Soil Microbial Community: Understanding the Belowground Network for Sustainable Grassland Management

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Soil microbial community: understanding the belowground network for sustainable grassland management

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Key points

1. In addition to the use of conventional methodologies in soil microbial research, molecular techniques are now being applied to gain insights into the soil microbial community;
2. Plant diversity can exert impacts on soil microbial diversity (through root activities and plant litter etc.), but may in itself be significantly altered by soil properties;
3. Soil microbial diversity largely determines the stability of soil ecosystems under biotic and abiotic perturbations.
4. Management of soil microbial diversity can only be achieved through better understanding their structures and functions.

Keywords: microorganisms, decomposition, soil-plant systems

Introduction

Soil microorganisms play important roles in many ecosystem processes such as biogeochemical cycling of nutrients (nutrient transformations) and soil structural and hydrological properties (Lesser *et al*., 2004; Driver *et al*., 2005). A decline in soil productivity can often be attributed to a number of factors such as nutrient depletion, pests and diseases, drought, salinity and changes in climatic conditions. However, with the development of DNA and RNA methodologies there is an increasing understanding of the importance of the microbial community for soil health (Stocking, 2003). Thus, soil microbial properties are often used as indicators of soil quality. Traditionally, the belowground and aboveground components of soil-plant systems have been studied in isolation from one another (Wardle *et al*., 2004), due in the main to a lack of proper techniques. Consequently our understanding of the belowground ecosystem has been far poorer than of the aboveground ecosystem. Over the last ten years or so there have been substantial advances in soil microbial ecology, including the development of new methodologies, and the recognition of the linkage between belowground and aboveground components.

Methodologies

Progress in examining microbial diversity in soils has been limited due to difficulties in the accurate measurement of species richness and evenness in soil microbial communities (Wardle & Giller, 1997). Microbial diversity can be categorized into two groups: species diversity and functional diversity. Functional diversity is a component of overall soil diversity, which may provide a more practical and ecologically relevant measure of microbial diversity (Zak *et al*., 1994).

Species diversity consists of species richness, the total number of species present, species evenness, and the distribution of species (Øvreås, 2000). Methods measuring species diversity in soil include fatty acid methyl ester (FAME) and molecular-based techniques. The FAME method provides information on the microbial composition based on groupings of fatty acids (Frostegård *et al*., 1993). Treonis *et al*. (2004) combined microbial community phospholipid fatty acid (PLFA) analyses with an *in situ* stable isotope $^{13}CO_2$ labelling approach, to identify microbial groups actively involved in assimilation of root-derived C in limed grassland soils. Ritz *et al*. (2004) studied the relationship between vegetation composition and soil microbial structure in upland grassland, and found that PLFA and community level physiological profiles (CLPP) showed some association with vegetation composition, but denaturing gradient gel electrophoresis (DGGE) profiling did not.

Alternative methods for studying microbial species diversity are based on molecular techniques. Polymerase chain reaction (PCR) - based approaches, i.e. DGGE and terminal restriction fragment length polymorphism (T-RFLP), have been used extensively. Increasingly PCR targeting of the 16S rDNA are been used to study prokaryote diversity, whilst 18S rDNA and internal transcribed spacer regions (ITS) are used to study fungal communities in soils. In these methods, genomic DNA is extracted from soil samples and purified. Target DNA (16S, 18S or ITS) is amplified using universal or specific primers, and then differences among microbial communities in soil are further analyzed. Nicol *et al*. (2003) used a PCR-DGGE method to study spatial differences in community structure in grassland soils. Griffiths *et al*. (2003) investigated the effects of water stress upon the diversity of bacterial communities in the rhizosphere of established upland grassland, subjected to different watering regimes over a two-month period. Profiling using DGGE analyses of community 16S rRNA genes and rRNA transcripts did not reveal any changes relating to the moisture regimes, suggesting that bacterial communities in this soil were resistant to water stress. The technique is thought to be accurate and sensitive for assessment of species diversity of arbuscular mycorrhizal fungi.

The use of T-RFLP for the study of microbial diversity in soil has become widespread. This technique allows the detection of only the labeled terminal restriction fragment (Liu *et al*., 1997). It provides information on diversity, as each visible band represents a single operational taxonomic unit (Tiedje *et al*., 1999). The method can be automated to allow analysis of larger numbers of soil samples, but it can also lead to overestimation due to incomplete digestion by restriction digestion (Osborn *et al*., 2000). Klamer *et al*. (2002) used the T-RFLP technique to assess the effects of elevated atmospheric CO_2 levels. Although fungal biomass was enhanced in the high CO_2 concentration treatment, there was no significant influence on fungal composition and richness in the soil. The same technique (T-RFLP analysis) was also useful for identifying broad-scale, consistent differences in the bacterial communities in different soil locations over the natural micro scale heterogeneity of the soil in arid grassland (Kuske *et al*., 2002).

Microbial functional diversity is different from that obtained by measuring species diversity, in that it concerns the range and evenness of functions expressed *in situ* by the microbial community, rather than the species present in soils. Microbial functional diversity includes a vast range of activities comprising nutrient transformations, decomposition, plant growth promotion/suppression, modification of soil physical processes (Wardle *et al*., 1999), resistance/resilience to exotic stress or disturbance (Griffiths *et al*., 2001a), potential carbon utilization (Garland & Mills, 1991), and catabolic response profile (Degens & Harris, 1997).

Thus, different methods measuring the functions of the microbial community in soil have been developed for different functions.

Among the various approaches, Biolog substrate decomposition and catabolic profiling have become popular. Mäder *et al*. (2002), used the Biolog method to demonstrate that enhanced soil biodiversity and fertility in organic plots, might render these systems less dependent on external inputs such as fertilisers and pesticides. In temperate upland grassland ecosystems, potential substrate utilisation using the Biolog method was assessed across a gradient of three upland grassland types (unimproved, semi-improved and improved) (Grayston *et al*., 2001). Greater total carbon utilization by microbial communities from the improved grassland, suggested that there was more readily available carbon present in improved grasslands which stimulated bacterial growth. Therefore, plant species composition is an important factor governing microbial community structure in upland grasslands. With the Biolog method, Papatheodorou & Argyropoulou (2004), indicated that there were significant seasonal differences in functional diversity and evenness, and that the substrates responsible for the monthly differences were mainly carbohydrates and carboxylic acids.

Another popular approach is catabolic response profiles (CRPs) developed by Degens & Harris (1997). The method provides an easily interpretable and practical indicator of the diversity component of the soil microbiota (Sparling *et al*., 2000). Stevenson *et al*. (2004) used the method to show that in each pasture or forest category, catabolic responses showed a similar pattern, suggesting similarities in functional catabolic capability. They found that pasture soil communities had significantly higher relative responses to carbohydrate and amino acid substrates, and significantly lower relative response to carboxylic acid substrates than microbial communities from forest soils. Changes caused by exotic stresses in the catabolic response of the soil microbial community were determined in crop and pasture soils (Degens *et al*., 2001). The results showed that the crop soil with lower catabolic diversity reduced the resilience to applied stress compared with the pasture soil with higher catabolic diversity.

Substrate decomposition is also extensively used to assess functions of the microbial community in soil. The decomposition of a range of substrates added to soil is multiphasic. Hu & van Bruggenm (1997), demonstrated that multiphasic decomposition of cellulose is controlled by C and N availability, and the structure of the microbial community. Thus, given similar nutrient status, the pattern of substrate decomposition should reflect microbial community function. Griffiths *et al*. (2001a) found an interesting result that decomposition of grass shoot residues added to a petrol-contaminated soil, were significantly greater than in the uncontaminated soil, suggesting that microbial communities in soils contaminated with a particular pollutant could decompose that compound more rapidly than those in uncontaminated soils.

Soil microbial diversity and sustainability

The linkage between biodiversity and sustainability of terrestrial ecosystems has been studied mainly in aboveground systems. Diversity-sustainability relationships take various forms, e.g. hump-shaped, U-shaped, positive, negative and flat (non-significant) patterns, with no pattern predominating. Until recently, the hump-shaped relationship was the most widely observed pattern, in which plant diversity peaks at intermediate productivity levels under given conditions (Loreau *et al*., 2001). Sustainability is the maintenance of system productivity and its continued function, whilst using its functions to establish sustainable production in the face of continued increases in human population (Pankhurst, 1997; Kennedy, 1995). Soil

sustainability focuses more on soil functional stability, comprising resilience and resistance. Soil resistance is the inherent capacity to withstand exotic disturbance, whereas soil resilience is the capacity to recover after disturbance (Seybold *et al*., 1999).

There is some evidence that lower diversity is less resistant to stress and disturbance. Degens *et al*., (2001) compared soil capacity to withstand stress and disturbance in arable soil with lower catabolic evenness, and pasture soil with higher catabolic evenness. They found that increasing Cu concentration, salt stress, acidification, and wet-dry and freeze-thaw cycles caused greater decline in catabolic evenness in the arable soil than the pasture soil. Soil microbial diversity followed the classical 'hump-back' responses to the three stresses and two disturbances. Their results suggest that land use resulting in reduced catabolic evenness in soils, such as long-term cropping, might similarly exhibit a microbial community with reduced resistance to stress or disturbance. Griffiths *et al*. (2000) obtained similar results using progressive fumigation to reduce grassland soil microbial biodiversity, to measure the effects of different diversity upon the stability of key soil processes. Results suggest that more specific parameters decreased as biodiversity decreased, e.g. nitrification, denitrification and methane oxidation. The soils with the highest biodiversity were more resistant to the stress (brief heating to 40 ^{o}C and the addition of $CuSO_4$) than soils with impaired biodiversity. Griffiths *et al*. (2001a) determined the stability of three pairs of soils with different microbial diversities. The first pair of soils were planted for 5 years with either a single annual grass species or six annual grass species; a second pair were from 3 cm depth of a petrol-polluted soil, one undergoing remediation and the unpolluted control; the last two were agricultural and organic soils from intensively and extensively managed horticultural farms. The six soils were subjected to perturbation by adding 500µg Cu/g dry soil and heating to 40^{o}C for 18 hours. The results demonstrated that both grassland soils and the organically managed agricultural soil with high diversity had the greatest resistance to both Cu and heat; the intensively managed agricultural soil had intermediate resistance to the two applied perturbations; the two petrol-polluted soils had the least resistance. Zhou *et al*. (2002) also found no reduction in soils with extremely high chromium (III) contents (e.g. >20%). They suggested that the carbon-rich soils with high microbial diversity were able to counteract stress to maintain soil stability.

There are examples of experimental reduction in microbial diversity having no direct effects on soil functions. Griffiths *et al*. (2001b) studied soil diversity relationships for a range of soil processes by inoculating sterile agricultural soil with serially diluted soil suspensions prepared from the parent soil. No consistent effect of biodiversity on the soil processes (e.g. incorporation of thymidine, potential nitrification, nitrate accumulation, respiratory growth response, community level physiological profile and decomposition) was observed. One possible reason is that there was selection of microbial communities due to the initial dilution. Degens (1998), also investigated whether reducing the microbial diversity of a pasture soil using a fumigation treatment compromises the decomposition function of the microbial community. The results indicated that the decomposition function of the soil with reduced functional diversity can be diminished under optimum moisture conditions, but is not invariably reduced when assessed under sub-optimal moisture conditions. This suggested that decreases in the functional diversity of soil microbial communities may not consistently result in declines in soil functioning.

Top-down influence

All terrestrial ecosystems consist of aboveground and belowground components, but they have traditionally been investigated separately from each other (Wardle *et al*., 2004), except for the symbiotic relationship between plant roots and mycorrhizal fungi/nitrogen fixing bacteria (see next section). Top-down feedback is regarded as the impact of the plant community on soil microorganisms. Generally speaking, soil microorganisms are responsive to the nature of organic matter input (i.e. substrate quality and quantity). Plant species differ in their patterns of organic matter return to soils, thus individual plant species may have dominant impacts on soil microbial community and related biogeochemical processes (Wardle *et al*., 2004). For example, size, activity and catabolic diversity of the soil microbial biomass could be substantially affected by agricultural land use (i.e. vegetation composition), and microbial diversity is greater in native grassland than in arable and forest soils (Nsabimana *et al*., 2004). Grassland plant species may differ in the microbial communities around their roots, and this may help to explain why soils with different grassland species support different abundance of soil microbes and microbe-feeding fauna. Different traits of plant cultivars (genetic diversity) will also likely affect microbial community in the rhizosphere. However, there is little information available on grassland plant cultivars and soil microbial activities. The topic is important for two reasons: 1) the qualities of forage plants (chemical composition) may influence the soil microbial community, but this will also affect their usefulness as animal feeds and thus efficiency in nutrient cycling in grassland ecosystems; 2) the combination of different cultivars of forage plants may increase their tolerance to some diseases, particularly root diseases through the changes in microbial community. The later has been demonstrated in rice cultivation (Zhu *et al*., 2000b), but has not as yet been extensively investigated in grassland. It is recommended that the possible functions of genetic diversity in managed grassland ecosystem, such as soil microbial diversity, nutrient cycling and feed quality should be investigated.

Plant community structure affects the soil microbial community mainly through rhizosphere deposition (e.g. root exudates and mucilage). An *in vitro* study using artificial root exudates demonstrated that the addition of root exudates enhanced bacterial density and changed the community-level profiles (Baudoin *et al*., 2003). In temperate upland grassland, Bardgett *et al*., (1999) demonstrated that in the short term, the abundance and activity of soil microorganisms were regulated more by plant species traits than by the direct effect of nitrogen input. It was suggested that these effects were possibly linked to variations in root exudates and nutrient acquisition of different plant species. However, the effects of individual plant species on the soil microbial community may also be dependent on environmental factors such as soil fertility. By comparing improved (optimum fertiliser application) and unimproved (with minimal nutrient input) soil types, Innes *et al*. (2004) found that all tested plant species grown in the improved soil, enhanced fatty acids synthesised by bacteria, but negatively impacted the same group of fatty acids in the unimproved soil. Soil fertility-dependent effects of plant species on soil microbes, make general predictions on how plant communities influence soil microbial properties more difficult. Other biotic and abiotic factors may also contribute to the spatial variability of the association between plant and microbial communities (Ritz *et al*., 2004), and this needs further investigation.

Bottom-up influence

To understand the feedback mechanisms between aboveground and belowground components of the ecosystem, it is important to elucidate how soil organisms can influence the

aboveground community structure and functioning (de Deyn, 2003; Wardle *et al*., 2004). The bottom-up influence can be best illustrated by the symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and plant roots. Over 90% of land plants can form symbiotic associations with AMF (Smith & Read, 1997), and these associations can facilitate plant growth by increasing resource acquisition from the soil. It has been generally found that AMF can form symbiosis with a broad range of host plants (i.e. with low specificity) (Smith & Read, 1997). However, in recent years there has been increasing evidence of some degree of ecological specificity or functional compatibility existing between host plants and AMF (Zhu *et al*., 2000a; Smith *et al*., 2003). The responsiveness of host plants to AMF depends on the specific plant and fungal combinations (van der Heijden *et al*., 1998).

The ecological specificity (or functional compatibility) of AMF can have profound impacts on the aboveground plant community. Despite the specific mycorrhizal effects, it is clear that mycorrhizas exert a significant influence on plant community structure and dynamics in grasslands and other terrestrial ecosystems. The effects of mycorrhizal fungi on patterns of plant diversity and their underlying mechanisms are varied. In some plant communities, the presence and abundance of mycorrhizal fungi may regulate structure and the patterns of variation in mycorrhizal dependency among co-occurring plant species (Hartnett & Wilson, 2002). It is a frequent phenomenon that tolerant grasses prevail in poor soil conditions, but when mycorrhizal fungi are added, a broader plant community could overrun the grasses. By using two independent, but complementary ecological experiments, van der Heijden *et al*. (1998) demonstrated that the diversity of AMF is a major factor contributing to the maintenance of plant biodiversity and to ecosystem functioning. The plant species composition of microcosms that simulate European calcareous grassland fluctuate greatly with changing AMF taxa. Plant biodiversity, nutrient capture and productivity in macrocosms that simulate North American old-fields increase significantly with increasing AMF species richness. Seedling establishment within grassland communities can also be promoted by AMF. This was recently demonstrated by van der Heijden (2004), in a microcosm experiment in which the seedlings grew larger and obtained more P in the presence of AMF. These results emphasize the need to protect AMF and to consider these fungi in future management practices in order to maintain diverse ecosystems, and illustrates the potential role of belowground symbiotic systems in the promotion of species recruitment in grassland.

Biological nitrogen (N_2) fixation is another important component of grassland ecosystems, and the symbiotic association between legumes and rhizobia can provide substantial amounts of N to grassland. It has been estimated that the amounts of N fixation by various legumes can be over 500 kg N/ha per year (Carlsson & Huss-Danell, 2003). The actual amount of N fixed depends largely on the host plant species, environmental conditions, and management regimes as well as methods used to estimate N fixation. In grassland ecosystem, legumes often co-exist with non-legume plant species such as *Lolium perenne* (perennial ryegrass), and there may be an interspecific transfer of N from legumes to non-legumes. Due to its importance in the N economy of grassland, the belowground N transfer has received considerable attention in the last two decades (Laidlaw *et al*., 1996; Hogh-Jensen & Schjoerring, 2000). In a field experiment, Hogh-Jensen & Schjoerring (2000) demonstrated that as much as 50% of total aboveground N of *Trifolium repens* (white clover) could be transferred to associated *L. perenne*, with a corresponding value of 10% for *Trifolium pratense* (red clover). Interspecific N flow may occur through several pathways. Principally, N can be transferred from legumes to associated non-legumes through decomposition of donor plant debris, root exudation and sloughing-off of cortex cells from donor plants (Paynel *et al*., 2001), and directly through AMF interconnecting the root systems of the co-existing

plants (Haystead *et al*., 1988). By using a micro-lysimeter system, Paynel *et al*., (2001) showed that, irrespective of direct contact between *T. repens* and *L. perenne*, about 0.076 mg of N were transferred per plant from *T. repens* and *L. perenne* during a two-month experimental period. This N transfer is believed to be mainly through root exudates. However, in the long term, the decomposition of root debris from donor plants can also be substantial. The other major pathway for interspecific N transfer is through AMF hyphal networks connecting plant roots. Nevertheless, the evidence for hyphae-mediated N transfer between plants is inconclusive (Rogers *et al*., 2001), and may be partly due to a certain degree of ecological specificity between AMF and host plant species (Zhu *et al*., 2000a). Novel experimental designs and detection methods have yet to be developed to provide convincing evidence of N transfer through hyphal networks.

Challenges ahead

Although there is increasing evidence that belowground and aboveground community components are closely associated, and that belowground communities play an important role in grassland productivity and sustainability, the following issues need further investigation:

- Novel molecular techniques to quantify the key functional groups of soil microbiota response to key biogeochemical processes;
- Extrapolating laboratory-based results to field conditions and scaling-up factors;
- Novel agents to manipulate specific microbial groups to understand the functions of these groups;
- Linking long-term observational data to grassland sustainability under changing environments (including management practices and global changes).

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