

17 Soil Microbial Diversity in Grasslands and its Importance for Grassland Functioning and Services

Xavier Le Roux, Sylvie Recous and Eléonore Attard

17.1 Soil: a Hotspot of Biodiversity in Grasslands

A wide range of life forms exist on Earth. For instance, the total numbers of bird and plant species on Earth are around 10,000 and 300,000, respectively. Moreover, we know that some habitats, ecosystems or regions harbour high levels of biodiversity. For instance, Colombia harbours around 1800 bird species whereas 8000 tree species can be found in Brazil! Even at a smaller scale, biodiversity can be huge: a single hectare of forest in French Guyana can harbour 600 trees representing more than 200 tree species, whereas 10 to 50 plant species can be found within one hectare of permanent grassland. More generally, Earth harbours plenty of individuals of many different species: scientists have evaluated that around 200 billion (2×10^{11}) adult or sub-adult birds are present at a given time on planet Earth. Thus, understanding biodiversity dynamics, and the links that exist between living organisms including humans, represents one of the grand challenges of our time.

Astrophysicists could argue that such numbers are not so high and that they have to face a bigger challenge. Indeed, the Universe encompasses 10^{22} stars, including 4×10^{11} in our galaxy! In addition, stars

can hardly be seen clearly by the naked eye and cannot be approached to be observed. Astrophysicists thus have to work on objects to which they have no direct access. They develop large and advanced instruments to study them and develop refutable theories to analyse the functioning of the Universe.

One type of ecologist could, however, say that the numbers and difficulties raised by astrophysicists are not so challenging: soil microbial ecologists. Indeed, when you take 10 g of grassland soil in your hand, you handle around 10^{11} bacteria, encompassing 10,000 to 100,000 bacterial 'species', although the concept of bacterial species remains more open and debated than that used for higher organisms (Torsvik *et al.*, 1998; Gans *et al.*, 2005). Actually, when accounting for plants (plant roots), meso- and macro-fauna and microflora, soil is the reservoir of a huge diversity of living forms (Lavelle and Spain, 2002; Bardgett, 2005): around 3×10^8 animals can be found below ground in 1 m² of grassland! But knowledge on soil biodiversity is still sparse: in particular, soil microbial diversity is difficult to characterize and study, with more than 90% of bacterial taxa reluctant to be cultivated by humans. Thus, as for astrophysicists, working on grassland soil microbial diversity also implies working

on abundant and diverse objects that are hardly accessible, and developing advanced approaches and instruments and new theories to characterize those organisms and understand soil functioning. The big difference is that microbes are living objects, which makes the challenge even greater!

Let's stop the comparisons of numbers and research areas but let's remember this in the context of this book on grassland ecosystem functioning and services: the level of biodiversity is much higher in soil than in any other system we are used to considering when we are thinking about life on Earth. Both the number of species and organism abundance in grassland soil reach levels well above those of above-ground, higher organisms.

This leads to the first question: why is grassland soil biodiversity much higher than the diversity of plants and animals above-ground? A first explanation is that the size of soil organisms is often very small. Whereas an earthworm is large enough to be seen, most grassland soil organisms are too small to be seen with the naked eye: from a few microns for bacteria, 100 times smaller for viruses, to around 1 mm for nematodes. Concurrently, grassland soil is chemically and physically highly heterogeneous at a range of scales: there are soil aggregates of various sizes; the soil volume can be influenced by roots (rhizosphere), the amount or proximity to dead organic matter (detritosphere), or the activity of soil macrofauna such as earthworms, etc.

Grassland soil thus offers a range of diverse habitats to living organisms; in addition, all its porosity and particles create a huge surface to be colonized by life. Thus, this allows the co-existence of large numbers of species, and huge numbers of individuals, able to exploit the different 'microhabitats'. In such a complex ecosystem, the life of a small, weakly mobile organism can strongly differ among microhabitats. A significant proportion of bacteria are 'asleep', like the 'Sleeping Beauty' of the classic fairy tale, because environmental conditions at their exact location are not favourable to their activity:

those bacteria are waiting, not for the kiss of their Prince Charming, but that of a root (exudates), that of a soil organism (e.g. earthworm mucus) or an event, such as soil ploughing (e.g. when a temporary grassland is converted into a cropping system), that would bring more oxygen and energy to them and eventually awaken them (Lavelle and Spain, 2002). This is equivalent to a situation where the biomass of a grassland vegetation cover would include a substantial proportion of plant individuals present with no or very weak photosynthetic activity, but those plant individuals would be able to become active, after a few months or years, according to environmental changes. This would probably change much of grassland ecologists' approaches and theories! In addition, the evolutionary capacity of a number of grassland soil organisms is remarkable: with generation times ranging from a few hours to days and a striking ability to exchange genetic material (even between species, through 'horizontal transfer' of DNA); bacterial diversification and bacterial species radiation are fast. Given all those features, one can easily understand why grassland biodiversity is so high in soil. This leads to a second question: how and to what extent does such a huge biodiversity influence grassland soil functioning and the services delivered by grasslands to humans?

17.2 Importance of Soil Biodiversity for Grassland Functioning

Many studies have been performed to understand to what extent and through which mechanisms biodiversity plays a role in the functioning of grassland systems. Most of those studies focused on macroorganisms, often plants (Hooper *et al.*, 2005; Balvarena *et al.*, 2006). The results showed that high levels of biodiversity can enhance grassland ecosystem performance. In addition, those studies demonstrated that biodiversity can influence the stability of grasslands facing disturbances. Indeed, the presence of a diversity of species that can differ in their response to perturbations could decrease

the instability of grassland functioning following perturbations. However, fewer studies have focused on grassland soil organisms.

Beyond the methodological difficulties associated with the characterization of grassland soil biodiversity, many authors consider that the level of functional

redundancy in soil should be particularly high, given the high level of biological diversity. These authors thus assume that one can disregard soil biodiversity for understanding and predicting grassland ecosystem functioning. This assumption has been tested by different types of approaches (Fig. 17.1).

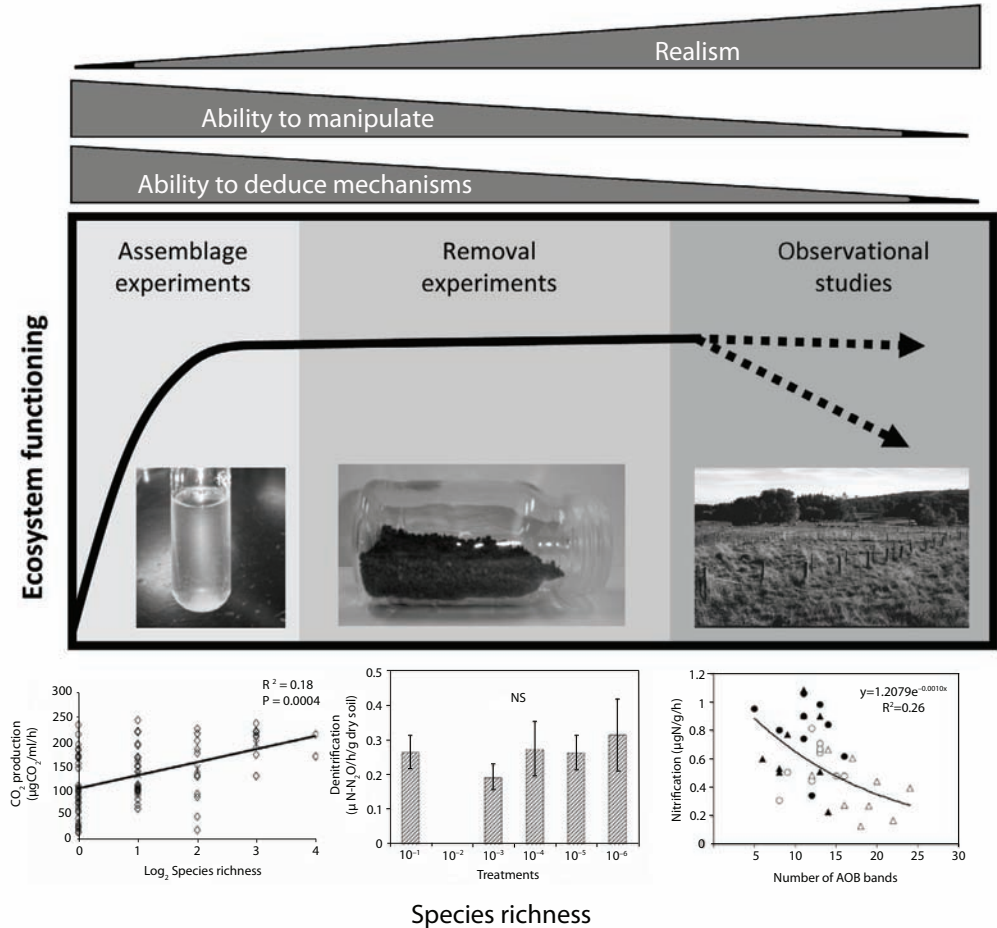


Fig. 17.1. Synthesis of different approaches used to analyse the relationships between the microbial diversity and functioning of grassland soils. (Left) an assemblage experiment (Salles *et al.*, 2009) demonstrates the positive effect of diversity for cultivated denitrifying bacterial taxa derived from bulk and rhizospheric soil; in particular, the authors demonstrated the importance of the functional dimension of diversity and of complementary effects. (Middle) A species removal approach via suspension–dilution applied on a native grassland soil community demonstrates the high level of functional redundancy in native, complex nitrifying and denitrifying communities (Wertz *et al.*, 2006, 2007). (Right) Observatory approaches show a broad spectrum of possible, apparent relationships between the microbial diversity of grassland soil and its functioning; in this case, analysing the co-variations of diversity, abundance and environmental conditions is a prerequisite to assess the actual role of biodiversity in changes in soil functioning (Patra *et al.*, 2005, 2006; Le Roux *et al.*, 2008). This figure has been modified according to an output of the EFS workshop ‘Microbial Diversity and Ecosystem Functioning’ held in Lunz, Germany, in 2007.

17.2.1 Species assemblage approaches

Some scientists have used a species assemblage approach to study the importance of microbial biodiversity for the functioning of grassland soil. As performed in ecology on higher organisms, such an approach consists of creating synthetic microbial communities and studying their performance (level of functioning, resistance and resilience to model disturbances) in relation to their diversity. Assemblage experiments are suitable to study biodiversity–functioning relationships in the context of (micro)-habitat colonization from a more or less diverse pool of taxa (Diaz *et al.*, 2003). These experiments are useful in analysing the mechanisms of interaction and complementarity that can exist between taxa and can shape biodiversity–functioning relationships.

Assemblage approaches have been used for litter-decomposing fungi (Setälä *et al.*, 2004), mycorrhiza (Van der Heijden *et al.*, 1998), detritivorous macro-fauna (Heemsbergen *et al.*, 2004) and bacteria isolated from grassland soil (Salles *et al.*, 2009), among other studies. Several of these studies reported a positive relationship between the level of soil functioning and the diversity level of the studied communities. They also stress that soil functioning depends mainly on functional diversity (i.e. the diversity of functional characteristics or traits of organisms) rather than on taxa number per se. Salles *et al.* (2009) even demonstrated that a simple index of functional diversity accounting for complementarity between bacterial taxa ('bacterial community niche') predicted threefold better the variations in community functioning than did taxa number.

However, the maximum number of species used in typical assemblage experiments is low, from a dozen to tens, and much lower than the number present in the native, complex microbial communities encountered in grassland soils.

17.2.2 Species removal approaches

Another approach used to study the importance of biodiversity for the func-

tioning of grassland soil and take account of the naturally high level of diversity is species removal. In such an approach, the native soil biodiversity is progressively eroded on purpose and the induced modifications of soil functioning are assessed. A study conducted by Griffiths *et al.* (2000) demonstrated that a function involving a broad range of soil organisms, i.e. organic matter decomposition, was not affected by a significant decrease in the diversity of the soil microbial community. Even more specialized bacterial communities, such as nitrifiers and denitrifiers, proved to be largely non-sensitive to a strong decrease in their diversity (Wertz *et al.*, 2006, 2007). In the latter studies, the authors showed that removing 99% of the bacterial taxa present in grassland soil slurry used to inoculate sterile grassland soil microcosms did not affect the functioning and resistance/resilience capacity of the nitrifying and denitrifying communities. Such results highlight the huge level of functional redundancy that exists within grassland soil bacterial communities.

Do such results imply that soil microbial diversity is so high that one can disregard it for a better understanding of, and to predict, soil function? This would be a misleading conclusion. First, the concept of functional redundancy is context dependent (Loreau, 2004). The huge diversity of the soil microbiota actually represents a large reservoir of genes and functions that can become useful when soil faces variable environmental conditions, and particularly extreme or new environmental conditions. This is clearly illustrated by the response of soil microbial communities to anthropogenic inputs of a pesticide: lindane. At the time of the first input of lindane in agroecosystems, no bacteria were able to degrade this new molecule: it is synthesized by humans so lindane had never been a selection pressure before for soil bacteria. After some decades, however, exposed soils were found to harbour bacteria able to degrade lindane. Some studies (Boubakri *et al.*, 2006) strongly suggest that a lindane-degradation gene appeared *de novo* through a reshuffling of

genetic material from different soil bacterial taxa: some bacteria might have 'gone shopping' in the huge reservoir of biological and genetic diversity of soil, to create a new gene allowing them to degrade lindane and thus cope with this new stressor. This illustrates well that the huge soil biodiversity is of paramount importance for soil functioning. More generally, humans are not always aware of the key role that soil biodiversity plays in the functioning of grassland soils and the services delivered by grasslands. But a challenge remains for soil microbial ecologists: how do we have to account for soil biodiversity, in particular soil microbial biodiversity, to model and predict changes in grassland soil functioning and services delivery?

17.3 How to Represent Grassland Soil Biodiversity in Models of Grassland Functioning and Service Delivery

Soil biodiversity plays a key role in the services delivered by grassland ecosystems, including recycling of organic detritus; maintenance of soil fertility; discrete and continuous soil ploughing throughout the year and maintenance of a good soil porosity and drainage capacity that can restrict soil erosion and promote crop yield; provision of organisms able to control crop pests or to stimulate plant growth; carbon sequestration and control of greenhouse gas emission; and runoff and sub-surface water purification. However, for ecologists who develop conceptual frameworks and models to understand the ecology of grassland ecosystems better and predict their functioning and services delivery, adequately representing soil biodiversity often remains a challenge. Can they disregard it? Or do they have to deal with the increasing view that more reductionism and increased application of modern molecular approaches of microbial communities are needed to improve the simulation of grassland soil functioning in response to environmental changes? Recent

studies have addressed this issue, creating guidelines for grassland ecosystem ecologists and modellers.

In a first study, Attard *et al.* (2011) assessed to what extent can changes in denitrification be predicted by representing the denitrifying community as a black box, i.e. without an adequate representation of the biological characteristics (abundance and composition) of this community. They analysed the effect of changes in land use on denitrifiers for two different agricultural systems: (i) crop/temporary grassland conversion and (ii) cessation/application of tillage. Changes in potential denitrifying activity, that were tightly linked to time-integrated N_2O emissions, were partly due to changes in denitrifier abundance but were not related to changes in the diversity of the denitrifier (nitrite reducer) community. Actually, changes in denitrification were more related to changes in soil environmental conditions than in denitrifier abundance: soil organic carbon explained more than 80% of the variance observed in denitrification at the crop/temporary grassland site, while soil organic carbon, water-filled pore space and nitrate explained more than 90% of the denitrification variance at the till/no-till site (Attard *et al.*, 2011). These results show that an accurate simulation of carbon, oxygen and nitrate availability to denitrifiers is more important, than an accurate simulation of denitrifier abundance and community structure, in adequately understanding and predicting changes in denitrification in response to land-use changes. Avoiding finely characterizing the diversity of the denitrifying community seems reasonable here. The authors noted that their conclusions probably hold for diverse microbial communities with high functional redundancy that realize a process through a facultative activity, as for denitrifiers. They stated that the balance between functional groups (i.e. NO_2^- , NO and N_2O reducers when studying denitrification) could also be more important for soil functioning than the accurate composition of each of these functional groups.

In another study, Attard *et al.* (2010) assessed to what extent understanding the changes in nitrification requires an adequate representation of the biological characteristics (abundance and composition) of the nitrifying community. They analysed the effect of changes in land use on nitrifiers (nitrite-oxidizing bacteria, NOB). Nitrification (nitrite oxidation) was strongly and positively correlated with the abundance of *Nitrobacter*-like NOB and was also strongly related to gross mineralization, a proxy of N availability. In contrast, nitrification was weakly and negatively correlated with the abundance of *Nitrospira*-like NOB. Nitrification was loosely correlated with the genetic structure of the *Nitrobacter*-like NOB community. These results demonstrate that *Nitrobacter*-

like NOB are the key functional players within the NOB community in soils with high N availability and high activity level, and that changes in nitrification are due to shifts between *Nitrospira*-like and *Nitrobacter*-like NOB rather than shifts of populations within *Nitrobacter*-like NOB. The results of this second study thus show that the coupling between the activity and abundance/community structure can be strong for less diverse soil microbial communities and obligatory activity (e.g. nitrifiers and nitrification).

This leads us to propose a general framework for guiding grassland or, more generally, ecosystem ecologists and modelers when they try to include some insights into the soil microbial component in their conceptual frameworks or models (Fig. 17.2).

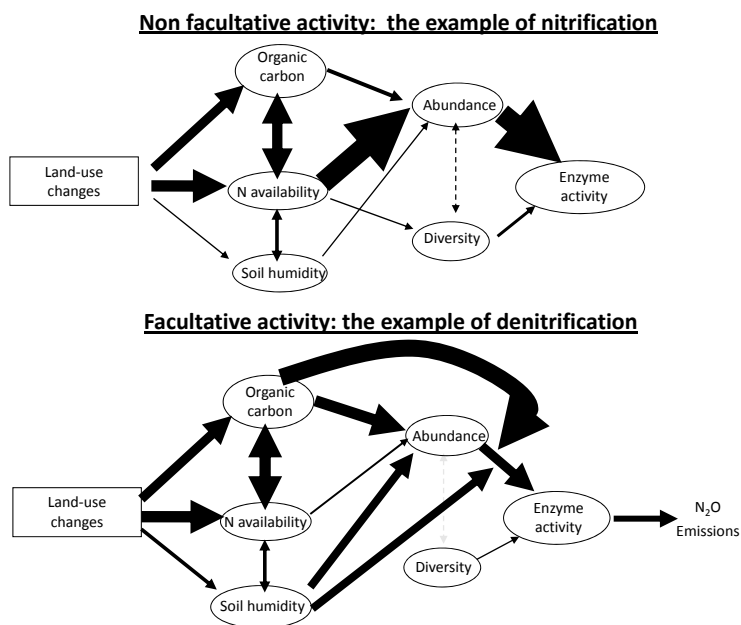


Fig. 17.2. Importance of soil bacterial diversity and abundance along with soil environmental conditions for predicting changes in soil functioning after changes in land use: adapted from the studies of Attard *et al.* (2010, 2011). Field situations involving crop/grassland conversion and cessation/application of tillage were studied. General linear analysis and path analysis were used to synthesize the observed relationships (the larger an arrow, the stronger the link between variables for (top) the nitrifying community and (bottom) the denitrifying community). The activity of the nitrite-oxidizing community is strongly linked to abundance and diversity (the key component of diversity being the balance between *Nitrobacter* and *Nitrospira*) that are themselves strongly linked to N availability. The activity of the denitrifying community is only partly explained by changes in denitrifier abundance and not related to the fine genetic structure of the denitrifying community; the activity is actually mainly explained by changes in organic carbon and, to a lesser extent, oxygen (through water-filled pore space) that influence the specific activity of denitrifiers.

Figure 17.2 demonstrates that there is no general rule for accounting for soil microbial diversity in models of grassland/ecosystem functioning. For some key functions like nitrification, which is a non-facultative activity (nitrifiers nitrify to acquire the energy needed to maintain their activity and to grow), accounting for the abundance of the functional group and key characteristics of their diversity is a major step forward in understanding and predicting changes in soil functioning. For instance, the ratio between *Nitrobacter*- and *Nitrospira*-like bacteria affects soil nitrite oxidizing activity, since *Nitrobacter*-like NOB are r-strategists favoured under high N availability, whereas *Nitrospira*-like NOB are K-strategists favoured under low N availability. For other important functions like denitrification, which is a facultative activity (denitrifiers reduce nitrate or nitrite under conditions of low oxygen availability, but behave as other heterotrophs with sufficient oxygen availability) realized by a broad range of organisms, representing in detail the changes in the abundance and diversity within the microbial functional group is, to some extent, useless. In this case, the activity depends much more on environmental conditions (organic carbon, oxygen and to a lesser extent nitrate availability) than on the microbial community characteristics. A good representation of soil environmental conditions is of paramount importance here.

More generally, the main objective of ecosystem ecologists in this context should be to account for the balance between soil microbial functional groups (e.g. NO_2^- , NO and N_2O reducers when studying denitrification) and/or between main groups differing in terms of their effect/response traits within a given functional group (e.g. *Nitrobacter*/*Nitrospira* within NOB). Given the wealth of diversity encountered in soil, this broad approach based on the abundance and activity of targeted microbial sub-groups is probably a more efficient way of understanding and predicting soil functioning and service or disservice delivery than the traditional characterization of the diversity/composition/genetic structure of whole functional groups. As Râmakrishna stated, 'knowledge leads to unity, but ignorance to diversity'. We can surely assess our real level of knowledge about the relationships between soil biodiversity and grassland functioning in our ability to provide comprehensive pictures of such functioning and biodiversity, disregarding useless details, and not only in our sole ability to apply advanced methodologies to deepen the characterization of the huge biodiversity harboured by grassland soils. This calls for a significant cooperation between, at least, soil microbial ecologists, grassland ecosystem ecologists and general/theoretical ecologists.

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