

Review

Culture-Independent Molecular Tools for Soil and Rhizosphere Microbiology

Vivian A. Rincon-Florez, Lilia C. Carvalhais and Peer M. Schenk *

Plant-Microbe Interactions Group, School of Agriculture and Food Sciences,
the University of Queensland, Brisbane, QLD 4072, Australia;
E-Mails: v.rinconflorez@uq.edu.au (V.A.R.-F.); l.carvalhais@uq.edu.au (L.C.C.)

* Author to whom correspondence should be addressed; E-Mail: p.schenk@uq.edu.au;
Tel.: +61-733-469-948; Fax: +61-733-651-699.

Received: 6 May 2013; in revised form: 21 June 2013 / Accepted: 25 June 2013 /

Published: 2 August 2013

Abstract: Soil microbial communities play an important role in plant health and soil quality. Researchers have developed a wide range of methods for studying the structure, diversity, and activity of microbes to better understand soil biology and plant-microbe interactions. Functional microbiological analyses of the rhizosphere have given new insights into the role of microbial communities in plant nutrition and plant protection against diseases. In this review, we present the most commonly used traditional as well as new culture-independent molecular methods to assess the diversity and function of soil microbial communities. Furthermore, we discuss advantages and disadvantages of these techniques and provide a perspective on emerging technologies for soil microbial community profiling.

Keywords: DNA microarrays; fatty acid analysis; molecular fingerprinting methods; next generation sequencing; plant-microbe interactions; rhizosphere biology; soil quality; soil microbial communities

1. Introduction

One of the biggest challenges in agriculture nowadays is to increase yield and sustainability of crop production as the global population is approaching nine billion people by 2050 [1]. According to projections of the Food and Agriculture Organization of the United Nations (FAO), the demand for

cereals will increase by 70%, and will double in developing countries [2]. To increase the yield of basic food grains, additional inputs for crop production are needed and new technologies are essential for managing soil nutrients as well as crop pests and diseases.

Soil quality is believed to be an integrative indicator of environmental quality, food security, and economic viability [3]. Soil quality was defined as “the capacity of a specific kind of soil to function, within natural managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” [4]. Therefore, the evaluation of soil quality will be closely associated with the role for which the soil was designated. Intensive soil exploitation particularly for agriculture has led to physical, chemical, and biological changes, as well as soil erosion that are compromising plant health and sustainability of crops. Soil quality has been decreasing dramatically due to the exacerbated anthropogenic disturbance that accelerates the degradation and desertification of soils [5].

Deficiencies of soil minerals that are not commonly included in fertilizers (e.g., sulfur) are becoming increasingly common and may reduce drastically plant growth and crop yields [6]. Mineral plant nutrition includes the supply, absorption, and utilization of essential nutrients for the growth and yield of crop plants [7]. Plants require at least 17 different minerals for adequate nutrition. Several factors including soil, plant species, microbial interactions, and environment can affect the acquisition of these nutrients.

Microbial communities play an important role in nutrient cycling by mineralizing and decomposing organic material, which are released into the soil as nutrients that are essential for plant growth. These communities can influence nutrient availability by solubilization, chelation, and oxidation/reduction processes. In addition, soil microorganisms may affect nutrient uptake and plant growth by the release of growth stimulating or inhibiting substances that influence root physiology and root architecture. It has been suggested that microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth [8,9], enhance nutrient availability and uptake [10–12], and improve plant health [12].

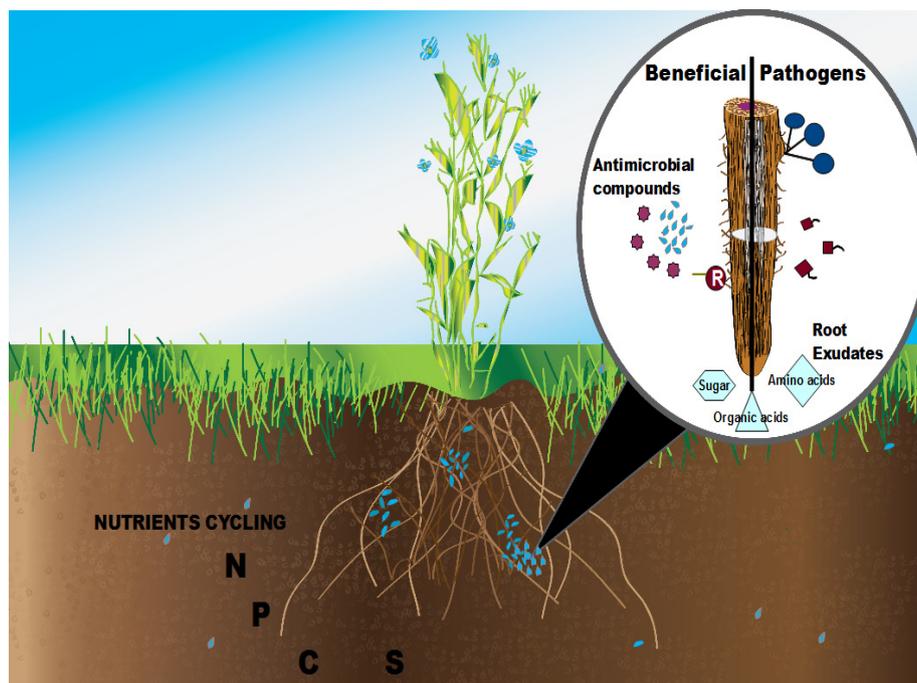
No single agricultural practice is sufficient to guarantee the quality of soils. However, changes in microbial communities could be used to predict the effects of soil quality by different environmental and anthropogenic factors. In addition, knowledge on soil microbial processes will provide insight into how agricultural practices such as tillage systems can be better managed to increase soil quality. In this review, we describe and discuss advantages and disadvantages of conventional and modern microbiological approaches to evaluate soil quality and access alternatives to increase crop yield.

2. Rhizosphere Plant-Microbe Interactions

The rhizosphere is one of the most complex environments with thousands of interactions that play crucial roles for plant health. Plants secrete up to 40% of photosynthates that reach the roots into the rhizosphere [12]. Because most of the soils are carbon deficient, these hot spots of carbon increase the microbial densities from 10 to 1000 times, compared to bulk soil [13]. The elevated concentration of microorganisms in this particular region is due to an exchange of nutrients between the plant and the different communities surrounding the root, which allows different types of associations (Figure 1). A number of factors have been shown to influence the quantity and quality of root exudates including

plant species [14], soil type [15,16], developmental stage [17], and nutritional status [18]. If specific elements associated to the release of such exudates are better understood, novel approaches to enhance beneficial microbial communities could be proposed.

Figure 1. Examples of plant-microbe interactions in the rhizosphere. Plant roots release exudates containing sugars, organic acids, and amino acids that may attract microbes. In exchange, they protect the plant against pathogens releasing antimicrobial compounds; or increase nutrient uptake. On the other hand, these carbon-containing compounds can also attract pathogens. They can compete for nutrients, infect the plant, and affect the rhizosphere microbial community.



Recent studies have revealed that plants are able to shape their rhizosphere microbiome [12,19,20]. Some plant species have been demonstrated to host specific communities and attract protective microorganisms to suppress pathogens in the rhizosphere [21]. Soil physical, chemical, and biological properties will also play an important role in the establishment of such plant-microbe interactions [12]. Although pathogens can severely affect plant health, certain beneficial bacteria and fungi that also thrive in the rhizosphere, or inside plant tissues, also known as endophytes, can compete with these pathogens for space and nutrients; therefore exert an antagonistic effect on them [22,23]. Root-associated beneficial soil bacteria are generally known as Plant Growth Promoting Rhizobacteria (PGPR).

PGPR grow in, on, or around root plant tissue and enhance plant growth, increase yield, protect plant against pathogens, and/or reduce abiotic or biotic stress [24]. Growth promotion can be achieved directly by the interaction between the microbe and the host, as well as indirectly, due to antagonistic activities against plant pathogens. Various interacting microbes produce phytohormones, which have been shown to inhibit or promote root growth, protect plants against biotic or abiotic stress, and improve nutrient acquisition by roots [25,26]. PGPR represent an environmentally sustainable alternative to

increase crop production and plant health as they have the potential to at least partially replace chemical fertilizers and pesticides and their use may then be reduced (Figure 1).

An interesting example of the role of microbial communities in plant nutrition and health is the interaction between rhizosphere fluorescent pseudomonas and plants. Plants reduce soil iron (Fe) availability by acquiring iron and releasing exudates which attract to the rhizosphere microbes that also utilizes Fe. In Fe-stressed environment, siderophore-producing bacterial populations are enriched, which then suppress pathogens such as fungi and oomycetes through competition for Fe. The plants, however, are able to utilize siderophores-bound iron, which enhances their growth [27]. Another instance applied to plant disease suppression is the ability of resident microbiota in suppressive soils or compost to prevent pathogen infection [28]. In a soil suppressive to the fungal pathogen *Rhizoctonia solani*, Proteobacteria, Firmicutes, and Actinobacteria were prominent taxa found to be involved in disease suppression [21]. There is also evidence to suggest that plants may use microbial communities to their own benefit to avoid infections [21].

The presence of potentially toxic compounds, low availability of essential minerals and pathogens in the soil often restrict crop production. To address these issues, numerous studies have focused on specific genes from plants and microbes that are involved in nutrient uptake and defense against pathogens [29–31]. Different molecular techniques have been used to conduct these studies. These methods range from DNA-based techniques [32,33], microscopic observation of labeled microorganisms colonizing roots [34,35], and incorporation of labeled nutrient substrates [36–38].

Using molecular methods to address research questions in soil environments is often challenging given the intrinsic characteristics of soil samples. The most common problems include presence of enzyme-inhibiting organic compounds such as humic and fulvic acids, as well as low extraction yields due to adsorption of nucleic acids to soil particles, incomplete cell lysis, and DNase and RNase contamination [39,40]. Extraction methods using bead-beating are the most used and they were shown to be so far the most efficient to overcome the problem of adsorption of nucleic acids to soil particles [41]. RNA-based studies are even more challenging because of the higher instability of RNA molecules compared to DNA. The ubiquity and stability of RNases make it difficult to obtain RNase-free environments. In addition, often mRNA is fragmented even before cell lysis, because of simultaneous transcription and translation occurring in archaeal and bacterial cells [42]. For approaches that focus on mRNA, such as microarray and metatranscriptomics analysis, often an rRNA subtraction step is advised as only up to 5% of extracted total RNA is comprised of mRNA. For this purpose, several methods have been developed and a comprehensive review describing methods and alternatives to deal with most methodological problems can be found elsewhere [43]. In the following sections, some of the methods that have been used as indicators of soil quality are briefly described and advantages and disadvantages are discussed.

3. Methods for Studying Microbial Communities

Robust indicators are necessary to monitor changes in soil quality. One of the advantages to study soil microorganisms is their rapid response to disturbances; therefore, they may provide instant information about soil health. During the last decade, new molecular, enzymatic, and organism-based techniques have been developed to diagnose soil health and complement existing physicochemical

properties [44]. These techniques have been systematically evaluated for their sensitivity and capacity to discriminate between types and uses of soils, as well as their ecological relevance. A summary of advantages and disadvantages inherent to each method is summarized in Table 1.

Table 1. Advantages and disadvantages of molecular methods described in this study to characterize soil microbial communities. Methods are sorted based on their ability to profile microbial biomass, diversity or activity. Chloroform Fumigation-Extraction (CFE); Phospholipid Fatty Acid Analysis (PLFA); Quantitative PCR (Q-PCR); Denaturing Gradient Gel Electrophoresis (DGGE); Temperature Gradient Gel Electrophoresis (TGGE); Single-Strand Conformation Polymorphism (SSCP); Terminal Restriction Fragment Length Polymorphism Fingerprinting (T-RFLP); Automated Ribosomal Intergenic Spacer Analysis (ARISA/RISA); Length-Heterogeneity PCR (LH-PCR); Random Amplified Polymorphic DNA (RAPD); Amplified Ribosomal DNA Restriction Analysis (ARDRA); Fluorescence *In Situ* hybridization (FISH); Fluorescein Diacetate (FDA); Stable-Isotope Probing (SIP).

| Methods | Advantages | Disadvantages | Ref. |
|-----------|---------------|---|--|
| BIOMASS | CFE | -Measurements of microbial biomass can be done in recently added and freshly decomposed substrates | -Clay soils may need to be corrected for the amount of chloroform C added to assess the concentration of biomass C [45,46] |
| | PLFA | -Sensitive detection and accurate quantification of different microbial groups -Rapid and efficient -Useful information on the dynamics of viable bacteria -Reproducible | -Time consuming -Low number of samples can be treated at the same time [47] |
| | Q-PCR | -Quick, accurate and highly sensitive method for sequence quantification that can also be used to quantify microbial groups -Relatively cheap and easy to implement -Specific amplification can be confirmed by melting curve analysis. | -Can only be used for targeting of known sequences. -DNA impurities and artifacts may create false-positives or inhibit amplification. [48] |
| DIVERSITY | DGGE/ TGGE | -Sensitive to variation in DNA sequences -Bands can be excised, cloned and sequenced for identification | -Time consuming -Multiple bands for a single species can be generated due to micro-heterogeneity -Can be used only for short fragments -Complex communities may appear smeared due to a large number of bands -Difficult to reproduce (gel to gel variation) [49] |

Table 1. Cont.

| | Methods | Advantages | Disadvantages | Ref. |
|-----------|----------------|--|---|------------|
| DIVERSITY | SSCP | <ul style="list-style-type: none"> -Community members can be identified -Screening of potential variations in sequences -Helps to identify new mutations | <ul style="list-style-type: none"> -Short fragments -Lack of reproducibility -Several factors like mutation and size of fragments can affect the sensitivity of the method | [50] |
| | T-RFLP | <ul style="list-style-type: none"> -Enables analyses of a wide array of microbes -Highly reproducible -Convenient way to store data and compare between different samples | <ul style="list-style-type: none"> -Artifacts might appear as false peaks -Distinct sequences sharing a restriction site will result in one peak. -Unable to retrieve sequences | [49,51,52] |
| | RISA/ ARISA | <ul style="list-style-type: none"> -High resolution when detecting microbial diversity -Quick and sensitive | <ul style="list-style-type: none"> -More than one peak could be generated for a single organisms -Similar spacer length in unrelated organisms may lead to underestimations of community diversity | [49] |
| | LH-PCR | <ul style="list-style-type: none"> -Results are reproducible -Easy and rapid -Efficient and reliable | <ul style="list-style-type: none"> -Limited by the bacterial species known in public databases -Not enough information is available for fragment length on databases to compare LH-PCR lengths with environmental microorganisms. | [52–54] |
| | RAPD | <ul style="list-style-type: none"> -Suitable for unknown genomes -Requires low quantities of DNA. -Efficient, fast and low cost | <ul style="list-style-type: none"> -Low reproducibility -Sensitive to reaction conditions | [55,56] |
| | ARDRA | <ul style="list-style-type: none"> -Highly useful for detection of structural changes in simple microbial communities -No special equipment required | <ul style="list-style-type: none"> -More applicable to environments with low complexity -Several restrictions are needed for adequate resolution -Labor- and time-intensive -Different bands can belong to the same group | [51] |
| DIVERSITY | FISH | <ul style="list-style-type: none"> -Allows detection and spatial distribution of more than one samples at the same time | <ul style="list-style-type: none"> -Autofluorescence of microorganisms -Accuracy and reliability is highly dependent on specificity of probe(s) | [57] |
| | DNA ARRAY | <ul style="list-style-type: none"> -Analyzes a vast amount of genetic information simultaneously | <ul style="list-style-type: none"> -Requires the construction of an array and access to a scanner -Issues with specificity/cross hybridization -Requires normalization -Sensitivity and reproducibility can be problematic -Limited by the presence of probes on the array | [58,59] |

Table 1. Cont.

| | Methods | Advantages | Disadvantages | Ref. |
|------------------|---|---|---|---------|
| DIVERSITY | Next Generation Sequencing (16S rRNA amplicon sequencing) | -Rapid method to assess biodiversity and abundance of many species/organizational taxonomic units simultaneously and at a considerable depth compared to the methods that have been available so far | -Relatively expensive -Replication and statistical analysis are essential -Computational intensive -Challenging in terms of data analysis | |
| | Next Generation Sequencing (metagenomics) | -Biodiversity can be studied in more detail -Captures polymorphism in microbial communities -Reveals the presence of thousands of microbial genomes simultaneously -Provides information about the functions of microbial communities in a given environment | -High cost -Data analysis is challenging and time-consuming -Difficult to use for low-abundance communities. -The high biodiversity in soil leads to many incomplete genomes -Current sequencing methods and computing power still in its infancy to the high biodiversity in soil | [60,61] |
| ACTIVITY | FDA | -Low-cost, easy and fast method to measure microbial activity for soil samples | -The measurement of soil microbes by FDA can be contaminated by external sources, e.g. plant matter | [62,63] |
| | SIP | -High sensitivity -Provides evidence on the function of microorganisms in a controlled experimental setup | -Incubation and cycling of the stable isotope might cause biases within the microbial communities | [64] |
| | Functional Gene Arrays (RNA-based) | -Analyzes a vast amount of genetic information simultaneously | -Requires the construction of an array and access to a scanner -Issues with specificity/cross hybridization -Requires normalization -Insufficient sensitivity and reproducibility can be problematic -Limited by the presence of probes on the array -Issues with RNA extraction from soil | [58,59] |
| | Next Generation Sequencing (Metatranscriptomics) | -Allows rRNA and/or mRNA profiling and quantification without prior knowledge of sequence -Provides a snapshot of microbial transcripts at the time of sampling that may allow deduction of microbial ecosystem function -Helps to understand the response of microbial communities to changes in their environment | -Many issues with isolation of RNA from soil -mRNA isolation and often amplification are required for gene expression analyses -Current sequencing methods, data bases and computing power are not sufficient yet to cover the high biodiversity in soil. | [43,65] |

3.1. Low to Medium Resolution Fingerprinting Methods Based on PCR Analysis

Since a few decades ago, molecular approaches have been used to investigate the diversity and composition of soil communities. DNA arrays, fatty acid analysis, fingerprinting, and *in situ* techniques (FISH) are commonly used to assess soil quality. Many genetic fingerprinting techniques are based on PCR amplification that provides information about the genetic structure of a community. They can be divided into two groups according to the differential electrophoretic migration on agarose or polyacrylamide gels: (1) migration depending on the size (T-RFLP, ARISA/RISA, RAPD, SSCP, LH-PCR) and (2) migration depending on the sequence (DGGE, TGGE; abbreviations are explained below). The generation of amplified fragments by selected primers can be used to evaluate the community structure of microbial populations. These approaches are particularly useful for comparing bacterial communities [66]. The main advantages and disadvantages of each method described below are summarized in Table 1 and further discussed in Section 4 to assist with methods choice and possible complimentary approaches. For example, all PCR based fingerprinting methods are likely to underestimate diversity, but are useful for tracking the dominant members of the community in complex environments such as soil. This is because PCR typically preferentially amplifies short fragments, with weak secondary structures (low GC contents), and from cells that provide easily extractable DNA [67]. Advantages of DGGE/TGGE, SSCP, ARISA/RISA, LH-PCR, RAPD in comparison to the other PCR-based fingerprinting methods include (i) fast evaluation of community changes, (ii) there is no need to clean-up of amplification products or digestion with restriction enzymes [53].

3.1.1. DGGE/TGGE

Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE) were developed to separate PCR-amplified ribosomal DNA fragments of DNA with the same length but with variation in nucleotide composition. Over the years these methods were adapted to analyze bacterial community structure. The separation principle for both methods is applying a linear gradient of DNA denaturing agents (such as a mixture of formamide and urea in DGGE), or temperature (TGGE) on polyacrylamide gels to influence the electrophoretic mobility of partially melted double-stranded DNA. Melting temperatures are associated to the sequence, and DNA fragments stop migrating when regions of base-pairs with the lowest melting temperature reach this temperature. This occurs due to a transition of conformation from helical to partially melted, and consequently the movement along the electric field will stop. A GC clamp (GC rich sequence) attached to the 5'-end is used as a special primer to anchor the PCR fragments and prevent them from completely dissociating. Soil bacterial dynamics, structure, and diversity are still being assessed through these methods but have also been increasingly replaced by the advent of high-throughput sequencing platforms. This is because DGGE and TGGE can only detect the most abundant organisms present in the bacterial community. In addition, interpretation can be misleading as a single band may represent multiple species and same species may be represented by multiple bands [68,69]. Taxon-specific primers combined with nested PCR have then been developed to profile microbial populations that occur in low abundance and appear to be able to show congruent results with more

thorough methods such as 16S rRNA gene amplicon pyrosequencing to profile low abundance microbial populations [70]. These methods have contributed to elucidate changes in microbial community structure in response to long-term effects of diverse minerals and molecules [71–73]. Different rhizosphere studies have utilized DGGE/TGGE methods to compare microbial communities under shifting conditions. For instance, Zhou and Wu (2012) identified changes in structure and composition of bacterial and fungal communities under different concentrations of the autotoxin [74]. In addition, molecular fingerprinting of microbial communities demonstrated changes in environmental conditions at a volcanic CO₂ vent reported by Frerichs *et al.* 2012 [75]. Another study reported the effect of genetically modified microorganisms introduced for rhizoremediation and their impact on native community structure of eubacteria, α and β -proteobacteria, actinobacteria, and acidobacteria using TGGE [76]. In general, gradient gel electrophoresis-based methods are more laborious compared to other fingerprinting methods using gel electrophoresis, such as RISA or RAPD (described below).

3.1.2. T-RFLP

Terminal restriction fragment length polymorphism fingerprinting (T-RFLP) uses either the 5' PCR primer, or both primers, labeled with a fluorescent dye. The labeled primer allows the tagging of amplification products that are then digested with one or several restriction enzymes, which result in labeled terminal restriction fragments whose sizes are determined by capillary methods or on a sequencing gel [77]. As different soil microbial communities will exhibit distinct combinations of restriction sites because of the variation on the sequences of the gene that has been amplified, “fingerprints” are obtained for particular assemblages of organisms. However, different DNA amounts may disturb the abundance and phylotypes in a T-RFLP profile. Dumber *et al.* suggested using the profile with the smallest total peak heights as a base to normalize the total peak heights. This method will produce a correction factor for each profile. Once each peak is adjusted, the different amounts of DNA will be compensated [78]. T-RFLP approaches have provided a better understanding of changes in the structure and composition of soil communities in a number of environments including soil [79–81]. Several studies involving crops have included this method. For instance, Hilton *et al.*, 2013, identified the cause of a decline in yield of oilseed rape (OSR) monocultures using T-RFLP and other methods. This study showed that two fungi that showed high similarity with plant pathogens were enriched in monocultures compared to OSR cultivated in a range of rotations [82]. Furthermore, it is well known that arbuscular mycorrhizal (AM) plays an important role in soil bacteria population and plant health. Using T-RFLP, Toljander *et al.*, 2007, identified changes in bacterial community composition in response to AM extraradical mycelia exudates *in vitro* [83]. Compared to the previously described methods, T-RFLP offers the advantage of allowing higher throughput.

3.1.3. SSCP

Similarly to DGGE/TGGE, single-strand conformation polymorphism (SSCP) is an electrophoresis method adapted to the analysis of microbial communities; however the separation is based on single-stranded DNA. Secondary structures formed with the single-stranded DNA are used to separate between products from different phylotypes. Unlike DGGE/TGGE or T-RFLP, in SSCP neither GC clamps nor restriction digestions are required. Typical problems with this technique are the occurrence

of three bands due to several conformations of one product. Recently, this technique has been used for the rapid profiling of soil microbial communities [84,85] and phylogenetic studies [86]. An interesting study on diversity and distribution of polyhydroxyalkanoate-producing bacteria has used SSCP as a culture-independent approach. Gasser *et al.*, 2009, concluded that the method helped to confirm that rhizosphere is an attractive reservoir for bacteria, which are producers of polyhydroxyalkanoate. However, at a strain level some incongruities between the culture-dependent and culture-independent (SSCP) methods were detected [87]. SSCP-based approaches have been also used as well on fungal communities. For example, Zachow *et al.*, 2009, used SSCP analysis to assess rhizosphere fungal diversity on the Canary Islands. In this study, *Trichoderma*-specific communities which play a major role in soil health exhibited low diversity [88]. An improved variation of this technique, namely the Capillary Electrophoresis-Single Strand Conformation Polymorphism (CE-SSCP) fingerprinting has been developed and used to profile communities with low complexity [85], but its application for highly diverse environments is still under evaluation. An alternative of this variation is polymerase chain reaction-free; however it has also been tested only in low diversity samples [89].

3.1.4. ARISA/RISA

The automated ribosomal intergenic spacer analysis (ARISA/RISA) method aims to monitor changes in microbial diversity, based on the variation in lengths of the Intergenic Transcribed Spacer (ITS) region between the 16S and 23S, as well as 18S and 28S rRNA-encoding genes, for bacteria and eukaryotes (in particular fungi), respectively. This method has been used to compare microbial community structure and estimate species richness of multiple samples from several environments including soils [90–95]. Zancarini *et al.*, 2012, studied the different responses of rhizosphere microbial communities under N availability and plant genotype. The influence of those two variables was determined by the ARISA method. According to the results, nitrogen availability affected bacterial communities only in presence of the plant [96]. This technique has also been used in agricultural fields. Baudoin *et al.*, 2009, used ARISA fingerprints to determine the effect of plant growth-promoting rhizobacterium (PGPR) *Azospirillum lipoferum* CRT1 on the structure of rhizobacterial communities in field-grown maize. Results showed that this method was able to distinguish from plant to plant variability, as well as to detect changes in native rhizobacteria communities after *Azospirillum* inoculation [97]. Two main limitations of this method have been raised. Firstly, the length of the spacer may vary in a single genome due to differences in multiple operons, which would cause an overestimation of richness. Secondly, spacer regions with the same length may be found in unrelated microbes. An *in silico* approach used hundreds of complete bacterial genomes to obtain a dataset of bacterial 16S-23S spacers and simulate ARISA profiles to evaluate the accuracy of richness estimations [98]. It revealed that ARISA is not suitable to estimate richness of highly diverse ecosystems such as soils. However, despite the fact that this method overestimates species richness, a correction can be applied to assess species richness in low diversity ecosystems [98].

3.1.5. LH-PCR

Amplicon Length-Heterogeneity PCR (LH-PCR) is a technique analogous to ARISA. This method is based on the natural differences between lengths of amplified gene fragments. So far it has been

used for analysis of 16S rRNA fragments. The length differences within the 16S rRNA genes or inter-genic spacer regions can be compared with databases in databases to identify the most probable identity of microbial groups. Available databases include Greengenes, SILVA, Integrated Microbial Genomes, or NCBI. LH-PCR can provide insight into the community structure without the construction of clone libraries and DNA sequencing analysis [53]. In soil, it has been successfully used to assess differences in soil bacterial community structures [54,99,100]. For instance, it has been used to characterize phylotypes in soil fungal communities. Wu *et al.*, 2008, demonstrated that soil fungal communities were affected by land and crop management practices [101]. The inherent limitations of this method are the same as ARISA.

3.1.6. RAPD

Random Amplified Polymorphic DNA (RAPD) is a technically simple method that was developed to analyze genetic relationships and genetic diversity [102]. Short (10-mer), single synthetic oligonucleotide primers of arbitrary nucleotide sequence are used to anneal at multiple locations on the microbial genomes. A range of amplicons that are characteristic of the template are then generated. Typically, very low annealing temperatures are used to allow promiscuous pairing of the primer (approximately 36 °C). For this reason, reproducibility of profiles is often an issue. This technique has been one of the most commonly-used molecular techniques to develop DNA markers. It is also less time consuming and cheaper than T-RFLP. RAPD is described as a useful tool for soil microbial community analysis to access soil microbial genetic structure [103–105] and genetic fidelity of micro-propagated plants [106].

3.1.7. ARDRA

Amplified Ribosomal DNA Restriction Analysis (ARDRA) generates restriction fragment profiles from the 16S rRNA gene amplicon of bacterial populations, or from the 18S gene of fungal populations. After amplification of a specific region, the product is digested using tetracutter restriction enzymes. The amplified products can be used as a pool for fingerprinting or as clone libraries to differentiate each sequence in further analyses [51]. Universal primers cannot provide much information on particular organisms in the sample but can be used to compare microbial community structure across samples. Alternatively, specific primers for certain microbial types can be used for phylogenetic analysis. ARDRA has been used as a tool for the classification of isolates with antagonistic properties against *Phytophthora capsici* [107], identification of clusters isolated from glacier soils [108], and confirmation of PGPB strain inoculation in strawberry between mother-plant and daughter-plant via stolon [109]. In addition, this method has been traditionally used to assess microbial diversity in a number of environments, including soils [107,110,111].

3.1.8. Q-PCR

Real-time quantitative PCR (Q-PCR) or reverse transcriptase Q-PCR (RT-PCR) is a technique that collects amplification data while the PCR occurs [112]. Different fluorescence chemistries are available, including SYBR green and TaqMan. The first dye binds to any double-stranded DNA. The

latter requires pre-designed probes that will be hydrolyzed given the 5' nuclease ability of the DNA polymerase during the extension step and fluorescence emission will be consequently higher. The PCR cycle where amplification is first detected is known as cycle threshold (CT) and can be identified when the background fluorescence is lower than the fluorescence intensity. Real-time has been used in several rhizosphere studies such as in the evaluation of soil acidobacterial communities' responses from soybean croplands and adjacent amazon forest [113]. In addition, the effect of long-term fertilization on the activity of ammonia oxidizers communities in the rhizosphere of a fluvo-aquic soil was also assessed through this method [114]. An interesting approach has been proposed to estimate relative abundances of the most common taxonomic groups of bacteria and fungi in the soil using taxon-specific real time primers [115]. An improvement of these primers was later proposed by increasing coverage without affecting their specificity [116]. The limitation for using this approach is that the taxonomic resolution is considerably low. Compared to other PCR-based techniques; Q-PCR provides quantitative data on gene and transcript abundances, and does not require post-PCR handling avoiding potential contamination of samples [117]. Q-PCR is usually restricted to a relatively low number of sequences as it requires specific primers, although high-throughput Q-PCR has also been developed [118].

3.2. Non-PCR Based Methods

3.2.1. CFE

Chloroform Fumigation-extraction (CFE) uses chloroform as a biocide. Microbial components are degraded by enzymatic autolysis and converted into extractable compounds. Following incubation with chloroform, the components are extracted using 0.5 M K₂SO₄. Total dissolved carbon can then be determined on a TIC/TOC analyzer. For N extraction, samples are required to be digested via Kjeldahl digestion. The C and N difference between fumigated vs. non-fumigated soils is the chloroform-labile C or N pool (EC) and it is proportional to microbial biomass C or N (C, N).

$$C = EC/k_{EC} \text{ or } N = EN/k_{EN}$$

Where k_{EC} and k_{EN} is soil-specific. It has been estimated as 0.45 and 0.54 respectively [119–121]. This method is one of the most commonly used to estimate soil microbial biomass carbon [122,123]. Biomass measurements are important to characterize soil basic properties as well as to prognosticate metal transport models from soil [45]. Soil microbial biomass has been estimated by CFE and combined with stable isotope analysis to identify the source of microbial biomass carbon and measure turnover rates of different molecular size compounds in soil [124]. In recent studies, microbial biomass C and N were measured using CFE to understand the influence of biological soil crust (BSCs) on microbial communities in sand dunes [125].

3.2.2. PLFA

Phospholipids derived from microbial cell membranes can be used to distinguish specific microbial taxa. These phospholipids contain unique fatty acids composed of different acyl chains and can be used as biomarkers for microbial groups. Phospholipid Fatty acid Analysis (PLFA) is widely used in

microbial ecology as chemotaxonomic markers of microorganisms. The technique is based on the premise that phospholipids are rapidly degraded, therefore phospholipids remaining should belong to living organisms. Buyer and Sasser [126] have developed a new procedure with increased throughput and therefore a large number of PLFAs can be analyzed. The method has been used to detect changes and structure of soil microbial communities [127–129]. The PLFA method has been used recently to understand the formation of soil organic matter (SOM) and the involvement of microbial cell-envelope fragments in this process. Results from PLFA provided a better understanding of SOM development and the relationship between microbial abundance and activity [130]. The use of PLFA to determine diversity indices has been criticized as the phylogenetic resolution of community characterization is low [131].

3.2.3. FDA

Fluorescein diacetate (FDA) has been used since early 80s as a measure of microbial activity [132]. FDA is hydrolyzed by free exoenzymes and membrane-bound enzymes that convert the colorless FDA in a colored fluorescein. Fluorescein can then be quantified by spectrophotometry at 490 nm wavelength [62]. This method is generally applied to estimate total microbial activity and has been proposed to be used as a biochemical/biological indicator of soil quality [63]. Recent publications have used this method in a subtropical coal mining dump as a bioindicator for revegetation practices [133], in a glacier forefield to measure the effect of reciprocal soil transfer on microbial activities along a temperature and soil moisture gradient in glacier forefield [134], and in a flooded soil to assess the impact of elevated temperature and carbon dioxide on soil enzyme activities in a tropical flooded rice plantation [135].

3.2.4. SIP

Stable-isotope probing (SIP) incorporates ^{13}C -labeled, ^{12}C -labeled or ^{15}N substrates into cellular biomarkers. SIP is applied to the identification of active microorganisms without the use of radioactive isotopes. The substrate can be purified from unlabeled nucleic acid by density-gradient centrifugation [64,136]. This is typically followed by molecular profiling or sequencing analyses by using one of the techniques described in the present review. This technique has been applied to detect spatial variation of active microorganisms related to the C flow in the rhizosphere [137]. In addition, fungal-bacterial interactions have been studied using SIP in an effort to understand the role of each community in the litter degradation of soils [138]. Interactions involving fungi and bacteria have been studied using SIP to better understand the role of each community in litter degradation of soils [138]. The method also allows to track different processes like matter fluxes and biochemical activities in microbial communities. Comparing with other methods, SIP can provide information about C fluxes involving microbial communities in the soil [64].

3.2.5. DNA Arrays

After 17 years since its first appearance, microarray techniques have evolved in gigantic steps [139–141]. Thousands of microarray papers are published annually and the number keeps growing. A DNA array

is solid surface that has been spotted with an arrangement of DNA samples. Spots containing DNA, cDNA, or oligonucleotides (DNA chips) typically represent genes with known and unknown function as well as non-coding RNA. DNA or reverse-transcribed RNA is then hybridized onto these spots and information on thousands of genes can be simultaneously collected. Various reports on genome-wide transcriptional profiling of soil microbes as well as numerous studies assessed changes in bacterial diversity after a disturbance or treatment through microarrays [142–147]. For example, the PhyloChip was used for microbial community profiling of disease suppressive soils [21].

Microarray applications on soil microbial communities include profiling of taxonomic groups and functional genes or RNA (cDNA from mRNA or rRNA). DNA microarrays containing functional microbial genes are also known as Functional Gene Arrays (FGA), and a typical example is the GeoChip 3.0 that harbors 57,000 genes with known function from many different species and gene variants from 292 functional gene families [148]. mRNA-based expression profiling aims to identify and characterize differentially expressed genes, some of which can be grouped into clusters based on similar or coordinated gene expression patterns. These “regulons” may belong to specific signaling or biochemical pathways in microorganisms. However, most FGAs have been used for microbial DNA and the profiling of the presence of certain genes rather than gene expression (mRNA) [149,150]. It should be mentioned that the use of microarrays for soil microbial communities is limited to the sequences provided as probes. Typically, microarray data and other methods are verified and further analyzed for the presence of certain microbes, certain microbial genes or individual differentially expressed candidate genes. This can be done by either nucleic acid blot hybridization or by Q-PCR.

3.2.6. FISH

Fluorescence *in situ* hybridization (FISH) is a technique that has been used since the late 80s. A fluorescent molecule or fluorochrome is conjugated with an oligonucleotide probe. In microbiology, 16S rRNA is generally used as a probe due to its genetic stability and high copy number. The fluorescent probe binds to a complementary sequence that can therefore be detected using fluorescence microscopy. Nowadays, FISH is a widely used tool in several fields in microbiology including ecology, phylogenetics, and diagnostics [57]. For example, the presence of live bacteria was detected in root segments of *Arabidopsis thaliana* by CARD-FISH, which is a variation of FISH that improve its sensitivity through the use of horseradish peroxidase-labeled probes in combination with catalyzed deposition. This study provided a better understanding of the influence of soil type, plant development stage and genotype as key factors to mold the root microbiome [20].

3.3. High-Throughput Sequencing Technologies

High-throughput sequencing approaches (also referred to as next generation sequencing; NGS) are increasingly being used for estimates of microbial diversity in complex environments such as soils in a culture-independent manner. Due to advances in nanotechnology and bioinformatics, alternative technologies have been created to increase the throughput of DNA and RNA sequencing have emerged. Such technologies play a major role in metagenomic (DNA-based), and metatranscriptomic (RNA-based) approaches, which provide a comprehensive picture of potential and active functions of microbial communities, respectively [43,151]. The most widely-used platforms for massive parallel

sequencing for assessing soil microbial diversity are Roche 454 Genome Sequencer (Roche Diagnostics Corp., Branford, CT, USA), HiSeq 2000 (Illumina Inc., San Diego, CA, USA), and AB SOLiD™ System (Life Technologies Corp., Carlsbad, CA, USA). Other commonly used high throughput sequencing systems that have been applied to other approaches including metatranscriptomics and whole genome re-sequencing are also described below for comparison, which includes Ion Personal Genome Machine (Life Technologies, South San Francisco, CA, USA), Heliscope (Helicos Bioscience Corp., Cambridge, MA, USA), and PacBio RS SMRT system (Pacific Bioscience, Menlo Park, CA, USA). A comparative summary of the main features of these platforms is shown in Table 2.

Table 2. Comparative summary of high-throughput sequencing platforms.

| Technology | Cost | Read length | Run time | Error rate | Output per run | Notes | Ref. |
|------------|----------|-------------------------|--------------|------------|----------------------|--|----------------|
| 454 | Low | Up to 1000 bp (GS FLX+) | 23 h | Low | 0.7 Gb | -Provides one of the longest reads, which makes it ideal for studies aiming at measuring microbial diversity as it provides a more refined taxonomic assignment. -The calibration base calling cannot interpret long stretches of the same nucleotide | [152,153] |
| Illumina | Low | 2×100 bp | 3 to 11 days | Low | 120 Gb to 600 Gb | -Error rates increase past 32 bp -Provides the highest output of reads, which makes it suitable for metatranscriptomics studies, which require considerable sequencing depth for detection of rare transcripts. | [152] |
| SOLiD | High | 50 bp | Up to 8 days | Moderate | 150 Gb | -Offers 99.9% accuracy | [152,154, 155] |
| PGM | Moderate | 400 bp | 3 h | High | From 20 Mb to 400 Mb | -Inadequate for SNP or mutation analysis | [156–158] |
| HeliScope | High | 35 bp | 30 days | High | Over 1 Gb per day | -Single molecule sequencing has higher raw error rates | [154,155] |
| SMRT | Low | 1100 bp | 2 h | High | 230 Gb | -It has the ability to observe and capture kinetic information | [159] |

3.3.1. Roche 454 FLX Pyrosequencer

This system is based on the detection of pyrophosphate (PPi) that is released during DNA synthesis. The intensity of the visible light that it generates is proportional to the number of nucleotides incorporated. The FLX instrument applies 100 flows of each nucleotide. The resulting reads of the GS FLX Titanium XL + yield up to 700 Mb of data. The read length provided by the latest 454 platform can come to 1000 base pairs (bp). Due to reasonably longer sequences compared to the other

high-throughput sequencing platforms (see below sections) and higher output compared to conventional cloning sequencing approaches, pyrosequencing allows the detection of rare bacterial and archaea genera. Recently, this method has been extensively used to characterize composition and diversity of soil microbial communities [20,107,160–163] and has also been applied to understand the effect of heavy metals and disturbances on soil microbial communities [162,164,165]. The particularity of this method is that it provides reads that are long enough (average 500 bp but up to 1000 bp) to assign probable taxonomic identity up to genus level and rare groups can be detected as thousands of reads can be obtained per sample. For instance, Lundberg *et al.* (2012) [20] provided new insights into the bacterial communities associated to *Arabidopsis thaliana* through 16S rRNA gene amplicon pyrosequencing. A comparison of the composition of microbial communities colonizing the rhizosphere with the endophytic compartment showed an enrichment of Actinobacteria and Proteobacteria in the endophytic compartment in both soil types tested [20]. In farming systems, pyrosequencing has been used to evaluate the effect of conventional and organic systems on bulk soil bacterial communities [166]. Another study found differences in soil bacterial community composition in the rhizosphere of plants with activated jasmonate signaling pathway [137]. However, compared to other methods it is more costly and analytically more challenging to assess changes in microbial community structure.

3.3.2. Illumina Genome Analyzer

The Illumina genome analyzer platform is based on parallel, fluorescence-based readout of millions of immobilized libraries that are sequenced using reversible terminator chemistry. Nowadays, Illumina offers four sequencers: HiSeq 2500, Hi Seq 2000, Genome Analyzer IIx and MiSeq platform. The most powerful of them is the HiSeq 2500, which delivers up to 600 Gb of data with a maximum of six billion reads per run, and a read length of approximately 2×100 bp [167]. Compared to the Roche 454 FLX pyrosequencer, Illumina sequencers have shorter reads but a much higher throughput, which makes them very appropriate for gene expression studies of complex environments such as soils. This method has enabled the characterization of a number of microbial communities [168] and demonstrated a low cost access to DNA from organisms with low relative abundances [169]. The problem with performing microbial diversity studies with this platform is that although the output is much higher than pyrosequencing, the length of reads are shorter, which turns the taxonomic assignment less accurate. Still, a few studies have used this method to measure diversity in microbial communities. For instance, soil management practices such as tillage was reported to affect microbial diversity in a subtropical Acrisol [170]. Another study documented distinct fungal community compositions in green roofs and five city parks in New York City, with only 54% of taxonomic group overlap between green roof media and park soils [171]. A combined approach using metagenomic analysis and functional assays provided evidence to suggest that soil resource availability and soil stratification had an effect on functional diversity and to a lower degree on taxonomic diversity [172].

3.3.3. Applied Biosystems Sequencing by Oligonucleotide Ligation and Detection (SOLiD) Sequencer

Similarly to the 454 platform, the Sequencing by Oligonucleotide Ligation and Detection (SOLiD) system uses emulsion PCR to produce clone libraries. However, different from the other technologies, SOLiD uses a DNA ligase as well as a unique approach to sequence amplified DNA fragments. In brief, a fluorescently-labeled probe hybridizes to its complementary sequence. DNA ligase is added to join the dye-labeled probe to the primer. Fluorescence imaging determines the identity of the ligated probe. This technology generates billions of short sequence reads (2×60 bp) at once (120 Gb of data). In a recent study, SOLiD was used to sequence the genome of the *Pectobacterium* sp strain SCC3193, which is known for causing soft rot and blackleg disease in different plants. This platform was used to correct the homopolymer and assembly errors obtained by 454 sequencing [173]. The main disadvantage of this platform is the difficult assemblage of short reads, which also applies for the Illumina platform. However, its two-base sequencing technology provides the highest accuracy of all platforms. It is widely used for transcriptomics and epigenomics.

3.3.4. Ion Personal Genome Machine (PGM)

This platform uses semiconductor sequencing technology. Each time a nucleotide is incorporated into the DNA a proton is released, and the subsequent change in pH is measured by pH-sensitive field effect transistor. Therefore, no labeled nucleotides are used and synthesis is detected directly. It offers shorter run times when compared to systems based on fluorescence detection. Currently, it delivers 400 bp reads in four hours. A microbial ecology sequencing platform has been recently proposed to assess bacterial and archaeal community dynamics using the PGM [174]. This platform was also used in a study which showed that diesel biodegradation was affected by modifying the microbial community structure [175]. Furthermore, consistent microbial community shifts were observed when diesel and nutrients were added to Arctic soils. Actinobacteria and Proteobacteria became dominant in low-organic matter and high-organic matter, respectively [176].

3.3.5. Heliscope Single Molecule Sequencer

The main innovation of this platform is the direct sequencing of DNA/RNA fragments, therefore no amplification is needed. It involves fragmenting the template DNA and hybridizing on disposable glass flow cells. Each of the 25 channels on one standard flow cell can be addressed individually for the addition of samples. Once the flow cells have been prepared, they are inserted in the HeliScope Sequencing system along with all the reagents necessary for sequencing by synthesis and imaging. This system can generate billions of reads per run that range from 25 to 35 bp [154], and the data output is over one Gb per day. Heliscope might contribute to genome biology through direct sequencing of nucleic acids. For example, Kapranov *et al.* [177] obtained sequence information for counting abundance of short RNA (sRNAs) and discovery of new sRNAs through the HeliScope sequencer in cultured cells. This technique counts with a high error rate that goes between 3 to 4%. Compare to other methods it manage a higher cost than other platforms and still not popular in the NGS market [152].

3.3.6. Pacific Biosciences SMRT DNA Sequencer

Pacific Biosciences launched in 2010 a single-molecule real-time sequencing platform [156]. This platform uses a structure called Zero Mode Waveguide or ZMW. This structure allows the observation of a single nucleotide of DNA being incorporated by the DNA polymerase fixed at the ZMW. Each nucleotide has been marked with different fluorescent dyes. A detector reads the fluorescent signal of the nucleotide incorporated. SMRT has been used mainly in genome sequencing, re-sequencing and methylation detection [178]. English *et al.* [179] presented new software (PBJelly) for upgrading genome assemblies based on long-read sequence from Pacific Bioscience RS. This software will improve annotation problems in gap-associated regions. Further improvements have been done for genome assembly. Chin *et al.* [180] discussed the use of short reads to correct errors in the long SMRT reads. This assembly requires at least two different libraries and a variety of sequencing runs. In recent publications, the use of SMRT has been applied to analyze BAC clone that carries MATE1 gene associated with aluminum tolerance in maize [181].

4. Choice of Methods and Complimentary Approaches

Soil is one of the most complex microbial environments where colonization of microhabitats enables co-existence of thousands of species with essential ecosystem functions, including biomass and nutrient cycling, mineralization and detoxification [182–185]. Culture-independent methods are regarded as to provide a more accurate picture of microbial communities because most microbes from soil currently cannot be cultivated. However, the use of different culture-independent techniques will depend on the experimental design and the main focus of the research. The preference of one technique over another is subjective to the researcher's hypothesis and resources. Having knowledge of the different advantages and disadvantages of the methods will increase the possibility to obtain better data and acquire more information from the samples. In most cases, ecosystem functions of microbial communities need to be revealed to increase our understanding of the microbiota and their interaction with their environments. However, this is easier said than done. Some direct functional insights can come from enzymatic measurements of the soil, biochemical analyses or be derived from the effect of soil microbes on their environment (e.g., degradation of organic matter, nitrogen fixation, phosphorus solubilization, plant growth promotion/inhibition, mineralization, greenhouse gas emissions, filtration, detoxification of polluted soils, *etc.*). Most methods described above focus on profiling soil microbial biomass and diversity (Table 1). These soil attributes are relatively easy to measure and have been widely associated with "soil health" [186,187]. For example, agricultural soils with low microbial biomass and diversity are linked to yield decline in sugarcane [188]. However, soil microbial biomass, biodiversity and even DNA-based metagenomics approaches may not provide a direct measure of current microbial activities in the soil. This is because a large proportion of the microorganisms present in soil maybe dormant and even the quantification of microbial DNA maybe misleading as extracellular DNA can be adsorbed on soil particles where its integrity can be maintained [189] Microbial activity profiles, based on actual metabolic activity are therefore more likely to reveal important microbial ecosystem functions. FDA, SIP, FGAs probed with cDNA, and RNA-based next generation sequencing (metatranscriptomics or RNA-seq) may complement well

biochemical methods and the indirect measures of microbial biomass and diversity. The choice of methods is further limited by the availability of samples, resources including equipment and funding to carry out the experiments. Most PCR-based fingerprinting methods are limited to a fraction of microbial communities but are relatively cost-efficient. Recent progress in next generation sequencing (NGS) allows a much more comprehensive analysis and costs have significantly decreased in recent years. At present, the combination of more traditional methods with more inclusive new techniques may provide the most powerful approach. It has been shown that the use of conventional methods plus new platforms will diminish the error rate in some of the next generation sequencing methods and help the data to be more robust. For instance, the use of pyrosequencing and DGGE fingerprinting has helped for the assessment of bacterial composition in a mangrove environment, providing robust data at a reasonable cost [70]. Similarly, Gözdereliler *et al.* [190] adopted pyrosequencing and 16S rRNA gene DGGE to investigate shifts in community structure and composition following exposure to different herbicide concentrations.

NGS technologies have revolutionized research on environmental microbiology and have a great potential to shed light into relevant questions in agriculture and soil biology. These technologies have had a high impact on genome research in terms of feasibility and scale. However, the large amount of data that has been generated will pose analytical challenges especially with regard to bioinformatics. Furthermore, data processing and interpretation of results will require additional studies to avoid misinterpretation of the data. Table 2 provides a summarized description of the different sequencing platforms presented previously.

Finally, controlled experiments can be carried out on soil microbial communities to test hypotheses about microbial ecosystem function. For example, a study involving SIP assessed how plant-fixed carbon is translocated belowground under elevated atmospheric carbon dioxide [191] and 16S rRNA amplicon sequencing of plants treated with defense hormones has revealed that plants are able to alter their rhizosphere bacterial community to recruit potentially beneficial microbes that assist in plant defense [160].

5. Conclusions

Nowadays, the scientific community is focusing on major worldwide concerns such as food security, energy sustainability and climate change. Researchers have been motivated to develop novel approaches towards sustainability of agricultural practices. The recognition of the rhizosphere as a critical interface for soil and plants has led to major research in this area, hence new technology and new projects have been developed to face challenges posed by such a complex and diverse environment.

The intricate network of interactions occurring in the rhizosphere requires high-throughput techniques to deal with all emerging data in a reasonable timeframe. However, the large number of organisms involved and the highly dynamic nature of the rhizosphere itself makes it challenging to provide the level of detail afforded by molecular techniques. Because of the complex nature of the rhizosphere, the use of different techniques is necessary to propose appropriate soil management strategies. However, it is important to take into consideration the pros and cons of the various molecular methods that are available to assess soil health to avoid biased and distorted interpretations of microbial diversity.

Microbial community structure in soils, in particular rhizosphere soil, is considered of great importance to assess soil quality to achieve high crop yields. The development and selection of molecular methods that increasingly lead to the acquisition of data that better reflect soil quality is imperative to evaluate agricultural practices that will contribute to the increase of food production in a sustainable manner.

Acknowledgements

We wish to thank the Australian Research Council and the Grains Research & Development Corporation for financial support.

Conflict of Interest

The authors declare no conflict of interest.

References and Notes

1. Evans, A. The feeding of the nine billion. Available online: <http://www.chathamhouse.org/sites/default/files/public/Research/Energy,%20Environment%20and%20Development/r0109food.pdf> (accessed on 6 May 2013).
2. FAO. World agriculture: Towards 2030/2050. Available online: http://www.fao.org/fileadmin/user_upload/esag/docs/Interim_report_AT2050web.pdf (accessed on 6 May 2013).
3. Herrick, J.E. Soil quality: An indicator of sustainable land management? *Appl. Soil Ecol.* **2000**, *15*, 75–83.
4. Karlen, D.L.; Mausbach, M.J.; Doran, J.W.; Cline, R.G.; Harris, R.F.; Schuman, G.E. Soil quality: A concept, definition, and framework for evaluation (a guest editorial). *Soil Sci. Soc. Am. J.* **1997**, *61*, 4–10.
5. Lal, R. Soil degradation by erosion. *Land Degrad. Dev.* **2001**, *12*, 519–539.
6. White, P.J.; Brown, P.H. Plant nutrition for sustainable development and global health. *Ann. Bot.* **2010**, *105*, 1073–1080.
7. Fageria, N.K.; Baligar, V.C.; Jones, C.A. *Growth and Mineral Nutrition of Field Crops*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2011.
8. Compant, S.; Clément, C.; Sessitsch, A. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* **2010**, *42*, 669–678.
9. Lugtenberg, B.; Kamilova, F. Plant-growth-promoting rhizobacteria. *Ann. Rev. Microbiol.* **2009**, *63*, 541–556.
10. Adesemoye, A.O.; Kloepper, J.W. Plant–microbes interactions in enhanced fertilizer-use efficiency. *Appl. Microbiol. Biotechnol.* **2009**, *85*, 1–12.
11. Yang, J.; Kloepper, J.W.; Ryu, C.M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* **2009**, *14*, 1–4.
12. Berendsen, R.L.; Pieterse, C.M.J.; Bakker, P.A.H.M. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **2012**, *17*, 478–486.

13. Smalla, K.; Sessitsch, A.; Hartmann, A. The rhizosphere: “Soil compartment influenced by the root”. *FEMS Microbiol. Ecol.* **2006**, *56*, 165.
14. Dennis, P.G.; Miller, A.J.; Hirsch, P.R. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiol. Ecol.* **2010**, *72*, 313–327.
15. Berg, G.; Smalla, K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiol. Ecol.* **2009**, *68*, 1–13.
16. Bulgarelli, D.; Rott, M.; Schlaeppi, K.; van Themaat, E.V.L.; Ahmadinejad, N.; Assenza, F.; Rauf, P.; Huettel, B.; Reinhardt, R.; Schmelzer, E.; *et al.* Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature* **2012**, *488*, 91–95.
17. Houlden, A.; Timms-Wilson, T.M.; Day, M.J.; Bailey, M.J. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. *FEMS Microbiol. Ecol.* **2008**, *65*, 193–201.
18. Carvalhais, L.C.; Dennis, P.G.; Fedoseyenko, D.; Hajirezaei, M.R.; Borriss, R.; von Wirén, N. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. *J. Plant Nutr. Soil Sci.* **2011**, *174*, 3–11.
19. Badri, D.V.; Vivanco, J.M. Regulation and function of root exudates. *Plant Cell Environ.* **2009**, *32*, 666–681.
20. Lundberg, D.S.; Lebeis, S.L.; Paredes, S.H.; Yourstone, S.; Gehring, J.; Malfatti, S.; Tremblay, J.; Engelbrektson, A.; Kunin, V.; del Rio, T.G.; *et al.* Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **2012**, *488*, 86–90.
21. Mendes, R.; Kruijt, M.; de Bruijn, I.; Dekkers, E.; van der Voort, M.; Schneider, J.H.; Piceno, Y.M.; DeSantis, T.Z.; Andersen, G.L.; Bakker, P.A.; *et al.* Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **2011**, *332*, 1097–1100.
22. Nihorimbere, V.; Ongena, M.; Smargiassi, M.; Thonart, P. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnol. Agron. Soc. Environ.* **2011**, *15*, 327–337.
23. Raaijmakers, J.M.; Paulitz, T.C.; Steinberg, C.; Alabouvette, C.; Moëgne-Loccoz, Y. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* **2009**, *321*, 341–361.
24. Vessey, J.K. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* **2003**, *255*, 571–586.
25. Berg, G. Plant–microbe interactions promoting plant growth and health: Perspectives for controlled use of microorganisms in agriculture. *Appl. Microbiol. Biotechnol.* **2009**, *84*, 11–18.
26. Davies, P.J. The plant hormones: Their nature, occurrence, and functions. In *Plant Hormones—Biosynthesis, Signal Transduction, Action*, 3rd ed.; Davies, P.J., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2010; pp. 1–15.
27. Lemanceau, P.; Mazurier, S.; Avoscan, L.; Robin, A.; Briat, J.f. Reciprocal interactions between plants and fluorescent *Pseudomonas* in relation to iron in the rhizosphere. In *Molecular Microbial Ecology of the Rhizosphere*, de Bruijn, F.J., Ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2013; pp. 1181–1189.

28. Hadar, Y.; Papadopoulou, K.K. Suppressive composts: Microbial ecology links between abiotic environments and healthy plants. *Ann. Rev. Phytopathol.* **2012**, *50*, 133–153.
29. Damiani, I.; Baldacci-Cresp, F.; Hopkins, J.; Andrio, E.; Balzergue, S.; Lecomte, P.; Puppo, A.; Abad, P.; Favery, B.; Hérouart, D. Plant genes involved in harbouring symbiotic rhizobia or pathogenic nematodes. *New Phytol.* **2012**, *194*, 511–522.
30. Dechorgnat, J.; Patrit, O.; Krapp, A.; Fagard, M.; Daniel-Vedele, F. Characterization of the *NRT2.6* gene in *Arabidopsis thaliana*: A link with plant response to biotic and abiotic stress. *PLoS One* **2012**, *7*, e42491.
31. Hann, D.; Boller, T. Microbial effectors and their role in plant defense suppression. In *Effectors in Plant-Microbe Interactions*; Martin, F., Kamoun, S., Eds.; Wiley-Blackwell: Chichester, UK, 2012; pp. 33–52.
32. López-Fuentes, E.; Ruíz-Valdiviezo, V.M.; Martínez-Romero, E.; Gutiérrez-Miceli, F.A.; Dendooven, L.; Rincón-Rosales, R. Bacterial community in the roots and rhizosphere of hypericum silenoides juss. 1804. *Afr. J. Microbiol. Res.* **2012**, *6*, 2704–2711.
33. Doornbos, R.F.; Geraats, B.P.J.; Kuramae, E.E.; van Loon, L.; Bakker, P.A.H.M. Effects of jasmonic acid, ethylene, and salicylic acid signaling on the rhizosphere bacterial community of *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* **2011**, *24*, 395–407.
34. Compant, S.; Mitter, B.; Colli-Mull, J.G.; Gangl, H.; Sessitsch, A. Endophytes of grapevine flowers, berries, and seeds: Identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microb. Ecol.* **2011**, *62*, 188–197.
35. Lecomte, J.; St-Arnaud, M.; Hijri, M. Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. *FEMS Microbiol. Lett.* **2011**, *317*, 43–51.
36. Jones, D.L.; Nguyen, C.; Finlay, R.D. Carbon flow in the rhizosphere: Carbon trading at the soil–root interface. *Plant Soil* **2009**, *321*, 5–33.
37. Marschner, P.; Crowley, D.; Rengel, Z. Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis—model and research methods. *Soil Biol. Biochem.* **2011**, *43*, 883–894.
38. Pellegrini, A.; Corneo, P.E.; Camin, F.; Ziller, L.; Tosi, S.; Pertot, I. Studying trophic interactions between a plant pathogen and two different antagonistic microorganisms using a ¹³C-labeled compound and isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2012**, *26*, 510–516.
39. Opel, K.L.; Chung, D.; McCord, B.R. A study of PCR inhibition mechanisms using real time PCR. *J. Forensic Sci.* **2010**, *55*, 25–33.
40. Arbeli, Z.; Fuentes, C.L. Improved purification and PCR amplification of DNA from environmental samples. *FEMS Microbiol. Lett.* **2007**, *272*, 269–275.
41. Lakay, F.M.; Botha, A.; Prior, B.A. Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. *J. Appl. Microbiol.* **2007**, *102*, 265–273.
42. Proshkin, S.; Rahmouni, A.R.; Mironov, A.; Nudler, E. Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science* **2010**, *328*, 504–508.
43. Carvalhais, L.C.; Dennis, P.G.; Tyson, G.W.; Schenk, P.M. Application of metatranscriptomics to soil environments. *J. Microbiol. Methods* **2012**, *91*, 246–251.

44. Killham, K. Integrated soil management—moving towards globally sustainable agriculture. *J. Agric. Sci.-Lond.* **2011**, *149*, 29–36.
45. Alessi, D.S.; Walsh, D.M.; Fein, J.B. Uncertainties in determining microbial biomass C using the chloroform fumigation–extraction method. *Chem. Geol.* **2011**, *280*, 58–64.
46. Ocio, J.; Brookes, P. An evaluation of methods for measuring the microbial biomass in soils following recent additions of wheat straw and the characterization of the biomass that develops. *Soil Biol. Biochem.* **1990**, *22*, 685–694.
47. Kaur, A.; Chaudhary, A.; Kaur, A.; Choudhary, R.; Kaushik, R. Phospholipid fatty acid—a bioindicator of environment monitoring and assessment in soil ecosystem. *Curr. Sci. Bangalore* **2005**, *89*, 1103.
48. Smith, C.J.; Osborn, A.M. Advantages and limitations of quantitative PCR (qPCR)-based approaches in microbial ecology. *FEMS Microbiol. Ecol.* **2009**, *67*, 6–20.
49. Kirk, J.L.; Beaudette, L.A.; Hart, M.; Moutoglis, P.; Klironomos, J.N.; Lee, H.; Trevors, J.T. Methods of studying soil microbial diversity. *J. Microbiol. Methods* **2004**, *58*, 169–188.
50. Konstantinos, K.V.; Panagiotis, P.; Antonios, V.T.; Agelos, P.; Argiris, N.V. PCR–SSCP: A method for the molecular analysis of genetic diseases. *Mol. Biotechnol.* **2008**, *38*, 155–163.
51. Nocker, A.; Burr, M.; Camper, A.K. Genotypic microbial community profiling: A critical technical review. *Microb. Ecol.* **2007**, *54*, 276–289.
52. Okubo, A.; Sugiyama, S. Comparison of molecular fingerprinting methods for analysis of soil microbial community structure. *Ecol. Res.* **2009**, *24*, 1399–1405.
53. Mills, D.E.K.; Entry, J.A.; Gillevet, P.M.; Mathee, K. Assessing microbial community diversity using amplicon length heterogeneity polymerase chain reaction. *Soil Sci. Soc. Am. J.* **2007**, *71*, 572–578.
54. Ritchie, N.J.; Schutter, M.E.; Dick, R.P.; Myrold, D.D. Use of length heterogeneity PCR and fatty acid methyl ester profiles to characterize microbial communities in soil. *Appl. Environ. Microbiol.* **2000**, *66*, 1668–1675.
55. Fritsch, P.; Rieseberg, L.H. The use of random amplified polymorphic DNA (RAPD) in conservation genetics. *Mol. Genet. Approaches Conserv.* **1996**, *1996*, 54–73.
56. Newbury, J.; Ford-Lloyd, B. The use of RAPD for assessing variation in plants. *Plant Growth Regul.* **1993**, *12*, 43–51.
57. Moter, A.; Göbel, U.B. Fluorescence *in situ* hybridization (FISH) for direct visualization of microorganisms. *J. Microbiol. Methods* **2000**, *41*, 85–112.
58. Li, E.S.Y.; Liu, W.T. DNA microarray technology in microbial ecology studies-principle, applications and current limitations. *Microbes Environ.* **2003**, *18*, 175–187.
59. Everett, K.; Rees-George, J.; Pushparajah, I.; Janssen, B.; Luo, Z. Advantages and disadvantages of microarrays to study microbial population dynamics - a minireview. *N. Z. Plant Prot.* **2010**, *63*, 1–6.
60. Manichanh, C.; Chapple, C.E.; Frangeul, L.; Gloux, K.; Guigo, R.; Dore, J. A comparison of random sequence reads *versus* 16S rDNA sequences for estimating the biodiversity of a metagenomic library. *Nucleic Acids Res.* **2008**, *36*, 5180–5188.
61. Podar, M.; Abulencia, C.B.; Walcher, M.; Hutchison, D.; Zengler, K.; Garcia, J.A.; Holland, T.; Cotton, D.; Hauser, L.; Keller, M. Targeted access to the genomes of low-abundance organisms in complex microbial communities. *Appl. Environ. Microbiol.* **2007**, *73*, 3205–3214.

62. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951.
63. Green, V.; Stott, D.; Diack, M. Assay for fluorescein diacetate hydrolytic activity: Optimization for soil samples. *Soil Biol. Biochem.* **2006**, *38*, 693–701.
64. Dumont, M.G.; Murrell, J.C. Stable isotope probing—linking microbial identity to function. *Nat. Rev. Microbiol.* **2005**, *3*, 499–504.
65. Carvalhais, L.C.; Dennis, P.G.; Tyson, G.W.; Schenk, P.M. Rhizosphere metatranscriptomics: Challenges and opportunities. In *Molecular Microbial Ecology of the Rhizosphere*; de Bruijn, F.J., Ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2013; pp. 1137–1144.
66. Ranjard, L.; Poly, F.; Nazaret, S. Monitoring complex bacterial communities using culture-independent molecular techniques: Application to soil environment. *Res. Microbiol.* **2000**, *151*, 167–177.
67. McGrath, K.C.; Thomas-Hall, S.R.; Cheng, C.T.; Leo, L.; Alexa, A.; Schmidt, S.; Schenk, P.M. Isolation and analysis of mRNA from environmental microbial communities. *J. Microbiol. Methods* **2008**, *75*, 172–176.
68. Dowd, S.E.; Sun, Y.; Secor, P.R.; Rhoads, D.D.; Wolcott, B.M.; James, G.A.; Wolcott, R.D. Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. *BMC Microbiol.* **2008**, *8*, 43.
69. Nübel, U.; Garcia-Pichel, F.; Muyzer, G. PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl. Environ. Microbiol.* **1997**, *63*, 3327–3332.
70. Cleary, D.F.R.; Smalla, K.; Mendonca-Hagler, L.C.S.; Gomes, N.C.M. Assessment of variation in bacterial composition among microhabitats in a mangrove environment using DGGE fingerprints and barcoded pyrosequencing. *PLoS One* **2012**, *7*, e29380.
71. Wakelin, S.; Mander, C.; Gerard, E.; Jansa, J.; Erb, A.; Young, S.; Condrón, L.; O’Callaghan, M. Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures. *Appl. Soil Ecol.* **2012**, *61*, 40–48.
72. Caliz, J.; Montserrat, G.; Martí, E.; Sierra, J.; Cruañas, R.; Garau, M.A.; Triadó-Margarit, X.; Vila, X. The exposition of a calcareous mediterranean soil to toxic concentrations of Cr, Cd and Pb produces changes in the microbiota mainly related to differential metal bioavailability. *Chemosphere* **2012**, *89*, 494–504.
73. Babin, D.; Ding, G.C.; Pronk, G.J.; Heister, K.; Kögel-Knabner, I.; Smalla, K. Metal oxides, clay minerals and charcoal determine the composition of microbial communities in matured artificial soils and their response to phenanthrene. *FEMS Microbiol. Ecol.* **2013**, doi:10.1111/1574-6941.12058.
74. Zhou, X.; Wu, F. P-coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of *Fusarium oxysporum* f. Sp. Cucumerinum owen. *PLoS One* **2012**, *7*, e48288.
75. Frerichs, J.; Oppermann, B.I.; Gwosdz, S.; Möller, I.; Herrmann, M.; Krüger, M. Microbial community changes at a terrestrial volcanic CO₂ vent induced by soil acidification and anaerobic microhabitats within the soil column. *FEMS Microbiol. Ecol.* **2012**, *84*, 60–74.
76. Carcer, D.A.; Martin, M.; Mackova, M.; Macek, T.; Karlson, U.; Rivilla, R. The introduction of genetically modified microorganisms designed for rhizoremediation induces changes on native bacteria in the rhizosphere but not in the surrounding soil. *ISME J.* **2007**, *1*, 215–223.

77. Osborn, A.M.; Moore, E.R.; Timmis, K.N. An evaluation of terminal-restriction fragment length polymorphism (T-RFLP) analysis for the study of microbial community structure and dynamics. *Environ. Microbiol.* **2000**, *2*, 39–50.
78. Dunbar, J.; Ticknor, L.O.; Kuske, C.R. Phylogenetic specificity and reproducibility and new method for analysis of terminal restriction fragment profiles of 16s rRNA genes from bacterial communities. *Appl. Environ. Microbiol.* **2001**, *67*, 190–197.
79. Aiken, J.T. Terminal restriction fragment length polymorphism for soil microbial community fingerprinting. *Soil Sci. Soc. Am. J.* **2011**, *75*, 102–111.
80. Tipayno, S.; Kim, C.-G.; Sa, T. T-RFLP analysis of structural changes in soil bacterial communities in response to metal and metalloid contamination and initial phytoremediation. *Appl. Soil Ecol.* **2012**, *61*, 137–146.
81. Gough, H.L.; Stahl, D.A. Microbial community structures in anoxic freshwater lake sediment along a metal contamination gradient. *ISME J.* **2011**, *5*, 543–558.
82. Hilton, S.; Bennett, A.J.; Keane, G.; Bending, G.D.; Chandler, D.; Stobart, R.; Mills, P. Impact of shortened crop rotation of oilseed rape on soil and rhizosphere microbial diversity in relation to yield decline. *PLoS One* **2013**, *8*, e59859.
83. Toljander, J.F.; Lindahl, B.D.; Paul, L.R.; Elfstrand, M.; Finlay, R.D. Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* **2007**, *61*, 295–304.
84. Stefanis, C.; Alexopoulos, A.; Voidarou, C.; Vavias, S.; Bezirtzoglou, E. Principal methods for isolation and identification of soil microbial communities. *Folia Microbiol.* **2013**, *58*, 61–68.
85. Rossmann, B.; Müller, H.; Smalla, K.; Mpiira, S.; Tumuhairwe, J.B.; Staver, C.; Berg, G. Banana-associated microbial communities in Uganda are highly diverse but dominated by Enterobacteriaceae. *Appl. Environ. Microbiol.* **2012**, *78*, 4933–4941.
86. Badin, A.L.; Mustafa, T.; Bertrand, C.; Monier, A.; Delolme, C.; Geremia, R.A.; Bedell, J.P. Microbial communities of urban stormwater sediments: The phylogenetic structure of bacterial communities varies with porosity. *FEMS Microbiol. Ecol.* **2012**, *81*, 324–338.
87. Gasser, I.; Müller, H.; Berg, G. Ecology and characterization of polyhydroxyalkanoate-producing microorganisms on and in plants. *FEMS Microbiol. Ecol.* **2009**, *70*, 142–150.
88. Zachow, C.; Berg, C.; Müller, H.; Meincke, R.; Komon-Zelazowska, M.; Druzhinina, I.S.; Kubicek, C.P.; Berg, G. Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): Relationship to vegetation zones and environmental factors. *ISME J.* **2009**, *3*, 79–92.
89. Nai, Y.H.; Zemb, O.; Gutierrez-Zamora, M.L.; Manefield, M.; Powell, S.M.; Breadmore, M.C. Capillary electrophoresis ribosomal RNA single-stranded conformation polymorphism: A new approach for characterization of low-diversity microbial communities. *Anal. Bioanal. Chem.* **2012**, *404*, 1897–1906.
90. Fuhrman, J.A.; Steele, J.A.; Hewson, I.; Schwalbach, M.S.; Brown, M.V.; Green, J.L.; Brown, J.H. A latitudinal diversity gradient in planktonic marine bacteria. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7774–7778.
91. Danovaro, R.; Luna, G.M.; Dell’Anno, A.; Pietrangeli, B. Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal

- intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. *Appl. Environ. Microbiol.* **2006**, *72*, 5982–5989.
92. Sepehri, S.; Kotlowski, R.; Bernstein, C.N.; Krouse, D.O. Microbial diversity of inflamed and noninflamed gut biopsy tissues in inflammatory bowel disease. *Inflamm. Bowel Dis.* **2007**, *13*, 675–683.
 93. Mathew, R.P.; Feng, Y.; Githinji, L.; Ankumah, R.; Balkcom, K.S. Impact of no-tillage and conventional tillage systems on soil microbial communities. *Appl. Environ. Soil Sci.* **2012**, doi:10.1155/2012/548620.
 94. Quilliam, R.S.; Marsden, K.A.; Gertler, C.; Rousk, J.; DeLuca, T.H.; Jones, D.L. Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. *Agric. Ecosyst. Environ.* **2012**, *158*, 192–199.
 95. Pascal, J.; Pierre-Alain, M.; Virginie, N.; Toan, T.D. Utilization of microbial abundance and diversity as indicators of the origin of soil aggregates produced by earthworms. *Soil Biol. Biochem.* **2013**, *57*, 950–952.
 96. Zancarini, A.; Mougel, C.; Voisin, A.-S.; Prudent, M.; Salon, C.; Munier-Jolain, N. Soil nitrogen availability and plant genotype modify the nutrition strategies of *Medicago truncatula* and the associated rhizosphere microbial communities. *PLoS One* **2012**, *7*, e47096.
 97. Baudoin, E.; Nazaret, S.; Mougel, C.; Ranjard, L.; Moëgne-Loccoz, Y. Impact of inoculation with the phytostimulatory PGPR *Azospirillum lipoferum* crt1 on the genetic structure of the rhizobacterial community of field-grown maize. *Soil Biol. Biochem.* **2009**, *41*, 409–413.
 98. Kovacs, A.; Yacoby, K.; Gophna, U. A systematic assessment of automated ribosomal intergenic spacer analysis (ARISA) as a tool for estimating bacterial richness. *Res. Microbiol.* **2010**, *161*, 192–197.
 99. Chaudhary, D.R.; Saxena, J.; Lorenz, N.; Dick, L.K.; Dick, R.P. Microbial profiles of rhizosphere and bulk soil microbial communities of biofuel crops switchgrass (*Panicum virgatum* L.) and jatropha (*Jatropha curcas* L.). *Appl. Environ. Soil Sci.* **2012**, 906864.
 100. Wallenius, K.; Rita, H.; Mikkonen, A.; Lappi, K.; Lindstrom, K.; Hartikainen, H.; Raateland, A.; Niemi, R.M. Effects of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. *Soil Biol. Biochem.* **2011**, *43*, 1464–1473.
 101. Wu, T.; Chellemi, D.O.; Graham, J.H.; Roskopf, E.N. Assessment of fungal communities in soil and tomato roots subjected to diverse land and crop management systems. *Soil Biol. Biochem.* **2008**, *40*, 1967–1970.
 102. Hadrys, H.; Balick, M.; Schierwater, B. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Mol. Ecol.* **1992**, *1*, 55–63.
 103. Amorim, J.; Vidal, R.; Lacerda-Junior, G.; Dias, J.; Brendel, M.; Rezende, R.; Cascardo, J. A simple boiling-based DNA extraction for RAPD profiling of landfarm soil to provide representative metagenomic content. *Genet. Mol. Res.* **2012**, *11*, 182–189.
 104. Li, Y.; Ying, Y.X.; Zhao, D.Y.; Jin, S.; Ding, W.L. Genetic diversity analysis on rhizosphere soil microbial population of *Panax ginseng* and *Panax quinquefolium* by RAPD. *Chin. Tradit. Herb. Drugs* **2010**, *41*, 1871–1875.

105. Gao, Y.; Miao, C.Y.; Xia, J.; Mao, L.; Wang, Y.F.; Zhou, P. Plant diversity reduces the effect of multiple heavy metal pollution on soil enzyme activities and microbial community structure. *Front. Environ. Sci. Eng.* **2012**, *6*, 213–223.
106. Singh, S.K.; Rai, M.K.; Sahoo, L. An improved and efficient micropropagation of *Eclipta alba* through transverse thin cell layer culture and assessment of clonal fidelity using RAPD analysis. *Ind. Crop. Prod.* **2012**, *37*, 328–333.
107. Yang, M.-M.; Xu, L.-P.; Xue, Q.-Y.; Yang, J.-H.; Xu, Q.; Liu, H.-X.; Guo, J.-H. Screening potential bacterial biocontrol agents towards *Phytophthora capsici* in pepper. *Eur. J. Plant Pathol.* **2012**, *134*, 811–820.
108. Liu, G.-X.; Hu, P.; Zhang, W.; Wu, X.; Yang, X.; Chen, T.; Zhang, M.; Li, S.-W. Variations in soil culturable bacteria communities and biochemical characteristics in the Dongkemadi glacier forefield along a chronosequence. *Folia Microbiol.* **2012**, *57*, 485–494.
109. Guerrero-Molina, M.F.; Winik, B.C.; Pedraza, R.O. More than rhizosphere colonization of strawberry plants by *Azospirillum brasilense*. *Appl. Soil Ecol.* **2012**, *61*, 205–212.
110. Lee, M.S.; Do, J.O.; Park, M.S.; Jung, S.; Lee, K.H.; Bae, K.S.; Park, S.J.; Kim, S.B. Dominance of *Lysobacter* sp in the rhizosphere of two coastal sand dune plant species, *Calystegia soldanella* and *Elymus mollis*. *Antonie van Leeuwenhoek* **2006**, *90*, 19–27.
111. Hagerberg, D.; Manique, N.; Brandt, K.K.; Larsen, J.; Nybroe, O.; Olsson, S. Low concentration of copper inhibits colonization of soil by the arbuscular mycorrhizal fungus *Glomus intraradices* and changes the microbial community structure. *Microb.Ecol.* **2011**, *61*, 844–852.
112. Higuchi, R.; Fockler, C.; Dollinger, G.; Watson, R. Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions. *Biotechnology* **1993**, *11*, 1026–1030.
113. Navarrete, A.A.; Kuramae, E.E.; Hollander, M.; Pijl, A.S.; van Veen, J.A.; Tsai, S.M. Acidobacterial community responses to agricultural management of soybean in Amazon forest soils. *FEMS Microbiol. Ecol.* **2013**, *83*, 607–621.
114. Ai, C.; Liang, G.; Sun, J.; Wang, X.; He, P.; Zhou, W. Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. *Soil Biol. Biochem.* **2013**, *57*, 30–42.
115. Fierer, N.; Jackson, J.A.; Vilgalys, R.; Jackson, R.B. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. *Appl. Environ. Microbiol.* **2005**, *71*, 4117–4120.
116. Bacchetti De Gregoris, T.; Aldred, N.; Clare, A.S.; Burgess, J.G. Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa. *J. Microbiol. Methods* **2011**, *86*, 351–356.
117. Heid, C.A.; Stevens, J.; Livak, K.J.; Williams, P.M. Real-time quantitative PCR. *Genome Res.* **1996**, *6*, 986–994.
118. McGrath, K.C.; Dombrecht, B.; Manners, J.M.; Schenk, P.M.; Edgar, C.I.; Maclean, D.J.; Scheible, W.R.; Udvardi, M.K.; Kazan, K. Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of *Arabidopsis* transcription factor gene expression. *Plant Physiol.* **2005**, *139*, 949–959.

119. Beck, T.; Joergensen, R.; Kandeler, E.; Makeschin, F.; Nuss, E.; Oberholzer, H.; Scheu, S. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biol. Biochem.* **1997**, *29*, 1023–1032.
120. Brookes, P.; Landman, A.; Pruden, G.; Jenkinson, D. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol. Biochem.* **1985**, *17*, 837–842.
121. Joergensen, R.G. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{ec} value. *Soil Biol. Biochem.* **1996**, *28*, 25–31.
122. Setia, R.; Verma, S.L.; Marschner, P. Measuring microbial biomass carbon by direct extraction—comparison with chloroform fumigation-extraction. *Eur. J. Soil Biol.* **2012**, *53*, 103–106.
123. Philippot, L.; Ritz, K.; Pandard, P.; Hallin, S.; Martin-Laurent, F. Standardisation of methods in soil microbiology: Progress and challenges. *FEMS Microbiol. Ecol.* **2012**, *82*, 1–10.
124. Malik, A.; Blagodatskaya, E.; Gleixner, G. Soil microbial carbon turnover decreases with increasing molecular size. *Soil Biol. Biochem.* **2013**, *62*, 115–118.
125. Liu, Y.; Li, X.; Xing, Z.; Zhao, X.; Pan, Y. Responses of soil microbial biomass and community composition to biological soil crusts in the revegetated areas of the tengger desert. *Appl. Soil Ecol.* **2013**, *65*, 52–59.
126. Buyer, J.S.; Sasser, M. High throughput phospholipid fatty acid analysis of soils. *Appl. Soil Ecol.* **2012**, *61*, 127–130.
127. Chodak, M.; Gołębiewski, M.; Morawska-Płoskonka, J.; Kuduk, K.; Niklińska, M. Diversity of microorganisms from forest soils differently polluted with heavy metals. *Appl. Soil Ecol.* **2013**, *64*, 7–14.
128. Jiang, Y.; Sun, B.; Jin, C.; Wang, F. Soil aggregate stratification of nematodes and microbial communities affects the metabolic quotient in an acid soil. *Soil Biol. Biochem.* **2013**, *60*, 1–9.
129. Sipila, T.P.; Yrjala, K.; Alakukku, L.; Palojarvi, A. Cross-site soil microbial communities under tillage regimes: Fungistasis and microbial biomarkers. *Appl. Environ. Microbiol.* **2012**, *78*, 8191–8201.
130. Schurig, C.; Smittenberg, R.H.; Berger, J.; Kraft, F.; Woche, S.K.; Goebel, M.-O.; Heipieper, H.J.; Miltner, A.; Kaestner, M. Microbial cell-envelope fragments and the formation of soil organic matter: A case study from a glacier forefield. *Biogeochemistry* **2013**, *113*, 595–612.
131. Frostegard, A.; Tunlid, A.; Baath, E. Use and misuse of PLFA measurements in soils. *Soil Biolol. Biochem.* **2011**, *43*, 1621–1625.
132. Swisher, R.; Carroll, G.C. Fluorescein diacetate hydrolysis as an estimator of microbial biomass on coniferous needle surfaces. *Microb.Ecol.* **1980**, *6*, 217–226.
133. Finkenbein, P.; Kretschmer, K.; Kuka, K.; Klotz, S.; Heilmeyer, H. Soil enzyme activities as bioindicators for substrate quality in revegetation of a subtropical coal mining dump. *Soil Biol. Biochem.* **2013**, *56*, 87–89.
134. Zumsteg, A.; Bååth, E.; Stierli, B.; Zeyer, J.; Frey, B. Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. *Soil Biol. Biochem.* **2013**, *61*, 121–132.

135. Bhattacharyya, P.; Roy, K.; Neogi, S.; Manna, M.; Adhya, T.; Rao, K.; Nayak, A. Influence of elevated carbon dioxide and temperature on belowground carbon allocation and enzyme activities in tropical flooded soil planted with rice. *Environ. Monit. Assess.* **2013**, 1–13.
136. Radajewski, S.; Ineson, P.; Parekh, N.R.; Murrell, J.C. Stable-isotope probing as a tool in microbial ecology. *Nature* **2000**, *403*, 646–649.
137. Lu, Y.; Abraham, W.R.; Conrad, R. Spatial variation of active microbiota in the rice rhizosphere revealed by *in situ* stable isotope probing of phospholipid fatty acids. *Environ. Microbiol.* **2007**, *9*, 474–481.
138. Bodé, S.; Fancy, R.; Boeckx, P. Stable isotope probing of amino sugars—a promising tool to assess microbial interactions in soils. *Rapid Commun. Mass Spectrom.* **2013**, *27*, 1367–1379.
139. Zhou, J.; Kang, S.; Schadt, C.W.; Garten, C.T., Jr. Spatial scaling of functional gene diversity across various microbial taxa. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 7768–7773.
140. He, Z.; Gentry, T.J.; Schadt, C.W.; Wu, L.; Liebich, J.; Chong, S.C.; Huang, Z.; Wu, W.; Gu, B.; Jardine, P.; *et al.* Geochip: A comprehensive microarray for investigating biogeochemical, ecological and environmental processes. *ISME J.* **2007**, *1*, 67–77.
141. Yergeau, E.; Kang, S.; He, Z.; Zhou, J.; Kowalchuk, G.A. Functional microarray analysis of nitrogen and carbon cycling genes across an antarctic latitudinal transect. *ISME J.* **2007**, *1*, 163–179.
142. Fan, B.; Carvalhais, L.C.; Becker, A.; Fedoseyenko, D.; von Wiren, N.; Borriss, R. Transcriptomic profiling of *Bacillus amyloliquefaciens* fzb42 in response to maize root exudates. *BMC Microbiol.* **2012**, *12*, 116.
143. Hayden, H.L.; Mele, P.M.; Bougoure, D.S.; Allan, C.Y.; Norng, S.; Piceno, Y.M.; Brodie, E.L.; DeSantis, T.Z.; Andersen, G.L.; Williams, A.L.; *et al.* Changes in the microbial community structure of bacteria, archaea and fungi in response to elevated CO₂ and warming in an Australian native grassland soil. *Environ. Microbiol.* **2012**, *14*, 3081–3096.
144. Russo, S.E.; Legge, R.; Weber, K.A.; Brodie, E.L.; Goldfarb, K.C.; Benson, A.K.; Tan, S. Bacterial community structure of contrasting soils underlying bornean rain forests: Inferences from microarray and next-generation sequencing methods. *Soil Biol. Biochem.* **2012**, *55*, 48–59.
145. Cruz-Martinez, K.; Rosling, A.; Zhang, Y.; Song, M.Z.; Andersen, G.L.; Banfield, J.F. Effect of rainfall-induced soil geochemistry dynamics on grassland soil microbial communities. *Appl. Environ. Microbiol.* **2012**, *78*, 7587–7595.
146. Zysko, A.; Sanguin, H.; Hayes, A.; Wardleworth, L.; Zeef, L.A.H.; Sim, A.; Paterson, E.; Singh, B.K.; Kertesz, M.A. Transcriptional response of *Pseudomonas aeruginosa* to a phosphate-deficient lolium perenne rhizosphere. *Plant Soil* **2012**, *359*, 25–44.
147. Khudyakov, J.I.; D’haeseleer, P.; Borglin, S.E.; DeAngelis, K.M.; Woo, H.; Lindquist, E.A.; Hazen, T.C.; Simmons, B.A.; Thelen, M.P. Global transcriptome response to ionic liquid by a tropical rain forest soil bacterium, *Enterobacter lignolyticus*. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E2173–E2182.
148. He, Z.; Deng, Y.; van Nostrand, J.D.; Tu, Q.; Xu, M.; Hemme, C.L.; Li, X.; Wu, L.; Gentry, T.J.; Yin, Y.; *et al.* Geochip 3.0 as a high-throughput tool for analyzing microbial community composition, structure and functional activity. *ISME J.* **2010**, *4*, 1167–1179.
149. Waldron, P.J.; Wu, L.; van Nostrand, J.O.Y.D.; Schadt, C.W.; He, Z.; Watson, D.B.; Jardine, P.M.; Palumbo, A.V.; Hazen, T.C.; Zhou, J. Functional gene array-based analysis of

- microbial community structure in groundwaters with a gradient of contaminant levels. *Environ. Sci. Technol.* **2009**, *43*, 3529–3534.
150. Wu, L.; Kellogg, L.; Devol, A.H.; Tiedje, J.M.; Zhou, J. Microarray-based characterization of microbial community functional structure and heterogeneity in marine sediments from the gulf of Mexico. *Appl. Environ. Microbiol.* **2008**, *74*, 4516–4529.
151. Simon, C.; Daniel, R. Metagenomic analyses: Past and future trends. *Appl. Environ. Microbiol.* **2011**, *77*, 1153–1161.
152. Loman, N.J.; Constantinidou, C.; Chan, J.Z.; Halachev, M.; Sergeant, M.; Penn, C.W.; Robinson, E.R.; Pallen, M.J. High-throughput bacterial genome sequencing: An embarrassment of choice, a world of opportunity. *Nat. Rev. Microbiol.* **2012**, *10*, 599–206.
153. Mardis, E.R. Next-generation DNA sequencing methods. *Ann. Rev. Genomics Hum. Genet.* **2008**, *9*, 387–402.
154. Shokralla, S.; Spall, J.L.; Gibson, J.F.; Hajibabaei, M. Next-generation sequencing technologies for environmental DNA research. *Mol. Ecol.* **2012**, *21*, 1794–1805.
155. Magi, A.; Benelli, M.; Gozzini, A.; Girolami, F.; Torricelli, F.; Brandi, M.L. Bioinformatics for next generation sequencing data. *Genes* **2010**, *1*, 294–307.
156. Loman, N.J.; Misra, R.V.; Dallman, T.J.; Constantinidou, C.; Gharbia, S.E.; Wain, J.; Pallen, M.J. Performance comparison of benchtop high-throughput sequencing platforms. *Nat. Biotechnol.* **2012**, *30*, 434–439.
157. Egan, A.N.; Schlueter, J.; Spooner, D.M. Applications of next-generation sequencing in plant biology. *Am. J. Bot.* **2012**, *99*, 175–185.
158. Diaz-Sanchez, S.; Hanning, I.; Pendleton, S.; D'Souza, D. Next-generation sequencing: The future of molecular genetics in poultry production and food safety. *Poult. Sci.* **2013**, *92*, 562–572.
159. Schadt, E.E.; Turner, S.; Kasarskis, A. A window into third-generation sequencing. *Hum. Mol. Genet.* **2010**, *19*, R227–R240.
160. Carvalhais, L.C.; Dennis, P.G.; Badri, D.V.; Tyson, G.W.; Vivanco, J.M.; Schenk, P.M. Activation of the jasmonic acid plant defence pathway alters the composition of rhizosphere bacterial communities. *PLoS One* **2013**, *8*, e56457.
161. Dohrmann, A.B.; Kuting, M.; Junemann, S.; Jaenicke, S.; Schluter, A.; Tebbe, C.C. Importance of rare taxa for bacterial diversity in the rhizosphere of Bt- and conventional maize varieties. *ISME J.* **2013**, *7*, 37–49.
162. Lami, R.; Jones, L.C.; Cottrell, M.T.; Lafferty, B.J.; Ginder-Vogel, M.; Sparks, D.L.; Kirchman, D.L. Arsenite modifies structure of soil microbial communities and arsenite oxidization potential. *FEMS Microbiol. Ecol.* **2013**, *84*, 270–279.
163. Eilers, K.G.; Debenport, S.; Anderson, S.; Fierer, N. Digging deeper to find unique microbial communities: The strong effect of depth on the structure of bacterial and archaeal communities in soil. *Soil Biol. Biochem.* **2012**, *50*, 58–65.
164. Sutton, N.B.; Maphosa, F.; Morillo, J.A.; Al-Soud, W.A.; Langenhoff, A.A.M.; Grotenhuis, T.; Rijnaarts, H.H.M.; Smidt, H. Impact of long-term diesel contamination on soil microbial community structure. *Appl. Environ. Microbiol.* **2013**, *79*, 619–630.
165. Suleiman, A.; Manoeli, L.; Boldo, J.; Pereira, M.; Roesch, L. Shifts in soil bacterial community after eight years of land-use change. *Syst. Appl. Microbiol.* **2013**, *36*, 137–144.

166. Li, R.; Khafipour, E.; Krause, D.O.; Entz, M.H.; de Kievit, T.R.; Fernando, W.D. Pyrosequencing reveals the influence of organic and conventional farming systems on bacterial communities. *PLoS One* **2012**, *7*, e51897.
167. Sequencing systems. Available online: <http://www.illumina.com/systems/sequencing.ilmn> (accessed on 6 May 2013).
168. Fierer, N.; Leff, J.W.; Adams, B.J.; Nielsen, U.N.; Bates, S.T.; Lauber, C.L.; Owens, S.; Gilbert, J.A.; Wall, D.H.; Caporaso, J.G. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 21390–21395.
169. Bartram, A.K.; Lynch, M.D.J.; Stearns, J.C.; Moreno-Hagelsieb, G.; Neufeld, J.D. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end Illumina reads. *Appl. Environ. Microbiol.* **2011**, *77*, 3846–3852.
170. Dorr de Quadros, P.; Zhalnina, K.; Davis-Richardson, A.; Fagen, J.R.; Drew, J.; Bayer, C.; Camargo, F.A.; Triplett, E.W. The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. *Diversity* **2012**, *4*, 375–395.
171. McGuire, K.L.; Payne, S.G.; Palmer, M.I.; Gillikin, C.M.; Keefe, D.; Kim, S.J.; Gedallovich, S.M.; Discenza, J.; Rangamannar, R.; Koshner, J.A.; *et al.* Digging the new york city skyline: Soil fungal communities in green roofs and city parks. *PLoS One* **2013**, *8*, e58020.
172. Uroz, S.; Ioannidis, P.; Lengelle, J.; Cébron, A.; Morin, E.; Buée, M.; Martin, F. Functional assays and metagenomic analyses reveals differences between the microbial communities inhabiting the soil horizons of a norway spruce plantation. *PLoS One* **2013**, *8*, e55929.
173. Koskinen, J.P.; Laine, P.; Niemi, O.; Nykyri, J.; Harjunpää, H.; Auvinen, P.; Paulin, L.; Pirhonen, M.; Palva, T.; Holm, L. Genome sequence of *Pectobacterium* sp. Strain SCC3193. *J. Bacteriol.* **2012**, *194*, 6004–6004.
174. Whiteley, A.S.; Jenkins, S.; Waite, I.; Kresoje, N.; Payne, H.; Mullan, B.; Allcock, R.; O'Donnell, A. Microbial 16S rRNA ion tag and community metagenome sequencing using the Ion Torrent (PGM) platform. *J. Microbiol. Methods* **2012**, *91*, 80–88.
175. Bell, T.H.; Yergeau, E.; Juck, D.; Whyte, L.; Greer, C. Alteration of microbial community structure affects diesel biodegradation in an arctic soil. *FEMS Microbiol. Ecol.* **2013**, *85*, 51–61.
176. Bell, T.H.; Yergeau, E.; Maynard, C.; Juck, D.; Whyte, L.G.; Greer, C.W. Predictable bacterial composition and hydrocarbon degradation in arctic soils following diesel and nutrient disturbance. *ISME J.* **2013**, *7*, 1200–1210.
177. Kapranov, P.; Ozsolak, F.; Milos, P.M. Profiling of short RNAs using helicob single-molecule sequencing. In *Next-Generation microRNA Expression Profiling Technology*; Springer-Verlag: Berlin, Germany, 2012; pp. 219–232.
178. Myllykangas, S.; Buenrostro, J.; Ji, H.P. Overview of sequencing technology platforms. In *Bioinformatics for High Throughput Sequencing*; Springer-Verlag: Berlin, Germany, 2012; pp. 11–25.
179. English, A.C.; Richards, S.; Han, Y.; Wang, M.; Vee, V.; Qu, J.; Qin, X.; Muzny, D.M.; Reid, J.G.; Worley, K.C.; *et al.* Mind the gap: Upgrading genomes with pacific biosciences RS long-read sequencing technology. *PLoS One* **2012**, *7*, e47768.

180. Chin, C.-S.; Alexander, D.H.; Marks, P.; Klammer, A.A.; Drake, J.; Heiner, C.; Clum, A.; Copeland, A.; Huddleston, J.; Eichler, E.E.; *et al.* Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* **2013**, *10*, 563–569.
181. Maron, L.G.; Guimarães, C.T.; Kirst, M.; Albert, P.S.; Birchler, J.A.; Bradbury, P.J.; Buckler, E.S.; Coluccio, A.E.; Danilova, T.V.; Kudrna, D.; *et al.* Aluminum tolerance in maize is associated with higher MATE1 gene copy number. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 5241–5246.
182. Bradford, M.A.; Newington, J.E. With the worms: Soil biodiversity and ecosystem functioning. *Biologist* **2002**, *49*, 127–130.
183. Lavelle, P.; Decaëns, T.; Aubert, M.; Barot, S.; Blouin, M.; Bureau, F.; Margerie, P.; Mora, P.; Rossi, J.-P. Soil invertebrates and ecosystem services. *Eur. J. Soil Biol.* **2006**, *42*, S3–S15.
184. Barrios, E. Soil biota, ecosystem services and land productivity. *Ecol. Econ.* **2007**, *64*, 269–285.
185. Dominati, E.; Patterson, M.; Mackay, A. A framework for classifying and quantifying the natural capital and ecosystem services of soils. *Ecol. Econ.* **2010**, *69*, 1858–1868.
186. Xu, L.; Ravnskov, S.; Larsen, J.; Nilsson, R.H.; Nicolaisen, M. Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing. *Soil Biol. Biochem.* **2012**, *46*, 26–32.
187. Avidano, L.; Gamalero, E.; Cossa, G.P.; Carraro, E. Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. *Appl. Soil Ecol.* **2005**, *30*, 21–33.
188. Garside, A.; Bell, M.; Robotham, B.; Magarey, R.; Stirling, G. Managing yield decline in sugarcane cropping systems. *Int. Sugar J.* **2005**, *107*, 16–26.
189. Pietramellara, G.; Ascher, J.; Borgogni, F.; Ceccherini, M.; Guerri, G.; Nannipieri, P. Extracellular DNA in soil and sediment: Fate and ecological relevance. *Biol. Fertil. Soils* **2009**, *45*, 219–235.
190. Gözdereliler, E.; Boon, N.; Aamand, J.; de Roy, K.; Granitsiotis, M.S.; Albrechtsen, H.-J.; Sørensen, S.R. Comparing metabolic functionalities, community structures, and dynamics of herbicide-degrading communities cultivated with different substrate concentrations. *Appl. Environ. Microbiol.* **2013**, *79*, 367–375.
191. Drigo, B.; Pijl, A.S.; Duyts, H.; Kielak, A.M.; Gamper, H.A.; Houtekamer, M.J.; Boschker, H.T.; Bodelier, P.L.; Whiteley, A.S.; van Veen, J.A.; *et al.* Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO₂. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 10938–10942.