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REVIEW

Examining assumptions of soil microbial ecology in the monitoring of ecological restoration

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Abstract

1. Global interest in building healthy soils combined with new DNA sequencing technologies has led to the generation of a vast amount of soil microbial community (SMC) data.

2. SMC analysis is being adopted widely for monitoring ecological restoration trajectories. However, despite the large and growing quantity of soil microbial data, it remains unclear how these data inform and best guide restoration practice.

3. Here, we examine assumptions around SMC as a tool for guiding ecosystem restoration and evaluate the effectiveness of using species inventories of SMC as a benchmark for restoration success.

4. We investigate other approaches of assessing soil health, and conclude that we can significantly enhance the utility of species inventory data for ecological restoration by complementing it with the use of non-molecular approaches.

KEYWORDS

biodiversity monitoring, ecosystem functioning, microbial community composition, restoration ecology, soil microbes

1 | INTRODUCTION

The question of how to restore ecosystems is increasingly important as the planet is swept with anthropogenic disturbances. In practice, rehabilitation and ecological restoration outcomes fail to meet targets more often than they succeed (Crouzeilles et al., 2016), particularly for diverse native vegetation on heavily altered substrates (Cross & Lambers, 2017). The factors determining rehabilitation and restoration outcomes are complex and depend on a suite of factors, biotic and abiotic, contemporary and historical (Nsikani, van Wilgen, & Gaertner, 2018). Indeed, for some ecosystems, particularly those which experience severe soil disturbance, degradation or contamination, return to a pre-disturbed state may not be possible (Webster et al., 2018).

Soil microbial communities (SMCs) have long been proposed as indicators of success for monitoring the spectrum of ecological restoration (Harris, 2003, 2009) including plantation forests (Banning et al., 2011), post-mining areas, oil and gas activities (Zhang et al., 2020), invasive species management (Chen et al. 2020) and soil stabilization (Rodríguez-Caballero et al. 2012). As soil microbial functioning is the basis for all successful restoration and rehabilitation, SMC inventories have become the de facto metric for describing soil health during restoration events (e.g. Maestre, Solé, & Singh, 2017), regardless of disturbance type. In most cases, these inventories are collected along restoration trajectories to establish a 'benchmark' for restoration success (e.g. Gellie, Mills, Breed, & Lowe, 2017; Yan et al., 2018).

But how informative are SMC species inventories for determining soil health? Given that the ability to inventory SMC depends upon next-generation sequencing technology (see Williams, Nevill, & Krauss, 2014), our understanding of SMC assemblage and functionality is still developing (Yang, Wagg, Veresoglou, Hempel, & Rillig, 2018). Yet the number of studies based on quantifying SMCs is ever increasing; in the past 6 months alone, the search terms 'soil microb* community AND restoration' returned 1231 papers (Web of Science, August 8, 2020). Does this deluge of data improve estimates of restoration success, or does our understanding still lag too far behind the technology for these approaches to be useful?

There is little doubt as to the importance of SMC in ecosystem functioning. But the value of SMC species inventories as a metric for evaluating restoration success is unclear. In this paper, we examine questions about the role of SMC in ecological restoration: (a) How impacted are SMC by anthropogenic disturbance and can they 'recover' naturally? (c) Are SMC species inventories a good metric of soil health? (c) Can soil microbial amendments accelerate SMC 'recovery'? And (d) what are the appropriate technologies for measuring SMC composition and function? Our goal is to provide practitioners and researchers with an overview of the underlying assumptions and potential limitations of a sequencing-based approach for assessing SMC restoration, and suggest potential complementary methods to improve the utility of such an approach.

2 | HOW ARE SMC DISTURBED BY ANTHROPOGENIC ACTIVITY?

At first glance, the use of SMCs to inform restoration success is intuitive because SMC can be easily disrupted and degraded by anthropogenic activities (Allison & Martiny, 2008). However, it is difficult to predict the effect of disturbances on SMC because the magnitude and direction of responses depend on the type and severity of the disturbance event (De Vries et al., 2012). Predicting effects of disturbance on SMC is made even more difficult as most anthropogenic activities involve multiple disturbance agents. For example agriculture has a unique disturbance signature of altered soil chemical and physical properties and altered plant communities (Pimentel et al., 1995). Mining, in contrast, can involve both soil degradation or pollution and complete soil removal (Ghose, 2004). Clearly, such different sources of disturbance would affect SMC in different ways and to different extents. For example, an open quarry or waste rock landform lacking sufficient topsoil cover would require different restoration strategies than a tailings storage facility. In such cases of differing disturbance, it will likely be challenging to determine what factors have driven changes in SMC and how these communities can be restored.

One of the most commonly studied factors associated with anthropogenic disturbance is soil chemistry (Leff et al., 2015). Addition of nitrogen (N) and phosphorus (P) can cause shifts in SMC (Suzuki, 2009). For example increased soil N and P concentrations can reduce the levels of *Glomeromycota*, fungal plant symbionts that are known for their role in facilitating plant nutrient uptake (Treseder, 2004). The extent to which these effects are direct or plant mediated is unclear, as plant communities also influence SMC composition through symbioses (Martinez-Garcia, Richardson, Tylianakis, Peltzer, & Dickie, 2015), and altered carbon input into the soil (Lange et al., 2015). Mineral N supplements can supress soil microbial respiration, affecting decomposition rates and soil carbon pools (Ramirez, Craine, & Fierer, 2012). Similar to nutrient addition, heavy metal toxicity affects bacterial and fungal communities differently, reducing bacterial activity more than fungal (Rajapaksha, Tobor-Kapłon, & Bååth, 2004). Metal toxicity inhibits certain enzymes (Belyaeva, Haynes, & Birukova, 2005) and reduces microbial activity, resulting in interrupted metabolic cycles in SMC (Shi, Bischoff, Turco, & Konopka, 2002).

In addition to chemical changes, anthropogenic activity affects SMC through substrate modifications. These disturbances vary in intensity from tillage in agriculture to soil removal in surface mining. SMC responses to tillage vary, but fungi are generally more affected by substrate modification than bacteria (Frey, Elliott, & Paustian, 1999). The impact of mining on SMC can be more severe as it represents the combined effects of substrate and plant removal. In many cases, sites can also be contaminated with heavy metals, causing near sterile conditions (Baker & Banfield, 2003). The removal of topsoil for mining has clear detrimental effects on SMC diversity, microbial biomass and soil metabolic cycles. These effects are often compounded when soil is stockpiled for extended periods (Golos & Dixon, 2014) and can still be detected 100 years after rehabilitation efforts have ceased (Poncelet, Cavender, Cutright, & Senko, 2014).

Topsoil is a valuable resource in restoration and is usually harvested before mining activities and stockpiled for later restoration use (Golos & Dixon, 2014). The quality and functionality of a stockpiled topsoil are affected by many factors including the size and depth of topsoil stockpiles, duration of storage, soil physical and chemical characteristics, vegetation cover and climate (Abdul-Kareem & McRae, 1984). Vegetation cover on topsoil stockpiles can increase SMC activity and diversity (Muñoz-Rojas, Martini, Erickson, Merritt, & Dixon, 2015; Pandey et al., 2017). Significant effects of topsoil storage on the biomass, size, activity and composition of the SMC have been recorded, which have implications for nutrient cycling (Harris, Birch, & Short, 1993).

The multifactorial nature of disturbance makes it hard to predict how SMC are affected, and whether they are a reliable indicator of restoration success. The idiosyncratic responses exhibited by bacteria, fungi and other microbes compound this complexity. Additionally, biases in sampling may interfere with ability to detect changes (Box 1). These problems make it difficult to design useful molecular inventories. For example if arbuscular mycorrhizal (AM) fungi were compromised by deforestation but saprotrophic fungi were not, then an assay targeting general fungal communities may not detect changes as AM fungi represent such a small fraction of general fungi in the soil. Conversely, assays that are too fine may also fail to detect changes. In this case, assays targeting bacteria involved in the nitrogen cycle would miss changes to bacteria responsible for carbon cycling.

BOX 1: Sampling bias in SMC description

Sampling design may preclude accurate SMC assessment. Heterogeneity within and among sites may require intensive sampling both spatially and temporally than is feasible for most sites. For example, sampling during the growing season may yield results that are qualitatively and quantitatively different from those in dormant periods (Šťovíček, Kim, Or, & Gillor, 2017). SMC in bulk soil may also vary with rhizosphere conditions including nutrient availability and plant root interactions (Huang et al., 2014). Finally, traditional soil sampling depths (<15–20 cm) may provide a biased examination of SMC, as the rooting depth of vegetation in most ecosystems greatly exceeds these depths (Canadell et al., 1996). In such cases, significantly deeper sampling may be required to obtain a complete picture of SMC diversity and composition (Pickles & Pither, 2014).

3 | CAN SMC REASSEMBLE NATURALLY?

Even though SMCs are affected by anthropogenic processes, they are rarely the target of restoration themselves. Rather, the ability of SMC to reassemble to a pre-disturbance state is an explicit assumption in restoration events. This can be problematic if SMC fail to recover and impair ecosystem recovery. But even if they do return to a reference state, this may not reflect successful ecosystem recovery. Without an explicit understanding of the relationship between SMC and ecosystem functioning, using SMC inventories may fail to reflect restoration success.

Is it necessary that SMC be indistinguishable from ‘reference’ SMC in order to have successful restoration? To assess SMC recovery, it is important to define the recovery trajectory. There is limited evidence in the literature for what constitutes SMC ‘recovery’ and, importantly, forms the basis for reinstating predetermined plant cover (McDonald, Gann, Jonson, & Dixon, 2016). Studies have shown that SMCs change as site successional processes occur, but that they rarely achieve predisturbance conditions (e.g. Banning et al., 2011). While recovering SMC can achieve similar levels of species richness or composition compared with ‘reference’ SMC, they appear to rarely achieve both (e.g. McKinley, Peacock, & White, 2005). Alternatively, if recovery represents a return to the same level of functionality as ‘reference’ SMC, then there is evidence that SMC recover these processes only partially (Kumaresan et al., 2017). It is not clear whether functional gene assays are informative proxies for determining plant-relevant SMC recovery, given the complexity of SMC on soil and ecosystem processes (Graham et al., 2016).

If assays reveal that SMC recovery failed to achieve ‘reference’ status, is it possible that the altered SMC is functionally equivalent to the reference community? If recovery after disturbance assumes a different trajectory, then SMC may differ compositionally, yet still be ‘functional’ for the contemporary ecosystem. Because SMC composition is strongly affected by identity and diversity of vegetation (e.g. Andersen, Grasset, Thormann, Rochefort, & Francez, 2010), it may be unrealistic for SMC to return to reference status if vegetation communities differ from pre-disturbance (Wardle & Peltzer, 2017). Recovery in a functional sense may be delayed or thwarted indefinitely.

It has been argued that heavily altered landscapes can pass a theoretical tipping point where the recovery of the pre-disturbance state becomes unachievable or even undesirable (Perring et al., 2015). Under such circumstances, the identification of restoration targets and goals may be difficult (Radeloff et al., 2015). While the idea of a fully functional ‘alternative’ state can be appealing for restoration practitioners and governments, they may lower restoration standards (e.g. Murcia et al., 2014), while representing a ‘Get-Out-of-Jail-Free-Card’ for industry (Simberloff, Murcia, & Aronson, 2015). For belowground restoration, the ‘alternative states’ debate pertains to whether the goal is taxonomic or functional resemblance to reference states. Given the hyper-taxonomic diversity of SMC, the SMC of reinstated ecosystems may be functionally equivalent but compositionally divergent (Louca et al., 2018). Whether or not these differences are likely to be of ecological significance remains unclear.

4 | DOES SMC DIVERSITY INDICATE A HEALTHY ECOSYSTEM?

It is thought that the diversity of SMC is a key indicator of soil health in both natural and managed ecosystems (Schimel & Schaeffer, 2012). For example higher microbial diversity significantly improved the soil carbon (C) cycling in a labelled 13C microcosm experiment (Maron et al., 2018), as well as nitrogen (N) cycling mediated by soil bacteria and arbuscular mycorrhiza (Nelson, Martiny, & Martiny, 2016). While there is a relationship between SMC diversity and many ecosystem functions (Sahu et al. 2017), there is little evidence that plant communities and SMC are always tightly coupled (Van Nuland, Ware, Bailey, & Schweitzer, 2019). Even if SMC never recover to a reference state, it may not affect ecosystem recovery.

It is difficult to link specific ecosystem processes to specific microbial communities. For instance, Bier et al. (2015) reported that about one quarter of soil microbial diversity studies have tried to link the microbial community structure to microbial processes. The vast majority of these links are not well understood and remain unclear (Jansson & Prosser, 2013). Therefore, it is difficult to link specific traits of ecosystem health to microbial community members, and the enzymatic activity measurements are still imperfect to picture changes in microbial community composition (Baldrian, 2019).

If soil ecosystem functioning is the additive effect of the functions of a diverse SMC, then the presence of a taxon should confer certain functions, and thus SMC diversity could function as an indicator for ecosystem recovery. However, there is evidence that SMC diversity is not the main factor affecting soil functioning. Graham et al. (2014) argued the physical and chemical environment was as important for N-cycling as SMC diversity. The presence of a specific microbial taxon may not guarantee functionality; a taxon may be represented in an inventory but non-functional due to dormancy, death or suboptimal environmental conditions (Blagodatskaya & Kuzyakov, 2013). Alternatively, increased SMC diversity may not manifest as more functional due to high levels of functional redundancy in the SMC (Nannipieri et al., 2003). While such redundancy may help buffer functioning against disturbance (Mendes et al., 2015), it means that diversity alone is an unreliable measure of SMC recovery. Currently, we do not know how much functional diversity exists in the SMC and how this diversity differs across systems or conditions (Jansson & Prosser, 2013).

5 | CAN SMC RECOVERY BE ACCELERATED THROUGH SOIL AMENDMENT AND INOCULANTS?

If SMCs are important for ecosystem recovery, there may be reason to introduce soil microbes to accelerate positive change (Neuenkamp, Prober, Price, Zobel, & Standish, 2019, Policelli, Horton, Hudon, Patterson, & Bhatnagar, 2020). The use of microbes in ecosystem restoration can range from simple ‘growth enhancer’ claims to more directed applications, for example to ameliorate polluted soils. Inoculants have successfully alleviated salinity stress (Ahmad et al., 2018) and heavy metal contamination (Fashola, Ngole-Jeme, & Babalola, 2016). Inoculation with metal-tolerant microbes has improved plant establishment (Ahmad et al., 2018) and has been advocated for in the remediation of heavy metal-contaminated sites (Fashola et al., 2016) while conferring plant growth promotion (Mishra, Singh, & Arora, 2017).

There are many reasons why microbial inoculants may fail to achieve desired outcomes, including lack of local adaptation and post-application evolution of microbial inoculants in the field (Hart, Antunes, & Abbott, 2017). Further, successful inoculant establishment may represent an invasive species threat (Hart et al., 2019). If the invasive microbe replaces or occludes resident taxa, ecosystem functioning may be compromised. Sequencing-based technologies may be useful not only for identifying candidate inoculants and evaluating the SMC response to inoculation in the field, but also for monitoring resident SMC responses.

5.1 | Local adaptation

For plants, locally adapted genotypes may be critical for restoration success (Breed et al., 2018). The same may be true for SMC, though how important this is remains unclear (Austin, Vivanco, González-Arzac, & Pérez, 2014; Rúa et al., 2016). Even if commercial products are able to establish in the field, they may not be resilient to natural conditions compared to local SMC (Griffiths & Philippot, 2013, Moreira-Grez et al., 2019).

5.2 | Unintended consequences

Soil inoculation can lead to changes in the structure of indigenous microbial communities (Trabelsi & Mhamdi, 2013), resulting in potentially synergistic or antagonistic interactions with native SMC. This aspect of microbial inoculant use is understudied, largely because there have been few longitudinal studies tracking inoculant fate in the field. More importantly, such changes and/or replacement of native microbial guilds can have a negative effect on native plant fitness (Moreira-Grez et al., 2019). Consequences of shifts in SMC as a result of inoculants could have long-lasting and unintended ecosystem-level consequences.

5.3 | Post-application evolutionary changes in microbial inoculants

Microbial inoculants, largely a product of directed selection, are also subject to natural selection once they are applied in the field where they may lose or gain traits over time. If inoculants do change over time or conditions, it is also feasible that there could be gene transfer among the inoculant and local SMC (Braga, Dourado, & Araújo, 2016) resulting in rapid spread of novel traits. Depending on the trait, this could result in reduced plant performance or rapid growth of undesired species through enhanced interactions with invasive plant species (Rout et al., 2013).

6 | ARE CURRENT MOLECULAR TECHNIQUES INFORMATIVE ENOUGH TO ASSESS DAMAGE AND RECOVERY OF SMC?

Taxonomic approaches, or amplicon metagenomics, provide information on SMC taxa present and, to an extent, their relative abundance (Frac, Hannula, Belka, & Jędryczka, 2018). However, sequencing alone provides no indication of the functionality or activity of the microbial community (Gellie et al., 2017, Yan et al., 2018). The link between SMC composition and ecosystem processes is still being elucidated; SMC status as an indicator for ecosystem recovery remains ambiguous.

The problem of SMC composition versus function has been addressed by the development of in silico approaches which has facilitated taxonomic surveys by matching barcoding sequences to known genome sequences in a database. These approaches infer metagenomic function, and there are several software packages in the public domain, for example PICRUSt (Langille et al., 2013), Tax4Fun (Aßhauer, Wemheuer, Daniel, & Meinicke, 2015), Piphillin (Iwai et al., 2016), Vikodak (Nagpal, Haque, & Mande, 2016), BURRITO (McNally, Eng, Noecker, Gagne-Maynard, & Borenstein, 2018), CowPI (Wilkinson et al., 2018) and FUNGuild (Nguyen et al., 2016). These packages can use barcoded tag sequences from taxonomic databases such as Greengenes (DeSantis et al., 2006), Silva (Pruesse et al., 2007) or RDP (Cole et al., 2013). The output can be used to identify genes that are part of soil biological processes (e.g. Mickan et al., 2018; O'Brien et al., 2019).

Perhaps the most informative assay to SMCs as appropriate indicators of ecosystem status will be through metagenomic approaches. Knowing which genes or proteins are actively transcribed is the best way to gauge whether soil ecosystem functioning has been restored. These approaches give a more comprehensive view of SMC functioning through identification of microbial metabolites, transcripts and proteins. There are only a limited number of studies using these approaches as they pertain to SMC. Those that do, however, are able to achieve a much more nuanced understanding of SMC identity and functioning (Box 2). For example Bastida, Selevsek, Torres, Hernandez, and Garcia (2015) used proteomics to show that microbial biomass did not reflect functioning. Such a distinction would have been overlooked without the ability to measure the activity of cell growth cycle proteins and extracellular enzymes. While these approaches will be the key to understanding how SMCs respond to land use, their use is currently limited in soil ecosystems (Chiapello, Zampieri, & Mello, 2020), mostly due to technical issues such as there are too few databases for soil microbial transcripts and proteins (Nesme et al., 2016; Starke, Jehmlich, & Bastida, 2019) meaning most proteins are currently unidentifiable. Further, protein extraction from soil is challenging due to inhibitory effects of humic acids (Qian & Hettich, 2017) as well as the context-specific extraction protocols associated with different proteins (Taylor & Williams, 2010). As much as this approach is promising, the majority of proteins are lost during extraction, or are unidentifiable.

BOX 2: Case study: Soil microbial community (SMC) monitoring and mine site restoration

The measurement of SMC as a tool for monitoring and estimating restoration success was first proposed by Harris (2003). We continue producing vast quantities of sequence data with little understanding of its functional meaning. Rather than continue to generate data and simply assume correlation with ecological functioning, we call for greater focus on the function of microbial communities. For example, marker gene and shotgun metagenome sequencing show that soil handling after mining changes SMC composition but preserves metabolic functioning at the metagenome level (Kumaresan et al., 2017). This study demonstrates that relying only on phylogenetic information may lead to incorrect inference of SMC status, and multiple lines of evidence are needed to provide a meaningful tool for assessing restoration trajectory (Figure 1). Please see Figure 2 for study site.

All photos by Adam Cross and Kingsley Dixon.

7 | ALTERNATIVE AND COMPLEMENTARY METHODS TO MOLECULAR APPROACHES

The most common method, species inventories through amplicon sequencing, is at best an indirect estimation of SMC activity and functionality (Yan et al., 2018). To improve the utility of this method in assessing SMC recovery, it may be useful to incorporate other approaches that measure SMC physiological activity, many that predate the molecular era (Figure 1), in order to measure the fundamental makeup of soil biota. Although many of these approaches have been abandoned for more high resolution methods, they can complement molecular information and increase our understanding on the soil processes associated with SMC.

7.1 | Phospholipid fatty acid analysis

Phospholipid fatty acid (PLFA) analysis is a method of assessing microbial taxonomic and functional diversity that involves the extraction, fractionation, methylation and chromatography of the phospholipid component of soil lipids (Muñoz-Rojas, Erickson, Dixon, & Merritt, 2016). Changes in the phospholipid profile can be related to the variation in the abundance of microbial groups (Nannipieri et al., 2003). PLFAs degrade quickly upon cell death and so are considered to be strictly representative of the living SMC (Quideau et al., 2016). PLFA analysis can, however, only be used to detect coarse changes in

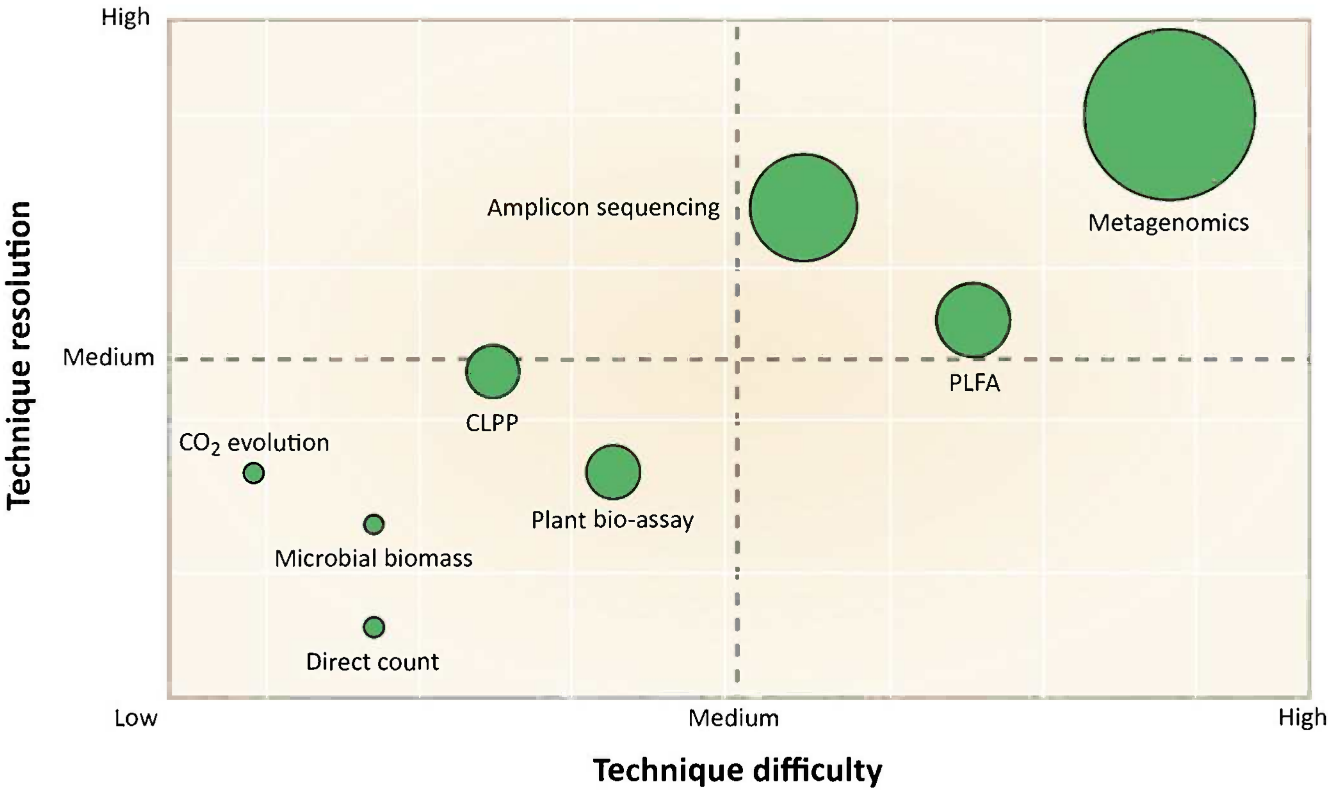

FIGURE 1 Potential approaches for assessing SMC composition, abundance and functioning. Technique resolution is the relative amount of information obtained. Technique difficulty is the relative complexity of the method. Circle size indicates relative cost. CLPP = community-level physiological profiling, PLFA = phospholipid fatty acid analysis

community structure, as it does not permit detection to a taxonomic level (Nannipieri et al., 2003). Data on microbial biomass are produced, and the ratio of fungi to bacteria present within the soil can be measured to provide insights into the SMC, as fungi and bacteria thrive in different soil conditions (Strickland & Rousk, 2010). This approach is suitable where the abundance or biomass of large taxonomic groups is relevant. However, this method for assessing soil functionality does not provide any information on finer taxonomy, genes present or microbial activity.

7.2 | Microbial biomass

A simple, inexpensive method of assessing microbial abundance is through the determination of microbial biomass. Microbial cells are removed from soil through chloroform fumigation or irradiation with gamma- or microwaves (de Aquino Moura, da Silva Garrido, da Silva Sousa, Simões Cezar Menezes, & de Sá Barretto Sampaio, 2018), and the carbon content assessed. Similar to PLFA, this method would be suitable for studies assessing biomass recovery of large taxonomic groups and where costs are a limiting factor. This method for assessing soil functionality is low in resolution, providing only a measure of microbial abundance but no information on SMC structure or activity.

7.3 | Direct counts

Counts of cultured cells or colonies can give a simple and quick indication of microbial diversity. However, most environmental microbes are not culturable, and therefore will not be present in plate counts (Oliver, 2005). This approach gives a quick but coarse estimate of microbial diversity. This method for assessing the microbial community does not provide information on SMC structure or activity. It would be best used in cases where the presence of specific, desirable taxa is required, such as the presence of rhizobacteria compatible with target vegetation.

7.4 | CO_2 evolution

CO_2 is released during microbial respiration; therefore, the production of CO_2 can be used as an indicator of microbial activity and relative soil fertility (Haney, Hossner, & Haney, 2008; Muñoz-Rojas et al., 2016). CO_2 evolution is relatively simple and inexpensive to measure; the Solvita test or 1-day CO_2 test involves measuring the production of CO_2 24 hr after re-wetting dried soil (Muñoz-Rojas et al., 2016). This method would be well used similar to microbial biomass, where identity is secondary. For example, when restoration is attempting to restore basic microbial functioning, after contamination or soil removal. This

FIGURE 2 Top: mine site in southwest Australian biodiversity hotspot. Middle left: the same mine site three years after replacement of topsoil. Middle centre: plant re-establishment underway after replacement of topsoil. Middle right: tailings from the same site where topsoil (red soil) was used as an SMC inoculant. Bottom left: soil crusts have complex microbiomes. Bottom centre: direct seeding on a site undergoing restoration. Bottom right: soil microbial amendments and seed adjuvants

method for assessing soil functionality does not provide any information on taxonomy, genes present within or community structure of the microbiome.

7.5 | Community-level physiological profiling

Community-level physiological profiling most often refers to data collected with the use of Biolog EcoPlates: 96-well plates containing 31 different carbon sources and a blank in triplicate with a redox dye indicator (Weber and Legge 2010). When the microbial community is inoculated into each well, cell respiration results in a colour change within the well that can be read photometrically (Weber and Legge 2010). This method for assessing soil functionality is coarse (Waterhouse, Adair, & Boyer, 2014) and is limited to carbon-degrading microbes. It would be a good technique to use when attempting to assess basic nutrient cycling in previously devastated soils, but does not provide any information on taxonomy or genes present.

7.6 | Plant bioassays

Perhaps the simplest and most informative method for assessing SMC status for plant restoration is through growth assays. The microbial community can be extracted to be used as an inoculant, thereby eliminating the effects of soil chemistry on plant establishment and growth. Although this method for assessing soil functionality represents SMC as a ‘black box’ (it does not provide any information on taxonomy, microbial community structure, genes present or microbial activity), by encompassing the full complexity of the SMC, it gives an informative representation of SMC status. This approach is cheap and easy to use and would suit almost any scenario. Ultimately, the ability of a SMC to facilitate plant establishment is the end goal of any restoration event.

7.7 | Other approaches

To improve restoration outcomes, determine restoration trajectories and evaluate restoration success, we need a more 'integrated' approach using multiple methods, not just SMC inventories. Proximal soil sensing with visible near-infrared and mid-infrared spectra might facilitate such integrated assessments of soil health and function (Stenberg et al. 2010; Cécillon et al., 2009). These spectra provide an integrative measure of the soil, a 'fingerprint' of its molecular composition. Many soil physicochemical properties have been modelled with spectra to accurately estimate their concentrations (Soriano-Disla, Janik, Viscarra Rossel, Macdonald, & McLaughlin, 2014). And, there is research that has related soil spectra to bacterial abundance measured with traditional PLFA profiling (Zornoza et al., 2008), microbial biomass (Chodak, 2011, Zornoza et al., 2008), enzyme activity (Zornoza et al., 2008) and respiration (Chodak, 2011). More recently, vis–near-infrared spectra and other easily obtainable environmental data were used with machine learning to develop spectro-transfer functions to explain and infer bacterial abundance and diversity based on 16S rRNA gene metabarcoding (Yang et al., 2019). Another advantage of the spectroscopic technique is that it is rapid and inexpensive so that many measurements can be made across space and time in a cost-efficient manner (Viscarra Rossel et al. 2011).

8 | CONCLUSIONS

The recovery of functional SMC is essential for ecological restoration, yet our ability to determine 'disturbed' or 'recovered' SMC remains limited. Incorporating SMC measurement and monitoring into study designs is challenging, because we do not yet have a metric which represents the diverse functional and compositional complexity inherent in SMC. But do we need to understand the complexity of SMC to manage them effectively in restoration? Regardless of the technology or the approach used, the problem facing global soil health remains the same: we are degrading and simplifying soil biodiversity at an unprecedented rate. We cannot afford to wait until science opens up the black box of soil microbial ecology.

Until soil metagenomic approaches become more accessible and comprehensive, practitioners will be forced to rely on incomplete surrogates. In the meantime, a more informative approach may be to incorporate multiple physiological metrics in addition to standard DNA sequence-based species inventories. The focus should shift from attempting to compositionally recreate 'reference' SMC to creating functionally robust SMCs that provide ecosystem functioning and confer ongoing ecological resilience in restored ecosystems.

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