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Microbial catabolic activity: methods, pertinence and potential interest for improving microbial inoculant efficiency

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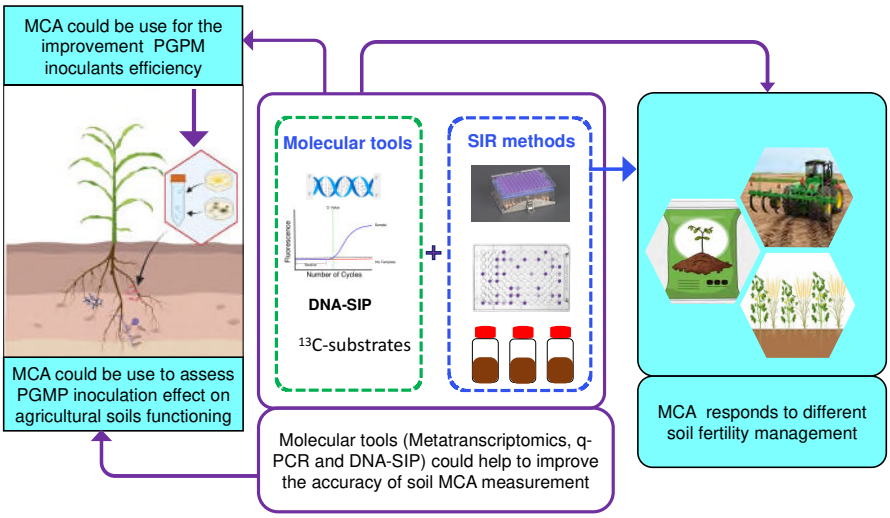
Abstract

Microbial catabolic activity (MCA) defined as the degrading activity of microorganisms toward various organic compounds for their growth and energy is commonly used to assess soil microbial functions potential. For its measure, several methods are available including multi-substrates induced respiration (MSIR) measurement which allow to estimate functional diversity using selected carbon substrates targeting specific biochemical pathways. In this review, the techniques used to measure soil MCA are described and compared with respect to their accuracy and practical use. Particularly the efficiency of MSIR-based approaches as soil microbial functions indicators was discussed by (i) showing their sensitivity to different agricultural practices including tillage, amendments and cropping systems (ii) and by investigating their relationship with soil enzyme activities and some soil chemical properties (pH, soil organic carbon, cation exchange capacity). We highlighted the potential of these MSIR-based MCA measurement to improve microbial inoculants composition and to determine their potential effects on soil microbial functions. Finally, we have proposed ideas for improving MCA measurement notably through the use of molecular tools and stable isotope probing which can be combined with classic MSIR methods.

Keywords: microbial catabolic activity; CLPP; soil quality indicators; soil function; microbial inoculant efficiency



Graphical abstract describing the interrelation between the different parts and the concepts developed in the review

1. Introduction

Soil fertility is often evaluated by various physico-chemical and biological parameters, which can measure the impact of land use and management practices, and ecosystem restoration [1, 2]. The biological indicators include microbial biomass which provide information on the size of the microbial component responsible for most soil biological processes [3], the microbial diversity which largely estimates the composition/structure of the soil microbial community and the microbial catabolic activity (MCA). The MCA is the degrading activity of heterotrophic microorganisms toward different type of organic substances to ensure their needs for energy and nutrients. Therefore, it can be used to assess soil functional capacities related to organic matter (SOM) decomposition, nutrients recycling and ecosystem functions in general. Therefore, several methods have been developed and used to assess the soil MCA.

Basal respiratory rate (often measured as CO_2 evolution) which reflects the decomposition of organic substances by microorganisms under aerobic conditions [4] as well as the physiological response of soil to amendment with an excess of a carbon and energy source proposed by Anderson et Domsch [5] are univariates methods used to assess the soil MCA. Likewise, isothermal microcalorimetry (IMC) is also used to assess soil microbial activity [6–8]. IMC is based on the measurement of heat released from soil microorganisms, which is reported to be related to several catabolic processes such as CO_2 release [9, 10], O_2 consumption [11], and dehydrogenase activity [12]. Soil MCA can be further characterized by multivariate soil activity profiling methods which can offer higher degree of sensitivity and probably a better discriminative resolution toward the functional status of soils. These include enzymatic activities assays are based on the degradation capabilities of specific substrates, indicating the capacity of soil microbiota to carry out different specific functions [13], either by extra- or intra-cellular enzymatic pathways. In addition, many multi substrates-induced respiration (MSIR) techniques have been established to assess soil MCA potential after the addition of carbon substrates from different biochemical classes (carbohydrates, amino acids, carboxylic acids, polymers, etc.) [14–17]. These methods allow the determination of soil Community-Level Physiological Profiles (CLPP), which provide relevant insight on the capacity of soil microorganisms to catabolize different types of carbon substrates for their growth and energy [18]. Determining the soil CLPP allows the detection of shifts in soil functional diversity in response to fertility management strategies (amendment, tillage, crop rotation etc.) used to improve plant productivity [19–22]. The developments of molecular tools (based on microbial DNA and RNA) have also made considerable advances for apprehending soil catabolic activity through the quantification of the expression of genes controlling specific catabolic processes. Therefore, MCAs are likely key microbial functional indicator that can be useful for soil health assessment in different frameworks including soil restoration/remediation, fertility diagnostic or microbial inoculation that may shape soil microbial communities functioning.

Furthermore, the inoculation of soils or seeds with plant growth promoting microbes (PGPM) is considered as an eco-friendly soil fertility management strategy of modern agriculture [23, 24]. Microbial inoculants have been proposed to improve plant growth/yield and soil fertility owing to soil biological activities including N_2 fixation, nutrient mineralization solubilization/mobilization (e.g., N, P, K), phytohormone-production, abiotic stress relief and disease suppression [25–30]. However, some PGP traits validated under *in vitro* conditions may not be reproduced under uncontrolled (i.e. field) conditions due to many factors, including competition with indigenous microbiota, crop specificity, edaphic and environmental conditions [29]. Several studies have shown that soil microbial inoculation can impact the native soil microbial diversity and composition, suggesting that soil microbial inoculants can persist under

specific conditions [31–35]. In addition, little is known regarding the effect that microbial inoculants may have on the functional capacity of native soil microorganisms [36]. MCA measurement tools may provide insightful information describing the potential implications of these inoculations in terms of soil global catabolic potential and carbon dynamics (mineralization and sequestration), beyond the improvement of plant growth. This is of particular interest for the current global warming context where preserving more carbon into the soil can contribute to climate change mitigation as an important ecofriendly agricultural practice [37].

Despite the wide range of approaches used to asses soil MCA, critical reviews on their efficiency, applicability and convenience remain scarce. Blagodatskaya et Kuzyakov [38] reviewed the different approaches used to estimate the active and potentially active fraction of soil microbiota including those based on process rates such as catabolic activity measurement. Chapman et al. [39] made a descriptive synthesis of three different CLPP techniques based on soil respiration activity (Biolog™, Multi-SIR and MicroResp™). Nevertheless, a critical review summarizing the different MCA approaches including MSIR methods, enzyme activities-based methods as well as molecular tools, and analyzing their sensitivity to different soil managements could help to better assess soil functions in different contexts. Moreover, MCA measurements are likely useful for assessing the functional capacities of microbial inoculants and those of the resident communities of soil after inoculation. In this review: (i) we provide a critical description of the different techniques used to measure soil MCA and elucidate their potential to asses soil functioning in different agroecosystems, (ii) we also discuss the potential of MSIR-based MCA methods to improve the efficiency of soil inoculum during formulation and deployment in agroecosystems and (iv) provide insights towards combining MSIR methods with molecular tools (i.e., RT-qPCR, /meta-transcriptomics) and stable isotope probing techniques to increase their accuracy.

2. Description of different methods used to measure soil MCA

Soil MCA potential is commonly studied by different techniques that enable to describe the soil CLPP by assessing directly or indirectly its potential to mineralize different substrates from various biochemical classes. Indeed, several techniques are based on the measurement of the soil respiration rate after individual addition of multiple substrates. Chapman et al. [39] reviewed the three major different CLPP techniques based on soil respiration measurement: The Biolog™, the Multi-SIR and the MicroResp™. In this review, we describe available methods used for measuring MCA including those that are not based on respiration rate measurement (enzyme activities, expression of genes involved in specific catabolic processes).

2.1. Methods based on substrate induced respiration (SIR)

Respiration-based methods for soil MCA characterization have been developed and a wide range of substrates with ecological relevance have been used and commercialized in microtiter plates (Biolog™ and MicroResp™). This has allowed rapidity and convenience for analyzing physiological response of soils from various ecosystems to addition of different classes of substrates. However, a common denominator between the Biolog™, the Biolog EcoPlate™ and the BD-Oxygen-Biosensor method is the need for a prior extraction of the soil microflora by preparing dilutions from soils before their incubation in the presence of substrates [16, 17, 40] **(Table 1).** Therefore, these methods depend on the growth of extracted microbial populations during the incubation time, increasing measurement bias between measured MCA activity and the initial total soil functioning status. These limitations were overcome by the development of the Multi-SIR [15] and the MicroResp™ [14] methods that use whole soil, avoiding the need of a pre-extraction step.

Moreover, these whole soil based techniques offer the possibility to determine the specific contribution of bacterial and fungal component in the global soil catabolic activity by adding antibiotics to substrate solutions [41, 42]. Although closer to natural conditions, these also have some limitations.

In the MicroResp™ system, soil moisture variations and the differential release of non-specific respiration-related CO_2 by soils with different carbonate levels (e.g. originating from different geographical regions) could bias the respiratory results [43]. Nevertheless, carbon radiolabeled (^{14}C) substates can overcome this issue to increase the specificity of respiration-related CO_2 release but this MicroResp™ experiment is more complex to set up and is more expensive. There is also the problem of CO_2 retention in the solid phase of the soil-substrate mixture, particularly when the pH is greater than 6, which can underestimate the respiration [44, 45]. Oren and Steinberger. [45] recommended, particularly for calcareous soils, to determine experimentally the CO_2 retention levels, the pH changes induced by substrates addition (particularly for organic acids) and the abiotic CO_2 release rates to calculate the correction factor to apply. However, this extends considerably time and cost particularly when analyzing a large number of samples. The differential sorption of charged substrates (divalent/trivalent) in different soils could also have a significant impact on their availability and therefore on CO_2 output [46], which calls for caution when interpreting data, especially in the case of multi-site studies. The low solubility in water of some complex and recalcitrant substrates make them difficult to be tested in multi-SIR methods while they are ecologically relevant, particularly in the plant rhizosphere. Indeed, a part from simple substrates, plant rhizodeposition provides variable quantity of recalcitrant compounds such as fatty acids, sterols cellulose, hemicelluloses, lignin and polyphenols [47, 48] which can be degraded by specialized microbes (mainly by fungi). Therefore, using only simple water-soluble substrates can lead to miss important catabolic activities that could be particularly important in this soil niche.

2.2. Methods not based on soil respiration

The isothermal microcalorimetry (IMC) method is based on measurement of heat production within a soil sample after glucose addition [8, 10, 49, 50]. IMC is described as an integrative technique to accurately describe soil MCA [51]. Indeed, heat production occurs in almost all biological processes that take place within microbial cells, proportionally to the rate of the corresponding physical and chemical processes. Importantly, soil heat output is reported to correlate with microbial biomass, basal respiration rate and enzymatic activities [9, 12]. Sparling [10] and Raubuch and Beese [11] have found a strong correlation between soil heat measured by IMC and respiratory activity measured respectively by CO_2 release and O_2 consumption. Therefore, IMC gives an estimation of the soil MCA as the heat flow signal is related to the sum of all chemical and physical processes including respiration and fermentation. However, Thiele-Bruhn et al. [51] suggest that use of IMC is limited by the relatively high cost of the instrumentation. In addition, heat release might be non-specific to microbial respiration as other chemical processes may also lead to heat release/dispel and thus, the final heat is the net balance between all exo-thermic and endo-thermic processes occurring in a given biological system [49, 52].

Enzyme activities are reliable indicators of soil MCA as they are provide relevant information about soil ability to carry out biogeochemical reactions [53]. This includes hydrolytic enzymes involved in various biochemical cycles: C (cellobiohydrolase, β-glucosidase, β-xylosidase, β-cellobiopyranosid etc.), N (N-acetyl-β-glucosaminidase, Leucine aminopeptidase, Urease etc.) P (Phosphomonoesterase, Phosphodiesterase), S (Arylsulfatase etc.) but also oxidative enzymes like dehydrogenase activity which is often correlated with respiratory activity [13]. Indeed, multiple enzyme

activities assays are commonly used to estimate the soil functional response to various agro-ecological factors [54–57]. As for CLPP approaches, functional diversity estimates can be derived from these enzymatic activities assays through a functional profiling using diversity indices or statistical ordinations methods like PCA and NMDS used to compare different soil treatments [54, 58, 59]. However, the existence of abiontic enzymes in soil, which could be stabilized in the soil during several years after cell death, constitutes a limit for this technique for the determination of soil microbial function status. For example, by using sterilization by microwave irradiation, it was shown that between 34 and 75% of the beta-glucosidase activity measured in different soils did not come from active cells, and then were attributed to the abiontic enzymes pool stabilized in the soils [60]. Therefore, the interpretation of enzyme activities measured from soil as the MCA potentials should be done with particular precaution.

Different approaches based on nucleic acids are used to estimate specific microbial catabolic activities (**Table 1**). This includes real time RT-PCR, microarray and metagenomics. Indeed, the catabolic activity related to the degradation of soil pollutants has been studied in soil inoculated with different bacterial strains using quantitative PCR (q-PCR). This enabled to estimate the abundance and the expression of genes involved in alkanes degradation: *Alk-H*, *Alk-B*, *CYP153* [61–63], catechol degradation : *catA* [64] or protocatechuate degradation : *pcaH* [65]. Importantly, these studies often revealed positive correlations between the abundance and the expression of the tested genes, and the degradation rate of hydrocarbons measured by gas chromatography. Quantitative RT-PCR can also be used to assess the expression of genes involved in soil C and nutrients (N, P, S…) recycling, considered as proxies of potential specific microbial catabolic activities. For example *phoC* and *phoD* genes are frequently used for he assessment of soil phosphatase activity [66–69]. Moreover, a high-throughput quantitative-PCR-based chip including 72 primer pairs targeting 64 microbial functional genes involved in C, N, P, S cycles have been developed by Zheng et al [70] for the estimation of soil potential catabolic activity. In another hand, Metagenomics-based tools are also used to estimate soil MCA. Indeed, the C-and nutrients-cycling genes, organic contaminant degradation genes can be profiled either by gene array *e.g.* GeoChip tool [71–75] which cover in its fifth version more than 1500 functional gene families, by metagenomics [76, 77] or metatranscriptomics sequencing [38, 78, 79], which can be used to decipher the whole soil catabolic genome or transcriptome. The **Table 1** shows examples of genes targeted with metagenomics to characterize soils MCA potential. However, it is worthe noting that the expression and the abundance of genes related a catabolic process cannot be systematically translated into a relevant proxy of the specific activity as others steps (transcription and translation etc.) are need before the proteins (the enzymes) are synthesized to realize the function.

Table 1: Description of the different techniques used to evaluate soil microbial catabolic activity. It includes methods based on respiration measurement, heat release, enzyme activities and molecular tools like metatranscriptomics and real time polymerase chain reaction (q-PCR)

Principle of the methods	Methods	Detection technique	Type of samples	References
Measurement of the microbial respiration	Biolog™	Colorimetry	Soil extract	[17]
	Biolog-EcoPlate™	Colorimetry	Soil extract	[40]
	BD Oxygen Biosensor System	Fluorimetry	Soil extract	[16]
	Multi-SIR	Gaz Chromatography or Spectroscopy	Whole soil	[15]
	MicroResp™	Radioactivity/ Colorimetry	Whole soil	[14]
Measurement of heat released by soil microorganisms	Isothermal Microcalorimetry (IMC)	Calorimetry	Whole soil	[9, 10]
Measurement of specific enzymatic activities (cellobiohydrolase, β-glucosidase, *N*-acetylglucosaminidase, acid/alkaline phosphatases, Leucine aminopeptidase, Urease etc.)	Multiple hydrolytic enzyme assays	Colorimetry/ Fluorimetry	Whole soil	[54, 57–59]
Quantification of expression/genes controlling the recycling of C, N, P, S etc. and the degradation of specific/complex organic compounds (alkanes, catechol, protocatechuate etc.)	q-PCR targeting different genes: *amyA* (α-amylase), *chiA* (endochotinase), *phoX* (alkaline phosphatase), *phoC* (acid phosphatase), *phoD* (alkaline phosphatase), *ppx* (exopolyphosphatase), *naglu* (α-N-acetylglucosaminidase), *dsrA* (sulfite reductase α subunit), *Alk-H, Alk-B*	Fluorimetry	DNA extracted from whole soil	[67, 69, 66, 68, 70, 63, 80]

	(alkanes degradation), *nirK, nirS, nosZ (denitrification)*			
	RT-qPCR of alkanes degrading gene *(CYP153)*	Fluorimetry	mRNA extracted from whole soil	[61]
	Microarray of C, N, P, and S cycling genes expression	DNA hybridation (GeoChip)	mRNA extracted from whole soil	[72–75]
	Metatranscriptomics of C and N	DNA sequencing	mRNA extracted from whole soil	[38, 78, 79]05/07/2023 16:15:00
	Metagenomics of C cycling genes: alpha-amylase *(AmyA),* beta-galactosidase *(lacZ),* xylan 1,4-beta-xylosidase *(xynB),* beta-galactosidase *(lacA)* etc.	DNA sequencing	DNA extracted from whole soil	[76, 77]

2.3. Analysis of MSIR data to better describe the soil potential capacity

The different MCA techniques described in this section are based on the estimation of the CO_2 release rate/the heat release after substrates addition or the marker genes abundance/expression in the soil microbiome. Therefore, the soil catabolic activity in regard to a specific substrate can be expressed as the total amount of CO_2 released per gram of soil [54, 81], the amount of heat produced per gram of soil [50] over a defined incubation period, the gene expression or the number of specific gene copies per gram of soil [62, 63]. However, in the study of Creamer et al. [81], the different substrates responded differently to soil treatment (arable, grassland and forest) suggesting that this strategy can be suitable only when evaluating the catabolic activity related to a limited number of substrates/soil function as in remediation-oriented studies in which the objective is to determine the capacity of soil/microbial matrix to degrade specific organic pollutants or carbon or a specific nutrient cycle. Conversely, for studies aiming to assess the impact of fertility management type, land use, global pollution on a wide range of soil catabolic processes related to several soil functions ensuring different ecosystem services, global aggregative indices are needed for the comparison of different situations (e.g., organic fertilization vs chemical fertilization, inoculated soil vs non inoculated soil, tillage vs no tillage, crop rotation vs monoculture, forest soil vs arable soil etc.). Therefore, it could also be relevant to analyze the substrates use rate by grouping them per biochemical guilds (carbohydrates, carboxylic acids, amino acids, polymers, amines/amides etc.). Moreover, the data obtained from the different substrates/specific function are often used to calculate aggregative quantitative variables. These include the AWCD (Average Well Color Development) index, based on gel dye coloration which indicates the total activity of a soil sample in a plate containing a specific number of substrates (95 for the Biolog™ and 36 for Biolog EcoPlate™), diversity indices calculated using the CO_2 release values or functional gene abundances (e.g. Shannon, Simpson-Yule, Richness, catabolic evenness) which give pondered values estimating diverse MCA potentialities, and the multivariate discriminant analyses such as principal component or canonical variance analyses [50, 82] . Altogether, as analyzing MCA data with only one approach may result in different conclusions for the same set of data, we recommend to perform a gradual analysis starting with the calculation of global diversity indicators (e.g. Shannon, AWCD, Simpson …), then calculating the consumption rate of the different biochemical groups of substrates (e.g. amino acid, carbohydrate, carboxylic acid…) before attempting to interpretate the consumption rate of specific substrates or genes abundance/expression involved in specific activities with particular interests.

3. Could MSIR methods be considered as relevant indicators of soil microbial functioning?

The soil MCA could be estimated by different approaches we described in the precedent section. Among them, those determining CLPP and based on soil respiration (MSIR) seem very interesting due to their convenience and the fact that they measure effective catabolic process despite their limitations. Here, we discussed their relevance through their (i) relationships with other soil function indicators like enzymes activities and some edaphic factors with known influence on soil microbial activity (pH, soil organic carbon: SOC and cation exchange capacity: CEC) and (ii) their capacity to distinguish soils with different uses and fertility managements.

3.1. Relationship between MSIR methods and other soil bio-chemical and chemical parameters

3.1.1. Soil enzymes activities

Soil microbial catabolic processes are mediated by enzymatic activities (for example hydrolysis reactions). Several studies found a relationship between soil enzymatic activities and SIR. For instance, simultaneous increases were noticed in both enzymatic activities (phosphatase, β-glucosidase and urease) and Multi-SIR in a fallow soil (21-year) compared to soil from an adjacent cultivated plot [83]. Moreover, Pignataro et al. [54] found positive relationships between several enzymatic activities (cellulase, chitinase, β-glucosidase, phosphatase, arylsulphatase, xylosidase, acetate esterase and L-leucine aminopeptidase) and the soil respiration induced by various organic substrates (carboxylic acids, amino acids, carbohydrates and amide) measured by MicroResp™. In the study of Grządziel et al. [84], an increase of the AWCD, Shannon diversity index and substrate richness index calculated from Biolog EcoPlate™ data were also accompanied with an increase in dehydrogenase activity in high pH soils. However, Moscatelli et al. [59] found that there was no correlation between the Shannon diversity indices based on enzymatic activities and on soil MCA measurement by MicroResp™. An opposite variation pattern was found in diversity indices (Simpson-Yule and Catabolic versatility) calculated with data from MicroResp™ and enzyme activities used to characterize soil microbial activity [54]. One possible explanation is that these two approaches could target different phases of the organic matter decomposition, i.e. enzymatic hydrolysis would basically focus on incomplete decomposition of the complex organic molecules whereas catabolic assays would provide information on the complete mineralization of simple and complex substrates to CO_2 [54, 59]. Therefore, combining the enzymatic activity and multi-SIR assays like MicroResp™ would be more reliable for the assessment of soil functional status given the complementarity between the two approaches.

3.1.2. Soil edaphic parameters

Soil MSIR change was noticed along geographical climatic gradient [41] likely due to edaphic parameters which are subject to spatio-temporal variations. Soil microbes are of fundamental importance for bio-geochemical cycling and as such it is important to understand how the functional capacity of soil microbiota is affected by variation of such parameters. In this perspective, SOC was reported to be positively correlated with microbial catabolic diversity. For example, when comparing different land use types (pasture, indigenous vegetation, cereal/maize/horticultural cropping), a positive correlation ($r^2 = 0.45$, $p < 0.05$) was found between the differences in catabolic evenness measured using SIR of 25 substrates and the differences in SOC of these treatments [85]. Similarly, SOC content was positively correlated ($r^2 = 0.77$ and 0.72, respectively) with richness (R) and Shannon-Weaver (H) indices calculated from Biolog EcoPlate™ data [86]. Moreover, the same relationship was found between SOC and the consumption rate of different substrates (assessed by CO_2 release) after their addition to soil, when evaluating either the impact of coppicing in the MCA of forest soils [54], or comparing the effect of fertilization type (organic vs mineral) on soil physico-chemical and microbiological characteristics [87], as well as comparing the respiration profiles of soils sampled across Europe [81]. Lagomarsino et al.[88] reported a higher catabolic response in soil fine fractions and micro-aggregates compared to coarse fractions and macro-aggregates using the MicroResp™. Importantly, the relative increased functional diversity within the fine fraction pool was accompanied by an increased SOC content relatively to the coarse fraction.

Moreover, soil microbial diversity and activity are widely recognized to respond to pH variation [89–91]. Overall, in agricultural soils, increased pH, within specific range, may have a positive effect on soil MCA. When assessing the potential catabolic function of seven agricultural sites in Australia using the MicroResp™ method, Wakelin et al. [91] found that the soil pH (varying between 4.3 to 7.4) was the dominant driver of substrates mineralization with a

significant correlation (r =0.604, p =0.001). This general observation was also confirmed in several other studies assessing the impact of different soil management/amendment regimes on microbial catabolic activity. MicroResp™ data showed that the increase in soil pH (from 6.3 to 7.3) subsequent to cattle manure amendment, was associated with an increase in Shannon diversity index calculated, compared to the chemical fertilization [92]. Using the Multi-SIR approach, pH was found to be the main predictor (with positive effect) of the microbial functional evenness measured in soil sampled from five grassland sites [93]. Moreover, pH showed positive correlations with the Average Well Color Development (AWCD = $\sum$(C−R)/n, with C = Optical Density (OD) reading of the well, R=OD reading of the control well, and n=the number of substrates), the Shannon diversity and the substrate richness indices, calculated from a Biolog EcoPlate™ assay performed on 8 contrasting-pH soil types (pH ranging from 4 to 7.5) [84]. These studies agree about the positive effect of increasing pH on soil MCA when considering total catabolic diversity indices (mainly Shannon index, AWCD and Richness index). However, the influence of pH is less clear if substrates are considered individually or grouped by class/guild. Mineralization of galactose, gamma amino butyric acid and N acetyl glucosamine were negatively correlated with the pH, conversely to other substrates belonging to carboxylic acid class (citric acid, malic acid and α-Ketoglutaric acid) which showed a positive correlation based on a redundancy discriminant analysis [81]. Conversely, McDaniel and Grandy [94] found that the soil pH was positively correlated with N-containing and complexes substrates and negatively correlated with carboxylic acids. These different findings suggest that even though the influence of pH on MSIR is widely demonstrated, this influence depends on the nature of the substrates mineralized by soil microorganisms.

The effect of soil cation exchange capacity (CEC) on MSIR-based MCA is not frequently directly studied. Nevertheless, a positive correlation was found between the CEC and the soil respiration response to the addition of malic and citric acids [81]. This positive relation was further demonstrated by Bongiorno et al. [22] who reported a positive correlation between the total substrate induced respiration measured by MicroResp™ and the CEC of different soils sampled across different European long-term experiments. Moreover, in a study where soil physico-chemical and microbiological characteristics were investigated in soils with contrasting electric conductivity, it was shown that the CEC was correlated with the SOC content [95]. Importantly, this increased CEC was accompanied with an increased basal respiration suggesting a possible correlation between CEC and the MCA in these soils.

3.2. Fertility management techniques influencing the soil MSIR-based MCA

The different techniques described in section 2 have been used to evaluate MCA shifts in response to various agricultural practices (**Table 2**). As described in 3.1.2, the SOC content and pH are the two physico-chemical factors directly impacting soil MSIR [59, 81, 87, 91]. As such, the land management types and agricultural systems that influence soil pH and SOC content would consequently affect the soil MSIR-based MCA.

3.2.1. Soil amendment

Soil MSIR was reported to be sensitive to different types of agricultural land amendments. Application of different types of organic matter to soil (including pig manure, pig slurry, sewage sludge, and crop residues) resulted in an increase of soil functional diversity indices calculated using their substrates utilization rates [3, 20, 21, 96]. However, the effect of mineral fertilizers on MSIR is less evident although it has been shown to be linked to a lower taxonomic and functional bacterial diversity and species richness [97]. Indeed, even if soil NPK fertilization leads to an increase in the utilization rates of some substrates used in the MicroResp™ assays, the catabolic diversity index calculated from

data did not vary significantly between fertilized and control treatment [3]. Moreover, it was reported a decrease of the utilization rate with some substrates (amino acids, amines/amides) measured with the Biolog EcoPlate method, under N (Urea) fertilization compared to no fertilized control treatment [98], This finding suggests that the effect of this type of inorganic amendment could depends on the quality of the applied mineral fertilizer and the targeted specific catabolic activity. Therefore, future studies should clearly investigate this question specially by selecting different mineral fertilizer formulations and test relevant catabolic activities responses for agricultural soils. A recent study has tested the effect of inorganic amendment (NPK) alone or in combination with organic amendment (manure and straw) on soil microbial biomass and functioning using MicroResp™ [3]. Importantly, the authors concluded that combining organic and inorganic fertilization resulted in a higher soil metabolic activity and functional diversity than the inorganic treatment.

3.2.2. Agricultural system management

The management of agricultural systems has an impact on soil MCA. Several studies reported a negative effect of soil tillage on MCA, compared to no tillage [21, 22, 99, 100]. Indeed, tillage can decrease the soil microbial activity through the destruction of fungal mycelium and soil aggregates, which harbor active microbiota. In addition, there is probably the effect of tillage on soil aeration and water infiltration which could also affect the microbial respiration. The species of cover crop also impacted the soil MCA. For example, a higher soil catabolic activity, particularly the mineralization of complex amino acids and carboxylic acids has been reported with rye compared to oat [20]. Furthermore, an increase in MCA was noticed with the duration of *Rehmannia glutinosa* monoculture with a higher AWCD index in third and second year of monoculture compared to the newly planted plot [101]. The change in substrate utilization rate by stimulated microbial communities was attributed to the release of a large number of low molecular weight root exudates such as sugars, carboxylic acids and amino acids [102]. Rotation of *Medicago sativa* L., with *Elsholtzia splendens* and *Sedum plumbizincicola* was reported to increase soil AWCD index based on substrate utilization rate compared to *Medicago sativa* L. monoculture, with the highest MCA recorded when alternating the three species [103]. This finding could be related to the selection of rhizosphere microbiota by root exudates. Similar results were previously described in sugarcane cultivation system, where rotations with pasture species (either forage peanut: *Arachis pintoi* Krapov. & W. C. Gregory, *Brachiaria decumbens* Stapf, or *Digitaria decumbens* Stent) demonstrated a higher substrate utilization rate and AWCD index compared to sugarcane monoculture [104].

Table 2: Non-exhaustive list of fertility management methods influencing soil MCA. AWCD means average well color development =∑(C−R)/n, with C = Optical Density (OD) reading of the well, R=OD reading of the control well, and n=the number of substrates

Factors	Treatments	Techniques	Main results	Variation in soil MCA (%)	References
Soil amendment	Organic amendment (cattle manure, Pig manure and Pig slurry) vs conventional amendment (calcium-ammonium-nitrate fertilizer, mineral fertilization and pig slurry)	MicroResp™	Increased substrates utilization rate in soil from organic amendment plots treatment compared to soil from conventional treatments	Up to +20 % increase of substrate utilization rate	[20, 22, 92]
	Dairy sewage sludge	Biolog EcoPlate™	Increased AWCD, Shannon and richness indices in amended compared to non-amended and chemical fertilized treatments	+9 % for Shannon index; +47 % for AWCD	[96]
	Crop residues and quality	Biolog EcoPlate™	Increased AWCD with retention of residues compared to control treatment.	Up to +57 % increase of AWCD index	[21]
	Mineral vs organic amendments	MicroResp™	Separation between organic and mineral treatments in the PCA performed with substates induced respiration rate data		[50, 87]
	Inoculation with rhizobacteria (*Pantoe sp, Rhizobium sp)*	Real time PCR and Hydrocarbon concentration measurement	Increased abundance and expression of alkane hydroxylase genes (*CYP153* and *Alk-H*), and decreased hydrocarbon content in the rhizosphere of *Lolium multiflorum* inoculated *with Pantoea sp,* compared to those of no-inoculated treatments	Up to -84 % decrease of hydrocarbon content. Up to +530 % increase of *Alk-H* abundance	[61, 62]
	N fertilization	Biolog EcoPlate™	Decreased utilization rate of specific substrates (amino acids and amines/amides, Tween 40, Tween 80) in N fertilized treatment compared to control	Up to -60 % decrease of substrate utilization rate	[98]
	NPK fertilization	Real time PCR and Hydrocarbon concentration measurement	Increased abundance and expression of alkane hydroxylase genes (*CYP153*), and higher hydrocarbon degradation in fertilized treatment compared to control	+123 % increase of gene abundance, +215 % of gene expression and -61 % decrease of soil hydrocarbon content	[105]

Agricultural system management	No tillage + maize-soybean rotation	Biolog EcoPlate™	Increased AWCD diversity index with no tillage. Greater AWCD recorded when combining no tillage and maize-soybean rotation	Up to +150 % increase of AWCD index in no-tilled soils compared to tilled ones	[100]
	Conventional tillage vs direct drilling	Biolog™	Increased richness and Shannon diversity index in conventional tillage compared to direct drilling plot.	Up to +68 % and + 6 % increase of Richness and Shannon diversity indices	[99]
	Reduced tillage vs conventional tillage	MicroResp™	Increased Shannon diversity index in reduced tillage compared to conventional tillage	6 % increase of Shannon diversity index	[22]
	Cover Crops type or quality (Rye vs Oat)	MicroResp™	Higher substrates-induced respiration rates in plot where rye is used as cover crop compared to Oat for specific substrates (L-Arginine, Alanine, Oxalic acid and Lignin)	Up to +20 % increase of utilization rate	[20]
	Crop rotation: Sugarcane monoculture vs rotation with pastures species; *Medicago sativa* monoculture vs rotation with *Elsholtzia splendens* and *Sedum plumbizincicola.*	Biolog™ /Biolog EcoPlate™	Higher substrates-induced respiration and AWCD index in crop rotation plots compared to monocultures	Up to +70 % increase of AWCD index in sugarcane rotations. Up to +7% increase of Shannon index in *Medicago sativa* rotation	[103, 104]
	Rehmannia glutinosa monoculture duration (Two- and three-years monocultures plots vs control newly planted plot)	Biolog EcoPlate™	Increased AWCD index over growing seasons of monoculture.	Up to +66% and +133% increase of AWCD index in 2- and 3-years monocultures compared to newly planted plot	[101]

3.3. Specificities of MSIR-based MCA as an indicator of soil microbial functioning

We have described how different MSIR methods are used to assess soil microbial functioning in response to different soil amendments and various agricultural systems. The **Table 2** highlight that these MCA measurement allowed the detection of changes in soil catabolic activity/functional diversity and to discriminate between different situations or treatments such as tillage vs non tillage [21, 22, 99, 100], organic amendment vs chemical fertilization [20, 22, 50, 87, 96], monoculture vs crops rotation [103, 104]. Therefore, the ability of MSIR assays to distinguish these different soil agroecosystems and soil managements makes it a good candidate as an indicator of soil microbiological status, especially since MSIR-based MCA methods present some specificities compared to approaches like enzymatic activities. Enzymatic activities measurements are widely used to characterize soil functional status [13]. Apart from fluorescein diacetate (FDA) hydrolysis and dehydrogenase activities, which can give an estimate of the total soil microbial activity, most enzymatic activity assays which are used to assess soil quality are linked to the dynamic of specific enzymes often linked to agronomical or environmental interests. For example, beta-glucosidase, amylase and xylanase are indicators of C metabolism, urease, N-acetylglucosaminidase and leucine aminopeptidase are indicators of N cycling, phosphatases and arylsulfatase are indicators of P and S metabolism, respectively. In contrast, MSIR assays provide appropriate information about the activity of more diverse microbial processes, through CO_2 evolution measurement. Furthermore, MSIR based techniques provide functional information related to the complete mineralization of SOM including simple and complex organic substances, leading to the release of CO_2 whereas measurements of enzyme activities are often related to an incomplete decomposition of the SOM, particularly the decomposition of complex organic substances [54, 59]. In addition, enzymatic methods measure activities of both living microbial cells and soil-immobilized enzymes. The latter could therefore report legacy microbial activity providing from dead microbial cell stabilized in the soil environments [13, 53, 106] whereas activity measured by MCA seems to be more accurately estimate microbial function at real-time as the CO_2 efflux excludes theatrically non-living cells. This suggests that MSIR techniques could be of particular interest for an accurate assessment of soil microbial functioning in short/mean time scale context such as for plant rhizosphere and/or microbial inoculation effect, as in addition, root exudation is characterized by a high content of simple organic substrates (sugars, amino acids, organic acids, carboxylic acids, etc.) [48, 107, 108], which are similar to that used in MSIR assays.

4. Using MSIR-based MCA to improve microbial inoculants to sustain crop production

4.1. Influence of root exudation on the MSIR in the plant rhizosphere

The plant rhizosphere hosts an active microbial community. A higher microbial activity was largely reported in the rhizosphere compared to bulk or unplanted soils [109–111]. This rhizosphere microbial activity has been revealed using different MSIR measurement approaches including Microresp™, Biolog EcoPlate™ and Biolog™ which indicate a higher carbon consumption rate compared to the bulk soil. Using Biolog™ plate, Söderberg et al. [112] reported significant differences in the AWCD index between the rhizosphere and bulk soils of pea plants under controlled conditions. Concordantly, a higher substrate utilization rate was found in the rhizosphere compared to bulk soil in different plant species (*Triticum aestivum*, *Brassica juncea, Solanum tuberosum, Festuca rubra, Centaurea jacea*) using MicroResp™ and Biolog EcoPlate™ techniques for the determination of the soil CCLP profiles [113, 114]. Moreover, the rhizosphere effect on soil MCA is plant species-dependent as the substrates induced respiration were higher in the rhizosphere of *Plantago lanceolata* and *Rumex acetosa* than in those of *Leucanthemum vulgare*

and *Centaurea jacea* [113]. In addition, the respiration rates induced by carbohydrates and carboxylic acids were higher in rhizosphere soil of wheat intercropped with faba bean compared to that of wheat monoculture [115]. Several mechanisms could explain the variations of MCA between these two soil niches (rhizosphere and bulk) and across plant species. First, it this may be related to root exudates which vary quantitatively and/or qualitatively according to plant species, plant genotype and plant developmental stage [116–119]. Indeed, root exudation is linked to increased C resources availability in the vicinity of plant root, as compared to the bulk soil. Therefore, this could make the microbial community in the rhizosphere more physiologically active than that of bulk soil in which C resources are less abundant. Moreover, the higher microbial biomass (MBC) in the rhizosphere compared to bulk soil [120, 121] could partially explain the higher MCA in the rhizosphere. Indeed, we can advocate that a global increase of MBC would result in broadening the size of the microbial populations involved in different mineralization processes, even if we recognize that some species could be suppressed by root exudates through counter-selection mechanisms. Consistent with this hypothesis, Shannon diversity and richness indices based on MSIR data and MBC increased simultaneously when passing from pasture to agroforestry soils [122]. Mureva and Ward, (2017) reported that both MBC and the AWCD index derived from Biolog EcoPlate data were increased in grassland soils compared to soils sampled from adjacent shrub-encroached plots. In addition, Song et al. [3] reported that soil microbial biomass is the key predicting factor of soil microbial catabolic activity and functional diversity . There is also a probability of a higher relative adaptation of the rhizosphere community to metabolize the substrates frequently used in MSIR assays, as plant root exudates may include similar molecules such as carbohydrates, amino acids, organic acids[107, 124] which should be taken into account when comparing between soil niches. Furthermore, this exudation-driven effect on soil MCA can be explained by its selection effect on the soil microbial community that could lead to a differential mineralization capacity between rhizosphere and bulk soils. For example, due to the higher availability of low molecular weight molecules, which are the preferential carbon source of heterotrophic bacteria, the rhizosphere is enriched in gram-negative bacteria (which are preferentially copiotroph) compared to bulk soil that is more taxonomically diversified, including oligotrophs that are not competitive in the rhizosphere [112, 125–127]. These two groups of bacteria with opposite preferential ecological strategies (*r*-strategy for copiotroph *i.e.* fast reproduction rate vs *K*-strategy for oligotroph *i.e.* low reproduction rate) could have variable substrates consumption profiles. Accordingly, this variation in catabolic potentiality between these two different niches has been evidenced in a recent meta-analysis with rhizosphere having higher potential in organic compound conversion (cellulolysis, xylanolysis, chitinolysis, ureolysis, etc.), denitrification and methanol oxidation than bulk soil [128].

4.2. Suitability of MCA to assess plant inoculant efficiency

Soil microbiota drives several ecological processes including organic matter (OM) mineralization and nutrient cycling. A high microbial functional diversity is key element to ensure delivery of ecosystem services, particularly in nutrient poor soils with low OM content. Indeed, when resources are scarce, a high soil catabolic diversity enables the utilization of a wide range of carbon sources (including complex/recalcitrant organic matters), consequently allowing nutrient recycling for the benefit of both plants and soil microorganisms. A recent study also demonstrated that plants grown under low nutrient conditions selected for a higher proportion of PGP bacteria than equivalent plants fertilized with NPK [129]. Therefore, we could consider designing microbial inoculum to boost functional capacity of soils to support plant growth. Indeed, a recent study has reported significant differences in the consumption capacity of different substrates among different *Rhizobium* strains isolated from lentil nodules [130]. It is possible that a synthetic

community of strains with complementary PGP effect (including enhanced catabolic activity) would be more efficacious and resistant than those with similar PGP properties but exhibiting fewer catabolic capabilities, particularly when they are inoculated into soils with low organic matter content. When isolating PGP bacteria/fungi, it could be therefore interesting to consider their catabolic capabilities with respect to different carbon source classes available in a given soil, in addition to the commonly tested PGP effects. From a theoretical standpoint, MCA profiling of bacterial/fungal strains could help in designing consortia (combination of strains or species) for a better efficiency of inoculants. Indeed, the advantages of inoculating soil or plant with a consortium of bacteria/fungi compared to single strain inoculation for plant growth improvement are well documented [131–135]. These advantages are related to the multitude of mechanisms developed by PGPM, producing multiple positive effects (biological N-fixation, nutrient solubilization, disease suppression, phytohormone production, etc.), which can be synergistic in some cases. However, even if the inoculation by consortia could present several benefits, consortium formulation is more challenging compared to that of a single strain inoculum. The compatibility and the interaction of the different microbial strains/species in the inoculum must be tested to ensure their survivability and their efficiency. Then, MCA profiling (in regard to carbohydrates, carboxylates, amino acids, soil complex molecules, enzymatic activities…) of the candidate strains should be performed to help selecting bacterial candidates with contrasted C source preferences/catabolic capabilities to avoid inhibition that might be caused by competition for resources within the inoculum or in the targeted environment [136–138]. The advantage of this strategy could be particularly relevant for the inoculation of soils with low level of nutrients and poor organic matter. Such a strategy recently tested by Kumar et al. (2021) has the potential to be widely used as a key step during the protocol of inoculum composition and assessment. A practical diagram explaining how microbial MCA could be used to improve the formulation of the microbial mixture in a consortium is proposed in **Fig. 1.**

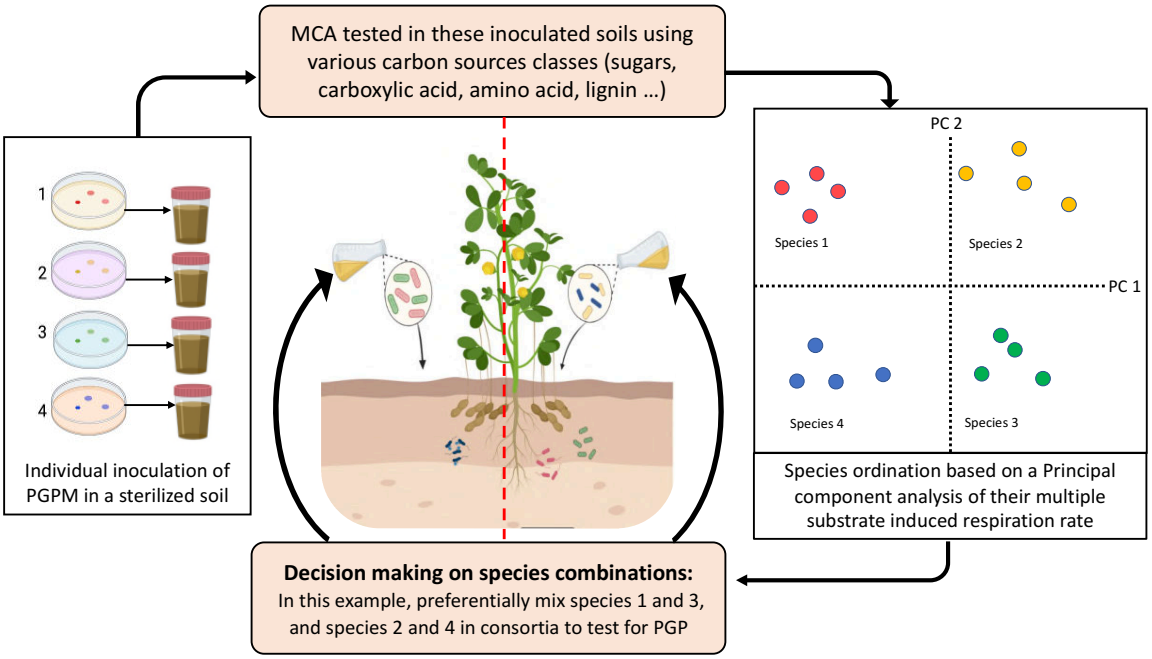


Figure 1*: Conceptual idea explaining how MCA profiling of microbial strains could help in the formulation on efficient consortia with can improve plant growth. In this example, we assume that microbial species 1 and 2 are P solubilizing*

bacteria, and Specie 3 and 4 are N-fixing bacteria in addition common other PGP traits (for examples AIA production ACC deaminase activity etc). Therefore, combining species 1 and Species 3 could enable to have a consortium with P solubilizing and N-fixing capacity along with other PGP trait, but importantly these two strains have more could be more compatible in a mixture thanks to their opposite profile in terms of substrate consumption preferences and then have more chance to against nutrient stresses they may face in the environment.

4.3. Utility of MCA as a tool to evaluate the effect of PGPM in soil functioning

The effects of introducing plant growth promoting rhizobacteria may extend to ecosystem functions beyond the host plant growth improvement [139]. Effects include the modification of resident microbiome structure and functioning, the alteration of plant root exudation, which can influence soil aggregation and SOM dynamic [140], the modification of pH, nutrient status, erodibility, and water holding capacity of soil. Particularly, the potential legacy effects of the introduced microbes on the diversity and functioning of the native communities and consequently on the ecosystem services in which they contribute in the mid- and long-term is of great interest. A meta-analysis performed by Mawarda et al. [141] reported that 86% of the 108 studies reviewed concluded a significant effect of microbial inoculation on the diversity/composition of the resident soil microbial communities. Furthermore, several studies reported a positive influence of microbial inoculation on soil enzymatic activities. For example, inoculation with bacteria increased the rhizosphere activities of several enzymes such as catalase [26, 142], urease, invertase, acid and alkaline phosphatase [26, 143, 144]. Similarly, fungal inoculation increased soil dehydrogenase, invertase, catalase, urease, protease, phosphatase, beta-glucosidase activities [145–147]. These increased enzymatic activities following fungal inoculation might be related to the mycorrhizal hyphae development, which increased the SOC and nutrients content (total N, NO_3^--N and available P etc.), and hence affecting positively microbial activities in inoculated soils [147]. However, few studies have investigated the effect of PGPM inoculations on soil indicators related to complete organic matter mineralization such as catabolic diversity or basal respiration [145, 148]. Such assessments could complement the enzymatic assays as they may target different catabolic processes. For instance, soil inoculation with an arbuscular mycorrhizal fungi (*Glomus intraradices*) impacted differently the utilization rate of substrates 12 months later (increase for ketoglutaric acid, and decrease for phenylalanine, citrate, glutamine, ascorbic acid, formic acid, and cyclohexane) [149]. Moreover, inoculation of the same AMF in a sterilized soil stimulated the growth (shoot, root, total biomass and root/shoot ratio) of sorghum plant and increased soil catabolic diversity (catabolic evenness) whereas in non-sterilized soil, the growth promoting effect due to inoculation was less apparent with a decreased soil catabolic diversity [150]. In addition, the authors report a negative correlation between catabolic diversity indices (richness and evenness) and the density of the fungal inoculum suggesting a potential perturbation caused by mycorrhizal colonization on the functioning of soil native microbial community. Catabolic diversity (AWCD and Shannon indices) was reported to be less sensitive than enzymatic activities in monitoring changes caused by faba bean (*Vicia faba)* inoculation with rhizobia (*Rhizobium leguminosarum bv. viciae*) on soil functioning in a field study [36]. Then, this discrepancies between the two approaches makes necessary the monitoring of soil functions after microbial inoculation using MSIR measurement, in addition to enzymatic activity assays in different time scales. Particularly, the use of MSIR-based MCA methods could allow a higher accuracy and provide additional information for evaluating the influence of microbial inoculation on C dynamics and balance in soil agro-systems, which are important parameters to consider for the mitigation of climate change impact.

5. Using molecular and isotopic approaches to better characterize soil MCA

As mentioned above, the MCA measurement offers several benefits and advantages to assess soil functional capabilities in different soil managements and beyond, in different agro-environmental contexts. Nevertheless, they present some limitations because of the complexity of soil (e.g. composition: organic vs mineral vs microbial, and properties: pH, CO_2 retention potential in liquid etc.) which can lead to overestimate or underestimate the microbial activity, particularly when derived from respiration rates. Moreover, considering the various DNA/RNA based approaches targeting variable catabolic processes and variable genes that can be functionally redundant or not inclusive, it seems necessary to go toward a more standardized methods to enable reproducibility and comparability of results from different studies. Therefore, we propose here some strategies which can be developed to improve MCA measurement by combining molecular and isotopic tools (transcriptomics, qPCR and DNA-SIP) widely used in microbial ecology with MSIR approaches. This may improve the accuracy and the efficiency of MCA measurement in soil or other microbial matrices and allow a better estimation of their functional diversity.

5.1. Potential of MSIR, transcriptomics and q-PCR combination for measuring soil MCA

The development of molecular tools such as metagenomics enables researchers to study the diversity of genes associated with metabolic pathways and to predict soil functions [151–154]. Multi-SIR techniques such as MicroResp™, use mainly sugars (e.g. fructose, galactose, glucose, sucrose, starch…), carboxylic acids (e.g. salicylic acid, malic acid, acetic acid, citric acid, alpha-ketoglutaric acid, gamma-aminobutyric acid, oxalic acid…), amino acids (e.g. aspartic acid, phenylalanine, arginine, glycine, lysine, valine, leucine, alanine …) and the amino sugar (*N*-Acetylglucosamine) as substrates [14, 50, 155]. In another hand, available resources such as the carbohydrate-active enzymes database (CAZy: http://www.cazy.org) [156, 157] and The MEROPS database (http://www.merops.ac.uk) [158] offer the possibility to find enzymes for degradation of sugars and carboxylic acids in samples. Then, the sequences of their coding genes could be found using the NCBI *tblastn* tool and the *Kyoto Encyclopedia of Genes and Genomes* (KEGG). This substrate degrading genes database should be complemented by a representative set of recalcitrant substrates degrading genes (cellulose, hemicelluloses, lignin, polyphenols etc.) to be ecologically relevant for soil MCA assessment. Therefore, meta-transcriptomics could allow to measure the expression of genes controlling microbial enzymes related to selected catabolic processes and can be exploited to predict the substrate utilization profile of soils in a semi-quantitative approach. Furthermore, RT q-PCR could be applied to estimate the expression of these genes to measure more accurately soils mineralization potential toward these substrates **(Fig. 2).** A comparable strategy has been used by [70] who developed a high-throughput quantitative-PCR-based chip named QMEC (for Quantitative Microbial Element Cycling) which contained 72 primers pairs assessing functional genes involved in C, N, P, S cycling and methane metabolism. For that, mRNA isolated from the total soil RNA extracted will be transform to cDNA, hybridized, sequenced and mapped against the catabolic gene database. This will enable to determine the expression profile of the soil samples regarding their microbial catabolic activities **(Fig 2).** These approaches could have advantages compared to the current MSIR methods such as the possibility to increase the number of substrates and the possibility to consider recalcitrant substrates that are often difficult to tested in MSIR-based method because of their low water solubilities, their high throughput potential, but they could also help overcome biases related to the microbial cultivation and non-respiratory/mineral CO_2 release for respectively Biolog™ and basic MicroResp™ methods.

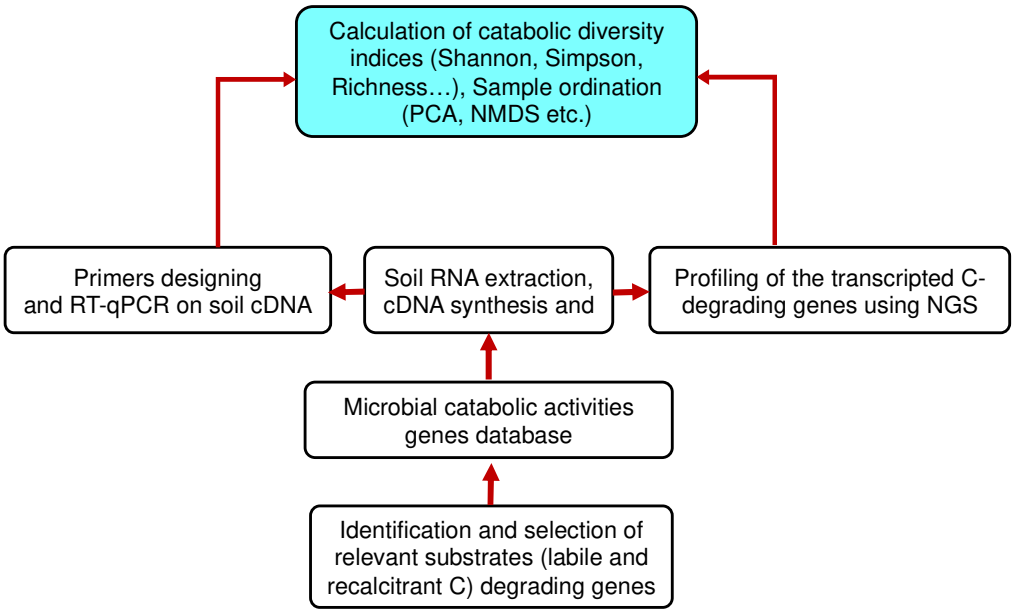


Figure 2: *Graphical workflow describing the different steps for using molecular methods (q-PCR, NGS) to gain accuracy in soil catabolic diversity assessment. The method would be based on exploitation mRNA extracted from soil to derive the expression of genes responsible of catabolic processes. The targeted degradation processes will be selected on the basis of the substrates commonly used in classical MSIR techniques (MicroResp™, Biolog ™) and those that are ecologically relevant and naturally present in soils*

5.2. Potential of DNA-SIP and SIR combination for soil MCA measurement

The most widely used MCA measurement techniques are based on the direct or indirect measurement of the amount of CO_2 released by soil microorganisms when amended with different carbon sources (MSIR). Interestingly, substrate consumption by soil microorganisms leads also to an immobilization of a proportion of substrate-derived C into the microbial biomass, which depends on their carbon use efficiency (CUE). Therefore, MCA intensity could be estimated through the amount of substrate-derived C immobilized in the whole microbial biomass. This strategy could be achieved by the combination of ^{13}C-labeling of the substrates, DNA stable isotope probing technology (DNA-SIP) and q-PCR **(Fig. 3)**. Indeed, substrate ^{13}C-labeling has allowed to trace the incorporation of different organic substrate C within the soil microbial diversity biomass. Several labeled substrates have been used including simple molecules (glucose, methanol) [159–161], root exudates [162, 163] and cellulose [164, 165]. These approaches were able to identify the main bacterial or fungal taxa incorporating the labeled substrates after separation of the ^{13}C DNA extracted from whole soil DNA, using a density-gradient centrifugation [166] and 16S gene or 18S rRNA gene sequencing. Similarly, the, the incubation of soil with selected ^{13}C-labeled substrates, followed by DNA extraction and fractionation combined with subsequent q-PCR of the microbial communities (using 16S and 18S r-RNA genes) could give an estimation of the proportion of the microbial biomass derived from specific substrates and then the multi-substrate utilization profile (MCA) in different soils. This strategy may overcome the limitations of CO_2 but also could provide additional information on the CUE if coupled with $^{13}CO_2$ respiration measurement.

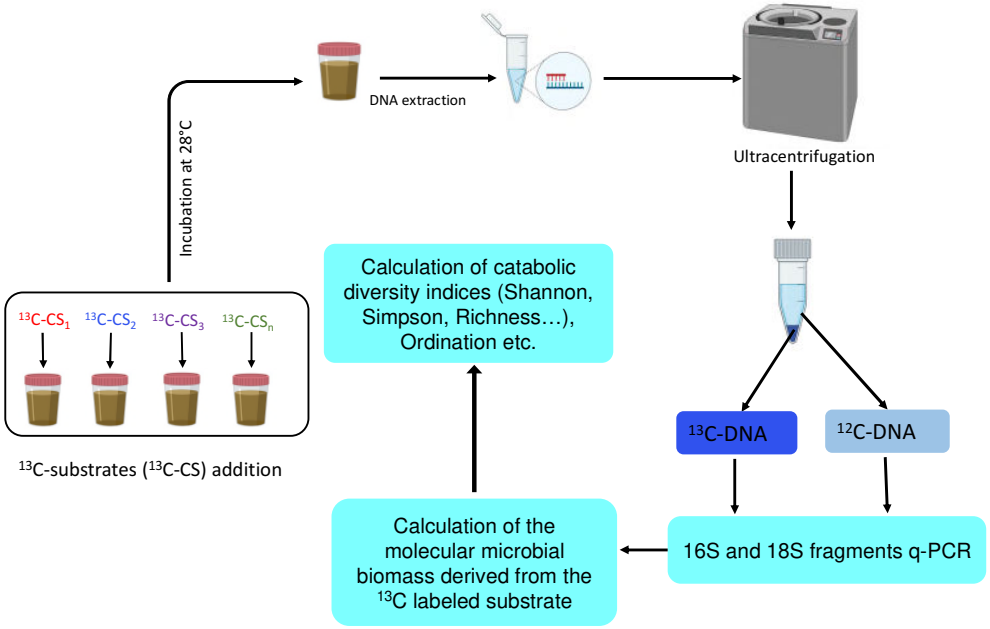


Figure 3: *Descriptive diagram of the combination of ^{13}C -substrate labeling and MCA principle to characterize soil microbial catabolic activity. The method would be based on the measurement of the ^{13}C labeled DNA amount after soil amendment and incubation with ^{13}C labeled substrate. The quantity of specific substrate-derived DNA will give an estimation of the assimilation capability and then the catabolic potential of the microbial community toward the corresponding substrate.*

6. Concluding remarks and perspectives

This review highlights the utility of soil MCA measured using MSIR methods as a relevant indicator of soil functions given that it responds to different types of fertility management like organic amendments, mineral fertilizations, crop rotation etc. Advantages and specificities of MSIR-based MCA methods and enzymatic activities assays which are the two main approaches used to assess soil functions were discussed. Moreover, molecular techniques relying on the study of genes involved in specific catabolic pathway are emerging and could potentially be more investigated. This suggests that the potential utilization rate of selected substrates (used in MicroResp™ or Biolog, which are the more used MSIR techniques) and other complex and ecologically relevant substrates present in soils, could be derived from soil microbial nucleic acids through the combination of the MSIR with either molecular approaches or substrate ^{13}C-labeling and DNA-SIP to assess soil MCA more accurately.

Within the nutrient supply ecosystem function of soils, considering MCA could likely enable to improve the efficiency of microbial bio-stimulants during formulation and inoculum assays. For example, formulation of microbial inoculant consortium should consider catabolic diversity of microbial strains which may help to improve their persistence/competitiveness in the soil and then their efficiency through a better adaptation for C resources exploitation. Moreover, PGPR inoculation effect on soil physico-chemical and biological parameters should be assessed prior to any commercialization. Indeed, even if effects of soil/plant microbial inoculation on microbial community structure and composition is now demonstrated [141], the potential effects on SOM persistence remain poorly understood, particularly in the long term as well as if there is a resilience in ecosystem services which can be impacted [139]. Future

studies should address these questions and MCA seem to be a relevant indicator to be take into account given that it is directly linked to soil C and nutrient dynamics.

Furthermore, root C exudation increased soil MCA in the rhizosphere compared to bulk soil and studies aiming to determine the effect of inoculation on soil microbial diversity/activities, focused on rhizosphere community by sampling soil around the root (see meta-analysis of Mawarda et al. [141]). Thereby, the specific effect of the inoculated microbes on soil activity and particularly on soil microbial catabolic activities could be mixed with those related to the potential indirect effect caused by the modification of plant production and physiology (PGP effect) and their repercussion on root exudation rate. Therefore, future studies should determine the direct effect of inoculated microbes on soil microbial diversity and MCA and distinguish it from such indirect effect.

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Papa Mamadou Sitor NDOUR, Adnane BARGAZ, and Karim LYAMLOULI conceptualized the paper, Papa Mamadou Sitor NDOUR and Adnane BARGAZ wrote the original draft. All authors contributed to the editing, review and validation of the manuscript.

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