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Microbial Abundances in Salt Marsh Soils: A Molecular Approach for Small Spatial Scales

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The rate of biological decomposition greatly determines the carbon sequestration capacity of salt marshes. Microorganisms are involved in the decomposition of biomass and the rate of decomposition is supposed to be related to microbial abundance. Recent studies quantified microbial abundance by means of quantitative polymerase chain reaction (qPCR), a method that also allows determining the microbial community structure by applying specific primers. The main microbial community structure can be determined by using primers specific for 16S rRNA (*Bacteria*) and 18S rRNA (*Fungi*) of the microbial DNA. However, the investigation of microbial abundance pattern at small spatial scales, such as locally varying abiotic conditions within a salt-marsh system, requires high accuracy in DNA extraction and qPCR methods. Furthermore, there is evidence that a single extraction may not be sufficient to reliably quantify rRNA gene copies. The aim of this study was to establish a suitable DNA extraction method and stable qPCR conditions for the measurement of microbial abundances in semi-terrestrial environments.

DNA was extracted from two soil samples (top 5 cm) by using the PowerSoil DNA Extraction Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) and applying a modified extraction protocol. The DNA extraction was conducted in four consecutive DNA extraction loops from three biological replicates per soil sample by reusing the PowerSoil bead tube. The number of *Fungi* and *Bacteria* rRNA gene copies of each DNA extraction loop and a pooled DNA solution (extraction loop 1 - 4) was measured by using the qPCR method with taxa specific primer pairs (*Bacteria*: B341F, B805R; *Fungi*: FR1, FF390).

The DNA yield of the replicates varied at DNA extraction loop 1 between 25 and 85 ng μL^{-1} and decreased at extraction loop 4 with values between 11 and 37 ng μL^{-1}. The DNA extraction purification factor (260/230 nm ratio) decreased with DNA extraction loops while DNA extraction quality (260/280 nm ratio) was constant. The number of *Fungi* rRNA gene copies ranged from 6 to 21 $\cdot 10^7$ gene-copies g (dry wt) of $soil^{-1}$ after four DNA extraction loops while the number of *Bacteria* rRNA gene copies ranged from 399 to 643 $\cdot 10^7$ gene-copies g (dry wt) of $soil^{-1}$. The cumulative number of gene copies increased from the first to the fourth DNA extraction loop in a linear way in *Fungi* ($r^2 > 0.99$) and towards an asymptote in *Bacteria* ($r^2 > 0.98$) .

We propose that determining microbial abundances and community structure by one DNA extraction followed by qPCR is sufficient for relative comparisons at small spatial scales. Still, the determination of the number of gene copies in qPCR runs can be influenced by several sources of inaccuracy, e.g., PCR inhibition, primer specific PCR annealing failure, or low qPCR efficiency values when calculating the number of gene copies employing a standard curve. Therefore, further investigations are necessary to determine accuracy limiting factors in more detail and to verify the qPCR results by means of the phospholipid fatty acid method (PLFA).