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# How do grassland management history and bacterial micro-localisation affect the response of bacterial community structure to changes in aboveground grazing regime?

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**Abstract**

In a context of frequent intensification or de-intensification of management in grasslands, a better understanding of how quickly soil microbiota responds to changes in management is required. The kinetics of changes in the structure of the bacterial community (using ribosomal intergenic spacer analysis) was studied in grassland mesocosms after changes of aboveground grazing regime, taking into account bacteria micro-localisation by separating the bacteria located inside stable aggregates (inner soil fraction) and the bacteria easily washed out, i.e. mainly located in macropores (outer soil fraction). Four treatments were used: (i) control grazed mesocosms, (ii) control ungrazed mesocosms, (iii) application of grazing on previously ungrazed mesocosms, (iv) cessation of grazing on previously grazed mesocosms. Each grazing event was simulated by application of synthetic sheep urine and plant clipping. Application of grazing led to a change in the structure of the whole soil bacterial community within 5 months, whereas changes were observed only 12 months after cessation of grazing. Changes in plant species composition and soil organic carbon content observed after cessation of grazing were found to be possible drivers of the changes in the bacterial community structure. However, after application of grazing, changes of the bacterial community structure occurred prior to changes in plant species composition and soil organic carbon content, suggesting that supply of urine and/or impact of labile carbon were likely the main drivers of changes. After 12 months, the application of grazing significantly affected the bacterial community structure in both inner and outer soil fractions. Conversely, 12 months after cessation of grazing, community structure was affected only for bacteria located in the outer fraction. This study shows that the bacterial community structure responded faster and more deeply after application than after cessation of grazing, and may be driven by different environmental factors between both scenarios. This study also shows that, 2 years after the changes in grazing regime, the bacterial community structure was determined by both the past and new grazing regimes.


*Keywords:* Bacterial diversity; Herbivory; Plant species composition; Prairie; Ribosomal intergenic spacer analysis; Soil fractionation; Microbial community

## 1. Introduction

In grassland ecosystems, management practices induce disturbance regimes (i.e. repeated disturbances in time) that deeply affect the soil system and its microbial component. For instance, tillage (Calderon et al., 2001), mineral fertilisation (McCaig et al., 1999; Clegg et al., 2003; Grayston et al., 2004) and organic fertilisation (Bol et al., 2003; Bittman et al., 2005) have been shown to strongly influence the activity and composition of the soil microbiota. In particular, aboveground grazing regime by cattle or sheep deeply affects C and N cycling mainly via urine/dung deposition and sward defoliation. This, in turn, has been shown to affect the composition of soil microbial community on the long term, typically on a few years to decades in fertilised grasslands (Bardgett et al., 1997, 2001; Clegg, 2006) and unfertilised grasslands (Patra et al., 2005, 2006). Actually, demographic and socio-economical constraints often lead to intensification or de-intensification in

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grazed grasslands over time scales shorter than decades but information on the response of the soil bacterial community in this context is very scarce. For instance, Bardgett et al. (2001) have analysed the response of the structure of the soil bacterial community after cessation of grazing on a long term (after 5, 10 and 20 years). Conversely, after urine application (which is one aspect of intensification), Rooney et al. (2006) have surveyed the genetic structure of the soil bacterial community on a short term (after 10 and 50 days) in soil microcosms. However, the response of soil bacterial community to both intensification and de-intensification has never been studied during a course of time from short term (a few months) to medium term (a few years). Furthermore, to what extent the bacterial micro-localisation can influence the kinetics of the response of the bacterial community has not been studied yet. This should be taken into account, because depending on their micro-localisation in soil, microorganisms are likely exposed differently to modifications of environmental conditions after a change in management regime (Ranjard and Richaume, 2001).

The aim of our study was to characterise the changes in the genetic structure of the eubacterial community by applying ribosomal intergenic spacer analysis (RISA) to DNA extracted from whole soil and from soil fractions corresponding to inner and outer soil fractions. This study was conducted in grassland mesocosms originating from grazed and ungrazed sites, over 2 years after reciprocal shifts of grazing regime. Four treatments were compared : (i) application of grazing on previously ungrazed mesocosms, (ii) cessation of grazing on previously grazed mesocosms, (iii) control grazed mesocosms, and (iv) control ungrazed mesocosms. Simulated grazing regime corresponded to plant clipping and application of synthetic sheep urine five times per year. The specific objectives were:

(1) To test the hypothesis that application of a disturbance regime on a previously undisturbed system would lead to a faster response than cessation of the disturbance regime on a previously disturbed system, as observed in other ecosystems (Garbeva et al., 2006; Salles et al., 2006).
(2) To analyse to what extent the changes in the bacterial community structure could be explained by the changes in plant species composition and/or soil organic carbon content.
(3) To test whether the changes in grazing regime affect differently the structure of the bacterial community according to its micro-localisation in soil, assuming that the impact on bacteria in the stable aggregates, which are less exposed to modifications in environmental conditions, would be lower than that on bacteria located in the macropores.
(4) To evaluate whether the bacterial community structure was mainly determined by the present, the past or by both present and past disturbance regimes during the 2-year period after changes in grazing regime.

## 2. Materials and methods

### 2.1. Experimental design

In May 2002, intact soil blocks with plant cover were sampled in a semi-natural grassland in Theix (45°43′N, 3°1′E, at 870 m a.s.l., France), in plots that had experienced either intensive grazing by sheep (IG, five grazing events per year) or light grazing by sheep (LG, one grazing event per year) for 14 years without mineral N fertilisation. IG and LG plots experienced the same initial soil characteristics (53% sand, 22% loam, 25% clay; pH 5.6) and the same climate (Le Roux et al., 2003). After 14 years, management led to different total soil organic carbon content in the 0–20 cm layer: 4300 g C $m^{-2}$ as soil organic matter plus 450 g C $m^{-2}$ as root and rhizome biomass in LG plots as compared to 3450 plus 150 g C $m^{-2}$ in LG plots (Klumpp et al., 2007). One hundred and four mesocosms (L: 50 cm × *w*: 50 cm × *d*: 40 cm) were placed in a 5-side stainless-steel box with drain holes at the bottom (see details in Klumpp et al., 2007). The mesocosms then experienced two contrasted grazing regimes during 1 year:

Grazing (G treatment): plants were clipped to 5 cm above soil surface and synthetic sheep urine supplied five times per year for the mesocosms originating from the intensively grazed plot.
No grazing (ungrazed U treatment) for the other mesocosms.

Soil moisture was monitored and kept around 40% (ca. 70% of water holding capacity) by regular watering. Simulation of grazing by cutting corresponded to an annual mean of biomass removal of approximately 600 g C $m^{-2}$ $year^{-1}$. In order to simulate N returns at grazing, 90% of the N removed by cutting was replaced a few days later by applying artificial urine. Artificial urine had mean total N content of 0.7 g N $L^{-1}$ and consisted of 80% urea, 10% hippuric acid, 5% allantoin N and 5% creatine N adjusted to pH of 7 with NaOH (Doak, 1952). The annual amount of N application represented 250 kg N $ha^{-1}$.

In late April 2003, eight mesocosms (4 G and 4 U) were sacrificed to characterise the genetic structure of the soil microbial community at the beginning of the experiment. At the same date, grazing regime was reversed for half of the remaining mesocosms: 24 grazed mesocosms were submitted to a shift of grazing regime, i.e. from grazing to no grazing, GU treatment; and 24 ungrazed mesocosms were submitted to the reciprocal shift from no grazing to grazing, UG treatment. Grazing regime was left unchanged for the 48 other mesocosms: 24 control grazed mesocosms, GG treatment; and 24 ungrazed mesocosms, UU treatment.

### 2.2. Soil sampling

Soil was sampled just before the change in grazing regime (April 2003), and then 1.5 months (June 2003), 5