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Original article

Optimising whole-soil multiple substrate-induced respiration (MSIR) of soil microbiota for large scale surveillance and monitoring

Thomas Peham [a,b,*], Alexander Bruckner [a]

[a] University of Natural Resources and Life Sciences Vienna, Gregor Mendel Straße 33, 1180 Vienna, Austria
[b] University of Innsbruck, Institute of Ecology, Technikerstraße 25, 6020 Innsbruck, Austria

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ABSTRACT

Multiple substrate-induced respiration is a method for characterising and assessing the functional diversity of soil microbiota. In this procedure, an array of simple organic substrates is added to soil samples, and the resulting multiple respiration values are used to give a functional description of the microbial community. In this study, we tested 44 substrates and five substrate concentrations for their ability to discriminate land use types and individual sites, specifically in large scale surveillance and monitoring programs. We assessed the concentrations with the coefficient of variation and found only little differences (less than 0.8 units) in the discriminative power of sites. Therefore we recommend using the amount equivalent to the substrate-induced respiration. In practice, most substrates performed well with respect to number of re-measurements, linearity of measurement curves and retail price. A Principle Components Analysis of all 44 substrates successfully ordinated land use types in distinct clusters and identified sites of unusual soil condition (e.g. especially wet or freshly fertilised sites). The discriminative power of 26 substrates was high (> 60% contribution to total variance) and substrates were equally appropriate to differentiate land use. Four optimum substrates were identified (threonine, malonic acid, quinic acid and pantothenic acid) that together explained 86% of the empirical data variation and yielded an almost identical ordination of sites as the full substrate set. Thus, the number of substrates in future studies can be considerably reduced. A resemblance matrix based on root exudates was highly (76%) correlated to a non-exudate matrix, indicating that root exudates were not better suited for community-level profiling than others. We discuss current measurement systems and suggest using more than just several grams of whole soil samples per measurement to adequately represent field conditions.

1. Introduction

Multiple substrate-induced respiration (MSIR) is a method for characterising and assessing the functional diversity of soil microbiota, and is one of several techniques for generating community-level physiological profiles (CLPPs). Usually, an array of carbon substrates of different chemical status (amino acids, amines, carbohydrates, carboxylic acids) is added to soil samples and the amount of produced CO_2 represents, for each substrate, the catabolic response of the whole microbial community. Multivariate analyses of the data set of all substrates can be used to discriminate sites, land use types, and various anthropogenic impacts on soil (e.g. [7,11,37]).

In a critical assessment, Ritz et al. [27] selected 21 out of 183 candidate methods for large scale soil monitoring programs, and concluded that MSIR is an appropriate indicator. We discern two practical problems with this method, both of which limit its applicability in large scale studies.

First, the number of substrates tested in the various other studies was large [4,25], and measurement campaigns were therefore laborious and expensive. The criteria for inclusion of specific substrates in the experiments were rarely given, and the substrate sets varied considerably among authors and papers, respectively. Degens and Harris [4] made their choice according to the strength and variability of the catabolic responses. Campbell et al. [9] suggested that the use of a lower number of "ecologically relevant" substrates (especially root exudates), might be more efficient and less expensive. Therefore, before MSIR can be adopted

* Corresponding author. University of Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria. Tel.: +43 512 507 39201; fax: +43 512 507 6190.
E-mail address: thomas.peham@uibk.ac.at (T. Peham).

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in large scale programs, a small set of substrates with high discriminative power should be identified.

Second, recommendations for optimal substrate concentrations differ from author to author (compare e.g. [4] and [8]). Further, the results of Lalor et al. [6] indicated that the concentration level affects the sensitivity of MSIR methods when differentiating among sites.

In this paper, we demonstrate that MSIR is a simple, reliable and inexpensive method to discriminate among the main land use types of temperate regions. The number of substrates may be considerably reduced without loss of discriminative power. We address practical aspects of substrate selection specifically for large scale surveillance and monitoring, recommend optimised concentrations, and discuss the applicability of various measurement systems.

2. Materials and methods

2.1. Study sites

The study sites were situated in Lower Austria, a warm-temperate, fully humid region with warm summers in Central Europe [29]. Five sites for each of three types of land use (arable land, grassland and forest) were chosen. The forest samples were further differentiated in litter and mineral horizons, giving a total of 20 site samples. Sites were selected to represent the wide range of soil conditions and cultivation practices of Lower Austria (see Table 1 for details).

Four of the five forest sites were located in the Austrian Natural Forest Network; they were analysed and described in Hackl et al. [12]. The fifth forest was chosen because of its extraordinary soil type (Andosol) and thick humus layer [5]. The grasslands were selected from a classification study by Lichtenecker et al. [1,2]. The field sites were located at the College for Agriculture Edelhof near Zwettl and selected because of the long term soil and cultivation data available.

The soil types were classified according to the World Reference Base of Soil Resources [20] and the plant communities according to Mucina et al. [28].

2.2. Field sampling and sample treatment

From April 16 to May 3, 2008, the top 5 cm of soil and forest litter were sampled within five replicate 1 m^2 plots along a transect at each site with a garden trowel. The samples were manually pooled to form composites and homogenised. Large debris was removed (roots, twigs, cones, etc.). The samples were cooled, immediately transferred to the laboratory, and kept in a cooling chamber at 2–4 °C until further treatment, for no more than one week. The soil samples were sieved to 2 mm and the forest litter samples to 4 mm, and stored in polyethylene bags at −17 °C.

pH, total organic carbon (C_{org}), conductivity,NO_3^-, NO_2^-, Cl^-, PO_4^{3-}, SO_3^{2-}, N_{tot} and C/N were determined according to Austrian standards (Ö-Norm L1062, L1080, L1082, L1083, L1084, L1085, L1092, L1099 und EN ISO 10304, respectively): pH in a suspension of 10 g soil in 25 ml 0.01 M $CaCl_2$; carbonate content with the Scheibler method; C_{org} in a combustion analyser (LECO SC 444) and corrected for carbonate; conductivity in a suspension of 10 g soil in 25 ml deionised water; water soluble nutrients in a percolate of 10 g soil in 50 ml deionised water, N_{tot} with the Kjeldahl method; the C/N as the ratio of C_{org} and N_{tot}. The water holding capacity (WHC) was measured according to Öhlinger [33]: 50 g of soil were saturated with water for 1 h, the surplus water was drained in

Table 1
Site characteristics of a study on MSIR of soil microbiota. Data on the soil from forest 1 according to Delvaux et al. [5]; soils and plant communities from forests 2 to 5 according to Hackl et al. [12]; meadows according to Lichtenecker et al. [1,2]; soils from fields according to the Austrian Soil Map 1:25.000, Bl. 19-4N; crops according to unpublished farm records. All other data are own measurements. EC: electrical conductivity, For Hum: forest litter layer, For Min: forest mineral soil, Grass: meadow, ND: not determined.

Site	Plant community/ crop	Soil type	pH ($CaCl_2$)	EC ($\mu S\ cm^{-1}$)	C_{org} ($mg\ g^{-1}$)	Total N ($mg\ g^{-1}$)	C/N ratio	Cl^- ($mg\ g^{-1}$)	NO_2^- ($mg\ g^{-1}$)	NO_3^- ($mg\ g^{-1}$)	PO_4^{3-} ($mg\ g^{-1}$)	SO_4^{2-} ($mg\ g^{-1}$)	Elevation (m a.s.l.)	Geographical location
For Hum 1	Culto-Piceetum	Andosol-Cambisol	3.0	56	362.9	14.6	24.9	31.0	ND	18.3	121.1	36.7	930	48°23N′ 15°03′E
For Hum 2	Luzulo-Fagenion	Cambisol	4.1	90	288.7	12.8	22.5	36.9	ND	6.5	211.2	47.0	550	48°32′N 15°33′E
For Hum 3	Euphorbio saxatilis-Pinetum nigrae	Leptosol	6.1	173	352.7	13.8	25.5	14.8	10.2	26.6	ND	19.0	554	47°59′N 16°10′E
For Hum 4	Carpinion	Planosol	6.3	232	157.2	10.9	14.5	46.3	7.5	23.7	32.1	62.9	270	47°58′N 16°41′E
For Hum 5	Pruno-Fraxinetum	Fluvisol	6.9	289	123.0	9.0	13.7	55.9	5.3	30.1	9.1	277.3	160	48°00′N 16°42′E
For Min 1	Culto-Piceetum	Andosol-Cambisol	3.2	62	121.6	6.1	20.0	11.1	ND	4.6	ND	26.0	930	48°23N′ 15°03′E
For Min 2	Luzulo-Fagenion	Cambisol	3.3	97	195.3	10.4	18.7	27.9	ND	2.9	93.0	52.8	550	48°32′N 15°33′E
For Min 3	Euphorbio saxatilis-Pinetum nigrae	Leptosol	6.6	321	259.9	12.4	21.0	13.9	9.8	20.1	ND	25.8	554	47°59′N 16°10′E
For Min 4	Carpinion	Planosol	6.1	108	73.1	6.5	11.3	11.3	6.0	24.8	3.7	24.1	270	47°58′N 16°41′E
For Min 5	Pruno-Fraxinetum	Fluvisol	7.0	203	56.3	4.2	13.3	8.8	11.4	59.9	2.4	36.8	160	48°00′N 16°42′E
Grass 1	Arrhenaterion-Poetosum trivialis	Cambisol	6.7	169	56.3	4.5	12.5	7.0	9.8	22.6	0.9	13.9	700	48°23′N 15°18′E
Grass 2	Phyteumo-Triseton	Cambisol	5.1	211	39.3	3.9	10.1	281.3	ND	18.1	4.1	85.8	760	48°24′N 15°17′E
Grass 3	Caricion davallianae	Cambisol	5.9	78	117.6	9.1	12.9	28.8	1.6	16.0	ND	65.8	760	48°24′N 15°17′E
Grass 4	Arrhenateretalium of unspecific phytosociological rank	Cambisol	5.3	25	34.5	3.1	11.1	2.5	0.3	13.0	ND	6.7	850	48°24′N 15°17′E
Grass 5	Kolerio-phleetatio phleoides	Leptosol	6.1	64	48.9	3.6	13.4	4.7	6.9	14.1	ND	8.5	720	48°25′N 15°19′E
Field 1	Oat	Gleysol	5.9	69	15.9	1.9	8.6	1.9	ND	94.9	27.1	5.9	610	48°36′N 15°12′E
Field 2	Fodder (grass and clover), organic	Gleysol	5.6	31	30.9	2.7	11.6	2.6	0.5	13.4	4.7	4.9	610	48°36′N 15°13′E
Field 3	willow short rotation forest, organic	Cambisol	6.4	78	28.2	2.8	10.1	2.4	1.6	35.4	13.4	7.3	610	48°36′N 15°13′E
Field 4	Wheat	Cambisol	5.4	86	23.3	2.5	9.3	1.6	0.3	186.5	19.7	6.8	610	48°36′N 15°13′E
Field 5	Wheat	Cambisol	4.7	65	19.5	1.8	10.9	1.8	0.2	126.8	5.0	7.9	610	48°36′N 15°13′E