Soil Biology and Biochemistry 122 (2018) 203–210

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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

# Ley grassland under temperate climate had a legacy effect on soil organic matter quantity, biogeochemical signature and microbial activities

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ARTICLE INFO

*Keywords:*
Temporary grassland management
Microbial activities
Potential $N_2O$ emission
Soil organic matter biogeochemistry
Long-term field experiment

ABSTRACT

Temporary (ley) grassland introduced into cropping cycles has been advocated as being beneficial for the delivery of ecosystem services by agricultural soils. The management of these temporary grasslands has unknown effects on soil organic matter (SOM) concentrations and biogeochemical properties of the cropland soils following the grassland phase. Here, we investigated the legacy effect of differently managed temporary grasslands, i.e. change of soil properties lasting beyond three years of crop. We assessed soil organic carbon (SOC) quantity and SOM biogeochemical signature (composition of soil neutral carbohydrates and lignin), as well as microbial activities (potential C and N mineralization and denitrification). We used a long-term field experiment on Cambisol with temperate climate in western France, where temporary grassland management practices differed in terms of duration (3 or 6 years) and presence or absence of N fertilisation. Topsoil (10 cm) samples were collected after a 3-yr crop rotation (maize, wheat, barley).

Our results showed that N fertilisation during the grassland phase was necessary to maintain soil C and N concentrations beyond three years of crop. Temporary grassland management may affect microbial activities as indicated by contrasting polysaccharide and lignin composition. It had however, no effect on potential $CO_2$ and $N_2O$ emissions during laboratory incubations. The biogeochemical signature of SOM was close to continuous grassland only in treatments with 6 yrs of fertilized temporary grassland. We thus, conclude that the legacy effects of a grassland phase on SOC quantity and properties of SOM depend on its management.

## 1. Introduction

Agricultural soils of the temperate regions have lost much of their organic carbon since the 1960s due to the intensification of crop production (Lal, 2004). Nowadays, rebuilding soil organic matter (SOM) stocks has become a priority because SOM greatly improves soil properties and functions. Moreover, SOM storage may be able to counteract increasing atmospheric greenhouse gas concentrations (Chabbi et al., 2017; Smith, 2016). In this context, grassland soils with high belowground C stocks can be carbon sinks (Conant et al., 2001). Therefore, the introduction of temporary or ley grassland in cropland cycles may be a suitable negative emission technology (Smith, 2016), if it increases soil C sequestration as a legacy effect (i.e. soil C lasts beyond the following cropland phase).

Temporary grasslands were shown to have several beneficial effects on soil quality related to SOM increase (Studdert et al., 1997; Franzluebbers et al., 2014). Moreover, they reduce nitrate leaching as compared to continuous cropping systems (Kunrath et al., 2015). Recent work also showed that introduction of temporary mowed grassland in the cropping cycle led to higher contribution of SOM in macro-aggregates as compared to permanent arable land (Panettieri et al., 2017). This effect was observed as a legacy effect after 3 yrs of continuous cropping, and explained by higher amounts of root litter input during the grassland phase (Armas-Herrera et al., 2016; Attard et al., 2016).

*Abbreviations:* SOM, Soil organic matter; SOC, Soil organic carbon; TG, temporary grasslands; TG3N +, temporary grassland with 3 years of fertilized ley; TG6N +, temporary grassland with 6 year of fertilized ley; TG6N-, temporary grassland with 6 years ley without fertilisation; CC, permanent cropland; GG, permanent grassland; POM, particulate organic matter; IN, inorganic nitrogen; ON, organic nitrogen; IP, inorganic phosphorus; OP, organic phosphorus; V, vanillyl; S, syringyl; Co, *p*-coumaryl; Ac, acid; Al, aldehyde; C5, pentoses; C6, hexoses; MB-C, microbial carbon; MB-N, microbial nitrogen; CLPP, community-level physiological profiles; AWCD, average well color development; Cmin, carbon potential mineralization; Nmin, nitrogen potential mineralization; *k*, carbon mineralization constant rate; PD, potential denitrification; PCA, principal component analysis

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https://doi.org/10.1016/j.soilbio.2018.04.018
Received 13 October 2017; Received in revised form 13 April 2018; Accepted 17 April 2018

However, in order to secure the C sink function of grasslands, judicious management is necessary (Smith et al., 2015). Indeed, management of temporary grasslands in terms of cutting or grazing, fertilisation and duration, influences the decomposition and stabilisation processes of SOM through its impact on C, N and P coupling and decoupling (Acharya et al., 2012; Attard et al., 2016; Conant et al., 2001; Crème et al., 2016; Klumpp et al., 2009; Lemaire et al., 2015; Rumpel et al., 2015). Beneficial effects of temporary grasslands may depend on the interplay between environmental constraints, soil microorganisms and SOM characteristics. To date the effect of temporary grassland management on these variables is still unknown.

In this study, we investigated the legacy effect (i.e. changes observable after 3 years of crop) of differently managed temporary grasslands on SOM and microbial properties. The aim of the study was to better apprehend the impact of temporary grassland management on SOM biogeochemical composition, nutrient forms as well as potential microbial activities. We analyzed SOM molecular signatures of soil neutral carbohydrates and lignin, susceptible to be affected by grassland management (Crème et al., 2017). These two macromolecules were chosen because their nature and fate in soil are contrasting (Koegel-Knabner and Rumpel, 2018). As biomarkers, their quantity and composition may give valuable information on degradation and stabilisation processes of SOM. Non-cellulosic carbohydrates have a low degree of polymerization and may be plant or microbial derived (Murayama, 1984; Koegel-Knabner, 2017, 2002; Kuzyakov et al., 2000; Rumpel and Dignac, 2006). In contrast, comparatively more recalcitrant compounds such as aromatic lignins are exclusively plant-derived. They are mineralizable only by a restricted number of decomposers (white-rot fungi) (Frei, 2013; Koegel-Knabner, 2017, 2002). In addition, we determined SOM C:N:P stoichiometry, which may change in response to grassland management (Crème et al., 2016). We also quantified the amount of particulate organic matter and potential microbial activities involved in $CO_2$ and $N_2O$ emissions and nutrient release. We hypothesised (i) that temporary grassland has legacy effects on SOM quantity, molecular composition and microbial activities, observable after a 3 year crop rotation following the end of the grassland phase; (ii) that the magnitude of the legacy effect would depend on the length of the grassland phase (6 versus 3 years); and (iii) that the magnitude of the legacy effect would be sensitive to nitrogen fertilisation applied during the grassland phase.

## 2. Materials and methods

### 2.1. Site description

The experimental site is the long term observatory on environmental research called “agrosystems biogeochemical cycles and biodiversity” (SOERE ACBB) situated at the INRA research station in Lusignan (46°25′12.91″ N; 0°07′29.35″ E), western France.

The mean annual temperature is 12 °C and the mean annual precipitation is around 750 mm. The experiment was established in 2005 on a Cambisol with a silty-loam texture and a bulk density of 1.43 kg $m^{-3}$ (Chabbi et al., 2009; Moni et al., 2010). The profile can be divided into two main domains: three red-brown upper horizons characterised by a loamy texture overlying three lower clayey rubefied horizons rich in kaolinite and iron oxides (Ducloux and Chesseron, 1988). The lower rubefied horizons were classified as Paleo-Ferralsol from the late Mesozoic period, while the upper horizons were classified as Cambisol formed from Ferralsol horizons under temperate climate (Ducloux and Chesseron, 1988).

At this site, in 2005, a long-term field experiment was established consisting of ley grassland introduction in the cropping cycle with 5 different treatments in terms of fertilisation and duration of the grassland phase (Table 1): TG3N + corresponds to 3 yrs of fertilized ley, TG6N + to 6 yrs of fertilized ley and TG6N- is a 6 yrs ley without fertilisation. In addition, two control treatments with permanent cropland (CC) consisting of maize-wheat-barley rotation and with permanent grassland (GG) were established. Treatments were replicated 4 times in 4 separated blocks. Before the beginning of the experiment, the whole surface had been homogenized by 3 years of cropping (barley, barley and wheat).

The species sown for CC in April 2005, was *Zea mays* L. (maize) with a density of 8.5 seeds $m^{-2}$. In October 2005, *Triticum aestivum* L. (wheat) was sown with density of 150 seeds $m^{-2}$. In October 2006, *Hordeum vulgare* L. (barley) was sown with density of 165 seeds $m^{-2}$. This crop rotation of 3 yrs was repeated when the soil was under permanent cropland (CC). For ley grassland, this crop rotation was alternated with a grassland phase (TG). To establish grassland, a species mixture containing *Dactylis glomerata* L. (cocksfoot), *Festuca arundinacea* Schreb (tall fescue) and *Lolium perenne* L. (rye-grass) was sown.

The grasslands were harvested 3–4 times a year depending on their productivity. The biomass was removed. Nitrogen was the only element added through mineral fertilizer application. The N-fertilizer amount needed to account for biomass removal was determined each year by soil analyses. In consequence, fertilisation varied with years and ranged between 36 and 160 kg N $ha^{-1}$ $year^{-1}$ for cropland, and between 170 and 380 kg N $ha^{-1}$ $year^{-1}$ for fertilized grassland phases according to the residual soil N amount determined at the end of the previous growing season. It is important to note that unfertilized temporary grassland (TG6N-) differed from the other treatments only with regards to fertilisation during the grassland phase.

### 2.2. Soil sampling

The top 10 cm soil was sampled in March 2014 for each plot (i.e. 5 treatments × 4 blocks), nine years after implantation of the experiment. All soils except GG soils had been under cropland rotation for the last three years (year 7–9, Table 1). Two soil cores (8 cm diameter) were randomly taken from each plot, and mixed to yield one composite sample per plot. Fresh sub-samples were used for biological analysis. The remaining soil was air-dried, and aliquots were ground for chemical analysis.

### 2.3. Soil elemental composition

Total carbon (C) and total nitrogen (N) concentrations were determined using an elemental analyzer (CHN NA 150, Carlo Erba). No carbonate was present in the soil, therefore soil C is considered to be exclusively organic.

Nitrate-N ($NO_3^-$) and ammonium-N ($NH_4^+$) were determined colorimetrically after extraction of soil with a 0.5 M KCl solution (1:5 weight/volume) (Page et al., 1982).

Total soil phosphorus (P) was determined with the ignition method (Walker and Adams, 1958) using the modifications introduced by Ivanoff et al. (1998). Briefly 0.5 g of dry soil was ignited for 1 h at 550 °C before extraction during 24 h by shaking with 1 M sulphuric acid ($H_2SO_4$) for total P. A second (unignited) replicate of 0.5 g dry soil was extracted with 30 mL of 1 M $H_2SO_4$ for 24 h for inorganic phosphorus (IP) determination. The suspensions were centrifuged at 17000 g for 10 min and the supernatants analyzed. The orthophosphate content in each extract was measured as acid molybdenum complex colorimetrically with detection at 880 nm (Murphy and Riley, 1962). The organic phosphorus (OP) content was determined by difference.

### 2.4. Particulate organic matter

Particulate organic matter (POM) was separated from soil by physical fractionation (Zimmermann et al., 2007 modified by Poeplau et al., 2013). Briefly, 30 g of soil were dispersed in 150 mL of deionized water using an ultrasonic probe with a calibrated energy output of 22 J $mL^{-1}$ to break down macro-aggregates. The soil suspension was