Agriculture, Ecosystems and Environment 127 (2008) 286–293

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

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

Short communication

Molecular and functional responses of soil microbial communities under grassland restoration

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ARTICLE INFO

Article history:
Received 13 December 2007
Received in revised form 4 April 2008
Accepted 10 April 2008
Available online 9 June 2008

Keywords:
Grassland
Management intensification
Soil restoration
Soil microbial biomass
Microbial functional diversity

ABSTRACT

The influence of ageing grassland on microbial community structure in different long-term grassland regimes compared to tillage in neighbouring fields was investigated to evaluate whether grassland restoration can be considered as a specific type of management for soil conservation in northern France. Microbial community structure was examined by analyzing the distribution of total and labile organic matter, the size of bacterial and fungal populations, and bacterial metabolic fingerprints and fungal genetic fingerprints. Results showed a gradual positive increase of total microbial biomass between the intensive management reference site and the six grassland soils, and that soil organic matter storage is associated with changes in microbial biomass. There was a large increase in fungal and bacterial populations in the permanent grassland, but bacteria were more weakly affected by agricultural management practices than the fungi. Although potential functional diversity shifts in the bacterial community seemed to be related to the ageing grassland gradient, we were not able to highlight any significant difference in bacterial genetic diversity between the sites. There was, however, a strong relationship between fungal genetic diversity and the ageing grassland. Finally, an increase in microbial activities (% mineralization) was observed according to the age of the meadow. Among agricultural management practices, grassland restoration may have a positive impact in maintaining the soil status.

1. Introduction

Intensive agricultural management regimes are believed to be a major factor contributing to the degradation of soil properties (Bending et al., 2000) and leading to a decrease of soil organic matter (SOM) (Tiessen et al., 1982, 1994; Fliebach et al., 2007; Schjønning et al., 2007), a reduction in structural stability (Reganold et al., 1987) and a change in soil microbial diversity and activity (Lawlor et al., 2000; Lupwayi et al., 2001; Nicol et al., 2003; Oehl et al., 2004; Walker et al., 2004; Govaerts et al., 2007).

Due to the climatic conditions and the depth of the soils, the silty soils of northern France (Paris basin) explain a large part of the wealth of the agricultural economy in the French plains, with a high production of field crops, but the soil quality status and fertility are now deteriorating (Arrouays and Pelissier, 1994; Balesdent, 1996; Le Bissonnais and Arrouays, 1997; Tessier et al., 1998). The cultivation of arable crops for long periods, the intensification of management practices with weak organic amendments, and the specific soil texture are leading to a decline in the organic matter under silty arable soils. Soil organic matter is involved in the physical and chemical properties of soil and in the nutrient availability for microbial and plant growth (Arias et al., 2005). Consequently, change in the SOM status directly affects the soil microbial diversity and the soil stabilization, leading to the appearance of runoff and erosion (Arrouays and Pelissier, 1994).

The restoration of the physico-chemical properties and biological characteristics of soil has become of interest in order to preserve the soil ecosystem and promote sustainable agriculture (Dobson et al., 1997; Kindscher and Tieszen, 1998; Samson and Knopf, 1994). Alternative farming practices based on natural biological processes with lower chemicals inputs, such as inorganic (phosphate minerals, oxides) and organic matter amendments (e.g. plant material, animal residues, manure, sewage sludge, municipal waste), are privileged (Mäder et al., 2002; Reganold et al., 1987). However, amendments may only be effective in the short- or mid-term (Crecchio et al., 2001). Moreover, periodic and long-term addition of organic amendments may impact on the chemical and biological soil properties, promoting pathogen introduction and

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doi:10.1016/j.agee.2008.04.008

Table 1
Soil properties, organic matter content and microbial biomass

Agricultural management	pH	Total carbon (g/kg)	C/N	Microbial carbon ($\mu g\ g^{-1}$)	Microbial carbon/total organic carbon (%)
Intensive culture	6.0	9.47 ± 0.21 [a]	8.97	179.53 ± 39.53 [a]	1.90
TM2001	5.6	14.21 ± 0.79 [b]	10.38	306.99 ± 31.13 [b]	2.16
RM2000	6.2	12.51 ± 0.16 [b]	10.14	178.95 ± 24.08 [a]	1.43
RM1998	6.9	22.09 ± 0.55 [c]	13.46	430.75 ± 68.33 [c]	1.95
RM1995	6.6	19.60 ± 0.87 [c]	10.29	416.20 ± 25.66 [c]	2.12
TM1994	6.1	25.22 ± 0.77 [c]	11.42	555.59 ± 172.95 [cd]	2.20
PM1968	5.9	33.27 ± 0.80 [d]	11.21	557.54 ± 55.72 [d]	1.68

Results are presented as arithmetic means ± standard deviation of triplicate analysis of three composite samples. Letters between brackets (a–d) show the ANOVA groups.

movement in soils or leading to heavy metal pollution, for example (Zelles et al., 1992; Crecchio et al., 2004; Marschner et al., 2003; Saison et al., 2006).

Due to bacteria–root association and bacteria–clay particle adsorption, grassland pastures have been described as resilient soils (i.e. soils capable of retrieving their functionalities after a perturbation). Including restoration grasslands with native plant species in the rotation of crops might reduce degradations due to conventional farming, acting positively on microbial biomass and decomposition rates (Bardgett and Shine, 1999; Loiseau et al., 1995; Balesdent et al., 1996). The understanding of the microbial dynamics during the transition from conventional to grassland practices or when grassland is maintained long-term in silty soils may provide information about the impact of soil management in northern France. The soil microbial community regulates the dynamics of organic matter, leads to its decomposition and controls nutrient cycling (Zeller et al., 2001). Changes in soil microbial biomass and activities have been extensively and rapidly observed under different soil management practices and other disturbances (Chandler et al., 1995; Frostegård et al., 1993; Kandeler et al., 1996), but most of these results are correlated with their pedoclimatic context (Angers et al., 1995; Feller et al., 1996; Wander et al., 1994; Breland and Eltun, 1999). As the pedoclimatic context of north western France is not described in these studies, research integrating physico-chemical soil properties with microbial communities needs to be conducted in the silty soils of this particular region.

In order to evaluate whether grassland restoration can be considered as a specific type of management for soil conservation in northern France, this study investigates the influence of ageing grassland on microbial community structure (distribution of total and labile organic matter; metabolic and genetic fingerprints) in different long-term grassland regimes compared to tillage in neighbouring fields. In particular, changes in size distribution and in the metabolic and genetic profiles of the soil microbial community were studied.

2. Materials and methods

2.1. Field sites

The experimental sites are located at the Lycée Agricole d'Yvetot in northwestern France. The "Haute-Normandie" region is dominated by an oceanic and temperate climate characterized by mild temperatures, rainfall with a mean of 800–900 mm yr^{-1} and narrow seasonal ranges. The soil, representative of the Paris Basin, is classified as silty (e.g. loess) soil containing 15% clay, 65% silt, and 20% sand. The site affords a rare opportunity to compare microbial-pedologic linkages in seven adjacent fields with well-known soil management practices, including one field with long-term arable cropping (>10 yr), one with long-term grassland (>25 yr) and several with restored grassland. The six permanent or temporary grasslands were implanted with perennial rye-grass and clover at various times: 1968, 1994, 1995, 1998, 2000, 2001; these grasslands and the cultivable field (plowland without grassland rotation) are presented in Table 1. The permanent grassland (PM1968) has a long-term history of monoculture (25 yr pasture), and is considered as a reference site with permanent cover and no tillage. In contrast, the intensive management (ploughing/fertilization) reference site has a history of long-term cropping (wheat, maize, flax or beet). Two fields with arable (2 yr wheat–maize rotations) and grassland (4–8 yr) rotation management are considered as temporary grasslands (TM1994 and TM2001). Finally, three restoration grasslands (RM1995, RM1998, and RM2000) are characterized by grassland establishment after a cropping period of at least 8 yr.

2.2. Soil sampling

Soil samplings were carried out in spring 2002 from the 7 silty fields with the same soil type and topographical features.

Each field was divided into three subplots (equal rectangle of 2 m × 40 m). Samples were collected in triplicate from the surface horizon (0–10 cm) in four randomly chosen plots from each of the three subplots. For each subplot, the samples were combined into bulk composite samples. This sampling procedure is justified by previous spatial variability measurement (data not shown). Field-moist soils were sieved to 2-mm particle size and split into three replicate subsamples. The soil collected was used immediately or was stored at 4 °C for no longer than 24 h before sieving, for microbial biomass, activity and diversity analyses.

2.3. Soil chemical properties

Soil pH was measured in air-dried samples with a glass electrode in a 1:2.5 soil:water slurry. Moisture content was recorded after drying at 105 °C for 24 h. Total organic carbon and nitrogen were quantified by a conventional dry combustion method using an automatic analyzer (FISONS NEA 1108).

2.4. Microbial biomass, microbial respiration and qCO_2

Microbial biomass C was estimated by the fumigation-extraction method (Vance et al., 1987; Wu et al., 1990) with 24 g of moist sieved soil for the fumigated and 24 g for the non-fumigated treatment, both extracted with 0.5 M K_2SO_4 and then vacuum filtered through 0.45 μm Millipore filters. Soluble organic C in the K_2SO_4 extracts was measured using the high temperature combustion method (Shimadzu TOC 5050A Carbon Analyzer). The quantity of K_2SO_4-extractable C from non-fumigated soil was used as a measure of labile soil organic C. Microbial biomass C is the difference between the organic C extracted from fumigated soil and the organic C extracted from non-fumigated soil. Microbial biomass C was expressed in mgC kg^{-1} dry soil. Soil microbial activity (e.g. soil microbial respiration) was evaluated by the soil organic C mineralization status over 28 days in controlled