B).

The effect of TOC, POXC, and TN concentration on enzyme activities for gross N mineralization followed a similar pattern, with ARYL slightly affecting N immobilization. Additionally, variations in pH–water and CEC values impacted GLU activity; however, no correlations with this flux were observed (Figure 3B).

Since a significant correlation was previously discovered with ARYL activity (Table 2), ARYL was used as a proxy for potential nitrification in the generalized path analysis to prevent co-linearity in the Structural Equation Modeling (SEM) (Figure 3C). Agricultural practices induced significant but indirect variations in ARYL through changes in the soil's physicochemical properties. A negative relationship was observed between GLU and ARYL activities for the three tested generalized models.

In this study, the two defined practice types antagonistically affected the soil's physicochemical properties, soil enzyme activities, and gross N fluxes. While HF-LI techniques negatively impacted gross N mineralization and nitrification and increased soil N supply, LF-HI procedures positively influenced the soil pH and CEC and increased the soil N availability for wheat.

4. Discussion

4.1. Disentangling the Effects of Conservative Practices from the Effects of Crop Itineraries

During the last several decades, conventional agricultural practices (i.e., monoculture, frequent conventional tillage, high chemical inputs) have affected soil's biological, chemical, and physical qualities [42–44]. The consensus on the effects of conventional practices on soils has promoted new forms of agriculture to mitigate the risks of decreased crop productivity, a loss in soil fertility, and environmental impacts. Among the emerging practices, the introduction of leguminous plants within crop rotations and tillage reduction have appeared as the most suitable methods to maintain soil fertility and preserve microbial communities [44–51]. Nevertheless, this study based on farm plots demonstrated that the introduction of leguminous plants, tillage reduction, or a combination of these conservative practices did not significantly affect soil microbial functions. These results can be connected to how these conservative practices were implemented. Implementing these practices generates changes in how farmers design crop itineraries, with a potential cascading effect on the soils' physicochemical and biological properties. Thus, disentangling the effects of agricultural practices and crop itineraries from the impacts of conservative practices on soil enzyme activity and gross N fluxes is essential to modify the implementation of conservative practices. The primary findings of this study, which explain the minimal direct impact of conservative practices on enzyme activity, are the decoupling between the impacts of conservative practices and crop itinerary effects or the agricultural practices connected to using conservative methods. The studied conservative practices (reduced tillage and faba bean introduction) directly enhanced soil OM content through TOC, POXC, and TN. These three soil characteristics, which constitute a part of the trophic resources for soil biota, positively correlated with the levels of ARYL and GLU activities. In contrast, enzyme activity and the N soil cycle were strongly regulated by the effects of the agricultural practices (e.g., the use of pesticides, N inorganic fertilization, liming, and crop rotation) on soil physicochemical properties (pH and CEC), i.e., the physical environment of the soil biota.

4.2. The Effects of the Conservative Practices

This study's results indicated that the impact of the studied conservative practices (faba bean introduction and reduced tillage) on soil microbial activity and N gross fluxes were mainly mediated by changes in the soil C and N supplies. Several studies conducted in different agro-pedoclimatic contexts reported increased soil C and N supplies (as well as their availability for subsequent crops) when these conservative practices are applied [19,47,52–54]. Even though enzymatic activities were only slightly affected in this study, there is a trend toward an increase in these activities in treatments such as faba bean and reduced tillage. This observation is consistent with those reported in the literature [27,55]. Regarding the impact of this increase on ARYL activity, several authors have demonstrated that heterotrophic organisms use TOC as energy sources and TN as substrates to release more assimilable N to cultivated wheat [56–58]. In addition, ARYL activity was significantly and positively driven by soil pH and CEC. Although CEC did not exhibit significant changes directly associated with these practices, pH was significantly and directly impacted by reduced tillage. Variations in pH lead to significant changes

in microbial dynamics and nitrification rates. Indeed, soil pH is a key factor driving the niche partitioning of AOB and AOA communities in soils. According to a recent study, AOA grew preferentially in acidic soil with low nutrient availability, while AOB grew preferentially in soils with relatively high pH and NH_3 concentrations [59,60]. Karmarkar and Tabatabai [61] showed that certain organic acids inhibit or activate soil nitrification. Therefore, increasing soil pH may induce significant beneficial changes in the habitat of soil microorganisms and the associated enzyme systems, which are reflected in N transformations [36,62]. As previously reported by Ajwa and Tabatabai [63], factors related to soil fertility, such as TOC, TN, and POXC, positively influence GLU activity. Furthermore, a recent study found that increasing microbial biomass, POXC, and GLU enzyme activity through a varied agricultural system managed with NT could improve soil health [64].

For the direct link observed between ARYL activity and gross N fluxes, this study's results support the observations of several authors who highlighted the participation of ARYL in the soil N cycle [36,65–67]. ARYL catalyzes the hydrolysis of an N-terminal amino acid from peptides, amides, or arylamides [36]. The released amino acids are used as substrates for other amidohydrolase activities involved in N mineralization and nitrification [56]. The second path illustrated the effect of GLU activity on gross N mineralization and nitrification. This outcome is expected given that soil organic content is the most important soil property influencing the enzymes responsible for the degradation of carbohydrates [68,69]. Furthermore, GLU activity provides essential C skeletons and energy sources for the growth of heterotrophic soil microorganisms, initiates processes leading to the mineralization and stabilization of N [66], and may explain the observed negative correlation between GLU and ARYL activities. This antagonist link may be due to the sequential release of these two enzymes into the soil.

4.3. The Direct and Indirect Effects of Crop Itineraries on Gross N Fluxes

The lack of a significant direct impact of the introduction of leguminous plants or tillage reduction raised various questions about implementing conservative agricultural methods in this study. The agricultural survey revealed that tillage reduction was associated with more pesticide use, primarily herbicides, which ultimately challenged its use as a conservative practice [70]. According to Preissel et al. [71], the advantages of introducing legumes as the crop that precedes wheat were highlighted in this study, particularly when conventional tillage was used. Notably, introducing legumes within crop rotation seems to be associated with less pesticide usage and inorganic N fertilization, which enhances its suitability as a sustainable agricultural practice [50,72,73].

Pesticide treatment, inorganic N fertilization, and the previous crop (primarily the presence of faba bean) were considered HF practices and were applied annually in the farm plots. The negative effects of these practices on soil pH and CEC observed in this study were also reported by Iturri et al. [74]. High inorganic N fertilization accelerates soil acidification [75–77], as applying ammoniacal N fertilizer decreases the CEC, base saturation, and amount of exchangeable Ca^{2+} and Mg^{2+} , resulting in low pH values [78]. In addition, the significant variation in soil pH may be due to the incorporation of faba bean residues into the soil [79]. Butterly et al. [80] reported that the chemical properties of the legume residues may influence soil pH due to the crops' alkalinity and N content. Legumes can initially cause soil acidification because they rely on N_2 fixation and take up more cations than anions, resulting in a net export of protons [81].

Nevertheless, adding residues can counteract this effect by decomposing organic anions and N. The decarboxylation of organic anions may be a major cause of the increases in soil pH [82,83]. Moreover, the absence of leguminous plants as a preceding crop, pesticide treatment, and inorganic N fertilization positively affected potential N immobilization through the pH and CEC. According to several authors, the mineralized N-NH_4^+ from high-quality inputs was immobilized by the microbial communities or absorbed by plants, thereby out-competing its absorption in the soil [84–86].

This study revealed that HF–LI practices have no direct impact on the biological functions of soil. There are two possible reasons for this. The first reason is that the high frequency of these practices selects specific well-adapted microbial communities, thereby limiting the changes in N fluxes and enzyme activity. The second reason may be that the low intensity of these practices cannot significantly alter the optimal soil property levels for crop productivity (Figure 4). Conversely, liming treatment and organic matter inputs are ancient practices that occur at low frequency to improve the soil's organic state and acidity.

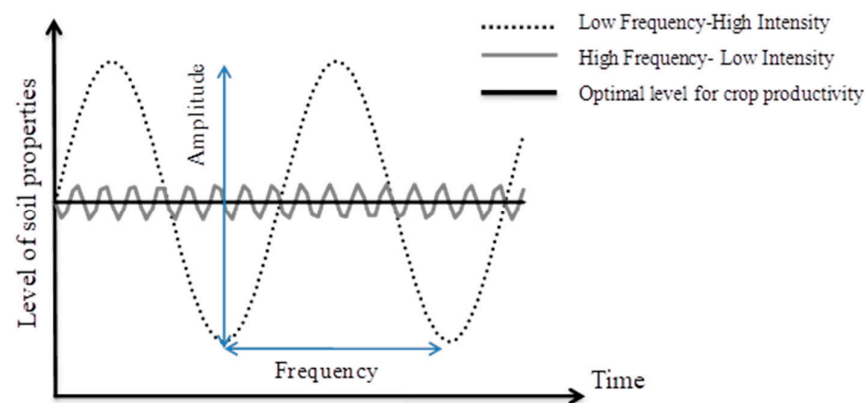


Figure 4. Schematic variability of the optimal level for crop productivity of given soil factors such as pH, cation exchange capacity (CEC), and total organic carbon (TOC) in response to low-frequency-high-intensity practices (organic matter input + liming) versus high-frequency-low-intensity practices (pesticide treatments + inorganic N fertilization + no leguminous plant as a previous crop).

The present study applied significant amounts of liming every six to seven years and organic matter every three to four years (20–30 tonnes for each treatment). Soil pH and CEC were positively influenced by LF–HI practices and negatively influenced by HF–LI practices. Consistent with the present results, Ekenler and Tabatabai [87] revealed that liming application significantly affected soil pH, influencing ARYL activity. These authors concluded that soil liming and tillage systems affect N cycle enzymes and N availability in soil. In this study, OM inputs increased soil pH and CEC, which is consistent with the results of several authors who demonstrated significant pH increases in acid soils under various kinds of OM inputs [88,89]. Agegnehu et al. [90] reported that adding OM increased soil pH and CEC by approximately 10% and 22%, respectively. Thus, LF and HF inputs enabled the soil properties to deviate significantly from their optimal crop productivity levels while giving the soil biota enough time to adapt to this high amplitude. The LF–HI practices, particularly those related to the N cycle, directly and indirectly influenced soil function. Moreover, the high variation amplitude may enable these molecules to cross the ecological thresholds for soil microflora, consequently impacting soil biological functions. At the same time, the low application frequency provides time for soil biota to establish a new equilibrium with the soil characteristics.

5. Conclusions

This multifactorial or multisite study aimed to evaluate the combined effect of two conservative practices (legume introduction in the rotation and reduced tillage) and demonstrated that faba bean crop introduction and soil tillage reduction indirectly impact soil functions by enhancing soil OM. LF–HI and HF–LI practices also impact soil enzyme activity and gross N fluxes by changing soil pH and CEC. These agricultural practices indirectly and simultaneously impact soil functions by modifying the microbial habitat and altering trophic resources.

Therefore, this study has demonstrated the need to consider the soil tillage methods based on the legume crop placement in the rotation. While stubble plowing might be performed before the sowing of a cereal of the legume crop three years earlier, plowing could be recommended

when implementing the legume (faba bean) as the preceding crop in the rotation. However, implementing these innovative practices cannot be established without considering the resulting modifications to the farm's technical itineraries, such as changing the crops in the rotation, residue management, the use of pesticides, and tillage mode.

Finally, the findings indicate that microbial communities participate in the C and N biogeochemical cycles through enzymatic activities and soil nitrogen fluxes. These results are important for revising existing agricultural methods and proposing new practices that may alter soil microbial functions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture13061223/s1>, Table S1. Enzymes and substrates used for enzyme activities assays; Figure S1. General conceptual model of hypothesized direct and indirect relationships between gross nitrogen flux and agricultural practices through soil physicochemical properties and soil enzyme activities; Figure S2. Preliminary multiple correspondence analysis of organic matter inputs during the last four years preceding sampling date. Data were collected from farmers. The first axis was well correlated to organic matter inputs of the 2013–2014 and 2011–2012 crop years. The second axis was correlated to organic matter inputs of the 2010–2011 crop year; Figure S3. Hierarchical clustering of the thirty-six sites based on their organic matter inputs (modalities presented in Figure S2) during the last four years preceding the sampling date. Data were collected from farmers; Figure S4. Correlation circle of soil physicochemical properties of agricultural sites chosen for this study. CEC: cation exchange capacity, pH: pH measured in a 1:2.5 soil in water suspension using a glass electrode, TOC: total organic carbon, POXC: active carbon, TN: total nitrogen.

Author Contributions: A.A. experiment execution, data analysis, presentation of results and original draft manuscript preparation; W.R.-A. contribution on the original draft manuscript preparation; review, and editing; S.R. data acquisition, data analysis and review; C.B. data acquisition; M.A. and I.T.-G. identification of the research topic, resources, review, editing and supervision. All authors have read and agreed to the published version of the manuscript.

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