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Responses of soil properties and crop yields to different inorganic and organic amendments in a Swiss conventional farming system

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ABSTRACT

In agro-ecosystems, fertilization practices are crucial for sustaining crop productivity. Here, based on a 50-year long-term experiment, we studied the influence of fertilization practices (inorganic and/or organic) and nitrogen (N) application rates on (i) soil physicochemical properties, (ii) microbial and earthworm communities and (iii) crop production. Our results showed that soil organic carbon content was increased by incorporation of crop residues (+2.45%) and farmyard manure application (+6.40%) in comparison to the use of mineral fertilizer alone. In contrast, soil carbon stock was not significantly affected by these fertilization practices. Overall, only farmyard manure application improved soil physicochemical properties compared to mineral fertilization alone. Soil microbial population was enhanced by the application of organic amendments as indicated by microbial biomass and phospholipid-derived fatty acids contents. The fertilization practices and the N application rates affected significantly both the biomass and composition of earthworm populations, especially the epigeic and endogeic species. Finally, farmyard manure application significantly increased crop yield (+3.5%) in comparison to mineral fertilization alone. Crop residue incorporation rendered variable but similar crop yields over the 50-year period. The results of this long-term experiment indicate that the use of organic amendments not only reduces the need for higher amount of mineral N fertilizer but also improves the soil biological properties with direct effects on crop yield.

1. Introduction

In agro-ecosystems, fertilization practices influence soil quality and crop productivity. Depending on the nature of the applied fertilizer (organic or inorganic), modifications of soil properties have been observed over the long-term under different pedoclimatic conditions (Rasmussen et al., 1998). The specialization of farming activities has led to the spatial segregation of crop and livestock productions, thus causing a drastic decrease in the use of animal manure as organic amendment in many conventional farms in Switzerland (Vullioud et al., 2004) and more generally in western Europe (Chesworth, 2008). As a result, the necessity for maintaining soil organic carbon (SOC) at sufficient levels in arable fields has become an important issue.

Soil organic carbon is a crucial parameter for soil fertility as it enhances soil physical, chemical and biological properties (Birkhofer et al., 2008; Lützow et al., 2006). Indeed, an increase of SOC content may promote crop yields through increased nutrient supply (Haynes and Naidu, 1998; Maltas et al., 2013) and improved water retention capacity (Edmeades, 2003). Furthermore, SOC contributes to the attenuation of environmental impacts of farming activities so that, for example, soil erosion is reduced (Six et al., 2002) and nutrient leaching is minimized (Drinkwater et al., 1998). Soil organic matter (SOM) is also the most important terrestrial pool for carbon (C) sequestration (Lal, 2002), and its management is therefore relevant for the mitigation of climate change (Lal, 2004).

The dynamic of soil organic carbon (SOC) is directly related to the amount of C supplied to the soil and the rate of SOM decomposition (Lal, 2002). In agro-ecosystems, fertilization practices influence SOC content by modifying both C inputs and

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losses (Follett, 2001). In general, the application of organic amendments such as crop residues and/or farmyard manure increases significantly SOC (Diacono and Montemurro, 2010; Lützow et al., 2006; Maltas et al., 2013), whereas the long-term application of only inorganic fertilizers often has the opposite effect (Edmeades, 2003). The decomposition rate of SOM is influenced by many factors such as (i) the chemical composition and the molecular structure of organic matter (OM) (Kögel-Knabner, 2002); (ii) the physical protection of OM within soil aggregates (Six et al., 2002) and/or (iii) the soil biological activity (Condron et al., 2010). Since processes involved in the accumulation and/or the mineralization of SOM can be particularly slow (Diacono and Montemurro, 2010; Rasmussen et al., 1998), the relative importance of soil properties and farm practices on SOC dynamics should be evaluated in long-term experiments.

Green manure and/or crop residues incorporation have been proposed as alternative cropping systems in order to reduce SOC loss when farmyard manure is unavailable (Drinkwater et al., 1998; Zhao et al., 2009). However, these practices are relatively recent and most of the studies regarding the effect of crop residue incorporation on SOC have been performed on relatively short timescales, generally over a decade or two. The long term effect of crop residues incorporation is therefore still under discussion (Liu et al., 2014; Powlson et al., 2011) especially if pedoclimatic conditions are taken into account (Poeplau et al., 2015).

Soil biological communities are particularly sensitive to agricultural practices sustaining SOC (Mäder et al., 2002), as soil SOC is their principal feeding substrate. Biological processes are crucial for the maintenance of soil fertility due to their role in nutrient cycling. For instance, soil biota plays a major role in the mineralization of the SOM, in the fixation of atmospheric nitrogen, or in the reduction of nutrient losses by immobilizing temporarily nutrients in the biomass. In addition, particular biological communities enhance plant nutrient uptake (*e.g.* mycorrhizal fungi) (Johansson et al., 2004) or improve soil texture (*e.g.* earthworms) (Bertrand et al., 2015). As consequence, diversified biological communities in agroecosystems can offer a panel of ecological services that ultimately enhance the sustainability of crop production (Altieri, 1999).

Nevertheless, intensive agricultural practices in conventional farming systems negatively impact the soil biological communities of agroecosystems due to the disturbance induced by chemical fertilizer, pest control measures or soil tillage (Bertrand et al., 2015; Geisseler and Scow, 2014; Zhao et al., 2014). In Switzerland, the DOK ("*biologisch-Dynamische, Organisch-biologische und Konventionelle*") experiment (Mäder et al., 2002) compares the long term effects of different organic and conventional farming systems, but little is still known about the comparative impact of different variants of conventional fertilization management on SOC and biological properties. Therefore, the objectives of the present study were to evaluate the long-term influence of fertilization practices on (i) soil C storage and soil physic-chemical properties, (ii) microbial and earthworm communities and (iii) crop yields. In this study, results of the oldest long-term field experiment in Switzerland, which started in 1963, are presented. In a conventional farming system, the effects of two different organic amendments (farmyard manure application and crop residues incorporation) were compared to the conventional use of mineral fertilizers alone.

2. Materials and methods

2.1. Site description and experimental design

The experiment was established in 1963 by the Swiss Research Station Agroscope in Changins (46°24′5.28″N, 06°14′7.47″E, altitude: 445 m) on a Calcaric Cambisol (FAO classification) characterized by 196 g kg^{-1} of clay, 345 g kg^{-1} of sand, 20.3 g kg^{-1} of SOC and 7.3 of pH in the plough layer (0–20 cm). During the experimental period 1963–2013, mean annual rainfall and temperature were, respectively, 1004 mm and 9.5 °C. Before the establishment of the experiment, the area was covered with grassland (alfalfa field). Winter wheat was planted one year before the beginning of the experiment as a buffer crop. The experimental design has undergone some modifications since its establishment. The original design of the experiment (1963–1970) was a randomized block with three main fertilization practices (FP) and four replications. The three FP were: (i) mineral fertilizers alone (*MIN*), (ii) crop residues incorporation with reduced mineral fertilization (*RES*) and (iii) cattle farmyard manure application (10 t ha^{-1} $year^{-1}$) with reduced mineral fertilization (*FYM*). In 1971, two different levels of mineral nitrogen (N) fertilization were introduced as sub-treatments to all three fertilizer practices, thus converting the experimental design into a split-plot one, where the size of each subplot was 55 m^2 (5 m x 11 m). A dose of 120 kg N ha^{-1} (*N120*), considered optimal according to the Swiss fertilization guidelines for wheat crop (Sinaj et al., 2009), and a limiting dose of 50 kg N ha^{-1} (*N50*) were applied one or two times during the growth period as ammonium nitrate (NH_4NO_3) according to crop type.

2.2. Fertilization and agronomic practices

The crop rotation changed twice over the whole experimental period. Initially a 'wheat-maize-wheat' rotation was established for the 1963–1972 period, followed by the integration of sugar beet from 1972 to 2008, which was then finally replaced by rapeseed in 2008 (Table 1).

At harvest, crop residues were systematically removed from the soil in the case of *MIN* and *FYM* main-treatments, but were incorporated with the plough layer (0–20 cm) for the *RES* main-treatment. *FYM* (composted cattle manure originating from loose housing) was applied at the rate of 10 t ha^{-1} $year^{-1}$ every two or four years (Table 1) and incorporated into the soil with the plough before planting. Finally, the soil was prepared with a rotary harrow (5 cm) for planting.

Phosphorus (P) and potassium (K) fertilizer rates were determined according to the Swiss fertilization guidelines (Sinaj et al., 2009) and were the same for all treatments. The recommended P and K fertilizer rates from the Swiss fertilization guidelines were adjusted (reduced) to account for the contribution from organic amendments (*RES* and *FYM*). The average amounts of P and K applied as mineral fertilizers are reported in Table 1. Triple superphosphate [$Ca(H_2PO_4)_2{\cdot}H_2O$] and salt of potash (KCl) were applied prior to planting for the summer crops (maize, sugar beet) and during the growing period for other crops (winter wheat and rapeseed). Herbicides were applied depending on weed pressure, and standard phytosanitary protection was applied according to integrated crop protection principles (Häni et al., 1990).

2.3. Soil sampling and analyses

Soils were sampled in early August 2013, after the harvest of winter wheat, from the plough layer (0–20 cm). Ten cores with a diameter of 2.5–3 cm were randomly taken within each sub-plot. Plant residues were removed from the soil and the individual samples mixed to form a composite sample per plot. Samples were oven-dried at 40 °C during 48 h, sieved at 2 mm and analysed for different soil properties (Table 2).

Soil bulk density was measured in early April 2014. Samples were taken from the central part of each plot away from any wheel track. A single pit was dug for each replicate and 6 cm diameter

Table 1
Applied phosphorus and potassium fertilization (kg ha^{-1}) over the experimental period.

Period	Crop	MIN		RES		FYM		
		P	K	P	K	P	K	Manure application
1963–1971	Winter Wheat	39	125	39	125	39	125	30 t/3 years
	Maize	39	125	39	125	39	125	
1972–1975	Sugar beet	44	349	31*	166*	31	166	40 t/4 years
	Winter Wheat	35	100	31*	125*	22	83	
	Maize	52	166	35*	50*	26	166	
	Spring Wheat	35	100	35*	100*	35	100	
1976–1995	Sugar beet	44	249	39*	199*	24	174	40 t/4 years
	Winter Wheat	31	100	26*	0*	11	25	
	Maize	44	174	39*	125*	44	125	
	Winter Wheat	31	100	26*	66*	26*	66*	
1996–2007	Sugar beet	52	224	48*	33*	39	100	40 t/4 years
	Winter Wheat	17	75	0*	0*	0	0	
	Maize	52	224	48*	33*	39	100	
	Winter Wheat	17	75	0*	0*	0*	0*	
Since 2008	Rapeseed	52	224	48*	33*	39	100	20 t/2 years
	Winter Wheat	17	75	0*	0*	0*	0*	
	Maize	52	224	48*	33*	39	100	
	Winter Wheat	17	75	0*	0*	0	0	

Asterisks (*) indicate that crop residues of the preceding year were incorporated into the soil after their harvest.

cylinders were inserted into the soil at 2–8 and 12–18 cm below soil surface. Soil samples were oven-dried at 105 °C during 72 h and weighed (FAL et al., 2004). The values of both depth increments (2–8 cm and 12–18 cm) were averaged for further calculations.

During the same time, moist soil samples from the plough layer were collected for the estimation of microbial nutrient biomass and the determination of phospholipid fatty acids (PLFAs). The early spring period (April) is considered ideal for inter-comparison of treatments with regards to soil biology in agro-ecosystems (Kaiser et al., 1995). Samples were kept moist and sieved at 2 mm. Samples for microbial biomass estimation were stored at 4 °C and those used for PLFA determination were frozen at −20 °C until laboratory analysis.

2.4. Soil microbial biomass nutrients and microbial community structure

Microbial biomass carbon (C_{mic}), nitrogen (N_{mic}) and phosphorus (P_{mic}) were measured according to the fumigation-extraction procedure (Vance et al., 1987). Total C and N of both fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 solution according to a soil:solution ratio of 1:10.

Table 2
Effect of the treatments on soil properties in the plough layer (0–20 cm) in 2013.

Analyses	FP	MIN		RES		FYM		*p*-value		
	N	N50	N120	N50	N120	N50	N120	FP	N	FP × N
Organic properties										
SOC[a]	[$g.kg^{-1}$]	15.10 a	15.50 a	15.35 b	16.00 b	16.20 b	16.30 b	<0.001	n.s	n.s
N_{tot}[a]	[$g.kg^{-1}$]	1.85	1.91	1.79	1.83	1.97	1.95	n.s	n.s	n.s
C/N ratio	[−]	7.64	7.88	8.23	8.38	8.21	8.79	n.s	n.s	n.s
C stock[b]	[$Mg.ha^{-1}$]	37.37	37.96	37.85	38.67	39.25	40.62	n.s	n.s	n.s
Physical properties										
Bulk density	[$g.cm^{-3}$]	1.47	1.48	1.50	1.49	1.44	1.46	n.s	n.s	n.s
Chemical properties										
pH[a]	[H_2O]	7.75	7.70	7.66	7.80	7.75	7.56	n.s	n.s	n.s
CEC[a]	[$meq.kg^{-1}$]	106.11	102.77	103.79	107.46	113.28	108.68	n.s	n.s	n.s
P_{tot}[c]	[$g.kg^{-1}$]	0.98	0.96	0.96	0.91	0.96	0.96	n.s	n.s	n.s
P_{org}[c]	[$g.kg^{-1}$]	0.35	0.33	0.32	0.32	0.33	0.33	n.s	n.s	n.s
P_{AAE}[d]	[$mg.kg^{-1}$]	102.24 b	84.70 b	81.25 a	61.16 a	99.53 b	83.18 b	0.021	n.s	n.s
K_{tot}	[$g.kg^{-1}$]	15.19	16.56	16.32	15.97	15.60	15.98	n.s	n.s	n.s
K_{AAE}[d]	[$mg.kg^{-1}$]	185.60 b	168.83 b	135.40 a	106.77 a	148.33 ab	157.40 ab	0.008	n.s	n.s

Displayed values are averages of four replicates. *p*-values of each factor are computed according to an ANOVA performed after the fitting of a linear mixed effects model. The abbreviation "n.s" stands for "not significant". Letters refer to the results of Tukey's HSD test and are only displayed if the significance threshold ($p < 0.05$) is reached. In addition, uppercase letters refer to the pairwise comparison of N rates and lowercase letters to fertilization practices.

[a] SOC, total N, pH-water and CEC are measured according to the Swiss standard methods (FAL et al., 2004).

[b] Soil C stock of the upper soil layer was estimated using the minimum Equivalent Soil Mass (ESM) correction (Lee et al., 2009).

[c] Total and organic-P are measured after soil incineration at 550 °C during 1 h and extraction of the ashes with 0.5 M H_2SO_4 (Saunders and Williams, 1955).

[d] P- and K-AAE are extracted with ammonium acetate and EDTA according to the Swiss standard methods (FAL et al., 2004).

Carbon and N determination were obtained by a TOC/TN auto analyser (Shimadzu analyser TOC-V CPH + TNM-1). Phosphorus was extracted with a 0.5 M $NaHCO_3$ solution (pH 8.5) according to a soil:solution ratio of 1:20 and then measured by a colorimetric method using a sulfomolybdic reagent (Olsen et al., 1954). All the values for C_{mic}, N_{mic} and P_{mic} were calculated using the coefficient factors k_C, k_N and k_P, respectively 0.45, 0.45 and 0.4 (Jenkinson et al., 2004).

Determination of soil microbial community structure was based on lipid biomarkers using the PLFA technique (White et al., 1979). In brief, lipids were extracted from 4 g of lyophilised soil with a solution of chloroform, methanol and phosphate buffer. Lipids were separated into neutral, glyco-, and phospholipids on silica solid phase extraction cartridges. The phospholipids were then trans-esterified in fatty acid methyl esters (FAMEs) and detected by gas chromatography (Varian CP3800, USA) with reference to commercial standards (Supelco, USA).

2.5. Earthworms population

In early April 2014, earthworms were sampled using a mustard powder suspension (Lawrence and Bowers, 2002). Concentrated mustard powder solution (10% m/v) was prepared the day before sampling and stored in a cool place. The solution was then diluted at 0.6% (m/v) prior to application and frequently mixed in order to avoid any settling of mustard powder. Sampled surface was 1 m^2, delimited by a quadrat, and located in the middle of each microplot. Twenty litres of mustard powder solution was applied on each quadrat in 2 steps at interval of 15 min. Earthworms appearing on soil surface were collected during 30 min, rinsed and stored in a 70% (v/v) ethanol solution. Remaining earthworms in the soil were estimated by digging up one fourth of the sampled surface (Fründ and Jordan, 2004), and separately stored in ethanol solution. Earthworms were further identified according to Bouché (1972) and classified according to the main ecomorphological groups (*epigeic*, *endogeic* and *anecic*). The number of individuals and biomass were measured for each of the ecomorphological groups.

2.6. Data analyses

To avoid any inter-annual variations in absolute yield due to different crops in the rotation, a crop yield index was used to compare the results (Maltas et al., 2013). Thus, results of grain yields were expressed as a percentage of the control (*MIN-N120*) according to Eq. (1) and were reported as relative grain yields.

$$Relative\ yield = \frac{Absolute\ yield\ of\ a\ \ microplot}{Absolute\ yield\ of\ MIN - N120} \times 100 \qquad (1)$$

Statistical analyses were carried out using R statistical software (R Core Team, 2014). The analysis of variance (ANOVA) took into consideration the split plot design of the experiment. Assumptions of normality and homoscedasticity were visually checked and in case of violations, variables underwent Box-cox transformation using the *MASS* package (Venables and Ripley, 2002). Treatment means were further compared by means of *a posteriori* Tukey HSD tests and differences were considered significant at $p < 0.05$ using the *Agricolae* package (de Mendiburu, 2015). Ordinations of relative abundance of PLFA (Fig. 2) and earthworm biomasses (Fig. 4) were performed through principal component analysis (PCA) with the *vegan* package (Oksanen et al., 2015). The evolution of relative crop yield was investigated by using a simple moving average (SMA) curve. As inter-annual variations were quite important, SMA allowed a better trend analysis by removing a certain environmental noise, such as local climatic variations. The filter of SMA curve took into consideration the current year and the three previous ones so to show the evolution of crop yields at the timescale of crop rotation.

3. Results

3.1. Soil physicochemical properties

Soil organic carbon (SOC) content ranged on average from 15.1 g kg^{-1} (*MIN-N50*) to 16.3 g kg^{-1} (*FYM-N120*) (Table 2), indicating overall a significant effect of the FP ($p < 0.001$). Compared to the *MIN* treatment, SOC content increased significantly by 2.45% and 6.2% in the *RES* and *FYM* treatments, respectively. However, SOC was not significantly affected by N application rates (Table 2), despite higher SOC contents observed on average in N optimal conditions (*N120*). In opposition to SOC content, soil C stock of the upper soil layer (0–20 cm) was rather comparable among the different FP after correction according to the minimum Soil Equivalent Mass concept (Lee et al., 2009). This poor differentiation is due to similar but nevertheless varying soil bulk densities within treatments (Table 2). However, a positive trend of organic amendments on C stock remains visible despite its non-significance ($p = 0.075$). The effects of FP and N-application rates on total N contents and C/N ratios (Table 2) were not different ($p > 0.05$). Nevertheless, according to average values, the C/N ratio, which is an indicator of SOC quality, was increased under *RES* and *FYM* by respectively +7.0% and +9.0% in comparison to *MIN*.

The effects of FP and N application rates were not significant ($p > 0.05$) for the soil pH, CEC, P_{tot}, P_{org}, K_{tot} (Table 2). However, available fractions of P (P_{AAE}) and K (K_{AAE}) were both significantly affected by FP, with lower values reported in case of *RES* (Table 2).

3.2. Microbial biomass nutrient immobilization and community structure

Despite an increasing trend of microbial biomass nutrient immobilization in response to organic amendments (Fig. 1), no significant differences were observed among treatments. In comparison to *MIN*, the *RES* and *FYM* fertilization increased the C and N microbial biomass on average from 7 to 10% and 16–21%, respectively. Furthermore, the correlation between microbial biomass C (C_{mic}) and both SOC and C stock was relatively high and significant (r-SOC = 0.58, $p = 0.003$; r-C stock = 0.66, $p < 0.001$). The bacterial community ($p = 0.046$), fungal community ($p = 0.049$) and total PLFA content ($p = 0.042$) were significantly affected by FP (Table 3). The microbial community structure remained stable in all treatments and the bacterial community was largely dominating, as suggested by low fungi-to-bacteria (F/B) ratios (Table 3). Interestingly, the F/B ratio was relatively well correlated ($r = 0.53$; $p = 0.009$) with the amount of microbial biomass C per unit of SOC (C_{mic}-to-C_{org} ratio), which remained stable across all the treatments and averaged 2.93% (data not shown). Also, the relative abundance of arbuscular mycorrhizal fungi (AMF) and the Positive Gram-to-Negative Gram ratio (G+/G- ratio) appeared to be significantly impacted by an interaction effect between FP and N application rates ($p = 0.027$ and $p = 0.038$, respectively). Interestingly, the observed interaction effect was similar for both variables ($r = 0.70$, $p < 0.001$) and their response to N application rates clearly differentiated *MIN* from the other treatments with organic amendments (Table 3). Regarding the G+/G- ratio, the interaction effect appeared to be mostly induced by variations of the relative abundance of the Gram positive community, as indicated by a marginal significance ($p = 0.061$).

The PLFA profiles of respective treatments was analysed by conducting PCA on the relative abundance of 31 biomarkers

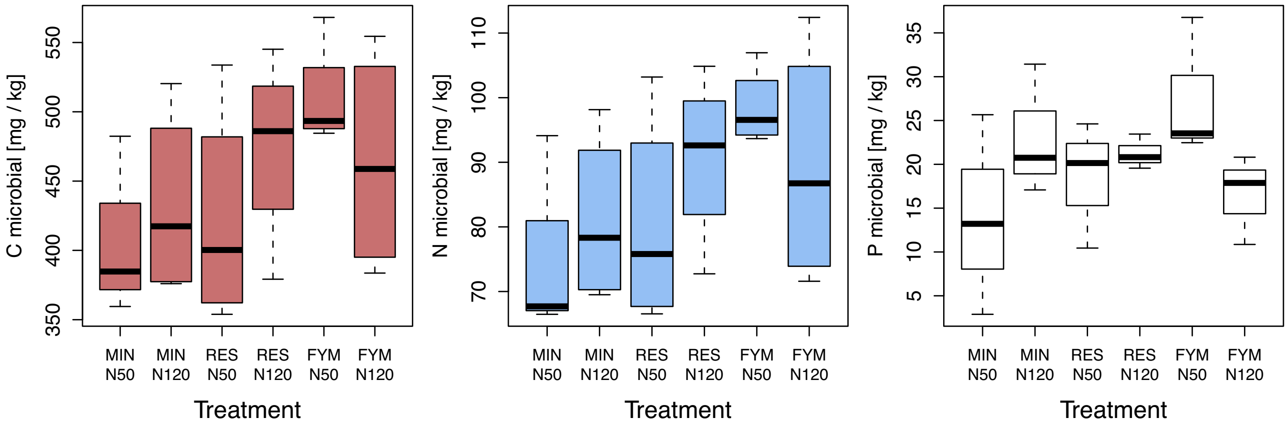

Fig. 1. Boxplots of carbon (C), nitrogen (N) and phosphorus (P) content in microbial biomass in the plough layer (0–20 cm) for the different treatments. Boxes are median and 25th and 75th percentiles. Whiskers are non-outlier ranges.

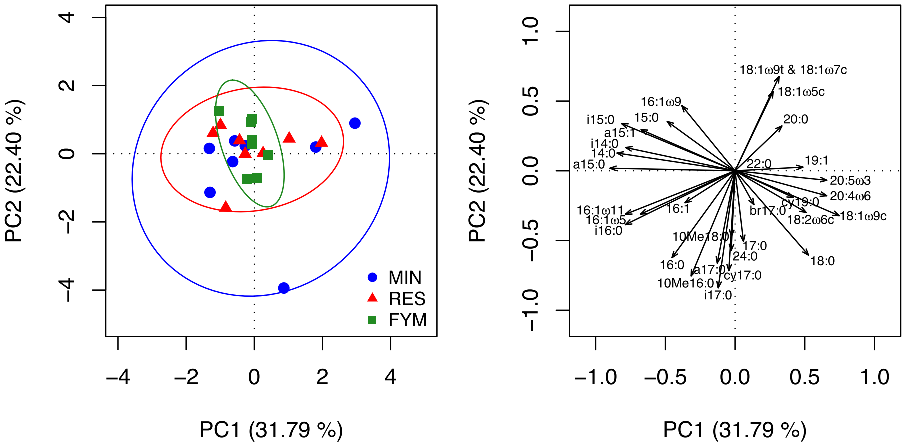

Fig. 2. Principal component analysis (PCA) of relative abundance of phospholipid fatty acids (PLFAs) (left) and loading values for individual PLFA (right). The 95% confidence ellipses of the site scores are displayed according to the different fertilization practices. Percentage values on PC1 and PC2 indicate the respective variance explained by the first two PCA axes.

(Fig. 2). The PC1 and PC2 explained 31.79% and 22.40% of the overall variance. PLFA profiles did not clearly separate the different FP according to their respective centroids, but dispersion of the sites was distinctly increased in case of *MIN* and *RES*.

3.3. Characteristics of the earthworm population

The main characteristics of the earthworm community are summarized in Fig. 3. Total biomass on average varied from 61 g/m^2 (*MIN-N50*) to 93 g/m^2 (*FYM-N120*) while the number of individuals varied from 184 (*MIN-N50*) to 273 (*FYM-N120*). On average, earthworm biomass tended to increase under organic amendment fertilizer treatments and was +7% in *RES* and +15% in *FYM* compared to MIN treatment. The effect of N application rates was statistically significant ($p = 0.044$) indicating a reduction of earthworm biomass on average by 25–35% for N-limiting conditions (*N50*).

The population structure (Fig. 3) showed that anecic species were the most abundant in all treatments (55–70% of the total biomass), followed by endogeic species (25–35% of the total biomass), whilst epigeic species represented the smallest eco-morphological class. Their low presence (*ca.* 1% of the biomass) was observed only in the plots amended with organic matter. This distinction of the effects of FP is noticeable in the PCA performed on the relative biomass of eco-morphological classes (Fig. 4). The PC1 and the PC2 represented respectively 66.47% and 33.30% of the overall variance. PC1 revealed the opposition between the anecic and the endogeic species, the two dominant eco-morphological classes. PC2 was fully loaded with the proportion of epigeic species, suggesting its ecological relevance for discriminating the FP with or without organic matter. The effect of the FP was significant for the proportion of endogeic species ($p = 0.018$), with a higher proportion of this eco-morphological class observed in *FYM* (*ca.* 35% of the total earthworm biomass). The biomass of anecic species was also sensitive to FP ($p = 0.027$) as their biomass decreased significantly in *FYM*.

3.4. Crop yields

The relative yields of different crops during the entire experimental period are reported in Table 4. Independently from the FP, N-limiting conditions decreased the relative crop yield by *ca.* 10% ($p < 0.001$) for almost all the crops. However, the differentiation between the two N-application rates took about a decade to be fully established (Fig. 5). In total, crop yield was significantly impacted by FP ($p < 0.001$, Table 4). Without any

Table 3
Microbial community structure according to PLFA indicators for different fertilization practices (FP) and mineral nitrogen (N) additions.

Microbial community	FP	MIN		RES		FYM		*p*-value		
	N	N50	N120	N50	N120	N50	N120	FP	N	FP×N
Total PLFA	[nmol.g^{-1}]	16.98 a	17.77 a	21.18 ab	22.14 ab	22.45 b	26.10 b	0.045	n.s	n.s
Bacterial PLFA[a]	[nmol.g^{-1}]	10.26 a	10.69 a	13.37 ab	13.62 ab	14.13 b	16.36 b	0.046	n.s	n.s
Fungal PLFA[b]	[nmol.g^{-1}]	3.01 a	2.95 a	3.38 ab	3.89 ab	3.72 b	4.18 b	0.049	n.s	n.s
Fungi/Bacteria ratio	[–]	0.29	0.29	0.25	0.29	0.27	0.26	n.s	n.s	n.s
Gram+/Gram- ratio	[–]	1.37	1.63	1.55	1.34	1.56	1.35	n.s	n.s	0.038
Gram+[c]	*[% PLFA]*	*13.84*	*17.17*	*17.82*	*13.55*	*16.42*	*15.57*	*n.s*	*n.s*	*n.s*
Gram-[d]	*[% PLFA]*	*13.77*	*13.42*	*15.16*	*14.20*	*13.73*	*15.10*	*n.s*	*n.s*	*n.s*
Actinobacteria[e]	*[% PLFA]*	*12.98*	*14.84*	*14.13*	*13.16*	*13.28*	*12.85*	*n.s*	*n.s*	*n.s*
AMF[f]	*[% PLFA]*	*3.95*	*4.55*	*4.69*	*4.14*	*4.58*	*4.45*	*n.s*	*n.s*	*0.021*
Saprophytic fungi[g]	*[% PLFA]*	*13.41*	*12.37*	*11.32*	*13.46*	*12.18*	*11.69*	*n.s*	*n.s*	*n.s*
Unspecific PLFA[h]	*[% PLFA]*	*21.78*	*23.52*	*20.92*	*20.97*	*20.59*	*21.18*	*n.s*	*n.s*	*n.s*

P-values of each factor are computed according to an ANOVA. The abbreviation “n.s” stands for “not significant”. Letters refer to the results of Tukey’s HSD test and are only displayed if the significance threshold ($p < 0.05$) is reached. In addition, uppercase letters refer to the pairwise comparison of N rates and lowercase letters to fertilization practices.

The upper part of the table refers to absolute PLFA contents (expressed as nmol.g^{-1}) and ratios of ecological groups (unitless). The lower part of the table shows the proportion of specific microbial groups (expressed as mole ratios, *i.e.* as percentage of the total PLFA content). Microbial groups were determined according to the sum of different fatty acid methyl esters (FAMEs). The respective FAMEs and the corresponding literature are detailed hereunder.

[a] Bacterial community: Gram +, Gram –, Actinobacteria, 14:0, 15:0, 16:1, br17:0, 17:0, 18:1ω9t, 18:1ω7c, 19:1.

[b] Fungal PLFA: AMF and Saprophytic fungi.

[c] Gram positive bacteria (Gram +): i14:0, i15:0, a15:0, a15:1, i16:0, i17:0 and a17:0 (Frostegård and Bååth, 1996; Zelles, 1999; Potthoff et al., 2006).

[d] Gram negative bacteria (Gram –): 16:1ω9c, 16:1ω11c, 18:1ω5c, cy17:0 and cy19:0 (Zelles, 1999; Ruess and Chamberlain, 2010; Brockett et al., 2012).

[e] Actinobacteria: 10Me16:0 and 10Me18:0 (Frostegård et al., 1993).

[f] Arbuscular mycorrhyzal fungi (AMF): 16:1ω5c (Frostegård et al., 1993).

[g] Saprophytic fungi: 18:2ω6c and 18:1ω9c (Frostegård and Bååth, 1996; Potthoff et al., 2006).

[h] Unspecific PLFA: 16:0, 18:0, 20:0, 22:0, 20:4ω6 and 20:5ω3.

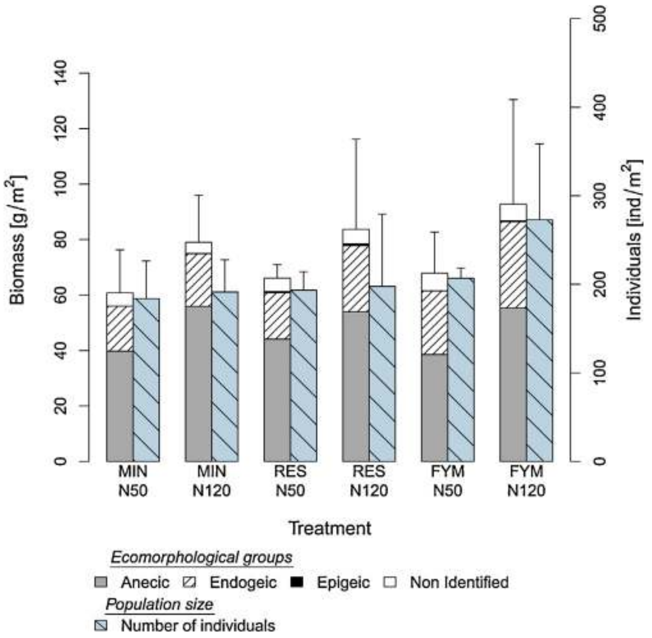

Fig. 3. Earthworm biomass and abundance. Error bars represent the standard deviation for the sum of all fractions. The un-identified fraction consists of juveniles and fragments of earthworms that could not be attributed to any eco-morphological class.

distinction of crop types, *MIN* and *RES* fertilization practices presented similar crop yields for both N application rates, whereas *FYM* was significantly higher (*ca.* +4%).

During the 50 year experimental period, three distinctive periods could be observed: (i) from 1963 to 1977 crop yields in both *RES* and *FYM* increased, (ii) from 1978 to 1985, all crop yields decreased in comparison to *MIN-N120* and (iii) from 1985 and on, crop yields remained relatively constant in all treatments. The differentiation between N-limiting (*N50*) and N-optimal (*N120*) conditions progressively increased over the second period.

4. Discussion

4.1. Influence of fertilization practices on soil C stock and physicochemical properties

After 50 years, fertilization practices induced considerable modifications in soil properties, especially regarding the SOC content. In 1963, at the start of the experiment, the SOC content of the plough layer was relatively high (20.3 g kg^{-1}) (Vullioud et al., 2004). However, after the introduction of agricultural management (*e.g.* soil tillage, crop rotation and fertilization management), SOC content decreased gradually in all treatments, suggesting a progressive mineralization of SOM as observed by other researchers (Lal, 2002). Interestingly, regardless of the SOC trend over time, different fertilization practices led to highly significant differences in SOC contents in the plough layer after 50 years. In this study, organic amendments, such as the incorporation of crop residues (*RES*) and farmyard manure (*FYM*) application, sustained SOC content at higher levels (+2.45% in *RES* and +6.2% in *FYM*) compared to mineral application alone (*MIN*) (Edmeades, 2003). This effect was restricted to the upper soil layer and no difference was observed in the underlying horizon (data not shown), suggesting that the effects of FP on SOC content was primarily restricted to the plough layer (Syswerda et al., 2011). During the experimental period, farmyard manure was applied at a fixed rate (10 tons ha^{-1} year^{-1}) whereas the amount of incorporated crop residue was influenced by the crop type, the crop biomass and its inter-annual variation ranging, on average, between 4 and 12 tons ha^{-1} year^{-1}. Based on simple estimates, *FYM* treatment received higher OM inputs than *RES*, thereby influencing directly their respective SOC contents. In addition, both organic amendments presented different chemical (*e.g.* C/N ratio) and structural (*e.g.* lignin and cellulose contents) characteristics, affecting the dynamics of the humification and mineralization processes of organic compounds

Table 4
Average yield per crop type.

Crop	Realized crop rotation	FP:	MIN		RES		FYM		*p*-value		
		N:	N50	N120	N50	N120	N50	N120	FP	N	FP × N
Wheat (after wheat)	3*		n.a	100	n.a	96.65	n.a	100.13	n.c	n.c	n.c
Maize	13*		92.76 aA	100 aB	91.62 aA	99.62 aB	95.93 aA	104.05 aB	0.045	<0.001	n.s
Wheat (after maize)	13*		80.26 aA	100 aB	83.71 aA	103.87 aB	83.71 aA	105.00 aB	n.s	<0.001	n.s
Sugar beet	9		96.21 aA	100 aB	95.74 aA	98.36 aB	100.45 bA	103.00 bB	0.002	0.008	n.s
Wheat	9		89.89 aA	100 aB	89.87 aA	98.73 aB	95.22 aA	101.62 aB	n.s	0.028	n.s
(after sugar beet)	(7**)		(81.64) aA	(100) aB	(85.81) aA	(98.49) aB	(88.63) aA	(103.68) aB	(n.s)	(<0.001)	(n.s)
Rapeseed	2		89.60 aA	100 aB	87.12 aA	103.73 aB	95.20 aA	104.73 aB	n.s	0.001	n.s
Wheat (after rapeseed)	2		89.91	100	92.02	93.18	98.50	104.00	n.s	n.s	n.s
Average	14		89.72 aA	100 aB	90.16 aA	100.07 aB	94.07 bA	103.50 bB	0.003	<0.001	n.s
1963–2013	(14**)		(88.31) aA	(100) aB	(89.49) aA	(100.09) aB	(92.88) bA	(103.87) bB	<0.001	<0.001	n.s

Single asterisks (*) indicate that among the total number of realized crop rotation, 3 of them had no value for N50, as this fertilization level was not yet introduced in the experimental design. Double asterisks (**) indicate that values from years 1973 and 1977 were removed, as harvest was strongly impacted by lodging due to bad incorporation of crop residues and/or bad meteorological conditions. Corresponding values are presented under brackets. The abbreviations "n.a", "n.c" and "n.s" stand respectively for "not available", "not computable" and "not significant". Letters refer to the results of Tukey's HSD test and are only displayed if the significance threshold ($p < 0.05$) is reached. In addition, uppercase letters refer to the pairwise comparison of N rates and lowercase letters to fertilization practices. FP: fertilization practice; N: mineral nitrogen fertilization addition.

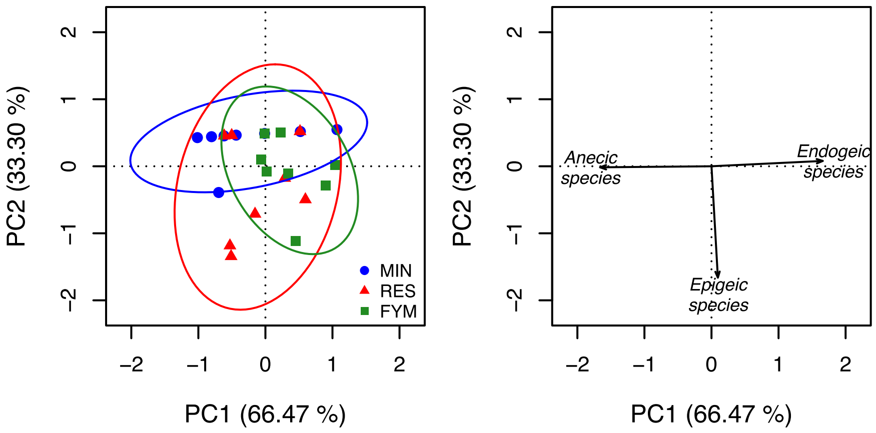

Fig. 4. Principal component analysis (PCA) of earthworm biomass (left) and loading values of the respective eco-morphological groups (right). The 95% confidence ellipses of the site scores are displayed according to the different fertilization practices. Percentage values on PC1 and PC2 indicate the respective variance explained by the first two PCA axes.

via soil microbial activity (Diacono and Montemurro, 2010; Kögel-Knabner, 2002; Lützow et al., 2006). Organic matter in crop residues is generally more easily degradable than that of manure (Damon et al., 2014; Lupwayi et al., 2006) and may therefore have less effect on SOC content. Despite these dissimilarities of the OM supplied in *RES* and *FYM*, SOC contents in these two treatments were not statistically different, suggesting the possibility to increase of SOC content in stockless farms by incorporating crop residues.

However, the observed changes for SOC content were not associated with a significant differentiation of soil C stock in the plough layer (Table 2), despite a trend emerging among the FP ($p = 0.075$). In terms of C sequestration, this result suggested that FP had a modest influence even after 50 years in our experimental conditions. Other studies with similar organic matter inputs showed greater effect of the FP on soil C stock, even during a shorter experimental period (see the comparison of European long term experiments by Körschens et al., 2013). Such result highlights the preponderance of pedoclimatic conditions for enhancing C sequestration through fertilization practices (Wiesmeier et al., 2015).

It is also generally accepted that N-fertilization helps soil C sequestration by increasing biomass crop residues (Liebig et al., 2002; Paustian et al., 1992). However, no significant effect of N application rates was observed in the present study either on SOC content or soil C stock (Table 2) in accordance with other studies (Khan et al., 2007). It is possible that N-fertilization stimulates microbial activity (Fig. 1; Khan et al., 2007) and/or accumulates more labile organic forms (Stevens et al., 2005) enhancing SOC mineralization.

The organic amendments used in this experiment provided significant amounts of P and K due to their presence in livestock feed and crop residues (Damon et al., 2014; Lupwayi et al., 2006). However, the P and K content of organic amendments was taken into account for the calculation of P and K fertilization needs (Sinaj et al., 2009), which explains why total P and K were not affected by the fertilization practices (Table 2). The beneficial effect of *FYM* treatment on available P and K fractions (Diacono and Montemurro, 2010; Zhao et al., 2009) was once again confirmed, whereas *RES* treatment exhibited significantly lower plant available fractions (Table 2). The results from *RES* fertilization practice was unexpected as many authors have found that P and K of crop

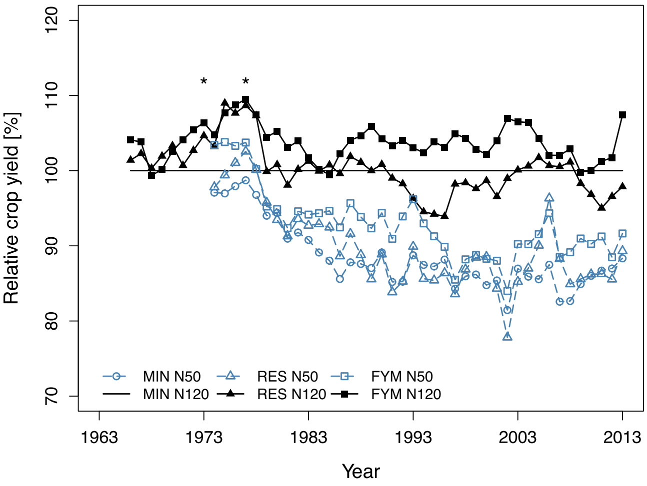

Fig. 5. Relative crop yield evolution (4-year moving average). The horizontal line represents reference yield (100%) set as *MIN-N120*. Simple moving average (SMA) curve was plotted by applying a linear filter based on the current year and the previous three ones. The smoothing coefficient corresponds therefore to the crop rotation period. Asterisks (*) indicate years when the harvest was strongly influenced by lodging (see Table 4). The influence of these years was consequently minimized by calculating the average value of the previous three years.

residues is quickly released after their incorporation in the soil (Damon et al., 2014; Lupwayi et al., 2006; Noack et al., 2012). There may be many reasons for the results presented herewith. Despite the lack of accurate information for the proper estimation of the respective cumulative balances, it was hypothesized that the method of estimation of P and K content in organic amendments was not accurate enough and led to significant differences in the long term with regards to their available fractions (see Section 4.4). In addition to the inherent chemical composition variability, the incorporated amount of crop residues could be highly variable from one year to another, making the estimation of their P and K contribution difficult and approximate. Also, the contribution of crop residues was estimated according to a mean plant uptake based on a “reference yield”, calculated as an average value in Switzerland for each crop type (Sinaj et al., 2009) and not based on specific conditions of each crop under the experimental conditions. In the long term, all these estimations and their related uncertainties are likely to have led to a decrease of the P and K soil fertility in *RES* treatment.

4.2. Influence of fertilization management on soil microbial and earthworm community

Organic amendments had a positive impact on soil microbiology, as suggested by the increased amount of microbial C, N and P in the fertilization practices *RES* and *FYM* (Fig. 1). This trend was also further confirmed by (i) significant correlations between microbial biomass and SOC (see Section 3.2), and (ii) the significant increase of the total PLFA content (Table 3). Consequently, our results corroborate the idea that soil microbial biomass is related to the SOC content in the soil as well as to the amount of OM supplied (Bünemann et al., 2004; Damon et al., 2014; Zhao et al., 2009). In response to the significant increase of SOC content (Table 2), the size of both microbial and fungal communities increased, but without any modification of their relative proportions, as suggested by the fungi-to-bacteria (F/B) ratio (Table 3). Also, the amount of C immobilized by microbial biomass did not vary sensibly among the different treatments, but was well-correlated to the F/B ratio (see Section 3.2). Soils with higher F/B ratios can sequestrate higher C amounts in the microbial biomass, as the fungi community presents a longer turn-over than the bacterial community and is likely to immobilize more C (Strickland and Rousk, 2010). Thus, our results supported the existence of a feedback between the structure of the microbial community and the immobilization of C substrate by soil microorganisms.

The deeper investigation of the microbial structure through PLFA analyses revealed only minor influence of the FP on the overall PLFA profiles (Fig. 2) and the relative composition according to the principal ecological groups (Table 3). These results are consistent with those of Kätterer et al. (2014), who reported only slight alteration of microbial structure in the long-term despite the use of various organic amendments. The similarity of soil microbial communities could be explained by the chosen sampling date. In the case of *RES* and *FYM*, the latest OM addition dated back to respectively 8 months and 2 years. As shown by the F/B ratios, soil microbial communities were largely dominated by the bacterial community (Table 3), which are characterized by a short turn-over and thus a high resilience to disturbance induced by fertilization management. Nevertheless, PLFA profiles according to the different FP presented a decreasing variability in the case of *FYM* and *RES* compared to *MIN* (Fig. 2). We interpret this result as consequence of the establishment of more specific microbial communities when OM was incorporated (Hartmann et al., 2015). In addition, the variability of PLFA profiles in relation to the FP was also proportional to the variable quality of the OM supplied in the respective treatments: in *FYM*, only farmyard manure was incorporated since the onset of the experiment whereas *RES* was amended with crop residues of various nature. This observation is consistent with the results of Wander et al. (1995), who stated that the variability of PLFA profiles was as important as the variability among farming systems.

In contrast to fertilization practices, the response of microbial community to N application rates was less clear. A trend of increased microbial biomass was observed with increasing fertilization rates in the case of *MIN* and *RES*, but this effect disappeared in *FYM* (Fig. 1). Zhao et al. (2014) showed that the biomass and the structure of the microbial community was influenced by N-fertilization, but over a larger range of N application rates (from 0 to 300 kg N ha^{-1}) compared to our experiment. However, the impacts of fertilizer application rates on soil microbial biomass (Geisseler and Scow, 2014) and microbial composition (Rousk et al., 2010) is often associated to the induced modification of soil pH. Accordingly, in our study, the dependence of soil microbial biomass to soil pH was confirmed ($r_{Cmic} = 0.66$; $p < 0.001$) but no influence of the N application rates could be assessed as soil pH was well-buffered due to the location of the experimental plots on calcareous deposits.

Although FP and N application rates had no direct effect on soil microbial composition, both factors induced a significant interaction effect on the proportion of AMF and the structure of the bacterial community, as suggested by the modifications of the G+/G- ratio (Table 3). Soil and PLFA analyses performed in this on-field experiment were inadequate for explaining such relationship among microbial communities in response to fertilization management. However, it corroborated with the observations made in pot experiments (Leigh et al., 2011) showing the existence of interactions between AMF and soil bacterial communities (Johansson et al., 2004). In particular, interaction between AMF and bacterial communities may have an implication on soil N and C cycles (Herman et al., 2012; Nuccio et al., 2013).

Despite a great variability, earthworm biomass and abundance were quantitatively similar with those reported in other conventional farming systems in Switzerland (Pfiffner and Mäder, 1997). Although it is already well-established that organic amendments exert a positive effect on earthworms, as they feed on organic matter (Bertrand et al., 2015; Bouché, 1972; Curry and Schmidt, 2007), the effect of fertilization practices on total earthworm biomass remained relatively weak from the onset of the experiment. Nevertheless, a trend similar to SOC emerged among the treatments: *FYM* recorded the highest earthworm biomass whereas *MIN* presented the lowest values. These results are in agreement with many studies which point out the beneficial effect of various organic amendments on earthworm communities (Eriksen-Hamel et al., 2009). Furthermore, N application rates in this study appeared to be a significant factor influencing earthworm biomass ($p = 0.039$). N-fertilization is known to enhance earthworm populations up to a certain dose (Edwards and Lofty, 1982) if soil pH is not altered (Iordache and Borza, 2010). Similarly to SOC, increased root biomass under improved N-conditions would be beneficial as it may increase the amount of SOM. A higher N-fertilization could also imply a reduction of the soil C/N ratio, suggesting that SOM is more easily decomposed by soil micro-organisms, stimulating earthworm activity in return.

The impact of fertilization practices on the earthworm community structure was assessed as well. The different eco-morphological classes regroup earthworms according to their morphology, behaviour and feeding habits (Bouché, 1972). Detrivorous species, which feed on relatively fresh OM, encompass the anecic and epigeic species, whereas endogeic species are considered as geophageous, as they feed on humified OM (Bertrand et al., 2015; Curry and Schmidt, 2007). According to results presented here (Figs. 3 and 4), the farmyard manure application had a significant and positive impact on the geophageous (or endogeic) species compared to *MIN* and *RES* (Figs. 3 and 4). This corresponds to results reported by Simonsen et al. (2010), who also found that manure application was a relevant factor controlling the abundance of endogeic species in croplands. Also, the presence of endogeic species tended to be inversely correlated to bulk density ($r = -0.41$; $p = 0.047$). The burrowing activity of this particular ecomorphological group is likely to have had an influence on soil physical properties in this experiment. In addition, the presence of epigeic species was a discriminating factor between *MIN* and the treatments with organic amendments (Fig. 4), confirming their sensitivity to SOC, as they feed on raw organic matter (Bouché, 1972; Curry and Schmidt, 2007).

Although conventional soil management represented rather adverse conditions for the earthworm community (*e.g* conventional ploughing, pest management and use of mineral fertilizer) (Eriksen-Hamel et al., 2009; Pelosi et al., 2013; Pfiffner and Luka, 2007), there were evidences in this study of long-term effects of fertilization management on the total earthworm biomass and the relative proportion of earthworm functional groups, reflecting the different amount and degree of humification of SOM.

4.3. Long-term effects on crop yield

During the experimental period, crop yield was influenced by both fertilization practices (*MIN*, *RES* and *FYM*) and N application rates (*N50* and *N120*) (Table 4). The most important factor was the N-application rate as differences between N-limiting (*N50*) and non-limiting (*N120*) conditions were found to increase progressively once this fertilization was introduced in 1972 (Fig. 5). The decrease in crop yield after the introduction of *N50* was visible from the first rotation in all treatments and suggested limited residual effects of the N- fertilization prior to the start of this experiment (Sieling et al., 2006).

The effect of organic amendments was highly significant over the whole experiment and particularly clear in the case of farmyard manure application. At both N application rates, relative crop yields of *FYM* were higher than the other treatments over the whole experimental period with an average increase of *ca.* 4% (Table 4). Since the N application rates were not adjusted for the contribution of organic amendments in the experimental design, the yield increase could be attributed directly to the effect of additional N and perhaps indirectly to the improved soil conditions associated with farmyard manure application (Haynes and Naidu, 1998). In addition, when crop types were also considered, it appeared that the positive effect of *FYM* was reported as significant only with sugar beet ($p = 0.002$) and maize ($p = 0.045$) and could not be assessed during years cropped with wheat. As N application rates were constant and based on wheat needs, it depicted a greater impact of the incorporation of farmyard manure for crop types that were probably N-limited at both application rates, as their N requirements were higher than wheat needs (Sinaj et al., 2009).

By contrast, RES presented on average similar crop yields with *MIN* (Table 4). In this study, the similarity between crop yields on *MIN* and *RES* appeared to be likely influenced by P and K fertilization history. Indeed, the evolution of relative crop yield over the experimental period showed an interesting pattern. From 1963–1977, the increase in crop yields of *RES* and *FYM* fertilization practices coincided quite well with the period during which the contribution of P and K in organic amendments was not accounted for (Table 1). After 1978, the P and K application rates were adjusted to account for the contribution of organic amendments, which could explain the relative decrease in crop yields and more fluctuating crop yields for *RES* during this period. Interestingly, during the same period under N-optimal application rates, crop yields in *RES-N120* decreased even further by 1.4% compared to the reference (*MIN-N120)* which could be related to the relatively more PK limiting conditions, as suggested by soil analyses (Table 2).

4.4. Limitations of this study

In this long-term field experiment, interpretations of the results were hindered by several factors inherent to the experimental design itself. The main issue concerned the consideration of P and K from organic amendments over the whole experimental period. During the first decade (1963–1971), the respective contributions of crop residues and farmyard manure were not taken into consideration (Table 1). After 1972, regular modifications of the mineral P and K fertilization were carried out in order to equilibrate the cumulative balance of these respective elements. These modifications were made according to the successive revisions of the Swiss fertilization guidelines (Sinaj et al., 2009), which provided estimates of nutrient uptake of grain and crop residues for a given reference yield. From a practical perspective, it was a good opportunity to verify whether crop residues management could be handled in this way, with simple estimates. However, in a research context, it would have been preferable to couple this method with annual measurements of nutrient content of crops residues and farmyard manure during the entire experimental period, so that the “expected” and the “actual” cumulative nutrient balance could be compared.

5. Conclusions

The maintenance of SOC content is a key factor for preserving soil fertility and sustainability of cropping systems. Over a 50 year-long study period, here we showed the systematic incorporation of crop residues (*RES*) and farmyard manure application ($10\,t\,ha^{-1}$ $year^{-1}$) (*FYM*) promoted a significantly higher SOC content compared to mineral fertilizers alone (*MIN*), but without sensible differences in soil C stock. Both organic amendments improved soil biological properties, in particular the total microbial biomass but not the community structure, with a more pronounced effect in the *FYM* practice. In contrast, N application rates had an impact on total earthworm biomass, whereas the fertilization practice affected the structure of the earthworm community. In the long term, *FYM* increased also significantly crop yields compared to *MIN*. In the absence of farmyard manure, crop residue incorporation should be considered as a viable alternative for sustaining SOC content and promoting soil biological properties. However, particular attention should be paid to the correct estimation of their contribution in the PK fertilization in order to maximize their beneficial effect on crop yields.

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