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SHORT COMMUNICATION

Choice of methods for soil microbial community analysis: PLFA maximizes power compared to CLPP and PCR-based approaches

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Summary

Polyphasic studies that used phospholipid fatty acid analysis (PLFA) in conjunction with community level physiological profiling (CLPP) or PCR-based molecular methods were analyzed in order to evaluate the power of each strategy to detect treatment effects on soil microbial community structure (MCS). We found no studies where CLPP or PCR-based methods differentiated treatments that were not also differentiated by PLFA. In 14 of 32 studies (44%), PLFA differentiated treatments that were not resolved by CLPP analysis. In 5 of 25 studies (20%), PLFA differentiated treatments that were not resolved by PCR-based methods. We discuss PLFA, CLPP, and PCR-based methods with respect to power to discriminate change in MCS versus potential for characterization of underlying population level changes.

The response of soil microbial community structure (MCS) to perturbation is of interest to researchers seeking biologically relevant variables in experimentally or naturally altered ecosystems. For the current discussion, MCS is defined as the number and relative abundance of microbial populations in soil. Three strategies for the elucidation of treatment effects on MCS dominate contemporary Microb. Ecol.: community level physiological profiling (CLPP), phospholipid fatty acid analysis (PLFA), and PCR-based methods such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), and randomly amplified polymorphic DNA (RAPD) (Øvreås, 2000). Independent assessments have indicated that each approach returns similar results with respect to the demonstration of

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treatment effects (Widmer et al., 2001; Ritchie et al., 2000). The relative power of each to elucidate treatment effects has rarely been compared. In one study, PLFA was demonstrated to be more sensitive than CLPP and a PCR-based method (guanine plus cytosine ratio) to changes in MCS across a gradient of grassland management intensities (Grayston et al., 2004). In another study, the ability of PLFA and a molecular method, length heterogeneity PCR (LH-PCR), to resolve the effects of tillage and ground cover on MCS were compared using discriminant analysis (Dierksen et al., 2002). In that study, the inclusion of molecular data into the discriminant analysis did not improve predictive power of the analysis above that which was achieved using PLFA data alone. This study raises the hypothesis that using a polyphasic approach to detect change in MCS is no more useful than PLFA data alone. Here, we tested this hypothesis by searching for studies that used PLFA in conjunction with CLPP or PCR-based methods in order to evaluate the question: Has CLPP or a PCR-based method been used to detect a treatment effect on MCS that was not also detectable by PLFA?

Searches of the Web of Science and CSA Illumina databases with various combinations of the words PLFA, FAME, CLPP, fatty acids, T-RFLP, Biolog®, DNA, PCR, 16s, rDNA, DGGE, TGGE, gel electrophoresis, soil, community structure, and polyphasic returned 53 studies that used PLFA in conjunction with CLPP or PCR-based methods to identify treatment effects on MCS. While not exhaustive, the highest impact factor soils journals were among the journals included (see references in Table 1). Therefore, the sample should represent the current state of knowledge. Papers in which PCR-based methods were used to track specific populations either by DGGE band excision and sequencing or by the use of primer sets specific to phylogenetic groups were not considered to be demonstrations of change in MCS unless including a general test of significant difference (or correlation) at the total community level.

No studies were found where CLPP or PCR-based analyses were used to differentiate a treatment effect on soil MCS that was not also identified by PLFA of the same samples. Conversely, in 14 of 32 studies (44%), PLFA differentiated treatments that were not resolved by CLPP analysis of the same samples. In 5 of 25 studies (20%), PLFA differentiated treatments that were not resolved by a PCR-based method. These studies are arranged categorically in Table 1. In the five studies where PCR-based methods were unable to detect differences detected by PLFA, the specific PCR-based methods used were LH-PCR, DGGE (twice), RISA, and DNA RAPD (Dierksen et al., 2002; Thirup et al., 2003; Leckie et al., 2004; Ritz et al., 2004; Suhadolc et al., 2004). If the MCS changes detected by PLFA are real in all cases, our analysis implies that studies using only CLPP or a PCR-based method incur a type II error rate of approximately 44% and 20%, respectively.

Of the three general strategies for detecting MCS changes, PCR-based methods are used in a higher proportion of studies than PLFA or CLPP (Fig. 1), probably because PCR-based methods offer the greatest potential for characterization of underlying population level changes. However, the power of PCR-based methods to resolve treatment effects on the total soil microbial community may be limited compared to PLFA because less statistically relevant information can be gained from pattern analysis of PCR-generated fingerprint patterns than from PLFA profiles. One explanation of this is that in a typical DGGE analysis, 20–50 detectable and quantifiable bands may vary in intensity by one or two orders of magnitude (due to detection and imaging limitations), while in a typical PLFA profile more than 70 continuous variables (PLFA peaks) can be detected in concentrations ranging over at least 3 orders of magnitude. Further, quantitative estimates of population densities gleaned from community level analyses must be considered carefully due to so-called "PCR bias" introduced by the exponential amplification of DNA targets. Rarefaction analysis of molecular data allows estimates of relative population abundance within a sample (e.g. Basiliko et al., 2003). Still, quantification of change in the abundance of individual populations requires support from additional analyses, such as species/group specific quantitative PCR (Yu et al., 2005).

CLPP produces large numbers of continuous variables and so should be highly sensitive to change in MCS. However, CLPP requires growth of microbes on carbon substrates in microtiter plates (i.e. metabolism). Many organisms present in soil will not grow in the wells and, conversely, organisms growing in the wells may not have been active in the soil. Also, not all substrates catabolized by soil microbes are represented. Thus, CLPP probably loses sensitivity due to a bias toward underrepresenting metabolic diversity.

It hence appears that PLFA offers the most powerful approach to demonstrating change in MCS, and that monophasic studies relying on CLPP or PCR-based methods are prone to high type II error rates. On the other hand, PLFA offers limited insight into changes in specific microbial populations. While certain PLFAs can be used as biomarkers for specific populations (White and Ringelberg, 1998), the resolution of population level change

Table 1. Authors, year, and treatment type of polyphasic studies in which PLFA was used in conjunction with a molecular method or CLPP

Studies in which PLFA resolved a treatment effect that molecular methods did not		Studies in which PLFA resolved a treatment effect that CLPP did not	
Dierksen et al. (2002)	Methods comparison	Bååth et al. (1998)	Contamination
Leckie et al. (2004)	Plant cover type/nutrient availability	Bossio et al. (2005)	Land use change
Ritz et al. (2004)	Spatial structure	Bundy et al. (2004)	Contamination
Suhadolc et al. (2004)	Heavy metals	Entry et al. (2004)	Land use
Thirup et al. (2003)	Introduced bacteria and fungicide	Grayston et al. (2001)	Cover-type gradient
Studies in which PLFA and CLPP resolved the same treatment effect		Ibekwe and Kennedy (1998)	Methods comparison
Bossio et al. (2005)	Plant cover type	Ibekwe et al. (2001)	Fumigants
Cahyani et al. (2003)	Succession	O'Donnell et al. (2001)	Cover type/fertilizers
Cahyani et al. (2004)	Succession	Pankhurst et al. (2001)	Salinity/alkalinity
Clegg et al. (2003)	Plant cover type	Priha et al. (2001)	Land use
Ebersberger et al. (2004)	Global change	Priha et al. (1999)	Cover type
Griffiths et al. (1997)	Contamination	Soederberg et al. (2002)	Cover type
Griffiths et al. (1999)	Nutrient availability	Thirup et al. (2003)	Succession
Hernesmaa et al. (2005)	Plant cover type	Yao et al. (2000)	Land use
Ibekwe et al. (2002)	Plant cover type	Studies in which PLFA and molecular methods resolved the same treatment effect	
Ibekwe et al. (2001)	Fumigants	Broughton and Gross (2000)	Productivity gradient
Kandeler et al. (2000)	Contamination	Certini et al. (2004)	Spatial structure
Landeweert et al. (2003)	Methods comparison	Collins and Cavigelli (2003)	Elevation gradient
MacNaughton et al. (1999)	Contamination	Ellis et al. (2001)	Methods comparison
Miethling et al. (2000)	Plant cover type/spatial structure	Fang et al. (2001)	Cover type
Patra et al. (2005)	Plant cover	Fritze et al. (2000)	Contamination
Ritchie et al. (2000)	Methods comparison	Kelly et al. (1999)	Contamination
Suzuki et al. (2005)	Plant cover type	Macdonald et al. (2004)	Cover type
Turpeinen et al. (2004)	Contamination	Miethling et al. (2000)	Land use/inoculation
Widmer et al. (2001)	Methods comparison	Pankhurst et al. (2002)	Spatial structure
Yang et al. (2003)	Succession	Pietikaeinen et al. (2000)	Fire
		Ritz et al. (2004)	Spatial structure
		Schutter et al. (2001)	Land use
		Schutter and Dick (2001)	Carbon substrates
		Siciliano and Germida (1998)	Inoculants
		Siciliano et al. (1998)	Cover type
		Thompson et al. (1999)	Contamination
		Widmer et al. (2001)	Methods comparison

within communities is coarse due to several factors including: (1) overlap exists in the PLFA composition of microorganisms; (2) determination of signature PLFAs for specific microbes requires their isolation in pure culture; and (3) PLFA patterns for individual populations can vary in response to environmental stimuli. Therefore, where population level information is needed, PCR-based methods offer avenues for hypothesis testing not available through PLFA.

In some situations, it is more important to employ the most powerful method of demonstrating a treatment effect on MCS than it is to use a method that provides information on underlying changes in microbial populations. Statistical power depends on effect size, variability, and sample size, and could also be related to the number of response variables in a multivariate analysis. Hence there are a number of possible reasons for the observed difference in power for

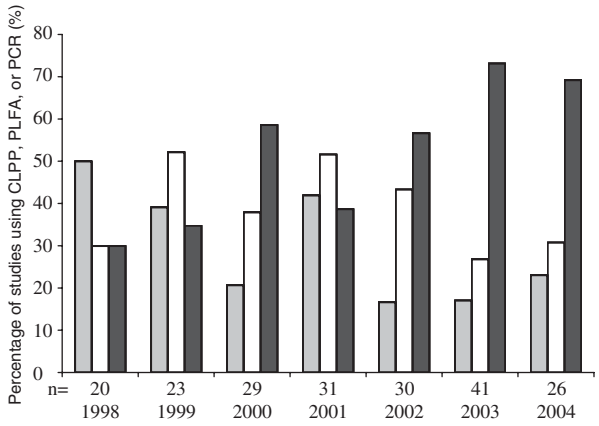

Figure 1. Percentage of soil microbial community structure studies using CLPP (light gray bars), PLFA (open bars), and PCR-based methods (dark gray bars), by year, from 1998 to 2004. Results were compiled from a search of CSA Illumina database. The search terms "soil" and "microbial community structure" returned 250 studies, of which 200 (80%) used one of the three major strategies for the determination of microbial community structure.

the various strategies, including the number of response variables a method yields, data type (continuous vs. presence/absence), and cultivation or PCR biases. Especially in situations where it is important to minimize type II errors (such as questions involving pollution effects), a hierarchical analysis approach may be warranted: first, PLFA would be used to detect treatment differences, and subsequently (if an effect was found) a PCR-based method for resolving particular populations involved in the detected response. For example, Callaway et al. (2004a) used PLFA to show that microbial communities differed between rhizospheres of native grasses and an invasive weed. Then Callaway et al. (2004b) used a PCR-based method to explore the effects of differences in microbial communities between rhizospheres of the weed grown in its native soil and in soil from its invaded range in a situation where further identification of populations could have been valuable (Callaway et al., 2004b). Particularly in soil ecology, lack of power is a significant problem (e.g. Klironomos et al., 1999) that can be exacerbated by a choice of method that does not maximize power.

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