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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