Applied Soil Ecology 108 (2016) 147–155

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization

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ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form 19 July 2016
Accepted 1 August 2016
Available online xxx

Keywords:
Soil management
Microbial functional responses
Carbon content

ABSTRACT

Agricultural practices such as tillage, cover crops, and nitrogen (N) fertilization affect physico-chemical and biological soil parameters. However, these factors were often studied separately and their combined effects remain unclear, especially with respect to soil microbial functional diversity and carbon (C) and N content. Thereafter, we aim to assess the links between cropping systems and functional response of microbial communities by using a large range of soil chemical and biological measurements. A 5-yr field experiment was conducted in Northern France using a combination of three factors: i) no-till (NT) *vs.* conventional tillage (CT); ii) with or without winter cover crops (bare fallow; cover crops with a low prevalence of legumes; cover crop with a high prevalence of legumes); and iii) with or without N fertilization.

C and N inputs from cover crops and crop residues, C and N content, enzyme activities, and microbial functional diversity in the topsoil (0–10 cm) were measured over an industrial crop rotation: wheat, pea, corn, wheat, flax. No-till combined with any of the cover crops was characterized by increased total soil organic C and N contents by more than 20% between 2010 and 2015. Dehydrogenase and urease activities were significantly greatest under NT, irrespective of the presence of cover crops. Cover crops without N fertilization under no-till led to higher microbial functional activity (faster carbohydrate and phenolic compound degradation) and diversity. Bare fallow had lower soil microbial functional diversity and C and N contents compared with soil under NT and cover crops. On the other hand, NT associated with cover crops allowed to maintain the soil in both C and N, and to promote microbial activities without N fertilization. In conclusion, winter cover crops and/or NT are sustainable agricultural practices resulting in a greater soil quality index. These results demonstrate that NT and use of standard cover crops or cover crops with legumes for 5 years under a low biomass return in industrial crop production have a positive effect on: i) upper soil C content and microbial enzymes, irrespective of N fertilization regime; ii) soil microbial functional diversity in the absence of N fertilization.

1. Introduction

Alternative farming practices such as direct seeding mulch-based cropping systems are often employed to reduce the depressive effects of intensive farming on soil fertility. These practices are known to improve ecological relations between plants, soil, and microorganisms (Chaussod 1996; Alvarez et al., 2002; Marinari et al., 2006), while conventional tillage (CT) depletes physico-chemical properties of soil (Chen et al., 2009; Mangalassery et al., 2015). Nevertheless, the response of soil carbon (C) and nitrogen (N) as well as microorganisms to different farm management patterns are still poorly understood, being dependent on many factors such as pedoclimate and crop rotation. Furthermore, the combined effects of different agricultural practices on the chemical and biological properties of soil received little attention so far (Acosta-Martinez et al., 2011).

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http://dx.doi.org/10.1016/j.apsoil.2016.08.004
0929-1393/

Although no-till (NT) with cover crops has been shown to increase soil enzyme activities (SEA), microbial functional diversity (Gomez et al., 2004; Nannipieri and Eldor, 2009; Lagomarsino et al., 2009; Nautiyal et al., 2010; Mangalassery et al., 2015; Mbuthia et al., 2015), and abundance (Mathew et al., 2012), there is still a lack of information for low organic-input cropping systems (Mbuthia et al., 2015), especially for industrial crop rotations. Indeed, C inputs from cover crops and crop residues shift soil moisture (Jordan, 2004; Bayer et al., 2006), temperature (Kladivko, 2001), and pH (Reganold et al., 1987), inducing short- and medium-term responses from microbial communities. These responses result in an increase of SEA such as dehydrogenase, alkaline phosphatase, and urease, with consistent results across subtropical (Staben et al., 1997), semi-arid (Nautiyal et al., 2010), mediterranean (Marinari et al., 2006; Lagomarsino et al., 2009), and temperate (Salinas-Garcia et al., 1997; Grayston et al., 2001) biomes. In addition, cover crops have been shown to induce priming effects in rhizosphere and detritusphere hotspots, by affecting the turnover of the soil organic matter (SOM) (Kuzyakov, 2010; Kuzyakov and Blagodatskaya, 2015), hence altering soil C and N contents. Increased lignin and cellulose contents, C/N ratio, as well as rhizodeposition (i.e. organic compounds released by roots during plant growth) are also considered as main factors affecting microbial communities (Marinari et al., 2000; Kusliene et al., 2014; Bais et al., 2006; Mangalassery et al., 2015). In low organic restitution cropping systems, one of the main challenges is to compensate the low biomass returned by cover crops originated-C and NT system, thus maintaining C and N contents and improving microbial functions (Mbuthia et al., 2015).

In this way, cover crop species have to be selected according to agronomic objectives, and the potential of different mixtures for improving soil quality needs to be assessed. Indeed the species composition of cover crops is a key factor determining the potential benefits to the soil following their incorporation/mulching. Cover crops enriched in leguminous species were associated with increased soil N content, likely due to N-fixation (Mbuthia et al., 2015). Moreover, the last study reported that vetch cover crops, in comparison with wheat cover crops or bare fallow, improved soil microbial responses such as microbial biomass N or microbial respiration rates. However, according to the level of available N, the inputs of fresh C may negatively correlate with C sequestration through an induced speed-up of C decomposition (Fontaine et al., 2004). Thereby, while the addition of N through fertilizers is commonly described to increase SOM stocks by increasing the rate of residues returned to the soil, long-term application of N fertilization may also cause a SOM depletion by stimulating mineralization.

In the present study, a 5-year experiment is used to investigate, under two N fertilization rates, the combined influence of tillage and cover crops on SEA, soil microbial functional diversity (functional activities and diversity indices), total soil organic carbon (TOC), and nitrogen (TN). We hypothesized that the decrease of soil quality in an industrial crop rotation under conventional soil management could be prevented by no tillage associated with cover crops (with low/high prevalence of legumes). Specifically in these systems, N inputs originated from leguminous cover crops could maintain C and N contents as well as microbial functional activities and diversity despite the absence of N fertilization.

2. Materials and methods

2.1. Site description

The field experiment was conducted at “La Woestyne” experimental site, in Northern France (50°44′N, 2°22′E, 40 m above sea level). The average annual air temperature and total precipitation between 2010 and 2015 were 11 °C and 620 mm, respectively, with rainfall relatively homogeneous across seasons. Soil particle size composition is characterized by 668 g kg^{-1} silt, 212 g kg^{-1} clay and 120 g kg^{-1} sand (silty clay loam). The main characteristics of the soil before the beginning of the experiment are shown in Table 1.

Table 1
Main characteristics of soil (0–10 cm) before the start of the experiment.

Parameters (units)	
pH in H_2O	6.28
CEC[a] (cmol + kg^{-1})	11.7
P_2O_5[b] (g P kg^{-1})	0.13
Organic C (g C kg^{-1})	13.1
Total N (g N kg^{-1})	1.48
Soil C:N ratio	8.85

[a] Cation-exchange capacity (Metson method).
[b] Available phosphorus (Olsen method).

2.2. Experimental design

Prior to the experiment’s establishment, the field was conducted under chisel plough and rotary power system, fertilized conventionally, and cultivated with wheat (*Triticum aestivum*). In 2010, the experimental field was split into twelve treatments with three replicated plots placed randomly: two N fertilization regimes (without (N0) or with (Nx) N fertilization), two tillage systems (no-till (NT) or conventional tillage (CT)), and three cover crops modalities (bare fallow (bf), with cover crop enriched in leguminous species (lcc) or with standard cover crop (cc)). The 18 plots which received the treatment N0 measured 7 × 8 m while the 18 plots which received the treatment Nx measured 14 × 8 m. A 7-m width corridor separated the Nx and N0 plots in order to avoid N contamination.

During the 5-year period of the experiment, the main crop rotation pattern was wheat, pea (*Pisum sativum*), corn (*Zea mays*), wheat and flax (*Linum usitatissimum*). Wheat in 2010 and 2013 was sown in mid October at a row spacing of 12.5 cm using an AS 400 drill (Alpego, Italia) and harvested in late July, Green pea was sown in mid April at a row spacing of 17 cm using a Turbosem drill (Herriau, France) and harvested in early July. Maize was sown in mid May at a row spacing of 75 cm with a Maxima drill (Kuhn, France) and harvested in late September. Flax was sown in early April at a row spacing of 12.5 cm using an AS 400 drill (Alpego, Italia) and harvested in mid July. Only wheat straws were returned as main crop residues since corn and flax were cultivated for forage and fiber respectively. The Nx regime was determined according to the N budget method (Machet et al., 1990) for each rotation crop, and the fertilizer was composed of 50% urea, 25% ammonia and 25% nitrate. Since the beginning of the experiment, 108 kg N ha^{-1} were added to corn, 160 kg N ha^{-1} to wheat, and 80 kg N ha^{-1} to flax. The N0 plots received no N fertilization since 2010. Cover crops “cc” and “lcc” were composed of non-leguminous species: oats (*Avena sativa*), phacelia (*Phacelia tanacetifolia*), flax (*Linum usitatissimum*), as well as leguminous species: vetch (*Vicia sativa*), faba bean (*Vicia faba*), and Egyptian clover (*Trifolium alexandrinum*), but the lcc mixture was particularly characterized by an increase of legume seeding rates. The cc/lcc plots received 160/60 seeds m^{-2} of oats, 600/200 seeds m^{-2} of phacelia, 80/80 seeds m^{-2} of flax, 25/50 seeds m^{-2} of vetch, 20/30 seeds m^{-2} of faba bean, and 230/400 seeds m^{-2} of Egyptian clover. All cover crop seeds were mixed and simultaneously sown in line. Each year, cover crops were sown immediately after the harvest of the previous crop and were terminated by grinding following a frost period. Before the main crops were sown, cover crop residues were

buried by a conventional moldboard plough to a depth of 30 cm in CT plots and left on the ground in NT plots. Crop protection against weed development and pests was ensured conventionally according to recommended local practices, and all the treatments received the same molecules and doses (see Supplementary Table 1). Neither potassium-phosphate nor other elements were applied throughout the experiment.

2.3. Analysis of nitrogen and carbon inputs from cover crop and main crop residues

During the experiment, 3×1 m row of the main crop were yearly sampled at the time of harvest, within each plot. The part of the plant which is commonly returned to the soil through crop residues was separated from the rest of the aerial vegetative part (i.e. exported part). Samples were oven-dried at 65 °C for 3 days, weighed, and then ground into powder for subsequent NC analysis using a Flash Elemental Analyzer 1112 series (Thermo Electron, Germany). Similarly, 3×1 m^2 of the entire vegetative part of cover crops were yearly sampled within each plot just before sowing the following main crop to measure the total aboveground biomass and NC contents using the same method. The cumulative organic inputs of N and C from both main crop residues and cover crops were calculated as the sum of C and N returned to the soil since 2010.

2.4. Soil sample collection and storage

In March 2015, six 10-cm deep soil cores were randomly collected using a 5-cm diameter auger in each of the three replicate plots by treatment. Soil sampling occurred 1 year after the last plowing in CT and 3 months after the death by frost of cover crop species in cc plots in order to avoid direct effects of soil disturbance and living roots on the measured parameters. We chose to sample to 10 cm since previous studies reported it was an appropriate depth to assess the effects of N additions on microbial communities (Zeng et al., 2016), even in plowed soil (Sun et al., 2015). The six soil cores were composited together as a single sample for each replicate plot. Soil samples were immediately put into polyethylene bags, placed into coolers for transportation to the laboratory, and, after homogenization, they were divided into two subsamples. The first one was stored at −20 °C and returned to 4 °C within 5 days prior to analysis of SEA (Schinner et al., 2012). The second one was stored at 4 °C before the determination of community level physiological profiles (CLPP), total organic carbon (TOC), and total nitrogen (TN).

2.5. Soil pH, total organic carbon (TOC) and total nitrogen (TN)

Soils were 2 mm-sieved and oven-dried at 45 °C for 48 h. Soil pH was measured using a pH meter FE20-FiveEasy™ (Mettler Toledo, Switzerland) and a ratio of 1:5 (mass/volume) of soil to distilled water shaken for 45 min. For TOC and TN, sieved soil was finely ground with a ball-mill (Retsch, MM400) and analyzed using a CN elemental analyzer (Flash EA 1112, Thermo Electron, Germany). Since analysis performed at time of the experiment setup revealed that the soil was free of carbonate, the soil total C was assumed to be equal to the TOC. Soil TOC and TN analyzes from samples taken at the beginning of the experiment with the same sampling method as shown in 2.4 were used to calculate TOC and TN change over the 5-year period.

2.6. Soil dehydrogenase and urease enzyme activities

Before SEA measurements, visible organic residues were removed. Soil dehydrogenase activity was measured as described by Casida et al. (1964). Soil sub-samples were adjusted with $CaCO_3$ to a final mass ratio of 100:1 (soil:$CaCO_3$) using 5.94 g of fresh soil. Then, 1 mL of a solution of 3% 2,3,5-triphenyl tetrazolium chloride and 2.5 mL of ultrapure water was added. After mixing, the tubes were incubated at 37 °C for 24 h. The resulting triphenylformazan (TPF) was then extracted with 30 mL of pure methanol by stirring for 1 min. The solution was filtered in a dark room and the intensity of TPF was measured at 485 nm (Eon spectrophotometer, BioTek Instruments Inc., USA) following Tabatabai (1994). Urease activity was determined using the method of Alef and Nannipieri (1998). A mixture of 5 g of fresh soil with 2.5 mL of 79,9 mM urea was incubated at 37 °C for 2 h before adding 50 mL of 2.0 M KCl. After 30 min of agitation, the suspension was filtered and a solution comprising 1 mL of filtrate, 9 mL of deionized water, 2 mL of 0.1% sodium dichloroisocyanurate and 5 mL of a mixture of 1,06 M sodium salicylate/0.3 M sodium hydroxide/deionized water (1:3/1:3/1:3, vol/vol/vol) was prepared. After decanting for 30 min at room temperature, ammonium was measured at 690 nm using the spectrophotometer.

2.7. Community level physiological profiles (CLPP)

The analysis of CLPP was completed within 5 days after sampling (Schinner et al., 2012) using Biolog EcoPlates™ (BiologInc., Hayward, CA) containing 31 different carbon sources plus a control well, in two replications (Huynh, 2009). Five g of fresh, homogenized and organic residue-free soil was incubated with 750 μL of sterile distilled water for 48 h at 26 °C. Then, 50 mL of physiological saline were added and the samples were stirred at 500 rpm for 30 min. The samples were centrifuged at 800 rpm for 10 min and recovered supernatants were centrifuged again at 3000 rpm for 5 min. Then, 150 μL of the supernatant was inoculated into each well of the Biolog EcoPlates and incubated at 25 °C. Data were recorded every 24 h for 196 h at 590 nm and the data recorded at the end of the exponential phase (96 h) were used for further statistical analysis. The optical density (OD) for each well was calculated by subtracting the control well values from each plate to the OD value of the well (Garland and Mills, 1991). Microbial activity in each microplate was expressed using average well color development (AWCD) and calculated following the method of Garland and Mills (1991). The Shannon index representing the soil functional diversity was calculated using an OD of 0.25 as threshold for positive response (Garland, 1997).

2.8. Statistical analysis

All statistical analyzes were performed using R software v. 3.1.2 (R Development Core Team, http://www.R-project.org, 2014). Data for each parameter were analyzed using a non-parametric Kruskal-Wallis test. Multiple comparisons among treatments used the Conover post-hoc test ($P < 0.05$) and the *PMCMR* package (Pohlert, 2016). Values in figures and tables correspond to the average of 3 data ($n = 3$) ± standard error. For statistical analyzes, the 31 Biolog substrates were grouped into (1) phenolic compounds, (2) amines, (3) amino acids, (4) polymers, (5) carboxylic acids, and (6) carbohydrates. Finally, a redundancy analysis (RDA) was run to visualize the relationships between the 35 functional variables (31 Biolog substrates, 2 enzymatic activities, 2 functional diversity indices), the 7 environmental variables (C, N and C:N ratio inputs from residues, soil TOC, TN, pH and C:N ratio) and the 36 plots, using the *vegan* package (Oksanen et al., 2015). A Monte Carlo permutation test (999 permutations) was used to assess the significance of environmental variables in accounting for the observed variance of the plots. A post-hoc permutation test was further implemented using the *envfit* function in *vegan* (999 permutations) to seek the individual significance of each

environmental and functional variable in accounting for the observed variance of plots.

3. Results

3.1. Carbon and nitrogen inputs from cover crop and main crop residues

In the absence of N fertilization (N0), both C and N inputs from main crops residues were significantly higher in conventional tillage with standard (CTcc) and leguminous cover crop (CTlcc) than in no till with standard cover crop (NTcc) and under bare fallow (NTbf) (Table 2). N input was also higher in conventional tillage with winter bare fallow (CTbf) than in NTbf. These differences disappeared under Nx treatments, into which only the C:N ratio of the main crop residues was significantly higher in NTcc and no till with leguminous cover crops (NTlcc) than in CTcc and CTlcc.

N, C, and C:N inputs from cover crops did not differ among treatments, irrespective of the fertilization level.

3.2. Soil chemical analyzes

Among N0 treatments, soil TOC contents increased between 2010 and 2015 in NTcc and NTlcc, whilst it did not change in NTbf, CTbf, and CTlcc, and it even decreased in CTcc. Soil TN increased in NTcc and NTlcc, showed little change in CTlcc, and decreased in NTbf, CTbf and CTcc (Fig. 1).

Under Nx fertilization, soil TOC increased in NTcc, NTlcc, and, to a lesser degree, in NTbf; it showed little change in CTlcc and CTcc, and decreased in CTbf. Regarding soil TN, the greatest (positive) values were found in NTcc and NTlcc, whilst the lowest (negative) ones were recorded in CTbf and CTcc.

The soil C:N varied from 9.5 to 10.9 and pH varied from 6.7 to 6.9 but they did not differ among treatments (results not shown).

3.3. Soil enzyme activities

A clear trend was found towards greater dehydrogenase and urease values in NT than in CT treatments, irrespective to N fertilization (Fig. 2). All NT treatments showed significantly higher

Table 2
Carbon and nitrogen input from main crop residues and cover crop aboveground biomass over 5 years.

N rate	Crop	Parameter	H (*P*)	NTbf (Mg ha^{-1})	CTbf (Mg ha^{-1})	NTcc (Mg ha^{-1})	CTcc (Mg ha^{-1})	NTlcc (Mg ha^{-1})	CTlcc (Mg ha^{-1})
N0	Main crop	N input	13.44(0.019)	0.03 ± 0.002[†]a	0.04 ± 0.003bc	0.03 ± 0.001ab	0.05 ± 0.005c	0.03 ± 0.002abc	0.04 ± 0.004c
		C input	12.88(0.024)	2.1 ± 0.12a	2.95 ± 0.29ab	2.22 ± 0.14a	3.16 ± 0.31b	2.67 ± 0.09ab	3.21 ± 0.13b
		C:N input	NS	79.1 ± 2.82	77.94 ± 2.46	82.98 ± 4.17	68.8 ± 4.41	78.95 ± 1.67	75.66 ± 5.36
	Cover crop	N input	NS			0.22 ± 0.02	0.11 ± 0.02	0.22 ± 0.03	0.2 ± 0.005
		C input	NS			1.95 ± 0.189	1.08 ± 0.17	1.96 ± 0.19	1.79 ± 0.04
		C:N input	NS			8.89 ± 0.271	9.9 ± 0.32	9.03 ± 0.31	8.9 ± 0.08
Nx	Main crop	N input	NS	0.06 ± 0.004	0.06 ± 0.004	0.06 ± 0.004	0.06 ± 0.003	0.06 ± 0.004	0.08 ± 0.004
		C input	NS	4.24 ± 0.27	4.48 ± 0.27	4.82 ± 0.10	4.38 ± 0.23	5.29 ± 0.45	4.74 ± 0.07
		C:N input	11.76(0.038)	73.25 ± 1.51ab	69.53 ± 0.69ab	82.07 ± 6.24b	71.88 ± 6.31ab	82.01 ± 1.55b	60.67 ± 2.96a
	Cover crop	N input	NS			0.23 ± 0.02	0.21 ± 0.02	0.22 ± 0.04	0.21 ± 0.02
		C input	NS			2.03 ± 0.18	1.89 ± 0.14	1.95 ± 0.25	1.89 ± 0.19
		C:N input	NS			8.82 ± 0.10	9.15 ± 0.20	8.87 ± 0.33	9.01 ± 0.13

H: value of the Kruskal-Wallis test with the *P* value of significance in brackets (NS: non-significant). Letters give the result of the Conover's post-hoc test at $P < 0.05$. CT: conventional tillage, NT: no-till, bf: bare fallow, cc: cover crop, lcc: leguminous cover crop, N0: no nitrogen fertilization, Nx: conventional nitrogen fertilization.
[†] Standard error of the mean.

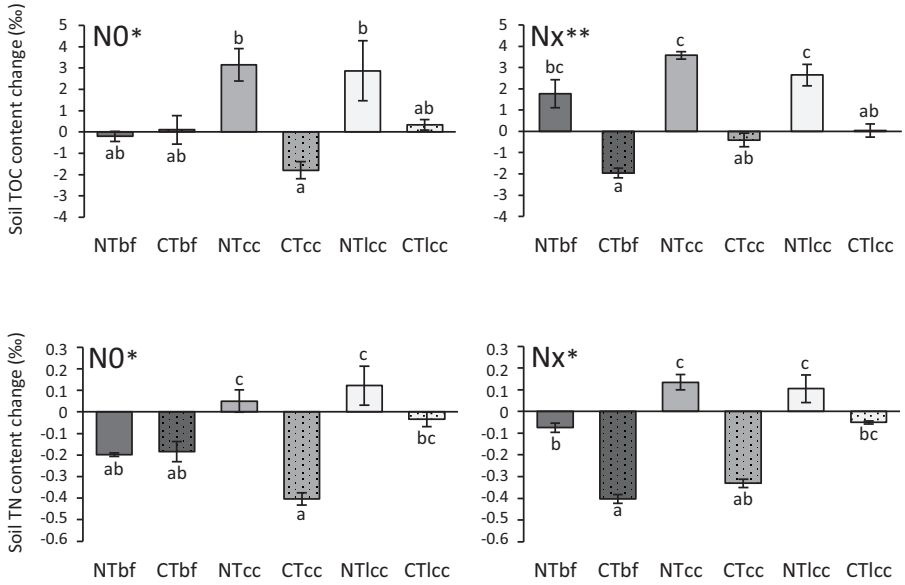

Fig. 1. Changes in soil total organic carbon (top) and total nitrogen (bottom) over the 5-year period. Stars indicate the significance level of the Kruskal-Wallis test ($^{*}P < 0.05$, $^{**}P < 0.01$). Bars with the same letter are not significantly different according to a Conover post-hoc test ($P < 0.05$). TOC: total organic carbon, TN: total nitrogen.

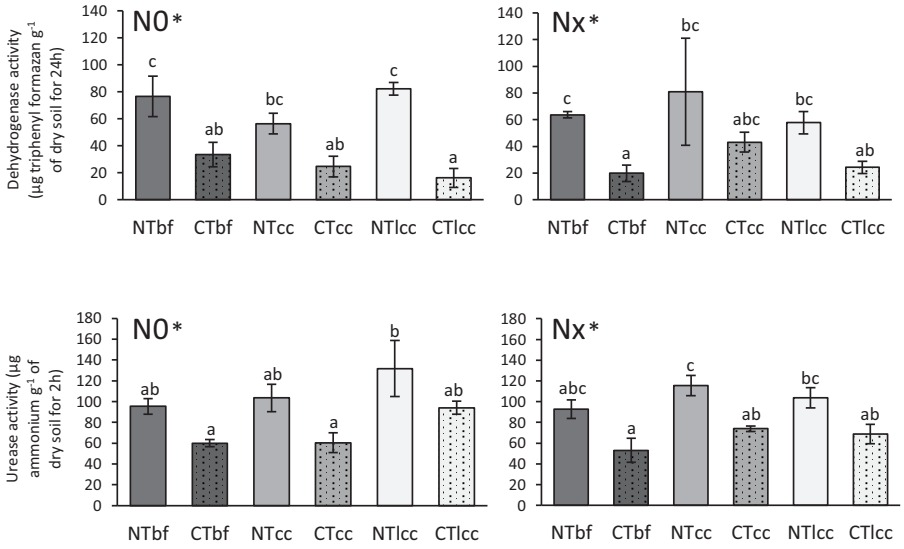

Fig. 2. Enzyme activities of dehydrogenase (top) and urease (bottom). Stars indicate the significance level of the Kruskal-Wallis test (*$P < 0.05$). Bars with the same letter are not significantly different according to a Conover post-hoc test ($P < 0.05$).

dehydrogenase activity than CTlcc (under N0) or CTbf (under Nx). Urease activity was greater in NTlcc than in CTbf and CTcc under N0, and greater in NTcc than in CT plots under Nx.

3.4. Community level physiological profiles

3.4.1. AWCD and shannon functional diversity

In the absence of fertilizer (N0), both average well color development (AWCD) and the Shannon index were the lowest in bare fallow treatments (NTbf and CTbf) (Fig. 3). In cover crop treatments, the Shannon index was similar in both CT and NT plots, whilst CTlcc showed lower AWCD values than the three other treatments. No statistically significant differences among treatments were observed under Nx.

3.4.2. Substrate used by the microbial communities

Among N0 treatments, significant differences were found for phenolic compounds, polymers, and carbohydrates, which were metabolized faster by microbial communities from cover crop treatments. Phenolic compounds were used faster by NTcc than by NTbf communities. Polymers were used faster in CTcc and NTcc than in CTbf and, to a lesser extent, in NTbf communities. Carbohydrates were used faster in NTcc and NTlcc than in bare soil treatments (NTbf and CTbf). In contrast, a lack of difference was found among treatments under Nx (data not shown).

3.5. Relationships between soil chemical and biological parameters

The RDA diagram defined by the first two canonical axes (adjusted $R^2 = 0.25$; cumulated explained variance by 'species data': 40.0%) revealed the relationships between experimental treatments, "functional" and environmental variables (Fig. 4). The variance in the 42 variables (31 Biolog substrates, AWCD, Shannon index, dehydrogenase activity, urease activity, and 7 environmental variables) was significantly related to differences in TOC, TN, N

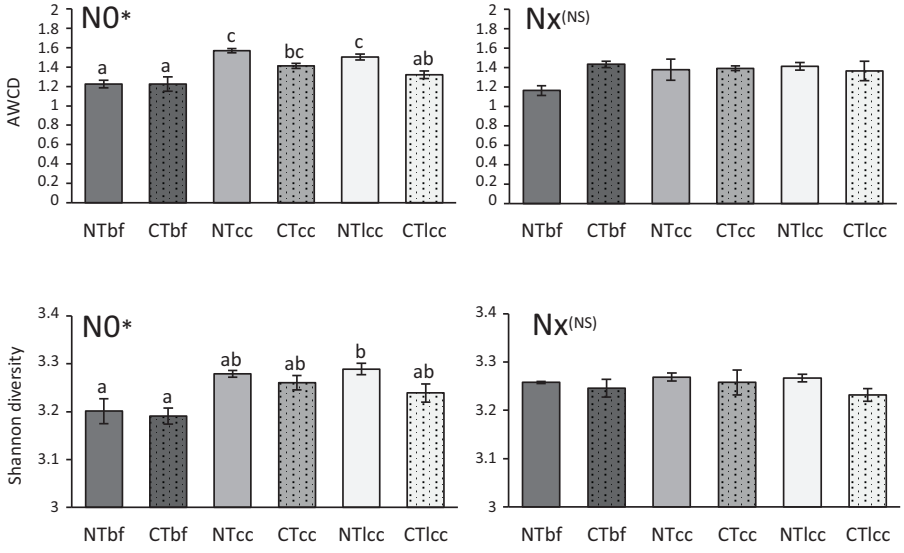

Fig. 3. Microbial functional diversity assessed by total degradation activity (top) and Shannon diversity (bottom) calculated from substrate utilization pattern (31 substrates). Stars indicate the significance level of the Kruskal-Wallis test (NS: non-significant, *$P < 0.05$). Bars with the same letter are not significantly different according to a Conover post-hoc test ($P < 0.05$). AWCD: average well color development.

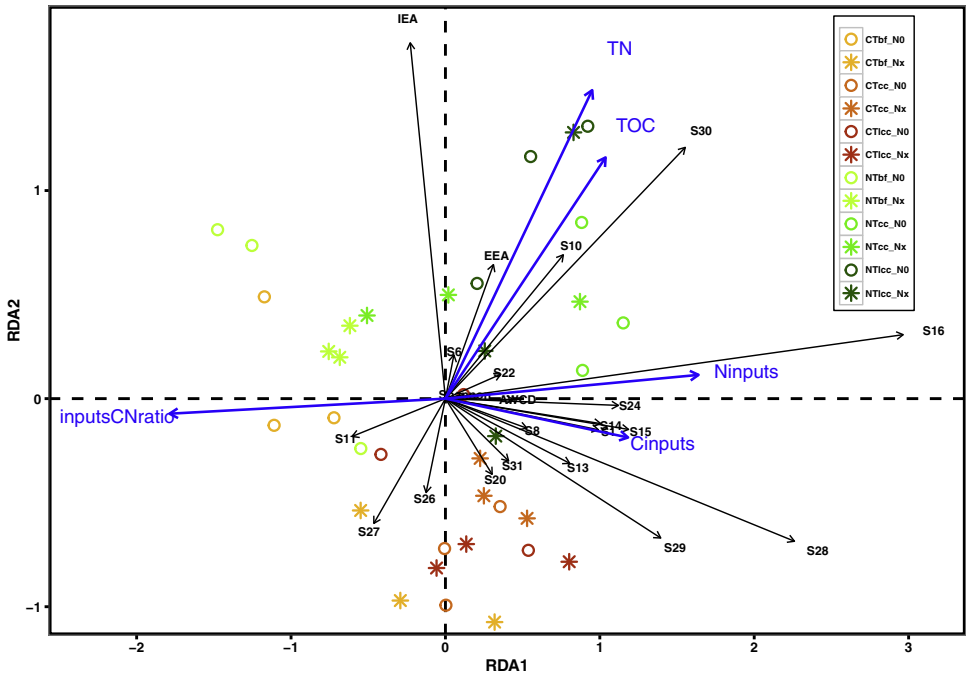

Fig. 4. Redundancy analysis (RDA) of microbial data using soil and input properties as environmental parameters (blue arrows). For the sake of simplicity, only the variables that significantly correlated with canonical axes according to a post-hoc permutation test ($P < 0.05$) are shown. TN: total nitrogen, TOC: total organic carbon, Ninputs: cumulative amount of nitrogen input from crop and cover crop residues, Cinputs: cumulative amount of carbon input from crop and cover crop residues, inputsCNratio: C:N ratio of inputs from crop and cover crops residues. Biolog substrates are **carboxylic acids** (S1: Pyruvic Acid Methyl Ester, S2: D-Galacturonic Acid, S3: γ-Hydroxybutyric Acid, S4: D-Glucosaminic Acid, S5: Itaconic Acid, S6: α-Ketobutyric Acid, S7: D-Malic Acid), **carbohydrates** (S8: β-Methyl-D-Glucoside, S9: D-Galactonic Acid γ-Lactone, S10: D-Xylose, S11: i-Erythritol, S12: D-Mannitol, S13: N-Acetyl-D-Glucosamine, S14: D-Cellobiose, S15: Glucose-1-Phosphate, S16: α-D-Lactose, S17: D,L-α-Glycerol Phosphate), **amino acids** (S18: L-Arginine, S19: L-Asparagine, S20: L-Phenylalanine, S21: L-Serine, S22: L-Threonine, S23: Glycyl-L-Glutamic Acid), **amines** (S24: Phenylethylamine, S25: Putrescine), **polymers** (S26: Tween 40, S27: Tween 80, S28: α-Cyclodextrin, S29: Glycogen), **phenolic compounds** (S30: 2-Hydroxy Benzoic Acid, S31: 4-Hydroxy Benzoic Acid). AWCD: average well color development, IEA: intracellular enzyme activity (dehydrogenase), EEA: extracellular enzyme activity (urease). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

inputs, C:N ratio of inputs ($P < 0.001$), and C inputs ($P < 0.01$), as indicated by permutation tests. There was a clear trend towards stronger dispersion among N0 plots compared with Nx plots, suggesting a greater heterogeneity in the values of the response variables.

The first RDA axis (proportion of constrained variance explained: 63.7%) corresponded to a gradient of increasing C and N inputs (but decreasing input C:N ratio) and increasing AWCD/Shannon. It tended to separate bare fallow plots (negative scores) from no tillage-cover crop plots (most positive scores). Regarding substrate utilization preferences, this first axis mostly correlated positively with several carbohydrates (S16: α-D-Lactose, S15: Glucose-1-Phosphate, S14: D-Cellobiose, S13: N-Acetyl-D-Glucosamine, S8: β-Methyl-D-Glucoside) and polymers (S28: α-Cyclodextrin, S29: Glycogen), as well as the phenolic compound 2-Hydroxy Benzoic Acid (S30), the amine Phenylethylamine (S24), and the carboxylic acid Pyruvic Acid Methyl Ester (S1). It negatively correlated with one carbohydrate (S11: i-Erythritol).

RDA axis 2 (18.6%) was associated with a gradient of increasing soil TN, TOC, dehydrogenase activity, and urease activity, and decreasing soil C:N ratio and pH (the last two were non-significant according to permutations test). It clearly separated CT (negative scores) from NT (positive scores) treatments. Among the latter, plots with cover crops receiving no fertilizer exhibited the highest scores. This axis positively correlated with the carboxylic acid α-Ketobutyric Acid (S6), the phenolics 2-Hydroxy Benzoic Acid (S30), and the carbohydrate D-Xylose (S10). It negatively correlated with the polymers Tween 40 (S26) and Tween 80 (S27), the amino-acid L-Phenylalanine (S20), and the phenolic compound 4-Hydroxy Benzoic Acid (S31).

4. Discussion

The present controlled experiment is one of the first to shed light on the combined effects of soil tillage and cover crops, under two N fertilization rates, on chemical and biological properties over an entire industrial crop rotation including cereals (wheat and corn), pea, and flax over a 5 year-period. It reveals clear trends towards (i) increased soil TN and TOC contents, and increased enzymatic activities that are also associated with the use of more diverse substrates when NT with or without N fertilizer is applied compared with CT soils; (ii) increased soil C and N inputs and functional activity/diversity of soil microbes, which are associated with the use of more diverse and complex substrates when a cover crop is associated to NT, compared with winter bare fallow. However, the single time point sampling must be taken into account within the interpretation of the discussion and conclusions related to the results obtained. Hereafter, these findings and their relevance for sustainable agriculture are discussed.

4.1. No tillage coupled with cover crops increases soil C and N contents

Increased soil C and N contents were found in both NTcc and NTicc plots, independently from the species mixture used in cover crops and irrespective of N fertilization (Fig. 1), a beneficial impact already reported in the literature (Mazzoncini et al., 2011; Mbuthia et al., 2015). Consistently, C and N inputs from cover crops were similar between the two mixtures and among treatments, indicating that a winter cover crop can compensate for the organic C and N losses associated with main crop exportation (Table 2, Verzeaux et al., 2016). NT and cover crops may enhance the soil C

storage by reducing oxidative stress (Mangalassery et al., 2015) and improving aggregate stability (Martens, 2000), thus reducing the aggregate disruption induced by plowing (Six et al., 2000). Among CT plots, only the cover crops enriched in leguminous species (CTlcc) impeded the soil C and N depletion induced by intensive tillage. The use of a mulch enriched with leguminous species has indeed been shown to increase soil N and C contents in soil (Sanginga et al., 1997; Mancinelli et al., 2015), as a plausible consequence of the high N contained in their roots, stems and nodules. Consistently, the positive effect of cover crops disappears under bare fallow, since the soil N content decreased over the rotation's duration, irrespective of fertilization and tillage. The soil C content followed the same trend except in NT plots receiving N fertilizer, where the main crop residues also exhibited the higher C:N input. Interestingly, this was the only case in which fertilization was beneficial.

4.2. No tillage increases soil enzymatic activity

NT plots exhibited greater soil enzymatic activity than CT plots through increased dehydrogenase and urease, irrespective of N fertilization (Fig. 2). This is consistent with Das et al. (2014), who found that the dehydrogenase activity, which reflects microbial activity and oxidation-reduction reactions in soil, was 107% greater under NT than under CT. In association with cover crops, NT improved urease activity as compared with CT without cover crops, a result already reported by Hamido and Kpomblekou (2009). Overall, our results are consistent with Janusauskaite et al. (2013), since SEA were mainly impacted by soil tillage among other variables. This result is explained by the greater content of organic matter and nutrient availability associated with NT, the lack of disruption of soil layers, and the less oxidizing environment, which can stabilize the pool of extracellular enzymes (e.g. urease) (Melero et al., 2009; Mangalassery et al., 2015). Soil nutrient cycling involves enzymes that are related to certain soil properties such as moisture and organic matter content. Changes in soil enzyme activity may thus affect chemical and biological properties (Bergstrom et al., 1998) and explain the positive correlation between urease activity, TOC, and TN, and to a lesser degree, between dehydrogenase and TN (Costa et al., 2013).

4.3. Cover crops increase microbial substrate use diversity in the absence of N fertilization

Microbial substrate use diversity clearly differed between N0 treatments, being the lowest in bare fallow and the greatest in cover crop-NT plots (Fig. 3). This is explained by the high contents of soil TN and TOC in the latter compared with the other treatments, which may increase the diversity of substrate-richness and thus induce more microbial enzymes (Diosma et al., 2006; Govaerts et al., 2007). This is supported by the greater metabolism of phenolic compounds and carbohydrates (under NT) and polymers (under CT) as carbon sources in plots with a standard cover crop (Fig. 4). This beneficial effect of cover crops disappears in CT treatments, suggesting that microbial functional diversity is impaired by soil tillage-induced disturbance via aggregate disruption, compaction, or buried litter (Mangalassery et al., 2015). Remarkably, neither functional diversity nor substrate preference differed among Nx treatments, indicating a functional convergence of the soil microbial community under N fertilization.

4.4. No tillage together with cover crops ensures sustainable management of agricultural soils

RDA results highlighted the combined effect of N fertilization, plowing, and winter cover crops on soil chemical properties and biological activity. Overall, treatments including both cover crops and no tillage performed the best with respect to soil C and N contents, enzymatic activity and the diversity of substrates used as C sources, independently from N fertilization (see the top-right part of the RDA diagram on Fig. 4). In these conditions, carbohydrates were heavily used by microorganisms in the topsoil (0–10 cm), which is composed of a rich organic litter and contains sugars released from its decomposition and rhizodeposits, hence conferring greater availability of these compounds for soil microbes. Carbohydrates are also known for maintaining and stimulating soil microbial activities in the rhizosphere and detritusphere (Guinina and Kuzyakov, 2015).

Opposingly, conventionally-tilled bare fallow systems negatively impacted the soil, by reducing C and N contents and enzymatic activity (see the bottom-left part of the RDA diagram in Fig. 4). In bare fallows, the high C:N ratio of inputs (i.e. close to 80) is a consequence of the incorporation of only wheat straw residues, which is not compensated for by the low C:N ratio of cover crops returned to the soil. It has been indeed shown that available N and P released from decomposition of low C:N inputs (i.e. cover crop residues) is used by microorganisms for the decomposition of high C:N inputs (i.e. main crop residues) (Schimel and Haettenschwiler, 2007). It is likely that the nutrient release following cover crop decomposition increases the soil N content and microbial activity, which in turn improves the decomposition of high C:N ratio residues. This hypothesis is supported by the legacy effect of the C:N quality of organic residues on the ability of microbial communities to decompose the subsequent amendments (Marschner et al., 2015). The higher degradation of polymers (e.g. Tween 40, Tween 80, α-Cyclodextrin and Glycogen) observed in CT plots including cover crops and in CTbf with N fertilization is also consistent with this hypothesis, since these substrates represent more stable or recalcitrant C compounds. Especially tween 40 and tween 80 are molecules that do not resemble plant-derived polymers (Nunan et al., 2015), but characterize processed organic matter (Grandy and Neff, 2008). Their greater use may be a consequence of the greater availability of N following N fertilization in CTbf, decomposition of cover crops in CTcc/CTlcc (Marschner et al., 2015), and/or of the aggregate disruption under plowing, which eases the accessibility of old organic matter to microorganisms (Six et al., 2000). For other polymers, such as α-cyclodextrin and glycogen, the observed higher degradation in CT can simply reflect "young" polymers originating from fresh plant inputs (Nunan et al., 2015; Grandy and Neff, 2008).

The first axis of the RDA strongly separated the plots without cover crops (i.e. CTbf, NTbf, see the left part of the RDA diagram in Fig. 4) from others. These plots were characterized by a higher C:N ratio of inputs (only wheat straws were returned to the soil) and by a higher utilization of the carbohydrate i-Erythrytol. However, the plots which received cover crops (see the right part of the RDA diagram in Fig. 4) which were characterized by lower C:N ratio of inputs, and overall, by higher C and N inputs, significantly enhanced AWCD and Shannon diversity. In the same plots, the degradation of carboxylic acid Pyruvic Acid Methyl Ester (S1), the carbohydrates D-Cellobiose (S14), Glucose-1-Phosphate (S15), and α-D-Lactose (S16), the amino acid L-Threonine (S22), and the amine Phenylethylamine (S24), was improved. Frac et al. (2012) reported that an increase of soil diversity following organic inputs (i.e. cover crops in our study) resulted from the development of different microbiota. This is consistent with our study since uncovered plots are characterized by the degradation of one sugar alcohol (i-Erythrytol) compared with the large panel of substrates listed above which were preferentially degraded in covered plots.

5. Conclusions

In summary, without N fertilization, conventional tillage plus standard cover crops strongly decreased the soil CN content and markedly increased potential soil polymer degradation. Under the same N fertilization, no-tillage with standard and legume-enriched cover crops improved degradation activity of soil microorganisms (AWCD, carbohydrates and phenolic compounds degradation) and their functional diversity. These results indicate that, without N fertilization, cover crops are required to prevent C and N depletion and to maintain microbial functional activities and diversity.

Irrespectively of N fertilization, cover crops enriched with legumes prevented the strong decrease of C and N content caused by plowing. No-till combined with cover crops increases enzyme activities.

Thereby, under a crop rotation characterized by a low organic C input, a gradual reduction of synthetic N fertilizers is compensated with an increase of cover crop N input must be encouraged to reduce the risk of soil fertility depletion associated with conventional farming practices. However, it is known that soil microbial functional responses may vary seasonally, so, studies including sampling collections throughout seasons and years are required to investigate further the links between cropping systems and microbial communities' behavior at long term.

Acknowledgments

The authors thank all the students who have been involved in this work through their internships since 2009. Pierre Berrezig and Shân Williams (Université de Picardie Jules Verne) as well as Lyla Rothschild are thanked for their critical reading of this manuscript. This research was funded by Bonduelle and Syngenta companies within the framework of the collaborative project *VEGESOL*. The PhD studies of Elodie Nivelle are funded by a scholarship from the French Ministry of Higher Education and Research.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.08.004.

References

Acosta-Martinez, V., Mikha, M.M., Sistani, K.R., Stahlman, P.W., Benjamin, J.G., Vigil, M.F., Erickson, R., 2011. Multi-location study of soil enzyme activities as affected by types and rates of manure application and tillage practices. Agriculture 1, 4–21.

Alef, K., Nannipieri, P., 1998. Urease activity. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Acad. Press Harcourt Brace & Company, Publishers, London, pp. 316–320.

Alvarez, G., Chaussod, R., Cluzeau, D., Godden, B., Lemarié, C., Metzger L., Nicolardot, B., Parat, J., Salducci, X., 2002. Activités biologiques et fertilité des sols. Intérêts et limites des méthodes analytiques disponibles. « Etat et activités biologiques des sols ». ITAB, Online document.

Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57, 233–266.

Bayer, C., Martin-Neto, L., Mielniczuk, J., Pavinato, A., Dieckow, J., 2006. Carbon sequestration in two Brazilian Cerrado soils under no-till. Soil Tillage Res. 86, 237–245.

Bergstrom, D.W., Monreal, C.M., King, D.J., 1998. Sensitivity of soil enzyme activities to conservation practices. Soil Sci. Soc. Am. J. 62, 1286–1295. doi:http://dx.doi.org/10.2136/sssaj1998.03615995006200050020x.

Casida, L.E., Klein, D.A., Santoro, T., 1964. Soil dehydrogenase activity. Soil Sci. 98, 371–376.

Chaussod, R., 1996. La qualité biologique des sols: evaluation et implications. Etude et Gestion des Sols 3, 261–277.

Chen, H., Hou, R., Gong, Y., Li, H., Fan, M., Kuzyakov, Y., 2009. Effects of 11 years of conservation tillage on soil organic matter fractions in wheat monoculture in Loess Plateau of China. Soil Tillage Res. 106, 85–94. doi:http://dx.doi.org/10.1016/j.still.2009.09.009.

Costa, D., Freitas, H., Paulo, J., 2013. Influence of seasons and land-use practices on soil microbial activity and metabolic diversity in the Montado ecosystem. Eur. J. Soil Biol. 59, 22–30. doi:http://dx.doi.org/10.1016/j.ejsobi.2013.08.003.

Das, A., Lal, R., Patel, D.P., Idapuganti, R.G., Layek, J., Ngachan, S.V., Ghosh, P.K., Bordoloi, J., Kumar, M., 2014. Effects of tillage and biomass on soil quality and productivity of lowland rice cultivation by small scale farmers in North Eastern India. Soil Tillage Res. 143, 50–58. doi:http://dx.doi.org/10.1016/j.still.2014.05.012.

Diosma, G., Aulicino, M., Chidichimo, H., Balatti, P.A., 2006. Effect of tillage and N fertilization on microbial physiological profile of soils cultivated with wheat. Soil Tillage Res. 91, 236–243.

Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A., 2004. Carbon input to soil may decrease soil carbon content. Ecol. Lett. 7, 314–320.

Frac, M., Oszust, K., Lipiec, J., 2012. Community level physiological profiles (CLPP), characterization and microbial activity of soil amended with dairy sludge. Sensors 12, 3253–3268.

Garland, J.L., Mills, A.L., 1991. Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon source utilization. Appl. Environ. Microbiol. 57, 2351–2359.

Garland, J.L., 1997. Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbiol. Ecol. 24, 289–300.

Gomez, E., Garland, J., Conti, M., 2004. Reproducibility in the response of soil bacterial community-level physiological profiles from a land use intensification gradient. Appl. Soil Ecol. 26, 21–30.

Govaerts, B., Mezzalama, M., Unno, Y., Sayre, K.D., Luna-Guido, M., Vanherck, K., 2007. Influence of tillage, residue management, and crop rotation on soil microbial biomass and catabolic diversity. Appl. Soil Ecol. 37, 18–30.

Grandy, S.A., Neff, J.C., 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci. Total Environ. 404, 297–307.

Grayston, S.J., Griffith, G.S., Mawdsley, J.L., Campbell, C.D., Bardgett, R.D., 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biol. Biochem. 33, 533–551. doi:http://dx.doi.org/10.1016/S0038-0717(00)00194-2.

Guinina, A., Kuzyakov, Y., 2015. Sugars in soil and sweets for microorganisms Review of origin, content, composition and fate. Soil Biol. Biochem. 90, 87–100.

Hamido, S.A., Kpomblekou, A.K., 2009. Cover crop and tillage effects on soil enzyme activities following tomato. Soil Tillage Res. 105, 269–274. doi:http://dx.doi.org/10.1016/j.still.2009.09.007.

Huynh, T.M.D., 2009. Impact des métaux lourds sur les interactions plante/ver de terre/microflore tellurique. Océan, Atmosphère. Université Paris-Est, France.

Janusauskaite, D., Kadziene, G., Auskalniene, O., 2013. The effect of tillage system on soil microbiota in relation to soil structure. Pol. J. Environ. Stud. 22, 1387–1391.

Jordan, C.F., 2004. Organic farming and agroforestry: alleycropping for mulch production for organic farms of southeastern United States. Agrofor. Syst. 61/62, 79–90.

Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil Tillage Res. 61, 61–76.

Kusliene, G., Rasmussen, J., Kuzyakov, Y., Eriksen, J., 2014. Medium-term response of microbial community to rhizodeposits of white clover and ryegrass and tracing of active processes induced by 13C and 15N labelled exudates. Soil Biol. Biochem. 76, 22–33. doi:http://dx.doi.org/10.1016/j.soilbio.2014.05.003.

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: concept & review. Soil Biol. Biochem. 83, 184–199. http://dx.doi.org/10.1016/j.soilbio.2015.01.025.

Kuzyakov, Y., 2010. Priming effects: interactions between living and dead organicmatter. Soil Biol. Biochem. 42, 1363–1371.

Lagomarsino, A., Moscatelli, M.C., Di Tizio, A., Mancinelli, R., Grego, S., Marinari, S., 2009. Soil biochemical indicators as a tool to assess the short-term impact of agricultural management on changes in organic C in a Mediterranean environment. Ecol. Indic. 9, 518–527.

Machet J.M., Dubrulle P., Louis P., 1990. Azobil®: a computer program for fertiliser N recommendations based on a predictive balance sheet method. In: Scaife A. Proceedings of the 1 st Congress of the European Society of Agronomy, 5th–7th December 1990, Paris, France.Colmar, France: ESA/INRA/CEC.

Mancinelli, R., Marinari, S., Brunetti, P., Radicetti, E., Campiglia, E., 2015. Organic mulching, irrigation and fertilization affect soil CO2 emission and C storage in tomato crop in the Mediterranean environment. Soil Tillage Res. 152, 39–51. doi:http://dx.doi.org/10.1016/j.still.2015.04.001.

Mangalassery, S., Mooney, S.J., Sparkes, D.L., Fraser, W.T., Sjogersten, S., 2015. Impacts of zero tillage on soil enzyme activities, microbial characteristics and organic matter functional chemistry in temperate soils. Eur. J. Soil Biol. 68, 9–17.

Marinari, S., Masciandaro, G., Ceccanti, B., Grego, S., 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. Bioresour. Technol. 72, 9–17.

Marinari, S., Mancinelli, R., Campiglia, E., Grego, S., 2006. Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy. Ecol. Indic. 6, 701–711.

Marschner, P., Hatam, Z., Cavagnaro, T.R., 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol. Biochem. 88, 169–177.

Martens, D.A., 2000. Plant residue biochemistry regulates soil carbon cycling and carbon sequestration. Soil Biol. Biochem. 32, 361–369. doi:http://dx.doi.org/10.1016/S0038-0717(99)00162-5.

Mathew, R.P., Feng, Y., Githinji, L., Ankumah, R., Balkcom, K.S., 2012. Impact of No-tillage and conventional tillage systems on soil microbial communities. Appl. Environ. Soil Sci. 2012, 1–10. doi:http://dx.doi.org/10.1155/2012/548620.

Mazzoncini, M., Sapkota, T.B., Bàrberi, P., Antichi, D., Risaliti, R., 2011. Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. Soil Tillage Res. 114, 165–174. doi:http://dx.doi.org/10.1016/j.still.2011.05.001.

Mbuthia, L.W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biol. Biochem. 89, 24–34. doi:http://dx.doi.org/10.1016/j.soilbio.2015.06.016.

Melero, S., Lopez-Garrido, R., Murillo, J.M., Moreno, F., 2009. Conservation tillage: short-and long-term effects on soil carbon fractions and enzymatic activities under Mediterranean conditions. Soil Tillage Res. 104, 292–298.

Nannipieri, P., Eldor, P., 2009. The chemical and functional characterization of soil N and its biotic components. Soil Biol. Biochem. 41, 2357–2369.

Nautiyal, C.S., Chauhan, P.S., Bhatia, C.R., 2010. Changes in soil physico-chemical properties and microbial functional diversity due to 14 years of conversion of grassland to organic agriculture in semi-arid agroecosystem. Soil Tillage Res. 109, 55–60. doi:http://dx.doi.org/10.1016/j.still.2010.04.008.

Nunan, N., Lerch, T.Z., Pouteau, V., Mora, P., Changey, F., Kätterer, T., Giusti-Miller, S., Herrmann, A.M., 2015. Metabolising old soil carbon: Simply a matter of simple organic matter? Soil Biol. Biochem. 88, 128–136.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2015. Vegan: Community Ecology Package Version 2. 3-2. http://cran.r-project.org/.

Pohlert, T., 2016. PMCMR: Calculate Pairwise Multiple Comparisons of Mean Rank Sums Version 4.1. http://cran.r-project.org/.

Reganold, J.P., Elliott, L.F., Unger, Y.L., 1987. Long-term effects of organic and conventional farming on soil erosion. Nature 330, 370–372.

Salinas-Garcia, J.R., Hons, F.M., Matocha, J.E., 1997. Long-term effects of tillage and fertilization on soil organic matter dynamics. Soil Sci. Soc. Am. J. 61, 152–159.

Sanginga, N., Dashiell, K., Okogun, J.A., Thottapilly, G., 1997. Nitrogen fixation and nitrogen contribution in promiscuous soybeans in Southern Guinea Savanna of Nigeria. Plant Soil 195, 257–266.

Schimel, J.P., Haettenschwiler, S., 2007. Nitrogen transfer between decomposing leaves of different N status. Soil Biol. Biochem. 39, 1428–1436.

Schinner, F., Ohlinger, R., Kandeler, E., Margesin, R., 2012. Methods in Soil Biology. Springer.

Six, J., Elliott, E., Paustian, K., 2000. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol. Biochem. 32, 2099–2103. doi:http://dx.doi.org/10.1016/S0038-0717(00)00179-6.

Staben, M.L., Bezdicek, D.F., Smith, J.L., Fauci, M.F., 1997. Assessment of soil quality in conservation reserve program and wheat-fallow soils. Soil Sci. Soc. Am. J. 61, 124–130.

Sun, R., Zhang, X.X., Guo, X., Wang, D., Chu, H., 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. Soil Biol. Biochem. 88, 9–18. doi:http://dx.doi.org/10.1016/j.soilbio.2015.05.007.

Tabatabai, M.A., 1994. Soil enzymes. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. Soil Sci. Soc. Am. J., Madison, pp. 775–833.

Verzeaux, J., Alahmad, A., Habbib, H., Nivelle, E., Roger, D., Lacoux, J., Decocq, G., Hirel, B., Catterou, M., Spicher, F., Dubois, F., Duclercq, J., Tetu, T., 2016. Cover crops prevent the deleterious effect of nitrogen fertilisation on bacterial diversity by maintaining the carbon content of ploughed soil. Geoderma 281, 49–57. doi:http://dx.doi.org/10.1016/j.geoderma.2016.06.035.

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biol. Biochem. 92, 41–49.