Discriminatory power of MicroResp™ analyses across variable spatial scales in semiarid ecological zones

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Abstract

Indirect assessment of enzymatic activity potential via substrate induced respiration is a common tool used to evaluate variability in soil microbial activity induced by environmental or management variables. The MicroResp™ method for total soil sample catabolic profiling has been employed to estimate divergence in microbial activities under contrasting conditions. Here we evaluate the potential for the method to discriminate across samples exposed to similar ecological conditions at distinct geographical locations, and across samples exposed to distinct soil management conditions. Samples used in this report came from four spatially distinct arid or semi arid locations in New Mexico, USA and Jordan. For all scales of comparison, similar conditions such as rhizosphere and non-rhizosphere soils, disturbance levels, soil depth and distance from plants were considered as factors. Catabolic profiles vary within and between sampling locations partially as a function of soil characteristics as induced by proximity to plants, sampling depth and land disturbance status.

Hypothesis

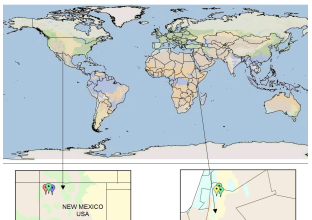
Microbial catabolic profiles (community level physiological profiling by substrate induced respiration - CLPP-SIR) in arid soils are sensitive to relatively minor climatic variations and also to changes in soils' physico-chemical parameters induced by human activities.

Methods

Soil Sampling

The project compared microbial activity profiles at four arid locations, two in New Mexico, USA and two in the Al-Badia region of Jordan. Soil was sampled at three undisturbed sites at the Jornada Experimental Range in the Northern Chihuahuan (NC) desert and on undisturbed areas and lands remediated following surface coal mining at Farmington, NM on the tablelands of the Arizona/New Mexico Plateau (ANMP). Similar soil sampling was also conducted on two distinct arid zone locations in Al- Badia representing semi-desert steppe rangelands in Jordan, one grazed location located on the research station of the Jordan University of Science and Technology one undisturbed location at the Al-Khanasri research station. Each sampling site was centered on a four-wing saltbush (*Atriplex* spp) plant and within a 1 m radius around the plant. At each site, rhizosphere (root) and non-rhizosphere soil samples were collected. Non-rhizosphere soils were sampled, along three transects per site, at 30 cm and 60cm from the central *Atriplex* plant. For each sample point on each transect we collected the topsoil crust (0.5cm), the 0.5cm to 5cm, and the 5cm to 25cm layers. Rhizosphere soil (soil loosely adhering to roots) was collected for each plant species within 100 cm radius of the central *Atriplex* shrub.

Physical and chemical parameters of soils were estimated using standard methodology at an external laboratory.



Undisturbed Remediated 1985 Remediated 2006 Grazed

Key to sites and disturbance regimes:

Disturbance	Location
Undisturbed	Northern Chihuahua (NC) desert (Jornada Experimental Range), NM, USA
Undisturbed	Tablelands of the Arizona/New Mexico Plateau, (Farmington), NM, USA
Remediated following surface coal mining since 1985	Tablelands of the Arizona/New Mexico Plateau, (Farmington), NM, USA
Remediated following surface coal mining since 2006	Tablelands of the Arizona/New Mexico Plateau, (Farmington), NM, USA
Grazed	Al-Badia Jordan University of Science and Technology (JUST), Jordan
Undisturbed	Northern Al-Badia Jordan University of Science and Technology (JUST), Jordan
Undisturbed	Northern Al-Badia, Al-KHANASRI, Jordan

Community level physiological profile - Substrate induced respiration (CLPP-SIR)

For each soil sample, respiration due to microbial activity was measured using the microtitre-plate based respiration system, MicroResp® as described in the MicroResp™ Technical Manual (Macaulay Institute, Craigiebuckler, Aberdeen, AB 15 8QH, Scotland, UK) and by Campbel et al., 2003. The MicroResp™ system consists of a 96-well "Deepwell" plate in which moisture corrected soil samples (at the soil's field capacity for water) amended with various substrates are placed. The plate is separated from a 96 well microtiter plate containing a colorimetric CO_2 detection gel by a PTFE lined porous rubber seal. The system is incubated at 25° C for 6 hours followed by spectrophotometry readings carried out at 570 nm.

However, CO_2 may be released abiotically even in the absence of microbial activity, especially common when acid substrates are added to soils high in $CaCO_3$ in arid zones. Calibration and correction for this abiotic CO_2 was carried out as described by Oren and Steinberger (2008). For this, fumigated soil aliquots were tested using the MicroResp protocol as described above. The hourly CO_2 production rate obtained here was deducted from the total fresh soil rate to obtain the biotic hourly respiration rate.

Microresp substrates

Simple Sugars	Amino acids	Carboxylic acids	Polymeric sugar	Fatty acid ester polymer
Glucose	L-Alanine	Malic	Cellulose	Tween 80
Fructose	L-Cisteine	Ascorbic		
Mannose	L-Lysine	Fumaric		
	L-Histidine	Protocatechuic		
	L-phenylalanine			

Data Analysis

Soil Microbial community diversity based on catabolic activity from different locations was compared using analysis of variance (ANOVA) and principal component analyses (PCA) using both Minitab® 16 and GenStat® Release 11.1 software.

Results

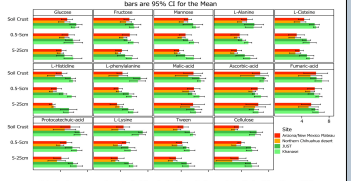
1. Discriminating across locations

Potential for C-substrates (MicroResp CLPP-SIR) to discriminate among sampling locations (non-rhizosphere soils)

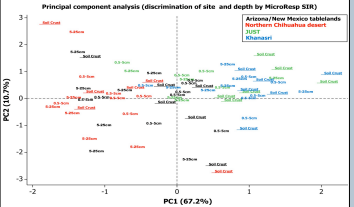
Substrate	Significance of factors (α=0.05)			
	Location	Sample depth	Distance from central Atriplex	Adjusted R^2
L-Cisteine	<0.001***	<0.001***	0.331 ns	71%
L-Histidine	<0.001***	0.004***	0.176 ns	71%
Tween	<0.001***	0.001***	0.424 ns	70%
L-Lysine	<0.001***	<0.001***	0.977 ns	65%
L-Alanine	<0.001***	<0.001***	<0.001***	64%
Cellulose	<0.001***	0.302 ns	0.858 ns	62%
L-Phenylalanine	<0.001***	0.004***	0.865 ns	59%
Protocathecuic acid	<0.001***	0.001***	0.936 ns	59%
Mannose	<0.001***	<0.001***	0.019 *	57%
Fructose	<0.001***	<0.001***	0.416 ns	48%
Glucose (basal respiration)	<0.001***	0.003 **	0.747 ns	39%
Fumaric acid	0.015 ns	0.368 ns	0.028 *	22%
Malic acid	0.043 *	0.552 ns	0.209 ns	13%
DI Water	0.053 ns	0.275 ns	0.513 ns	13%
Ascorbic acid	0.329 ns	0.064 ns	0.192 ns	8%

NOTES:
¹ Balanced ANOVA analyses

Substrate induced respiration in non-rhizosphere soils - Sampling site and depth
bars are 95% CI for the Mean



Principal component analysis (discrimination of site and depth by MicroResp SIR)



Principal component analysis of MicroResp CLPP-SIR profiles of non-rhizosphere soils across tested locations.

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Protocatechuic-acid	0.902	-0.098	-0.102	-0.056	0.083	0.338
L-Cisteine	0.896	0.143	-0.041	0.205	0.182	-0.045
L-Lysine	0.888	0.104	-0.247	-0.065	-0.067	0.103
L-phenylalanine	0.883	0.159	-0.088	0.119	-0.011	0.006
Tween	0.875	0.204	-0.245	-0.097	0.004	-0.076
Mannose	0.865	0.198	0.307	0.064	-0.027	0.057
L-Histidine	0.862	0.169	-0.176	0.089	0.221	-0.233
Fructose	0.853	0.079	0.362	-0.066	-0.12	-0.146
Glucose	0.841	0.063	0.163	-0.021	-0.468	0.033
L-Alanine	0.793	0.306	0.32	0.186	0.215	0.174
Cellulose	0.761	0.38	-0.32	-0.114	-0.18	-0.085
Water	0.739	-0.196	0.237	-0.523	0.211	-0.084
Malic-acid	0.738	-0.584	-0.011	0.127	0.024	-0.216
Fumaric-acid	0.698	-0.595	-0.188	-0.083	0.024	0.183
Ascorbic-acid	0.648	-0.664	0.049	0.211	-0.104	-0.04
Variance	**10.084**	**1.599**	**0.723**	**0.479**	**0.463**	**0.339**
%Variance	**0.672**	**0.107**	**0.048**	**0.032**	**0.031**	**0.023**
Cumulative %Var.	**0.672**	**0.779**	**0.827**	**0.859**	**0.89**	**0.913**

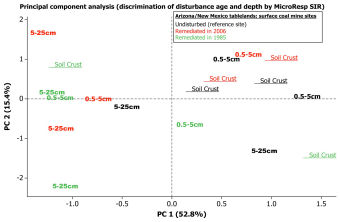
2. Discriminating across disturbance status

Capability of the tested C-substrate (MicroResp CLPP-SIR) for discriminating remediation status (non-rhizosphere soils) [1, 2]

Substrate	Significance of factors (α=0.05)			
	Sample depth	Distance from central Atriplex	Age of remediation	Adjusted R^2
L-Alanine	0.001***	0.007 **	0.001***	77%
Mannose	<0.001***	0.333 ns	0.001***	77%
L-Cisteine	0.057 ns	0.85 ns	0.033 *	41%
L-Histidine	0.011 *	0.837 ns	0.288 ns	40%
Fructose	0.04 *	0.834 ns	0.68 ns	38%
Glucose (basal respiration)	0.073 ns	0.519 ns	0.145 ns	28%
Tween	0.064 ns	0.744 ns	0.23 ns	24%
Water	0.447 ns	0.151 ns	0.107 ns	21%
Protocathecuic acid	0.138 ns	0.448 ns	0.349 ns	13%
L-Lysine	0.076 ns	0.965 ns	0.631 ns	12%
L-Phenylalanine	0.258 ns	0.307 ns	0.924 ns	0%
Fumaric acid	0.641 ns	0.217 ns	0.453 ns	0%
Malic acid	0.985 ns	0.239 ns	0.349 ns	0%
Cellulose	0.636 ns	0.968 ns	0.305 ns	0%
Ascorbic acid	0.878 ns	0.476 ns	0.429 ns	0%

NOTES:
¹ Balanced ANOVA analyses
² Surface coal mine: Reference undisturbed site, 1985 remediated and 2006 remediated (Arizona/New Mexico tablelands)

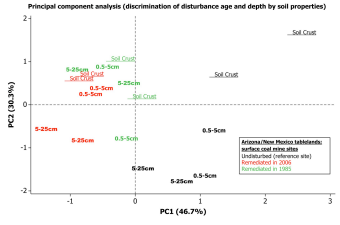
Principal component analysis (discrimination of disturbance age and depth by MicroResp SIR)



Principal component analysis of MicroResp CLPP-SIR profiles of non-rhizosphere soils; soil disturbance analysis

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Fructose	0.874	0.263	-0.038	-0.093	0.245	-0.115
Glucose	0.865	0.09	-0.247	0.126	-0.292	-0.024
Protocatechuic-acid	0.832	-0.166	-0.109	-0.171	-0.399	0.001
Water	0.828	0.034	-0.35	0.198	-0.004	0.187
Mannose	0.817	0.356	0.225	0.045	-0.279	-0.099
Fumaric-acid	0.774	-0.512	-0.137	0.154	-0.079	0.074
L-Histidine	0.772	0.153	0.452	-0.169	0.14	0.142
L-Lysine	0.739	0.068	-0.495	-0.079	0.228	-0.295
L-Alanine	0.695	0.249	0.543	0.134	-0.198	-0.209
L-phenylalanine	0.689	-0.002	-0.138	-0.561	-0.073	0.397
Tween	0.669	0.553	-0.203	-0.159	0.318	-0.032
L-Cisteine	0.65	0.128	0.531	0.163	0.249	0.409
Malic-acid	0.603	-0.702	0.004	0.21	0.17	-0.006
Cellulose	-0.043	0.689	-0.269	0.629	-0.034	0.159
Ascorbic-acid	0.65	-0.665	0.095	0.273	0.146	-0.069
Variance	**7.917**	**2.3165**	**1.4341**	**1.0529**	**0.7199**	**0.5712**
%Variance	**0.528**	**0.154**	**0.096**	**0.07**	**0.048**	**0.038**
Cumulative % var.	**0.528**	**0.682**	**0.778**	**0.848**	**0.896**	**0.934**

Principal component analysis (discrimination of disturbance age and depth by soil properties)

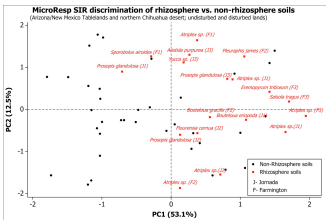


Principal component analysis of Soil Parameters of non-rhizosphere soils; soil disturbance analysis.

Variable	PC1	PC2	PC3	PC4	PC5
% Organic matter	0.952	0.17	-0.098	0.198	0.092
P(NaHCO$_3$, extracted; mg kg^{-1})	0.924	0.172	0.092	0.275	0.016
Available K (ppm)	0.799	0.416	-0.105	-0.289	0.279
CEC	0.799	-0.482	-0.032	0.208	-0.192
SAR	0.33	-0.886	0.003	-0.129	0.059
EC (mS cm^{-1})	0.536	-0.75	0.005	-0.333	-0.07
pH	-0.456	-0.714	0.349	0.254	0.284
% $CaCO_3$ equivalents	0.310	0.324	0.879	-0.133	-0.078
Variance	**3.7361**	**2.4275**	**0.9254**	**0.4519**	**0.2186**
% Variance	**0.467**	**0.303**	**0.116**	**0.056**	**0.027**
Cumulative % Variance	**0.467**	**0.77**	**0.886**	**0.942**	**0.969**

3. Discriminating between rhizosphere or non-rhizosphere

MicroResp SIR discrimination of rhizosphere vs. non-rhizosphere soils
(Arizona/New Mexico Tablelands and northern Chihuahua desert; undisturbed and disturbed lands)



Principal component analysis of MicroResp CLPP-SIR profiles of Rhizosphere vs. Non-Rhizosphere soils
(Arizona/New Mexico tablelands and northern Chihuahua desert)

Variable	PC1	PC2	PC3	PC4	PC5	PC6
Water	0.879	0.048	0.105	0.159	0.131	0.02
Fructose	0.837	-0.332	0.136	-0.1	-0.057	0.261
Glucose	0.832	-0.085	0.298	0.091	-0.021	-0.226
Fumaric-acid	0.787	**0.472**	0.02	0.137	-0.175	-0.161
Mannose	0.761	-0.204	0.367	-0.139	0.252	-0.099
Ascorbic-acid	0.746	**0.533**	-0.059	0.14	0.159	0.066
Protocatechuic-acid	0.743	0.062	-0.002	-0.02	-0.557	-0.258
L-Lysine	0.738	-0.231	0.126	0.426	-0.093	0.326
L-phenylalanine	0.709	0.291	-0.33	-0.135	-0.037	-0.084
L-Cisteine	0.707	-0.115	-0.381	-0.244	0.199	0.254
L-Alanine	0.691	-0.202	0.406	-0.39	0.185	-0.128
Tween	0.688	**-0.544**	-0.18	0.117	-0.287	0.159
L-Histidine	0.683	-0.111	-0.447	-0.439	-0.024	-0.083
Malic-acid	0.68	**0.63**	-0.06	0.147	0.21	0.11
Cellulose	0.267	**-0.554**	-0.389	0.445	0.334	-0.387
Variance	**7.970**	**1.877**	**1.079**	**0.939**	**0.774**	**0.615**
% Variance	**0.531**	**0.125**	**0.072**	**0.063**	**0.052**	**0.041**
Cumulative %Var	**0.531**	**0.656**	**0.728**	**0.791**	**0.843**	**0.884**

Summary

1. The CLPP profiles were clearly distinguishable across the tested geographical locations. The more clayey Jordan soils induced greater respiration rates for all tested substrates. Aminoacids, sugars and the fatty acid ester polymer have induced significant differences in the respiration rates. While respiration induced by the tested organic acids were was the greatest the variability associated with their use precluded them to act as good discriminators across sites.

Discrimination across sampling depths was also best described by the aminoacids, sugars and the fatty acid ester polymer. While the crust of the Jordanian soils were the most actively respiring the same was not as necessarily true for the New Mexico soils.

The two distances from the center of each sampling unit (30 and 60cm) did not induce any significant differences in catabolic profiles.

2. Disturbance status has been evaluated here for the surface coalmine remediated areas in the Arizona/New Mexico tablelands. L-alanine, commonly exudated in the rhizosphere, was the only substrate able to statistically differentiate between sample depth, distance from plant and age of remediation. Remediation age was also predicted by mannose and L-cistine. Again, other substrates induced more respiratory activity (see the PCA table) but also greater intra-site variability and thus did not allow for statistically significant differentiation among the remediated and natural sampling points.

3. Rhizosphere soils were clearly more capable of utilizing easily accessible sugars and organic acids, common root exudates. This was true for both natural and disturbed systems. There is also possible that SIR varied consistently across sampling locations but may have been more similar within each sampling unit even as plant species varied.

Fast degradable substrates can distinguish between rhizosphere and non-rhizosphere soils across small scales. Slightly more stable compounds such as aminoacids or fatty acids esters are better descriptors on non-rhizosphere soils catabolic potential differences across small to large scales while cellulose degradation did induce significantly differential respiration only across large spatial scales.

While there is also a possibility that host plant species may induce catabolic profile variability our data set did not allow it to be tested.

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