A Short Review about Using MicroResp Method for the Assessment of Community Level Physiological Profile in Agricultural Soils

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Abstract

MicroResp is a colorimetric method developed by Campbell *et al.,* (2003), used for assessing the community level physiological profile of the microbial population. MicroResp can be used to assess soil health, pollution induced community tolerance, also for toxicity testing, pesticide degradation profiles, bioremediation evaluation and water ecology and toxicity. The aim of the present review was to look over the results of recent papers and to highlight the importance and efficiency of the MicroResp method in assessing the physiological profile of the microbial community. The method advantages and limitations were also assessed. We focused on agricultural soil in order to deepen our understanding about changes of microbial community induced by agricultural practices. To achieve this goal, academic literature was analyzed using an academic database. There were set a total of six keywords, used to make a search algorithm, achieving five search terms. For each search, the first four articles of interest were chosen to be reviewed. After the searches for each of the terms, between 72 and 210 articles were found, 20 of them being chosen for final evaluation. Following the undertaken research, it can be stated that MicroResp method is an important tool to assess the physiological profile of the microbial community, featuring a series of advantages that place it ahead of other competing methods.

Keywords: *Community level physiological profile, MicroResp, Soil, Functional diversity.*

Introduction

Soil is an important resource which supports a large number of ecosystem goods and services from which humankind benefits (Laishram *et al.*, 2012). Today soil face a large range of threats from human activities and global change like erosion, sealing, salinization, compaction, pollution with heavy metals, hydrocarbons and xenobiotics, loss of organic matter, atmospheric pollution, acidification and climate change (Chapman *et al.*, 2007; Sassi *et al.,* 2012).

In soil the microbial community plays an important role in decomposition and stabilization of organic matter as well in immobilization and mineralization of nutrients and presents a great diversity. It can also intervene in plant growth and nutrient uptake by releasing certain substances which can stimulate or inhibit, influencing root physiology and architecture (Bérard *et al.*, 2012; Vivian *et al.*, 2013). There is little knowledge about the composition and diversity of microbial communities and how the microbiota can affect the fertility and productivity of the soil (Grayston *et al*., 2001). However, the microbial community composition depends primarily on the land-use, pH and soil organic matter (Creamer *et al*., 2016), plant species (Gartzia Bengoetxe *et al.,* 2016) and also environment factors such as water regime can affect microbial activity (Pailler *et al*., 2014; Sandor *et al*., 2011).

One of the parameters used to assess the impact of land use in soil microbial community size, activity, composition and diversity is the soil microbial respiration, which can be used as indicator of soil fertility, which if drops indicates a decrease in soil quality and health (Burton *et al.*, 2010).

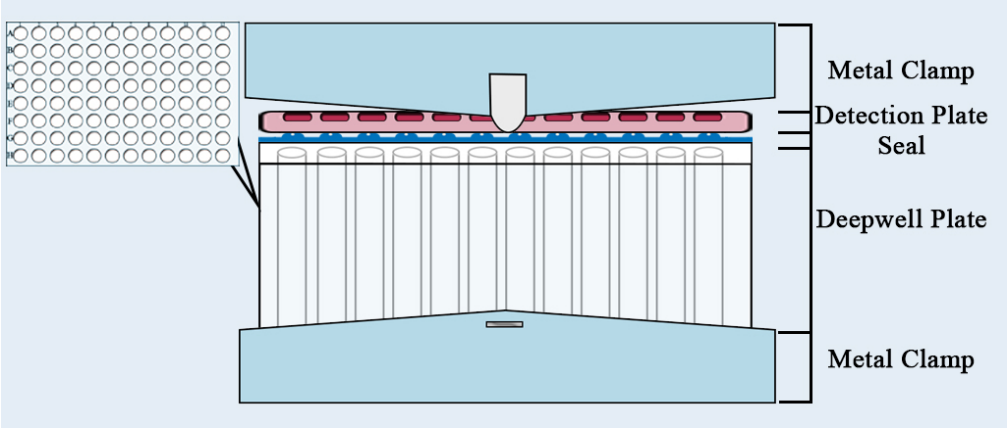

Figure 1. MicroResp system components

Table 1. Carbon sources used in MicroResp

Carbon sources				
Carbohydrates	**Carboxylic acids**	**Amino acids**	**Amino sugar**	**Phenolic acid**
D-trehalose	Citric Acid	L-arginine	N-acetylglucosamine	Protocatechuic acid
D-galactose	L-malic acid	γ-aminobutyric acid		
L-arabinose	Oxalic Acid	L-lysine		
D-glucose	α- ketoglutaric acid	L-alanine		
D-fructose		L-cysteine		

Source: (Campbell *et al.*, 2003)

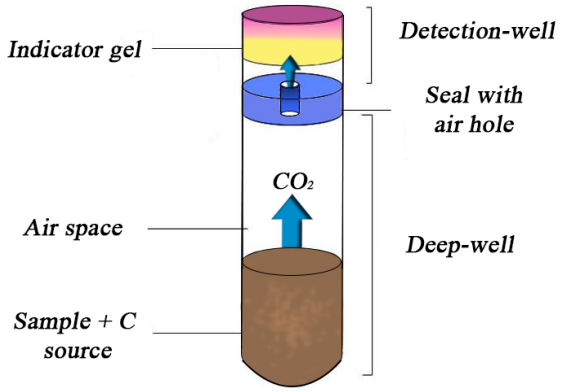

Figure 2. Deep-well and detection plate schematic working principle
Source: (Campbell *et al.*, 2003)

To assess soil respiration and community level physiological profile, MicroResp is a suitable method. MicroResp is a colorimetric whole soil method, based on 96-well microtitre plate and 16 carbon substrates which are used by the microbial community from the soil (Chapman *et al.*, 2007).

The main objectives of this study were to assess the results of reviewed papers and the importance and efficiency of the MicroResp method in determining the physiological profile of the microbial community, its advantages and also its limitations.

MicroResp Technique

Soil microbial community and activity monitoring can be a powerful tool for understanding applied ecological context (Frąc *et al.*, 2012). To assess the health and activity a basic parameter is the carbon dioxide level, which is released by the soil microbial community during

Table 2. Search algorithm and results

Number	Keywords	**Total Results**	**Results of interest**
1	„Community level physiological profile“ AND Microresp AND Soil	89	4
2	Microresp AND Soil AND Microbiota	72	4
3	Microresp AND Soil And „Functional diversity“	210	4
4	Microresp AND Soil AND Fertilizer	150	4
5	Microresp AND Soil AND Wheat	136	4

Table 3. Objectives and results of assessed studies about fertilizers and CLPP

No.	**Objectives**	**Results**	**Source**
1	Effect of cattle manure with and without biodynamic preparation in comparison with mineral fertilization (+ straw incorporation) on functional diversity of the soil microbial community.	Carbon source catabolic profile was clearly different between cattle manure treatments and those with mineral treatments, the discrimination was mainly based on amino acids and neutral sugars.	Sradnick *et al.,* 2013
2	Assess the genetic composition of fungal community by molecular tools and the functional diversity of soil microbial community by MicroResp in poplar plantations grown under elevated CO_2 with or without fertilization.	The microbial catabolic activity was directly influenced by the soil nutrient availability and plant inputs, and the poplar species significantly affected C utilization rates. They found higher utilization rates of C compounds in soils without N limitation, and under elevated [CO2], there was a decrease of microbial catabolic activity in N limited soils whereas in fertilized soils the substrate utilization was enhanced, in particular in *P. alba* and *P. nigra.*	Lagomarsino *et al.*, 2007

Table 4. Objectives and results of assessed studies about pollution and CLPP

No.	**Objectives**	**Results**	**Source**
1	Development and evaluation of a MicroResp-based method which can be used as an ecotoxicological assay to measure the impact of metals, namely copper (Cu), on soil function.	The MicroResp assay can be simply modified to assess the ecotoxicology of soil contaminants, such as metals, on soil catabolic function. The modified process includes experimentally determined soil moisture assessment for optimum microbial activity. Contaminants are mixed into soil samples at a range of concentrations, each sample is then dispensed into a column of eight wells in 96 well format (deep) plates. Moisture and glucose are added to the samples at levels to provide maximum response, and released CO_2 is measured.	Wakelin *et al.*, 2013
2	Assess SIR, CLPP and PICT using the MicroResp method to characterize microbial communities after exposure to environmental concentrations of metals in a long-term field study.	MicroResp can be used in PICT-bioassays to assess heavy metal (Cd) impact to soil microbial communities. Dose response curves for soil Cd and soil microbial glucose mineralization were obtained on microrespirometric ecotoxicological bioassays with Cd, making it possible to calculate half maximal effective concentration (EC_{50}). EC_{50} values were positively correlated with Cd concentrations in soil plots.	Bérard *et al.*, 2014
3	Developed a protocol based on the substrate-induced respiration of a river biofilm community, using the Micro-Resp technique, in a pollution-induced community tolerance approach	The results show that MicroResp can be used in bioassays to assess the toxicity toward biofilm communities of a wide range of metals (Cu, Zn, Cd, Ag, Ni, Fe, Co, Al and As). Moreover, a community-level physiological profile based on the mineralization of different carbon substrates was established.	Tlili *et al.*, 2011

decomposition of carbon substrates (Campbell *et al.*, 2003).

A method which can be used to assess the soil microbial community is MicroResp which is a colorimetric technique that allows the measurement of CO_2 from whole soil resulting from the utilization of different carbon sources by the microbial population (Sassi *et al.*, 2012). However, MicroResp consists of two microtiter plates placed face to face, one is the deep-well plate and the other is the detection plate (Campbell *et al.*, 2003). In the deep-well plate soil samples and carbon

Table 5. Objectives and results of assessed studies about crops and CLPP

No.	Objectives	Results	Source
1	Assess the effects on soil labile organic C and N pools and microbial metabolic profiles and diversity in temperate climate with treatments of winter cover crops.	Growth of winter crops increased soil labile organic matter and microbial metabolic diversity compared with the control. There are no differences in labile organic matter between the legume crops and non-legume crops. Legume crops had lower SIR and higher H than non-legume crops. The microbial metabolic profiles are different under crop treatments against those of control. Moisture, pH, crop biomass and extractable organic N were the most important factors in determining pattern of the microbial metabolic profiles.	Zhou *et al.*, 2012
2	This study used soil microcosms to investigate the impact of water status (saturated and non saturated) and straw application (10 g kg^{-1} soil) on soil microbial composition and activity (MicroResp™ method).	Results suggest that (1) the size of microbial communities in paddy soil is more limited by carbon substrate availability rather than by the anaerobic conditions due to waterlogging and (2) that soil water status is more important as a control of fungal growth and microbial community activity.	Pan *et al.*, 2015

Table 6. Objectives and results of assessed studies about forest soils and CLPP

No.	Objectives	Results	Source
1	Assess the changes of microbial functional diversity and other soil chemical and biochemical properties following forest coppicing.	Enzyme activities and CLPP increased in coppiced plots indicating higher decomposition processes promoted by plant debris and rhizodepositions released after cutting.	Pignataro *et al.*, 2012
2	1. compare the ability of the Degens and Harris approach and the MicroResp™ approach to distinguish between soils from previously mined (rehabilitated) and non-mined Western Australian forest ecosystems. 2. assess the similarity in CLPP profiles between 3- and 16-year-old forest rehabilitation (mound and furrow) and adjacent non-mined forest soil.	Results suggest that the aspect of microbial heterotrophic function measured in this study takes up to 3 years to re-establish in the furrows and between 3–16 years in the mounds of post-mined rehabilitation soils. Results also indicated that the MicroResp™ was substantially better than the Degens and Harris approach in distinguishing between treatments, this is likely to be due to differences in substrate concentrations and soil water potentials between approaches.	Lalor *et al.*, 2007
3	Explore the effects of repeated burning on the broader soil microbial community and determine if the changes observed were also associated with changes in functional diversity.	Microbial biomass in the 2 year burn treatments was 50% less than both the control and 4-year burn treatments. There was also concomitantly less respiratory activity which mirrored the known changes in soil C and substrate quality. The short term (6 h) SIR of arginine, lysine, galactose and trehalose were significantly inhibited in the 2 year burn soils. The data suggest that a 4-year burn is a more sustainable practice for maintaining the original structure and function of the forest belowground ecosystem.	Campbell *et al.*, 2008
4	The aim of this study was to determine which substrates provided the highest discriminatory power for the CLPP assay; either individually or grouped into functional types.	Differences in soil CLPP between rehabilitation and non-mined forest soils were found to persist (up to 26 years post-mining) despite recovery of total microbial respiratory activity. The proportional response to complex substrates was consistently lower in rehabilitation than non-mined forest soil, suggesting a decreased functional diversity. Differences in CLPP between rehabilitation and non-mined forest soils were also due to variable responses to several carboxylic acids. Prescribed burning did not influence soil CLPP.	Banning *et al.*, 2012

sources are inserted, and the detection plate is a system which detects the evolved carbon dioxide using cresol as an indicator (Campbell *et al.*, 2003). The carbon sources used in MicroResp method are 16 and were selected to be ecologically relevant to soil and to be able to dissolve in water (Table 1).

The two plates are sealed face to face using a silicone rubber such that each well from the deep-well plate communicates with one from the

detection plate and is held together firmly with a metal clamp like in Fig. 1 (Chapman *et al.*, 2007).

The principle of indicator gel from detection plate is based on the chemical formula: $CO_2 + H_2O + HCO_3^- \rightarrow 2CO_3^- + 3H^+$

Cresol indicator changes its color from pink to yellow with decreasing pH of the gel. The advantage of this method is that the indicator is not in direct contact with the sample (Drage *et al.*, 2012).

Materials and methods

In order to make this review Google Scholar scientific database was used, which is an academic web search engine released in 2004, that indexes

Table 7. Objectives and results of assessed studies about CLPP

No.	Objectives	Results	Source
1	1. to determine C saturation concentrations with the MicroResp method by testing a gradient of C additions with a wide range of substrates. 2. to evaluate the potential influence of the amount of SOC on this threshold.	The soil organic carbon (SOC), total activity and catabolic evenness were similar for C substrate addition above 10% C added of SOC, suggesting microbial respiratory metabolism saturation. Below this threshold, CLPP were significantly altered, especially when the amount of SOC was low. This threshold corresponded to 4 up to 10 mgC mL^{-1}soil water which is 66-87% lower than used in the original approach.	Lerch *et al.*, 2013
2	It was investigated the relationship between soil physicochemical properties of microbial community structures (bacterial and fungal) and catabolic function.	Soil pH was identified as the key habitat-selective physicochemical soil property associated with variation in biological diversity and profiles of organic substrate utilization. With decreasing pH, the catabolism of common low molecular weight organic compounds (especially cysteine and aspartic acid) declined, however catabolism of two others (lysine and arginine) increased. The genetic structure of the bacterial communities in soil strongly correlated with pH and that of soil fungi with pH and sand percent.	Wakelin *et al.*, 2008
3	Determine the impacts of sample storage on microbial activity and community structure in paddy field soil.	Basal respiration was unusually increased in the drained soil after storage, but recovered after a 7-day re-incubation. In contrast, the impact on flooded soils was minimal. Further, the soil community-level physiological profile (CLPP) was affected by storage, but microbial community structure remained mostly unchanged. Sequencing also showed that α diversity of the flooded paddy soil was higher than that in the drained soil.	Wang *et al.*, 2014
4	1. compare soil microbial biomass, activity and community structure in vegetable systems between greenhouse and open field conditions under both conventional and organic management strategies. 2. determine whether soil chemical parameters and microbial properties were correlated.	Four treatments were examined and the impact on soils and their microbial communities: (1) organic management in greenhouses (Or-Gr) and (2) open fields (Or-Op) and (3) conventional management in greenhouses (Co-Gr) and (4) open fields (Co-Op). Or-Gr had significantly higher total, bacterial (both Gram-positive and negative), and fungal PLFA concentrations ($P < 0.05$) than the other treatments. Generally, soil quality followed the series Or-Gr > Or-Op > Co-Gr > Co-Op. Organic and greenhouse management had a significant interaction effect. Findings suggest that greenhouse management should be promoted for food security.	Ge *et al.*, 2013
5	Investigate the potentially ameliorative effects of lime, charcoal, and urea additions on soil nitrification and carbon substrate utilization (using the MicroResp method).	Multivariate analysis of the C source utilization data revealed that lime altered C substrate utilization more than urea or charcoal in these highly acidic soils. Results suggest that acidtolerant nitrifiers do exist in these soils and have potential for high activity, and pH (lime addition) and N-substrate (urea) most often increased nitrification. However, no single factor controlled nitrification in every soil, suggesting an interaction between abiotic and nitrifier community composition as a result of land use and soil type interactions.	Yao *et al.*, 2011
6	Identify possible impacts of timing and the type of tillage implement used in a strategic tillage (ST) on a long-term no-till (NT) farm with regards to soil productivity, physical, chemical and biological properties.	ST with either a chisel cultivator or a disc chain has great potential to assist in weed management as it did not statistically influence crop productivity or the physical, chemical and biological properties of the soil, regardless of the tillage timing. Further research is needed.	Liu *et al.*, 2016 a

No.	Objectives	Results	Source
7	Examine the influence of chisel and offset disc tillage on soil microbial properties of this long-term no-till (NT) Calcisol.	Relative to the NT, chisel tillage led to significant increases in microbial biomass carbon (+34.4%), abundances of Alphaproteobacteria (+74.6%), Bacteroidetes (+113.7%) and Firmicutes (+36.5%), and the utilization of D+ cellubiose (+178.4%) as well as mannitol (+167.2%) at 0–10 cm depth. In contrast, the influence of offset disc tillage was restricted to an increased abundance of Alphaproteobacteria (+64.6%) at 0–10 cm depth. Overall, one-time strategic tillage using either chisel or offset disc had a minor positive influence on soil biological attributes of the NT Calcisol 13 months after tillage.	Liu *et al.*, 2016 b
8	1. to determine the distribution of organic matter, microbial functions and diversity in soil and physically isolated fractions. 2. to determine how soil microbial functions and diversity were related to the accessibility and decomposability of organic C pool at the small scale.	Organic C availability drove microbial activity and functional diversity in particle and aggregate-size fractions, with the highest degree of soil functioning potential found in soils with the largest amount of organic matter. This pattern was dependent on the organic matter quality and accessibility, as well as microbial selection and distribution. Fine fractions, micro-aggregates and free particulate organic matter (F-POM) resulted to have specific physical and chemical characteristics, which induced a high degree of functional diversity.	Lagomarsino *et al.*, 2012
9	1. assess if commonly used restoration treatments (hydroseeding, fertilization and irrigation) affect soil microbial functional diversity and processes related to soil functioning (basal respiration, total N and P and in situ N availability rate). 2. assess what portion of plant effects on processes related to soil functioning is mediated indirectly by microbial functional diversity.	Results indicate that the restoration of recently built slopes can potentially be improved with treatments that promote plant compositional shifts, such as fertilization, or alter soil function, such as the enhancement of soil microbial functional diversity. They also highlight that plant-soil interactions are an important process that can be manipulated for restoration purposes in early-successional stages, especially in nutrient-poor semi-arid ecosystems.	Pablo *et al.*, 2011

academic journals, conference paper, books, dissertations, theses, abstracts and other academic literature.

Study selection was accomplished in two stages, firstly a number of seven keywords were set and used to make a search algorithm (Table 2). In the second stage of literature screening the search was made in the Google Scholar database using the five search algorithm set at the first stage and then, for each search algorithm, four relevant articles were set for inclusion.

Further, for inclusion of studies for data extraction, the screened studies had to assess microbial community from soil using as an indicator the soil respiration, which was assessed by MicroResp method, the article had to be written in English and the article should not be older than ten years.

Results and discussion

Table 2 presents the five search terms and the number of output results in the academic database.

Twenty studies were selected for data extraction and after analysis were divided into five categories:

- CLPP and fertilizers;
- CLPP and pollution;
- CLPP and crops;
- analysis of CLPP in forest soils;
- other studies to understand CLPP in soil

Table 3 presents the objectives and results for reviewed papers which assess the interaction between CLPP and fertilizers.

Table 4 presents the objectives and results for reviewed papers which assess the interaction between CLPP and pollution.

Table 5 presents the objectives and results for reviewed papers which assess the interaction between CLPP and crops.

Table 6 presents the objectives and results for reviewed papers which assess the CLPP in forest soils.

Table 7 presents the objectives and the results for reviewed papers which assess other ways to understand CLPP in soils.

Conclusions

Soil health is important for humanity because supports a large number of ecosystem goods and services. One way to maintain soil health is to study soil microbial community which is an indicator of soil wellness.

MicroResp is a method which can be used in range studies for understanding soil microbial community like studies on the effect of pollution on the community, effect of fertilizers, effect of plant or other treatments and soil management. However, this method can be used as it is or with small modifications which are more relevant for the aim of the research, like using other carbon sources.

After reviewing all the studies can be affirmed that MicroResp is an appropriate method, rapid and sensitive for determination of microbial community functional diversity.

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