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Article

Introducing Grasslands into Crop Rotations, a Way to Restore Microbiodiversity and Soil Functions

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Abstract: The aims of this study were to investigate (i) the influence of aging grassland in the recovery of soil state by the comparison of permanent grassland, two restored grasslands, two temporary grasslands, and a continuous crop in the same pedoclimatic conditions, (ii) the extent and the persistence of the potential changes following a grassland/or cropland phase. We hypothesized that the level of microbial communities and enzyme activities could achieve a profile close to that of permanent grassland after the introduction of grassland for a few years in crop rotations. Soil biophysicochemical properties were studied. Our results indicated that the abundance of microbial communities and enzyme activities were positively correlated to soil C and N contents and negatively correlated to soil pH. The changes in microbial abundance level were strongly linked to the changes in functional level when grasslands are introduced into crop rotations. We also showed that a continuous crop regime had a stronger legacy on the soil biota and functions. By contrast, the legacy of a grassland regime changed quickly when the grassland regime is interrupted by recent culture events. A grassland regime enabled the restoration of functions after more than five cumulative years in the grassland regime.

Keywords: enzyme activities; microbial communities restoration; crop rotation; grassland; function level; grassland/cropland legacy

1. Introduction

During the last decade, nutrients deficiency has been considered as the main cause of poor productivity and crop failure. A study of the current trends in agronomic practices has suggested that the soil nutrients deficiency is further aggravated by continued use of high-yielding crop varieties, intensive cropping patterns, and the use of relatively poor fertilizers [1]. Soils are a non-renewable resource at a human time scale, and some adverse effects of degradative processes on soil quality are irreversible [2]. Thus, soils need to be recognized and valued for their productive capacities as well as their contribution to food security and the maintenance of key ecosystem services driven by soil biodiversity [3]. Among soil biodiversity, microorganisms are particularly abundant, given that just one

gram of soil contains as many as 10^{10}–10^{11} bacteria, including 6000–bacterial species and up to 200 m of fungal hyphae [4]. They represent the unseen majority and comprise a large portion of the genetic diversity of soil [4,5]. Bacteria and fungi communities play key roles in soil ecosystems because they constitute a major part of the biomass contributing to various biogeochemical cycles [6–8]. These soil microorganisms play a key role in the decomposition and mineralization of organic matter (OM) [9,10]. Indeed, they control and produce most of the enzymes involved in the breakdown of OM and influence soil carbon (C), nitrogen (N), phosphorus (P), and sulfur (S) cycling. The abundance and the diversity of soil microbial communities are also important in plant health, soil fertility, and wider ecosystem functioning [11–15].

Agricultural practices can affect directly and indirectly soil microbial communities and their functions [16,17]. Amongst these impacts, reduction in microbial diversity is often viewed as major threats to the future [18,19]. Today, agricultural practices should tend to sustainable agriculture to guarantee or improve crop yields and soil quality, thus limiting greenhouse gases emissions [20]. Among sustainable practices, farmers are reconsidering an old method of adding grasslands within arable crop rotations. This type of farming may be a way to reconcile agricultural productivity with concerns about environmental quality [21,22] by integrating crop-livestock farming systems [23]. Alternation of grassland and cropland has been indicated as more efficient than permanent cropping systems in terms of organic C sequestration, nutrient availability, and increasing biodiversity of the agrosystems [24–26]. Grasslands have been described as resilient soils, although soil cultivation results in well-documented losses of OM and soil structure [27–29].

The establishment of grasslands on previously cultivated soils can result in the recovery of some key soil properties and might act positively on soil microbial communities [30–34]. The analysis of soil microbial communities in cultivated soil and grassland found higher bacterial diversity in grassland as compared to highly disturbed cultivated soils [34–36]. McKinley et al. [37], Bach et al. [38] also documented that stress Phospholipid Fatty Acids (PLFAs) ratios indicators (saturated: monounsaturated and iso: anteiso) were highest in cultivated soils and decreased in restored grasslands. Authors explained that the reductions in these ratios during soil restoration by the alleviation of nutrient stress as grassland established on formerly cultivated soils, likely as a result of increased C substrate availability in the soil. The analysis of microbial communities showed differences in microbial abundance and diversity in response to time since grassland implementation [34,36,39,40]. Plassart et al. [41] also documented that a strong relationship was found between fungal genetic diversity and the aging of grasslands. Furthermore, various studies have reported changes in the soil functions on sites with an arable cropping history after which a grassland was established [32,41–43]. In fact, adding grasslands within arable crop rotations increase C mineralization, potential catabolic activity, microbial respiration, and soil N transformations (i.e., net N mineralization rates and net nitrification rates), indicating a strong activity of microorganisms [30,41–43].

The intimate connection between soil state, microbial communities, and enzyme activities emphasizes the importance of considering the role of microbial communities during soil restoration by the introduction of grasslands into crop rotations. Therefore, the objectives of this study were to investigate (i) the influence of aging grassland in the recovery of soil state by the comparison of different grassland regimes between permanent grassland and conventional crop in the same pedoclimatic context, (ii) the extent and the persistence of the potential changes of microbial communities and enzyme activities following a grassland/or cropland phase. We hypothesized that microbial communities could achieve an abundance level and functional state close to that of permanent grassland after the introduction of grassland for a few years into crop rotations. To achieve our objective, we analyzed (i) physicochemical properties, (ii) microbial community (indicators of total, bacterial and fungal abundance), and (iii) soil functions (14 enzyme activities involved in C, N, P, and S cycles) during the adding of grassland into crop rotations.

2. Materials and Methods

2.1. Soil Samples

The experimental site is located in northwestern France, in the Normandy area (at Yvetot, 35 km northwest from Rouen), under temperate oceanic climate (+10 °C mean annual temperature and 850 mm annual precipitation). This site was used in several previous studies and is considered as a reference site in the Normandy area [41,44–47] as well as in France [48–50].

Six plots with different land use and grassland regimes were studied. Their land uses, cropping history, and cumulative years under grasslands are presented, respectively, in Tables 1 and 2 for the 20 years before the sampling date in 2010. The permanent grassland (PG) is considered as a reference plot and has not been changed during at least the last 20 years. In contrast, continuous crop (CC) has a history of long-term cropping (wheat, maize, flax, or beet) with intensive management (plowing/fertilization). Two restored grasslands (RGI and RGII) characterized by grassland establishment after a cropping period of at least 8 years. These 2 restored grasslands differed significantly in the number of cumulative years under grassland. RGII cumulated 11 years of grassland, while RGI cumulated 5 years under grassland. We can also note that these 2 plots were replanted at the same time and have been managed in the same way since 2008. Temporary grasslands (TG and TC) were characterized by an alternating grassland period of 4 to 8 years and a corn/wheat rotation. In 2010, TC was sown with wheat after a previous corn crop, which is often used in the region as the head of rotation. The 2 temporary grasslands were managed differently in 2010. The plot TG is the oldest grassland after plot PG. The 6 plots were developed on the same native Luvisol, silty loam soil, within similar pedological and climatic contexts (Table 2).

Table 1. Description of the studied plots.

Plots	Abbreviations	Clay (g/kg)	Silt (g/kg)	Sand (g/kg)	Agricultural Practices	Land Use at the Sampling Time	Plots Area (ha)	Cumulative Years under Grassland since 20 Years
Permanent grassland	PG [1]	163	633	204	Permanent grassland no tillage	Grassland	3	20 years
Restored grasslands	RGI [2]	141	665	194	Restored grassland	Grassland	2	5.5 years
	RGII [3]	126	665	210	Restored grassland	Grassland	3	11 years
Temporary grasslands	TC [4]	141	656	203	Temporary grassland, switched to crop in 2010	Wheat crop	3	14 years
	TG [5]	147	651	202	Temporary grassland	Grassland	3	14 years
Continuous crop	CC [6]	133	671	196	Continuous crop and fertilization	Wheat crop	4	0 year

[1] Permanent Grassland (PG): 20 years of pasture, permanent cover and no tillage. [2] Continuous Crop (CC): permanent arable cropping. [3] Restored Grassland I (RGI): cumulate 5.5 years under grassland over a period of 20 years. [4] Restored Grassland II (RGII): cumulate 11 years under grassland over a period of 20 years. [5] Temporary Grassland (TG): cumulate 14 years under grassland over a period of 20 years. [6] Temporary crop (TC): this treatment is comparable to TG, but it switched to culture in the year of sampling.

Table 2. Description of the cropping history of the plots since 1991.

Plots	Permanent Grassland	Restored Grasslands		Temporary Grasslands		Continuous Crop
Years	**PG**	**RGI**	**RGII**	**TC**	**TG**	**CC**
2010	grassland	grassland	grassland	*Triticum aestivum*	grassland	*Triticum aestivum*
2009	grassland	grassland	grassland	*Zea mays*	grassland	*Zea mays*
2008	grassland	*Triticum aestivum*	*Triticum aestivum*	grassland	grassland	*Triticum aestivum*
2007	grassland	*Zea mays*	*Vicia faba*	grassland	grassland	*Zea mays*
2006	grassland	*Triticum aestivum*	*Linum usitatissimum*	grassland	grassland	*Triticum aestivum*
2005	grassland	*Zea mays*	*Triticum aestivum*	grassland	*Triticum aestivum*	*Linum usitatissimum*
2004	grassland	*Triticum aestivum*	*Zea mays*	grassland	*Zea mays*	*Triticum aestivum*
2003	grassland	*Zea mays*	grassland	*Triticum aestivum*	grassland	*Beta vulgaris*
2002	grassland	grassland	grassland	*Zea mays*	grassland	*Triticum aestivum*
2001	grassland	grassland	grassland	grassland	grassland	*Zea mays*
2000	grassland	grassland	grassland	grassland	*Triticum aestivum*	*Triticum aestivum*

Table 2. *Cont.*

Plots	Permanent Grassland	Restored Grasslands		Temporary Grasslands		Continuous Crop
Years	**PG**	**RGI**	**RGII**	**TC**	**TG**	**CC**
1999	grassland	*Pisum sativum*	grassland	grassland	*Zea mays*	*Beta vulgaris*
1998	grassland	*Triticum aestivum*	grassland	grassland	grassland	*Triticum aestivum*
1997	grassland	*Zea mays*	grassland	grassland	grassland	*Linum usitatissimum*
1996	grassland	*Triticum aestivum*	grassland	grassland	grassland	-
1995	grassland	*Zea mays*	grassland	grassland	grassland	-
1994	grassland	*Linum usitatissimum*	*Triticum aestivum*	grassland	grassland	-
1993	grassland	*Zea mays*	*Zea mays*	*Triticum aestivum*	grassland	-
1992	grassland	*Triticum aestivum*	*Triticum aestivum*	*Zea mays*	*Triticum aestivum*	-
1991	grassland	*Zea mays*	*Pisum sativum*	grassland	*Zea mays*	-

The sampling protocol followed the recommendations of the RMQS BioDiv national program [51], as described in Peres et al. [48] and Trap et al. [49]. For each plot, we designed a square with 10-m sides (10 m × 10 m) subdivided into 4 equal squares (5 m × 5 m) [49]. In March 2010, 12 soil cores were randomly collected in each sampling area, using an 8 cm diameter auger on the first 15 cm soil depth to form one composite sample. Four composite soil samples were, therefore, available for each plot (Figure S1). The soil samples were sieved at 2 mm and stored at 4 °C prior to analysis. Biological analyses were performed on the freshly sieved soils within 24 h after sampling, except for PLFAs and ergosterol. Indeed, for these 2 parameters, fresh soil samples were homogenized, sieved at 2 mm, and frozen at −80 °C until analyses. The main physicochemical characteristics (i.e., total carbon, total nitrogen, pH water, CEC, and P_2O_5) were measured on each individual air-dried soil sample, according to the normalized methods by the Soil Analysis Laboratory (LAS, INRA, Arras, France).

2.2. Microbial Biomass C Measurement

Soil total microbial biomass C (MBC) was determined using the chloroform fumigation extraction method according to Wu et al. [52] and Jenkinson et al. [53]. Briefly, soluble organic carbon was extracted with a 0.05 N K_2SO_4 solution from chloroform-fumigated (16 h) and non-fumigated soil samples (30 g, with 4 replicates). After centrifugation (10 min 3000× *g*), the soluble organic carbon in the extracts was measured by persulfate-UV oxidation with a Dohrman DC-80 Total Organic Carbon Analyzer (Rosemount Analytical, Santa Clara, California, USA). MBC was calculated as a difference in the C content in the fumigated and non-fumigated samples (EC) using the k_{EC} coefficient (MBC = EC/k_{EC}). k_{EC} = 0.45 was used to calculate MBC.

2.3. Phospholipid Fatty Acids (PLFAs) Analysis

After freeze-drying, PLFA were extracted using a modified method of Bligh and Dyer [54–56]. Fatty acids were extracted from 2 g of soil by a single-phase mixture of chloroform-methanol-pH 4 citrate buffer (1:2:0.8, *v*/*v*/*v*) shaken at 300 rpm for 1.5 h and centrifuged 15 min at 1500 rpm. The supernatant was retained, and the soil was re-extracted as before. Phase splitting in the combined supernatants was obtained by adding citrate buffer and chloroform (overnight separation). The $CHCl_3$ layer was dried under N_2 at ambient temperature, re-dissolved with chloroform, and purified on silica cartridges (chromabond® 3 mL/500 mg SiOH, Macherey and Nagel, Düren, Germany). The methanolic fraction containing phospholipids was evaporated under N_2. Before analysis by gas chromatography-mass spectrometry (GC-MS), PLFAs had to be transformed into their less polar fatty acid methyl ester (FAME) derivatives. The derivatization was performed online in GC injector by tri methyl sulfonium hydroxide (TMSH) [57]. FAMEs were analyzed with GC/MS (4000 GC/MS, Varian, Inc., Walnut Creek, CA, USA) equipped with a BPX70 column (60 m, 0.25 mm i.d., 0.25 mm df., SGE). The FAME identification and quantification were performed using as standards: 37 component FAME mix from Supelco,

methyl nonadecanoate (19:0, used as internal standard) from Fluka and Br1 Mix, methyl-13-methyl tetradecanoate (i15:0), methyl 15-methyl hexadecanoate (i17:0), methyl vaccinate (18:1w7c), methyl cis-9, 10-methyleneoctadecanoate (cy19:0) from Larodan, Solna, Sweden. The PLFAs i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0 were chosen to represent Gram-positive bacteria while 16:1ω7c, 17:1ω7c and cy19:0 were used as an indicator of Gram-negative bacteria [58,59]. The PLFAs 18:1ω9c and 18:2ω6c were designated as representatives of fungi [56,60].

2.4. Ergosterol Analysis

Two extraction methods were used for the determination of ergosterol. A modified method of Montgomery et al. [61] was used for the alkaline ergosterol extraction (total ergosterol, i.e., free and esterified ergosterol). Briefly, 1 g of fresh soil was mixed with 8 mL of methanol and 2 mL of 2 M NaOH. The extraction was assisted by a microwave oven (MarsX®,CEM, Mathews, NC, USA), under the following conditions: 2 × 15 s with 15 min cooling intermission (2450 MHz, 495 W output). After the treatment, the soils and solvents of each reactor were recovered by 2 × 4 mL of MeOH. For the step of liquid extraction, we added 3 × 4 mL of pentane, and each addition was shaken by vortex for 10 s and allowed to separate the 2 layers. The combined pentane extracts were evaporated and taken to dryness under a stream of N_2 gas. The residues were then made up to 2 mL in methanol, vortexed, and filtered at 0.2 µm nylon Acrodisc® filter (Gelman Sciences, Ann Arbour, MI, USA). The second ergosterol extraction method consisted of the non-alkaline ergosterol extraction (free ergosterol) using the Gong et al. [62] modified protocol. Briefly, 4 g of fresh soil was combined with acid-washed glass beads (4 g of 400–800 µm) and 6 mL methanol. After shaking (10 s and then shaken for 1 h at 320 rpm), the soil-methanol mixture was centrifuged (10 min at 8000 rpm) and the supernatant was then filtered (0.2 µm nylon Acrodisc® filter. For both extraction methods, 4 replicates were performed for each plot. Prior to HPLC quantification, ergosterol was separated by a RP using Lichrospher C18, connected to a Varian Prostar 230 pump and a Prostar 330 PAD detector, Merck KGaA, Darmstadt, Germany). Samples (20 µL) were injected and eluted (with 90% acetonitrile 10% MeOH at a flow rate of 1.2 mL mn^{-1}). Ergosterol was detected at 282 nm with a retention time of 3.2 min.

Ergosterol and PLFA could be both considered as bioindicators of fungal biomass but since they corresponded to different lipid classes, they could be affected variously by agricultural practices [63]. Moreover, the 18S rDNA amplification was also used to address the abundance of fungi in soils. These 3 methods might seem redundant, but since they each have their own strengths and weakness, they should be considered as complementary [64].

2.5. Total DNA Extraction and Quantification

Nucleic acids were extracted from 0.5 g of soil using a FastDNA SPIN Kit for soil (MP-Biomedicals, Santa Ana, CA, USA) according to the instructions of the manufacturer. Four replicates were performed for each plot. DNA was quantified by a fluorimetric measurement using Hoeschst fluorochrome at 360/460 nm excitation/emission wavelengths with a Fluorescent DNA Quantitation Kit (Biorad, Hercules, CA, USA).

2.6. Real-Time PCR Amplification

The 18S rDNA amplifications for fungal biomass estimation by 18S rDNA real-time qPCR were carried out with a total volume of 50 µL. The qPCR mix was prepared as follows: 10 ng of soil microbial DNA, 25 pmol of each primer [FU18S1 5′-GGAAACTCACCAGGTCCAGA-3′ and Nu-SSU-1536 5′-ATTGCAATGCYCTATCCCCA-3′ [65]], 25 µL of qPCR Mastermix for SYBR Green I Master mix (Roche, Basel, Switzerland) and 2.5 mg mL^{-1} BSA (GeneON Bioscience, Ludwigshafen, Germany). A dilution series containing known amounts of *Fusarium graminearum* genomic DNA (assuming that it was representative of the total fungal genomes) was used as the standard for the quantification

of the sample dsDNA. The amplification protocol (40 cycles of PCR, 20 s at 95 °C, 30 s at 62 °C, and 30 s at 72 °C) was performed using LightCycler 480 real-time PCR system (Roche, Basel, Switzerland). The efficiency of the qPCR ranged from 93% to 98%.

The 16S rDNA amplifications for bacterial biomass estimation by 16S rDNA real-time PCR were carried out under the same conditions of the 18S rDNA PCR except for the primers [63f 5′-CAGGCCTAACA CATGCAAGTC-3′ [66] and BU16S4 5′ CTGCTGCCTCCCGTAGG-3′ derived from 341F [67]] and the amplification protocol (40 s at 95 °C, 45 s at 64 °C and 30 s at 72 °C). The efficiency of the qPCR ranged from 98% to 102%. A dilution series of *Pseudomonas aeruginosa* (assuming that it is representative of the bacterial genomes). DNA was used as the standard, and the results were expressed in µg of bacterial and or fungal dsDNA per µg of dry soil. Two independent qPCR assays were performed for the bacterial and fungal dsDNA estimation. Four replicates were performed for each plot.

2.7. Enzyme Assays

A total of 14 enzyme activities involved in C, N, P, and S nutrient cycling: Deshydrogenase (DEH) [68], Fluorescein diacetate hydrolase (FDA) [69], β-Glalactosidase (GAL) [70], β-Glucosidase (GLU) [49], Cellulase (CEL) [49], Laccase (LAC) [71], Lipase (LIP) [72], Xylanase (XYL) [73], Arylamidase (ARYLN) [74], N-acetylglusosaminidase (NAG) [49], Urease (URE) [70], Arylsulphatase (ARYLS) [70], Acid phosphatase (ACP), and Alkalin phosphatase (AKP) [49], were quantified by spectrophotometry, using commercial substrate analogs for each enzyme (Table 3). Four replicates were performed for each plot. Enzyme activities were expressed as an international enzyme unit (nmole of hydrolyzed or oxidized substrate min^{-1} $g_{dry\ soil}{}^{-1}$).

Table 3. Enzymes and substrates used for enzyme activity assays.

Enzymes	Abbreviations	E.C. Numbers	Substrates (Buffer pH and Concentration)
Global enzymatic activities			
Dehydrogenase	DEH	1.1.1.1	2,3,5 triphenyltetrazolium chloride (pH 7; 0.12 M)
Fluorescein diacetate hydrolase	FDA		Fluorescein diacetate (pH 7.6; 1000 µg mL^{-1})
C cycle enzymatic activities			
β-galactosidase	GAL	3.2.1.23	*p*-NP-β-D-galactopyranoside (pH 7; 0.02 M)
β-glucosidase	GLU	3.2.1.21	*p*-NP-β-D-glucopyranoside (pH 6; 50 mM)
Cellulase	CEL	3.2.1.4	*p*-NP-β-D-cellobioside (pH 6; 10 mM)
Laccase	LAC	1.10.3.2	2,2′-azino-bis-(3 ethylbenzothiazoline-6-sulfonate) (pH 5; 0.5 mM)
Lipase	LIP	3.1.1.3	*p*-NP-palmitate (pH 6.5; 1 mM)
N-acetylglucosaminidase	NAG	3.2.1.30	*p*-NP-N-acetylglucosaminide (pH 6; 10 mM)
Xylanase	XYL	3.2.1.8	Xylan (pH 5.5, 12 g L^{-1})
N cycle enzymatic activities			
Arylamidase	ARYLN	3.4.11.2	L-leucine b-naphtylamide (pH 8; 2 mM)
Urease	URE	3.5.1.5	Urea (pH 7; 0.05 mM)
S cycle enzymatic activities			
Arylsulfatase	ARYLS	3.1.6.1	*p*-NP-sulfate (pH7; 25 mM)
P cycle enzymatic activities			
Acid phosphatase	ACP	3.1.3.2	*p*-NP-phosphate (pH 5; 50 mM)
Alkaline phosphatase	AKP	3.1.3.1	*p*-NP-phosphate (pH 9; 50 mM)

E.C: Enzyme Commission numbers.NP: nitrophenyl.

2.8. Statistical Data Analysis

Statistical differences among the plots on physicochemical parameters (total C, total N, pH_{water}, CEC, P_2O_5), microbial abundance (MBC, total, bacterial and fungal PLFAs, total and free ergosterol, total dsDNA, bacterial and fungal dsDNA) and enzyme activities (GAL, GLU, CEL, LAC, LIP, XYL, ARYLN, NAG, URE, ARYLS, ACP, AKP, DEH, and FDA) were compared by multiple comparisons of means using the non-parametric post hoc test (“nparcomp” package in R), with a significance level of $p < 0.05$. The calculations of the correlations between the different parameters were carried out using a Spearman’s rank correlation procedure, and the significance level of these correlations were tested

using a pairwise *t*-test. To examine the relationships between different physicochemical characteristics and both microbial abundance and enzyme activities, multiple linear regression was carried out. Two principal component analyses (PCAs) were performed on the data of abundance of microbial communities and enzyme activities. For each PCA, we collected the coordinates on the first and second axes for each sample and calculated Euclidean distances. Euclidean distances were calculated using the permanent grassland (PG) as the reference of the more abundant and highest diversified microbial communities. The Euclidean distances are commonly used to analyze the magnitude of modification of abundance/composition and functional profiles of soil microbial communities [17,75–78]. The significance of the difference between plots was evaluated using the Kruskal–Wallis test. The calculations were performed using R [79].

3. Results

3.1. Soil Physicochemical Characteristics

The different plots of the field experiment showed the same texture of silty loam soil but showed different physicochemical characteristics (Table 4). Across the six plots, the total C and N contents ranged between 10.76–15.74 g kg^{-1} DW $_{soil}$ and 1.08–2.46 g kg^{-1} DW $_{soil}$, respectively. The total C and N contents differed significantly between PG and CC plots; the highest values were obtained for PG plot when the lowest ones were obtained for CC plot. PG plot also differed from the other plots.

Table 4. Physicochemical characteristics of plots.

Plots	Permanent Grassland	Restored Grasslands		Temporary Grasslands		Continuous Crop
	PG	RGI	RGII	TC	TG	CC
Total C (g kg^{-1})	25.74 ± (0.96) ^{a}	11.11 ± (0.34) ^{d}	14.30 ± (0.22) ^{c}	13.89 ± (0.53) ^{c}	18.60 ± (0.81) ^{b}	10.67 ± (0.34) ^{d}
Total N (g kg^{-1})	2.46 ± (0.07) ^{a}	1.14 ± (0.03) ^{d}	1.41 ± (0.02) ^{c}	1.44 ± (0.06) ^{c}	1.81 ± (0.06) ^{b}	1.08 ± (0.04) ^{d}
C/N ratio	10.47 ± (0.18) ^{a}	9.76 ± (0.06) ^{b}	10.15 ± (0.11) ^{a}	9.61 ± (0.18) ^{b}	10.25 ± (0.09) ^{a}	9.85 ± (0.13) ^{b}
Moisture (%)	13.83 ± (0.55) ^{a}	10.53 ± (0.29) ^{c}	9.58 ± (0.19) ^{d}	10.45 ± (0.25) ^{b}	11.78 ± (0.56) ^{b}	9.35 ± (0.25) ^{d}
pH_{water}	5.46 ± (0.05) ^{b}	5.57 ± (0.05) ^{b}	6.04 ± (0.05) ^{c}	6.27 ± (0.05) ^{a}	5.51 ± (0.05) ^{b}	6.43 ± (0.05) ^{a}
CEC ($cmol^{+}$ kg^{-1})	8.08 ± (0.26) ^{a}	5.46 ± (0.37) ^{d}	6.92 ± (0.31) ^{b}	7.76 ± (0.05) ^{c}	7.29 ± (0.20) ^{b}	7.04 ± (0.30) ^{b}
P_2O_5 (g kg^{-1})	0.13 ± (0.03) ^{bc}	0.09 ± (0.01) ^{d}	0.13 ± (0.01) ^{c}	0.15 ± (0.01) ^{ab}	0.19 ± (0.02) ^{ab}	0.20 ± (0.02) ^{a}

Mean ± (SD). Different letters indicate significant differences among plots ($p < 0.05$, n = 4).

Based on the total C and N contents, plots can be classified in the following order PG > TG > RGII ≈ TC > RGI ≈ CC. The C/N ratio of the six plots was roughly the same, with an average of ten. Concerning moisture, the mean value observed was about 10 g kg^{-1} (Table 4). Moisture differed significantly between the PG plot, which was the most humid soil and CC plot, which was the less humid plot. PG and CC plots differed significantly from other plots. For pH_{water}, only a slight effect was observed, and three groups can be distinguished from less to greater acid plot: TC ≈ CC > RGII > PG ≈ RGI ≈ TG. Cation Exchange Capacity (CEC) was higher for PG, while the lowest was obtained for RGI. The P_2O_5 was partly affected by land uses, the highest value was obtained for CC, which was not different from the temporary grasslands (TG and TC) while the lowest value was obtained for PG.

3.2. Microbial Communities' Abundance

For the study of microbial communities, we have chosen to analyze total, bacterial, and fungal abundances by using different and complementary methods through the analysis of MBC, cell-bound lipid (total, bacterial and fungal PLFAs), total, and free ergosterol and nucleic acids (total ds DNA, bacterial, and fungal ds DNA). The results are presented in Table 5. Considering all these parameters, the highest values were recorded for the PG plot, which was significantly different from the other plots, except for the free ergosterol content, which was not different between PG and TG. Conversely, the lowest values were observed for the CC and, in most cases, in the TC Plot.

Table 5. Total, bacterial, and fungal biomasses of plots.

Plots	Permanent Grassland	Restored Grasslands		Temporary Grasslands		Continuous Crop
Abundance of Microbial Communities	**PG**	**RGI**	**RGII**	**TC**	**TG**	**CC**
Total microbial biomass						
Total dsDNA (μg g^{-1})	25.82 ± (4.37) [a]	12.22 ± (3.24) [c]	16.49 ± (0.28) [b]	9.76 ± (1.79) [b]	15.70 ± (3.62) [bc]	13.24 ± (3.11) [b]
Total microbial PLFA (nmol g^{-1})	717.05 ± (70.2) [a]	184.26 ± (4.00) [c]	259.41 ± (29.1) [b]	102.66 ± (31.3) [d]	262.33 ± (84.5) [bcd]	141.33 ± (26.8) [d]
Microbial biomass carbon (mg C kg^{-1})	987.78 ± (88.4) [a]	250.52 ± (13.4) [d]	348.50 ± (6.03) [c]	339.32 ± (23.3) [c]	570.32 ± (63.1) [b]	234.39 ± (16.8) [d]
Bacterial biomass						
Bacterial dsDNA (μg g^{-1})	6.91 ± (1.70) [a]	3.30 ± (1.12) [bc]	3.96 ± (0.50) [b]	2.91 ± (0.39) [c]	3.28 ± (1.7) [abc]	2.93 ± (1.20) [bc]
Total bacterial PLFA (nmol g^{-1})	348.88 ± (29.7) [a]	72.66 ± (0.82) [c]	105.34 ± (13.4) [b]	41.62 ± (17.0) [d]	110.76 ± (41.7) [bcd]	53.78 ± (9.79) [d]
Fungal biomass						
Fungal dsDNA (μg g^{-1})	1.78 ± (0.25) [a]	1.55 ± (0.84) [abc]	1.34 ± (0.19) [ab]	0.65 ± (0.12) [c]	1.13 ± (0.58) [abc]	0.84 ± (0.29) [bc]
Total fungal PLFA (nmol g^{-1})	22.68 ± (5.21) [a]	9.95 ± (0.33) [c]	13.10 ± (1.57) [b]	1.95 ± (0.72) [e]	12.04 ± (4.44) [bc]	5.12 ± (1.10) [d]
Total ergosterol (μg g^{-1})	2.08 ± (0.60) [a]	0.99 ± (0.22) [b]	1.21 ± (0.21) [b]	0.82 ± (0.06) [b]	3.12 ± (0.68) [a]	0.65 ± (0.22) [b]
Free ergosterol (μg g^{-1})	1.28 ± (0.23) [a]	0.77 ± (0.08) [b]	0.75 ± (0.45) [abc]	0.48 ± (0.04) [c]	1.06 ± (0.10) [a]	0.51 ± (0.04) [c]

Mean ± (SD). Different letters indicate significant differences among plots ($p < 0.05$, n = 4).

To reduce the dimensionality of the data set, a PCA was performed to compare the plots (Figure 1). The first axis (PC1) and the second axis (PC2) explained 54.8% and 17.5% of the total variability, respectively. PCA clearly illustrated how the data set of the microbial communities classified the plots. The distribution of the plots along the first axis showed that the RGI, RGII, and TG plots were clustered and occupied an intermediate position between the two contrasted land uses. Indeed, this cluster was placed in the middle between grassland (PG) and croplands (CC and TC), which occupied the most extreme positions of this "PCA gradient".

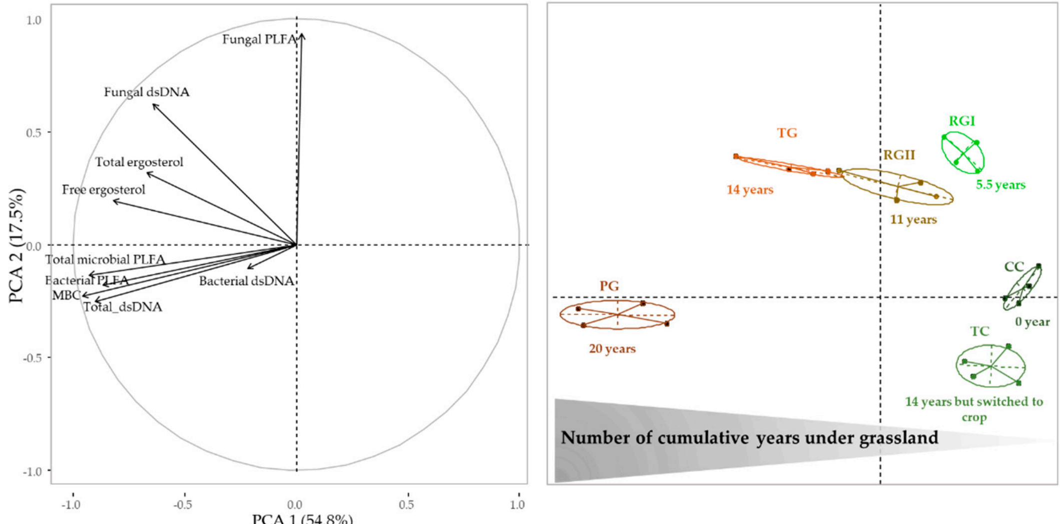

Figure 1. Principal component analysis and correlation circle of the abundance parameters of plots microbial communities.

3.3. Enzyme Activities

To evaluate the functions of microbial communities, 14 enzyme activities involved in different biogeochemical cycles were measured: for C cycle (GAL, GLU, CEL, LAC, LIP and XYL), for N cycle (ARYLN, NAG, and URE), for S cycle (ARYLS), for P cycle (ACP and AKP), and two global enzymes (DEH and FDA). The PG plot showed the highest values of all studied enzyme activities, and it was in most cases significantly different from the other plots except for AKP and GLU (Table 6). CC plot showed weak values of enzyme activities in comparison to the others plots. Overall, the enzyme activity values of the other treatments were intermediate and ranged between the PG and CC activity values. The pattern of variation of enzyme activities in the TG plot was similar to that of PG, while in

most cases, enzyme activities values in the CC plot were very close to those observed in RGI and TC.

Table 6. Enzyme activities of plots.

Plots	Permanent Grassland	Restored Grasslands		Temporary Grasslands		Continuous Crop
Enzyme Activities (nmole min^{-1} $g_{dry\ soil}^{-1}$)	PG	RGI	RGII	TC	TG	CC
Global enzyme						
DEH	0.20 ± (0.10) ^{a}	0.04 ± (0.02) ^{b}	0.04 ± (0.03) ^{bc}	0.04 ± (0.03) ^{bc}	0.08 ± (0.04) ^{ab}	0.00 ± (0.01) ^{c}
FDA	3.00 ± (0.30) ^{a}	1.56 ± (0.04) ^{b}	2.56 ± (0.54) ^{a}	1.30 ± (0.84) ^{b}	2.12 ± (0.46) ^{a}	1.08 ± (0.25) ^{b}
C cycle enzymes						
GAL	4.65 ± (0.28) ^{a}	2.07 ± (0.28) ^{b}	2.20 ± (0.36) ^{b}	1.48 ± (0.12) ^{c}	3.42 ± (0.36) ^{d}	1.35 ± (0.16) ^{e}
GLU	9.65 ± (0.81) ^{b}	3.36 ± (0.52) ^{e}	7.97 ± (0.44) ^{c}	5.94 ± (0.67) ^{d}	12.10 ± (0.94) ^{a}	5.36 ± (0.65) ^{d}
CEL	1.96 ± (0.24) ^{a}	0.77 ± (0.23) ^{b}	0.94 ± (0.14) ^{b}	0.81 ± (0.16) ^{b}	1.75 ± (0.26) ^{a}	0.65 ± (0.09) ^{b}
LAC	8.03 ± (1.35) ^{a}	5.80 ± (1.47) ^{ab}	2.57 ± (1.30) ^{c}	1.41 ± (0.58) ^{c}	3.53 ± (1.88) ^{bc}	2.73 ± (0.63) ^{c}
LIP	2.82 ± (0.26) ^{a}	1.68 ± (0.16) ^{b}	1.46 ± (0.32) ^{b}	1.06 ± (0.16) ^{c}	1.72 ± (0.17) ^{b}	0.74 ± (0.19) ^{c}
XYL	0.08 ± (0.01) ^{a}	0.01 ± (0.01) ^{c}	0.01 ± (0.00) ^{c}	0.02 ± (0.01) ^{bc}	0.04 ± (0.01) ^{b}	0.00 ± (0.01) ^{c}
N cycle enzymes						
ARYLN	3.94 ± (0.39) ^{a}	1.32 ± (0.09) ^{c}	1.66 ± (0.08) ^{b}	1.49 ± (0.11) ^{c}	1.90 ± (0.32) ^{b}	2.04 ± (0.24) ^{b}
NAG	5.78 ± (0.72) ^{a}	2.03 ± (0.41) ^{cd}	2.70 ± (0.20) ^{d}	1.97 ± (0.64) ^{cd}	4.20 ± (0.46) ^{b}	1.36 ± (0.31) ^{c}
URE	79.43 ± (10.03) ^{a}	25.84 ± (2.46) ^{d}	34.63 ± (2.01) ^{c}	30.01 ± (3.57) ^{cd}	54.71 ± (3.32) ^{b}	21.18 ± (0.36) ^{e}
S cycle enzyme						
ARYLS	7.27 ± (0.23) ^{a}	2.70 ± (0.11) ^{b}	3.32 ± (0.22) ^{c}	2.70 ± (0.10) ^{b}	5.48 ± (0.33) ^{d}	1.83 ± (0.24) ^{e}
P cycle enzymes						
ACP	42.72 ± (1.25) ^{a}	10.73 ± (7.23) ^{e}	20.86 ± (1.92) ^{c}	17.74 ± (0.91) ^{d}	30.60 ± (2.29) ^{b}	10.81 ± (0.90) ^{e}
AKP	4.30 ± (1.13) ^{c}	2.65 ± (0.39) ^{d}	7.90 ± (0.56) ^{a}	3.52 ± (1.11) ^{cd}	5.24 ± (1.17) ^{cb}	6.15 ± (0.48) ^{b}

Mean ± (SD). Different letters indicate significant differences among plots ($p < 0.05$, n = 4).

The comparison of the enzyme activities among the plots using PCA is presented in Figure 2. The first axis (PC1) and the second axis (PC2) explained 73.2% and 11.6% of the total variability, respectively. The plots were ranked as follows: PG > TG > RGII > TC ≈ RGI > CC based on the first axis. The distribution of the plots along the first axis reflects the duration in numbers of years cumulated under grassland. The RGI, TC, and CC plots were almost aggregated. Interestingly, the plots CC and TC were under culture at the time of sampling, while RGI was under a grassland regime but had a history of five years of continuous crop. Conversely, TC, which was closed to CC on the "PCA gradient", was under continuous crop at the time of sampling but after 14 years cumulated under grassland. In this Figure, the separation of the plots is slightly different in comparison to those observed in Figure 1 for abundance parameters of microbial communities.

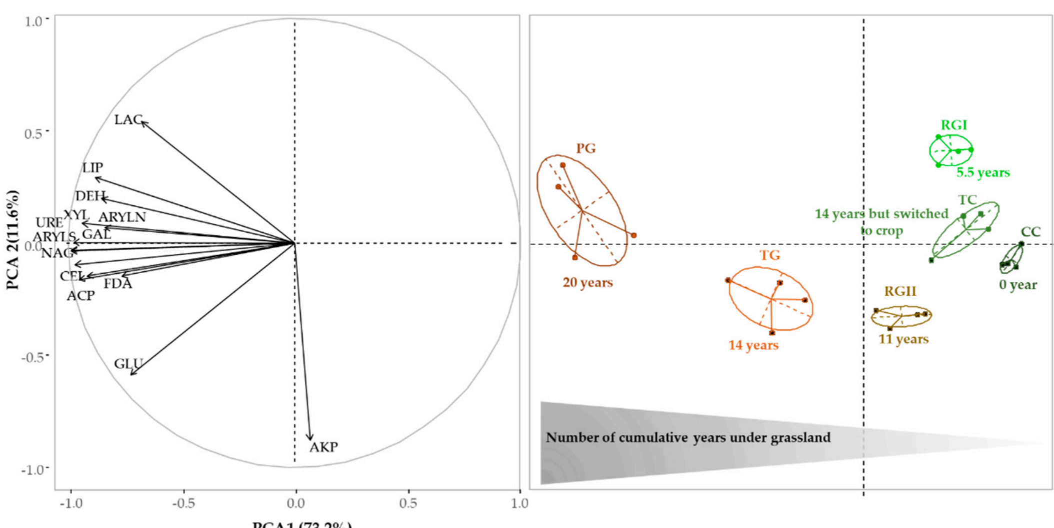

Figure 2. Principal component analysis and correlation circle of plots enzyme activities.

3.4. Relationship between Soil Physicochemical Characteristics, Microbial Communities, and Enzyme Activities

To test which physicochemical characteristics (total C, total N, pH_{water}, CEC, P_2O_5) shape microbial community and enzyme activities, Spearman's rank correlation was used (Figure 3). The results showed that with the exception of bacterial dsDNA and fungal PLFA, all the variables were positively correlated with the total C ($r \geq 0.58$, $p < 0.001$) and N ($r \geq 0.51$, $p < 0.01$) contents and were negatively correlated with pH_{water} ($r \geq -0.55$, $p < 0.001$). There was significant correlation between the CEC and total biomass ($r \geq 0.66$, $p < 0.0001$) and bacterial biomass ($r = 0.43$, $p < 0.01$), while fungal PLFA was strongly negatively correlated with CEC ($r \geq -0.81$, $p < 0.0001$) and in lesser extent with P_2O_5 ($r \geq -0.48$, $p < 0.01$). Concerning enzyme activities, except for LAC and AKP activities, all enzyme activities were positively correlated with the total C ($r \geq 0.55$, $p < 0.001$) and total N ($r \geq 0.51$, $p < 0.001$) contents (except for ARYLN and AKP). In most cases, enzyme activities were highly negatively correlated with $pH_{water,}$ ($r \geq -0.53$, $p < 0.001$, except for ARYLN and AKP). A positive relationship was shown between enzyme activities and CEC ($r \geq 0.50$, $p < 0.01$), except for GAL, LAC, LIP, and AKP. Beyond LIP and ARYLN activities, which were positively correlated to P_2O_5, no correlations were shown between P_2O_5 and any of the measured enzymes.

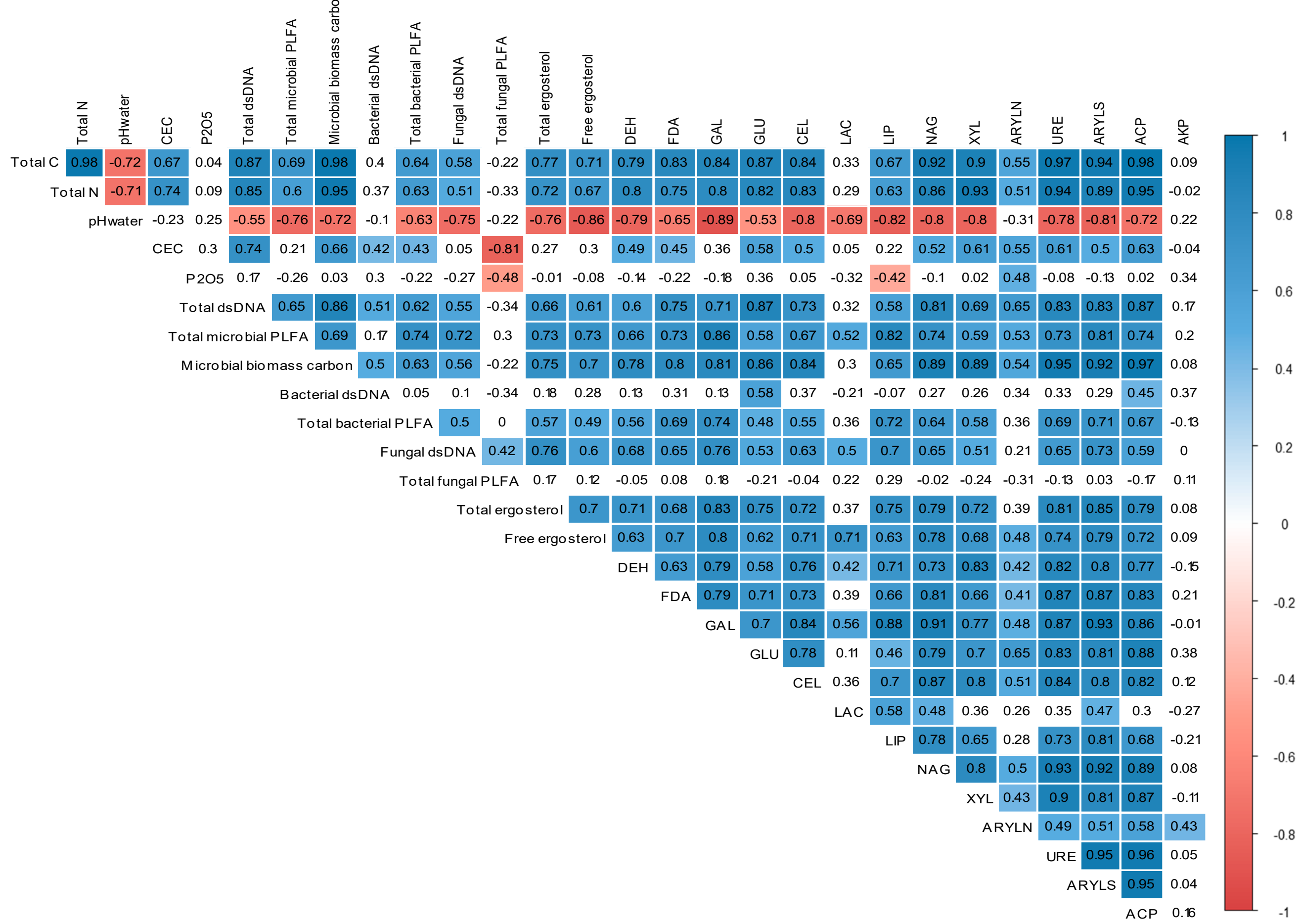

Figure 3. Correlation matrix between physicochemical parameters, microbial abundance, and enzyme activities. Red and blue color indicate statistically significant negative and positive correlations, respectively.

We further found that, except for AKP activity and in most cases LAC activity, all enzymes involved in C, N, S, and P cycling were strongly correlated with each other. For instance, the GAL and CEL activities were strongly correlated with the NAG, URE, ARYLS, and ACP activities (Figure 3). Among all soil parameters, the strongest correlations were

obtained for C and N contents and microbial biomass carbon and NAG, XYL, URE ARYLS, and ACP activities (r ≥ 0.90, $p < 0.0001$)

3.5. Relationships between Abundance of Microbial Communities and Enzyme Activities Levels Following the Introduction of Grassland into Crop Rotation

To explore the changes in abundance and functional profiles of microbial communities following the introduction of grassland into crop rotation, Euclidean distances were calculated as described in Section 2. These distances were used to assess the magnitude of the changes on the abundance of microbial communities and the functions profiles in comparison to the reference plot (PG) (Figure 4).

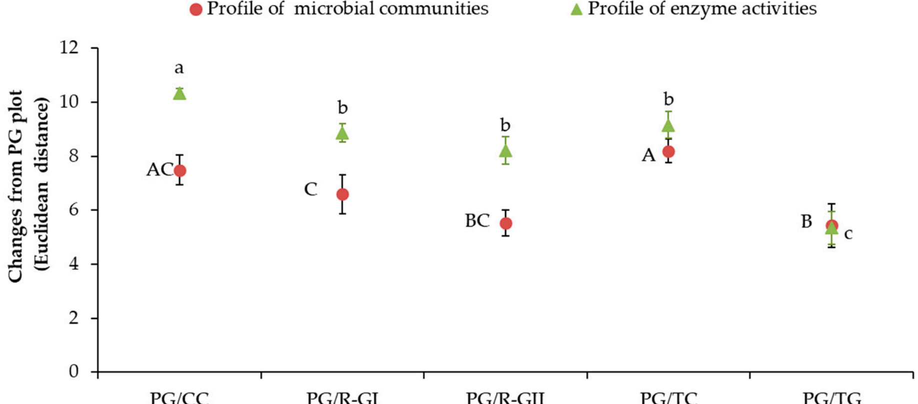

Figure 4. Changes on the levels of the abundance of microbial communities and enzymes activities expressed by Euclidean distance using the PG soil state as a reference state. Different letters indicate a significant difference between plots using Kruskal–Wallis test ($p < 0.05$, n = 4). Upper letters indicate a significant difference in the abundance profile, and lowercase letters indicate a significant difference in enzyme profile.

The highest the Euclidean distance is, the highest is the difference between two plots. For example, the large distances between PG and CC (distance ≈ 10) for the functional profile indicated that the cultivation had a large impact on enzyme activities. The analyses of distances showed significant changes in the abundance and functional profiles of microbial communities between PG and TC plot and between PG and CC plot. While these profiles in TG are closer to those of PG. The plots TC and TG have cumulated 14 years in grassland, but they differ in the land use at sampling day because the TC plot was re-cultivated the year of sampling (2010). Euclidean distance results showed that cultivation led to a significant change in soil microbial communities. The changes in the abundance and functions between PG and RGI, as well as between PG and RGII, were intermediate between those observed for PG/TC and PG/TG (Figure 4). Correlation analysis showed a positive correlation (r = 0.74, $p < 0.0001$, Figure S2) between the changes of microbial abundance profile and the changes on enzyme activities profile when grassland is introduced into crop rotations.

4. Discussion

The restoration of the physicochemical properties and biological characteristics of soil has become of interest to preserve the soil state and promote sustainable management [80]. In accordance with our expectations, the sustainable soil management, including the implementation of grassland in crop rotations, influenced the soil properties, microbial communities, and enzyme activities, but the variations depended on the number of cumulative years under grasslands of each plot and the type of land use at the time of sampling.

4.1. Influence of Soil Characteristics in Shaping the Whole Microbial Community and Enzyme Activities

This study aimed to assess how agricultural managements influenced the role of soil characteristics in shaping the belowground microbial communities and their enzyme activities. Microbial communities were associated with soil physicochemical characteristics determined by C and N content and soil pH. Indeed, when physicochemical characteristics are considered together as a grouping factor (Table S1), they explained between 55% and 97% of the variation in microbial communities and between 64% and 98% of the variation in enzyme activities ($p < 0.0001$), respectively. In our study, C and N contents were in relation with the cumulative years under grassland, whereas soil pH appeared to be in relation with land use (grassland or cropland) at the sampling time (wheat crop in TC, CC, Figure S3). Overall, all groups of microorganisms (bacteria and fungi) assessed using different methods were strongly related to soil properties, which help explaining the differences observed between plots communities. This is unsurprising as many edaphic conditions are co-correlated, and several studies are now beginning to shed light on the composition of different microbial communities and relationships with edaphic properties across various soils [17,81,82]. The effects of land-use change on soil conditions, which drive microbial community composition, was particularly demonstrated through this study, which investigated different grassland regimes between continuous grassland and continuous crop. Continuous cropping without OM restitution is known to deplete soil C and N contents [22,83]. On the opposite, permanent grassland contributes to C storage through both reduced soil disturbance and slow degradation dynamics of OM [26,84,85], with positive effects on microbial communities [17,60]. More generally, shifts in soil organic status resulting from land use (plant residues amounts and qualities, root exudates, etc.) induce modifications in microbial communities [86], confirming the relationships between land use and soil biota [87]. Furthermore, a stronger effect of soil pH on the microbial communities was already observed by Bissett et al. [88] and Kuramae et al. [89]. In our study, soil pH was negatively correlated with all the variables that describe microbial communities. Combining soil analyses with soil microbial communities revealed that the microbial communities are non-randomly distributed across soils but determined by environmental factors. More specifically, soil properties, notably the C and N contents and pH, appeared to be the main drivers of soil microbial community composition and distribution at the European scale [86,90–92].

Soil enzymes have been reported as useful bioindicators of soil-state because they provide information on the soil's ability to perform biogeochemical reactions [93]. The measured enzyme activities are involved in the decomposition of different substrates with varying complexity related to the C, N, P, and S cycles [94]. The highest enzyme activities were recorded in PG plots and the lowest in CC plots, while intermediate activities were observed in the plots that represented intermediate situations. We investigated enzyme activities in connection with soil chemical properties. Overall, the majority of the enzyme activities were negatively correlated with the soil pH but positively correlated with C and N contents. Several studies showed the same relationships between C and N contents and enzymes activities [95–99] because C and N cycles were also strongly coupled in soils [100–103]. Concerning AKP activity, several authors showed a positive correlation between C and N content and AKP activity [104,105]. This was not the case in our study because AKP was not correlated with any of the soil characteristics. However, a large study conducted by Margalef et al. [106] showed that climatic conditions were the first predictive variables of AKP variation in soil and to a lesser extent N content and soil pH. The lack of correlation between LAC activity and C and N contents could be due to that LAC is strongly linked to OM quality than quantity [107]. This activity is also specifically produced following exposure to xenobiotics, unlike hydrolases, which are constitutively expressed [44,108]. Indeed, the mechanism by which soil microbial communities maintain homeostasis in a fluctuating resource environment is based on regulating enzyme expression to optimize the responsiveness of substrate acquisition [109,110], confirming that OM

is the most driver of enzyme activities [100,111,112]. In our study, enzymes activities were significantly affected by soil pH; this result agrees with several studies showing that pH is an important factor in describing enzyme activities across soils [113,114]. Various soil enzymes have different optimal soil pH ranges. This is not always reflected by the association between the enzyme activities and pH because soil pH can affect the enzyme activities indirectly by changing nutrient availability, the soil OM content, and the composition of the microbial communities [59,98,115,116].

4.2. Relationships between Microbial Communities and Soil Enzyme Activities

We further found that the enzymes involved in C, N, S, and P cycling were strongly correlated with each other (Figure 3), which reflected the close link between soil biogeochemical cycles [103,117]. The study conducted by Ullah et al. [99] showed notably that the activities of C-cycling enzymes had a direct positive relationship with the activities of N-cycling enzymes, and soil C availability increased N-cycling enzymes. Beyond the relationships showed between enzyme activities involved in the different biogeochemical cycles of C, N, P, and S, our study highlighted strong relationships between microbial communities' level and functional profile in soils. Indeed, the changes in the abundance of microbial communities were strongly linked to the changes in enzymes activities profile under the introduction of grassland into crop rotations. Furthermore, our study also confirmed the strong functional redundancy of soil microbial communities [99,118,119]. Indeed, several enzymes were linked to both bacterial and fungal abundance at the same time (Figure 3). This functional redundancy is common for basic mediated processes by hydrolases activities and far less redundant for processes mediated by oxidase activities (LAC). Indeed, LAC activity, which is involved in the mineralization of recalcitrant organic pollutants, was only linked to fungal biomass underling that "rare" or "less redundant" functions are mediated by specific groups of microorganisms [108,119]. In our study, the variation of AKP activity was not explained by any measured parameters, and it was not correlated to them. We suggested that this enzyme activity could be linked to stocking density when plots are in grasslands. Indeed, some authors explained that phosphatase activity increased with grazing intensity [120,121].

4.3. Transition of Microbial Abundance and Functional States Following the Introduction of Grassland into Crop Rotation

Several studies have emphasized the modification in microbial communities, and enzyme activities in soil under different land uses [17,122,123]. The analysis of the introduction of grassland into crop rotation expressed by Euclidean distances revealed that the cumulative years in grassland were the primary factor that determined the abundance and functional states of microbial communities. Permanent grassland and continuous crop are two types of land use that have distinct effects on the soil biota [83,85,124–126]. In our study, the intermediate conditions between these two contrasted land uses showed that the level of microbial abundance of TG tended to return to the level of grassland (PG). Indeed, excluding TC plot, which was cultivated at the time of sampling, Euclidean distances showed that microbial abundance and functional states tended to resiliency with an increasing number of years cumulated in grassland. This observation is consistent with those of Van Eekeren et al. [32], who found that soil OM, earthworm abundance, soil structure, and major functions of the soil biota are restored after crop conversion to grassland. In our study, the restoration of the abundance of microbial communities and enzyme activities at a level equivalent to grassland required more than five years without disturbance (TG). In the meantime, the restoration of the soil biota and its functions in the grassland phase was only temporary due to the following crop phase, which can explain the behavior of RGI and RGII. In other words, a continuous crop regime apparently imposes a stronger legacy on microbial communities and enzyme activities (RGI), in contrast with the after-effect of a grassland regime, which appears to be readily "labile" when a grassland regime is interrupted by cultivation (TC). Most of the studies giving information about the potential legacy's effects of grassland/cropland rotation systems on soil biophysicochemical

properties and yields showed controversial findings [83,85,124,127]. Such practices, linked to grassland or cropland phases, can have "positive or negative legacy" effects resulting from the interactions between several factors such as (i) soil properties, (ii) the duration and the applied management under the grassland phase, (iii) the management practices applied for crop phase [32,83,85,125,128,129]. While most studies have focused on the evaluation of soil biophysicochemical parameters when introducing grasslands into crop rotations, only few studies have addressed the effect of such practices on soil ecosystem services. Indeed, recent studies showed a stronger beneficial grassland legacy effect on soil structure maintenance and biodiversity conservation. By contrast, water and pathogen regulation and forage production were not affected by the legacy of grassland during the rotation [130,131].

Our results suggest that the introduction of grassland into crop rotations can restore the microbial communities and functional soil states. A continuous culture regime has a stronger legacy on the abundance of microbial communities (RGI). By contrast, the legacy of the grassland regime changes quickly when the grassland regime is interrupted by recent culture events (TC). A grassland regime enables resilience by the restoration of functions. However, as observed for TG, more than five cumulative years under grassland are required to observe this resilience. Our results highlighted the strong relationships between microbial communities and enzyme activities and the strong functional redundancy of microbial communities that could explain the observed resiliency. Finally, including grasslands in crop rotations has strong consequences for the microbial communities and the sustainability of their functionality. Such biological studies could help to optimize crop rotations (e.g., frequency and duration of temporary leys) for both agricultural productivity and ecosystem services. This work confirms the potential benefits offered by the introduction of grassland into cropping systems and could be largely used in the context of diversification of agricultural systems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture11100909/s1, Figure S1: Plots positions in the area of the study (A) and sampling protocol (B), Figure S2: Correlation between microbial abundance and enzyme activities profiles, Figure S3: Principal component analysis and correlation circle of plots physicochemical, Table S1: Physicochemical characteristics explaining the variation of the abundance of microbial communities and enzyme activities by multiple regression analysis.

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